SECTION A6.5 **Annex Point IIA6.5**	**6.5 Chronic Toxicity** *6.5.1 chronic toxicity study in rodents*	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA [redacted], 2003, coumatetralyl - Waiver for chronic toxicity study in dogs, chronic/carcinogenicity toxicity study in rodents, and multigeneration reproductive toxicity study in rodents, MO-03-006927	Official use only
Other existing data [...] **Limited exposure [ ]**	**Technically not feasible [X]** **Scientifically unjustified [...]** **Other justification [ ]**	
Detailed justification:	A waiver for a long-term rodent study for coumatetralyl is scientifically justified based on: - the lack of mutagenic/genotoxic effects, X - the absence of any other effects that may lead to non-genotoxic carcinogenesis, X - the absence of any carcinogenic effects following long-term administration of warfarin, a coumarin compound, in humans, X - the absence of potential for long-term exposure of the public population, - the low risk of exposure during manufacturing and use, - the absence of residues in plant foodstuffs and water. The practical difficulties of long-term administration of coumatetralyl are such that an attempt at a study would almost be certain to fail and would be unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*February 2005*	
Evaluation of applicant's justification	*The justification for non-conduction of a long-term study in rats is overall acceptable, based on practical difficulties and on the low risk of exposure due to the physico-chemical properties of the formulation and the use pattern.* *The Danish CA has comments to some of the arguments:* - *The arguments on lack of mutagenic potential of coumatetralyl are not relevant for non-carcinogenic effects that may be seen in long-term exposure studies.* - *The Danish EPA agrees that the results from the 16-week study in rats indicate a low potential for any other effects than those on the blood clotting.*	

Conclusion	*Waiving of this study is accepted by RMS but will result in reference to exposure conditions in the Annex I listing of coumatetralyl, as the prerequisites for waiving is dependent upon the nature of the formulated product Racumin paste and the recommended use pattern instructions given on that specific fomulation.*
Remarks	*Bone protein depletion in women after 10-12 years continuous therapeutic use of the anticoagulant warfarin reported by WHO is mentioned in the waiver as being the only long term effect of warfarin. The mechanism of action of the warfarin and coumatetralyl being similar, a similar effect of coumatetralyl cannot be excluded.* *However, this long-term effect is considered unlikely to occur due to the restricted exposure of humans to Racumin paste.*

SECTION A6.6.1(01)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (01) In-vitro gene mutation study in bacteria (Salmonella typhimurium-reverse mutation assay)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1986, ENE 11183B (c. n.: coumatetralyl) - Salmonella/microsome test to evaluate for point mutagenic effect, [redacted], Report No. 15070, 1986-09-15 (unpublished), MO-03-004013	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	—	
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/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No, methods used in this study were comparable with the OECD-Guideline 471.	
2.2	**GLP**	No, GLP was not compulsory at the time the study was performed.	X
2.3	**Deviations**	Yes The Salmonella typhimurium strain TA 102 or E. coli WP2 strain were not used (TA 102 or E. coli WP2 strains have an AT base pair at the primary reversion site to detect certain oxidising mutagens, cross-linking agents and hydrazines). The recommended exposure concentrations are at least five concentrations of the active substance with approximately half log intervals between test points for an initial experiment (OECD-Guideline 471). In this study, the intervals between tested concentrations correspond to a factor 5. As described in the OECD-Guideline 471, equivocal results should be clarified by further testing preferably using a modification of experimental conditions. In this study the same experimental procedure was used for the first test and for the repeat test. Historical controls were not documented	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier.	
3.1.1	Lot/Batch number	Batch no. 233 569 571	X
3.1.2	Specification	As given in section 2 of dossier.	

SECTION A6.6.1(01)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (01) In-vitro gene mutation study in bacteria (Salmonella typhimurium-reverse mutation assay)
3.1.2.1 Description	Fine white powder
3.1.2.2 Purity	99.6 %
3.1.2.3 Stability	The batch was analytically examined and approved for a minimum of the test period. The stability test in the solvent did not detect a relevant indication of a change in the active ingredient.
3.2 Study Type	Bacterial reverse mutation test
3.2.1 Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100
3.2.2 Deficiencies / Proficiencies	—
3.2.3 Metabolic activation system	S9 mix Livers of at least six adult male Sprague Dawley rats were used to prepare the S9 mix. For enzyme induction the animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw five days before preparation of S9 mix. Following sacrifice livers were collected, homogenised and centrifuged at 9000 g. The supernatant (S9 fraction) was diluted with a cofactor solution. The amount of S9 fraction in S9 mix is indicated in percent.
3.2.4 Positive control	Sodium azide (for TA 1535); nitrofurantoin (for TA 100); 4-nitro-o-phenylene diamine (for TA 1537 and TA 98); 2-aminoanthracene (for all strains)
3.3 Administration / Exposure; Application of test substance	
3.3.1 Concentrations	First test: 20, 100, 500, 2500, 12500 µg/plate Repeat test: 125, 250, 500, 1000, 2000 µg/plate (for TA 1535) 250, 500, 1000, 2000, 4000, 8000 µg/plate (for TA 100, TA 1537, TA 98)
3.3.2 Way of application	Dissolved in medium (solvent: DMSO)
3.3.3 Pre-incubation time	—
3.3.4 Other modifications	—
3.4 Examinations	See tables in appendix for examinations and results. First test: with and without metabolic activation (30 % S9-mix) Repeat test: with and without metabolic activation (10 % and 30 % S9-mix)

SECTION A6.6.1(01)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (01) In-vitro gene mutation study in bacteria (Salmonella typhimurium-reverse mutation assay)
3.4.1 Number of cells evaluated	Not applicable.
	4 RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.1.1 without metabolic activation	No See table A6.6.1a-1, A6.6.1a-2 and A6.6.1a-3
4.1.2 with metabolic activation	No See table A6.6.1a-1, A6.6.1a-2 and A6.6.1a-3
4.2 Cytotoxicity	Yes A strong cytotoxicity was observed effects from 250 µg/plate onwards. See table A6.6.1a-1, A6.6.1a-2 and A6.6.1a-3
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The mutagenicity of the test substance coumatetralyl was evaluated with the Salmonella/microsome test, also termed the Ames Test, as described by Ames et al. (Proc. Nat. Acad. Sci. 70: 2281-2285, 1973 and Mutation Res. 31: 347-364, 1975). The study was done according to the OECD-Guideline 471 with slight deviations as described in section 2.3 (see above).
5.2 Results and discussion	There was no indication of a mutagenic effect for coumatetralyl. The mutant counts were neither doubled in dose correlation nor increased to a biologically relevant degree in comparison to negative control.
5.3 Conclusion	No indications of mutagenic effects were found in the Salmonella/microsome test for coumatetralyl.
5.3.1 Reliability	2
5.3.2 Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*(P. 6 and 7 of report copy is of poor quality)* *2.2 GLP: There is a Quality Assurance statement, which is, however, not signed by Quality Assurance Unit (but only the translator).* *3.1.1 Lot/Batch number: 233 5**96** 571*

SECTION A6.6.1(01)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (01) In-vitro gene mutation study in bacteria (Salmonella typhimurium-reverse mutation assay)

Results and discussion	*Applicant's version adopted*
Conclusion	*Applicant's version adopted*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*The study from 1986 was conducted in accordance with generally accepted scientific principles and is found acceptable with respect to the four strains used.* *The test does not follow the updated OECD guideline from 1997 in which the inclusion of a fifth strain is recommended (Salmonella typhimurium strain TA 102 or E. coli WP2) in order to detect certain oxidising mutagens, cross-linking agents and hydrazines that may not be detected by the other Salmonella typhimurium strains. However, there is no indication from the kinetics of the substance these effects types could occur.*

Table A6_6.6.1a-1: Table for gene mutation assay: first test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]	Number of mutant cells *(mean ± standard deviation)*							
	TA 1535		TA 100		TA 1537		TA 98	
	-S9	+S9 (30%)	-S9	+S9 (30%)	-S9	+S9 (30%)	-S9	+S9 (30%)
0	17 ± 4	10 ± 2	65 ± 7	110 ± 14	5 ± 1	15 ± 2	20 ± 4	34 ± 4
20	22 ± 4	12 ± 2	72 ± 10	99 ± 13	6 ± 3	8 ± 4	18 ± 4	39 ± 1
100	27 ± 5	14 ± 2	76 ± 10	107 ± 15	6 ± 1	9 ± 2	18 ± 4	41 ± 2
500	24** ± 5	14** ± 1	80** ± 10	85** ± 5	6** ± 2	12** ± 4	16** ± 2	55** ± 4
2500	4** ± 2	0**	62** ± 4	60** ± 15	3** ± 2	9** ± 2	10** ± 2	32** ± 10
12500	0**	0**	b**	b**	b**	b**	b**	b**
Positive control	1311 ± 82	—	333 ± 20	—	35 ± 2	—	95 ± 9	—
2-Amino-antharacene	—	420 ± 64	—	2039 ± 72	—	145 ± 19	—	1430 ± 100

** bacteriotoxic effect observed by titer determination *b* reduction in background growth

Table A6.6.1a-1: Table for gene mutation assay: repeated test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]	Number of mutant cells *(mean ± standard deviation)*							
	TA 1535		TA 100		TA 1537		TA 98	
	-S9	+S9 (30%)	-S9	+S9 (30%)	-S9	+S9 (30%)	-S9	+S9 (30%)
0	11 ± 3	12 ± 3	55 ± 7	78 ± 12	6 ± 1	8 ± 1	13 ± 4	23 ± 4
125	17 ± 4	12 ± 1	—	—	—	—	—	—
250	15** ± 3	10** ± 2	68 ± 8	89 ± 15	6 ± 2	9 ± 3	15 ± 3	23 ± 4
500	17 **± 6	10** ± 3	59 ± 10	83 ± 4	7 ± 1	6 ± 2	13 ± 4	26 ± 5
1000	10** ± 3	11** ± 2	63 ± 8	71 ± 3	7 ± 2	6 ± 3	12 ± 2	13 ± 2
2000	0**	0**	54** ± 10	44** ± 5	6 ± 1	9 ± 2	12 ± 3	15 ± 3
4000	—	—	26** ± 4	42** ± 9	5 ± 1	5 ± 1	5** ± 1	4** ± 2
8000	—	—	b**	b**	b**	b**	0**	0**
Positive control	737 ± 97	—	253 ± 23	—	36 ± 4	—	146 ± 21	—
2-Amino-antharacene	—	340 ± 23	—	860 ± 20	—	32 ± 2	—	543 ± 56

** bacteriotoxic effect *b* reduction in background growth

Table A6.6.1a-3.: Table for gene mutation assay: repeated test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]	Number of mutant cells *(mean ± standard deviation)*							
	TA 1535		TA 100		TA 1537		TA 98	
	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)	-S9	+S9 (10%)
0	16 ± 2	8 ± 3	61 ± 7	60 ± 3	7 ± 1	7 ± 2	11 ± 2	28 ± 5
125	20 ± 3	5 ± 1	—	—	—	—	—	—
250	20** ± 4	6** ± 2	66** ± 7	69** ± 6	5** ± 2	8** ± 2	13** ± 2	20** ± 2
500	20 **± 3	8** ± 3	69** ± 11	70** ± 9	5** ± 2	7** ± 1	13** ± 2	24** ± 2
1000	17** ± 3	6** ± 2	70** ± 6	54 ± 10	6** ± 1	7** ± 2	11** ± 1	18** ± 8
2000	4** ± 2	1** ± 1	54** ± 8	54** ± 10	3** ± 1	3** ± 1	10** ± 2	4** ± 2
4000	—	—	15** ± 5	0**	6** ± 2	6** ± 1	4** ± 1	0**
8000	—	—	0**	0**	4** ± 1	*b***	0**	0**
Positive control	819 ± 48	—	335 ± 15	—	27 ± 4	—	93 ± 10	—
2-Amino-antharacene	—	**468 ± 12**	—	**2621 ± 91**	—	**347 ± 43**	—	**2082 ± 325**

** bacteriotoxic effect observed by titer determination

b reduction in background growth

SECTION A6.6.1(02)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (02)In-vitro mitotic recombination assay in yeast (Saccharomyces cerevisiae)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], ENE 11183B (c. n.: coumatetralyl) – Test on S. cerevisiae D7 for the induction of mitotic recombination, [redacted], Report No. 15071, 1986-09-15, (unpublished), MO-03-004056	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	—	
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/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Methods used in this study were comparable with the OECD-Guideline 481.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	X
2.3	**Deviations**	Yes The OECD-Guideline 481 recommended for the initial experiment the use of growing cells. In case the first experiment is negative, a second experiment should be carried out using stationary phase cells. In this study the cells used were not specified.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier.	
3.1.1	Lot/Batch number	Batch no. 233 596 571	
3.1.2	Specification	As given in section 2 of dossier.	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	99.6 %	
3.1.2.3	Stability	The batch used was analytically examined and approved for a minimum of the test period. The stability test in the solvent did not detect any relevant indication of a change in the active ingredient.	
3.2	**Study Type**	Mitotic recombination assay with Saccharomyces cerevisiae	
3.2.1	Organism/cell type	Yeast: Saccharomyces cerevisiae D7	

SECTION A6.6.1(02)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (02)In-vitro mitotic recombination assay in yeast (Saccharomyces cerevisiae)
3.2.2 Deficiencies / Proficiencies	—
3.2.3 Metabolic activation system	S9 mix Livers of at least six adult male Sprague Dawley rats were used to prepare the S9 mix. For enzyme induction the animals received a single intraperitoneal injection of Aroclor 1254, at a dose of 500 mg/kg bw five days before preparation. Following sacrifice, livers were collected, homogenised and centrifuged at 9000 g. The supernatant (S9 fraction) was diluted with a cofactor solution. The amount of S9 fraction in S9 mix is indicated in percent.
3.2.4 Positive control	Methylmethanesulphonate (only without S9 mix) Cyclophosphaminde (only used with S9 mix)
3.3 Administration / Exposure; Application of test substance	
3.3.1 Concentrations	First and repeat test: 0, 625, 1250, 2500, 5000, 10000 µg/ml
3.3.2 Way of application	Dissolved in medium (the solvent for coumatetralyl and for methylmethanesulphonate was DMSO and for cyclophosphamind Soerensen buffer (pH 7.4).
3.3.3 Pre-incubation time	16 hours
3.3.4 Other modifications	—
3.4 Examinations	
3.4.1 Number of cells evaluated	—
	4 RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.1.1 without metabolic activation	No See table A6.6.1b-1 and A6.6.1b-2
4.1.2 with metabolic activation	No See table A6.6.1b-1 and A6.6.1b-2
4.2 Cytotoxicity	Yes Coumatetralyl was cytotoxic at concentrations up to 10000 µg/ml over the entire concentration range tested, both with and without S9 mix. See table A6.6.1b-1 and A6.6.1b-2
	5 APPLICANT'S SUMMARY AND CONCLUSION

SECTION A6.6.1(02)	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.1 (02)In-vitro mitotic recombination assay in yeast (Saccharomyces cerevisiae)
5.1 Materials and methods	The test for possible induction of mitotic recombinants by coumatetralyl employed Saccharomyces cerevisiae D7 as described by Zimmermann et al. (Mutation Res. 28, 381-388, 1975). The available literature already shows that yeast is a highly suitable organism for testing for mitotic gene conversion, mitotic crossing-over and point mutations. The test on Saccharomyces cerevisiae D7 is an effective supplement to the Salmonella/microsome test as described by Ames et al. (Proc. nat. Acad. Sci. 70, 2281-2285, 1973 and Mutation Res. 31, 347-364, 1975). The test largely followed the directions of Zimmermann et al. (in: A. Hollaender (ed.), Chemical Mutagens: Principles and methods for their detection, Vol. 3, Plenum Press New York 209-239, 1973). The methods used in this study were comparable with the OECD-Guideline 481 with slight deviations as described above (see section 2.3).
5.2 Results and discussion	Coumatetralyl induced cytotoxic effects over the entire range of concentrations used in this study. Nevertheless the complete range could be evaluated. The substance precipitated from 5000 µg/ml onwards. None of the five concentrations used in the first test produced a biologically relevant increase in recombinant counts in comparison to the respective negative control. This applies both to the test with and without S9 mix and was confirmed by the results of the repeat test. The positive controls clearly increased the recombinant count in comparison to the negative control, thus showing the sensitivity of the system.
5.3 Conclusion	No indication of induction of mitotic recombination on Saccharomyces cerevisea D7 could be found for coumatetralyl under the test conditions described above.
5.3.1 Reliability	2
5.3.2 Deficiencies	No

