4.4	**Ophthalmoscopic examination**	No treatment-related ophthalmological findings were observed	
4.5	**Blood analysis**		
4.5.1	Haematology	No treatment-related haematological findings were observed	
4.5.2	Clinical chemistry	Reductions (of the order of 3 – 45 %) in whole blood cholinesterase activity were frequently recorded throughout the study in animals receiving 500 ppm in comparison to respective controls. Additionally, top dose animals were found to have statistically significantly reduced (by approximately 26 and 34 % at weeks 52 and 104 respectively) brain cholinesterase levels compared to their respective controls. A 20 % decrease in brain cholinesterase activity was also seen at 104 w, in the 100 ppm group. No overt clinical/behavioural signs usually associated with cholinesterase inhibition were observed. No other treatment-related clinical chemistry changes were noted at any dose level.	X
4.5.3	Urinalysis	No treatment-related urinalysis findings were observed	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	No dose related changes were seen.	
4.6.2	Gross and histopathology	No treatment-related histopathological findings were observed	
4.7	**Other**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Beagle dogs (8 animals/sex/group) were dosed with bendiocarb (98.1 to 99 % purity) in the diet at 0, 20, 100 or 500 ppm (equivalent to an average dose of 0, 0.65, 3.12 and 16.24 mg kg^{-1} d^{-1}). The total duration of the study was 104 w with an interim kill performed after 52 w. Ophthalmoscopy, clinical biochemistry, haematology and whole blood cholinesterase activity determinations were conducted at various times during the study (weeks 14, 25, 52, 65, 79, 90, 96 and 103 for ophthalmoscopy, weeks 14, 25, 51, 79 and 103 for clinical biochemistry and haematology and weeks 7, 14, 25, 40, 43, 51, 79, and 103 for whole blood cholinesterase). Gross and histopathological examinations, together with brain cholinesterase activity determinations, were conducted after 52 and 104 w of treatment.	

5.2	**Results and discussion**	Two deaths occurred during the study, one in the control and one in the high dose groups, although neither was considered to be treatment-related. No treatment-related clinical signs of toxicity were noted. Reductions (of the order of 3 – 45 %) in whole blood cholinesterase activity were frequently recorded throughout the study in animals receiving 500 ppm in comparison to respective controls. Additionally, top dose animals were found to have statistically significantly reduced (by approximately 26 and 34 % at weeks 52 and 104 respectively) brain cholinesterase levels compared to their respective controls. A 20 % decrease in brain cholinesterase activity was also seen at 104 w, in the 100 ppm group. No overt clinical/behavioural signs usually associated with cholinesterase inhibition were observed at any dose level. No other treatment-related clinical chemistry changes were noted at any dose level. Additionally, no treatment-related haematological, ophthalmological, urinalysis or histopathological findings were observed. In summary, inhibition of cholinesterase activity was the only effect observed in this study. At 500 ppm (16.24 mg kg^{-1} d^{-1}), significant reduction in whole blood and brain cholinesterase activity was observed. At 100 ppm (3.12 mg kg^{-1} d^{-1}), whole blood activity was not adversely affected whereas brain activity was moderately decreased (20%) after 104 weeks. No effects were seen at 20 ppm (0.65 mg kg^{-1} d^{-1}). Overall, a NOEL of 20 ppm (0.65 mg^{-1} kg^{-1} d^{-1}) was established from this study based upon inhibition of cholinesterase activity.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL	100 ppm (3.12 mg^{-1} kg^{-1} d^{-1})	
5.3.2	NO(A)EL	20 ppm (0.65 mg^{-1} kg^{-1} d^{-1})	
5.3.3	Other	-	
5.3.4	Reliability	2	
5.3.5	Deficiencies	No	

Table A6.5-1 **Results of blood cholinesterase activity monitoring – Based on Dosage over 1 hour of treatment (feeding) expressed as percentage of post-treatment *vs* pre-treatment values (and % of inhibition)**

Dose (ppm)	Weeks of treatment							
	7	14	25	40	43	51	79	103
0	107.1	108.7	112.8	98.6 (-1.4%)	113.8	126.0	100.1	92.0 (-8%)
20	110.3	114.1	122.2	103.7	107.5	110.0	105.8	91.5 (-8.5%)
100	106.8	113.5	108.8	99.4	90.9 (-10%)	100.9	110.2	92.5 (-7.5%)
500	73.6 (-26%)	85.3 (-15%)	113.3	77.8 (-22%)	54.8 (-45%)	94.1 (-6%)	97.5 (-2.5%)	59.0 (-41%)

Table A6.5-2 **Results of brain cholinesterase activity monitoring – Expressed as % of recovery values (and % of inhibition)**

Dose (ppm)	Weeks of treatment	
	51	104
0	99.9	89.4 (-11%)
20	91.2 (-9%)	84.6 (-15%)
100	90.3 (-10%)	79.6 (-20%)
500	74.2 (-26%)	65.7 (-34%)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26th September 2006
Materials and methods	As described by the applicant.
Results and discussion	4.5.2. From week 14 to termination, serum cholesterol levels were consistently higher in dogs that received 500 ppm when compared with control values. 5.2. There was a slight but consistent increase in water consumption from week 36 in animals receiving 100 and 500 ppm, which sometimes reached significance.
Conclusion	
Reliability	2
Acceptability	Acceptable
Remarks	A LO(A)EL of 3.12 mg/kg/d and a NO(A)EL of 0.65 mg/kg/d were established by this study.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6 Genotoxicity studies

6.6.1 *In vitro* gene mutation study in bacteria

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1987) Technical Bendiocarb: Ames Bacterial Mutagenicity Test [redacted] Document A90615 6.6.1/01 16 July 1987 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**	Yes. Standard Ames test	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 20859/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	100%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Ames test	
3.2.1	Organism/cell type	*S. typhimurium* tester strains TA98, 100, 1535, 1537 and 1538	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Aroclor-induced rat liver S9	
3.2.4	Positive control	With S-9 mix: 2-aminoanthracene Without S-9 mix: 2-nitrofluorene, 9-aminoacridine, ENNG (N-ethyl-N'-nitro-nitrosoguanidine)	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	15, 50, 150, 500 and 1500 µg per plate bendiocarb (technical grade – purity 100 %)	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	18 h	
3.3.4	Other modifications	-	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	2 x 10^9 cells/ml	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No significant increases in the number of revertants were observed in any of the 5 strains	
4.4.2	With metabolic activation	No significant increases in the number of revertants were observed in any of the 5 strains	
4.2	**Cytotoxicity**	A preliminary toxicity test showed that concentrations in the range 500 – 5000 µg per plate were cytotoxic	X
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a standard Ames test conducted to QA and GLP, *S. typhimurium* tester strains TA98, 100, 1535, 1537 and 1538 were incubated with 15 – 1500 µg per plate bendiocarb (technical grade – purity 100 %) in DMSO with and without metabolic activation (Aroclor-induced rat liver S9). The highest concentration was selected on the basis of a preliminary toxicity test which showed that concentrations in the range 500 – 5000 µg per plate were cytotoxic, causing reductions in the number of revertant colonies, up to total lawn inhibition. Two independent experiments were performed using triplicate plating and negative and positive controls run concurrently.	
5.2	**Results and discussion**	No significant increases in the number of revertants were observed in any of the 5 strains, with and without metabolic activation, up to a concentration producing cytotoxicity. All the negative and positive controls produced appropriate responses.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.1/01-1. Summary of the reverse mutation tests with or without a liver metabolic activation system. Test 1.

Compound	µg/plate	S-9 mix	Mean no. of revertant colonies per plate				
			TA100	TA1535	TA98	TA1537	TA1538
DMSO	µg/plate	-	74	11	29	15	11
Bendiocarb	0*	-	93	10	29	12	9
	15	-	88	11	23	10	7
	50	-	85	9	24	12	10
	150	-	84	11	26	14	6
	500	-	63	8	15	9	7
	1500	-	40	7	9	5	-
Positive control		-	238	525	89	1573	73
DMSO		+	89	10	21	7	10
Bendiocarb	0*	+	93	9	16	9	8
	15	+	86	9	16	9	9
	50	+	77	8	14	8	9
	150	+	76	9	20	9	9
	500	+	54	8	13	7	8
	1500	+	60	5	9	5	7
Positive control		+	221	130	153	93	123

* Bacteria with buffer or S9 mix but no solvent or test compound

Table A6.6.1/01-2. Summary of the reverse mutation tests with or without a liver metabolic activation system. Test 2.

Compound	µg/plate	S-9 mix	Mean no. of revertant colonies per plate				
			TA100	TA1535	TA98	TA1537	TA1538
DMSO	µg/plate	-	84	11	34	9	10
Bendiocarb	0*	-	74	12	33	11	8
	15	-	99	10	27	9	10
	50	-	90	11	27	8	12
	150	-	84	9	26	9	12
	500	-	89	10	25	11	7
	1500	-	63	7	10	6	7
Positive control		-	454	438	122	1440	83
DMSO		+	84	11	24	11	10
Bendiocarb	0*	+	95	7	21	9	9
	15	+	98	9	23	9	8
	50	+	81	10	19	11	9
	150	+	80	11	21	10	8
	500	+	89	13	17	13	8
	1500	+	89	10	15	6	6
Positive control		+	485	134	179	165	189

* Bacteria with buffer or S9 mix but no solvent or test compound

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3rd October 2006
Materials and methods	2.3. The study deviates from the current OECD guideline 473 protocol in that four of the five recommended bacterial strains are used. One of the currently recommended strains that have an AT at the primary reversion site, i.e. *E. coli* WP2 uvrA, *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA 102, was not used. Therefore, the study may be less effective at detecting oxidising mutagens and cross-linking agents.
Results and discussion	4.2. The concentrations of bendiocarb tested in the preliminary study were 0, 5, 50, 500 and 5000 µg per plate. Cytotoxicity was apparent only at 5000 µg, not at 500 µg. The UK CA has added tables to summarise the results.
Conclusion	As described by the applicant.
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA notes that the strains of bacteria chosen would reduce the ability of the test to detect oxidising mutagens and cross-linking agents and so considers that the reliability is 2.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1981) The Microbial Mutagenicity Study of Bendiocarb (KNT) [redacted] Document A90483 6.6.1/02 August 1981 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**	Yes. Ames test (Japanese protocol)	
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	No data	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Not specified but bendiocarb is known as a beige-white powder	
3.1.2.2	Purity	98.8%	
3.1.2.3	Stability	Not specified but bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Ames test and rec. assay	
3.2.1	Organism/cell type	Ames test: *S. typhimurium* tester strains TA98, 100, 1535, 1537 and 1538 and *Escherichia coli* WP2 Rec. assay: H17 and M45 of *Bacillus subtilis*	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Ames test: Aroclor-induced rat liver S9	
3.2.4	Positive control	Ames test: 2-aminoanthracene, 2-nitrofluorene, 9-aminoacridine, ENNG (N-ethyl-N'-nitro-nitrosoguanidine), AF-2 (2-(2-furyl)-3-(5-nitro-2furyl) acrylamide Rec. assay: mitomycin C	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	Ames test: 5, 10, 50, 100, 500, 1000 and 5000 µg per plate bendiocarb (technical grade – purity 98.8 %) Rec. assay: 20, 50, 100, 200, 500, 1000, 2000 and 5000 µg/disk	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	Overnight for Ames test and rec. assay	
3.3.4	Other modifications	-	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Not specified	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No significant increases in the number of revertants were observed in any of the strains.	
4.4.2	With metabolic activation	No significant increases in the number of revertants were observed in any of the strains.	
4.2	**Cytotoxicity**	Concentrations of 5000 µg per plate were cytotoxic.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The genotoxic potential of bendiocarb was investigated using a standard reverse mutation Ames test and a repair test (rec-assay). In relation to the Ames test, *S. typhimurium* tester strains TA98, 100, 1535, 1537 and 1538 and *Escherichia coli* WP2 were incubated with 5 – 5000 µg per plate bendiocarb (technical grade – purity 98.8 %) in DMSO, with and without metabolic activation (Aroclor-induced rat liver S9). Duplicate plating was used and negative and positive controls were run concurrently. In relation to the rec-assay, strains H17 and M45 of *Bacillus subtilis*, which are impaired in their ability to repair deoxyribonucleic acid (DNA) damage, were incubated with 20 – 5000 µg per plate bendiocarb (technical grade – purity 98.8 %) in DMSO.	
5.2	**Results and discussion**	No significant increase in the number of revertant colonies was observed in any of the strains, with and without metabolic activation, up to a concentration (5000 µg per plate) that was cytotoxic to the bacteria. All the negative and positive control substances produced appropriate responses. No induction of zones of growth inhibition was observed up to the highest concentration employed, indicating that no DNA damage beyond the bacteria's capacity to repair had been produced by exposure to bendiocarb. All the negative and positive control substances produced appropriate responses. Overall, it was concluded that bendiocarb was not genotoxic to bacteria under the conditions of this study.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.1/02. Summary of reverse mutation tests with or without a liver metabolic activation system

Compound	µg/plate	S-9 mix	No. of revertant colonies per plate					
			TA100	TA1535	WP2 *hcr*	TA98	TA1537	TA1538
DMSO	µg/plate	-	117 112	9 4	20 26	29 30	8 16	5 8
Bendiocarb	5	-	126 151	12 4	18 15	33 35	12 16	12 14
	10	-	144 127	10 7	13 10	23 22	8 17	10 12
	50	-	117 116	8 8	13 17	26 29	9 14	13 13
	100	-	124 115	10 13	17 15	26 35	9 15	11 7
	500	-	96 114	4 7	16 22	25 21	16 6	14 16
	1000	-	106 83	5 9	17 19	29 31	3 4	9 5
	5000	-	* *	* *	9 12	* *	* *	* *
Positive control		-	419 438	1804 2352	190 209	446 396	>2000 >2000	432 311
DMSO		+	117 145	8 10	16 22	17 28	8 9	23 14
Bendiocarb	5	+	107 103	6 5	11 22	26 22	6 6	24 18
	10	+	132 111	12 7	16 23	27 33	5 8	17 17
	50	+	116 138	6 8	16 19	32 27	3 3	14 21
	100	+	123 137	7 7	19 28	28 19	8 7	16 19
	500	+	124 124	7 10	23 16	20 23	4 8	20 17
	1000	+	114 120	5 8	18 13	18 23	3 2	18 29
	5000	+	0* 16*	2 2	4 3	10 9	1* 0*	11 7

Positive control		+	462 516	191 124	501 539	109 93	99 86	81 91

* Toxic

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	3rd October 2006
Materials and methods	As described by the applicant.
Conclusion	As described by the applicant. The UK CA has added a table to summarise the results of the bacterial reverse gene mutation assay.
Reliability	1
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1982) Technical Bendiocarb: Induction of the Conversion and Mitotic Recombination in Yeast [redacted] Document A90499 6.6.1/03 August 1982 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**	No, but the study was conducted in line with good scientific practice	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4971/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	98.5%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Gene conversion and mitotic recombination	
3.2.1	Organism/cell type	Yeast *Saccharomyces cerevisiae* strain D7.	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Aroclor-induced rat liver S9	
3.2.4	Positive control	Ethyl methanesulphonate, cyclophosphamide monohydrate.	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	500, 1000, 2000, 4000 and 6000 µg per plate bendiocarb (technical grade – purity 98.5 %) in the presence and absence of metabolic activation. 375, 750, 1500, 3000 and 6000 µg/plate bendiocarb (technical grade 98.5%) in the presence of metabolic activation.	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	4 days	
3.3.4	Other modifications	-	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	1 x 10^7 cells/ml	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No statistically significant or dose-related increases in the frequency of gene conversion were seen in the absence of metabolic activation at up to cytotoxic concentrations (40 % – 60 % survival rate). The frequencies of mitotic recombination at 750 µg/ml and 3.0 mg/ml were slightly higher than the historical control range. However, there was no evidence of a dose response relationship at the 1.5 or 6.0 mg/ml levels (1.2 to 13.5% survival rate).	X
4.4.2	With metabolic activation	In the presence of metabolic activation, similar negative results and survival rates (44 % – 78 %) were obtained in the first experiment, but small increases (up to 2-fold compared with the negative control value) were noted at the 3 highest concentration levels in the second experiment. In the first mitotic recombination assay with metabolic activation, the results at the two highest dose levels were suggestive of a dose-response relationship, and at 6.0 mg/ml they were higher than the historical control range (2.5 to 37.4% survival rate). However, the second experiment did not indicate a dose-response relationship (44 to 78% survival rate).	X
4.2	**Cytotoxicity**	Concentrations of 8000 µg per plate were cytotoxic in initial tests.	

