Section A6.6.2 **Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (2)**

Annex Point IIA6.6 UDS-Test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/08/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.3
Annex Point IIA6.6

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (1)

HPRT assay

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1996b *[Monograph: 1996a]*): YRC 2894 - Mutagenicity study for the detection of induced forward mutations in the V79-HPRT assay in vitro [redacted] Report No. 25163, date: 1996-06-13.

PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.1 In vitro assays. B.6.4.1.3 Mammalian cell mutagenicity study

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes;

OECD guideline 476; US-EPA FIFRA PB 84-233295; Directive 88/302/EEC method B.17

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 V79-HPRT assay, YRC 2894 (thiacloprid) (purity: [redacted] dissolved in DMSO) was tested in V79 cell cultures at six concentrations of up to 500 µg/ml in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The maximum concentration used was stated to be the limit of solubility. Cells were exposed to YRC 2894 for 5 hours and cultured for an additional 4 and 7 days before sub-culturing in selective medium.

4 RESULTS AND DISCUSSION

No cytotoxicity was observed at the concentrations used in this study.

No reproducible biological or statistically significant increase in the mutation frequency was seen at any concentration. Appropriate positive controls (ethanemethanesulfonate and dimethylbenzanthracene) significantly increased the mutation frequency. Results were confirmed in independently repeated assays.

5 CONCLUSION

5.1 Conclusion YRC 2894 was considered to be non-mutagenic in the V79-HPRT Forward Mutation assay with and without metabolic activation.

[redacted]

5.1.1 Reliability [redacted]

Section A6.6.3

Annex Point IIA6.6

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (1)

HPRT assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/08/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.4 Genotoxicity studies - In-vivo mutagenicity study

Annex Point IIA6.6 Micronucleus test

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1995b *[Monograph: 1995c]*): YRC 2894 - Micronucleus test on the mouse [redacted] Report No. 24515, date: 1995-11-24. *PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.2 In vivo studies. B.6.4.2.1 Micronucleus study in the mouse*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; OECD guideline 474; US-EPA FIFRA, PB 84-233295; Directive 92/69/EEC method B.12
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1995 study, NMRI mice (5/sex/group) were administered YRC 2894 (thiacloprid) (purity: [redacted]) suspended in 0.5% aqueous Cremophoremulsion by intraperitoneal injection. Femoral bone marrow was prepared 16, 24 and 48 hours after administration as detailed below, 1000 polychromatic erythrocytes per animal were scored for micronuclei (see Table A6_6_4-1).

4 RESULTS AND DISCUSSION

Clinical signs of toxicity (rough fur, apathy, spasms and difficult breathing) were observed in YRC 2894-treated animals. Three of the animals died due to the acute intraperitonal toxicity of YRC 2894. The ratio of polychromatic to normochromatic erythrocytes was not affected by treatment with YRC 2894. No biologically or statistically significant increases in the number of micronucleated polychromatic erythrocytes were seen in YRC 2894 treated mice. Clear evidence of clastogenicity was seen with the positive control (cyclophosphamide).

5 CONCLUSION

5.1	**Conclusion**	This study did not reveal evidence of a clastogenic effect of YRC 2894 in an invivo system in mice.
		[redacted]
5.1.1	Reliability	[redacted]

Section A6.6.4 Genotoxicity studies - In-vivo mutagenicity study

Annex Point IIA6.6 Micronucleus test

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16/08/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_6_4-1 Study design

Group	Dose Level	Route of Administration	Sacrifice
Vehicle	---	Intra-peritoneal injection	24 h
YRC 2894 (thiacloprid)	60 mg/kg bw	Intra-peritoneal injection	16 h
			24 h
			48 h
			Replacement group
Cyclophosphamide	20 mg/kg bw	Intra-peritoneal injection	24 hours

Section 6.6.5 - 6.6.6 Annex Point IIA 6.6.5-6.6.6.	In vivo studies on mutagenicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	According to BPD, Annex IIA, in vivo studies on mutagenicity are only required if there would be a positive in vitro test on mutagenicity. Thiacloprid was not positive in any of the required mutagenicity tests. Therefore no in vivo test must be supplied.	
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	16/08/2006	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (1)

M34

S. typhimurium, Reverse mutation assay

Official use only

1 REFERENCE

1.1 Reference

Herbold, B. (2003a *[Addendum III: 2003b]*): YRC 2894-sulfonic acid amide. Salmonella/mircrosome test, plate incorporation and preincubation method. Unpublished Bayer Report No. T8063351/AT00750 (study date: October 2003).

Addendum III to PPP-Monograph; APPENDIX I: 2. Additional in vitro genotoxicity studies submitted for YRC 2894-Sulfonic acid Na-Salt (M30) and YRC 2894- Sulfonic acid amide (M34) – a)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 471 (adopted July 1997).

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

YRC 2894 (thiacloprid) - sulfonic acid amide ([redacted] in deionised water was evaluated in a bacterial mutagenicity assay over a range of concentrations using five strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 98, TA 100 & TA 102). The test material was assayed in the presence and in the absence of S9-mix prepared from the livers of Aroclor 1254-induced male Sprague-Dawley (SD) rats. The first trial used the standard plate incorporation protocol, over a dose range of 0 (vehicle) to 5000 µg per plate. The repeat trial was conducted using a pre-incubation protocol (preincubation period 20 minutes at 37°C). The incubation period for each experiment was 48 hours (at 37°C). Three plates were used for each experimental point. For each experiment, positive control substances were tested to validate the bacterial strain and to confirm the activity of the S9-mix used.

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (1)

M34

S. typhimurium, Reverse mutation assay

4 RESULTS AND DISCUSSION

Concentrations up to 158 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher concentrations, the test material only had a weak strain-specific bacteriotoxic effect and they could be used for assessment purposes. Precipitation was observed at 5000 µg per plate. None of the five strains exhibited an increase in mutant counts over those of the negative controls with or without metabolic activation. Appropriate results were observed with the vehicle and positive control substances.

5 CONCLUSION

5.1 Conclusion

The test material (metabolite M34) was not mutagenic in the presence or absence of metabolic activation in both the standard plate incorporation assay and the preincubation assay.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (1)

M34

S. typhimurium, Reverse mutation assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (2)

M02

S. typhimurium, Ames Test

Official use only

1 REFERENCE

1.1 Reference

Herbold, B. (1995c *[Monograph: 1995d]*): KKO 2254 - Salmonella/microsome test plate incorporation and preincubation method. Bayer AG, Report No. 24444, date: 1995-11-07.

PPP-Monograph Chapter: B.6.8 Other toxicological studies, B.6.8.1 Toxicity of metabolites – b) Genotoxicity (Study 1)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 471; US-EPA FIFRA PB 84-233295; Directive 92/69/EEC method B.14

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1995 study, the potential mutagenicity of M02 (purity: [redacted]; dissolved in DMSO) was investigated in S. typhimurium (TA 1535, 1537, TA 98, TA 100 and TA 102). Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The initial test was performed in triplicate using the plate incorporation procedure.

4 RESULTS AND DISCUSSION

No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. No bacteriotoxicity was observed. Appropriate positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene) produced significant increases in revertant colonies. The results were confirmed in a second independent assay using a modified preincubation (20 mins) salmonella/microsome test.

Section A6.6.7
Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (2)
M02
S. typhimurium, Ames Test

5 CONCLUSION

5.1 Conclusion

The test substance (metabolite M02) was considered to be non-mutagenic in this assay with and without metabolic activation in the plate incorporation as well as in the preincubation modification of the salmonella/microsome test.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (2)

M02

S. typhimurium, Ames Test

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (3)

M30

S. typhimurium, Ames Test

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1995d *[Monograph: 1995e]* [redacted]
Salmonella/microsome test plate incorporation preincubation method.
[redacted] Report No. 24454, date: 1995-11-08.

PPP-Monograph Chapter: B.6.8 Other toxicological studies, B.6.8.1 Toxicity of metabolites – b) Genotoxicity (Study 2)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes;

OECD guideline 471; US-EPA FIFRA PB 84-233295; Directive 92/69/EEC method B.14

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1995 study, the potential mutagenicity of M30 (purity: [redacted]; dissolved in DMSO) was investigated in S. typhimurium (TA 1535, 1537, TA 98, TA 100 and TA 102). Six concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). The initial test was performed in triplicate using the plate incorporation procedure.

4 RESULTS AND DISCUSSION

No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. No bacteriotoxicity was observed. Appropriate positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene) produced significant increases in revertant colonies. The results were confirmed in a second independent assay using a modified preincubation (20 mins) salmonella/microsome test.

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (3)

M30

S. typhimurium, Ames Test

5 CONCLUSION

5.1 Conclusion

WAK 6999 was considered to be non-mutagenic in this assay with and without metabolic activation in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.7.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation study in bacteria (3)

M30

S. typhimurium, Ames Test

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (4)

M30

Chromosome aberration test

Official use only

1 REFERENCE

1.1 Reference

[redacted]2003b *[Addendum II: 2003a]*): YRC 2894-sulfonic acid Na-salt. In vitro chromosome aberration test with Chinese hamster V79 cells. Unpublished[redacted]Report No. T3063329/ AT00745 (study date: August 2003 to October 2003).

Addendum II to PPP-Monograph; APPENDIX I: Additional in vitro genotoxicity studies submitted for YRC 2894-Sulfonic acid Na-Salt (M30) and YRC 2894- Sulfonic acid amide (M34) – 1. In vitro genotoxicity of YRC 2894-sulfonic acid Na-salt (M30) – b)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 473 (adopted July 1997).

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

The clastogenic potential of YRC 2894 (thiacloprid)-sulfonic acid Na-salt ([redacted]) was evaluated in an *in vitro* chromosome aberration test. Chinese hamster V79 cell cultures were exposed to the test substance at concentrations of 800, 1600 and 3200 µg/ml for 4 hours in the absence and presence of metabolic activation. The test material was dissolved in deionised water (soluble up to 80 mg/ml). The metabolic activation or S9-mix was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. Cytotoxicity was assessed pre-test and in the main study (mitotic and survival indices). Concentrations up to 3200 µg/ml did not change the pH in the medium but the osmolarity was slightly increased at 3200 µg/ml.

Cultures at all concentrations were harvested at 18 hours post-treatment. In addition, the cells treated with 3200 µg/ml were harvested at 30 hours post-treatment. An additional test was performed (without metabolic activation); the cells were exposed for 18 hours to 800, 1600 and 3200 µg/ml and then harvested. Colcemid was added two hours prior to the end of incubation to arrest the cells in metaphase. The cells were fixed, mounted on slides and stained (two slides per culture). The mitotic index was determined by counting a total of 1000 cells per culture. Approximately 200 metaphases per concentration were examined.