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*2.2 GLP: There is a Quality Assurance statement, which is, however, not signed by Quality Assurance Unit* *Applicant's version adopted.*

Results and discussion	*Applicant's version adopted.*
Conclusion	*Applicant's version adopted.*
Reliability	*2*
Acceptability	*Acceptable.*
Remarks	*The reliability is 2 (and not 1), because the growth phase of the cells treated was not specified.*

Table A6.6.1b-1: Table for mitotic recombination assay: first test for Saccharomyces cerevisiae D7

Concentration [µg/ml]	Number of colonies per plate *(mean ± standard deviation)*			
	with red and or pink content (mitotic crossing-over)		tryptophane-revertants (mitotic gene conversion)	
	-S9	+S9 (30%)	-S9	+S9 (30%)
0	**0.1 ± 0**	0.1 ± 0	34 ± 8	38 ± 6
625	0.3 ± 1	0.0 ± 0	16 ± 4	28 ± 8
1250	0.0** ± 0	0.4 ± 1	12 ± 4	17 ± 6
2500	0.2** ± 1	0.1** ± 0	13 ± 3	15 ± 5
5000	0.0** ± 0	0.1** ± 0	13 ± 3	5 ± 3
10000	0.0** ± 0	0.0** ± 0	2 ± 2	3 ± 1
Positive control	3.4# ± 1	1.8# ± 1	251# ± 28	148# ± 12

** cytotoxic effect observed by titer determination

positive effect

Table A6.6.1b-2: Table for mitotic recombination assay: repeat test for Saccharomyces cerevisiae D7

Concentration [µg/ml]	Number of colonies per plate *(mean ± standard deviation)*			
	with red and or pink content (mitotic crossing-over)		tryptophane-revertants (mitotic gene conversion)	
	-S9	+S9 (30%)	-S9	+S9 (30%)
0	**0.5 ± 1**	2.4 ± 1	33 ± 4	48 ± 10
625	1.4** ± 1	2.7** ± 1	16 ± 4	27 ± 7
1250	0.8** ± 1	1.9** ± 1	8 ± 3	23 ± 4
2500	1.2** ± 1	1.2** ± 1	10 ± 4	14 ± 3
5000	0.6** ± 1	0.0** ± 0	6 ± 2	9 ± 4
10000	0.5** ± 1	0.0** ± 0	4 ± 2	3 ± 2
Positive control	4.3# ± 2	7.0# ± 3	144# ± 4	385# ± 61

** cytotoxic effect observed by titer determination

positive effect

SECTION A6.6.2	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.2 In-vitro cytogenicity study in mammalian cells

	JUSTIFICATION FOR NON-SUBMISSION OF DATA Dominique Renault, 2004, Coumatetralyl - Waiver for genotoxicity studies, MO-04-001741	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [...]** **Other justification [ ]**	
Detailed justification:	The review of the available experimental data clearly showed that the genotoxic potential of coumatetralyl has been adequately investigated and complies with the requirements of the directive 98/8/EC. As recommended in this document, a three-stage testing strategy has been undertaken to assess the effects of coumatetralyl on • different test systems, including *in vitro* and *in vivo* methods, the large majority of the tests being performed according to GLP procedures and OECD guidelines, • different cell systems, including bacteria, yeast and mammalian somatic and germ cells, • different genotoxic end-points, including the induction of gene damage (i.e. gene mutation, gene conversion or crossing-over) and chromosome damage (i.e. structural and numerical chromosome aberrations). The global analysis of all the available genotoxicity data shows that coumatetralyl is devoid of any potential to induce: - gene mutation in both bacteria and mammalian cells according to the negative results of the Ames and the HPRT tests; - numerical and structural chromosome damage both *in vitro* and *in vivo* in somatic and germ cells according to the negative results of the mitotic recombination assay related to the negative results of the micronucleus test and dominant lethal experiment. Altogether, these informations provide a sufficient weight of evidence to conclude that coumatetralyl is devoid of any genotoxic potential. In addition, the analysis of the chemical structure confirms that this molecule has no structural alert for mutagenicity. In that respect, Bayer Environmental Science considers that there is no justification to further investigate coumatetralyl genotoxic potential as it is very unlikely to produce any additional valuable data.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the	

SECTION A6.6.2	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.2 In-vitro cytogenicity study in mammalian cells
	comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Evaluation of applicant's justification	*This justification for non-submission of data concerns the in vitro cytogenicity study in mammalian cells. This study is required in order to provide information on structural chromosome aberrations (clastogenicity) and possibly numerical chromosome aberrations (aneugenicity).* *The required documentation has been replaced by results from the following studies in the applicant's justification:* *1. In vitro mitotic recombination assay in yeast (Saccharomyces cerevisiae) (in accordance with OECD-Guideline 481 with slight deviations. Reliability: 2. Acceptability: Acceptable)* *2. In vivo mouse micronucleus test (according to OECD-Guideline 474 with some deviations. Reliability: 3. Acceptability: Acceptable)* *3. In vivo mouse dominant lethal test (with deviations from OECD-Guideline 478. Reliability: 3-4 Unknown. Acceptability: Unacceptable).* *Ad 1. Traditionally the in vitro mitotic recombination assay is considered a test for other genotoxic effects rather than chromosome aberrations. It is also a test in "lower" eukaryotes compared to mammalian cells. Although widely accepted by regulatory authorities, the test is not used in standard batteries.* *No indication of induction of mitotic recombination on Saccharomyces cerevisea D7 could be found for coumatetralyl under the test conditions described in test 1.* *Ad 2. Under the stated test conditions in test 2, the test substance did not show a clastogenic effect in the in vivo micronucleus assay.* *Ad 3. The dominant lethal test in mice was negative. However, the test suffers from major methodological deficiencies from guidelines.* *The waiver document contains the result from a QSAR program (DEREK) stating no alerts for mutagenicity.*
Conclusion	*The applicant's justification for not conducting an in in-vitro cytogenicity study in mammalian cells is acceptable with respect to testing for structural and numerical chromosome aberrations, because of the availability of the in vivo mouse micronucleus test. This in vivo test would have been an acceptable choice for a follow-up procedure anyway, in case an in vitro cytogenicity study in mammalian cells came out positive.*
Remarks	

SECTION A6.6.3	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

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1 REFERENCE

1.1	**Reference**	[redacted], V79/HPRT-test in vitro for the detection of induced forward mutations. Report No. AT00995, 2004-02-13 (unpublished), MO-04-001644
1.2	**Data protection**	Yes
1.2.1	Data owner	BAYER AG
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data on existing active substance submitted for the first time for entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, The study was performed according to the following guidelines: OECD Guideline 476, July 21, 1997, EEC Directive 2000/32/EC B.17 US EPA Guideline OPPTS 870.5300
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	0302
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.9%
3.1.2.3	Stability	Approved until April 2005.
3.2	**Study Type**	In-vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Chinese hamster V79 cells
3.2.2	Deficiencies / Proficiencies	6-Thioguanine resistance is generated by mutation in HPRT locus.
3.2.3	Metabolic activation system	S9 fraction from livers of Arochlor 1254-induced Sprague Dawley rats plus cofactor solution.
3.2.4	Positive control	Ethyl methanesulfonate (EMS) for non-activation trials. Dimethylbenzanthracene (DMBA) for trials with S9 mix.

SECTION A6.6.3	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

3.3 Administration / Exposure; Application of test substance	
3.3.1 Concentrations	With and without S9 mix: 0, 75, 150, 300, 450, 600, 750, 900 µg/ml. Duplicate cultures were treated with each concentration and each control. A preliminary cytotoxicity test with and without metabolic activation used concentrations between 20 and 1280 µg/ml. Cytotoxic effects were observed at ≥ 700 µg/ml with and without metabolic activation. Concentrations of ≤ 1280 µg/ml did not change the pH in medium. Slight osmolality changes were caused by concentrations >75 µg/ml. These changes were not considered to be of biological significance.
3.3.2 Way of application	Dissolved in DMSO.
3.3.3 Pre-incubation time	–
3.3.4 Other modifications	–
3.4 Examinations	
3.4.1 Number of cells plated	3×10^5 per Petri dish (∅ 60mm).
	4 RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.1.1 without metabolic activation	Negative.
4.1.2 with metabolic activation	Negative.
4.2 Cytotoxicity	Yes. Without metabolic activation: Yes, at concentrations ≥ 700 µg/ml. With metabolic activation Yes, at concentrations ≥ 700 µg/ml.

SECTION A6.6.3	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Coumatetralyl was evaluated for point mutagenic effects at the hypoxanthineguanine phosphoribosyl transferase locus (forward mutation assay) in V79 cell cultures after treatment with concentrations of up to and including 900 µg/ml, both with and without S9 mix.

The study was performed according to OECD Guideline 476, EEC Directive 2000/32/EC B.17, and US EPA Guideline OPPTS 870.5300.

5.2 Results and discussion

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to Coumatetralyl at concentrations of up to and including 900 µg/ml. Without and with S9 mix no substance precipitation occurred in the medium.

The means of the absolute cloning efficiency for the negative controls in the mutation experiments were 60.6% and 67.9% in the experiments without activation. In experiments with metabolic activation 58.4% and 59.6% were observed. These results demonstrate good cloning conditions for the experiments.

Coumatetralyl was tested in the V79/HPRT-test in concentrations ranging from 75 to 900 µg/ml without and with S9 mix. Under both activation conditions, clear cytotoxic effects were induced. Coumatetralyl induced no biologically relevant increases in mutant frequencies. The positive controls EMS and DMBA had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant frequencies as compared to the corresponding negative controls and thus demonstrated the sensitivity of the test system and the activity of the used S9 mix.

5.3 Conclusion

Coumatetralyl is considered to be non-mutagenic in the V79/HPRT forward mutation assay, both with and without metabolic activation

5.3.1 Reliability 1

5.3.2 Deficiencies No

EVALUATION BY COMPETENT AUTHORITIES

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	*February 2005*
Materials and Methods	*Applicant's version adopted.*
Results and discussion	*Applicant's version adopted.*
Conclusion	*Applicant's version adopted.*
Reliability	*1*
Acceptability	*Acceptable.*

SECTION A6.6.3	**6.6 Genotoxicity in vitro**
Annex Point IIA6.6	6.6.3 In-vitro gene mutation assay in V79-cells (HPRT-test)
Remarks	*None*

Table A6_6_1-1. A Table for gene mutation assay: HPRT-test

Treatment without S9 mix				
Mutant Frequency (Thioguanin-resistant mutants per 10^6 cells)				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	5.3	6.4	8.9	6.9
Vehicle control	7.0	3.1	10.0	5.9
75 µg/ml	16.8	5.8	2.6	5.1
150 µg/ml	2.9	4.5	5.2	3.6
300 µg/ml	7.9	2.6	6.3	–#
450 µg/ml	7.4	13.5	7.1	5.5
600 µg/ml	1.7	3.2	4.8	5.0
750 µg/ml	6.7	6.6	5.6	2.3
900 µg/ml	4.5	0.6	6.1	–#
Positive control	559.0*	572.0*	626.7*	585.9*

Table A6_6_1-1. B Table for gene mutation assay: HPRT-test

Treatment with S9 mix				
Mutant Frequency (Thioguanin-resistant mutants per 10^6 cells)				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	8.0	2.6	7.2	8.3
Vehicle control	3.4	4.3	10.0	8.1
75 µg/ml	4.0	1.8	5.7	9.1
150 µg/ml	5.1	3.1	9.4	10.3
300 µg/ml	1.8	0.8	10.0	9.5
450 µg/ml	–#	3.2	7.6	9.0
600 µg/ml	3.2	9.9	5.0	11.1
750 µg/ml	–#	0.0	7.2	26.0
900 µg/ml	–#	–#	7.6	4.2
Positive control	60.9*	86.3*	131.2*	132.7*

Dish lost due to contamination

* Significant increase, $\alpha = 0.05$, one-sided Dunnett Test

Table A6_6_1-1. C Table for gene mutation assay: HPRT-test

Treatment without S9 mix				
Survival to Treatment *(% of vehicle control)*				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	90.3	114.6	101.8	123.6
Vehicle control	100.0	100.0	100.0	100.0
75 µg/ml	93.7	105.0	87.1	90.4
150 µg/ml	117.0	89.2	115.6	86.9
300 µg/ml	110.3	124.9	90.6	106.5
450 µg/ml	90.5	105.0	68.3	92.2
600 µg/ml	82.8	69.1	69.6	90.4
750 µg/ml	71.1	66.7	31.9	39.7
900 µg/ml	7.7	7.4	9.0	19.7
Positive control	34.9	39.0	12.4	7.8

Table A6_6_1-1. D Table for gene mutation assay: HPRT-test

Treatment with S9 mix				
Survival to Treatment (% of vehicle control)				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	113.2	117.0	143.9	102.0
Vehicle control	100.0	100.0	100.0	100.0
75 µg/ml	104.8	97.6	102.6	94.1
150 µg/ml	101.1	90.5	110.2	88.1
300 µg/ml	114.6	114.3	98.7	93.5
450 µg/ml	84.7	110.7	92.9	90.3
600 µg/ml	72.2	71.1	55.9	64.0
750 µg/ml	20.6	13.7	22.2	25.0
900 µg/ml	3.4	1.5	5.4	8.1
Positive control	60.1	63.8	16.1	13.3

Table A6_6_1-1. E Table for gene mutation assay: HPRT-test

Treatment without S9 mix				
Relative Population Growth *(% of vehicle control)*				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	131.2	116.2	87.7	94.7
Vehicle control	100.0	100.0	100.0	100.0
75 µg/ml	83.7	109.4	69.4	90.5
150 µg/ml	84.5	93.3	76.8	78.7
300 µg/ml	88.2	90.2	78.4	95.3
450 µg/ml	125.6	103.4	94.4	97.9
600 µg/ml	95.0	37.1	76.9	108.0
750 µg/ml	50.5	46.5	33.4	26.9
900 µg/ml	7.6	6.9	10.9	19.4
Positive control	67.1	50.0	31.2	21.0

Table A6_6_1-1. F Table for gene mutation assay: HPRT-test

Treatment with S9 mix				
Relative Population Growth *(% of vehicle control)*				
Concentration [µg/ml]	1st trial		2nd trial	
	Culture 1	Culture 2	Culture 1	Culture 2
Negative control	118.8	96.4	107.3	101.3
Vehicle control	100.0	100.0	100.0	100.0
75 µg/ml	76.5	113.1	93.6	150.5
150 µg/ml	91.9	106.0	114.5	80.2
300 µg/ml	106.6	87.2	108.3	111.1
450 µg/ml	110.4	114.9	104.4	96.4
600 µg/ml	80.0	35.8	43.6	72.0
750 µg/ml	9.1	6.1	20.2	29.4
900 µg/ml	–#	–#	–$	8.4
Positive control	19.7	28.3	4.1	3.1

Not cloned due to cytotoxicity.

$ No calculation of relative population growth due to cell number < 1.5×10^6 at seeding.