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The ability of bendiocarb to induce gene conversion and mitotic recombination was tested in the yeast *Saccharomyces cerevisiae* strain D7. For the gene conversion analysis, based on the results of a preliminary toxicity test, the cells were incubated for 18 h with bendiocarb (technical grade 98.5%) dissolved in DMSO at concentration levels of 500 – 6000 µg ml^{-1} in the presence and absence of metabolic activation (a cofactor-supplemented post-mitochondrial fraction from the livers of male rats pretreated with Aroclor 1254). In the presence of metabolic activation, the experiment was repeated employing slightly different concentration levels, 375 – 6000 µg ml^{-1}. Similarly, for the mitotic recombination analysis, the cells were incubated for 18 h with 375 – 6000 µg ml^{-1} bendiocarb dissolved in DMSO, in the presence and absence of metabolic activation (Aroclor-induced rat liver S9). Again, in the presence of metabolic activation, the experiment was repeated employing slightly different concentration levels, 500 – 6000 µg ml^{-1}. Positive and negative controls were included in each experiment.	
5.2	**Results and discussion**	No statistically significant or dose-related increases in the frequency of gene conversion were seen in the absence of metabolic activation at up to cytotoxic concentrations (40 % – 60 % survival rate). In the presence of metabolic activation, similar negative results and survival rates (44 % – 78 %) were obtained in the first experiment, but small increases (up to 2-fold compared with the negative control value) were noted at the 3 highest concentration levels in the second experiment. However, given that these increased frequencies were within the historical negative control ranges and were seen at extremely toxic concentration levels (2.5 – 8.6 % survival rate), they were not regarded as representing a genuine positive mutagenic response. In addition, no reproducible, dose-related increases in the frequency of mitotic recombination were observed with and without metabolic activation at up to cytotoxic concentrations. All the negative and positive controls produced appropriate responses. Overall, it was concluded that bendiocarb showed no evidence of a genotoxic effect under the conditions of this study.	X X
5.3	**Conclusion**		
5.3.1	Reliability	2	
5.3.2	Deficiencies	None	

Table A6.6.1/03-1 *S. cerevisiae* D7 gene conversion in the absence of S9 mix.

Time	Substance	Concentration	Frequency of gene conversion	% survival
2.5 h	DMSO	50 µl/ml	$1.6/10^5$	100
	Ethyl methane-sulphonate	20 mg/ml	$44.4/10^5$	90

18 h	DMSO	50 µl/ml	$1.1/10^5$	100
	Bendiocarb	500 µg/ml	$1.3/10^5$	46
		1000 µg/ml	$1.4/10^5$	40
		2000 µg/ml	$1.4/10^5$	60
		4000 µg/ml	$1.6/10^5$	51
		6000 µg/ml	$1.5/10^5$	46

Table A6.6.1/03-2 *S. cerevisiae* D7 gene conversion in the presence of S9 mix. First experiment.

Substance	**Concentration**	**Frequency of gene conversion**	**% survival**
DMSO	50 µl/ml	$1.4/10^5$	100
Cyclophosphamide	20 mg/ml	$1.7/10^5$	140.1
Bendiocarb	375 µg/ml	$1.4/10^5$	37.4
	750 µg/ml	$1.6/10^5$	10.2
	1500 µg/ml	$3.2/10^5$	8.2
	3000 µg/ml	$3.2/10^5$	8.6
	6000 µg/ml	$2.7/10^5$	2.5

Table A6.6.1/03-3 *S. cerevisiae* D7 gene conversion in the presence of S9 mix. Second experiment.

Substance	**Concentration**	**Frequency of gene conversion**	**% survival**

DMSO	50 µl/ml	$1.5/10^5$	100
Cyclophosphamide	20 mg/ml	$0.7/10^5$	266
Bendiocarb	500 µg/ml	$1.1/10^5$	59
	1000 µg/ml	$1.4/10^5$	44
	2000 µg/ml	$1.3/10^5$	72
	4000 µg/ml	$1.5/10^5$	78
	6000 µg/ml	$1.7/10^5$	53

Table A6.6.1/03-4. *S. cerevisiae* D7 recombinogenic activity in the absence of S9 mix.

Substance	**Concentration**	**Frequency of mitotic recombination / 10^4 survivors**	**% survival**
DMSO	50 µl/ml	1.6	100
Ethyl methane-sulphonate	10 mg/ml	36.5	-
Bendiocarb	375 µg/ml	2.9	13.5
	750 µg/ml	3.9	5.3
	1500 µg/ml	2.9	1.2
	3000 µg/ml	4.2	6.2
	6000 µg/ml	2.8	1.9

Table A6.6.1/03-5. *S. cerevisiae* D7 recombinogenic activity in the presence of S9 mix. First experiment.

Substance	**Concentration**	**Frequency of mitotic recombination / 10^4 survivors**	**% survival**
DMSO	50 µl/ml	0.7	100
Cyclophosphamide	20 mg/ml	3.3	140.1
Bendiocarb	375 µg/ml	0.5	37.4
	750 µg/ml	2.4	10.2
	1500 µg/ml	1.8	8.2
	3000 µg/ml	3.2	8.6
	6000 µg/ml	6.3	2.5

Table A6.6.1/03-6. *S. cerevisiae* D7 recombinogenic activity in the presence of S9 mix. Second experiment.

Substance	**Concentration**	**Frequency of mitotic recombination / 10^4 survivors**	**% survival**
DMSO	50 µl/ml	0	100
Cyclophosphamide	40 mg/ml	5.9	266
Bendiocarb	500 µg/ml	1.9	59
	1000 µg/ml	3.9	44
	2000 µg/ml	5.4	72
	4000 µg/ml	2.5	78
	6000 µg/ml	2.6	53

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4th October 2006
Materials and methods	As described by the applicant.
Results and discussion	4.4.1. The UK CA has added a paragraph to give the results of the mitotic recombination assay. 4.4.2. In the presence of metabolic activation, small increases (up to 2-fold compared with the negative control value) were noted at the 3 highest concentration levels in the first experiment. However, survival in these groups was very low (2.5 to 8.2%). In the second experiment, only negative results with survival rates of 44% to 78% were obtained. 4.4.2. The UK CA has added a paragraph to give the results of the mitotic recombination assay.
Conclusion	5.2. In the presence of metabolic activation, small increases (up to 2-fold compared with the negative control value) were noted at the 3 highest concentration levels in the first experiment, but in the second experiment similar negative results and survival rates (44 % – 78 %) to the assay without metabolic activation were obtained. 5.2. The positive control compound, cyclophosphamide, was inactive in both repeats of the gene conversion assay in the presence of S9. However, the effectiveness of the S9 mix was demonstrated in the mitotic recombination assay, in which positive responses were achieved with cyclophosphamide with the same mix. The response to ethyl methanesulphonate proved the sensitivity of the strain to mitotic gene conversion events. The UK CA has added tables that summarise the results obtained.
Reliability	2
Acceptability	Acceptable
Remarks	Although there were some seemingly positive responses in this study, their general lack of dose-response relationships and reproducibility makes the results equivocal.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.2 *In vitro* cytogenicity study in mammalian cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1988) Technical Bendiocarb: Metaphase Chromosome Analysis of Human Lymphocytes Cultivated *in vitro* [redacted] Document A90616 6.6.2/01 13 June 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**	Yes. US EPA 84-2; OECD Guideline 473 (1983)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 20859/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Beige powder	
3.1.2.2	Purity	97.6%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Metaphase Chromosome Analysis	
3.2.1	Organism/cell type	Human lymphocytes Cultivated *in vitro*	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Aroclor-induced rat liver S9	
3.2.4	Positive control	Ethylmethane sulphonate and cyclophosphamide	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	17, 85 or 170 µg ml^{-1} in the absence of metabolic activation and 30, 150, 225 or 300 µg ml^{-1} in the presence of metabolic activation (Aroclor-induced rat liver S9) A repeat metaphase analysis without metabolic activation was conducted with the following lower concentration levels: 14.3, 71.5, and 143 µg ml^{-1}.	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	47 h	
3.3.4	Other modifications	-	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	1 x 10^6 cells/ml	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No statistically significant or dose-related increases in chromosomal aberrations were seen in the absence of metabolic activation at any concentration level. No reduction in the mitotic index was observed up to the highest concentration tested in the repeat experiment without metabolic activation.	
4.4.2	With metabolic activation	In the presence of metabolic activation, a statistically significant increase in the percentage of cells with chromosomal aberrations excluding gaps was noted at the 3 highest concentrations tested (4 %, 11.5 % and 5.5 % respectively, compared with 1.25 % in controls). In the repeat experiment reductions in the range 47 – 68 % were noted at the 3 highest concentrations in the presence of metabolic activation.	
4.2	**Cytotoxicity**	The concentration level of 170 µg ml^{-1} proved to be excessively toxic (producing a 75 % reduction in the mitotic index).	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a study conducted to GLP, the ability of bendiocarb to produce chromosomal aberrations was investigated in cultured human lymphocytes. Based on the results of a preliminary toxicity test, the lymphocytes were incubated with bendiocarb (technical grade 97.6%) dissolved in DMSO at concentration levels of 17, 85 or 170 µg ml^{-1} in the absence of metabolic activation and 30, 150, 225 or 300 µg ml^{-1} in the presence of metabolic activation (Aroclor-induced rat liver S9). Without metabolic activation, the cells were exposed to bendiocarb up to harvest at 24 h, whilst with metabolic activation, the cells were exposed for 2 h and harvested after 24 h. Positive and negative controls were included. In the absence of metabolic activation, the concentration level of 170 µg ml^{-1} proved to be excessively toxic (producing a 75 % reduction in the mitotic index) and, hence, this part of the experiment was eliminated. A repeat metaphase analysis without metabolic activation was therefore conducted with the following lower concentration levels: 14.3, 71.5, and 143 µg ml^{-1}.	

5.2	**Results and discussion**	No statistically significant or dose-related increases in chromosomal aberrations were seen in the absence of metabolic activation at any concentration level. However, in the presence of metabolic activation, a statistically significant increase in the percentage of cells with chromosomal aberrations excluding gaps was noted at the 3 highest concentrations tested (4 %, 11.5 % and 5.5 % respectively, compared with 1.25 % in controls). No reduction in the mitotic index was observed up to the highest concentration tested in the repeat experiment without metabolic activation, but reductions in the range 47 – 68 % were noted at the 3 highest concentrations in the presence of metabolic activation. All the negative and positive controls produced appropriate responses. It was concluded from this *in vitro* study that bendiocarb induced chromosomal aberrations in the presence of an exogenous metabolic activation system.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.2-1 Results of Metaphase Analysis – Chromosome Aberrations

A – Without metabolic activation

Test substance (µg/ml)	Mitotic index (%)	Aberrant cells (%)	
		Excluding gaps	Including gaps
Control: Dimethyl sulfoxide (10 µl/ml)	7.15	3.25	3.25
Bendiocarb 14.3	8.10	4.0	4.0
71.5	9.05	3.0	3.0
143	8.05	2.5	3.0
Positive control: ethylmethane sulphonate (500)	-	13.0*	13.0*
Positive control: ethylmethane sulphonate (1000)	-	21.0*	21.0*

* Statistically significant

B – With metabolic activation

Test substance (µg/ml)	Mitotic index (%)	Aberrant cells (%)	
		Excluding gaps	Including gaps
Control: Dimethyl sulfoxide (10 µl/ml)	8.35	1.25	1.25
Bendiocarb 30	7.60	2.5	2.5
150	2.70	4.0*	4.0*
225	4.40	11.5*	11.5*
300	3.65	5.5*	5.5*
Positive control: Cyclophosphamide (20)	-	20.5*	20.5*

* Statistically significant

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	10th October 2006
Materials and methods	As described by the applicant.
Conclusion	As described by the applicant.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.3 *In vitro* gene mutation assay in mammalian cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1988) Technical Bendiocarb: Unscheduled DNA Synthesis in Cultured Mammalian Cells [redacted] Document A90618 6.6.3/01 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**	Yes. US EPA 84-2; EPA TSCA 560/6-83-001 OECD Guideline 482 (1986)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 20859/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	96.4%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Unscheduled DNA synthesis	
3.2.1	Organism/cell type	Cultured human epithelioid (HeLa) cells	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Aroclor-induced rat liver S9	
3.2.4	Positive control	4-nitroquinoline-1 oxide (4NQO) without metabolic activation system 2-aminoanthracene (2AA) with metabolic activation system	
3.3	**Administration/ Exposure; Application of test substance**		

3.3.1	Concentrations	12 different concentrations ranging from 1.25 to 2560 µg ml^{-1} (its limit of solubility in culture medium)	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	96 h	
3.3.4	Other modifications	-	
3.4	**Examinations**	Autoradiography	
3.4.1	Number of cells evaluated	5 x 10^4 cells/ml	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No evidence of inducing UDS when tested up to cytotoxic concentrations under the conditions of this study.	
4.4.2	With metabolic activation	No evidence of inducing UDS when tested up to cytotoxic concentrations under the conditions of this study.	X
4.2	**Cytotoxicity**	Cytotoxicity (cell death and subsequent cell sloughing) was seen at the highest concentrations tested.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Bendiocarb was tested for its potential mutagenic activity by measuring its ability to induce UDS in cultured human epithelioid (HeLa) cells. Cells were incubated for 3 h with bendiocarb (technical grade 96.4%) dissolved in DMSO at 12 different concentrations ranging from 1.25 to 2560 µg ml^{-1} (its limit of solubility in culture medium) in the absence and presence of metabolic activation (Aroclor-induced rat liver S9). Two independent experiments were performed and negative and positive controls were run concurrently.	
5.2	**Results and discussion**	No reproducible, dose-related increases in net nuclear grain counts as assessed by autoradiography were observed, with and without metabolic activation. Cytotoxicity (cell death and subsequent cell sloughing) was seen at the highest concentrations tested and all negative and positive controls produced appropriate responses. It was concluded that bendiocarb showed no evidence of inducing UDS when tested up to cytotoxic concentrations under the conditions of this study.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11th October 2006
Materials and methods	As described by the applicant.
Results and discussion	4.4.2. In the first test with metabolic activation, a small but significant increase in the gross nuclear grain count was observed at 10 µg/ml bendiocarb. When the value was corrected for background labelling, no significant increase was apparent. A significant increase in the nuclear grain count also occurred at 40 µg/ml, with correction for background labelling. In the second test, there were no statistically significant increases at any dose.
Conclusion	As described by the applicant.
Reliability	1
Acceptability	Acceptable
Remarks	Although two statistically significant increases in nuclear grain count were detected in one test with metabolic activation, these were not dose-related nor reproducible. The UK CA considers that bendiocarb did not result in DNA damage in this test.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1982) The Assessment of Mutagenic Potential with NC 6897 in the Mouse Lymphoma Mutation Assay [redacted] Document A90491 6.6.3/02 15 January 1982 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**	No, but the study was conducted in line with good scientific practice	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4971/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	Not specified but based on the batch number (CR 4971/2) and the certificate number AS 09155 from 1981, purity should be 98.5% (as in study A90499)	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Study Type**	Mammalian cell gene mutation assay	
3.2.1	Organism/cell type	Mouse lymphoma L5178Y cells	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Aroclor-induced rat liver S9	
3.2.4	Positive control	Ethyl methanesulphonate (EMS) 2-acetylaminofluorene (2-AAF)	
3.3	**Administration/ Exposure; Application of test substance**		
3.3.1	Concentrations	Concentrations ranging from 0.2 to 25 µg ml^{-1}	