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (4)

M30

Chromosome aberration test

4 RESULTS AND DISCUSSION

The test substance was tested up to the limit of solubility in the solvent. Precipitation was not observed in the medium. Altered cell morphology was observed at 3200 µg/ml in the absence of metabolic activation. The mitotic indices were reduced at 3200 µg/ml after 18 hours of treatment without metabolic activation. There were no effects on the survival indices. The test material did not induce an increase in the number of metaphases with aberrations. A statistically significant value was observed for the highest concentration at 4 hours with in the presence of S-9 mix but was within the historical control values for this laboratory. Appropriate results were observed with the vehicle and positive control substances.

5 CONCLUSION

5.1 Conclusion

There was no evidence that the test material (metabolite M34) increased the numbers of aberrant metaphases in the absence or presence of metabolic activation.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (4)

M30

Chromosome aberration test

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (5)

M34

Chromosome aberration test

Official use only

1 REFERENCE

1.1 Reference

Herbold, B. (2003c *[Addendum II: 2003d]*): YRC 2894-sulfonic acid amide. In vitro chromosome aberration test with Chinese hamster V79 cells. Unpublished Bayer Report No. T9063352/AT00747 (study dates: July 2003-October 2003).

Addendum II to PPP-Monograph; APPENDIX I: Additional in vitro genotoxicity studies submitted for YRC 2894-Sulfonic acid Na-Salt (M30) and YRC 2894- Sulfonic acid amide (M34) – 2. In vitro genotoxicity of YRC 2894-sulfonic acid amide (M34) – c)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 473 (adopted July 1997).

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

The clastogenic potential of YRC 2894 (thiacloprid)- sulfonic acid amide was evaluated in an *in vitro* chromosome aberration test. Chinese hamster V79 cell cultures were exposed to the test substance at concentrations of 250, 500, 1000, 2000 and 4000 µg/ml for 4 hours in the absence and presence of metabolic activation. The test material was dissolved in deionised water (soluble up to 100 mg/ml). The metabolic activation or S9-mix was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. Cytotoxicity was assessed pre-test and in the main study (mitotic and survival indices). Concentrations up to 500 µg/ml did not change the pH in the medium and the osmolarity was not changed by concentrations up to 4000 µg/ml.

Cultures at all concentrations were harvested at 18 hours post-treatment. In addition, the cells treated with 1000, 2000 and 4000µg/ml were harvested at 30 hours post-treatment. An additional test was performed (without metabolic activation); the cells were exposed for 18 hours to 1000, 2000 and 4000µg/ml and then harvested. Colcemid was added two hours prior to the end of incubation to arrest the cells in metaphase. The cells were fixed, mounted on slides and stained (two slides per culture). The mitotic index was determined by counting a total of 1000 cells per culture. Approximately 200 metaphases per concentration were examined.

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (5)

M34

Chromosome aberration test

4 RESULTS AND DISCUSSION

The test substance was tested up to the limit of solubility in the solvent. Precipitation was not observed in the medium. Changes in pH were observed at 1000 µg/ml and above. There were no effects on the mitotic or survival indices. The test material did not induce an increase in the number of metaphases with aberrations. Appropriate results were observed with the vehicle and positive control substances.

5 CONCLUSION

5.1 Conclusion

There was no evidence that the test material (metabolite M34) increased the numbers of aberrant metaphases in the absence or presence of metabolic activation.

[redacted]

5.1.1 Reliability [redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (5)

M34

Chromosome aberration test

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (6)

M30

HPRT assay

Official use only

1 REFERENCE

1.1 Reference

Herbold B.(2003d *[Addendum II: 2003]*): YRC 2894-sulfonic acid Na-salt. V79/HPRT-Test in vitro for the detection of induced forward mutations. Unpublished Bayer Report No. T2063328/AT00744 (study date: July 2003 to October 2003).

Addendum II to PPP-Monograph; APPENDIX I: Additional in vitro genotoxicity studies submitted for YRC 2894-Sulfonic acid Na-Salt (M30) and YRC 2894- Sulfonic acid amide (M34) – 1. In vitro genotoxicity of YRC 2894-sulfonic acid Na-salt (M30) – a)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 476 (adopted July 1997).

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

YRC 2894 (thiacloprid)-sulfonic acid Na-salt ([redacted]) was evaluated for point mutations at the HPRT locus in V79 cell cultures in the absence and presence of metabolic activation. The S9-mix (metabolic activation) was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The test material was dissolved in deionised water (soluble up to 83 mg/ml) and tested at concentrations ranging from 100 µg/ml to 3200 µg/ml (limit of solubility under culture conditions). Two independent trials were performed. Vehicle and positive control substances were tested. Preliminary cytotoxicity tests were performed with concentrations of 12.5-3200 µg/ml (relative cloning efficiency).

4 RESULTS AND DISCUSSION

4.1 Deviations

The test substance was tested up to the limit of solubility in the solvent. Precipitation was not observed in the medium. There were no decreases in survival to treatment or in relative population growth. There were no biologically relevant increases in mutant frequencies above that of the vehicle controls. Appropriate results were observed with the vehicle and positive control substances.

Section A6.6.7
Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (6)
M30
HPRT assay

5 CONCLUSION

5.1 Conclusion

The test material (metabolite M30) was not mutagenic in the V79/HPRT assay in the absence and presence of metabolic activation.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (6)

M30

HPRT assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (7)

M34

HPRT assay

Official use only

1 REFERENCE

1.1 Reference

Herbold B. (2003e *[Addendum II: 2003e]*). YRC 2894-sulfonic acid amide. V79/HPRT-Test in vitro for the detection of induced forward mutations. Unpublished Bayer Report No. T0063353/AT00748 (study dates: July 2003-September 2003).

Addendum II to PPP-Monograph; APPENDIX I: Additional in vitro genotoxicity studies submitted for YRC 2894-Sulfonic acid Na-Salt (M30) and YRC 2894- Sulfonic acid amide (M34) – 2. In vitro genotoxicity of YRC 2894-sulfonic acid amide (M34) – a)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes;

OECD guideline 476 (adopted July 1997).

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

YRC 2894 (thiacloprid)-sulfonic acid amide [redacted]) was evaluated for point mutations at the HPRT locus (forward mutation assay) in V79 cell cultures in the absence and presence of metabolic activation. The S9-mix (metabolic activation) was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The test material was dissolved in deionised water (soluble up to 100 mg/ml) and tested at concentrations ranging from 125 µg/ml to 4000 µg/ml. Two independent trials were performed. Vehicle and positive control substances were tested. Preliminary cytotoxicity tests were performed with concentrations of 15-960 µg/ml (relative cloning efficiency). Concentrations up to 500 µg/ml did not change the pH in the medium and the osmolarity was not changed by concentrations up to 4000 µg/ml.

4 RESULTS AND DISCUSSION

The test substance was tested up to the limit of solubility in the solvent. Precipitation was not observed in the medium. There were no decreases in survival to treatment or in relative population growth. There were no biologically relevant increases in mutant frequencies above that of the vehicle controls. Appropriate results were observed with the vehicle and positive control substances.

Section A6.6.7
Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (7)

M34

HPRT assay

5 CONCLUSION

5.1 Conclusion

The test material was not mutagenic in the V79/HPRT assay in the absence and presence of metabolic activation.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.6.7

Annex Point III-0

Genotoxicity studies - In-vitro gene mutation assay in mammalian cells (7)

M34

HPRT assay

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7

Annex Point IIA6.7

Carcinogenicity study (2)

Mouse, 2 years

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]1998): YRC 2894 - Oncogenicity study in B6C3F1-mice. Administration in the food over 2 years[redacted] Report No.: 27247, date: 1998-03-05. Amendment Report-No. 27247A (Tox 3216), date: 1998-08-26. *PPP-Monograph Chapter: B.6.5 Chronic toxicity and carcinogenicity. B.6.5.2 Carcinogenicity in mice*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; OECD guideline 451; US-EPA FIFRA, § 83-2; Directive 67/548/EEC and 88/302/EEC method B.32
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1995/97 carcinogenicity study, B6C3F1 mice (50/sex/dose) were fed diet containing YRC 2894 (thiacloprid) (purity: [redacted]) at dose levels of 0, 30, 1250 or 2500 ppm for up to 107 weeks. In addition, two groups of mice (10/sex/dose) received 0 or 2500 ppm and were sacrificed after 1 year.

Clinical observations, body weight and food intakes were recorded at appropriate time points. Laboratory investigations (haematology, biochemistry and urinalysis) were performed at 53, 79 and 104/106 weeks. All animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, heart, liver, spleen, kidneys, adrenal glands, ovaries and testes. An extensive list of organs and tissue were subjected to microscopic examination.

The concentration, stability and homogeneity of the test material in the diet were acceptable. In ascending order of dose, daily intakes were equivalent to 0, 5.7, 234.1 and 546.4 mg/kg bw/day in males and 0, 10.9, 475.3 and 872.5 mg/kg bw/day in females, respectively.

4 RESULTS AND DISCUSSION

There was no evidence of a treatment-related increase in mortality rate (Table A6_7-1).

No treatment-related clinical signs were noted during the study. In 2500 ppm males, mean body weight was significantly reduced (up to 9%) and mean food intake was significantly increased (10%), indicative of

reduced food efficiency.

Small but significant increases in the mean leukocyte count were seen in 1250 and 2500 ppm males at all sampling points and in females at weeks 53 and 79.

No gross findings were observed in the interim animals or in those animals that died or were killed moribund during the study. Gross necropsy revealed an increased incidence of nodules in the ovaries at termination (1, 4, 4, and 6 at 0, 30, 1250 and 2500 ppm, respectively). Microscopic examination revealed that 9/15 of these nodules were caused by purulent abscesses/inflammation and 6/15 nodules were diagnosed as neoplastic lesions.

The main organ weight changes are presented in Table A6_7-2.

The microscopic investigations revealed centrilobular hepatocellular hypertrophy with an increase of hepatocellular fat storage (fatty change, intracytoplasmic vacuoles) in 2500 ppm males at the interim necropsy. There was also a decrease of sex-specific renal vacuoles in interim males at 2500 ppm males. Hypertrophy and vacuolation of the X-zone in the adrenal cortex was seen in 2 control females and all 2500 ppm females at the interim sacrifice.

The non-neoplastic microscopic findings at the final necropsy are presented in Table A6_7-3. Liver effects were evident at 1250 ppm and above but there were no changes in the thyroids. These liver effects are believed to be a consequence of chronic enzyme induction (as seen in the 14-week study). There was a significant increase in the incidence and severity of focal or diffuse vacuolisation of the adrenal X-zone in females at dose levels >1250 ppm. The persistence of this finding was considered to be due to delayed regression of the X-zone. There was a significant increase in the incidence and severity of focal or diffuse vacuolisation of the adrenal X-zone in females at dose levels >1250 ppm. The persistence of this finding was considered to be due to delayed regression of the X-zone.