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1987, ENE 11183B (c.n.: coumatetralyl) – Micronucleus test on the mouse to evaluate for clastogenic effect, [redacted], Report No. 15407, 1987-01-12, (unpublished), MO-03-004060	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access	—	
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/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No Methods used are comparable to OECD-Guideline 474.	
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.	X
2.3	**Deviations**	Yes <u>Housing conditions:</u> in test 2, the relative humidity exceeded the recommended 70%. Evaluation: at least 2000 immature erythrocytes per animal should be scored for the incidence of micronucleated polychromatic erythrocytes (OECD-Guideline 474). In this study 1000 polychromatic erythrocytes per animal were evaluated. The proportion of immature erythrocytes among total erythrocytes was not determined. Negative controls were not included for every sampling time and historical controls were not reported.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier.	
3.1.1	Lot/Batch number	Batch no. 233596571	
3.1.2	Specification	As given in section 2 of dossier.	
3.1.2.1	Description	White powder	
3.1.2.2	Purity	99.6 %	
3.1.2.3	Stability	The batch was checked by analysis and approved for the study period. The stability test under the conditions of administration did not show any indication of a relevant change in the active ingredient.	

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

3.1.2.4	Maximum tolerable dose	500 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Bor : NMRI (SPF Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	28 – 40 g (test 1) 30 – 43 g (test 2) These weights indicate that the animals were between approx. eight and twelve weeks old.
3.2.6	Number of animals per group	5 males + 5 females per dose
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	—
3.3.3	Post-exposure period	24, 48, and 72 h after treatment (first test) 48 and 72 h after treatment (second test)
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	First test: 750 mg/kg bw Second test: 500, 750, and 1000 mg/kg bw
3.3.6	Vehicle	Moistened with 0.5% Cremophor emulsion
3.3.7	Concentration in vehicle	50, 75, and 100 mg/ml
3.3.8	Total volume applied	10 ml/kg bw
3.3.9	Controls	Vehicle (negative control), cyclophosphamide (positive control). The positive control (20 mg/kg bw) was dissolved in de-mineralised water and administered as described above.
3.4	**Examinations**	

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

3.4.1 Clinical signs	Yes	
3.4.2 Tissue	Bone marrow	
	Number of animals:	all animals
	Number of cells:	1000 polychromatic erythrocytes were counted. 1000 normochromatic erythrocytes were counted.
	Time points:	24, 48, and 72 h after treatment (first test) 48 and 72 h after treatment (second test)
	Type of cells	erythrocytes in bone marrow
	Parameters:	incidence of polychromatic erythrocytes with micronuclei
		polychromatic/normochromatic erythrocytes ratio
		incidence of normochromatic erythrocytes with micronuclei
3.5 Further remarks	—	

4 RESULTS AND DISCUSSION

4.1 Clinical signs

In both tests, following administration of coumatetralyl up to 1000 mg/kg bw, the animals showed clinical signs including apathy, reduced motility, digging and grooming movements, bristling coats, staggering gaits, prostration on stomach, salivation and jumping spasms.

In the second test dyspnoea was also observed.

In the first test, two of the 40 animals treated with 750 mg/kg bw coumatetralyl died. In the second test, three of the 20 animals of the 1000 mg/kg bw dose group and one of the 20 mice of the 750 mg/kg bw dose group died.

4.2 Haematology / Tissue examination

In the first test the ratio of polychromatic to normochromatic erythrocytes was statistically significantly reduced within 48 hours after treatment with coumatetralyl. (see table A6.6.4-1 and A6.6.4-2)

4.3 Genotoxicity

No, (see table A6.6.4-1 and A6.6.4-2)

4.4 Other

—

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The mutagenicity of coumatetralyl was evaluated with the micronucleus test recommended as a sensitive in vivo mammalian test for chromosome damage as described by Salamone et al. (Muation Res. 74, 347-356, 1980).

The purpose of the study was to detect the potential induction of micronucleated polychromatic erythrocytes. A high incidence of these cells with micronuclei in comparison to the negative control would indicate that the substance tested has a chromosome damaging effect.

The study was done according to the OECD-Guideline 474 with some deviations (see 2.3 above). To verify the result of the first study, a repeat test was carried out.

5.2 Results and discussion

After the single oral administration of coumatetralyl at doses up to 1000 mg/kg bw the mice showed persistent clinical symptoms. In the first test two of the 40 animals treated with 750 mg/kg bw coumatetralyl died. In the second test three of the 20 animals of the 1000 mg/kg bw dose group and one of the 20 mice of the 750 mg/kg bw dose group died. Erythrocyte formation as measured by the ratio of polychromatic to normochromatic erythrocytes was affected.

The positive control resulted in an increase in polychromatic erythrocytes containing micronucleoli indicating the validity of the study. There was no relevant indication of a clastogenic effect for coumatetralyl after a single oral treatment with doses up to 1000 mg/kg bw.

5.3 Conclusion

Under the stated test conditions, the test substance did not show a clastogenic effect in the in vivo mammalian bone marrow assay.

5.3.1 Reliability: 2

5.3.2 Deficiencies: No

EVALUATION BY COMPETENT AUTHORITIES

EVALUATION BY RAPPORTEUR MEMBER STATE

Date: *February 2005*

Materials and Methods

2.2 GLP: There is a GLP statement (p. 1) and a Quality Assurance declaration (p. 19), but they are not signed by the responsible persons. (The translator has signed the QA statement).

2.3 Deviations: The study is divided into two tests. The first test had only one dose level but three sampling times (24, 48, and 72 h) and included a positive control. The dose (750 mg/kg) resulted in significant toxicity (mortality 2/40), and the test is considered reliable.

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

Materials and Methods	*14-15 weeks after the completion of the first test, the second test was initiated. The second test had three dose levels and two sampling times (48, and 72 h) but did not include a positive control. Like the first test the second test showed significant toxic effects (mortality 3/20 at 1000 mg/kg and 1/20 at 750 mg/kg), and the ratio of polychromatic to normochromatic erythrocytes was significantly ($p<0.05$) affected at the 48-hour sampling time in both tests.* *The exclusion of the positive control in the second test is not considered to compromise the second test considerably, because of 1) the clear clastogenic response of the positive control substance in the first test, 2) the use of mice of the same strain and origin for both tests, and 3) the comparable toxic effects observed in the two tests.* *It is doubtful whether the difference in relative humidity (45 - 47% in test 1, 55 – 76 % in test 2, 30 – 70 % (optimum 50 – 60 %) in OECD guideline 474) may have affected the study results.* *At least 2000 immature erythrocytes per animal should have been scored for the incidence of micronucleated polychromatic erythrocytes (OECD Guideline 474). In this study, 1000 polychromatic erythrocytes per animal were evaluated. There is no information about the historical control frequency for spontaneous micronucleus incidence for this animal strain and this laboratory. The incidence of micronucleated polychromatic erythrocytes (MPE) per 1000 polychromatic erythrocytes (PCE) is 1.3 (SD 1.1) when the negative control groups are combined under the assumption that that sample time after treatment with vehicle has no effect on the spontaneous frequency.* *The proportion of immature erythrocytes among total erythrocytes was not determined. The ration between normochromic (mature) and polychromic (immature) erythrocytes was determined instead.* *Negative controls were not included for every sampling time in the first test (only 24 h, not 48 and 72 h) and historical controls were not reported.*

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)

Results and discussion

The results from test 2, sample time 48 h, are not reported by the applicant. According to OECD guideline 474, this sampling time is more relevant than the 72-hour sample time. The results are presented in an additional table A6.6.4-3 (see below). There is a statistically significant increase ($p \leq 0.05$) in MPE per 1000 PCE at the highest dose level (1000 mg/kg). However, the effect is not considered biologically meaningful because:

1) *There was no evidence of a dose-related increase in MPE,*
2) *The incidence of MPE in the negative control group was relatively low (1.0 ± 0.8) compared to the negative control group in test 1 (1.2 ± 1.1) and in test 2, sample time 72 h (1.7 ± 1.4), and*
3) *There was no increase in MPE per 1000 PCE in the first test (750 mg/kg).*

The potential effect on the chromosomes is not completely clarified according to current guidelines, because the slide assessment was not extended to the effect that more than 2000 PCE were scored for MPE incidence per animal.

Overview of number of MPE per 1000 PCE (standard deviation):

Test/ Sample time

Negative control; Coumatetralyl: 1. Test: 0 and 750 mg/kg bw;

2. Test: (500, 750 and 1000 mg/kg bw); Positive control(only in 1. Test)

1. Test/ 24 hours

*1.2 (1.1); 1.1 (0.7); 11.7** (2.6)*

1. Test/ 48 hours

1.9 (1.0)

1. Test/ 72 hours

2.2 (1.2)

2. Test/ 48 hours

1.0 (0.8); 1.5 (1.4); 1.4 (1.3); 1.6 (0.9)*

2. Test/72 hours

1.7 (1.4); 1.5 (1.3); 1.4 (1.1); 1.0 (0.9)

Conclusion

Under the conditions of the study, the test substance is not considered to be clastogenic in this in vivo mammalian erythrocyte micronucleus test, but a clear negative response could not be demonstrated.

Reliability

3

SECTION A6.6.4	**6.6 Genotoxicity in vivo**
Annex Point IIA6.6	6.6.4 In vivo mammalian bone marrow assay (micronucleus test)
Acceptability	*Acceptable* *The study is considered acceptable despite a poor reliability indicator, because a clear positive response of the positive control in the first test demonstrated sufficient sensitivity of the test system, and because a repeat test is not likely to detect a statistically significant response at a 1.5-fold increase level over control using 7-10 animals/group with a background incidence of 1-1.5 MPE/1000 PCE (cf. Richold, M., Ashby, J., Bootman, J., Chandley, A., Gatehouse, D.G. and Henderson, L. (1990). In Vivo Cytogenetics Assay. In: D.J. Kirkland (Ed.) Basic Mutagenicity tests. UKEMS Recommended Procedures. UKEMS Sub-Committee on Guidelines for Mutagenicity Testing. Report, Part I, revised. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney, pp. 127-129.)*
Remarks	

Table A6.6.4-1 Table for micronucleus test in vivo (first test)

One oral treatment with coumatetralyl		Negative control (vehicle)	Dose 1	Positive control (cyclophosphamid)
Dose [mg/kg bw]		—	750	20
Number of evaluated polychromatic erythrocytes per animal		1000	1000	1000
Sampling time after last treatment (h)		24	24	24
Number of erythrocytes (average of animals investigated)	Normochromatic	1247	1347	1301
	Polychromatic	1000	1000	1000
	Polychromatic with micronuclei	1.2	1.1	11.7**
	Normochromatic with micronuclei (per 1000 normochromatic)	0.8	1.1	1.5
Ratio of erythrocytes	Polychromatic / normochromatic	1000/1247	1000/1347	1000/1301
Sampling time after last treatment (h)		—	48	—
Number of erythrocytes (average of animals investigated)	Normochromatic	—	2389*	—
	Polychromatic	—	1000	—
	Polychromatic with micronuclei	—	1.9	—
	Normochromatic with micronuclei (per 1000 normochromatic)	—	1.4	—
Ratio of erythrocytes	Polychromatic / normochromatic	—	1000/2389	—
Sampling time after last treatment (h)		—	72	—
Number of erythrocytes (average of animals investigated)	Normochromatic	—	1687	—
	Polychromatic	—	1000	—
	Polychromatic with micronuclei	—	2.2	—
	Normochromatic with micronuclei (per 1000 normochromatic)	—	0.7	—
Ratio of erythrocytes	Polychromatic / normochromatic	—	1000/1687	—

*p≤ 0.05 Wilcoxon-Rang test, referred to the control

** p≤ 0.01 Wilcoxon-Rang test, referred to the control

Table A6.6.4-2 Table for micronucleus test in vivo (second test)

One oral treatment with coumatetralyl		Negative control (vehicle)	Dose 1	Dose 2	Dose 3
Dose [mg/kg bw]		—	500	750	1000
Number of evaluated polychromatic erythrocytes per animal		1000	1000	1000	1000
Sampling time after last treatment (h)		72	72	72	72
Number of erythrocytes (average of animals investigated)	Normochromatic	919	1352	997	1228
	Polychromatic	1000	1000	1000	1000
	Polychromatic with micronuclei	1.7	1.5	1.4	1.0
	Normochromatic with micronuclei (per 1000 normochromatic)	1.4	0.5	0.7	0.4
Ratio of erythrocytes	Polychromatic / normochromatic	1000/919	1000/1352	1000/997	1000/1228

Table A6.6.4-3 Table for micronucleus test in vivo (second test)

One oral treatment with coumatetralyl		Negative control (vehicle)	Dose 1	Dose 2	Dose 3
Dose [mg/kg bw]		—	500	750	1000
Number of evaluated polychromatic erythrocytes (PCE) per animal		1000	1000	1000	1000
Number of animals evaluated		10	10	9	9
Sampling time after last treatment (h)		48	48	48	48
Number of erythrocytes (average of animals investigated)	Normochromatic per 1000 PCE	864	1447	1522	1841**
	PCE with micronuclei per 1000 PCE	1.0	1.5	1.4	1.6*
	Normochromatic with micronuclei per 1000 normochromatic	0.9	0.4	0.6	1.0

* $p \leq 0.05$ in non-parametric Wilcoxon rank test

** $p \leq 0.01$ in non-parametric Wilcoxon rank test

Section A6.6.5 **Annex Point IIA VI.6.VI.6.5**	6.6.5 Mutagenicity in other than bone marrow If negative in 6.6.4 but positive in-vitro tests then undertake a second in-vivo study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [x]** **Other justification [...]**	
Detailed justification:	The available in-vitro and in-vivo tests showed negative results therefore a second in-vivo study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow is scientifically unjustified.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed*	
Conclusion	*Accepted*	
Remarks		

Section A6.6.6 Annex Point IIA VI.6.VI.6.6	6.6.6 Germ cell effects If positive in 6.6.4 then a test to assess possible germ cell effects may be required	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [x]**	
Limited exposure [...]	**Other justification [...]**	
Detailed justification:	The available in-vitro and in-vivo tests showed negative results therefore a test to assess possible germ cell effects is scientifically unjustified.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed*	
Conclusion	*Accepted*	
Remarks		

Section A6.6.7 Annex Point III-0§	6.6.7 Tests on metabolites If the results are negative for the three tests 6.6.1, 6.6.2 and 6.6.3, then further testing is normally only required if metabolites of concern are formed in mammals	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...] Scientifically unjustified [x]**	
Limited exposure [...]	**Other justification [...]**	
Detailed justification:	The available in-vitro and in-vivo tests showed negative results and there are no metabolites of concern formed in mammals therefore further testing is scientifically unjustified and not required.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed.*	
Conclusion	*Justification accepted*	
Remarks		

SECTION A6.7 **Annex Point IIA6.7**	**6.7 Carcinogenicity study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA [redacted], 2003, coumatetralyl - Waiver for chronic toxicity study in dogs, chronic/carcinogenicity toxicity study in rodents, and multigeneration reproductive toxicity study in rodents, MO-03-006927	Official use only
Other existing data [...]	**Technically not feasible [X]** **Scientifically unjustified [...]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	A waiver for a long-term rodent study for coumatetralyl is scientifically justified based on: - the lack of mutagenic/genotoxic effects, - the absence of any other effects that may lead to non-genotoxic carcinogenesis, - the absence of any carcinogenic effects following long-term administration of warfarin, a coumarin compound, in humans, - the absence of potential for long-term exposure of the public population, - the low risk of exposure during manufacturing and use, - the absence of residues in plant foodstuffs and water. The practical difficulties of long-term administration of coumatetralyl are such that an attempt at a study would almost be certain to fail and would be unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*February 2005*	
Evaluation of applicant's justification	*The justification for non-conduction of a long-term study in rats is overall acceptable. However, the Danish CA has comments to some of the arguments:* *-The argument of structural relationship with warfarin, where no carcinogenic effects have been observed following long-term administration as anticoagulant medicine in humans is acceptable with respect to the*	

Evaluation of applicant's justification	*mechanism of action of the two compounds. However, coumatetralyl is not metabolised to any compound related to warfarin chemical structure. Carcinogenic effects of metabolites of coumatetralyl can thus not be excluded.* *However, due to the restrictions on human exposure, non-genotoxic carcinogenicity is of low concern. Potential for genotoxic carcinogenicity of metabolites is expected to be low because of the non-mutagenic effect of coumatetralyl itself.*
Conclusion	*Waiving of this study is accepted by RMS but will result in reference to exposure conditions in the Annex I listing of coumatetralyl, as the prerequisites for waiving is dependent upon the nature of the formulated product Racumin paste and the recommended use pattern instructions given on that specific fomulation.*
Remarks	

SECTION A6.8.1(01)	**6.8. Reproductive toxicity**
Annex Point IIA6.8.1	6.8.1(01) Developmental toxicity study in rabbits

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1996, Developmental toxicity study with Racumin in the rabbit, [redacted] Report No. R 6742, 1996-11-27 (unpublished), MO-03-004071
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	—
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/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Methods used in this study were in accordance with: the OECD-Guideline 414, 1981 the directive 67/548/EEC of 18th November 1987, pp. 24-26, US-EPA, Subdivision F, Vol. 43, Series 83-3 and 712-C-94-207.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no. 238 303 018
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.8 %
3.1.2.3	Stability	The stability of coumatetralyl in the vehicle was analysed and was found to be stable for at least two hours at room temperature. Additionally, the homogeneity and concentration of the test substance in the vehicle was determined. During the dosing period of the study, additional samples for confirmation of concentration, homogeneity, and stability were taken on one occasion.