3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Pre-incubation time	3 days	
3.3.4	Other modifications	-	
3.4	**Examinations**	Cell density measured by counting with a Neubauer haemocytometer	
3.4.1	Number of cells evaluated	3 x 10^5 cells/ml	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No dose-related, reproducible increases in the frequency of mutant colonies were observed.	
4.4.2	With metabolic activation	No dose-related, reproducible increases in the frequency of mutant colonies were observed.	
4.2	**Cytotoxicity**	An initial toxicity test showed a reduction in cell survival starting from a concentration of 100 µg ml^{-1}.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Mouse lymphoma L5178Y cells were exposed in 3 separate experiments to bendiocarb (technical grade) dissolved in DMSO at concentrations ranging from 0.2 to 25 µg ml^{-1}. The cells were treated for 3 h, with and without exogenous metabolic activation (Aroclor-induced rat liver S9) and assessed for gene mutations at the thymidine kinase (TK) locus. Triplicate plating was employed and positive and negative control substances were run concurrently. After treatment, the cells were grown for 3 d to allow expression of any mutations and selected after a growth period of 7 – 10 d in a selective medium. No separate scoring of small and large colonies was conducted. The concentration levels used were selected on the basis of an initial toxicity test that showed a reduction in cell survival starting from a concentration of 100 µg ml^{-1}.	
5.2	**Results and discussion**	No dose-related, reproducible increases in the frequency of mutant colonies were observed with and without metabolic activation, up to concentrations producing a reduction in cell survival by more than 50 % of the control levels. The positive and negative control substances produced appropriate responses. Overall, it was concluded that bendiocarb was not mutagenic in this test under the conditions of this study.	X
5.3	**Conclusion**		
5.3.1	Reliability	2	
5.3.2	Deficiencies	None	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11th October 2006
Materials and methods	As described by the applicant.
Results and discussion	5.2. The survival of cells without metabolic activation was 15 and 29% in two assays. With metabolic activation, cell survival was 62 and 80% in two assays. In the third repeats of the assays, cell survival as a percentage of the controls was not presented.
Conclusion	
Reliability	2
Acceptability	Acceptable.
Remarks	Trifluorothymidine was the selective agent used. Three experiments were conducted in the absence of S9 mix. Mutation frequencies higher than the historical control range for DMSO were detected at 20 µg/ml in the first experiment and at 5 µg/ml in the second. These results were not repeated in the third assay. However, in the latter, there was a dose-related increase in the mutants, although these were within the historical control range. Three experiments were also conducted in the presence of S9 mix. In one of these, the number of mutant colonies was greater than in the DMSO control, although there was no dose response, with them all being elevated to a similar extent. In the third experiment there was a greater than two-fold increase in the number and frequency of mutant colonies at 25 µg/ml, but this was well within the normal range for controls. The cell survival in the presence of S9 (62-80%) may indicate that higher concentrations of bendiocarb could have been applied. Overall, the UK CA agrees with the conclusion of the applicant that bendiocarb did not demonstrate mutagenic activity in these assays.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.4 *In vivo* mutagenicity study (bone marrow assay)

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1989) Technical Bendiocarb: Analysis of Metaphase Chromosomes from Rat Bone Marrow [redacted] Document A90620 6.6.4/01 3 January 1989 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**	Yes. US EPA 84-2; EPA TSCA 560/6-83-001 OECD Guideline 475 (1984); EEC Directive 79/831 Annex V (1982)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 20859/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	96.4%	
3.1.2.3	Stability	Not specified but bendiocarb is not known to decompose at room temperature	
3.1.2.4	Maximum tolerable dose	26 mg/kg	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River, UK	
3.2.4	Sex	Male/female	
3.2.5	Age/weight at study initiation	3 weeks; 45 – 50 g	
3.2.6	Number of animals per group	5M/5F per dose: 6, 24 and 48 h kill	

3.2.7	Controls animals	5M/5F	
3.3	**Administration/ Exposure**	Oral (gastric intubation)	
3.3.1	Number of applications	1	
3.3.2	Interval between applications	n.a.	
3.3.3	Postexposure period	48 h	
3.3.4	Type	Oral (gastric intubation)	
3.3.5	Concentration	2.6, 13.0 and 26.0 mg/kg	
3.3.6	Vehicle	1% methylcellulose	
3.3.7	Concentration in vehicle	0.026%, 0 13%, 0.26%	
3.3.8	Total volume applied	10 ml/kg bodyweight	
3.3.9	Controls	Vehicle (intraperitoneal)	
3.3.10	Vehicle	1% methylcellulose	
3.3.11	Concentration in vehicle	0.4%	
3.3.12	Total volume applied	10 ml/kg bodyweight	
3.3.13	Dose applied	10 ml/kg bodyweight	
3.3.14	Substance used as Positive Control	Cyclophosphamide 40 mg/kg	
3.3.15	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	No	
3.5	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Moderate to severe clinical signs of toxicity including pilo-erection, hunched posture, lethargy, body tremors and increased salivation were observed in the mid- (mainly slight or moderate) and top dose groups (mainly moderate or severe).	
4.2	**Haematology / Tissue examination**	n.a.	

4.3	**Genotoxicity**	No increase in the frequency of metaphases with chromosomal aberrations (including and excluding gaps) compared to controls was observed at any dose level, at any of the sampling times. Cyclophosphamide, the positive control substance, produced highly significant increases in the incidence of metaphases with aberrant chromosomes. Overall, it was concluded that bendiocarb was not clastogenic to the bone marrow of Sprague-Dawley rats under the conditions of this study.	
4.4	**Other**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a bone marrow cytogenetics assay, groups of 15 male and 15 female Sprague-Dawley rats were administered, by oral gavage, single doses of 0, 2.6, 13 or 26 mg kg^{-1} bendiocarb (technical grade 96.4 %) in 1 % w/v methylcellulose. Five males and 5 females from each group were sacrificed 6, 24 and 48 h after dosing, and bone marrow smears taken for cytogenetic analysis. A positive control group of 5 male and 5 female animals received a single intraperitoneal injection of 40 mg kg^{-1} cyclophosphamide in physiological saline, and was sacrificed 24 h post-dosing.	
5.2	**Results and discussion**	The dose levels used were selected on the basis of a preliminary toxicity test which indicated that the top dose (26 mg kg^{-1}) would be expected to kill approximately 10 % of the animals over a period of 48 h post-dosing (i.e. equivalent to the LD_{10}). However, in the study itself, deaths were observed at the mid- (1 male) and top dose levels (11 males and 8 females). Moderate to severe clinical signs of toxicity including pilo-erection, hunched posture, lethargy, body tremors and increased salivation were observed in the mid- (mainly slight or moderate) and top dose groups (mainly moderate or severe). No increase in the frequency of metaphases with chromosomal aberrations (including and excluding gaps) compared to controls was observed at any dose level, at any of the sampling times. Cyclophosphamide, the positive control substance, produced highly significant increases in the incidence of metaphases with aberrant chromosomes. Overall, it was concluded that bendiocarb was not clastogenic to the bone marrow of Sprague-Dawley rats under the conditions of this study.	x
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.4-1. Results of metaphase analysis – chromosome aberrations

Sampling time (hours)	Treatment	Dosage (mg/kg)	Incidence of aberrant cells (%)	
			Excluding gap damage	Including gap damage
6	Vehicle control	-	0.0	0.2
	Technical bendiocarb	26.0	0.0	0.0
24	Vehicle control	-	0.0	0.0
	Technical bendiocarb	26.0	0.2	0.2
	Cylophosphamide	40.0	16.2*	16.4*
48	Vehicle control	-	0.0	0.0
	Technical bendiocarb	26.0	0.0	0.0

* P<0.001

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	31st October 2006
Materials and methods	As described by the applicant
Results and discussion	Moderate to severe toxicity occurred in the 26.0 mg/kg group, including the deaths of 11 males and 8 females (the use of concurrently-dosed animals enabled assessment of 5 animals/sex/time point). This indicates that bendiocarb was systemically available and therefore, it can be assumed that the bone marrow has been reached.
Conclusion	The UK CA has added a table to summarise the results. Otherwise, as described by the applicant
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1982) A Micronucleus Study in Mice using Technical NC 6897, CR 4971/2 [redacted] Document A90496 6.6.4/02 March 1982 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**	No, but the study was conducted in line with good scientific practice	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4971/2	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	97.9%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature	
3.1.2.4	Maximum tolerable dose	Not reported	
3.2	**Test Animals**		
3.2.1	Species	Mice	
3.2.2	Strain	Charles River CD-1	
3.2.3	Source	Charles River, UK	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	7 weeks; 22 – 29 g	
3.2.6	Number of animals per group	5 per dose	
3.2.7	Controls animals	10M	
3.3	**Administration/ Exposure**	Intraperitoneal injection	

3.3.1	Number of applications	2	
3.3.2	Interval between applications	24 h	
3.3.3	Postexposure period	6 h	
3.3.4	Type	Intraperitoneal injection	
3.3.5	Concentration	0.625, 1.25 and 2.5 mg/kg bodyweight	
3.3.6	Vehicle	Propylene glycol	
3.3.7	Concentration in vehicle	4 mg/ml	
3.3.8	Total volume applied	Se 3.3.5 and 3.3.7	
3.3.9	Controls	Vehicle (intraperitoneal)	
3.3.10	Vehicle	Propylene glycol	
3.3.11	Concentration in vehicle	n.a.	
3.3.12	Total volume applied	Equivalent to highest dose of bendiocarb	
3.3.13	Dose applied	n.a.	
3.3.14	Substance used as Positive Control	Cyclophosphamide 75 mg/kg in physiological saline (5 mg/ml)	
3.3.15	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Femurs removed for bone marrow	
3.5	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No data	
4.2	**Haematology / Tissue examination**	Only bone marrow for erythrocyte examination (analysis for micronucleated polychromatic erythrocytes (PCE) per 1000 PCEs per animal)	

4.3	**Genotoxicity**	No information on mortalities or clinical signs of toxicity, including effects on body weight, was provided in the study report. No alteration of the PCE/NCE (PCE/normochromatic erythrocytes) ratio was seen in any treated group. No significant increases in micronucleated PCEs were observed at any dose level compared with controls. A statistically significant increase in micronucleated PCEs was noted in animals treated with the positive control substance, cyclophosphamide. Overall, although no cytotoxicity to the bone marrow (i.e. PCE/NCE ratio remained unaffected) was seen and no information on general toxicity was provided, consideration of the fact that the top dose employed was equivalent to 50 % of the intraperitoneal LD_{50} in the mouse, and evidence from the toxicokinetic data (wide distribution around the body) suggest that it is very likely that the bone marrow was exposed.	
4.4	**Other**	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a bone marrow micronucleus test, groups of 5 male CD-1 mice were administered intraperitoneal doses of 0.625, 1.25 or 2.5 mg kg^{-1} bendiocarb (technical grade – purity 97.9 %) in propylene glycol on two occasions, 24 h apart. The dose levels used were selected on the basis of an acute intraperitoneal study conducted in the mouse in which an LD_{50} value of 5 mg kg^{-1} had been determined. Ten males were treated with the vehicle only, and a positive control group of 5 males received a single intraperitoneal injection of 75 mg kg^{-1} cyclophosphamide in physiological saline. Femoral bone marrow cells were harvested 6 h after the second dose and analysed for micronucleated polychromatic erythrocytes (PCE) per 1000 PCEs per animal.	
5.2	**Results and discussion**	No information on mortalities or clinical signs of toxicity, including effects on body weight, was provided in the study report. No alteration of the PCE/NCE (PCE/normochromatic erythrocytes) ratio was seen in any treated group. No significant increases in micronucleated PCEs were observed at any dose level compared with controls. A statistically significant increase in micronucleated PCEs was noted in animals treated with the positive control substance, cyclophosphamide. Overall, although no cytotoxicity to the bone marrow (i.e. PCE/NCE ratio remained unaffected) was seen and no information on general toxicity was provided, consideration of the fact that the top dose employed was equivalent to 50 % of the intraperitoneal LD_{50} in the mouse, and evidence from the toxicokinetic data (wide distribution around the body) suggest that it is very likely that the bone marrow was exposed. In conclusion, this study provided reliable evidence that bendiocarb was not clastogenic to the bone marrow of CD-1 mice.	
5.3	**Conclusion**		
5.3.1	Reliability	2	X
5.3.2	Deficiencies	None	

Table A6.6.4-2. Results of metaphase analysis – chromosome aberrations

Treatment	Dose (mg/kg)	NCE with micronuclei/total	PCE with micronuclei/total	% PCE with micronuclei	PCE/NCE ratio
Vehicle control	-	0/1009	1/999	0.09	1.01
Bendiocarb	0.625	1/1094	0/1000	0.04	1.09
Bendiocarb	1.25	0/963	0/1000	0	0.96
Bendiocarb	2.5	1/1030	1/999	0.06	1.03
Cylophosphamide	75	0/1322	12/988*	1.26*	1.34

P<0.05

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1st November 2006
Materials and methods	As described by the applicant.
Conclusion	The UK CA has added a summary table of the results.
Reliability	3
Acceptability	Acceptable as supportive evidence.
Remarks	This study has not demonstrated that the administered bendiocarb reached the bone marrow of the treated mice, since no alteration in the PCE/NCE ratio was observed in any of the treated groups. Additionally, the study provided no information on mortalities or clinical signs of toxicity. The highest dose selected corresponded to 50% of the intra-peritoneal LD_{50} of bendiocarb in the mouse. Samples for analysis were collected at 6 hours after the second dose (the current OECD guideline 474 recommends that, in the case of two treatments being administered at an interval of 24 hours, bone marrow should be collected between 18 and 24 hours after the second treatment). However, the UK CA considers that the data requirement for A6.6.4 has been met by the *in vivo* chromosomal aberration assay submitted under A6.4.4-01.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.5 *In vivo* mutagenicity test for DNA damage

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Bendiocarb was positive in one *in vitro* test. Therefore two *in vivo* tests (see Point 6.6.4: rat bone marrow and mice micronucleus tests) were conducted and none of them gave any indication of genotoxicity. Based on the weight of evidence from this full *in vitro/in vivo* package and the results of the carcinogenicity studies, a second *in vivo* study in a tissue other than bone marrow should not be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7th November 2006
Evaluation of applicant's justification	Bendiocarb was negative in a battery of *in vitro* tests that comprised two bacterial reverse gene mutation assays, yest gene conversion/mitotic recombination, unscheduled DNA synthesis and mouse lymphoma assays. It was positive in an *in vitro* cytogenetics assay in the presence of metabolic activation. Therefore, an *in vivo* chromosome aberration assay was conducted, in which, in the presence of moderate to severe systemic toxicity, bendiocarb was negative. Two additional *in vivo* assays, a micronucleus test and a dominant lethal assay, were also negative and, in the absence of gene mutations in the Ames tests, provide supportive evidence for the lack of a genotoxic effect.
Conclusion	The UK CA agrees with the applicant that an *in vivo* mutagenicity test for DNA damage in a tissue other than bone marrow is not required.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.6 *In vivo* mutagenicity test re germ cell effects

		1. REFERENCE	Official use only
1.1	**Reference**	[redacted] (1977) A Test for the Induction of Dominant Lethal Mutations in Male Rats by Technical CR 4799/1 NC 6897 [redacted] Document A90380 6.6.6/01 April 1977 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 on new a.s. for first entry to Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, but the study was conducted in line with good scientific practice	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	CR 4799/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Not specified but bendiocarb is known as a beige/white powder (?)	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Not specified but bendiocarb is not known to decompose at room temperature	
3.1.2.4	Maximum tolerable dose	Not reported	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Charles River, UK	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	160 – 180 g (M) – 130 – 150 g (F)	
3.2.6	Number of animals per group	20 M – 10 F	
3.2.7	Controls animals	Yes	

3.3	**Administration/ Exposure**	Oral (diet)	
3.3.1	Number of applications	Daily for 13 weeks	
3.3.2	Interval between applications	n.a.	
3.3.3	Postexposure period	Mating for 7 days after the end of dietary exposure. Females killed and autopsied 14 days after mating	
3.3.4	Type	Oral (diet)	
3.3.5	Concentration	0, 10, 50 and 250 ppm in diet	
3.3.6	Vehicle	Powdered diet	
3.3.7	Concentration in vehicle	0, 10, 50 and 250 ppm in diet	
3.3.8	Total volume applied	Daily diet	
3.3.9	Controls	Vehicle	
3.3.10	Vehicle	Powdered diet only	
3.3.11	Concentration in vehicle	n.a.	
3.3.12	Total volume applied	Daily diet	
3.3.13	Dose applied	n.a.	
3.3.14	Substance used as Positive Control	None	
3.3.15	Controls	n.a.	
3.4	**Examinations**		
3.4.1	Clinical signs	Toxicity, bodyweight, food consumption	
3.4.2	Tissue	Organ weights, gross pathological examination, microscopic examination of abnormal gonads	
3.5	**Further remarks**	-	
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	No effects of treatment were seen on clinical signs of toxicity, body weight gain, food consumption, mating frequency and pregnancy rate.	
4.2	**Haematology / Tissue examination**	There was no effect of bendiocarb administration on numbers of *corpora lutea*, implantation sites, pre-implantation losses, viable foetuses and non-viable foetuses (early and late deaths) per pregnancy.	
4.3	**Genotoxicity**	Overall, there was no evidence in this study that bendiocarb exposure induced dominant lethal mutations in male Sprague-Dawley rats.	