The report considered these changes to be secondary to the liver effects producing increased synthesis of steroid hormones or altered kinetics. An increased number of eosinophilic, luteinised cells in the ovarian stroma or the surrounding adipose tissue were seen in females at 1250 and 2500 ppm. The incidence and severity of vacuolated cells in the medullary regions of mandibular and mesenteric lymph nodes were slightly increased at 1250 ppm and above.

At the interim necropsy an adenoma in the Harderian gland was found in a control female and a sarcoma of the skin was found in a 2500 ppm female. The incidences of ovarian tumour and the total number of tumours identified at the final necropsy are presented in Table A6_7-4 and A6_7-5.

An increased incidence of ovarian luteomas is evident at 30 ppm and above when compared to the control value. The single tumour at 30 ppm is within the historical control range for this strain of mice. These tumours are believed to be secondary to the to the known liver enzyme induction (e.g. aromatase) and the subsequent hormone imbalance.

The total incidence of tumours, the time of occurrence and the type of tumours observed in this study did not indicate any significant differences between treated and control mice. The same was true for the

number of animals with primary neoplasms, the number of animals with more than one primary neoplasm, the number of animals with metastases and the number of benign and malignant neoplasms per dose group and sex.

Based on the liver effects at the next highest dose, a NOAEL of 30 ppm was determined for the non-neoplastic effects seen in this study (equivalent to 5.7 and 10.9 mg/kg bw/day in males and females, respectively). There was no evidence of oncogenic activity in males but the incidence of ovarian tumours was significantly increased at 1250 ppm and above. Therefore, a NOEL of 30 ppm was determined for oncogenicity in females (equivalent to 10.9 mg/kg bw/day).

5 CONCLUSION

5.1 Conclusion

A NOAEL was established at 30 ppm for both sexes which is equivalent to 5.7 and 10.9 mg/kg bw in males and females, respectively. This was based on liver (cellular hypertrophy) and adrenal effects (hypertrophy and vacuolisation of X zone) at 1250 ppm. The total tumour incidence and the time of occurrence and type of tumours observed in this study do not indicate a primary oncogenic potential of YRC 2894.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.7 Carcinogenicity study (2)

Annex Point IIA6.7 Mouse, 2 years

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22/08/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.7 Carcinogenicity study (2)

Annex Point IIA6.7 Mouse, 2 years

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_7-1 Animal deaths

Dose (ppm)	0	30	1250	2500
Interim groups (52 weeks)				
Males				
Females				
Main groups (107 weeks)				
Males				
Females				

Key: a) Additional animals that died due to blood sampling from the retro-orbital venous sinus.

Table A6_7-2 Absolute and relative organ weights (mean values)

Parameter/dose	0 ppm	30 ppm	1250 ppm	2500 ppm
Males-Interim sacrifice				
Body weight (g).				
Abs[b] liver wt (mg).				
Rel[c] liver wt.				
Males-terminal sacrifice				
Body weight (g).				
Abs[b] liver wt (mg).				
Rel[c] liver wt.				
Females-Interim sacrifice				
Body weight (g).				
Abs liver wt (mg).				
Rel liver wt.				
Abs adrenal wt (mg).				
Rel adrenal wt (mg).				
Females-terminal sacrifice				
Body weight (g).				
Abs liver wt (mg).				
Rel liver wt.				

Key: a) * $p \leq 0.05$, ** $p \leq 0.01$. b) Abs = absolute. c) Rel = relative (mg/100 g).

Table A6_7-3 Non-neoplastic changes in animals scheduled for final necropsy

Dose	0	30	1250	2500	0	30	1250	2500
Sex	Males				Females			
Liver								
No examined								
Hepatocellular hypertrophy								
Fatty change								
Degeneration								
Necrosis								
Mesenteric lymph node								
No examined								
Vacuolisation								
Mandibular lymph node								
No examined								
Vacuolisation								
Adrenals								
No examined								
X-zone vacuolisation								
Grade 1								
Grade 2								
Grade 3								
Grade 4								
Mean grade								
X-zone atrophy								
Grade 3								
Grade 4								
Grade 5								
Mean grade								
Ovaries								
No examined								
Eosinophilic luteinized cells								

Key: a) ↑ indicates an increase (the statistical significance was not given in the report summary).
b) grades 1-5 (minimal, slight, moderate, marked and massive).

Table A6_7-4 Treatment-related neoplastic changes in animals scheduled for final necropsy

Dose (ppm)	0	30	1250	2500
OVARIES				
No. examined	[redacted]	[redacted]	[redacted]	[redacted]
luteoma (b)	[redacted]	[redacted]	[redacted]	[redacted]
luteoma (m)	[redacted]	[redacted]	[redacted]	[redacted]
cystadenoma (b)	[redacted]	[redacted]	[redacted]	[redacted]

Key: m = malign, b = benign

Table A6_7-5 Number of tumours in mice

Dose	**0**	**30**	**1250**	**2500**	**0**	**30**	**1250**	**2500**
Sex	Males				Females			
No examined	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Benign tumours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Malignant tumours	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Total	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A6.8.1 **Reproductive toxicity - Teratogenicity test (2)**

Annex Point IIA6.8 Rabbit

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1996): YRC 2894 - Developmental toxicity in rabbits after oral administration [redacted] Report No. 24709, date: 1996-01-26. *PPP-Monograph Chapter: B.6.6 Reproductive toxicity. B.6.6.3 Developmental toxicity study in rabbits*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; OECD guideline 414; US-EPA FIFRA §83-3; Directive 88/302/EEC method B.31
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1995 study, inseminated female Himalayan rabbits (24/dose level) were gavaged with YRC 2894 (thiacloprid) [redacted] purity in 0.5 % carboxymethylcellulose) from Day 6-28 *post coitum* at 0, 2, 10 or 45 mg/kg bw/d. Foetuses were delivered by caesarean section on Day 29 and examined by evisceration and for skeletal abnormalities by Alcian Blue/Alizarin Red differential staining.

4 RESULTS AND DISCUSSION

Two top dose animals (Day 23 and 28) and one 2 mg/kg bw/d animal aborted (Day 24). Alopoecia was noted with increased frequency in treated dams. Reduced faecal production, urine production and water consumption were noted in the majority of top dose animals. The number of females with decreased urination was increased slightly at 10 mg/kg bw/day. Light yellow discoloration of the urine was seen in 8/2 and 86/15 (frequency/animals) at 10 and 45 mg/kg bw/day, respectively. The report considered the incidence of light yellow urine at 10 mg/kg bw/day to be incidental. Light yellow discoloration may occur spontaneously in individual rabbits of this strain (historical control data provided). Food consumption was significantly lower in top dose animals throughout the dosing period and transiently (Day 6-11) in animals at 10 mg/kg bw/d. At 45 mg/kg bw/day, the animals lost weight from day 6-13 and their mean body weight was lower throughout dosing, significantly from Day 15. Body weight gain during the dosing period was significantly reduced at the top dose level. The animals in the 10 mg/kg bw/day group lost weight from Day 6-11 and their body weight gain was lower than the control group. Gross necropsy of top dose dams revealed hardened stomach contents and prominent intestinal vasculature.

Litter size was slightly lower in the top dose group due to increased abortion and significantly increased post-implantation loss. Incidences of early and late resorption were significantly increased at this dose level. The number of total resorptions was also increased at the top dose level. Litter size and placental weight were both significantly lower in the top dose group. Placental margin necrosis was noted with increased incidence at ≥10 mg/kg bw/d. The number of male foetuses was significantly lower at the top dose level. Foetal weight was significantly lower at ≥10 mg/kg bw/d, female foetuses were affected to a greater extent than males. At this dose level, 6 female foetuses were less than 30 g (including 1 foetus of 19.6 g) compared to only 2 female foetuses in the control group.

A slight increase in the number of malformed foetuses,largely attributable to an increased incidence of forelimb arthrogryposis was seen at 45 mg/kg bw/d. Arthrogryposis was defined as a persistent flexure of the limb in the region of the carpal joint. It is considered to be a consequence of restricted foetal movement in the uterus ([redacted], 1978) rather than compound-induced morphological changes. This finding was reported to be common in this strain and the incidence was within the historical range (0.0-5.6%).

Numerous skeletal findings indicative of reduced or delayed ossification were observed at the top dose level. The incidence of supernumerary 13th ribs with or without with supernumerary lumbar vertebra was also marginally increased at this dose level. No treatment-related skeletal findings were apparent at lower dose levels.

A maternal NOAEL of 2 mg/kg bw/d can be derived, based on bodyweight effects and reduced food intake at higher dose levels. A foetal NOAEL of 2 mg/kg bw/d can be derived based on decreased pup weight at higher dose levels.

5 CONCLUSION

Section A6.8.1 **Reproductive toxicity - Teratogenicity test (2)**

Annex Point IIA6.8 Rabbit

5.1 Conclusion

All effects of YRC 2894 on intrauterine development (abortions, total resorptions, decreased placental and fetal weights, retarded ossification, marginal increase in common malformations, possible increase in variations) correlated with systemic maternal toxicity so that a specific developmental toxicity of YRC 2894 is excluded. The NOAEL was thus 2 mg/kg bw per day for maternal and developmental toxicity.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.8.1 **Reproductive toxicity - Teratogenicity test (2)**

Annex Point IIA6.8 Rabbit

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/089/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8_1-1 Bodyweights and food consumption

Parameter	Time Point	Dose Level			
		0 mg/kg	2 mg/kg	10 mg/kg	45 mg/kg
Food consumption (g/d)	Day 6-11	[redacted]	[redacted]	[redacted]	[redacted]
	Day 24-29	[redacted]	[redacted]	[redacted]	[redacted]
Bodyweight (g)	Day 6	[redacted]	[redacted]	[redacted]	[redacted]
	Day 13	[redacted]	[redacted]	[redacted]	[redacted]
	Day 29	[redacted]	[redacted]	[redacted]	[redacted]
Weight gain (g)	Day 6-11	[redacted]	[redacted]	[redacted]	[redacted]
	Day 6-28	[redacted]	[redacted]	[redacted]	[redacted]
Alopoecia no. animals (observations)		[redacted]	[redacted]	[redacted]	[redacted]
Abortions		[redacted]	[redacted]	[redacted]	[redacted]