3.2 Test Animals

SECTION A6.8.1(01)	**6.8. Reproductive toxicity**
Annex Point IIA6.8.1	6.8.1(01) Developmental toxicity study in rabbits
3.2.1 Species	Chinchilla rabbit
3.2.2 Strain	Chbb:CH (Hybrids, SPF-quality)
3.2.3 Source	[redacted]
3.2.4 Sex	Females (mated)
3.2.5 Age/weight at study initiation	Weight: 2.822 – 4.963 kg Age: 4.5 – 6.5 months
3.2.6 Number of animals per group	24 females per group
3.2.7 Control animals	Yes
3.2.8 Mating period	Females were placed in cages with sexually mature males with the ratio 1male : 1 female, until copulation was observed.
3.3 Administration/ Exposure	Oral
3.3.1 Duration of exposure	
	Rabbit: day 6-27 post mating
3.3.2 Post-exposure period	None
	Oral
3.3.3 Type	Gavage
3.3.4 Concentration	Gavage: 0, 0.0125, 0.025, or 0.05 mg/kg bw
3.3.5 Vehicle	Bi-distilled water with 4% carboxymethylcellulose sodium salt
3.3.6 Concentration in vehicle	0, 0.003125, 0.00625 or 0.0125 mg/ml
3.3.7 Total volume applied	4 ml/kg bw
3.3.8 Controls	Vehicle
3.4 Examinations	
3.4.1 Body weight	Yes, daily
3.4.2 Food consumption	Yes Intervals: Days 0-6, 6-11, 11-15, 15 –19, 19-24 and 24 –28 of gestation.
3.4.3 Clinical signs	Yes, twice daily.

SECTION A6.8.1(01)	**6.8. Reproductive toxicity**
Annex Point IIA6.8.1	6.8.1(01) Developmental toxicity study in rabbits
3.4.4 Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of embryonic or foetal death and viable foetuses and number of resorptions
3.4.5 Examination of foetuses	
3.4.5.1 General	Litter Size, mean foetal weight of each litter and mean foetal weight of each group, incidence of runts (weight less than 19 g at section), sex ratio, external malformations, brain anomalies.
3.4.5.2 Skeleton	Yes
3.4.5.3 Soft tissue	Yes
3.5 Further remarks	—

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

Performance of mated females:

The number of mated females was 24 for each group. The number of pregnant females was 22 for the high dose level and 23 for all other groups.

Mortality:

Oral administration of coumatetralyl caused dose-related mortality from 0.025 mg/kg bw. At this dose level, three of 24 females died during the test period. At 0.05 mg/kg, 15 of 24 females died prior study termination as shown in table A6.8.1b-1.

No female rabbit died in the control group or at 0.0125 mg/kg bw.

Clinical signs:

No clinical signs were observed at 0.0125 mg/kg bw coumatetralyl.

For all females which died following administration of 0.025 or 0.05 mg/kg bw, bleedings or signs of bleedings from the vagina or from the mouth, nose and ears were observed one to four days prior to death (see table A6.8.1a -1.).

Bleedings or signs of bleedings were observed in one of the surviving females of the 0.025 mg/kg bw group and in two of the surviving females of the 0.05 mg/kg dose group. No other clinical signs occurred in female rabbits of these dose groups.

Body weight and food consumption:

In the dams with live fetuses on day 28 post coitum, the food consumption and the body weight development were not affected by treatment in any group. In some females which died at 0.05 mg/kg, slight body weight loss was ascertained during a few days

SECTION A6.8.1(01) **6.8. Reproductive toxicity**

Annex Point IIA6.8.1 6.8.1(01) Developmental toxicity study in rabbits

prior to death.

Three females which died at 0.05 mg/kg had reduced food consumption before death.

Reproduction:

No effect on the reproduction parameters (post implantation loss, number of implantations and foetuses) considered to be a primary reproductive effect of the administered test article were noted.

The statistically significant increase of the post implantation loss in the high dose group was caused by one dam with one live and ten dead foetuses at caesarean section. The presence of blood in the uterus of this animal was considered to be the cause of this increased percentage of dead foetuses (see table A6.8.1a -2.).

Pathology:

Similar abnormal findings were noted in the females which died before the end of the study. At 0.025 and 0.05 mg/kg bw the most frequent findings were lungs with foci and thoracic cavities, abdominal cavities and uteri containing blood. In addition isolated occurrence of pale or clay-coloured livers and blood in the vagina was noted.

At 0.025 mg/kg, three of the 21 surviving females the uteri contained blood and another female had dark red to brown discoloured foci in the lungs. At 0.05 mg/kg, two of the nine surviving females had blood in the uteri, two females had foci and foamy fluid in the lungs, in one female the abdominal cavity contained blood and another female the lungs had foci and were incompletely collapsed.

No substance-related effects were noted after dosing of 0.0125 mg/kg bw. (see table A6.8.1a -1.)

4.2 Teratogenic / embryotoxic effects

None of the foetal parameters – external and fresh visceral examination, sex ratios, body weights, skeletal examinations (bone and cartilage) – were affected by coumatetralyl. (see table A6.8.1a -3.)

4.3 Other effects

—

5 APPLICANT'S SUMMARY AND CONCLUSION

SECTION A6.8.1(01)	**6.8. Reproductive toxicity**	
Annex Point IIA6.8.1	6.8.1(01) Developmental toxicity study in rabbits	
5.1 Materials and methods	The study was conducted to determine the potential of coumatetralyl, administered orally by gavage, to induce maternal effects as well as to promote embryotoxicity, fetotoxicity, and/or teratogenicity in the rabbit. The methods used in this study were in accordance with the OECD-Guideline 414, 1981, the directive 67/548/EEC of 18 November 1987, pp. 24-26 and the US-EPA, Subdivision F, Vol. 43, Series 83-3 and 712-C-94-207. The dosages were based on the results of a dose range-finding study.	X
5.2 Results and discussion	From the dose level of 0.025 mg/kg bw per day, administration of coumatetralyl caused internal and external bleedings and mortality in dams. Based on these results the NOAEL for maternal toxicity was considered to be 0.0125 mg/kg bw per day. In one dam at the dose level of 0.05 mg/kg bw, nearly total post implantation loss (probably secondary due to intra-uterine bleeding) was ascertained. Therefore, with respect to foetal toxicity, the NOAEL was considered to be 0.025 mg/kg bw per day. Under the described conditions of this study, coumatetralyl did not reveal any teratogenic potential up to and including the dose level 0.05 mg/kg body weight per day.	
5.3 Conclusion		
5.3.1 LO(A)EL maternal toxic effects	Effects observed: internal and external bleedings, mortality LO(A)EL = 0.025 mg/kg bw	
5.3.2 NO(A)EL maternal toxic effects	NO(A)EL = 0.0125 mg/kg bw	
5.3.3 LO(A)EL embryotoxic / teratogenic effects	Embryotoxicity and/or foetotoxicity: Effects observed: nearly total post implantation loss in one dam (probably secondary due to intra-uterine bleeding in one dam) LO(A)EL = 0.05 mg/kg bw Teratogenicity: not applicable due to the results of the study.	X
5.3.4 NO(A)EL embryotoxic / teratogenic effects	Embryotoxicity and/or foetotoxicity: 0.025 mg/kg bw Teratogenicity: 0.05 mg/kg bw	X
5.3.5 Reliability	2	
5.3.6 deficiencies	No	
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*February 2005*	

SECTION A6.8.1(01)	**6.8. Reproductive toxicity**
Annex Point IIA6.8.1	6.8.1(01) Developmental toxicity study in rabbits

Materials and Methods	*Adopt applicant's version with the following addition:* *Ad 5.1 Materials and methods* *Each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy, and the maternal mortality should not exceed approximately 10 %. In this study, the mortality in highest dose group was 62.5%. Although the pregnancy rate in this group was 22/24, reproduction parameters were only included for the 8 animals with live pups at study termination.*
Results and discussion	*Applicant's version adopted*
Conclusion	*Ad 5.5.3 and 5.5.4: Teratogencity LOAEL > 0.05 mg/kg bw/day* *NOAEL 0.05 mg/kg bw/day*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	*The choice of the highest dose level of 0.05 mg/kg bw/day appears reasonable when based on a range-finding study in which 0/3 animals died at 0.040 mg/kg bw, 2/3 at 0.070 mg/kg bw and 3/3 at 0.100 mg/kg bw (none died at lower doses).* *The excess mortality of the highest dose group in the main study does not affect the establishment of maternal and fetal NOAELs and LOAELs mentioned in 5.3 Conclusion. Based on the results from this study, the test material is not likely to be a developmental toxicant at dose levels that do not exert marked maternal toxicity.*

Table A6.8.1A -1.Table for Teratogenic effects (separate data for all dosage groups)_in rabbits : Maternal effects

Parameter	Control data		Low dose 0.0125 mg/kg bw	Medium dose 0.025 mg/kg bw	High dose 0.05 mg/kg bw	Dose-response + / -
	Historical	Study				
Number of dams examined	See appendix	24	24	24	24	
Clinical findings during application of test substance	See appendix	None.	None.	Bleeding or signs of bleeding from the vagina and isolated from ear.	Bleeding or signs of bleeding from the vagina and isolated from ear, mouth and nose.	+
Mortality of dams	See appendix	0%	0%	12.5%	62.5%	+
Mean body weight gain gain (mean ± S.D.)	See appendix	Not affected.				-
Mean food consumption [g] (mean ± S.D.)	See appendix	Not affected.				-
Pregnancies*	See appendix	23/24	23/24	23/24	22/24	-

Parameter	Control data		Low dose 0.0125 mg/kg bw	Medium dose 0.025 mg/kg bw	High dose 0.05 mg/kg bw	Dose-response + / -
	Historical	Study				
Necropsy findings in dams at study termination (effected dams/number of dams examined at study termination)	See appendix	1/24 cyst containing hemorraghic fluid	2/24 uterine horn containing blood; raised area in the mucosa of the stomach	4/21 uteri containing blood; lung with many dark foci, dark red to brown discoloured.	6/9 uteri containing blood; lungs with many foci and foamy fluid; abdominal cavity filled with blood.	+
Necropsy findings in dams dead before end of test (effected dams/number of dams examined)	See appendix			3/24 lungs with foci; uteri, thoracic caveties and abdominal caveties contained blood.	15/24 lungs with foci; uteri, thoracic caveties and abdominal caveties contained blood.	+

pregnant animals / number of animals inseminated

* difference against control $p \leq 0.05$ significant (Fisher's Exact-test)

** difference against control $p \leq 0.01$ significant (Fisher's Exact-test)

Table A6.8.1A 2. Table for Teratogenic effects in rabbits: Litter response (Caesarean section data)

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical	Study	0.0125 mg/kg bw	0.025 mg/kg bw	0.05 mg/kg bw	+ / -
Corpora lutea *total/number of dams*	See appendix	270/23	287/23	235/20	95/8	-
Implantations *total/number of dams*	See appendix	246/23	274/23	218/20	82/8	-
Resorptions (embryonic) *total/number of dams*	See appendix	10/23	14/23	10/20	1/8	+ see remark
Resorptions (foetal) *total/number of dams*	See appendix	4/23	6/23	5/20	4/8	+ see remark
Total number of foetuses	See appendix	232	254	203	77	+ see remark
Pre-implantation loss *% of corpora lutea*	See appendix	8.9	4.5*	7.2	13.7	+ see remark
Post-implantation loss *% of implantation sites*	See appendix	5.7	7.3	6.9	18.3**	+ see remark
Number of litters	**See appendix**	23	23	20	8	+ see remark
Foetuses / litter ***(total/litter)***	**See appendix**	232/23	254/23	203/20	77/8	-
Live foetuses / litter *(total/litter)*	**See appendix**	**232/23**	**254/23**	**203/20**	**67/8**	**+ see remark**
Dead foetuses / litter *(total/litter)*	**See appendix**	**0/23**	**0/23**	**0/20**	**10/8**	+ **see remark**
Foetus weight *[g] (mean ± S.D.)*	**See appendix**	33 ± 3.3	32.3 ± 2.7	33.2 ± 5.5	33.8 ± 4.0	-
Placenta weight *[g] (mean ± S.D.)*	**Not determined.**					
Crown-rump length *[mm] (mean ± S.D.)*	**Not determined.**					
Foetal sex ratio *[ratio m/f]*	**See appendix**	**118/114**	**119/135**	**102/101**	**44/33**	-

* difference against control $p \leq 0.05$ significant (Fisher's Exact-test)

** difference against control $p \leq 0.01$ significant (Fisher's Exact-test)

Remark: results were not considered as a primary reproductive effect of the administered test substance (see also section 4.1 above)

Table A6.8.1A-3. Table for Teratogenic effects in rabbits: Examination of the fetuses

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical	Study	0.0125 mg/kg bw	0.025 mg/kg bw	0.05 mg/kg bw	+ / -
Number of foetuses examined	**See appendix**	**232**	**254**	**203**	**67**	
External anomalies*						
Omphalocele		**0/232**	**0/254**	**0/202**	**1/67**	-
Runt (small foetus)	**See appendix**	**1/232**	**0/254**	**1/203**	**1/67**	-
Microphthalmia	**See appendix**	**0/232**	**0/254**	**1/203**	**0/67**	-
Visceral anomalies*	**See appendix**					
Liver, several foci and discoloured		**0/232**	**1/254**	**1/203**	**0/67**	-
Liver,, darkred-black discoloured	**See appendix**	**0/232**	**0/254**	**0/203**	**1/67****	-
Brain, extremely soft, white discoloured	**See appendix**	**0/232**	**0/254**	**0/203**	**1/67****	-
Hydronephrosis	**See appendix**	**0/232**	**0/254**	**1/203**	**0/67**	-
Skeletal anomalies*	**See appendix**					
Bipartite sternebra no.5		**2/232**	**1/254**	**0/203**	**0/67**	-
Flying rib no. 13	*See appendix*	**0/232**	**0/254**	**1/203**	**0/67**	-

* **foetuses affected/total number of foetuses**

** additionally, the dam had 10 dead foetuses. All dead foetuses had dark red/black livers. Six of the dead foetuses had extremely soft brains which were white discoloured.