4.4	**Other**	A statistically significant increase (14 vs. 7 in controls) in the number of pregnancies with one or more early deaths was seen at the low dose only, and, consequently, an increase (1.24 vs. 0.74 in controls) in the incidence of non-viable implants per pregnancy was noted at the same dose level. Given the absence of a dose-response relationship, these findings are considered to be of no toxicological significance, but a chance finding. A total of 5 males (2, 1 and 2 at the low, mid- and top dose levels respectively) were apparently non-fertile. With the exception of 1 male from the low dose group, this infertility was correlated with large areas of atrophic seminiferous tubules. However, again, given the absence of a dose-response relationship and the fact that, according to the authors, higher incidences of male infertility have been recorded naturally in the same strain of rats, these findings are not regarded as being of any toxicological significance.	X
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 20 male Sprague-Dawley rats were administered 0, 10, 50 or 250 ppm bendiocarb (technical grade – purity 99 %) in the diet (approximating to 0, 0.4, 2 and 10 mg kg^{-1}) for 13 consecutive weeks and then mated (1 male to 1 female) with untreated, mature virgin females for 7 d. Pregnant and non-pregnant females were then sacrificed 14 d after mating, and analysed for numbers of *corpora lutea*, implantation sites, live and dead foetuses and early and late resorptions. Each male (all groups) was subjected to a gross pathological examination, and those with abnormal gonads were also examined histopathologically.	
5.2	**Results and discussion**	No effects of treatment were seen on clinical signs of toxicity, body weight gain, food consumption, mating frequency and pregnancy rate. There was no effect of bendiocarb administration on numbers of *corpora lutea*, implantation sites, pre-implantation losses, viable foetuses and non-viable foetuses (early and late deaths) per pregnancy. A statistically significant increase (14 vs. 7 in controls) in the number of pregnancies with one or more early deaths was seen at the low dose only, and, consequently, an increase (1.24 vs. 0.74 in controls) in the incidence of non-viable implants per pregnancy was noted at the same dose level. Given the absence of a dose-response relationship, these findings are considered to be of no toxicological significance, but a chance finding. A total of 5 males (2, 1 and 2 at the low, mid- and top dose levels respectively) were apparently non-fertile. With the exception of 1 male from the low dose group, this infertility was correlated with large areas of atrophic seminiferous tubules. However, again, given the absence of a dose-response relationship and the fact that, according to the authors, higher incidences of male infertility have been recorded naturally in the same strain of rats, these findings are not regarded as being of any toxicological significance. Overall, there was no evidence in this study that bendiocarb exposure induced dominant lethal mutations in male Sprague-Dawley rats.	X
5.3	**Conclusion**		
5.3.1	Reliability	2	
5.3.2	Deficiencies	None	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1st November 2006
Materials and methods	As described by the applicant.
Results and discussion	4.4. The statement that higher incidences of male infertility have been recorded naturally in the same strain of rats is incorrect. The study authors state that an incidence of 4% has been observed in a colony of Wistar rats. 5.2. The statement that higher incidences of male infertility have been recorded naturally in the same strain of rats is incorrect. The authors state that an incidence of 4% has been observed in a colony of Wistar rats.
Conclusion	
Reliability	2
Acceptability	Acceptable as supportive evidence.
Remarks	The UK CA notes that no signs of toxicity were noted in the treated animals, and that no justification for the choice of doses was provided in the study. However, the number of applications was extensive (daily for 13 weeks).
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.6.7 Test for metabolites formed in mammals

	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, if *in vitro* assays are negative, further testing is only required if metabolites of concern are formed in mammals. Bendiocarb was positive in one *in vitro* test. Therefore two *in vivo* tests (see Point 6.6.4: rat bone marrow and mice micronucleus tests) were conducted and none of them gave any indication of genotoxicity. Furthermore, no metabolite of concern is formed in mammals. Metabolites formed in mammals are assessed in all mammalian toxicity studies performed with bendiocarb. Additionally, no evidence of carcinogenicity has been seen in long-term studies with bendiocarb. Further tests on this compound should therefore not be required	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1st November 2006
Evaluation of applicant's justification	No metabolite of concern was identified in mammals. Three *in vivo* mutagenicity tests in mammals were negative, although there are questions about the suitability of the doses selected in two of these studies. Two carcingenicity studies were also negative; however, the low survival rates in one of these may have reduced its sensitivity.
Conclusion	The UK CA considers the justification for non-submission of data acceptable.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.7 Carcinogenicity study

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1981a) NC 6897 Toxicity and Tumorigenicity to Rats in Long-Term Dietary Administration (Final Report – Reproductive Phase and Main Phase) Main Phase = 104 Weeks [redacted] Document A90427 6.7/01 February 1981 Unpublished [redacted] (1980a) Determination of Bendiocarb (NC 6897) Dietary Concentrations in a Long-Term Toxicity and Tumorigenicity Study in Rats [redacted] Document A90428 6.7/02 June 1980 Unpublished [redacted] (1981b) NC 6897 Toxicity and Tumorigenicity to Rats in Long-Term Dietary Administration (Addendum to Final Report – Histopathology) [redacted] Document A90430 6.7/03 May 1981 Unpublished [redacted] (2005) Historical Control Incidence of Survival in Sprague Dawley CD Rats at Huntingdon Life Sciences LTD (1982-1987) [redacted] Document M-265313-01-1 6.7/04 [redacted] (2005) Historical Control Incidence of Lenticular Opacitiesl in Sprague Dawley CD Rats at Huntingdon Life Sciences LTD (1982-1987) [redacted] Document M-265496-01-1 6.7/05	
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**	Follows OECD Guideline 453.	

2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4799/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Not specified but bendiocarb is known as a beige/white powder	
3.1.2.2	Purity	96.5%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	Anglia Laboratory Animals (formerly Carworth Europe), Alconbury, UK	
3.2.4	Sex	Male/Female	
3.2.5	Age/weight at study initiation	9 weeks; 134 – 167 g (main study)	
3.2.6	Number of animals per group	50M/50F (main study)	
3.2.6.1	at interim sacrifice	10M/10F per group (20M/20F from control group)	
3.2.6.2	at terminal sacrifice	All surviving animals	
3.2.7	Control animals	100M/100F (main study)	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	104 weeks	
3.3.2	Interim sacrifice(s)	After 52 weeks	
3.3.3	Final sacrifice	After 104 weeks	
3.3.4	Frequency of exposure	Daily	
3.3.5	Postexposure period	None – all surviving animals were sacrificed at the end of 104 weeks dietary administration.	
3.3.6	Type	Oral (dietary)	
3.3.7	Concentration	0, 10*, 20, 200 ppm in diet (* Group 2 dietary level increased from 2 to 10 ppm after 2 weeks of main phase.)	
3.3.8	Vehicle	Diet	
3.3.9	Concentration in vehicle	0, 10*, 20, 200 ppm in diet.	

3.3.10	Total volume applied	Daily diet
3.3.11	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Body weight	Yes; at commencement and then weekly
3.4.2	Food consumption	Yes; weekly
3.4.3	Water consumption	Yes; over a five day period during weeks 6, 12 and 25 for 10 cages of males (50M) and 10 cages of females (50F) for control and higher dose
3.4.4	Clinical signs	Yes; daily for the first 4 weeks and at weekly intervals thereafter
3.4.5	Macroscopic investigations	Yes
3.4.6	Ophthalmoscopic examination	During weeks 13, 26 and 52, the eyes of all surviving rats of each sex in each of Groups 1 (Control) and 4 (200 ppm) were examined using a Keeler indirect ophthalmoscope. During week 66, the eyes of all surviving rats of each sex in each of Groups 1 (Control) and 4 (200 ppm) and all surviving males receiving 20 ppm, were similarly examined. During week 80 the eyes of all surviving male animals from each group and females from Groups 1 (Control) and 4 (200 ppm) were examined, and during week 91 the eyes of all surviving male animals from each group, and females from Groups 1 (Control), 3 (20 ppm) and 4 (200 ppm) were similarly examined. During week 102, ophthalmoscopic examination was carried out on all surviving rats from all groups.
3.4.7	Haematology	Yes During weeks 13, 26, 51 and 102. 10M/10F from control and high dose groups Parameters: packed cell volume, haemoglobin, red cell count, reticulocyte count, MCHC, MCV, total white cell count, platelet count, thrombotest.
3.4.8	Clinical chemistry	Yes During weeks 13, 26, 51 and 102. 10M/10F from control and high dose groups Parameters: plasma urea, plasma glucose, total serum protein, SGPT, SGOT, calcium, serum cholesterol Cholinesterase activity determination: whole blood – during weeks 2, 14, 26, 39, 51 and 103; 10M/10F from each group and 15M/15F from control group (AchE activity was measured within 30 minutes after sampling and then again after 24 hours) brain – in weeks 52 and 104 kills; 10M/10F from each group and 15M/15F from control group
3.4.9	Urinalysis	Yes. During weeks 13, 27, 51 and 103. 10M/10F from control and high dose groups Parameters: volume, pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigments, urobilinogen, haemoglobin
3.4.10	Pathology	Yes

3.4.10.1	Organ weights	Yes At interim sacrifice: 10M/10F from each group and 20M/20F from control group At terminal sacrifice: All surviving animals Organs: adrenals, brain, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids, uterus, heart.	
3.4.11	Histopathology	Yes Rats dying during the study and all surviving animals from control and high dose groups. Organs: adrenals, brain, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids, uterus, aorta, bone with marrow, bone marrow, caecum, duodenum, eye, ileum, heart, jejenum, lungs, lymph nodes, mammary gland, mid-colon, middle ear, nasal cavity, oesophagus, pancreas, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, stomach, tongue, trachea, urinary bladder.	
3.4.12	Other examination	-	
3.5.	Statistics	Kruskal Wallis, Jonckheere test, Chi square test, variance analysis and Student's 't' test, Williams' test	
3.6	Further remarks	-	
		4. RESULTS AND DISCUSSION	
4.1	**Body weight**	Body weight gains were comparable in all groups at the end of the study.	
4.2	**Food consumption**	After week 2, food intake of all treated rats remained generally similar to that of controls – in the first 2 weeks of the main phase food consumption of male rats receiving 20 or 200 ppm bendiocarb was much less than that of the control rats.	
4.3	**Water consumption**	No significant difference from controls.	
4.4	**Clinical signs**	No overt clinical signs	
4.5	**Macroscopic investigations**	No effects noted	
4.6	**Ophthalmoscopic examination**	Ophthalmoscopic examinations carried out at different intervals during the study revealed a biologically significant increased incidence of lenticular opacities in the top dose males starting from week 52 and in the mid-dose males starting from week 80. At week 102, the incidence of these lesions was also increased in the top dose females (see Table A6.7-3).	
4.7	**Haematology**	No significant difference between controls and rats receiving 200ppm bendiocarb.	
4.8	**Clinical chemistry**	No significant difference between controls and rats receiving 200ppm bendiocarb.	

		Whole blood AChE activity was significantly reduced (by 11 – 37 %) among top dose males and females at weeks 2, 14, 26 and 51 and in top dose males (by 8 – 31 %) only at weeks 39 and 103. No treatment-related depression of whole blood AChE activity was noted in low and mid-dose rats except for the mid-dose males at 26 weeks (by 36 %). Brain cholinesterase activity was also significantly decreased (by 23 – 24 %) at 52 w in the top dose males and females respectively, and to a lesser extent (by 10 %) in the mid-dose females. At week 104, a marginal inhibition (9 – 10 %) of brain cholinesterase activity was noted among all groups of treated males and in the mid- and top dose females (10 – 12 %).	
4.9	**Urinalysis**	No significant difference in quantity or composition of urine excreted by controls and rats receiving 200ppm bendiocarb.	
4.10	**Pathology**	No indication of any reaction to treatment with bendiocarb	
4.11	**Organ weights**	No indication of any reaction to treatment with bendiocarb	
4.12	**Histopathology**	No indication of any reaction to treatment with bendiocarb	
4.13	**Other examinations**	None	
4.14	**Time to tumours**	No treatment-related tumour findings were seen at the end of the study.	
4.15	**Other**	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a 2-year carcinogenicity study the effects of bendiocarb were studied in Sprague-Dawley derived rats that had previously been part of a fertility study in which their parental animals had already been exposed to bendiocarb. Weanling animals (50/sex/treated group and 100/sex in the control group) selected from the F_1 litters were given 0, 10, 20 or 200 ppm (equivalent to doses of approximately 0, 0.4, 0.8 or 8 and 0, 0.5, 1 or 10 mg kg^{-1} d^{-1} for males and females, respectively) bendiocarb (96.5 % purity) in the diet for 104 w. The dose levels used were selected on the basis of whole blood cholinesterase inhibition in rat 90-day dietary work. The group of rats administered 10 ppm bendiocarb was given a lower dose of 2 ppm (equivalent to 0.08 and 0.1 mg kg^{-1} d^{-1} for males and females, respectively) for the first 2 w of the study. The dietary level of bendiocarb was increased for this group of animals in an attempt to define the repeat-dose no-effect level more clearly. Clinical signs were examined daily during the first 4 w and at weekly intervals thereafter. Blood and urine samples were collected at termination for the standard haematological, clinical chemistry and urinalysis investigations. Whole blood and brain AChE activities were also determined in 10 males and 10 females from each treated group and in 15 males and 15 females from the control group.	