** significantly different to control ($p \leq 0.05$), ** ($p \leq 0.01$)*

Table A6_8_1-2 Reproduction data

Parameter		Dose Level			
		0 mg/kg	2 mg/kg	10 mg/kg	45 mg/kg
Mated females evaluated					
No of females with implantations (x)					
Females with viable foetuses (y)					
Abortions					
Total resorptions					
Corpora lutea (mean) (x)					
Corpora lutea (mean) (y)					
Implantations (mean) (x)					
Implantations (mean) (y)					
Live foetuses (mean (y)					
Pre-implantation loss (x)					
Pre-implantation loss (y)					
Post-implantation loss (x)					
Post-implantation loss (y)					
Early resorptions (x)					
Early resorptions (y)					
Late resorptions (x)					
Late resorptions (y)					
Male foetuses (% litter mean)					
Female foetuses (% litter mean)					
Gravid uterus (g)					
Mean placental weight (g)					
Placental margin necrosis					
Mean foetal weight (g)	Combined				
	Males				
	Females				

Key: a) 2 females with uterine anomalies. b) 1 female with a uterine anomaly. c) 1 aborted female excluded from the calculations. d) 2 aborted females excluded from the calculations. e) * significantly different to control ($p \leq 0.05$), ** ($p \leq 0.01$)

Table A6_8_1-3 External and skeletal findings

Finding % foetal (litter) incidence	Dose Level			
	0 mg/kg	2 mg/kg	10 mg/kg	45 mg/kg
Total malformations (%)	[redacted]	[redacted]	[redacted]	[redacted]
Arthrogryposis	[redacted]	[redacted]	[redacted]	[redacted]
5th right medial phalanx i.o.	[redacted]	[redacted]	[redacted]	[redacted]
Unossified	[redacted]	[redacted]	[redacted]	[redacted]
5th left medial phalanx i.o.	[redacted]	[redacted]	[redacted]	[redacted]
Unossified	[redacted]	[redacted]	[redacted]	[redacted]
1st right metacarpal i.o	[redacted]	[redacted]	[redacted]	[redacted]
1st left metacarpal i.o	[redacted]	[redacted]	[redacted]	[redacted]
Calcaneus i.o bilateral	[redacted]	[redacted]	[redacted]	[redacted]
1st cervical vertebral body	[redacted]	[redacted]	[redacted]	[redacted]
8th caudal vertebral arch	[redacted]	[redacted]	[redacted]	[redacted]
15th caudal vertebral body	[redacted]	[redacted]	[redacted]	[redacted]
13th caudal vertebral body i.o.	[redacted]	[redacted]	[redacted]	[redacted]
Pubis i.o. bilateral	[redacted]	[redacted]	[redacted]	[redacted]
Hyoid i.o.	[redacted]	[redacted]	[redacted]	[redacted]
13th rib with vertebra	[redacted]	[redacted]	[redacted]	[redacted]
13th rib present	[redacted]	[redacted]	[redacted]	[redacted]

** significantly different to control ($p \leq 0.05$), ** ($p \leq 0.01$)*

io = incomplete ossification

Section A6.8.2
Annex Point IIA6.8

Reproductive toxicity - Two generations reproduction study (1)

Rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]1997). A two-generation dietary reproduction study in rats using technical YRC 289[redacted] [redacted]eport No. 8385, date: 1997-12-08. *PPP-Monograph Chapter: B.6.6 Reproductive toxicity. B.6.6.1 Multigeneration study in rats. B.6.6.1.2 Two generation dietary reproduction study in rats*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD guideline 416; US-EPA FIFRA §83-4; US-EPA TSCA 40 CFR Sect. 798.4700
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1995-6 study, 7 week old Sprague-Dawley rats (30/sex/dose level) were administered YRC 2894 (thiacloprid) ([redacted]% purity) in the diet at 0, 50, 300 or 600 ppm. Animals were mated after 10 weeks to produce F1 pups. Following weaning, selected F1 pups were administered YRC 2894 in the diet for 10 weeks prior to mating and production of the F2 generation.

Determinations of body weights, food consumption, clinical signs, oestrous cycling, mating, fertility, gestation length and litter size were performed during the study. Offspring were examined for treatment-related effects on sex ratio, pup viability, bodyweight gain and clinical signs. Gross necropsy evaluations were performed on all adults and pups. Histopathological examination of reproductive organs, liver, pituitary, thyroids and all gross lesions was performed on all F_0 and F_1 adults.

4 RESULTS AND DISCUSSION

Dietary analyses revealed satisfactory test substance stability and concentration. Four F0 300 ppm females and three 600 ppm females were sacrificed or found dead on Day 23-24 of gestation due to dystocia. These animals also exhibited pallor, perineal and vaginal staining. Small numbers of deaths in other groups are not attributable to the effects of treatment. No clinical signs of toxicity were noted in surviving animals. The body weight changes in parental animals are presented in Table A6_8_6-2. No consistent effect on food consumption was seen in F0 animals. Food consumption was

X

significantly increased in F1 animals of both sexes at 600 ppm during the pre-mating period. No effects on food consumption were seen during gestation in dams of either generation.

The gestation length and litter data are presented in Table A6_8_6-3. No effects were seen on fertility or reproductive performance. Gestation length was increased in some F0 animals at 300 and 600 ppm due to dystocia, however no significant effect was seen on the mean gestation length for these groups. The decreased live birth index in F1 pups may be attributable to the low number of stillborn pups in the control group. The number of F2 pups noted to be 'weak' was increased in all treated groups. Pups were considered to be 'weak' if there was reduced movement or the pups were lethargic, as compared to control group pups of the same age. The report stated that the increased incidence of 'weak' pups in the treated-dose groups was not considered toxicologically significant or compound related for the following reasons: a) a dose-response relationship is not evident in the F1 or F2 pups, b) there is no statistical difference between the number of 'weak' pups/litter in the treated and control groups, c) although 'weak' pups were noted in a one-generation study at 1000 ppm, none were seen at 300 ppm (see Section B.6.8.4 b).

Red foci were noted on the livers of dystocic dams sacrificed or found dead. No gross treatment-related effects were noted at necropsy of surviving adults or pups. Relative and absolute liver weights were significantly increased in adult males and females of both generations at ≥300 ppm. Relative and absolute F0 thyroid weights were significantly increased at 600 ppm in both sexes and in females only at 300 ppm. Relative thyroid weight was increased in F1 females only at 600 ppm. Relative ovary and testes weights were slightly (but significantly) increased in F1 adults at 600 ppm.

Microscopy revealed an increased incidence and severity of hepatocyte and thyroid follicular cell hypertrophy in both sexes at ≥300 ppm. The severity and incidence of these findings was also noted to be greater in F1 than F0 animals. hepatocellular necrosis was noted in the livers of dams sacrificed or found dead. No further changes attributable to treatment were noted in adults. Pups were not examined microscopically.

A NOAEL for parental toxicity of 50 ppm (equivalent to 2.6 and 2.7 mg/kg bw in males and females respectively) can be defined for this study based on bodyweight effects and histopathological findings in the thyroids and liver at 300 ppm. A NOAEL for reproductive toxicity of 50 ppm (equivalent to 2.6 and 2.7 mg/kg bw in males and females, respectively) can be defined for this study, based on dystocia and decreased pup weights at 300 ppm.

5 CONCLUSION

5.1 Conclusion

The NOAEL for parental and reproductive toxicity is 50 ppm (equivalent to 2.6 mg/kg bw in males) based on body weight effects and histopathological findings in thyroids (follicular cell hypertrophy), and in the liver (hepatocytomegaly, necrosis) at 300 ppm. The NOAEL for reproductive toxicity is 50 ppm based on possible effects on live birth index and pup weights at 300 ppm.

[redacted]

Section A6.8.2	**Reproductive toxicity -**
Annex Point IIA6.8	**Two generations reproduction study (1)**
	Rat
5.1.1 Reliability	[redacted]

Section A6.8.2 **Reproductive toxicity -**
Two generations reproduction study (1)

Annex Point IIA6.8 Rat

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/09/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8_6-1 Equivalent dose of YRC 2894 (thiacloprid)

Group	Equivalent dose (mg/kg bw/d)							
	M				F			
	0 ppm	50 ppm	300 ppm	600 ppm	0 ppm	50 ppm	300 ppm	600 ppm
F0	[redacted]							
F1	[redacted]							

Table A6_8_6-2 Bodyweights and food consumption

Time Point		Mean Bodyweight (g)							
		M				5.2 F			
		0	50	300	600	0	50	300	600
F_0									
Pre-mate	Day 0	[redacted]							
	Day 70								
	Gain								
Gestation	Day 0								
	Day 20								
	Gain								
Lactation	Day 0								
	Day 21								
	Gain								
F_1									
Pre-mate	Day 0	[redacted]							
	Day 70								
	Gain								
Gestation	Day 0								
	Day 20								
	Gain								
Lactation	Day 0								
	Day 21								
	Gain								

** significantly different to control ($p \leq 0.05$), ** ($p \leq 0.01$)*

Table A6_8_6-3 Litter Data

Parameter			Dose Level			
			0 ppm	50 ppm	300 ppm	600 ppm
F_0	Gestation length (d)					
	Range (d)					
F_1	Litter number					
	Litter size					
	Stillborn (%)					
	Live birth index (%)					
	Pups missing					
	Viability index (%)					
	Pup weight (g)	Birth				
		Day 4				
		Day 7				
		Day 14 M/F				
		Day 14 F				
		Day 21 M/F				
		Day 21 F				
	No. of weak pups (litters)					
F_2	Litter number					
	Litter size					
	Stillborn (%)					
	Birth index (%)					
	Live birth index (%)					
	5.3 Pups missing					
	Pup weight (g)	Birth				
		Day 4				
		Day 7				
		Day 14				
		Day 21				
	No. of weak pups (litters)					

** significantly different to control ($p \leq 0.05$), ** ($p \leq 0.01$)*

Table A6_8_6-4 Adult organ weights

Organ	F_0							
	M				F			
	0	50	300	600	0	50	300	600
Liver (g)	[redacted]							
(relative)	[redacted]							
Thyroid (g)	[redacted]							
(relative)	[redacted]							
	F_1							
Liver (g)	[redacted]							
(relative)	[redacted]							
Thyroid (g)	[redacted]							
(relative)	[redacted]							
Gonads (relative)	[redacted]							

** significantly different to control (p≤0.05), ** (p≤0.01)*

Table A6_8_6-5 Microscopic findings at necropsy

Finding	F0							
	M				F			
	0	50	300	600	0	50	300	600
Hepatocyte hypertophy	[redacted]							
Severity[a]	[redacted]							
Follicular hypertrophy	[redacted]							
Severity[a]	[redacted]							
	5.3.1.1 F1							
Hepatocyte hypertophy	[redacted]							
Severity[a]	[redacted]							
Follicular hypertrophy	[redacted]							
Severity[a]	[redacted]							

** significantly different to control (p≤0.05), ** (p≤0.01)*

Section A6.8.2
Annex Point IIA6.8

Reproductive toxicity - Two generations reproduction study (2)

Rat

Official use only

1 REFERENCE

Reference

[redacted] 1995): A two-generation reproduction range-finding study with YRC 2894 technical in rat[redacted] Report No. 24084, date: 1995-06-02.