SECTION A6.8.1(02)	**6.8.1 Teratogenicity Study (rabbit)**
Annex Point IIA6.8	6.8.1 (02) Developmental toxicity study in rabbits (Dose range-finding study)

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1 REFERENCE

1.1	**Reference**	[redacted], 1995, Dose Range-Finding Developmental Toxicity Study with Racumin in the Rabbit, [redacted] Report No. R 6487, 1995-12-08 (unpublished), MO-03-004084
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	–
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/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	–

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no. 238 303 018
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.8 %
3.1.2.3	Stability	The stability of coumatetralyl in the vehicle was analysed and was found to be stable for at least two hours at room temperature. The homogeneity and concentration of the test substance in the vehicle was determined.
3.2	**Test Animals**	
3.2.1	Species	Chinchilla Rabbit
3.2.2	Strain	Chbb: CH, Hybrids (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Females (mated)
3.2.5	Age/weight at study	Weight: 2595-4646 g

SECTION A6.8.1(02) Annex Point IIA6.8	6.8.1 Teratogenicity Study (rabbit) 6.8.1 (02) Developmental toxicity study in rabbits (Dose range-finding study)
initiation	Age: 12-20 weeks
3.2.6 Number of animals per group	Controls and 3 lowest dose groups: 5 per group Two highest dose groups: 3 per group
3.2.7 Control animals	Yes
3.2.8 Mating period	Females were placed in cages with sexually mature males in the ratio 1 male : 1 female until copulation had been observed. After mating, the females were removed and caged individually. The day of mating was designated day 0 post coitum.
3.3 Administration/ Exposure	Oral
3.3.1 Duration of exposure	Day 6-27 post coitum
3.3.2 Post-exposure period	None
3.3.3 Type	Gavage
3.3.4 Concentration	0, 0.016, 0.025, 0.040, 0.070 and 0.100 mg/kg bw
3.3.5 Vehicle	Bi-distilled water with 4% carboxymethylcellulose sodium salt
3.3.6 Concentration in vehicle	0, 0.004, 0.0063, 0.010, 0.0175 and 0.025 mg/ml
3.3.7 Total volume applied	4 ml/kg bw
3.3.8 Controls	Vehicle
3.4 Examinations	
3.4.1 Body weight	Yes, daily.
3.4.2 Food consumption	Yes Intervals: days 0-6, 6-11, 11-15, 15-19, 19-24, and 24-28 post coitum.
3.4.3 Clinical signs	Yes, twice daily.
3.4.4 Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of embryonic or foetal deaths and viable foetuses and number of resorptions
3.4.5 Examination of foetuses	
3.4.5.1 General	Sex, number of viable and dead foetuses, individual weight of foetuses, external abnormalities, gross necropsy of cranium, thorax and abdomen.

SECTION A6.8.1(02)	**6.8.1 Teratogenicity Study (rabbit)**
Annex Point IIA6.8	6.8.1 (02) Developmental toxicity study in rabbits (Dose range-finding study)
3.4.5.2 Skeleton	No
3.4.5.3 Soft tissue	No
3.5 Further remarks	The two highest dose groups were added to the study after no signs of toxicity were noted up to and including 0.040 mg/kg bw.

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

Performance of mated females:

Dose group	Pregnancies/number of does
vehicle control	2/5
0.016 mg/kg	3/5
0.025 mg/kg	4/5
0.040 mg/kg	3/5
0.070 mg/kg	3/3
0.100 mg/kg	3/3

Mortality:
At 0.070 mg/kg bw, two out of three does died after 9 and 19 test article administrations and the three animals in the 0.100 mg/kg group died after 9, 14, and 22 test article administrations, respectively.
No deaths occurred in the other dose groups. (See Table A6.8-4)

Clinical signs:

In the animals that died, bleeding from the nose, mouth and/or vagina was observed one or two days prior to death. In one doe that died in the highest dose group and in the surviving doe in the 0.70 mg/kg group, no signs or symptoms were observed. No signs or symptoms were observed in the other dose groups (See Table A6.8-4)

Body weight:

In the two highest dose groups, a slight loss of mean body weight from days 6 to 7 p.c. (after the first test article administration) as well as reduced mean body weight gain from days 6 to 11 p.c. were noted. This was considered to be an effect of the test article.
Mean body weight gain was similar in controls and the three lowest dose groups.

Differences between the mean corrected body weight gain (corrected for uterus weight) of the vehicle control group and the three lowest dose groups gave no indication for a test-article-related effect.

Food consumption:

In the two highest dose groups, a reduction in mean food consumption was noted in the day 6-11 and 11-15 intervals. The three lowest dose groups showed no difference compared to controls.

Reproduction:
No test-article-related effects on reproductive parameters were noted. The differences which existed were within the normal

SECTION A6.8.1(02)	**6.8.1 Teratogenicity Study (rabbit)**
Annex Point IIA6.8	6.8.1 (02) Developmental toxicity study in rabbits (Dose range-finding study)
	ranges of variation and considered to be incidental. (See Table A6.8.1a-5) Pathology: During scheduled necropsy on day 28 p.c., no abnormal findings were evident in any doe of any group, and in the does that died, no test-article-specific abnormal findings were noted. (See Table A6.81a-4)
4.2 Teratogenic / embryotoxic effects	Gross pathology: In controls, two out of 16 foetuses were runts. In the 0.016 mg/kg group, the left cerebral hemisphere was reduced in size in one foetus, and in another foetus, slight caudal dystopia of the left kidney was noted. In the 0.025 mg/kg group, false position of the left fore paw was noted in one foetus. In the 0.040 mg/kg group, the left cerebral hemisphere was reduced in size in one foetus. In the 0.070 mg/kg group, no abnormal findings were noted in the eight live foetuses of the single surviving doe. Sex ratio: The sex ratios of foetuses in all groups was similar. Body weight: No adverse effects of the test article on the mean body weights of the foetuses were noted. (See Table A6.81a -5)
4.3 Other effects	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	The purpose of this study was to assess the effects of Racumin on the maternal organism and on embryonic and foetal development in mated female rabbits in order to establish suitable dose levels for the main developmental toxicity study. Due to the preliminary nature of this study, the respective Guidelines for the testing of chemicals were not applicable.
5.2 Results and discussion	Treatment with Racumin caused the deaths of all three females at 0.100 mg/kg bw and two out of three females at 0.070 mg/kg bw. Bleeding from the vagina and/or nose and mouth were observed prior to death of these animals. At these dose levels, unrelated to dose, reduced mean food consumption and body weight gain were noted after initiation of treatment. At necropsy, no abnormal findings which were considered to be test article related were noted. Up to and including the dose level of 0.100 mg/kg bw, no deaths occurred and no signs of reaction to treatment were observed. Reproductive parameters showed no evidence of test-article-related effects.Up to and including the 0.070 mg/kg bw level, no effects on the foetal parameters external and fresh visceral examinations, sex ratio and body weights were noted.
5.3 Conclusion	Based on the results of this dose range-finding developmental toxicity study with Racumin in the rat, dose levels of 0.0125, 0.025, and 0.05 mg/kg bw/day were chosen for the main study.
5.3.1 Reliability	0 This study is not intended to yield data for actual risk assessment but to serve for dose range-finding purposes only.
5.3.2 Deficiencies	Not applicable.

EVALUATION BY COMPETENT AUTHORITIES	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*February 2005*
Materials and Methods	*Applicant's version adopted.*
Results and discussion	*Applicant's version adopted.*
Conclusion	*Applicant's version adopted.*
Reliability	*0 (The object of the study is to serve for dose range-finding purposes only.)*
Acceptability	*Acceptable (as a dose range-finding study).*
Remarks	

Table A6.81a -4. Table for Teratogenic effects: Maternal effects

Parameter	Control 0 mg/kg bw	Group 2 0.016 mg/kg bw	Group 3 0.025 mg/kg bw	Group 4 0.040 mg/kg bw	Group 5 0.070 mg/kg bw	Group 6 0.100 mg/kg bw	Dose -resp. + / –
Number of does examined	2	3	4	3	3	3	
Clinical findings during application of test substance *(affected does/number of does examined)*	–	–	–	–	Vaginal bleeding (2/3) Oro-nasal bleeding (1/3)	Ataxia, ventrolateral recumbency (1/3) Vaginal bleeding (2/3)	+
Mortality of does	0%	0%	0%	0%	67%	100%	+
Mean body weight gain[1] ***[g] (mean ± S.D.)***	-89.9 ± 141.4	189.4 ± 164.6	87.6 ± 160.8	140.9 ± 135.4	5	–	–
Mean food consumption[2] ***[g] (mean ± S.D.)***	226 ± 25.1	239 ± 15.4	228 ± 11.9	261 ± 17.8	193 ± 34.9	180 ± 46.9	+
Pregnancies	2/5	3/5	4/5	3/5	3/3	3/3	–
Necropsy findings in does dead before end of test *(affected does/number of does examined)*	–	–	–	–	Lungs with red foci (1/3) Lungs dark red dis-coloured, liver foci (1/3)	Lungs with red foci (1/3) Lungs dark red dis-coloured (2/3) Uterus contained blood (1/3)	+
Necropsy findings in does *(affected does/number of does examined)*	No abnormal findings at study termination.						–

[1]Body weight gain corrected for weight of uterus.

[2]DAYS 6-11 P.C.

Table A6.81a -5. Table for Teratogenic effects: Litter response (Caesarean section data)

Parameter	Control 0 mg/kg bw	Group 2 0.016 mg/kg bw	Group 3 0.025 mg/kg bw	Group 4 0.040 mg/kg bw	Group 5 0.070 mg/kg bw	Group 6 0.100 mg/kg bw	Dose-response + / –
Corpora lutea *total/number of dams*	9.5	8.3	9.3	10.3	12	–	–
Implantations *total/number of dams*	9.0	6.7	7.5	9.7	9	–	–
Resorptions (embryonic) *total/number of dams*	1.0	0.3	0.5	0.0	1	–	–
Resorptions (foetal) *total/number of dams*	0.0	0.0	1.0	0.3	0	–	–
Total number of foetuses	16	19	24	28	8	–	–
Pre-implantation loss *% of corpora lutea*	5.3	20.0	18.9	6.5	25.0	–	–
Post-implantation loss *% of implantation sites*	11.1	5.0	20.0	3.4	11.1	–	–
Number of litters	2	3	4	3	1	0	–
Foetuses / litter ***(total/litter)***	8.0	6.3	6.0	9.3	8	–	–
Live foetuses / litter *(total/litter)*	8.0	6.3	6.0	9.3	8	–	–
Dead foetuses / litter *(total/litter)*	0.0	0.0	0.0	0.0	0	–	–
Foetus weight *[g] (mean ± S.D.)*	32.3 ± 6.9	34.6 ± 6.2	34.1 ± 6.4	33.7 ± 4.7	34.3 ± 4.2	–	–
Foetal sex ratio *[ratio m/f]*	**0.45**	**0.90**	**2.00**	**1.15**	**1.67**	–	–

SECTION A6.8.1(03)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (03)Developmental toxicity study in rats

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1996, Developmental toxicity study with Racumin in the rat, [redacted], Report No. R 6741, 1996-11-21 (unpublished), MO-03-004091
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	—
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/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Methods used in this study were in accordance with: the OECD-Guideline 414, 1981 the directive 67/548/EEC of November 18, 1987, pp. 24-26, US-EPA, Subdivision F, Vol. 43, Series 83-3 and 712-C-94-207.
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no. 238 303 018
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.8 %
3.1.2.3	Stability	The stability of coumatetralyl in the vehicle was analysed and was found to be stable for at least two hours at room temperature. The homogeneity and concentration of the test substance in the vehicle was determined. During the dosing period of the study, additional samples for confirmation of concentration, homogeneity, and stability were taken on one occasion.
3.2	**Test Animals**	

SECTION A6.8.1(03)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (03)Developmental toxicity study in rats
3.2.1 Species	Wistar rat
3.2.2 Strain	HanIbm : WIST (SPF)
3.2.3 Source	[redacted]
3.2.4 Sex	Females (mated)
3.2.5 Age/weight at study initiation	Weight: 178 – 221 g Age: at least 11 weeks
3.2.6 Number of animals per group	25 females per dose group and 29 females for the vehicle control.
3.2.7 Control animals	Yes
3.2.8 Mating period	Females were paired overnight with sexually mature males in the ratio 1male : 1 female. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0 post coitum.
3.3 Administration/ Exposure	
3.3.1 Duration of exposure	Rat Day 6-20 Post mating
3.3.2 Post-exposure period	None
3.3.3 Type	**Oral** , Gavage
3.3.4 Concentration	0, 0.035, 0.070, and 0.140 mg/kg bw
3.3.5 Vehicle	Bi-distilled water with 4% Carboxymethylcellulose sodium salt
3.3.6 Concentration in vehicle	0, 0.0035, 0.007, and 0.014 mg/ml
3.3.7 Total volume applied	10 ml/kg bw
3.3.8 Controls	Vehicle
3.4 Examinations	
3.4.1 Body weight	Yes, daily
3.4.2 Food consumption	Yes Intervals: Days 0-6, 6-11, 11-16 and 16-21 of gestation.
3.4.3 Clinical signs	Yes, twice daily.

SECTION A6.8.1(03)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (03)Developmental toxicity study in rats

3.4.4	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of embryonic or foetal death and viable foetuses and number of resorptions
3.4.5	Examination of foetuses	
3.4.5.1	General	Sex, number of embryonic or foetal deaths and viable or dead foetuses, individual weight of foetuses, mean foetal weight of each litter and mean foetal weight of each group, external abnormalities, incidence of runts (foetuses weighing less than 2.5 g at section), brain anomalies.
3.4.5.2	Skeleton	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

Performance of mated females:

The number of mated females was 25 for each group. The number of pregnant females was 24 for control, 0.035 and 0.070 mg/kg bw/day groups and 25 for the high dose level.

Mortality:

Oral administration of coumatetralyl caused dose-related mortality at 0.07 and 0.14 mg/kg bw. At 0.07 mg/kg bw, 1 of 25 female was found dead on gestation day t(GD) 19. At 0.14 mg/kg bw, a total number of 8 of 25 females died between GD 15 and 17.

No death was reported in the control group or at 0.035 mg/kg bw. (See Table A6.8.1b-1)

Clinical signs:

In the control group one female rat showed hairless regions on the chest wall.

No clinical signs were observed at any time during the treatment period for the 0.035 mg/kg bw group.

In all females administered 0.07 or 0.14 mg/kg bw and which died during treatment profuse bleeding from the vagina was observed in days prior death. The other clinical signs observed in some females included ruffled fur, sedation, ataxia and/or hunched posture, body weight loss, pale eyes and were considered to be the consequence of the extensive loss of blood.

In the surviving female rats of the 0.07 mg/kg bw dose group no clinical signs occurred.