		Ophthalmoscopic examinations were conducted at weeks 13, 26, 52 (control and top dose only), 66 (control animals, top dose and mid-dose males), 80 (males from each group, control and top dose females), 91 (males from each group, control, mid-dose and top dose females) and 102 (all surviving rats from all groups). At necropsy, a full histopathology examination was conducted on all animals including decedents. Additional satellite groups of rats (30/sex in the control group and 15/sex/treated group), dosed as above, were included for the provision of blood and urine samples at different intervals during the study, and for interim sacrifices at 52 and 60 w of treatment. The blood and urine samples from these satellite groups were collected at weeks 13, 26 and 52 (10 males and 10 females from the control and the top dose groups only) for the haematological, clinical chemistry and urinalysis investigations. Whole blood AChE levels were determined from the blood samples collected from these satellite groups at weeks 2, 14, 26, 39 and 52 (in 10 males and 10 females from each treated group and in 15 males and 15 females from the control group). At 52 w, up to 10 males and 10 females from each treated group and up to 20 males and 20 females from the control group were sacrificed and subjected to detailed macroscopic and microscopic examinations, organ weight analysis and brain AChE investigations. The remaining animals were maintained on treatment up to week 60 when they were killed for further brain AChE investigations.	
5.2	**Results and discussion**	In brief, there were no treatment-related mortalities or clinical signs of toxicity. Survival rates at 104 w were 26, 18, 24, 16 % in males and 38, 24, 30, 42 % in females at 0, 2 – 10, 20 and 200 ppm respectively which was considered to be typical of rats at that time (historical survival range: 25.0-53.0% and 25.0-60.0% for males and females, respectively) and therefore adequate to assess the carcinogenic potential of the test compound. Body weight gains were comparable in all groups at the end of the study. Whole blood AChE activity was significantly reduced among top dose males and females throughout the study (by 8-37% and up to 28% in males and females, respectively). At week 104, levels of AChE were comparable to controls (-8% and +2% in males and females respectively). No toxicologically relevant depression of whole blood AChE activity was noted in low and mid-dose rats except for the mid-dose males at 26 weeks (by 36 %). As this effect was not reproducible at any other time point, this isolated finding was not considered to be treatment-related. Brain cholinesterase activity was also significantly decreased (by 23 – 24 %) at 52 week in the top dose males and females respectively, and to a lesser extent (by 10 %) in the mid-dose females. This effect was however less pronounced at week 104 where only a marginal inhibition (9 – 10 %) of brain cholinesterase activity was noted among all groups of treated males and in the mid- and top dose females (10 – 12 %).	X

		Ophthalmoscopic examinations revealed an increased incidence of lenticular opacities in the top dose males compared to controls starting from week 52. At week 102, incidences of these lesions were also increased in the treated males and in the top dose females compared to their respective controls. However, with the exception of the high dose males at week 52 slightly outside the historical range, those incidences remained within the range of historical control data (see Table A6.7-3) and in the absence of a clear dose-relationship the relationship to treatment is doubtful. As a conclusion, the NOAEL was considered to be 20 ppm (= 0.8/1 mg/kg bw/d in males/females) based on the inhibition of whole blood and brain AChE at 200 ppm. In addition, no treatment-related tumour findings were seen at the end of the study. Hence, it was concluded that dietary administration of bendiocarb up to a dose (8/10 mg/kg bw/d in males/females) producing a toxicologically significant inhibition of brain cholinesterase activity at 52 w, was not carcinogenic to rats.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.7-1 Table for Brain Cholinesterase Monitoring as % of Variation Compared to Controls

Affected	Controls		Low dose 2 – 10 ppm		Medium dose 20 ppm		High dose 200 ppm	
	Weeks after treatment							
	52	104	52	104	52	104	52	104
Males	-	-	-6	-9	+2	-10*	-24*	-9
Females	-	-	-6	0	-10	-12*	-23*	-10

* = P<0.01 (means of the sexes were pooled for statistical analysis)

Table A6.7-2 Whole Blood Cholinesterase Levels at % of Variation Compared to Control (<30 mins After Sampling)

Group	Sex	Weeks after treatment					
		2	14	26	39	51	103
Control	M	-	-	-	-	-	-
	F	-	-	-	-	-	-
Low dose (2 – 10 ppm)	M	-11	-9	+7	-7	+26	+22
	F	+10	+1	+3	+46	-11	+32
Medium dose (20 ppm)	M	-4	-8	-36*	-1	-11	+8
	F	-3	+2	-8	+36	-4	+31
High dose (200 ppm)	M	-37*	-28*	-20*	-31*	-25*	-8
	F	-28*	-17*	-11*	+16	-15*	+2

* = P<0.01

Table A6.7-3 Results of Lenticular Opacity

Week	Incidence of lenticular opacities (% of animals affected)							
	Group							
	1 ♂ Control	2 ♂ + 2–10 ppm	3 ♂ 20 ppm	4 ♂ 200 ppm	1 ♀ Control	2 ♀ + 2–10 ppm	3 ♀ 20 ppm	4 ♀ 200 ppm
52	1.1	N	N	16.7*	0.0	N	N	0.0
Historical control data[a] (52 weeks)	Mean : 3.8 % Range : 0.0 – 12.8				Mean : 2.3 % Range : 0.0 – 10.6			
102	8.8	30.0	42.9*	40.0*	6.8	0.0	9.5	19.0
Historical control data[b] (102 weeks)	Mean : 35.3% Range : 20.0 – 83.3				Mean : 21.7 % Range : 5.9 – 50.0			

\+ Group 2 dietary level increased to 10 ppm after 2 weeks of main phase
N Not examined
a = historical data from 11 studies
b = historical data from 18 studies
* = P<0.05 compared with controls

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27th September 2006
Materials and methods	As described by the applicant.
Results and discussion	5.2. The survival rates of males rats in the 10 ppm and 200 ppm groups were considerably lower than the historical control value range. The UK CA has added an asterisk to those values in the tables that reached statistical significance.
Conclusion	
Reliability	3
Acceptability	The study is unacceptable by itself because of the low survival rates at 104 weeks. However, it can be used in a weight-of-evidence approach.
Remarks	The survival rates of rats in all the groups were considerably lower than the survival rate of ≥50% for rats at 24 months stipulated by OECD guideline 453. Therefore, the UK CA does not consider that the negative result is valid by itself.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1981) A Chronic Toxicity and Carcinogenicity Study in Mice with Technical NC 6897 (Final Report) [redacted] Document A90445 6.7/06 March 1981 Unpublished [redacted] (1980b) Determination of Bendiocarb (NC 6897) Dietary Concentration in a Chronic Toxicity and Carcinogenicity Study in Mice [redacted] Document A90446 6.7/07 June 1980 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**	Follows OECD Guideline 453.	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4799/3	
3.1.2	Specification	Material of lower purity than specification due to accidental plant contamination resulting in the presence of 9% china clay and 0.5% dimethoate (or dimethoate decomposition products) in some samples.	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	92.7%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	Albino HaM/ICR Swiss CD-1	
3.2.3	Source	Charles River Breeding Laboratories, Wilmington, MA	
3.2.4	Sex	Male/Female	

3.2.5	Age/weight at study initiation	5 weeks; 21 – 27 g	
3.2.6	Number of animals per group	50M/50F	
3.2.6.1	at interim sacrifice	n.a.	
3.2.6.2	at terminal sacrifice	All surviving animals	
3.2.7	Control animals	100M/100F	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	A maximum of 104 weeks	
3.3.2	Interim sacrifice(s)	No interim sacrifice	
3.3.3	Final sacrifice	After a maximum of 104 weeks The precise date of terminal sacrifice was when survival of all groups of the same sex was less than 40% and below 20% in any one of those groups. Therefore all surviving males were sacrificed after Week 89 and the females after Week 94 of the treatment.	
3.3.4	Frequency of exposure	Daily	
3.3.5	Postexposure period	None – all surviving animals were sacrificed at the end of dietary administration.	
3.3.6	Type	Oral (dietary)	
3.3.7	Concentration	0, 50, 250 and 1250 ppm in diet	X
3.3.8	Vehicle	Diet	
3.3.9	Concentration in vehicle	0, 50, 250 and 1250 ppm in diet.	
3.3.10	Total volume applied	Daily diet	
3.3.11	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Body weight	Yes; weekly	
3.4.2	Food consumption	Yes; weekly	
3.4.3	Water consumption	Yes; 5 day period at weeks 6, 12 and 24	
3.4.4	Clinical signs	Yes; daily	
3.4.5	Macroscopic investigations	Yes	
3.4.6	Ophthalmoscopic examination	Yes; weeks 34 and 89 for all animals. Weeks 51 and 78 for high dose and control groups.	
3.4.7	Haematology	No	
3.4.8	Clinical chemistry	No	
3.4.9	Urinalysis	No	

3.4.10	Pathology	Yes	
3.4.10.1	Organ weights	Yes Rats dying during the study and all surviving animals from control and high dose groups. Organs: brain, heart, kidneys, liver, testes.	
3.4.11	Histopathology	Yes Rats dying during the study and all surviving animals from control and high dose groups. Organs: adrenals, brain, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroids, uterus, aorta, bone with marrow, caecum, duodenum, eye, ileum, heart, jejenum, lungs, lymph nodes, mammary gland, inner ear, nasal cavity, oesophagus, pancreas, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, spinal cord, stomach, tongue, trachea, urinary bladder, gall bladder, large intestine	
3.4.12	Other examination	-	
3.5.	Statistics	Rao, Bartlett's test, ANOVA, Scheffe, Sachs	
3.6	Further remarks	-	
		4. RESULTS AND DISCUSSION	
4.1	**Body weight**	Body weight gains were comparable in all groups.	
4.2	**Food consumption**	No significant difference except at Week 24 where the food consumed by mid-dose males was significantly higher than control. After Week 86 the mean food consumption was generally higher in all groups of both sexes.	
4.3	**Water consumption**	No significant difference from controls.	
4.4	**Clinical signs**	No overt clinical signs of toxicity	
4.5	**Macroscopic investigations**	No effects noted	
4.6	**Ophthalmoscopic examination**	No effects noted	
4.7	**Haematology**	n.a.	
4.8	**Clinical chemistry**	n.a.	
4.9	**Urinalysis**	n.a.	
4.10	**Pathology**	No macroscopic findings attributable to treatment effects.	
4.11	**Organ weights**	A statistically significant increase in absolute and relative testes weight was seen in the high-dose males, and a dose-related increase in absolute and relative kidney weight was observed in the treated females at all dose levels.	
4.12	**Histopathology**	The histopathological investigations did not reveal any treatment-related changes.	
4.13	**Other examinations**	-	
4.14	**Time to tumours**	No treatment-related tumour findings were seen at the end of the study.	

4.15	**Other**	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a 2-year chronic toxicity/carcinogenicity study, albino Swiss CD-1 mice (50/sex/treated group and 100/sex in the control group) were administered 0, 50, 250 or 1250 ppm (equivalent to doses of approximately 0, 8.06, 42.4, 211 and 0, 10.7, 56.8, 286 mg kg^{-1} d^{-1} for males and females, respectively) bendiocarb (92.7 % purity) in the diet. The dose levels used were selected on the basis of whole blood cholinesterase inhibition in a preliminary dietary study. The study was scheduled to be terminated after a maximum of 104 w of treatment, contingent upon survival. When the survival rate in all groups of the same sex was < 40 %, and < 20 % in any one of the groups, the study was terminated; this resulted in all surviving males being sacrificed after week 89 of treatment and the females after week 94. No blood and urine samples were collected for the standard haematological, clinical chemistry and urinalysis investigations. Also, no blood and brain AChE activities were determined. Ophthalmoscopic examinations were conducted at weeks 34, 51 (control and top dose only), 78 (control and top dose only) and 89 (all dose levels). At necropsy, the standard gross pathology, organ weight and microscopic examinations were conducted in all animals including decedents.	X
5.2	**Results and discussion**	There were no treatment-related deaths, clinical signs of toxicity effects on body weight gain, food consumption or ophthalmoscopic examinations. A statistically significant lower survival rate was noted in the high-dose females and in the low-dose males. Survival rate at termination were 38, 18, 28 and 32% in males and 31, 28, 36 and 20% in females at 0, 50, 250 and 1250 ppm respectively. Given the absence of a dose-response relationship, this lower survival rate observed in the high-dose females and in the low-dose males was regarded as being an incidental finding of no toxicological significance. At necropsy, no treatment-related gross changes were noted. A statistically significant increase in absolute (by 12%) and relative (by 2%) testes weight was seen in the high-dose males, and a dose-related increase, which did not attain statistical significance, in absolute (up to 17% of the control value) and relative (up to 16%) kidney weights was observed in the treated females at all dose levels. However, the histopathological investigations of these organs did not reveal any treatment-related changes and hence the weight differences were judged not to be of toxicological significance. There was no treatment-related effects observed at the histopathological examinations. Neoplastic and non-neoplastic lesions were those commonly observed in mice of this strain and age with comparable incidence and severity in control and treated groups..	X
		No treatment-related tumour findings were seen at the end of the study. The NOAEL was considered to be 1250 ppm (equivalent to 211 mg/kg/day male and 286 mg/kg/day for females).	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	Sample purity was lower than specification but is not considered to have adversely affected the study.	

Table A6.7-4 **Table of Carcinogenicity Study – Testes Effects and Effects on Female Kidneys**

Parameter	Control data Study		Low dose 50 ppm/		Medium dose 250 ppm/		High dose 1250 ppm/		Dose response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	37	32	9	14	14	18	16	10	76	74
Testes weight	0.228	-	0.236	-	0.210	-	0.268	-	+	na
Kidney weights	-	0.555	-	0.581	-	0.606	-	0.648	/	+

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28th September 2006
Materials and methods	3.3.7/5.1 The mean bendiocarb concentrations of the test diet samples were 79.5%, 79.8% and 87.7% of the nominal 50, 250 and 1250 ppm levels, respectively. At the nominal 50 ppm level, five samples contained virtually no bendiocarb and at week 84 the content was 316% of nominal. Homogeneity of bendiocarb within the feed was shown to be satisfactory at the 250 ppm and 1250 ppm levels, but there was some gradation in the 50 ppm feed. The equivalent doses presented were not adjusted for the actual analytically determined levels of bendiocarb. When adjusted for the mean bendiocarb contents of the test diet samples, the equivalent doses for males were 6.4, 33.8, 185.0 mg/kg/d and for females were 8.5, 45.3, 250.8 mg/kg/d for the 50, 250 and 1250 ppm feeds, respectively.
Results and discussion	5.2. At 18 months (week 78) the survival of all groups was >50% (range 58% to 76%). 5.2. The NO(A)EL for mice was in this study was 250 ppm (equivalent to 42/57 mg/kg/d for males/females). For males, this was defined by an increase in absolute and testicular weights at 1250 ppm after 89 weeks of treatment. For females, a reduced survival was apparent for animals receiving 1250 ppm from week 86 to the termination of the study at week 94, with a statistically significant reduction in survival at week 89.
Conclusion	
Reliability	1
Acceptability	Acceptable
Remarks	At 18 months the survival in all groups was >50%, in accordance with the stipulation in OECD guideline 453 for a negative result to be acceptable.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.8 Reproductive toxicity

6.8.1 Teratogenicity test

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1991) Technical Bendiocarb: Rat Oral Developmental Toxicity (Teratogenicity) Study [redacted] Document A90627 6.8.1/01 15 August 1991 Unpublished [redacted] (1990) Determination of Bendiocarb Concentrations in Methyl Cellulose Suspensions for a Preliminary Dose Range Finding Study and Teratology Study in the Rat [redacted] Document A90628 6.8.1/02 17 August 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 83-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 19306/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Light brown powder	
3.1.2.2	Purity	97.2%	
3.1.2.3	Stability	Stable as stored at room temperature in the dark and bendiocarb is not known to decompose at room temperature.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley	
3.2.3	Source	Charles River, Portage, Michigan	