PPP-Monograph Chapter: B.6.6 Reproductive toxicity. B.6.6.1 Multigeneration study in rats. B.6.6.1.1 Range-finding study in the rat

Data protection [redacted]

1.1.1 Data owner [redacted]

1.1.2 Companies with letter of access [redacted]

1.1.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

Guideline study

Yes;

US-EPA FIFRA §83-4

GLP [redacted]

Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 pilot study, 10 week old Sprague-Dawley rats (7/sex/dose level) were administered YRC 2894 (thiacloprid) [redacted] purity) in the diet at 0, 100, 400, or 1600 ppm for a minimum of 28 days prior to mating. Females were allowed to litter and were sacrificed following completion of lactation. The resulting F_1 litters were reduced to 8 pups (4 pups per sex) on Day 4 *post partum*. Three pups per sex were sacrificed at Day 21 *post partum* and the remaining pups sacrificed at Day 35 *post partum*. Histopathological examinations were conducted on the liver and thyroids from all parental and selected F_1 animals.

X

Dietary analyses revealed satisfactory stability of the test material, dietary concentrations were within ±15% of the nominal concentration. One 1600 ppm male was found dead on Day 40, gross necropsy did not reveal the cause of death. No treatment related clinical signs were noted in any animal. The parental body weight changes are presented in Table A6_8_2-1. Food consumption was consistently lower in both sexes at 1600 ppm prior to mating and in 1600 ppm females during gestation, values occasionally attained statistical significance.

Section A6.8.2 **Reproductive toxicity - Two generations reproduction study (2)**

Annex Point IIA6.8 Rat

4 RESULTS AND DISCUSSION

No treatment-related effects on reproductive performance were seen. The number of pup deaths (Day 0-4) was significantly increased at 1600 ppm, resulting in a slightly lower viability index for this dose group. Mean pup weight at this dose level was significantly lower from Day 4, pup weights at birth were comparable in all dose groups.

Hepatocyte hypertrophy, vacuolisation and 'ground glass' appearance of the hepatocyte cytoplasm were noted at necropsy in the 400 ppm and 1600 ppm dose groups. The incidence of hepatocyte glycogenic vacuolar change was decreased at 1600 ppm. Thyroid follicular cell hypertrophy was also noted at 1600 ppm. similar findings were noted in F0 and F1 animals.

A parental NOAEL of 100 ppm can be determined for this study, based on microscopic hepatic and thyroid changes in F0 and F1 animals at 400 and 1600 ppm. A reproductive NOAEL of 400 ppm can be determined for this study, based on decreased weight gain and survival in pups at 1600 ppm.

5 CONCLUSION

Conclusion 100 ppm was a NOAEL in this study based on liver (hepatocellular hypertrophy) and thyroid (elongated follicular cells) at 400 ppm.

[redacted]

5.1.1 Reliability [redacted]

Section A6.8.2
Annex Point IIA6.8

Reproductive toxicity -
Two generations reproduction study (2)
Rat

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/09/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_8_2-1 Bodyweights and weight gain

Time Point		Mean Bodyweight (g)							
		M				F			
		0	100	400	1600	0	100	400	1600
Pre-mate	Day 0	[redacted]							
	Day 28	[redacted]							
	Day 42	[redacted]							
	Weight gain	[redacted]							
Gestation	Day 0	[redacted]							
	Day 20	[redacted]							
	Weight gain	[redacted]							
Lactation	Day 0	[redacted]							
	Day 21	[redacted]							
	Weight gain	[redacted]							

** significantly different to control ($p<0.05$), ** ($p<0.01$)*

Table A6_8_2-2 Pup weight and mortality

Time Point	Pup Weight (g)			
	0 ppm	100 ppm	400 ppm	1600 ppm
Birth	[redacted]			
Day 4	[redacted]			
Day 11	[redacted]			
Day 21	[redacted]			
Day 28	[redacted]			
Day 35	[redacted]			
Weight Gain (%)	[redacted]			
Live birth index (%)	[redacted]			
Pup deaths (Day 0-4)	[redacted]			
Viability index (%)	[redacted]			

** significantly different to control ($p\leq0.05$), ** ($p\leq0.01$)*

Table A6_8_2-3 Microscopic findings

Finding		Incidence of microscopic findings							
		M				F			
		0	100	400	1600	0	100	400	1600
Hepatocyte hypertrophy	F_0								
Glycogenic vacuolar change of hepatocytes.									
'Ground glass' hepatocytes									
Hepatocyte cytoplasmic inclusions									
Thyroid follicular hypertrophy									
Hepatocyte hypertrophy	F_1								
Glycogenic vacuolar change of hepatocytes.									
'Ground glass' hepatocytes									
Hepatocyte cytoplasmic inclusions									
Increased mitotic figures (liver)									
Thyroid follicular hypertrophy									

Section A6.9 Neurotoxicity study (1)

Annex Point IIIA, VI, 1

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1 REFERENCE

1.1	**Reference**	[redacted] 1997): An acute oral neurotoxicity screening study with technical grade YRC 2894 in Fischer 344 rats. [redacted] Report No. 8158, date: 1997-05-12. *PPP-Monograph Chapter: B.6.7 Neurotoxicity studies. B.6.7.2 Acute neurotoxicity studies (Study 1)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; US-EPA FIFRA, Addendum 10; US-EPA 540/09-91-123, PB 91-154617
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1996 study, Fischer rats (12/sex/dose) were gavaged with a single analysed oral dose of 0, 22, 53 or 109 mg/kg bw YRC 2894 (thiacloprid) (purity: [redacted]%). The test material was suspended in deionized water containing 0.5% methylcellulose and 0.4% Tween 80.

Dose selection was based on a range finding study (5/sex/dose) using dose levels of 27, 36, 85, 244 or 526 mg/kg bw (analytically confirmed). The two highest dose levels induced tremors, decreased activity, repetitive chewing movements, cool-to-touch body, dilated pupils, clear lacrimation and 100 % mortality. At 85 mg/kg bw, slight repetitive chewing movements in both sexes, slight tremors in males and staining in some females (oral or nasal staining). Slight repetitive chewing movements were also seen in males at 35 mg/kg bw. The clinical signs were evident 2-4 hours after treatment and all survivors were normal within 24 hours. In general, the deaths occurred with 24 hours of dosing.

4 RESULTS AND DISCUSSION

In the main study, mortality, clinical observations and body weight were recorded. A functional observational battery of tests and automated measurements of activity in a figure-eight maze were evaluated prior to treatment and approximately 4 hours after dosing on days 0, 7 and 14. The behavioural tests were started at the time of the peak plasma concentration. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Only one interruption of a given beam was counted until the rat relocated and interrupted one of the other beams. Habituation was evaluated as a decrease in activity during the test session. All the rats received a gross examination at necropsy. The brain was removed and weighed. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system were examined microscopically (control and top dose groups only).

There were no deaths during the study. The clinical signs observed in both sexes at the top dose level included tremor, decreased activity, locomotor incoordination (ataxia), cool-to-touch-body, dilated pupils, urine staining and eyelid ptosis. In addition, females in the top dose group had red nasal and oral staining, clear nasal discharge and clear lacrimation. At 53 mg/kg bw, dilated pupils were seen in one female rat. The clinical signs occurred on the day of treatment and were resolved within 1-5 days. Mean body weight was slightly reduced in males at the top dose level only.

In the FOB, dose-related effects were evident in males and females at all three dose levels (only minimal effects at the low dose level). The effects were on the day of treatment and included gait incoordination, tremors, decreased activity, dilated pupils, ptosis of eyelids and reduced body temperature. The difference in toxicity between the FOB and clinical observations (recorded after the FOB and motor activity assessments) indicates the transient nature of the effects. There were no overt signs of toxicity at any dose level on day 7. On the first treatment day, there were compound-related decreases in motor and locomotor activity in 109 mg/kg bw males and in females at dose levels $\geq$22 mg/kg bw. All signs were reversible in males and females at the seven-day observation point. Habituation was not affected by treatment. A NOAEL of 53mg/kg bw was determined for motor and locomotor activity in males.

No compound-related gross lesions were detected in males or females at necropsy. Brain weight was not affected by treatment. Compound-related microscopic lesions were not seen in males or females at any dose level. Based on the transient clinical signs of toxicity, the NOAEL for this study was <22 mg/kg bw for both sexes.

5 CONCLUSION

5.1 Conclusion

In this study clinical signs of acute toxicity of YRC 2894 were seen after all doses so that no clinical NOAEL was established. The NOAEL for microscopic lesions is 100 mg/kg bw for males and females. No evidence of a specific neurotoxic potential was seen.

Section A6.9 Neurotoxicity study (1)

Annex Point IIIA, VI, 1

[redacted]

5.1.1 Reliability [redacted]

Section A6.9 Neurotoxicity study (1)

Annex Point IIIA, VI, 1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.9 Neurotoxicity study (2)

Annex Point IIIA, VI, 1

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1 REFERENCE

1.1	**Reference**	[redacted]1998): A special acute oral neurotoxicity study to establish a no-observed-effect level with technical grade YRC 2894 in Fischer 344 rats[redacted]Report No. 8158, date: 1998-05-04. *PPP-Monograph Chapter: B.6.7 Neurotoxicity studies. B.6.7.2 Acute neurotoxicity studies (Study 2)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; US-EPA FIFRA, Addendum 10; US-EPA 540/09-91-123, PB 91-154617
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1997 study, Fischer rats (12/sex/dose) were administered a single analysed oral dose (gavage) of 0, 3.1 or 11.0 mg/kg bw YRC 2894 (thiacloprid) (purity: [redacted]). The test substance was suspended in 0.5% methylcellulose and 0.4% Tween 80 in deionized water. Clinical observations and mortality were recorded. A functional observational battery of tests and automated measurements of activity in a figure-eight maze (females only) were evaluated approximately 4 and 7 hours after treatment, respectively. The behavioural tests were started at the time of the peak plasma concentration. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Only one interruption of a given beam was counted until the rat relocated and interrupted one of the other beams.

4 RESULTS AND DISCUSSION

There were no deaths and no clinical signs at any dose level. Small but significant decreases in mean body temperature were observed in females at 3.1 and 11.0 mg/kg bw. Since temperature effects were not seen at 53 mg/kg bw in the previous study, these small decreases were considered to be incidental. Thus, no compound-related effects were detected by the FOB evaluations. Compound-related decreases in motor and locomotor activity were observed in females at 11.0 mg/kg bw (see Table A6_9-1).

Based on the decreases in motor and locomotor activity and the clinical signs seen in the previous study, NOAELs of 11.0 and 3.1 mg/kg bw were determined for males and females, respectively.