The remaining females of the 0.14 mg/kg bw dose group showed transient bleedings from the vagina. (See Table A6.8.1b-1)

Body weight:

SECTION A6.8.1(03)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (03)Developmental toxicity study in rats

	In all dams which survived until study termination, body weight gain was not affected by treatment with coumatetralyl. In most females of the 0.07 and 0.14 mg/kg bw dose groups which died during the study, body weight loss was noted prior to death. Food consumption: No effect was reported. Reproduction: No effects on the reproduction parameters were considered to be a primary reproductive effect of the administered test article. None of the differences amongst the post implantation losses, the number of implantations and foetuses of the control group and any dose group were considered to be treatment related. The differences which existed were within the normal ranges of the historical control data and considered to be incidental. (See Table A6.8.1b-2) Pathology: Clay-coloured livers were observed in three out of eight females which died at the 0.14 mg/kg dose group. The single female which died at 0.07 mg/kg showed a clay-coloured liver, hemorrhagic rectum and colon and reddish discoloured foci in the thymus. All these findings were considered to be the consequence of the massive bleedings caused by the test substance. . (See Table A6.8.1b-1)
4.2 Teratogenic / embryotoxic effects	The slightly increased mean foetal body weights in all treated groups were within the normal range of the historical control data and considered to be incidental as there was no dose-relationship . None of the foetal parameters - external and fresh visceral examination, sex ratios, body weights, skeletal examinations (bone and cartilage) – were affected by treatment. (See Table A6.8.1b-3)
4.3 Other effects	—

SECTION A6.8.1	**6.8 Teratogenicity Study**
Annex Point IIA6.8.1	6.8.1(03) Developmental toxicity study in rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	The study was conducted to determine the potential of coumatetralyl, administered orally by gavage, to induce maternal effects as well as to promote embryotoxicity, fetotoxicity, and teratogenicity in the rat. The methods used in this study were in accordance with the OECD-Guideline 414 of 1981, the directive 67/548/EEC of 18 November 1987, pp. 24-26 and the US-EPA, Subdivision F, Vol. 43, Series 83-3 and 712-C-94-207. The dosages were based on the results of a dose range-finding study.	
5.2 Results and discussion	Administration of coumatetralyl caused profuse bleedings, symptoms of anaemia and mortality in dams treated at 0.07 and 0.140 mg/kg bw/day. Based on these results the NOEL for maternal toxicity was 0.035 mg/kg bw / day.	x
	No adverse effect and no indication of any teratogenic potential was observed in foetuses up to and including 0.140 mg/kg bw/day. Therefore the NOEL for foetal toxicity was 0.140 mg/kg bw per day.	x
5.3 Conclusion		
5.3.1 LO(A)EL maternal toxic effects	Effects observed: profuse bleedings, symptoms of anaemia and mortality in dams LO(A)EL = 0.070 mg/kg bw	
5.3.2 NO(A)EL maternal toxic effects	NO(A)EL = 0.035 mg/kg bw	
5.3.3 LO(A)EL embryotoxic / teratogenic effects	Not applicable due to results of study.	x
5.3.4 NO(A)EL embryotoxic / teratogenic effects	Embryotoxicity and/or foetotoxicity: NO(A)EL = 0.14 mg/kg bw *Teratogenicity: NO(A)EL = 0.14 mg/kg bw*	
5.3.5 Reliability	2	
5.3.6 Deficiencies	No	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*Adopt applicant's version with the following addition:* *Ad 5.1 Materials and methods* *Each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy, and the maternal mortality should not exceed approximately 10 %. In this study, the mortality in highest dose group was 32% leaving 17 dams for examination of teratogenic / embryotoxic effects.*
Results and discussion	*Ad 5.2 As no adverse effects were seen in dams at 0.035 mg/kg bw/day and in pups at 0.14 mg/kg bw/day, these levels can be regarded as NOAELs instead of NOELs.* *Ad 5.3.3 the LOAEL for teratogenicity is >0.14 in this study.*
Conclusion	*Applicant's version adopted.*
Reliability	*2*
Acceptability	*Acceptable.*
Remarks	*The choice of the highest dose level of 0.14 mg/kg bw/day appears reasonable when based on a range-finding study in which 0/5 animals died at 0.10 mg/kg bw, 1/5 at 0.16 mg/kg bw and 3/5 at 0.20 mg/kg bw.* *The excess mortality of the highest dose group in the main study does not affect the establishment of maternal and fetal NOAELs and LOAELs mentioned in 5.3 Conclusion. Based on the results from this study, the test material is not likely to be a developmental toxicant at dose levels that do not exert marked maternal toxicity.*

Table A6.8.1b-1. Table for Teratogenic effects in rats: Maternal effects

Parameter	Control data		Low dose 0.035 mg/kg bw	Medium dose 0.070 mg/kg bw	High dose 0.140 mg/kg bw	Dose-response + / -
	Historical	Study				
Number of dams examined	**See appendix**	25	25	25	25	
Clinical findings during application of test substance	See appendix	Hairless regions on the chest wall and abdomen (one female).	No clinical sign.	Female which died prior study termination: profuse bleedings from the vagina, ruffled fur, sedation, ataxia and/or hunched posture, body weight loss, pale eyes. Surviving females: no clinical sign.	Females which died prior study termination: profuse bleedings from the vagina, ruffled fur, sedation, ataxia and/or hunched posture, body weight loss, pale eyes. Surviving females: showed transient bleedings from the vagina.	+
Mortality of dams	**See appendix**	**0%**	**0%**	**4%**	**32%**	**+**
Mean body weight gain ***gain (mean ± S.D.)***	*See appendix*	**Not affected. Most females of the mid and high dose groups which died during the study, showed body weight loss prior to death.**				-
Mean food consumption **[g]** ***(mean ± S.D.)***	*See appendix*	**Not affected.**				-
Pregnancies#	See appendix	24/25	24/25	24/25	25/25	-

pregnant animals / number of animals inseminated

Table A6.8.1B-1. Table for Teratogenic effects: Maternal effects (continued)

Parameter	Control data		Low dose 0.035 mg/kg bw	Medium dose 0.070 mg/kg bw	High dose 0.140 mg/kg bw	Dose-response + / -
	Historical	Study				
Necropsy findings in dams dead before end of test (effected dams/number of dams examined)	See appendix	0/25	1/25 diaphragmatic liver hernia	1/25 clay-coloured liver; hemorrhagic rectum and colon; thymus with reddish discoloured foci.	4/25 clay-coloured liver; reddish discoloured pancreas.	+
Necropsy findings in dams (effected dams/number of dams examined)	See appendix	No abnormal findings at study termination.				-

Table A6.8.1B-2. Table for Teratogenic effects in rats: Litter response (Caesarean section data)

Parameter	Control data		Low dose 0.035 mg/kg bw	Medium dose 0.070 mg/kg bw	High dose 0.140 mg/kg bw	Dose-response + / -
	Historical	Study				
Corpora lutea *total/number of dams*	See appendix	334/24	339/24	312/23	256/17	-
Implantations *total/number of dams*	See appendix	318/24	315/24	284/23	240/17	-
Resorptions (embryonic) *total/number of dams*	See appendix	22/24	22/24	17/23	17/17	-
Resorptions (foetal) *total/number of dams*	See appendix	8/24	1/24	3/23	1/17	-
Total number of foetuses	See appendix	288	292	264	222	-
Pre-implantation loss *% of corpora lutea*	See appendix	4.8	7.1	9.0#	6.3	-
Post-implantation loss *% of implantation sites*	See appendix	9.4	7.3	7.0	7.5	-
Number of litters	See appendix	24	24	23	17	-
Foetuses / litter *(total/litter)*	See appendix	288/24	292/24	264/23	222/17	-
Live foetuses / litter *(total/litter)*	See appendix	288/24	292/24	264/23	222/17	-
Dead foetuses / litter *(total/litter)*	See appendix	0/24	0/24	0/23	0/17	-
Foetus weight *[g] (mean ± S.D.)*	See appendix	4.5 ± 0.2	4.7* ± 0.2	4.7* ± 0.2	4.7 ± 0.2	-
Placenta weight *[g] (mean ± S.D.)*	Not determined.					
Crown-rump length *[mm] (mean ± S.D.)*	Not determined.					
Foetal sex ratio *[ratio m/f]*	See appendix	135/153	125/167	114/150	98/124	-

* Dunnett-Test based on pooled variance significant at level 5%

\# Difference against control $p \leq 0,05$significant (Fisher's Exact-test)

Table A6.8.1B-3. Table for Teratogenic effects in rats: Examination of the fetuses

Parameter	Control data		Low dose	Medium dose	High dose	Dose-response
	Historical	Study	0.035 mg/kg bw	0.070 mg/kg bw	0.140 mg/kg bw	+ / -
Number of foetuses examined	See appendix	288	292	264	222	
External anomalies*	See appendix					
Agnathia, microstomia		1/288	0/292	0/264	0/222	-
Deformed head, cheilognathoschisis, palatoschisis, micromelia, polydactylia	See appendix	1/288	0/292	0/264	0/222	-
Runt (small foetus)	See appendix	0/288	0/292	1/264	0/222	-
Number of foetuses examined	See appendix	137	139	126	107	
Visceral anomalies*	See appendix					
Palatoschisis		1/137	0/139	0/126	0/107	-
Number of foetuses examined	See appendix	151	153	138	115	
Skeletal anomalies*	See appendix	1/151 ①	3/153 ②	2/138 ③	0/115	-

*foetuses affected/total number of foetuses

① The following abnormal findings were observed: Upper and lower jaw shortened, palatoschisis; left and right forepaws, 7 digits; left hind paws, 8 toes; right hind paw, 6 toes; incompletely ossified cranium; missing ossification of the left ulna, radius, humerus, tibia, fibula, femur, talus, metatarsalia and missing ossification of the right humerus, calcaneus, talus, metatarsalia, sternum, cervical vertebral column, thoracic vertebral bodies no. 1-3, dumbbell shaped thoracic vertebral bodies nos. 4-13, no visible cartilages.

② One animal with wavy ribs (nos. 9-11, right side); one animal with wavy ribs (nos. 3-5, 9 and 10, left side and nos. 10-12 (right side); one animal with bipartite sternebra no.2.

③ One animal with abnormally shaped sternebra nos. 3 and 4, one animal showed lacunae in the left and right os parietale; incompletely ossified os interparietale and os occipitale.

SECTION A6.8.1(04)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (04) Developmental toxicity study in rats (Dose range-finding study)

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1 REFERENCE

1.1	**Reference**	[redacted], 1995, Dose Range-Finding Developmental Toxicity Study with Racumin in the Rat, [redacted], Report No. R 6460, 1995-11-13 (unpublished), MO-03-004095
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data on existing active substance submitted under national legislation authorisation

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	–

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no. 238 303 018
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.8 %
3.1.2.3	Stability	The stability of coumatetralyl in the vehicle was analysed and was found to be stable for at least two hours at room temperature. The homogeneity and concentration of the test substance in the vehicle was determined.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	HanIbm : WIST (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Females (mated)

SECTION A6.8.1(04)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (04) Developmental toxicity study in rats (Dose range-finding study)
3.2.5 Age/weight at study initiation	Weight: 205-229 g Age: 12 weeks
3.2.6 Number of animals per group	20 mated females, 5 per group
3.2.7 Control animals	Yes
3.2.8 Mating period	Females were paired overnight with sexually mature males in the ratio 1 male : 1 female. The day on which spermatozoa were found in the vaginal smear or a vaginal plug was observed was designated day 0 post coitum.
3.3 Administration/ Exposure	Oral
3.3.1 Duration of exposure	*Day 6-20 post coitum*
3.3.2 Post-exposure period	None
3.3.3 Type	Gavage
3.3.4 Concentration	0, 0.10, 0.16, and 0.20 mg/kg bw
3.3.5 Vehicle	Bi-distilled water with 4% carboxymethylcellulose sodium salt
3.3.6 Concentration in vehicle	0, 0.010, 0.016, and 0.020 mg/ml
3.3.7 Total volume applied	10 ml/kg bw
3.3.8 Controls	Vehicle
3.4 Examinations	
3.4.1 Body weight	Yes, daily.
3.4.2 Food consumption	Yes Intervals: days 0-6, 6-11, 11-16 and 16-21 post coitum.
3.4.3 Clinical signs	Yes, twice daily.
3.4.4 Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Number of embryonic or foetal deaths and viable foetuses and number of resorptions
3.4.5 Examination of	

SECTION A6.8.1(04)	6.8.1 Teratogenicity Study (rodent)
Annex Point IIA6.8	6.8.1 (04) Developmental toxicity study in rats (Dose range-finding study)
foetuses	
3.4.5.1 General	Sex, number of viable and dead foetuses, individual weight of foetuses, external abnormalities.
3.4.6 Skeleton	No
3.4.7 Soft tissue	No
3.5 Further remarks	–

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

Performance of mated females:
All females became pregnant upon mating. All females that survived until caesarean section carried live foetuses.

Mortality:
At 0.16 mg/kg bw, one animal was found dead on day 15 p.c. At 0.20 mg/kg bw, three animals died. They were found dead on days 15, 15, and 16, respectively.No deaths were reported in the control group or at 0.10 mg/kg bw. (See Table 6.8.1b-4)

Clinical signs:
Vaginal bleeding occurred in all animals that died on study and in one surviving dam of the 0.20 mg/kg group. Ruffled coat and decreased activity were also noted in these animals.

No signs or symptoms were observed in controls and the low-dose group or the surviving dams of the mid-dose group. (See Table A6.8.1b-4)

Body weight:
A slight but dose-dependent reduction of mean body weight gain in the 0.16 and 0.20 mg/kg group between days 11 and 16 p.c. was considered a test-article-related effect.Mean body weight gain was similar in controls and the 0.10 mg/kg group.

Food consumption:
No effects were reported.

Reproduction:
No test-article-related effects on reproductive parameters were noted. The differences which existed were within the normal ranges of variation and considered to be incidental. (See Table A6_8-5)

Pathology:
No abnormal findings were observed in any female of any group at scheduled necropsy. Clay-coloured livers and enlarged adrenal glands were observed in two out of three dams which died in the 0.20 mg/kg dose group. These findings were considered to be the consequence of the vaginal bleeding and treatment-related stress. (See Table A6.8.1b-4)

4.2 Teratogenic / embryotoxic effects

Gross pathology:
No abnormal findings were noted in any foetus of any group

SECTION A6.8.1(04)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (04) Developmental toxicity study in rats (Dose range-finding study)
	during external examination. Sex ratio The sex ratios of foetuses in all groups were similar Body weight A slight, but dose-dependent reduction of the mean body weights of foetuses on an individual basis as well as on a litter basis was noted in the 0.16 mg/kg group (See Table A6.8.1b-5)
4.3 Other effects	–
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The purpose of this study was to assess the effects of Racumin on the maternal organism and on embryonic and foetal development in mated female rats in order to establish suitable dose levels for the main developmental toxicity study. Due to the preliminary nature of this study, the respective Guidelines for the testing of chemicals were not applicable.
5.2 Results and discussion	At 0.16 mg/kg bw, one of five dams died after nine test article administrations and three out of five dams died at 0.20 mg/kg bw, two after nine and one after ten test article administrations. Prior to death, bleeding from the vagina, ruffled coat, decreased activity or apathy was observed. These symptoms were also noted in one of the two surviving dams in the 0.20 mg/kg group. With one exception, clay-coloured livers and enlarged adrenal glands were found during necropsy of the dams that died. Further findings at 0.16 and 0.20 mg/kg bw included a slight but dose-dependent reduction of the mean body weight gain, concomitant with the described clinical symptoms and mortalities. No effects on the foetal parameters, external examinations and sex ratios were noted in any group. The mean body weights were slightly, yet dose-dependently reduced in the 0.16 mg/kg group.
5.3 Conclusion	Based on the results of this dose range-finding developmental toxicity study with Racumin in the rat, dose levels of 0.035, 0.07, and 0.14 mg/kg bw/day were chosen for the main study.
5.3.1 Reliability	0 This study is not intended to yield data for actual risk assessment but to serve for dose range-finding purposes only.
5.3.2 Deficiencies	Not applicable.
	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*Applicant's version adopted.*
Results and discussion	*Applicant's version adopted.*
Conclusion	*Applicant's version adopted.*

SECTION A6.8.1(04)	**6.8.1 Teratogenicity Study (rodent)**
Annex Point IIA6.8	6.8.1 (04) Developmental toxicity study in rats (Dose range-finding study)

Reliability	*0 (The object of the study is to serve for dose range-finding purposes only)*
Acceptability	*Acceptable (as a dose range-finding study)*
Remarks	

Table A6.8.1B-4. **Table for Teratogenic effects: Maternal effects**

Parameter	Control 0 mg/kg bw	Low dose 0.10 mg/kg bw	Medium dose 0.16 mg/kg bw	High dose 0.20 mg/kg bw	Dose-response + / –
Number of dams examined	5	5	5	5	
Clinical findings during application of test substance *(affected dams/number of dams examined)*	–	–	Vaginal bleeding (1/5)	Vaginal bleeding (4/5) Ruffled coat (4/5) Apathy (1/5) Decreased activity (3/5)	+
Mortality of dams	**0%**	**0%**	**20%**	**60%**	**+**
Mean body weight gain[1] ***[g] (mean ± S.D.)***	**24.2 ± 2.3**	**22.3 ± 7.0**	**18.8 ± 5.1**	**19.6 ± 2.4**	+
Mean food consumption ***[g] (mean ± S.D.)***	**Not affected.**				–
Pregnancies	5/5	5/5	5/5	5/5	–
Necropsy findings in dams dead before end of test *(affected dams/number of dams examined)*	–	–	Clay-coloured liver, enlarged adrenals (1/1)	Clay-coloured liver, enlarged adrenals (2/3)	+
Necropsy findings in dams *(affected dams/number of dams examined)*	No abnormal findings at study termination.				–

[1]Body weight gain corrected for weight of uterus.