3.2.4	Sex	Female (mated with males of the same strain)	
3.2.5	Age/weight at study initiation	8 – 10 weeks; 200 – 220 g	
3.2.6	Number of animals per group	25 – 30	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Time-mated – considered day 0 of pregnancy	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Day 6 – 15 of gestation	
3.3.2	Postexposure period	5 days	
3.3.3	Type	Oral (gavage)	
3.3.4	Concentration	0, 0.4, 2 or 10 mg kg^{-1} d^{-1} bendiocarb	
3.3.5	Vehicle	1% Methyl cellulose in distilled water	
3.3.6	Concentration in vehicle	0.004, 0.02, 0.1% w/v	
3.3.7	Total volume applied	Calculated from dose level/bodyweight	
3.3.8	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Body weight	Yes; initially and on Days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20	
3.4.2	Food consumption	Yes; on weigh days	
3.4.3	Clinical signs	Yes; daily	
3.4.4	Examination of uterine content	Yes; on day 20 Uterus was weighed and examined for number of implantation sites, number of live and dead foetuses, resorptions (early and late) and any abnormalities.	
3.4.5	Examination of foetuses		
3.4.5.1	General	Each viable foetus was sexed and weighed.	
3.4.5.2	Skeletal	Yes; 50% of foetuses	
3.4.5.3	Soft tissues	Yes; 50% of foetuses	
3.5	**Further remarks**	-	

		4. RESULTS AND DISCUSSION	
4.1	**Maternal toxic effects**	There were no maternal deaths. Clinical signs of maternal toxicity were restricted to the top dose group and consisted mainly of muscle twitching, body tremors, smacking of lips and salivation. Mean maternal body weight gain was significantly reduced (by 26 %) in the top dose group during the treatment period; thereafter, the rate of body weight gain in this dose group was similar to the controls. Overall, bodyweight gain of this group during gestation remained 11% lower than in the controls. Food consumption was also slightly reduced (by 12%) during the treatment period at the top dose. A tendency toward slightly lower food consumption was seen in the mid-dose group during the later stages of gestation. Mean water consumption was significantly increased during the treatment period and thereafter in the top dose group (by 15% in average compared to the controls). No significant macroscopic abnormalities were noted amongst the dams from any dose group.	X
4.2	**Teratogenic / embryotoxic effects**	There were no significant intergroup differences in the mean number of *corpora lutea* or implantations. An increase in the mean number of early resorptions was seen at the top dose (1.1 vs. 0.4 in controls). This resulted in a statistically significant increase in the total number of resorptions (1.3 vs. 0.4), also expressed as post-implantation loss (13.9 % vs. 3.0 %). The litter size at this dose level was consequently slightly reduced (although not statistically significantly) when compared with the controls (10.2 vs. 11.6). Litter weight was also slightly, but not statistically significantly, reduced at the top dose (by 14 %). However, mean foetal weight was not significantly reduced (-2 % compared to controls). No treatment-related effects were noted at lower dose levels. No external, skeletal or visceral abnormalities were observed at any dose level. Overall, a statistically significant increased incidence of embryonic death (early and late resorptions) was seen at a dose (10 mg kg^{-1} d^{-1}) at which there was also significant maternal toxicity consisting of clinical signs and markedly reduced body weight gain. No developmental effects were noted at 2 and 0.4 mg kg^{-1} d^{-1}. Therefore, the NOAEL for developmental effects was 2 mg kg^{-1} d^{-1}. There were no signs of maternal toxicity at 0.4 mg kg^{-1} d^{-1}, and the only effect seen at 2 mg kg^{-1} d^{-1} was a reduction in food consumption which in the absence of any effect on bodyweight, was not considered as an adverse effect.	
4.3	**Other effects**	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 25 – 30 pregnant Sprague-Dawley rats were administered by oral gavage 0, 0.4, 2 or 10 mg kg^{-1} d^{-1} bendiocarb (purity 97.2 %) in 1 % methylcellulose on day 6 to 15 of gestation. The dose levels used were selected on the basis of the results of a range finding study. On day 20 of gestation, dams were sacrificed and subjected to macroscopic examinations. For each animal, the uterus was weighed and examined for number of implantation sites, number of live and dead foetuses, resorptions (early and late) and any abnormalities. Each set of ovaries was examined for *corpora lutea*. Each viable foetus was sexed and weighed. Half of them were then subjected to external and visceral examinations and half to skeletal investigations.	

5.2	**Results and discussion**	There were no maternal deaths. Clinical signs of maternal toxicity were restricted to the top dose group and consisted mainly of muscle twitching, body tremors, smacking of lips and salivation. Mean maternal body weight gain was significantly reduced (by 26%) in the top dose group during the treatment period; thereafter, the rate of body weight gain in this dose group was similar to the controls. Overall, bodyweight gain of this group during gestation remained 11% lower than in the controls. Food consumption was also slightly reduced (by 12 %) during the treatment period at the top dose. A tendency toward slightly lower food consumption was seen in the mid-dose group during the later stages of gestation. Mean water consumption was significantly increased during the treatment period and thereafter in the top dose group (by 15% in average compared to controls). No significant macroscopic abnormalities were noted amongst the dams from any dose group. There were no significant intergroup differences in the mean number of *corpora lutea* or implantations. An increase in the mean number of early resorptions was seen at the top dose (1.1 vs. 0.4 in controls). This resulted in a statistically significant increase in the total number of resorptions (1.3 vs. 0.4), also expressed as post-implantation loss (13.9 % vs. 3.0 %). The litter size at this dose level was consequently slightly reduced (although not statistically significantly) when compared with the controls (10.2 vs. 11.6). Litter weight was also slightly, but not statistically significantly reduced at the top dose (by 14 %). However mean foetal weight was not significantly affected (-2% compared to control). No treatment-related effects were noted at lower dose levels. No external, skeletal or visceral abnormalities were observed at any dose level. Overall, a statistically significant increased incidence of embryonic death (early and late resorptions) was seen at a dose (10 mg kg^{-1} d^{-1}) at which there was also significant maternal toxicity consisting of clinical signs and markedly reduced body weight gain. No developmental effects were noted at 2 and 0.4 mg kg^{-1} d^{-1}. Therefore, the NOAEL for developmental effects was 2 mg kg^{-1} d^{-1}. There were no signs of maternal toxicity at 0.4 mg kg^{-1} d^{-1}, and the only effect seen at 2 mg kg^{-1} d^{-1} was a reduction in food consumption which in the absence of any effect on bodyweight, was not considered as an adverse effect.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	-	
5.3.2	NO(A)EL maternal toxic effects	2 mg kg^{-1} d^{-1}	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	-	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	2 mg kg^{-1} d^{-1}	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

Table A6.8.1-1 Table for Teratogenic Effects – Maternal Effects

Parameter	Control	Low dose (0.4 mg/kg)	Medium dose (2.0 mg/kg)	High dose (10mg/kg)	Dose response +/-
Number of dams examined	30	25	30	25	na
Clinical findings during application of test substance					
Body tremors	0	0	0	2	+
Lip smacking	0	0	0	11	+
Muscle twitching	0	0	0	22	+
Salivation post-dosing	0	0	0	9	+
Mortality of dams	0	0	1	0	-
Abortions	-	-	-	-	-
Bodyweight gain in g (% of control)					
Overall	165.3 (100)	171.9 (104)	165.2 (100)	146.9 (89)	+
GD 6-15	69.2 (100)	72.5 (105)	64.7 (93)	51.1 (74)	+
Food consumption g/rat/day (% of control)					
Overall	26.4 (100)	26.8 (102)	25.8 (98)	24.3 (92)	+
GD 6-15	26.8 (100)	27.2 (101)	26.2 (98)	23.6 (88)	+
Water consumption during treatment g/rat/day (% of control)	37.2 (100)	37.6 (101)	36.2 (97)	42.6 (115)	+
Pregnancies	27	24	23	23	-
Necropsy findings in dams dead before end of test	The few minor macroscopic changes noted at post mortem examination of the females did not indicate any treatment-related aetiology.				

Table A6.8.1-2 Table for Teratogenic effects – Litter Response (Caesarean section data)

Parameter	Control	Low dose (0.4 mg/kg)	Medium dose (2.0 mg/kg)	High dose (10mg/kg)	Dose response +/-
Corpora lutea	13.9	14.0	14.0	13.9	/
Implantations	12.0	13.0	12.6	11.5	/
Resorptions	0.4	1.2	0.9	1.3	+
Total no. of foetuses	314	282	270	234	na
Pre-implantation loss %	13.1	7.2	9.3	16.3	-
Post-implantation loss %	3.0	9.8	6.3	13.9*	+
Live foetuses/litter	11.6	11.8	11.7	10.2	-
Foetus weight (mean)	3.17	3.09	3.24	3.10	-
Foetal sex ratio %male	47.2	47.5	49.3	48.5	-

* statistically significant

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1st November 2006
Materials and methods	As described by the applicant.
Results and discussion	4.1. There was one maternal death in the 2 mg/kg/d group, but no cause of death was established and it was not considered to be treatment-related. 5.2. There was one maternal death in the 2 mg/kg/d group, but no cause of death was established and it was not considered to be treatment-related.
Conclusion	
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1981a) Technical NC 6897: Effects of Oral Administration upon Pregnancy in the Rabbit (5) Definitive study [redacted] Document A90442 6.8.1/03 April 1981 Unpublished [redacted] (1980) Determination of Bendiocarb (NC 6897) Concentrations in Aqueous Gum Tragacanth Suspensions for a Teratology Study in the Rabbit [redacted] Document A90471 6.8.1/04 October 1980 Unpublished [redacted] (2007) Technical NC 6897: Effects of Oral Administration upon Pregnancy in the Rabbit – Review of eye abnormalities [redacted] Document M-286514-01-1 6.8.1/05 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**	Follows OECD Guideline	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4799/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Fine white powder	
3.1.2.2	Purity	97.7 – 98.5%	
3.1.2.3	Stability	Stable at room temperature in a dry atmosphere	
3.2	**Test Animals**		

3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	Morton Rabbitries, Stansted, Essex
3.2.4	Sex	Sexually mature virgin female
3.2.5	Age/weight at study initiation	18 – 24 weeks; 3.88 – 3.98 kg
3.2.6	Number of animals per group	27 – 29
3.2.7	Control animals	Yes
3.2.8	Mating period	Artificial insemination
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of exposure	Day 6 – 28 post-mating
3.3.2	Postexposure period	Animals sacrificed on day 29
3.3.3	Type	Oral (gavage)
3.3.4	Concentration	0, 1, 2.5 or 5 mg kg^{-1} d^{-1} bendiocarb
3.3.5	Vehicle	0.5 % w/v aqueous gum tragacanth
3.3.6	Concentration in vehicle	Calculated from dose level
3.3.7	Total volume applied	Volume-dosage 5 ml/kg
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes, daily
3.4.2	Food consumption	No
3.4.3	Clinical signs	Yes, daily
3.4.4	Examination of uterine content	Yes The reproductive tract, complete with ovaries, was dissected out and the following recorded: a) number of corpora lutea in each ovary b) number of implantation sites c) number of resorption sites (classified as early or late) d) number and distribution of live and dead foetuses in each uterine horn e) weight and sex of individual foetuses f) individual placenta weights g) external abnormalities of individual foetuses
3.4.5	Examination of foetuses	
3.4.5.1	General	Each viable foetus was sexed and weighed.
3.4.5.2	Skeletal	Yes
3.4.5.3	Soft tissues	Yes

3.5	Further remarks	10 females from each group tested for whole blood cholinesterase activity on day 28	
		4. RESULTS AND DISCUSSION	
4.1	Maternal toxic effects	Twenty-six (i.e. 6, 10, 5 and 5 at 0, 1, 2.5 and 5 mg kg^{-1} d^{-1} respectively) dams died or were killed in extremis during the study. At necropsy, there was no evidence that these deaths were due to treatment. Findings included evidence of other causes such as tracheal intubation, respiratory tract infection and gastro-intestinal disorder. Three mid-, and 24 high dose dams showed a post-dosing response to treatment that included increased respiratory rate, salivation, muscular tremors, prostration and incontinence. In addition, one top dose dam developed convulsions and died within 3 h of dosing. No clinical signs of toxicity were observed amongst dams of the low dose group. Mean maternal body weight gain of the top dose animals was significantly depressed during treatment (by 36 %), whereas that of the mid-dose animals was reduced (by 15 %). Body weight gains of the low dose group were unaffected by treatment. A statistically significant, dose-related inhibition (31 % to 71 %) of whole blood cholinesterase activity was evident from the low to the high dose treated groups on day 28 of gestation. No significant macroscopic pathological abnormalities were noted amongst the dams from any dose group. One animal each from the control and the low dose groups were not pregnant. Two dams (one each from the high and mid-dose groups respectively) aborted on days 20 and 22 of gestation. In addition, one animal in each of these groups delivered prematurely on day 28 of gestation.	
4.2	Teratogenic / embryotoxic effects	Numbers of *corpora lutea*, implantations and viable foetuses, the extent of pre-implantation loss, and mean foetal and placental weights were similar across all groups. Post-implantation loss was statistically significantly increased in the mid- and high dose groups (15.4 % and 15.6 % respectively vs. 5.3 % in controls), although the values were well within the laboratory historical background control range (1 % – 27 %). Other litter parameters were similar amongst all groups. An increased incidence of anomalies in the appearance of the eyes was seen amongst the mid- and high dose foetuses (1.1 and 1.9 respectively vs. 0.0 in controls), but these findings were considered not to be related to treatment. There was a slight reduction in the degree of ossification in the high dose group illustrated by the size of the anterior fontanelle. However, other ossification parameters were similar to controls. No other abnormalities and no malformations were observed.	
4.3	Other effects	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	Groups of 27 – 29 pregnant (artificially inseminated) New Zealand white rabbits were administered by oral gavage 0, 1, 2.5 or 5 mg kg^{-1} d^{-1} bendiocarb (purity 99 %) in 0.5 % w/v aqueous gum tragacanth on days 6 to 28 of gestation. The day of insemination was designated day 0 of gestation. Dams were observed daily for mortalities and clinical signs of toxicity.	

		Body weight was recorded at initiation, every other day of gestation and at termination (day 29 of gestation). On day 28, 10 dams from each group were randomly selected for estimation of whole blood cholinesterase activity. On day 29, all the treated dams were sacrificed and subjected to macroscopic examinations. For each animal, the uterus was excised, weighed and examined for implantation sites, number of live and dead foetuses, resorptions (early and late) and any abnormalities. Ovaries were examined for *corpora lutea*. Each viable foetus was sexed, weighed and subjected to external, skeletal and visceral examinations.	
5.2	**Results and discussion**	Twenty-six (i.e. 6, 10, 5 and 5 at 0, 1, 2.5 and 5 mg kg^{-1} d^{-1} respectively) dams died or were killed in extremis during the study. At necropsy, there was no evidence that these deaths were due to treatment. Findings included evidence of other causes such as tracheal intubation, respiratory tract infection and gastro-intestinal disorder. Three mid-, and 24 high dose dams showed a post-dosing response to treatment that included increased respiratory rate, salivation, muscular tremors, prostration and incontinence. In addition, one top dose dam developed convulsions and died within 3 h of dosing. No clinical signs of toxicity were observed amongst dams of the low dose group. Mean maternal body weight gain of the top dose animals was significantly depressed during treatment (by 36 %), whereas that of the mid-dose animals was reduced (by 15 %). Body weight gains of the low dose group were unaffected by treatment. A statistically significant, dose-related inhibition (31 % to 71 %) of whole blood cholinesterase activity was evident from the low to the high dose treated groups on day 28 of gestation. No significant macroscopic pathological abnormalities were noted amongst the dams from any dose group. One animal each from the control and the low dose groups were not pregnant. Two dams (one each from the high and mid-dose groups respectively) aborted on days 20 and 22 of gestation. In addition, one animal in each of these groups delivered prematurely on day 28 of gestation. Numbers of *corpora lutea*, implantations and viable foetuses, the extent of pre-implantation loss, and mean foetal and placental weights were similar across all groups. Post-implantation loss was statistically significantly increased in the mid- and high dose groups (15.4 % and 15.6 % respectively vs. 5.3 % in controls), although the values were well within the laboratory historical background control range (1 % – 27 %). Other litter parameters were similar amongst all groups. An increased incidence of anomalies in the appearance of the eyes was seen amongst the mid- and high dose foetuses (1.1 and 1.9 respectively vs. 0.0 in controls), but these findings were considered not to be related to treatment. There was a slight reduction in the degree of ossification in the high dose group illustrated by the size of the anterior fontanelle. However, other ossification parameters were similar to controls. No other abnormalities and no malformations were observed.	