5 CONCLUSION

5.1 Conclusion

In this study a slight decrease in activity in the figure-eight maze test was seen in 11 mg/kg bw females only. Therefore, the overall clinical NOAEL following acute oral exposure is 11 mg/kg bw for males and 3.1 mg/kg bw for females.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.9 Neurotoxicity study (2)

Annex Point IIIA, VI, I

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_9-1 Summary of motor and locomotor activity (% different from controls)

Dose [mg/kg]	3.1	11.2

Females		
Motor	[redacted]	[redacted]
Locomotor	[redacted]	[redacted]

Key: * = $p \leq 0.05$; ANOVA.

Section A6.9 Neurotoxicity study (3)

Annex Point IIIA, VI, 1

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1 REFERENCE

1.1	**Reference**	[redacted] 1997): A subchronic dietary neurotoxicity screening study with technical grade YRC 2894 in Fischer 344 rats [redacted] Report No. 8162, date: 1997-06-03. *PPP-Monograph Chapter: B.6.7 Neurotoxicity studies. B.6.7.3 Short-term neurotoxicity studies*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; US-EPA FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In 1995 study, Fischer 344 rats (12/sex/dose) were fed diet containing YRC 2894 (thiacloprid) (purity; [redacted]) at concentrations of 0, 50, 400 and 1600 ppm for 13 weeks. Clinical observations, mortality, body weight and food consumption were recorded at suitable time points. A functional observational battery of tests and automated measurements of activity in a figure-eight maze were evaluated prior to the start of the study and during weeks 4, 8 and 13. Ophthalmologic examinations were performed prior to the start of treatment and during week 12. All rats received a gross examination at necropsy. The brain was removed and weighed. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were examined microscopically (6/sex/dose from the control and top dose groups).

Analysis of the diet indicated that the homogeneity and stability of the test material were acceptable and that the actual dietary concentrations were 0, 43.3, 357 and 1527 ppm. The mean daily intakes were equivalent to 0, 2.94, 24.2 and 101 mg/kg bw/day in males, and 0, 3.41, 27.9 and 115 mg/kg bw/day in females, at the nominal dose levels of 0, 50, 400 and 1600 ppm, respectively.

4 RESULTS AND DISCUSSION

No deaths occurred and no clinical signs of toxicity were observed during the study. There were significant reductions in mean body weight in both sexes at 1600 ppm. In both sexes, the mean food intake was reduced by 34-37% during the first week and by approximately 6-15% during many weeks of the remainder of the study. Significant reductions in mean food intake were also seen at 400 ppm in both sexes during first week of the study (8-10% less). No treatment-related effects were detected by the FOB evaluations. Motor and locomotor activities were not affected by treatment. There were no treatment-related ophthalmologic findings. The macroscopic and microscopic examinations did not detect any findings attributable to treatment. Brain weight was not affected by treatment.

Based on reduced food intake, a NOEL of 50 ppm was determined for this study (equivalent to 2.94 and 3.41 mg/kg bw/day in males and females, respectively). A NOEL of 1600 ppm was determined for neurotoxicity in both sexes (equivalent to 101 and 115 mg/kg bw/day in males and females, respectively).

5 CONCLUSION

5.1 Conclusion

All tests of FOB and on motor and locomotor activity did not reveal compound-related effects. No gross or microscopic lesions in neural tissues or in skeletal muscles were detected. The only effects that were evident in this study, decreased body weight and decreased food consumption, support 1600 ppm as the highest practical dose for testing. In summary, the present study established that the highest dietary concentration of 1600 ppm (equivalent to 101 mg/kg bw in males and to 115 mg/kg bw in females) was a NOAEL for subchronic neurotoxicity in both sexes.

[redacted]

5.1.1 Reliability [redacted]

Section A6.9 Neurotoxicity study (3)

Annex Point IIIA, VI, 1

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.10

Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (1)

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1 REFERENCE

1.1	**Reference**	[redacted]1994): Studies on the inhibition of thyroid peroxidase-catalysed reactions by YRC 2894 and its metabolites in vitr[redacted] [redacted]eport No. 23495A, date: 1994-11-24, *revised 1999-01-28.* *PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.2 Supplemental studies on the findings in the chronic toxicity/carcinogenicity study in rats (Study 1)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; No guidelines available
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

Mechanistic studies were performed to clarify the following findings: i) the increased incidence of thyroid adenomas, ii) increased incidence of uterine tumours.

Studies on the inhibition of thyroid peroxidase-catalysed reactions by YRC 2894 (thiacloprid) and its metabolites.

In vitro studies (non-GLP/no guidelines) were carried out to investigate the possibility that YRC 2894 (purity: [redacted] or its metabolites could exert a direct effect on thyroid peroxidase (TPO). Interactions of 435 and 870 µM YRC 2894 with TPO-catalysed reactions were evaluated using a partially purified fraction of hog thyroid glands as an enzyme source. TPO-catalysed guaiacol oxidation and iodine formation were used as measures for peroxidase activity. Plasma extracts from rats treated with 2000 ppm YRC 2894 for 14 days were also screened for an inhibitory effect on TPO-catalysed iodine formation.

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Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (1)

4 RESULTS AND DISCUSSION

The results show that YRC 2894 neither inhibited the TPO-catalysed guaiacol oxidation nor the TPO-catalysed iodine formation from iodide at a concentration of 435 µM (IC 50 values >870µM). The plasma extracts also had no inhibitory effect on the TPO-catalysed iodine formation. Therefore, it was concluded that YRC 2894 and its metabolites had no direct inhibitory effect on TPO.

5 CONCLUSION

5.1 Conclusion

No direct inhibitory effect of YRC 2894 or of its metabolites on the TPO was evident.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.10 **Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (1)**

Annex Point IIIA, VI, 7

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.10
Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (2)

Official use only

1 REFERENCE

1.1 Reference

[redacted]1998): YRC 2894 - Mechanistic studies on aromatase induction in rats (4 week feeding studies)[redacted]Report No.: 27717, date: 1998-07-27. Amendment report No. 27717B, date: 1998-09-07.

PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.2 Supplemental studies on the findings in the chronic toxicity/carcinogenicity study in rats (Study 2)

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No;

But in general agreement with OECD guideline 407

2.2 GLP

[redacted]

2.3 Deviations

[redacted]

3 MATERIALS AND METHODS

Studies on aromatase induction and toxicokinetics in rats.

Two serial studies (GLP/no guidelines) have been conducted to investigate the effect of dietary YRC 2894 (thiacloprid) (purity: [redacted]) administration on the activity of aromatase in male and female rats and to provide toxicokinetic data.

In the first study, Wistar rats (15/sex/dose) were administered diet containing YRC 2894 at concentrations of 0, 100, or 1000 ppm for 4 weeks. In the second study, Wistar rats (10 females/dose) administered diet containing YRC 2894 at concentrations of 0, 200 or 500 ppm for 4 weeks. Clinical observations, body weight and food intakes were recorded at appropriate time points. Vaginal smears were taken, examined microscopically and classified as diestrous, proestrous or estrous. Blood samples were taken from the orbital plexus on days 1, 8, 15, 22 and 28 (1st study). Female rats were necropsied in the diestrous phase. Male rats were necropsied at the end of treatment. Selected organs (brain, adrenals, ovaries and liver) were removed and weighed. The liver (1st & 2nd studies) and ovaries (1st study) were used for enzyme determinations. At necropsy, blood was taken from female rats (1st study) in the diestrous stage of the estrous cycle for hormone determinations. These hormone determinations are stated to be flawed because of systematic errors, therefore, they were not reported.

The concentrations in diet were confirmed by analysis. Homogeneity and stability have been shown to be acceptable in previous studies. The mean daily intakes in males were equivalent to 0, 6.7 and 66.7 mg/kg

bw/day at 0, 100 and 1000 ppm, respectively. In females, the mean daily were equivalent to 0, 6.6, 20.4, 47.5 and 60.4 mg/kg bw/day at dose levels of 0, 100, 200, 500 and 1000 ppm, respectively.

4 RESULTS AND DISCUSSION

No deaths occurred during the study. Lightly coloured faeces were observed in some animals at 1000 ppm. Significant reductions in mean body weight gain were observed in males (-27%) and females (-80%) at 1000 ppm and in females at 500 ppm (-39%). Food intake was not affected by treatment.

The plasma levels of YRC 2894 in male and female rats are presented in Table A6_10-1 and graphically in Figures 6.3 and 6.4. In 1000 ppm males, the mean plasma concentration had reached a plateau of approximately 60-64 nmol/ml for days 1-8 but a slight decrease was evident from day 7 onwards. In 1000 ppm females, the mean plasma concentration increased up to day 7 and reached a plateau around 80-100 nmol/ml. A ten-fold increase in dose resulted in 8-fold increase in the mean plasma concentration in males and a 14-fold increase in females. A decline in the plasma levels during the determination period, which would be expected as a consequence of enzyme induction, did not occur.

No macroscopic changes were detected at necropsy. Liver weights were increased at doses of ≥ 500 ppm.

The kinetic data revealed that despite enzyme induction, mean YRC 2894 plasma levels remained relatively stable in female rats at 100 ppm and 1000 ppm throughout the study. A slight decrease in mean plasma levels was detected in male rats at the top dose level. The higher plasma/dose ratio seen in females at 1000 ppm may indicate saturation of the liver metabolic capacity in females at high dose levels.

A dose-related increase in aromatase activity was detected in the liver: a 1.8-fold increase at 200 ppm, a 2.1-fold increase at 500 ppm and a 2.4-fold increase at 1000 ppm. A NOEL 100 ppm was determined for aromatase induction in (equivalent to 6.6 mg/kg bw/day). In the ovaries, which are the main site of steroid production and aromatase activity, no induction of aromatase by YRC 2894 was evident.

5 CONCLUSION

5.1 **Conclusion**

The toxicokinetic data of this study revealed that despite the known enzyme induction of YRC 2894 no significant decrease of YRC 2894 plasma levels was observed in female rats. Furthermore, an over proportional plasma level/dose ratio occurred at 1000 ppm. This which may indicate an overload of the metabolic liver capacity especially in females and at higher doses, which supports that findings are related to high-dose phenomena.

[redacted]

5.1.1 Reliability [redacted]

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Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (2)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1 Mean plasma concentrations of YRC 2894 (thiacloprid) (nmol/ml)

Dose	100 ppm		1000 ppm	
	Males	Females	Males	Females

Day 1	[redacted]	[redacted]	[redacted]	[redacted]
Day 8	[redacted]	[redacted]	[redacted]	[redacted]
Day 15	[redacted]	[redacted]	[redacted]	[redacted]
Day 22	[redacted]	[redacted]	[redacted]	[redacted]
Day 28	[redacted]	[redacted]	[redacted]	[redacted]

The mean hepatic aromatase activity was 9.7, 9.3, 16.4**, 19.2** and 23 pmol/g/min (** p<=0.01) at 0, 100, 200, 500 and 1000 ppm, respectively.