Table A6.8.1B-5. **Table for Teratogenic effects: Litter response (Caesarean section data)**

Parameter	Control 0 mg/kg bw	Low dose 0.10 mg/kg bw	Medium dose 0.16 mg/kg bw	High dose 0.20 mg/kg bw	Dose-response + / –
Corpora lutea *total/number of dams*	12.4	15.0	15.3	16.5	–
Implantations *total/number of dams*	11.6	14.0	14.0	14.5	–
Resorptions (embryonic) *total/number of dams*	0.4	1.2	1.75	0.0	–
Resorptions (foetal) *total/number of dams*	0	0	0	0	–
Total number of foetuses	56	63	49	29	–
Pre-implantation loss *% of corpora lutea*	6.5	6.7	8.2	12.1	+
Post-implantation loss *% of implantation sites*	3.4	10.0	12.5	0.0	–
Number of litters	5	5	4	2	–
Foetuses / litter ***(total/litter)***	11.2	12.6	12.3	14.5	–
Live foetuses / litter *(total/litter)*	11.2	12.6	12.3	14.5	–
Dead foetuses / litter *(total/litter)*	0.0	0.0	0.0	0.0	–
Foetus weight *[g] (mean ± S.D.)*	5.0	4.9	4.7**	4.5**	+
Foetal sex ratio *[ratio m/f]*	**0.75**	**0.70**	**0.69**	**0.71**	–

**p < 0.01 (Dunnett t-test)

SECTION A6.8.2 **Annex Point IIA6.8**	**6.8.2 Fertlity Study** 6.8.2 Two Generations reproduction study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA [redacted], 2003, coumatetralyl - Waiver for chronic toxicity study in dogs, chronic/carcinogenicity toxicity study in rodents, and multigeneration reproductive toxicity study in rodents, MO-03-006927	Official use only
Other existing data [...]	**Technically not feasible [X]** **Scientifically unjustified [...]**	
Limited exposure [ ]	**Other justification [ ]**	x
Detailed justification:	A waiver for a multigeneration study with coumatetralyl anticoagulant rodenticide is scientifically justified based on: - the long half-life of the active compound would result in high body levels following the pre-mating period thus rendering the animals susceptible to death by haemorrhage from the natural events of reproduction and parturition, - technical difficulties, - the absence of reproduction risk based on developmental toxicity studies in rats and rabbits and the rat sub-chronic toxicity study,	x
	- the absence of potential long-term exposure of the public population, - the low risk of exposure in manufacturing and use, - the absence of residues in plant foodstuffs and water.	x
	The practical difficulties of long-term administration of coumatetralyl are such that an attempt at a study would be certain to fail and would be unethical and contrary to Directive 86/609/EEC.	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*March 2005*
Evaluation of applicant's justification	*In addition to "Technically not feasible", the justification also includes arguments of "Limited exposure" and "Other justification", namely animal welfare.* *The justification for non-conduction of the two-generation study include several valid arguments:* *- Repeated exposure from the premating period would result in high internal doses of coumatetralyl and consequent increased susceptibility to death by haemorrhage in relation to the events of reproduction and parturition.* *- Achievement of a sub MTD level at which a potential reproductive effect can be seen is technically difficult,* *- No effects on reproduction or developmental parameters were seen in the developmental toxicity studies in rats and rabbits or in the rat sub-chronic toxicity study.* *In addition, the waiver refers to WHO data showing no human developmental effects in humans from use of anticoagulant rodenticide. Therapeutic use of warfarin is reported in the waiver to have shown to induce malformations in humans. However differences in metabolism between coumatetralyl and warfarin support the evaluation of low developmental toxicity potential of coumatetralyl.* *- The potential long-term exposure of the public population is low as argued in the risk assessment document.* *- Residue levels in plant foodstuffs and water are expected to be negligible due to the physical-chemical properties and the use pattern of the product.* *- Animal welfare considerations.*
Conclusion	*Waiving of this study is accepted by RMS but will result in reference to exposure conditions in the Annex I listing of coumatetralyl, as the prerequisites for waiving is dependent upon the nature of the formulated product Racumin paste and the recommended use pattern instructions given on that specific fomulation.*
Remarks	*The risk of exposure in manufacturing is not well documented, as the waiver only mentions use of worker protection or antidote treatment in case of unacceptable exposure. Actual levels of exposure during manufacturing are not reported. However, it is not compulsory to address manufacturing in the risk assessment.*

SECTION A6.9 **Annex Point IIIAVI.1**	**6.9 Neurotoxicity study**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA [redacted], 2003, coumatetralyl - Waiver for chronic toxicity study in dogs, chronic/carcinogenicity toxicity study in rodents, and multigeneration reproductive toxicity study in rodents, MO-03-006927	Official use only
Other existing data [...]	**Technically not feasible [X]** **Scientifically unjustified [...]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Acute, sub-chronic and developmental toxicity studies conducted with coumatetralyl active ingredient (a.i.) or formulations in rats and rabbits show typical clinical signs of anticoagulant mainly including bleeding, pallor and signs of haemorrhages. Considering all these studies, no particular finding can be attributed to a neurotoxic origin. Moreover, a review of the data available for anticoagulant rodenticides shows that no neurotoxicity has been evidenced for this class of compounds (WHO, 1995). BCS concludes that there is no justification to further investigate coumatetralyl effects by conducting neurotoxicity studies as these would be unethical for animal welfare reasons.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*March 2005*	
Evaluation of applicant's justification	*The critical effect seen in acute, sub-chronic and developmental studies with coumatetralyl in experimental animals is the delayed clotting and consequent haemorrhages. No effect on the nervous system was reported in any of the available studies. However, no specific examination of neurological effects was performed in any of the studies available, and an effect may not have been discovered. The lack of neurotoxicity in other anticoagulant rodenticides increases the case that coumatetralyl is probably not a neurotoxicant. In addition to the arguments put forward in the waiver, information that no neurological effects were seen following therapeutical use of warfarin in humans lead to the evaluation that the neurotoxicological potential of coumatetralyl is negligible.*	
Conclusion	*Applicant's justification is acceptable, as the probability of a neurotoxic effect is negligible. Also, the argument of animal welfare is acceptable.*	

Remarks	*The referenced document by Lautraite (2003) does not specifically address the neurotoxicity of coumatetralyl. However data on neurotoxicity is only regarded as additional data initiated by indications that the substance has neurotoxic properties.*

Section A6.10 Annex Point IIIA VI.7	**6.10 Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Coumatetralyl mode of action and the associated effects are well known, therefore no mechanistic studies are needed.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Although the mode of action leading to the desired effects of anticoagulation in rodents in well described, the mechanism of action of the molecule is not known in details. Especially in the developing animal, the mode of action is not known. Therefore, read across from structural and mechanistically the similar compound warfarin is included in the evaluation of the developmental toxicity of coumatetralyl.*	
Conclusion	*The argumens ts of the justification cannot be endorsed. However, the non-performance of mechanistical studies is acceptable, as other data are available that can be used in the risk assessment of coumatetralyl.*	
Remarks		

Section A6.11 Annex Point IIIA III-0§	**6.11 Other routes of administration (parenteral routes)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	No studies by alternative routes are available and furthermore studies on parenteral routes are not relevant as coumatetralyl was found to be well absorbed from the gastrointestinal tract. Therefore performing new studies is scientifically not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Arguments agreed*	
Conclusion	*Justification accepted*	
Remarks		

SECTION A6.12 **6.12 Medical Data**

Annex Point IIA6.9

Subsection		Official use only
	6.12 MEDICAL DATA IN ANONYMOUS FORM	
6.12.1 Medical surveillance data on manufacturing plant personnel if available	Since 1992, occupational medical surveillance of up to 47 workers exposed to coumatetralyl was performed yearly to every five years (depending on the hazards) on a routine basis. This surveillance did not reveal any unwanted effects in these workers The examinations included clinical and technical examinations and the following laboratory parameters: blood count, hemogram, coagulogram, AST, ALT, □-GT, creatinine, cholesterol, blood sugar, urinalysis, urinary sediment, occult blood (faeces). Biological monitoring regarding coumatetralyl has not been performed. During the production period of more than 20 years up to the year 2002 no accidents with coumatetralyl were registered in the Medical Department and no consultations due to contact with coumatetralyl were required. See Human case report: 6.12.a Direct observation of clinical cases and poisoning incidents	

SECTION A6.12 **6.12 Medical Data**

Annex Point IIA6.9

6.12.2 Direct observation, e.g. clinical cases, poisoning incidents if available	The data about direct observations cover a time span from 1994 to 2003. In the above time frame, 35 complaints/adverse incidents from Germany concerning products containing coumatetralyl were brought to the sponsor's attention. Of these, 17 were requests for information, among these 7 attempted suicides, and two alleged poisoning by others. Information on the active ingredient, possible effects and antidote was provided. Nothing is known about the clinical course in these cases. In eight cases, the product had been swallowed accidentally (max. 40 g) without any symptoms occurring. Two further cases were symptomatic: an adult showed a "Quick value" of 30% (slight coagulation inhibition) following oral absorption of two teaspoons of Racumin Plus and a child having ingested an unknown amount and required vitamin K1 therapy. No sequelae occurred. In seven cases, contact with eyes or skin had occurred, either by accident or by lack of personal protective equipment (e.g. distributing the powder with bare hands). In five cases, symptoms were reported. Three affected persons showed short-term nausea, one showed swelling and itching of the hands after washing, and in one subject irritation of the eyes occurred. The irritation is regarded as due to the product (definite correlation), the skin reaction as possible, and the nausea as unlikely but it is most probably a secondary reaction to the fear of being poisoned. See Human case report: 6.12.b Direct observation of clinical cases and poisoning incidents.
6.12.3 Health records, both from industry and any other available sources	There are no health records from industry or other sources available.
6.12.4 Epidemiological studies on the general population, if available	Epidemiological studies are not available.
6.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available	As coumatetralyl is a coagulation inhibitor (vitamin K1 antagonist) in cases of human poisoning coagulation problems can occur. The prothrombin time („Quick-Test") may be prolonged. However, a single ingestion of a commercial product is unlikely to produce severe effects on coagulation. Accordingly a search of TOXLINE and MEDLINE (key words: coumatetralyl/coumatetralyl poisoning) did not reveal published cases of human poisonings. See Human case report: 6.12.b Direct observation of clinical cases and poisoning incidents.

SECTION A6.12 **6.12 Medical Data**

Annex Point IIA6.9

6.12.6	Sensitisation/allergenicity observations, if available	Coumatetralyl is not known to be a human sensitizer. However, one allergic reaction with swelling and itching of the hands was reported. As only one case of skin reaction of seven dermal contact cases occurred, it is unlikely that only coumatetralyl caused the reaction. See Human case report: 6.12.b Direct observation of clinical cases and poisoning incidents.
6.12.7	Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known	As coumatetralyl is a vitamin K1 antagonist, the antidote of choice is vitamin K1. However, it should only be administered, if the prothrombin time is prolonged and slight bleeding is observed. In cases of prolonged prothrombin time without bleeding only observation of the patient is recommended. A dosage suggestion for vitamin K1 from literature would be 0.25-0.5 mg/kg body weight every 24 hours orally, subcutaneously or intravenously. In the unlikely case of severe bleeding the application of human coagulation factors (PPSB) may be required. In case of severe blood loss with hypovolemia or hemorrhagic shock, fresh frozen plasma (FFP) or fresh plasma must be given and transfusion should be considered (Steffens, 2003). See Human case report: 6.12.b Direct observation of clinical cases and poisoning incidents.
6.12.8	Prognosis following poisoning	The prognosis of single dose ingestion of coumatetralyl is good. Sequelae are not expected to occur (Steffens, 2003). See Human case report: 6.12.b Direct observation of clinical cases and poisoning incidents.

SECTION A6.12	**6.12 Medical Data**
Annex Point IIA6.12	6.12.a Direct observation of clinical cases and poisoning incidents

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]: Occupational Medical Experiences with 4- Hydroxy-3-(1,2,3,4- tetrahydro-L-naphtalenyl)-2H-L- benzopyran-2-one (Racumin). Bayer CropScience, 2003-08-01 (unpublished). MO-04-002569	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Substance**	Chemical name: 4-Hydroxy-3-(1,2,3,4-tetrahydro-l-naphtalenyl)-2H-l-benzopyran-2-one Trade name: Racumin Physical state: solid, powder Processing plant: [redacted] Production period: More than 20 years, until 2002 Amount produced: up to 10 t/a	
3.2	**Persons exposed**		
3.2.1	Sex	–	
3.2.2	Age/weight	–	
3.2.3	Known Diseases	–	
3.2.4	Number of persons	~ 47	
3.2.5	Other information	–	
3.3	**Exposure**	–	
3.3.1	Reason of exposure	Occupational	
3.3.2	Frequency of exposure	–	
3.3.3	Overall time period of exposure	–	
3.3.4	Duration of single exposure	–	
3.3.5	Exposure concentration/dose	–	
3.3.6	Other information	–	

SECTION A6.12	**6.12 Medical Data**
Annex Point IIA6.12	6.12.a Direct observation of clinical cases and poisoning incidents
3.4 Examinations	Laboratory examinations: blood count, haemogram, coagulogram, AST, ALT, □-GT, creatinine, cholesterol, blood sugar, urinalysis,urinary sediment, occult blood (faeces) Technical examinations: audiogram, ECG/ ergometry, visual acuity testing, spirography, chest X- ray
3.5 Treatment	–
3.6 Remarks	–
	4 RESULTS
4.1 Clinical Signs	No problems related to production or handling of coumatetralyl were reported.
4.2 Results of examinations	–
4.3 Effectivity of medical treatment	-
4.4 Outcome	–
4.5 Other	–
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	Occupational medical surveillance of workers exposed to Racumin, was performed regularly (yearly to every five years, depending on the hazards) on a routine basis, not directly related to exposures. The examinations included the laboratory parameters, clinical and technical examinations listed above. Biological monitoring regarding Racumin has not been performed.
5.2 Results and discussion	During the production period of more than 20 years up to the year 2002 no accidents with Racumin were registered in the Medical Department and no consultations due to work or contact with Racumin were required. The routine examinations did not reveal any adverse effects in the workers.
5.3 Conclusion	No problems related to handling or production of coumatetralyl were reported.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*Applicant's version adopted.*
Results and discussion	*Applicant's version adopted.*

SECTION A6.12	**6.12 Medical Data**
Annex Point IIA6.12	6.12.a Direct observation of clinical cases and poisoning incidents
Conclusion	*Applicant's version adopted.*
Remarks	

SECTION A6.12	**6.12 Medical Data**
Annex Point IIA6.12	6.12.b Direct observation of clinical cases and poisoning incidents

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]: Clinical Toxicology experience with coumatetralyl. [redacted], Bayer AG, 2003-07-25 (unpublished). MO-04-002564	
		2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)	
		3 MATERIALS AND METHODS	
3.1	**Substance**	Chemical name: 4-Hydroxy-3-(1,2,3,4- Tetrahydro-l-naphtalenyl)-2H-l-8 Benzopyran-2-one Trade name: Racumin Physical state: solid, powder	
3.2	**Persons exposed**		
3.2.1	Sex	–	
3.2.2	Age/weight	–	
3.2.3	Known Diseases	–	
3.2.4	Number of persons	35	
3.2.5	Other information	–	
3.3	**Exposure**	Oral (19 cases), dermal/mucosal (7), inhalation (1)	
3.3.1	Reason of exposure	Accidental; suicidal intent; poisoning by others; lack of protective equipment.	
3.3.2	Frequency of exposure	Single	
3.3.3	Overall time period of exposure	–	
3.3.4	Duration of single exposure	–	
3.3.5	Exposure concentration/dose	A maximum dose of ~40 g Racumin (~ 14 mg active ingredient) was estimated in one case of accidental ingestion.	
3.3.6	*Other information*	–	
3.4	**Examinations**	–	
3.5	**Treatment**	One child required Vitamin K1 therapy after ingestion of an unknown amount of Racumin.	