		Overall, the only developmental effect that was regarded as being adverse was an increased incidence of foetuses with incomplete ossification of the cranial bones observed at the top dose of 5 mg kg^{-1} d^{-1}. Based on these findings, a NOAEL of 2.5 mg kg^{-1} d^{-1} was determined for developmental toxicity from this study in rabbits. Severe maternal toxicity consisting of clinical signs, abortions, premature deliveries, reductions in body weight gain and inhibition of whole blood cholinesterase was observed at 5 mg kg^{-1} d1 and to a lesser extend at 2.5 mg.kg^{-1}.d^{-1}. The only maternal effect observed at 1 mg/kg/d was a decrease in whole blood cholinesterase activity.	
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	-	
5.3.2	NO(A)EL maternal toxic effects	< 1 mg kg^{-1} d^{-1} (based on inhibition of acethylcholinesterase activity)	X
5.3.3	LO(A)EL embryotoxic / teratogenic effects	-	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	2.5 mg kg^{-1} d^{-1}	
5.3.5	Reliability	1	
5.3.6	Deficiencies	No	

Table A6.8.1-3 Table for Teratogenic Effects – Maternal Effects

Parameter	Control	Low dose 1.0 mg/kg/d	Medium dose 2.5 mg/kg/d	High dose 5 mg/kg/d	Dose response +/-
Number of dams examined	29	29	27	27	n.a.
Clinical findings during application of test substance					
Hyperactivity	0	0	0	3.3	+
Increased respiratory rate	0	0	0.4	14.1	+
Muscular tremors	0	0	0	8.3	+
Incoordination	0	0	0.5	20.6	+
Mortality of dams	6	10	5	6	-
Abortions	-	-	3	6	+
Body weight gain kg (% of control)					
Overall GD0-28	0.51 (100)	0.55 (108)	0.45 (88)	0.40 (78)	+
During treatment GD6-28	0.33 (100)	0.38 (115)	0.28 (85)	0.21 (64)	+
Pregnancies	22	18	20	19	-
Acetylcholinesterase activity (% of inhibition)	-	31	55	71	+
Necropsy findings in dams dead before end of test	The few minor macroscopic changes noted at post mortem examination of the females did not indicate any treatment-related aetiology.				

n.a. = not applicable

Table A6.8.1-4 Table for Teratogenic Effects – Litter Response (Caesarean section data)

Parameter	Control		Low dose 1 mg/kg/d	Medium dose 2.5 mg/kg/d	High dose 5 mg/kg/d	Dose response +/-
	Historical range	Study				
Corpora lutea	9.5 – 13.2	11.7	11.2	12.4	11.6	-
Implantations	6.5 – 11.6	9.5	9.4	10.7	9.8	-
Resorptions	0 – 2.5	0.5	0.7	1.7	1.5	-
Pre-implantation loss %	6.0 – 35.7	19.1	15.4	13.7	15.5	-
Post-implantation loss %	1.0 – 27.2	5.3	7.6	15.4*	15.6*	-
Total no. of foetuses	-	197	157	181	157	-
Live foetuses/litter	5.5 – 10.2	9.0	8.7	9.1	8.3	-
Foetus weight (mean)	36.6 – 47.2	39.3	43.3	40.4	38.6	-
Placenta weight	4.7 – 7.1	5.7	6.3	5.8	5.5	-
Foetal sex ratio %male	-	47.2	47.5	49.3	48.5	-

* Statistically significant from control but within historical control range

Table A6.8.1-5 Table for Teratogenic Effects – Examination of the Foetuses

Parameter	Control		Low dose 1 mg/kg/d	Medium dose 2.5 mg/kg/d	High dose 5 mg/kg/d	Dose response +/-
	Historical range	Study				
Size of anterior fontanelle		-	-			
Small	6.4 – 55.8	18.3	19.1	27.6	14.6	-
Medium	38.5 – 77.6	75.6	73.2	63.0	63.1	-
Large	0.0 – 21.3	6.1	7.6	9.4	22.3	+

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7th November 2006
Materials and methods	As described by the applicant.
Results and discussion	As described by the applicant.
Conclusion	5.3.2. The UK CA does not consider that inhibition of whole-blood acetylcholinesterase activity is a marker of toxicity. Therefore, the NO(A)EL for maternal effects is 1 mg/kg/d.
Reliability	1
Acceptability	Acceptable
Remarks	The total number of foetuses with eye anomalies in the 2.5 and 5.0 mg/kg/d groups was 5, with incidences of 1.1 and 1.9 in these two groups, respectively. The litter incidences were 5.0 and 10.5, respectively.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.8.2 Two generation reproduction study

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1981b) Technical NC 6897 (CR 4799/1): Effects of Dietary Administration upon Reproductive Performance and Teratogenic Response of Rats Treated Continuously through Three Successive Generations [redacted] Document A90447 6.8.2/01 March 1981 Unpublished [redacted] (1979b) Determination of Bendiocarb (NC 6897) Dietary Concentration in a Multigeneration Study with Rats [redacted] Document A90448 6.8.2/02 June 1979 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**	The overall study design was based on the OECD 2-generation reproduction toxicity study guideline, with an extension to include the production of an F_3 generation. A second mating for the F_0, F_1 and F_2 generations and a teratological segment for each generation was also incorporated.	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement but was conducted to QA.	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	CR 4799/1	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Not specified but bendiocarb is known as a beige/white powder	
3.1.2.2	Purity	97.0 – 99.3%	
3.1.2.3	Stability	Stable over the period of its administration to the test animals	
3.2	**Test Animals**		
3.2.1	Species	Rat	

3.2.2	Strain	CD	
3.2.3	Source	Charles River, UK	
3.2.4	Sex	Male/female	
3.2.5	Age/weight at study initiation	Young adults; 177 – 182 g (M), 145 – 148 g (F)	
3.2.6	Number of animals per group	30M/30F	
3.2.7	Mating	See Table A6.8.2-1	X
3.2.8	Duration of mating	Maximum of 21 days	
3.2.9	Deviations from standard protocol	None, but see 2.1	
3.2.10	Control animals	30M/30F	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	See Table A6.8.2-1	X
3.3.2	Duration of exposure before mating	90 days	
3.3.3	Duration of exposure in general P, F1, F2 males, females	90 days prior to pairing, and throughout mating (1 male to 1 female for a maximum of 21 d), gestation and the lactation of two litters, F_{1A} and F_{1B}. This protocol was repeated on two further occasions, with two litters, from each of the second (F_2) and third (F_3) generations, being investigated. More specifically, from the second litters of the F_1 and F_2 generations (F_{1B}, F_{2B}), within each treatment group, 30 male and 30 female weanlings were selected at random for a further reproductive toxicity investigation that included a maturation period of 90 days and 2 successive matings.	
3.3.4	Type	Dietary	
3.3.5	Concentration	0, 10, 50 and 250 ppm in food	
3.3.6	Vehicle	Diet	
3.3.7	Concentration in vehicle	0, 10, 50 and 250 ppm in food	
3.3.8	Total volume applied	Daily diet	
3.3.9	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes, daily	
3.4.2	Body weight	Yes Males were weighed weekly throughout the study. Females were weighed weekly during the 90-day maturation phase, on Days 1, 3, 7, 14 and 21 of gestation, and on Days 1, 4, 10, 14, 21 and 25 of lactation.	

3.4.3	Food/water consumption	Yes; daily for food and weekly for water	
3.4.4	Oestrus cycle	The distribution and regularity of the oestrus cycle were monitored for 10 d prior to the first pairing of each generation and the second pairing of the F_1 and F_2 generations.	
3.4.5	Sperm parameters	Testes weight	
3.4.6	Offspring	Number and sex of pups, stillbirths, live births, presence of gross abnormalities, weight gain, physical or behavioural abnormalities (pinna unfolding, hair growth, tooth eruption, eye opening, auditory function and visual function).	
3.4.7	Organ weights P and F1, F2, F3	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thyroid, uterus.	
3.4.8	Histopathology P and F1	F_0 parents – ten males and ten females per group, and also any animals with suspect fertility. F_1 and F_2 parents – ten males per group, and also any with suspect fertility; all females except those selected for teratological examination. For full microscopic evaluation the following tissues were studied: Adrenals, all tumours, aorta, brain (cerebral cortex, medulla, cerebellum), bone marrow smear (after air drying fixation in anhydrous methanol), caecum, duodenum, eye and optic nerve (fixation in Davidson's fluid), heart, ileum, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), mammary gland (posterior), oesophagus, *ovaries, pancreas, *pituitary, *prostate, salivary gland, sciatic nerve, skeletal muscle, skin, spleen, stomach, *testes, thymus, thyroid, trachea, urinary bladder, *uterus. *These tissues, and the seminal vesicles were studied for some animals with suspect fertility from the F_0 generation and all animals with suspect fertility from the F_1 and F_2 generations. Other tissues preserved, but not routinely processed, included: Bone, colon, mammary gland (anterior), seminal vesicles, second eye, spinal cord (where indicated by nature of material or signs), tongue.	
3.4.9	Histopathology F1 not selected for mating, F2	F_1 and F_2 parents – ten males per group, and also any with suspect fertility; all females except those selected for teratological examination. F_{2B} offspring – five males and five females per group. F_{3B} offspring – ten males and ten females per group.	
3.5	**Further remarks**	-	
		4. RESULTS AND DISCUSSION	
4.1	**Effects**		
4.1.1	Parent males	No treatment-related mortalities or clinical signs of toxicity were observed amongst animals of all 3 generations. No treatment related macroscopic abnormalities were observed at necropsy of the F_0 parental animals.	

4.1.2	Parent females	No treatment-related mortalities or clinical signs of toxicity were observed amongst animals of all 3 generations. In the F_0 females, a statistically significant reduction (by 12 %) in body weight gain was seen during the F_{1A} gestation period at the mid- and high-dose levels. A statistically significant increase in the number of F_0 females that were acyclic or pseudo-pregnant associated with a prolonged pre-coital interval was observed at the top dose. However, no impact on reproduction performance which was similar in all groups. Gestation length was comparable among all groups of all 3 generations throughout each littering. No treatment related macroscopic abnormalities were observed at necropsy of the F_0 parental animals.
4.1.3	F1 males	Organ weight analysis of these animals revealed statistically significant increases in the relative thyroid gland weight of the F_1 top dose males (by 24 %) and in the relative seminal vesicles weight of the F_1 mid- and top dose males (by 29 and 16 % respectively). No treatment related macroscopic abnormalities were observed at necropsy of the F_1 parental animals, F_{1B} surplus and F_{1A} offspring.
4.1.4	F1 females	Bodyweight gain of females in the low and high dose groups (10 and 250 ppm) showed a marginal depression towards the end of the pre-mating period, but all groups gained weight at a comparable rate throughout the F_{2A} gestation period. During the first 14 days of the F_{2B} gestation period, bodyweight gain of females in the high dose group was slightly depressed, but thereafter the performance of all treated groups was similar to that of the controls. A significant reduction (6%) in water intake was seen in the top dose females of the F1 generation only throughout the pre-mating period. Gestation length was comparable among all groups of all 3 generations throughout each littering. No treatment related macroscopic abnormalities were observed at necropsy of the F_1 parental animals, F_{1B} surplus and F_{1A} offspring.
4.1.5	F2 males	A slight (statistically significant at the mid-, and top dose levels) increase in the relative adrenal gland weight was also seen in all treated F_2 males (by 12, 23 and 22 % at 10, 50 and 250 ppm respectively). An increased incidence of moderate or severe geriatric nephropathy was observed in the mid- and top dose groups of the F_2 generation (5/9 and 2/10 respectively vs. 0/10 on controls). Given that this lesion was only seen in the F_2 generation, it was considered likely that it represented a chance finding of no toxicological significance. Ophthalmoscopic examinations conducted in the F_2 parental animals revealed no treatment-related changes. No treatment related macroscopic abnormalities were observed at necropsy of the F_2 parental animals.
4.1.6	F2 females	Marginal depression in female bodyweight gain were seen in the low and high dose groups (10 and 250 ppm) before pairing, in the high dose group during the first gestation period (F_{3A}) and in the low dose group during the second gestation period (F_{3B}).

		A slight (statistically significant at the low, and mid-dose levels) decrease in relative uterus weight was observed in all treated F_2 females (by 20, 25 and 20 % at 10, 50 and 250 ppm respectively). A slight (statistically significant at the top dose level) reduction in absolute pituitary gland weight was observed in all treated F_2 females (by 9, 5, and 15 % at 10, 50 and 250 ppm respectively). Although statistically significant, in the absence of any associated histopathological changes, these organ weight changes are regarded as being of no toxicological significance. In addition, for the adrenal gland, the uterus and the pituitary gland weights, no dose-response relationship was noted. An increased incidence of moderate or severe geriatric nephropathy was observed in the mid- and top dose groups of the F_2 generation (5/9 and 2/10 respectively vs. 0/10 on controls). Given that this lesion was only seen in the F_2 generation, it was considered likely that it represented a chance finding of no toxicological significance. A very few F_2 females of the mid- and high-dose groups showed irregular oestrus cycles prior to the F_{3A} mating. Gestation length was comparable among all groups of all 3 generations throughout each littering. No treatment related macroscopic abnormalities were observed at necropsy of the F_2 parental animals.	
4.2	**Other**	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effects of bendiocarb on reproduction were investigated in a comprehensive 3-generation study in rats. The overall study design was based on the OECD 2-generation reproduction toxicity study guideline, with an extension to include the production of an F_3 generation. A second mating for the F_0, F_1 and F_2 generations and a teratological segment for each generation was also incorporated. Groups of 30 male and 30 female Sprague-Dawley rats (F_0 generation) were fed continuously 0, 10, 50 or 250 ppm (equivalent to doses of approximately 0, 1, 4 and 18 mg kg^{-1} d^{-1}) bendiocarb (purity 99 %) in the diet for 90 d prior to pairing, and throughout mating (1 male to 1 female for a maximum of 21 d), gestation and the lactation of two litters, F_{1A} and F_{1B}. This protocol was repeated on two further occasions, with two litters, from each of the second (F_2) and third (F_3) generations, being investigated. More specifically, from the second litters of the F_1 and F_2 generations (F_{1B}, F_{2B}), within each treatment group, 30 male and 30 female weanlings were selected at random for a further reproductive toxicity investigation that included a maturation period of 90 d and 2 successive matings.	