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Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (3)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1998a *[Monograph: 1998b]*): Investigation of the inhibition of Cytochrome P450 dependent monoxygenases in liver microsomes (in vitro) [redacted] Report No: 27719, date: 1998-07-27. *PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 1)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; No guidelines available
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In vitro investigations into the potential inhibition of cytochrome P450 dependent monooxygenases in liver microsomes.

In vitro investigations (non-GLP/no guidelines) were performed to examine whether YRC 2894 (thiacloprid) has any inhibitory effect on the enzymes involved in steroid degradation.

The potential inhibition of 7-ethoxycoumarin–deethylase (ECOD) by YRC 2894 was measured in liver microsomes of male rats, phenobarbital-pretreated male rats and male dogs. For the determination of the IC50 values, concentrations of 0, 0.1, 1, 10 and 100 µM YRC 2894 were tested. ECOD correlates with the cytochrome P450 subtypes 1A1, 2B1, 2D1 and others.

The inhibition of testosterone hydroxylation was also determined for male rats. The main hydroxylation and oxidation reactions for testosterone are catalysed by different cytochrome P450 subtypes. For the determination of the IC50 values, concentrations of 0, 10, 100, 500 and 1000 µM YRC 2894 were tested. Liver microsomes from female rats, which were treated with YRC 2894 for 2 weeks, were also used in this experiment. The main metabolites identified by HPLC were 16α, 2α, 6β and 7α hydroxylation products and androstendione.

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Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (3)

4 RESULTS AND DISCUSSION

YRC 2894 was found to be a very weak inhibitor of ECOD in all microsomal preparations. In both rats and dogs, 50 % inhibition could not be achieved and the IC50 was >100 µM. YRC 2894 did not inhibit the main hydroxylation and oxidation reactions of testosterone. The IC50 for all reactions is >1000 µM. However, YRC 2894 was shown to increase the metabolism of the steroid testosterone.

An increase in the metabolism of the steroid testosterone was observed in pre-treated female liver (Figure A6_10-1 and 2).

These tests did not reveal any inhibiting effect on enzymes involved in steroid degradation so that an increased metabolism/excretion of estradiol and compensatory feedback reactions are not involved. An induction of enzymes which catalyse the metabolism of testosterone to androstenedione was detected. Since androstenedione is a precusor of estradiol, it is possible that this pathway may contribute to the increased estradiol production in female rats.

5 CONCLUSION

5.1 Conclusion

This test demonstrated that YRC 2894 had no inhibitory effect on enzymes which are involved in testosterone metabolism. On the other side it was demonstrated that YRC 2894 had an influence on enzymes which catalyse the transformation of testosterone to estradiol.

[redacted]

5.1.1 Reliability

[redacted]

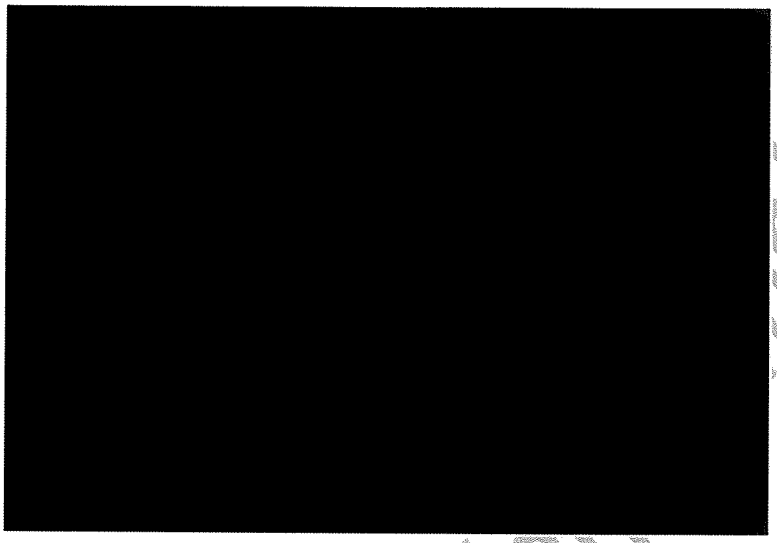
Section A6.10

Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (3)

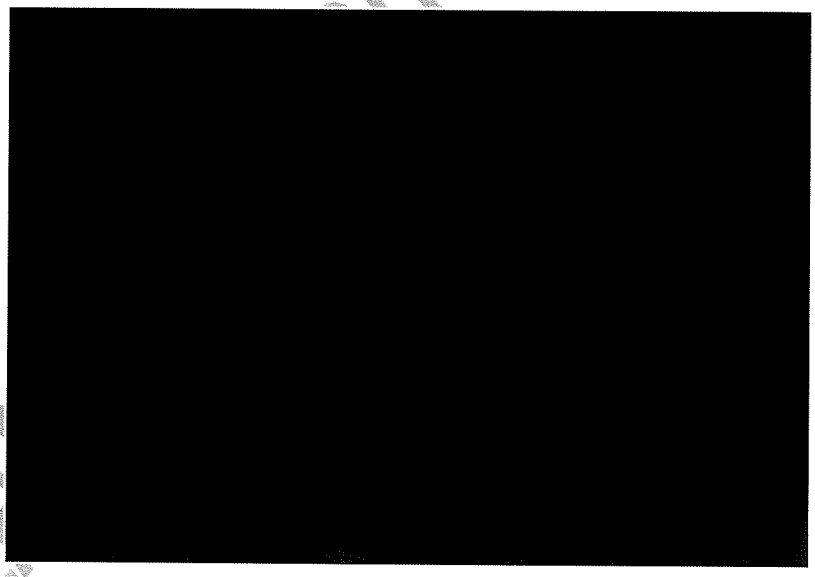
Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Figure A6_10-1 Inhibitory effects of YRC 2894 (thiacloprid) on the metabolism of testosterone



Key: i) 16α, 2α, 6β and 7α hydroxylation products of testosterone. ii) Andr = androstendione.

Figure A6_10-2 Effects on liver enzymes that catalyse the transformation of testoerone to androendione



Key: i) Andr = androstendione.

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (4)

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998a): A one-generation dietary reproduction study in rats using technical grade YRC 2894 to evaluate the reproducibility of dystocia and an increase in stillbirths in the P generation of a two-generation dietary reproduction study in rats [redacted] Report No. 8489, date: 1998-05-12.

PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 2)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes, X

OECD guideline 416; US-EPA FIFRA §83-4; US-EPA TSCA 40 CFR Sect. 798.4700

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

The purpose of this study was to determine the reproducibility of the dystocia and increase in stillbirths observed in the 2-generation reproduction study.

Sprague-Dawley rats (15 males and 30 female/dose) were administered diet containing YRC 2894 (thiacloprid) at concentrations of 0, 25, 300 or 1000 ppm. The animals were exposed to the test material throughout the entire study, which included a 10-week pre-mating period.

Clinical observations, body weight, food intake and the routine reproductive parameters were recorded at appropriate time points. In addition, the time between the initiation of labour and the first birth, and the time between the birth of pups was evaluated in some dams. Gross necropsy evaluations were performed on all adult females. Selected organs were removed and retained in fixative. The liver and thyroid were weighed. No histopathological examinations were performed.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equivalent to 0, 2, 20 and 69 mg/kg bw/day in males at dose levels of 0, 25, 300 and 1000 ppm, respectively. At the same dose levels in females, the daily intakes were 0, 2, 23 (20 & 35) and 75 (68 & 119) mg/kg bw/day, respectively (gestation and lactation intakes in brackets).

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (4)

4 RESULTS AND DISCUSSION

There were six unscheduled deaths in the top dose group. The cause of death was undetermined. One moribund female was sacrificed on day 134 (sperm positive with copulatory plug/no implants). One female was found dead on day 40 (prior to mating). Four pregnant females were found dead on days 98-105 (gestation days 22-24 with). Two of these animals had started to deliver at the time of death. One pregnant rat was found dead without ever having been observed in labour. Altogether 6 females were not pregnant (4 at the scheduled sacrifice, 1 died prior to mating and 1 was sacrificed moribund). Clinical signs of toxicity were observed in the top dose group and included paleness, laboured breathing and cold to touch. The mean body weights of 1000 ppm females were significantly lower during the last three weeks of the premating phase (5.8%), during gestation (4.9-10.4%) and during lactation days 0-4 (10-13%). At 1000 ppm, mean body weight gain was significantly reduced (16.6%) during gestation. No clear treatment-related effects on food consumption were noted during the study.

The main reproductive findings are presented in Table A6_10-1.

There were no treatment-related effects on time to insemination, mating, gestation length and pup gender. The time between the initiation of labour and birth or the time between births was not affected by treatment. There were treatment-related decreases in the pup viability index and of mean pup weight on day 4 at 1000 ppm. A greater number of top dose pups and litters were considered to be weak. This correlates with the decrease in the viability index.

There were no treatment related gross findings at necropsy. The organ weight changes are presented in Table A6_10-2.

In the top dose group, two dams that died were considered to be dystocic due to the delivery of pups followed by a long period of time with no further deliveries. One dam delivered 1 pup on gestation day 22 (13:15 hours) but delivered no further pups. This dam was found dead on the morning of gestation day 23 (12 pups found *in utero* at death). The second dam delivered 10 pups on gestation day 23 (7:40 hours). This dam was found dead on the morning of gestation day 24 (6 pups found *in utero* at death). A third dam considered to be dystocic was found dead on gestation day 24 (most dams deliver on gestation day 22 or 23) without ever having been observed in labour (9 pups found *in utero* at death). On day 4 of lactation, reduced viability and body weight were noted in the pups at 1000 ppm. There were significant increases in mean relative liver weight at 300 (9.6%) and 1000 ppm (31.6%). The dystocia and lack of initiation of labour may have been due to the marked toxicity seen at the top dose level (death, clinical signs, body weight effects and increased organ weights). A NOAEL of 300 ppm was determined for general toxicity. A NOEL of 300 ppm was determined for the reproductive effects.

5 CONCLUSION

5.1 Conclusion

The NOAEL for reproductive effects and for general toxicity was 300 ppm in this supplementary 1-generation study which, therefore, supports a NOAEL of 50 ppm in the discussed 2-generation study. In summary it can be stated that based on the results of the 2 studies with regard to

parental toxicity including dystocia and for reproduction toxicity, an overall NOAEL of 50 ppm (equivalent to 2.6mg/kg bw) can be established.