SECTION A6.12	**6.12 Medical Data**
Annex Point IIA6.12	6.12.b Direct observation of clinical cases and poisoning incidents
3.6 Remarks	–
	4 RESULTS
4.1 Clinical Signs	Reduced prothrombin time (Quick test, one case) Skin irritation (one case) Swelling and itching of the hands after washing (one case) Short-term nausea (three cases)
4.2 Results of examinations	–
4.3 Effectivity of medical treatment	No sequelae were reported after vitamin K1 therapy of one child that ingested coumatetralyl.
4.4 Outcome	–
4.5 Other	–
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	Between 1994 and 2003, 35 complaints/adverse incidents from Germany concerning products containing coumatetralyl have been brought to the attention of Bayer AG Clinical Toxicology and Product Safety.
5.2 Results and discussion	Of these 35 cases, 17 were requests for information, among these 7 attempted suicides, and two alleged poisoning attempts by others. Information on the active ingredient, possible effects and antidote was provided. Nothing is known about the clinical course in these cases. In 8 cases the product had been swallowed accidentally (max. 40 g) without any symptoms occurring. Two further cases were symptomatic: an adult showed a Quick value of 30% (slight coagulation inhibition) following oral absorption of two teaspoons of Racumin Plus and a child having ingested an unknown amount required vitamin K1 therapy. No sequelae occurred. In 7 cases, dermal or mucosal contact had occurred, either by accident or by lack of personal protective equipment (e.g. distributing the powder with bare hands). In 5 cases symptoms were reported (1 irritation of the eyes, 1 swelling and itching of the hands after washing, 3 short-term nausea).

The irritation is regarded as due to the product (definite correlation), the skin reaction as possible, and the nausea as unlikely result of coumatetralyl exposure, but most probably an anxiety reaction to the awareness of contact with a rodenticide.

In one case, the powder had been inhaled accidentally without symptoms occurring.

5.3 Conclusion –

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*February 2005*
Materials and Methods	*Applicant's version adopted* *Comment: In section 6.12.5 "Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available" it is mentioned that a search in the databases TOXLINE and MEDLINE did not reveal published cases of human poisoning. The search strategy is not evident, and other databases should have been consulted in order to make the data search comprehensive (e.g. EMBASE and HSDB).* *The applicant has subsequently sent in the following information on the bibliographic databases consulted: Embase, HCA, Medline, Biosis, Toxcenter, toxline, CABA, Ulidat, ESbiobase, IPA and Chemical Abstracts and explained that several keywords were combined including Coumatetralyl, human data, toxicity, poisoning, rodenticide.* *However, the results of these searches are not detailed.* *The review by WHO from 1995 (Environmental Health Criteria, 175. Anticoagulant Rodenticides) does not reveal any cases of human poisoning to coumatetralyl.*
Results and discussion	*Applicant's version adopted.*
Conclusion	*Applicant's version adopted.*
Remarks	

Section A6.12.3 Annex Point IIA VI.6.9.3	6.12.3 Health records, both from industry and any other available sources	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	There are no health records from industry or other sources available.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The statement that no health records have been taken in relation to the use of a compound as toxic as coumatetralyl is worrying.*	
Conclusion	*The justification is not acceptable. Data from work places must be provided at the latest for national registration.*	
Remarks		

Section A6.12.4 Annex Point IIA VI.6.9.4	6.12.4 Epidemiological studies on the general population, if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	Epidemiological studies on the general population are not available.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*No comments*	
Conclusion	*Accepted*	
Remarks		

Section A6.13 Annex Point IIIA VI.2	**6.13 Toxic effects on livestock and pets**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Coumatetralyl will not be used in places in which animals are housed, kept or transported nor exposure is possible via drinking water or feedingstuffs. Therefore, additional data considering toxic effects in livestock and pets is not needed.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2009*	
Evaluation of applicant's justification	*Although coumatetralyl may not specifically intended to be used in stables, exposure to rodenticides of livestock and pets cannot be excluded, e.g. in control of rodents in farms. The risk assessment will have to be restricted to formulations will be unaccessible to livestock and pets. Some information on the potential effects on livestock and pets can be extrapolated from the available data on laboratory animals.*	
Conclusion	*Justification accepted, but some information should be made available at product authorisation stage.*	
Remarks		

Section A6.14 Annex Point IIIA III-XI.2	**6.14 Other test(s) related to the exposure of humans**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Humans are not significantly exposed to neither degradation products, by-products, reaction products when Racumin® paste is used as recommended on its labels. Therefore other tests are considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed*	
Conclusion	*Accepted*	
Remarks		

. Section A6.15 Annex Point IIIA VI.4	**6.15 Food and feedingstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Coumatetralyl will not be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feeding stuff for livestock is prepared, consumed or stored. Therefore, additional tests are considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed*	
Conclusion	*Accepted*	
Remarks		

. Section A6.15 Annex Point IIIA VI.4	6.15.1 Food and feedingstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...] Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Coumatetralyl will not be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feeding stuff for livestock is prepared, consumed or stored. Therefore, additional tests are considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*Agreed*	
Conclusion	*Accepted*	
Remarks		

Section A6.15.1 Annex Point IIIA XI.1.1, 1.3, 1.6	6.15.1 Identification of the residues, degradation and reaction products and of metabolites in contaminated foods or feedingstuffs Identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. No residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs. Therefore, identification of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs are considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Section A6.15.2 Annex Point IIIA XI. 1.2, 1.3, 1.5. and 1.6	6.15.2 Behaviour of residues, degradation and reaction products and metabolites on treated or contaminated food or feeding stuffs Behaviour of the residues of the active substance, its degradation and reaction products and, where relevant, its metabolites on the treated or contaminated food or feeding stuffs including the kinetics of disappearance	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...] Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. No residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs. Therefore, behaviour of the residues of the active substance, its degradation and reaction products and, where relevant, its metabolites on the treated or contaminated food or feeding stuffs including the kinetics of disappearance is considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Section A6.15.3 Annex Point IIIA XI. 1.4	6.15.3 Exposure to humans or animals through food and feeding stuffs **Estimation of potential or actual exposure of the active substance to humans or animals through food and feeding stuffs and other means**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...] Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. No residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs. Therefore, estimation of potential or actual exposure of the active substance to humans or animals through food and feeding stuffs and other means is considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Section A6.15.4 Annex Point IIIA XI. 1.7	6.15.4 Proposed acceptable residues and the justification of their acceptability	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. Therefore no residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Section A6.15.5 Annex Point IIIA XI. 1.8	6.15.5 Any other available information that is relevant	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. Therefore no residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Section A6.15.6 Annex Point IIIA XI. 1.9	6.15.6 Summary and evaluation of data submitted under point 6.15	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	The active substance is not used in a manner which may cause contact with food or feedstuffs. Under normal use conditions the biocidal product (Racumin® Paste in a paper sachet) is placed in bait boxes or comparable equipments or in inaccessible rats "feeding places". Because of this use pattern no contact of coumatetralyl with food or feedstuffs and other products is possible. Therefore no residues, degradation and reaction products and metabolites of coumatetralyl could contaminate foods or feeding stuffs.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

. Section A6.16 Annex Point IIIA VI.3.5, XI.2	**6.16 Any other tests related to the exposure of the active substance to humans,** in its proposed biocidal products, that are considered necessary may be required	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	**Technically not feasible [...]** **Scientifically unjustified [...]**	
Limited exposure [...]	**Other justification [x]**	
Detailed justification:	Coumatetralyl will not be used in preparations for use where food for human consumption is prepared, consumed or stored, or where feeding stuff for livestock is prepared, consumed or stored. Therefore, additional tests are considered to be not necessary.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

. Section A6.17 Annex Point IIIA VI.6	**6.17 toxic effects from metabolites on treated plants** If the active substance is to be used in products for action against plants then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals shall be required	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [...]**	**Technically not feasible [...] Scientifically unjustified [...]** **Other justification [x]**	
Detailed justification:	Coumatetralyl will not be used in products for action against plants or in a way where it would come in contact with plants. Therefore no further studies are required.	
Undertaking of intended data submission [ ]		
	EVALUATION BY COMPETENT AUTHORITIES	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*29 April 2008*	
Evaluation of applicant's justification	*The RMS agrees with applicant in his arguments, however, these are related to the product Racumin Paste and not to other potential produt with different use pattern. Data on residues and reaction products may be relevant in risk assessment of other coumatetralyl products.*	
Conclusion	*Accepted for this CA report*	
Remarks	*Data may be relevant at produt authorisation stage*	

Document III-A

Study summaries, active substance

COUMATETRALYL

SECTION A7

CAS No. 5836-29-3

From Bayer Environmental Science

For use in Rodenticides (Product type 14)

Rapporteur Member State: Denmark

September 2005

SECTION A7.1.1.1.1 **HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS**

Annex Point IIA7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	R. Wilmes, 1983, Fate/Behaviour of Crop Protection Products in Water, Bayer AG, Report No. WLF HA 027 (unpublished), 1983-03-23, MO-03-002794	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active ingredient for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Method "Hydrolysis as a function of pH" (OECD guideline No. 111)	
2.1	**GLP**	No, GLP was not compulsory at the time the study was performed	
2.1	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Coumatetralyl (Racumin), analytical standard	
3.1.1	Lot/Batch number	No data	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99,8 % (Analytical standard)	
3.1.4	Further relevant properties	The following properties are given in the report: Volatility from water: 9.8 x 10^9 Solubility in water: 10 mg/l at 20 °C; Vapour pressure: 8.5 x 10^{-11} mbar at 20 °C (extrapolated value)	X
3.2	**Reference substance**		
3.2.1	Initial concentration of reference substance	No data	

3.3 Test solution	Solutions of coumatetralyl in aqua bidest. (active ingredient concentration: 2 – 2.5 µg/ml) were diluted with 0.1 M buffer solutions pH 4: citric acid/sodium hydroxide; pH 7: potassium dihydrogen phosphate/sodium hydroxide; pH 9: boric acid/sodium hydroxide) in a ratio of 1:1 (v/v). Three samples per pH were introduced into glass ampoules, the ampoules were sealed, and the samples were either incubated at 55 °C or kept in a refrigerator at 5 °C for five da ys.
3.4 Testing procedure	
3.4.1 Test system	Samples were introduced into glass ampoules and after sealing them they were either incubated at 55 °C or kept in a refrigerator at 5 °C. The system used for determining the concentrations of the test substance in the samples was a Hewlett Packard 1084 B high performance liquid chromatograph.
3.4.2 Temperature	Three samples per pH were either incubated at 55 °C or kept in a refrigerator at 5 °C.
3.4.3 pH	pH 4, pH 7 and pH 9
3.4.4 Duration of the test	Five days
3.4.5 Number of replicates	Three samples per pH were tested
3.4.6 Sampling	The samples incubated at 55 °C and the samples kept in a refrigerator were analysed for the concentration of active ingredient five days after the beginning of the test.
3.4.7 Analytical methods	The concentrations of coumatetralyl were determined using reversed phase HPLC under the following conditions: Column: LiChrosorb RP 18, 5 µm, 250 mm; Mobile phase: 40 % aqueous KCl solution (1 g/l) adjusted with HCl to pH 3 and 60 % acetonitrile ; Flow rate: 1.2 ml/min; Injection volume: 20 µl; Limit of determination: 150 µg/l; Recovery: 100 % (direct analysis of buffer solutions)
3.5 Preliminary test	Yes, see the data given above
	4 RESULTS
4.1 Concentration and hydrolysis values	Concentrations of the active ingredient in the samples after five days at the different pH values see table A7.1.1.1.1-1.
4.2 Hydrolysis rate	No hydrolysis rate constants assignable.

SECTION A7.1.1.1.1 Annex Point IIA7.6.2.1	HYDROLYSIS AS A FUNCTION OF pH AND IDENTIFICATION OF BREAKDOWN PRODUCTS
constant (k_h)	
4.3 Dissipation time	The half-lives (DT_{50}) at 22 °C for pH 4, pH 7 and pH 9 are >> 1 year
4.4 Concentration – time data	Active ingredient concentration: 2 – 2.5 µg/ml; Concentrations of the active ingredient in the samples after five days at the different pH values see table A7.1.1.1.1-1.
4.5 Specification of the transformation products	No transformation products.
	5 APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	The study was performed according to the OECD Method "Hydrolysis as a function of pH" at pH 4, pH 7, and pH 9 in citrate, phosphate and borate buffer solutions in order to determine the hydrolytic stability of the test substance in water. Solutions of coumatetralyl in aqua bidest were diluted with buffer solutions in a ratio of 1:1. Samples of these solutions were either incubated at 55 °C or kept in a refrigerator at 5 ° C for five days.
5.2 Results and discussion	The active ingredient content of the samples incubated at 55 °C was not observed to have changed, as compared with the refrigerated samples. The half-lives at 22 °C for pH 4, pH 7 and pH 9 are therefore >> 1 year.
5.2.1 kh	No hydrolysis rate constants assignable.
5.2.2 DT50	pH 4 >> 1 year at 22 °C; pH 7 >> 1 year at 22 °C; pH 9 >> 1 year at 22 °C
5.2.3 r2	-
5.4 Conclusion	In accordance with the OECD guideline (degradation after 5 days at 50 °C < 10 percent, corresponding to a half -life at 25 °C > 1 year), coumatetralyl is stable to hydrolysis.
5.4.1 Reliability	2-3

SECTION A7.1.1.1.1 **HYDROLYSIS AS A FUNCTION OF PH AND IDENTIFICATION OF BREAKDOWN PRODUCTS**

Annex Point IIA7.6.2.1

5.4.2 Deficiencies	Yes, no data about lot or batch number; Information incomplete about the test system (incubation equipment, method of sterilisation, measures for avoiding photolytic effects and to exclude oxygen); no result of analytical measurement at day 0 of the test but measurement of the sample kept at 5°C after 5 days (assuming no degradation)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2004.08.16
Materials and Methods	**Comment:** The water solubility (**see 3.1.4**) is stated to be much lower than that given in section A3 (**3.5**). However, even the low solubility quoted is sufficient for the test to be run.
Results and discussion	**Comment:** In the printed version, the results of the tests are not included in **Table A7.1.1.1.1-1**. However, they are visible in the electronic version. Adopted
Conclusion	Adopted
Reliability	2 (not GLP)
Acceptability	Acceptable
Remarks	no data about lot or batch number; Information incomplete about the test system (incubation equipment, method of sterilisation, measures for avoiding photolytic effects and to exclude oxygen); no result of analytical measurement at day 0 of the test but measurement of the sample kept at 5°C after 5 days (assuming no degradation)

Table A7.1.1.1.1-1: Concentration of active ingredient in relation to duration of the study and pH

[µg/ml]

Duration of study [days]	Citrate buffer pH 4		Phosphate buffer pH 7		Borate buffer pH 9	
	55 °C	5 °C	55 °C	5 °C	55 °C	5 °C
5	2.0	2.0	2.1	2.1	2.5	2.5