		For the F_0 (parental) generation and retained F_1, F_2 and F_3 animals, clinical signs of toxicity, mortalities, body weights and food and water consumption were recorded. The distribution and regularity of the oestrus cycle were monitored for 10 d prior to the first pairing of each generation and the second pairing of the F_1 and F_2 generations. Approximately 10 d after weaning of the first litters (A), the dams were re-mated with different males from the same dose group to produce the second litters (B). After the second set of mating, all males were dosed until termination (weaning of their respective second litters). At termination, 10 animals were subjected to macroscopic and microscopic examinations. Following the second set of mating, the females from each group were divided into 2, approximately equal-sized subgroups. One subgroup was killed on day 21 of gestation (teratological segment of the study), and the other was allowed to deliver and rear their pups (B) until weaning. The dams allowed to deliver their pups were used to provide information on gestation length, parturition, litter size, weight and viability of the offspring, incidence of abnormal offspring and post-natal development. After weaning of their respective second litters, these dams were sacrificed and subjected (only 10 animals) to macroscopic and microscopic examinations. All adult animals from the F_2 generation, except those females selected for the teratological segment of the study were subjected to ophthalmoscopic examinations between 31 and 35 w of age. All F_{3B} offspring were also examined ophthalmoscopically, but between 19 and 30 d of age. All the first litter offspring (i.e. F_{1A}, F_{2A} and F_{3A}), all surplus second litter offspring, i.e. F_{1B} and F_{2B} (those animals not selected for the next generation) and all third generation second litter offspring (F_{3B}) were sacrificed after day 25 post-partum and subjected to a macroscopic examination for external and gross internal abnormalities. Five males and 5 females per dose group from the surplus F_{2B} offspring and 10 males and 10 females per dose group from the F_{3B} offspring were also subjected to microscopic examinations.	
5.2	**Results and discussion**	No treatment-related mortalities or clinical signs of toxicity were observed amongst animals of all 3 generations. In the F_0 females, a statistically significant reduction (by 12 %) in body weight gain was seen during the F_{1A} gestation period at the mid- and high-dose levels. A significant reduction (6 %) in water intake was seen in the top dose females of the F_1 generation only throughout the pre-mating period. No treatment-related macroscopic abnormalities were observed at necropsy of the F_0, F_1 and F_2 parental animals. Organ weight analysis of these animals revealed statistically significant increases in the relative thyroid gland weight of the F_1 top dose males (by 24 %) and in the relative seminal vesicles weight of the F_1 mid- and top dose males (by 29 and 16 % respectively). A slight (statistically significant at the mid-, and top dose levels) increase in the relative adrenal gland weight was also seen in all treated F_2 males (by 12, 23 and 22 % at 10, 50 and 250 ppm respectively), and a slight (statistically significant at the low, and mid-dose levels) decrease in relative uterus weight was observed in all treated F_2 females (by 20, 25 and 20 % at 10, 50 and 250 ppm respectively).	

	A slight (statistically significant at the top dose level) reduction in absolute pituitary gland weight was observed in all treated F_2 females (by 9, 5, and 15 % at 10, 50 and 250 ppm respectively). Although statistically significant, in the absence of any associated histopathological changes, these organ weight changes are regarded as being of no toxicological significance. In addition, for the adrenal gland, the uterus and the pituitary gland weights, no dose-response relationship was noted. Histopathological examinations performed in the parental animals from all 3 generations did not show any treatment-related changes except for an increased incidence of moderate or severe geriatric nephropathy in the mid- and top dose groups of the F_2 generation (5/9 and 2/10 respectively vs. 0/10 in controls). Given that this lesion was only seen in the F_2 generation, it was considered likely that it represented a chance finding of no toxicological significance. Ophthalmoscopic examinations conducted in the F_2 parental animals revealed no treatment-related changes. Considering the reproduction-related parameters, prior to the F_{1A} mating, but not prior to the F_{1B} mating, a statistically significant increase in the number of F_0 females that were acyclic or pseudo-pregnant associated with a prolonged pre-coital interval was observed at the top dose. However, the reproductive performance of treated animals at the F_{2A} and F_{2B} matings was similar to that of the controls, and only a very few F_2 females of the mid- and high-dose groups showed irregular oestrus cycles prior to the F_{3A} mating. During the production of the F_{1B} offspring, a marginal (by 6 %), but not statistically significant, reduction in fertility rate (number pregnant/number mated) was seen at the top dose compared to controls. Gestation length was comparable among all groups of all 3 generations throughout each littering. In the offspring, a statistically significant reduced rate (by 8 %) of body weight gain prior to weaning of the F_{1A} litters was seen at the top dose, although other aspects of litter development, e.g. litter size, sex ratio, auditory and visual functions and necropsy findings were unaffected by treatment. In the F_{1B} litters, there was a slight (statistically significant at the top dose) reduction in the rate of body weight gain prior to weaning in all treated groups (by 9, 6 and 12 % at 10, 50 and 250 ppm respectively). However, in the absence of a dose-response relationship, this reduction is considered as being an incidental finding of no toxicological significance. In all treated F_{1B} litters, there was also a marginal, but not statistically significant, delay in physical development, as assessed by the onset of pinna unfolding, hair growth, tooth eruption and eye opening, but the overall pattern of physical development was essentially similar in all groups. No treatment-related macroscopic abnormalities were found at necropsy of the F_{1B} surplus and F_{1A} offspring.	

		Overall, it was concluded that dietary administration of bendiocarb to rats, throughout 3 successive generations up to a dose of approximately 18 mg kg^{-1} d^{-1} had no effect upon fertility. Although slight inter-group differences were recorded in certain fertility and developmental parameters, there was no evidence that these findings were consistent from one generation to another, nor was there any evidence of a consistent dose-response relationship. No significant parental toxicity was seen. Thus, a NOAEL for both systemic toxicity and effects on fertility of approximately 18 mg kg^{-1} d^{-1} was identified from this three-generation study.	
5.3	**Conclusion**		
5.3.1	LO(A)EL maternal toxic effects	-	
5.3.1.1	Parent males	-	
5.3.1.2	Parent females	-	
5.3.1.3	F1 males	-	
5.3.1.4	F1 females	-	
5.3.1.5	F2 males	-	
5.3.1.6	F2 females	-	
5.3.2	NO(A)EL	-	
5.3.2.1	Parent males	18 mg/kg bw/day (250 ppm in diet)	X
5.3.2.2	Parent females	18 mg/kg bw/day (250 ppm in diet)	X
5.3.2.3	F1 males	18 mg/kg bw/day (250 ppm in diet)	X
5.3.2.4	F1 females	18 mg/kg bw/day (250 ppm in diet)	X
5.3.2.5	F2 males	18 mg/kg bw/day (250 ppm in diet)	X
5.3.2.6	F2 females	18 mg/kg bw/day (250 ppm in diet)	X
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	8th November 2006
Materials and methods	3.2.7 and 3.3.1. Table A6.8.2-1 was not reproduced in the robust study summary.
Conclusion	5.3.2.1 to 5.3.2.6. NO(A)ELS were not defined during this study, as no significant toxicity occurred at the highest dose level. It is also noted that brain cholinesterase was not measured, which is the most sensitive parameter of toxicity.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.9 Neurotoxicity study

			Official use only
		1. REFERENCE	
1.1	**Reference**	[redacted] (1978) Examination of NC 6897 for Neurotoxicity in the Domestic Hen Schering Agrochemicals Ltd [redacted] Document A90423 6.9/01 October 1978 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**	The protocol followed was based on that of the United States Environmental Protection Agency (EPA) Working Group Draft § 162, 81-8 acute delayed neurotoxicity, dated 27 April 1977. When the study was in progress a second EPA draft protocol with the same reference number but dated 20 October 1977 was issued. As the study was in progress and because the protocol followed appeared to cover all the points raised in the second draft protocol (20 October 1977) no modifications to the study design were made.	
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	CR 4799/4	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Powder	
3.1.2.2	Purity	Not specified	
3.1.2.3	Stability	Not specified but bendiocarb is not known to decompose at room temperature	
3.2	**Reference Substance (positive control)**	TOCP (tri-ortho-cresyl phosphate)	
3.3	**Test Animals**		
3.3.1	Species	Hen	
3.3.2	Strain	*Gallus domesticus* (domestic hen)	

3.3.3	Source	Stanley Brown Ltd , Bucks	
3.3.4	Sex	Female	
3.3.5	Rearing conditions	Battery cage	
3.3.6	Age/weight at study initiation	ca. 14 months old; 1670 – 2500 g	
3.3.7	Number of animals per group	10	
3.3.8	Control animals	10	
3.4	**Administration**	Oral by gavage	
3.4.1	Exposure	Single dose	
3.4.2	Dose levels	0, 189, 378, 757 mg/kg (based on LD_{50} 757 mg/kg) TOCP 500mg/kg	
3.4.3	Vehicle	Corn oil	
3.4.4	Concentration in vehicle	20%	
3.4.5	Total volume applied	Calculated from 3.4.2 and bodyweight	
3.4.6	Postexposure period	21 days	
3.4.7	Anticholinergic substances used	Atropine (intra muscular injection at a 1% concentration in sterile water at 10 mg/kg bw)	
3.4.8	Controls	Vehicle	
3.5	**Examinations**	The parameters recorded included bodyweight, food consumption and clinical signs. Birds used in the neurotoxicity determination were observed daily for signs of ataxia and at termination of the study were sacrificed for histological examination of the brain, sciatic nerve and spinal cord.	
3.5.1	Body weight	Yes; prior to initiation and Days 7, 14 and 21 of observation period	
3.5.2	Signs of toxicity	Yes; daily	
3.5.3	Observation schedule	Daily	
3.5.4	Clinical chemistry	No	
3.5.5	Pathology	Yes	
3.5.6	Histopathology	Yes; brain, spinal cord and distal sciatic nerve	
3.6	**Further remarks**	-	
		4. RESULTS AND DISCUSSION	
4.1	**Body weight**	All group mean bodyweight changes with the exception of those birds dosed with TOCP were considered to be within normal limits. The bodyweight decrease of birds dosed with TOCP ranged from 75 to 820g. Food consumption was reduced in all birds dosed with bendiocarb for the first seven days after dosing. Although variable all other results are considered to be within normal limits.	

4.2	Clinical signs of toxicity	Signs of ataxia were observed in all birds dosed with TOCP and with one exception the signs observed were graded as being from moderate to severe. No positive ataxia was observed in any of the negative control birds or in birds dosed with bendiocarb.	
4.3	Clinical chemistry	-	
4.4	Pathology	No treatment-related abnormalities observed.	
4.5	Histopathology	Significant neuropathological changes were found in all birds dosed with TOCP at 500 mg/kg. The minor changes recorded in hens from all other groups including the vehicle controls were considered to be spontaneous in origin.	
4.6	Other	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	Materials and methods	**Range finding:** Small groups of birds were dosed orally with bendiocarb in corn oil at concentrations ranging from 2.0% – 40.0%. A total of 36 birds were used of which twenty were protected with atropine given by intramuscular injection as a 1% concentration in sterile water at 10 mg/kg bodyweight. **LD_{50} determination** **Without atropine protection:** Thirty adult hens were allocated to six groups each of five birds. One group was dosed orally with vehicle only (corn oil) to act as the control. The other five groups were dosed with bendiocarb at a 4% w/v concentration in corn oil. The dose levels used were 20, 40, 80, 160 and 320 mg/kg. The test compound was given as a single dose by oral gavage and the birds were observed for fourteen days after dosing. **With atropine protection:** Forty adult hens were allocated to eight groups each of five birds (two of these groups were included at a later date in an attempt to improve the spread of mortalities). One group was dosed with vehicle only (corn oil) to act as the control. The remaining seven groups were dosed with bendiocarb at a 20% concentration in corn oil and the birds were protected by atropine given by intramuscular injection at a 1% concentration in sterile water at 10 mg/kg bodyweight immediately prior to dosing. The dose levels used were 100, 200, 400, 800, 900, 1000 and 1200 mg/kg. The test compound was given as a single dose by oral gavage and the birds were observed for fourteen days after dosing. **Neurotoxicity assessment** Sixty adult hens were allocated to six groups each of ten birds. One group was dosed with vehicle only to act as the negative control and one group was dosed with TOCP at a 20% concentration in corn oil (500 mg TOCP/kg bodyweight) to act as the positive control. The remaining four groups were dosed with bendiocarb at a 20% concentration in corn oil and the birds were protected by atropine given as an intramuscular injection at a 1% concentration in sterile water at 10 mg/kg bodyweight. The dose levels used were 0, 189, 378, 757 mg/kg and TOCP 500 mg/kg. The test and positive control compounds were given as a single dose by oral gavage and the birds were observed for twenty-one days after dosing.	

5.2	**Results and discussion**	**LD_{50} determinations** Groups of birds were dosed with bendiocarb at various levels with and without atropine protection. The results of the LD_{50} determination without atropine protection gave a value of 137 mg/kg and with atropine protection the value was 757 mg/kg. In both parts of the study clinical signs observed included lethargy, loss of muscle function and balance, these being more marked in the 48-hour period immediately after dosing. As the birds recovered and the condition was clearly reversible it was considered that the signs observed were a result of toxicity and not acute delayed neurotoxicity. The LD_{50} value obtained with atropine protection was used to calculate the dose levels for the neurotoxicity assessment. **Neurotoxicity assessment** Six groups of ten birds were allocated to treatment. One group was dosed with vehicle only to act as the negative control in the event of spontaneous lesions being observed during histopathological examination. A positive control group of birds was dosed with TOCP at 500 mg/kg. Groups of birds were dosed with bendiocarb at 25% and 50% of the LD_{50} value and two groups were dosed with LD_{50} value (757 mg/kg). The assessment of ataxia based on a scoring system is a subjective examination and for this reason all the birds were examined histopathologically. Birds dosed with TOCP showed positive signs of ataxia which were confirmed by the results of the histological examination. One bird dosed with bendiocarb at 189 mg/kg was graded as showing doubtful signs of ataxia on Days 9 and 10. As no further signs of ataxia were observed the signs recorded were considered to be the result of mechanical injury and not related to neurotoxicity. No other signs of ataxia were observed in any of the birds dosed with bendiocarb and the few minor changes observed during the microscopic examination were considered to be spontaneous in origin. The results of this study indicate that dosing hens with bendiocarb at the dose level of 757 mg/kg and following protection with atropine resulted in no signs of ataxia or histopathological evidence of neurological lesions. The positive control compound (TOCP at 500 mg/kg) resulted in all birds in the group showing signs of ataxia which were confirmed by microscopic examination. Under the conditions of this study no signs of neurotoxicity were observed in birds dosed with bendiocarb at levels up to and including 757 mg/kg.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	-	
5.3.2	NO(A)EL	-	
5.3.3	Reliability	1	X
5.3.4	Deficiencies	No	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9th November 2006
Materials and methods	As described by the applicant.
Conclusion	As described by the applicant.
Reliability	2
Acceptability	Acceptable.
Remarks	Since this study did not include a period of forced activity nor a determination of brain and spinal cord neuropathy target esterase (NTE), both of which are required by OECD guideline 418, a full examination for neurotoxicity effects was not performed. Therefore, the UK CA considers that the reliability of this study is 2.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.10 Mechanistic study

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [✓]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	On the basis of the toxicological properties of bendiocarb, no mechanistic study should be required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9th November 2006
Evaluation of applicant's justification	The acute toxicity induced by bendiocarb is directly related to its mode of action as a reversible inhibitor of cholinesterase. No effects unrelated to this mode of action were observed, and it was negative in assays for carcinogenicity and reproductive toxicology. No hormonal or immunotoxic adverse effects were noted.
Conclusion	The justification for non-submission of mechanistic studies is acceptable.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

6.11 Other routes of administration

		1. REFERENCE	Official use only
1.1	**Reference**	[redacted] (1971a) The Toxicology of NC 6897: Acute Toxicity of Pure NC 6897 [redacted] Document A90940 6.11/01 January 1971 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**	No, but the study was conducted in line with good scientific practice.	
2.2	**GLP**	No, the study was conducted prior to the introduction of GLP as a standard requirement.	
2.3	**Deviations**	n.a.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Bendiocarb	
3.1.1	Lot/Batch number	Batch 15, 33	
3.1.2	Specification	As given in Section 2	
3.1.2.1	Description	Not specified but bendiocarb is known as a white/beige powder	
3.1.2.2	Purity	Specified as 'pure' without any additional information.	
3.1.2.3	Stability	Not specified, but bendiocarb is not known to decompose at room temperature	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar	
3.2.3	Source	No data	
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	M: 259-301 g F: 160-270 g	
3.2.6	Number of animals per group	6M, 16F	X
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Intraperitoneal	
3.3.1	Postexposure period	Not reported	