[redacted]

5.1.1 Reliability [redacted]

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (4)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/09/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1 Summary of the main reproductive findings and indicies

Dose (ppm)	0	25	300	1000

No. of animals mated.				
No. of animals delivered.				
No.of animals with implants.				
Mating index[a].				
Fertility index[b].				
Gestation index[c].				
Total No. implantations.				
Mean implantations.				
Total No. pups born.				
Total No. pups found dead.				
No. of litters with pups found dead.				
Mean litter size.				
Mean weight of viable pups at birth (g).				
Gestation length (days).				
Mean weight of viable pups on Day 4 (g).				
No. of weak pups				
No. of stillborn pups.				
Live birth index[d].				
Viability index[e].				
Birth index[f].				

Key: a) No. of inseminated females/No. of females co-housed with males x 100. b) No of pregnant females/No. of inseminated females x100. c) No. of females with live pups/No. of pregnant females x 100. d) No of live pups born per litter/total no. of pups per litter x 100. e) No, of live pups on Day 4 per litter/No of live pups born per litter x 100. f) Total No. of pups born per litter/Total No. of implantation sites per dam x 100. g) excluding dead animals. h) including dead animals. * = statistically significant.

Table A6_10-2 Organ weight changes (absolute and relative)

Dose (ppm)	TBW (g)	Liver (g)	Ovaries (g)	Thyroid (g)
0	[redacted]	[redacted]	[redacted]	[redacted]
25	[redacted]	[redacted]	[redacted]	[redacted]
300	[redacted]	[redacted]	[redacted]	[redacted]
1000	[redacted]	[redacted]	[redacted]	[redacted]

Key: a) relative organ weight in parenthesis (organ weight/body weight x 100).
b) TBW =terminal body weight.

Section A6.10

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (5)

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1 REFERENCE

1.1	**Reference**	[redacted]1998b): An experimental study to investigate the cause of dystocia and stillbirths in rats treated with technical grade YRC 2894[redacted]Report No. 8605, date: 1998-09-02. *PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 3)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD guideline 416; US-EPA FIFRA §83-4; US-EPA TSCA 40 CFR Sect. 798.4700
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

Study to investigate the cause of dystocia and stillbirths in rats.

The purpose of this study was to investigate possible mechanisms by which YRC 2894 (thiacloprid) may have produced dystocia and stillbirths in the two-generation study.

Sprague-Dawley rats (30 males and 155 females/dose) were treated with 0 or 1000 ppm YRC 2894 (purity: [redacted]) during a 10-week premating period, mating and gestation. Clinical observations, body weight and food intake were recorded at appropriate time points.

Four deaths occurred during the study at 1000 ppm. Rats treated with 1000 ppm had lower body weights than control animals.

The effects of the test material on parturition were investigated using functional and morphological tests presented below.

4 RESULTS AND DISCUSSION

Cervical extensibility

In one investigation, pregnant rats were sacrificed on gestation days 16 (6/dose), 21 (6/dose) and at term (2 treated and 4 controls). In a second independent investigation, pregnant rats (10/dose) were sacrificed on gestation day 21. The cervices were was collected prior to delivery. All foetuses were counted and weighed. Each cervix was suspended between two hooks in an oxygenated organ bath at 37° C. One hook was attached to the bottom of the organ bath the other was connected to a cervimeter, a special instrument for extensibility determinations. The cervix was stretched in 0.1 mm increments at 1-minute intervals. The resulting force was recorded by a computer connected to the cervimeter. The change in slope of extensibility was an indicator of cervical extensibility and a reduction of the slope served as a measure of an increase in extensibility.

No statistically significant difference in cervical extensibility between controls and treated animals was observed on gestation days 16 and 21 which was also valid for gestation day 22 although at that time the group size was lower.

Effects on the cervix

Twenty pregnant rats (10/dose) were used to evaluate cervical wet and dry weight. The cervix of the animals was removed on day 21 of gestation. The wet weight was determined after cleaning the cervix from surrounding connective tissue and fat. Dry weight was determined after lyophilization. There were no treatment-related effects on the cervical wet and dry weights.

Effects on the cervical collagen content

The effect of YRC 2894 on cervical collagen was tested from gestation day 13 until term using light-induced fluorescence measurements. The probe consisted of a fiberoptical probe and a sheath for isolating the optical fiber from the measuring site. This probe was inserted in the vagina with a sapphire window in contact with the surface of the external cervical os for measurement. A total of nine control rats and six treated rats were evaluated. Two measurements per time point were performed every other day starting on gestation day 13. On gestation day 22, the measurements were recorded at the time of delivery where possible. The rats that had not delivered and the rats that were in the delivery process at the time of the measurement were separated into two different groups: day 22 non-delivery and day 22 delivery.

The results indicated a cervical hardening in on gestation days 13, 15 and 17 for treated rats as compared with control animals. No differences were obvious on gestation days 19 and 21. However, marginal cervical hardening was noted on gestation day 22 in the non-delivery and delivery treatment groups. Since this slight hardening was seen in both the treated non-delivery and delivery groups, this marginal effect did not appear to have a negative effect on birth. In addition, the observed mid gestation changes did not appear to affect the birth process.

Contractile activity of isolated uterine and cervical tissue

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (5)

In this investigation, the effect of YRC 2894 administration on the contractile activity of isolated uterus and cervical tissue was tested using oxytocin (induces contraction) and isoproterenol (inhibits contractions).

Pregnant rats (4-6 rats/dose) were sacrificed on gestation days 16 and 22. The uterus and cervix were dissected free of the surrounding tissues and cut into rings of 4 mm width. These rings were mounted on stirrups in organ chambers containing Krebs-Henseleit solution at 37° C. The lower stirrup was fixed to the bottom of the organ chamber and the other was connected to an isometric force transducer. Passive tension was applied to the uterine and cervical rings up to basal tension at a weight of 2 grams. After the basal tension was stabilized either oxytocin (10^{-10}-10^{-6} M) or isoproterenol (10^{-11}-10^{-6} M) were added incrementally to the organ chamber and the contractility was measured in the control and YRC 2894-treated animals.

The concentration-contraction relationship of oxytocin was similar for both treated and control animals on gestation days 16 and 22. Although there was a tendency towards weaker activation of spontaneous rhythmic contractions by oxytocin in rats treated with YRC 2894, this was not considered biologically significant. Since the minimal oxytocin concentration produced more than a 50% increase in rhythmic activity in most of the uterine rings, the EC50 value could not be calculated.

The concentration-contraction relationship of isoproterenal was similar for both treated and control animals on gestation days 16 and 22. No statistically significant difference in the IC50 values was detected (isoproterenol-induced reduction of contractile activities in uterine and cervical tissue). A slight decrease in IC50 was observed on gestation day 22 as compared with day 16, but this was not statistically significant. Thus, it was summarised that basal rhythmic activity or the inhibition of activity induced by the ß-adrenergic agonist isoproterenol was not affected by YRC 2894.

Uterine electromyographic activity and intrauterine pressure

To measure the change in uterine electrical activity and intrauterine pressure in rats from gestation day 18 through delivery telemetric implants were used. For this purpose electrodes were sutured to the uterine wall, at approximately mid distance between the ovarian and cervical ends of the uterine horn. Each pair of electrodes consisted of a pickup electrode and a ground electrode which were approximately 2-3 mm apart. Intrauterine pressure was measured by placing a pressure transducer in the uterine cavity, which was also connected with the telemetric recording system.

Uterine electromyographic (EMG) activity (duration, amplitude and number of bursts, power density at different frequencies, integral of electrical activity) and pressure were recorded each day, beginning on gestation day 18 for 4 hours, continuous recordings were collected from gestation day 21 until delivery. The intrauterine pressure was determined as the strength of contraction multiplied with the time unit.

With regard to electrical burst results, despite some variability, this parameter and the resulting integral of electrical burst and maximum power density values showed no difference between YRC 2894-treated animals and controls through the duration of the pregnancy. In addition, intrauterine pressure was not different between treated and control

groups.

Alpha receptors

The uteri from pregnant rats (15/dose) sacrificed on day 21 of gestation were evaluated for alpha-1 adrenergic receptors. The mean alpha-1 receptor levels for the control and YRC-2894-treated dams were 3.85 and 3.89 pmol/mg protein, respectively. Exposure to YRC 2894 does not affect uterine alpha-1 adrenergic receptor levels.

Pathology

A routine gross necropsy was performed on the ten animals used for histological evaluation of the cervix and uterus. At necropsy, the following data was recorded for each dam: terminal body weight, ovary, cervical, vaginal and empty uterine weights and the number of foetuses.

No gross treatment-related findings were seen at necropsy. The terminal body weight was reduced at 1000 ppm. There was a significant decrease in the number of foetuses per litter in 1000 ppm dams used for the pathological investigations. When the number of foetuses/litter from the cervical extensibility studies and uterine contractility study were combined with the number of foetuses/litter used for the pathological investigations, a significant decrease in the number of foetuses/litter was also evident at 1000 ppm (Table A6_10-1).

Microscopy did not reveal any treatment-related effects on the cervix or uterus.

5 CONCLUSION

5.1 Conclusion

The results of this study did not indicate an effect of YRC 2894 (1000 ppm) on uterine contractility or cervical extensibility in vitro or in vivo. The in vitro assay on the contractile activity of uterine tissue did not reveal an influence of YRC 2894 on the tissue response to oxytocin or isoproterenol. Intrauterine pressure and electrical activity were also not affected by YRC 2894. Therefore, this study did not indicate a direct effect of YRC 2894 on birth functions. X

[redacted]

5.1.1 Reliability [redacted] X

Section A6.10

Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (5)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/09/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (5)

Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6_10-1 The combined data from the pathological investigations, the cervical extensibility studies and the uterine contractility uterine study

Dose	Number of litters	Mean number of foetuses
0 ppm	[redacted]	[redacted]
1000 ppm	[redacted]	[redacted]

Section A6.10

Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (6)

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998c): A reproduction study in rats to determine if administration of technical YRC 2894 from gestation days 18 to 21 will cause dystocia (study number II[redacted] Report No. 8481, date: 1998-05-04.

PPP-Monograph Chapter: B.6.8 Other toxicological studies, B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 4)

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No;

No guideline available

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

A reproduction study in rats to determine if the short-term administration of YRC 2894 (thiacloprid) to pregnant rats can induce dystocia (GLP study/no guidelines).

Pregnant Sprague-Dawley rats (30/dose) were initially gavaged with 0, 35 and 60 mg/kg bw/day YRC 2894 (purity: [redacted]) on gestation days 18-21. The vehicle was aqueous 0.5% (w/v) carboxymethyl cellulose with 0.4% (v/v) Tween 80. Because of severe toxicity and deaths in the 35 and 60 mg/kg bw/day dose groups, an additional dose level of 17.5 mg/kg bw/day was introduced into the study. This third group of animals was created from the 0, 35 and 60 mg/kg bw/day animals which had not reached gestation day 18 (not previously dosed with vehicle or YRC 2894). Dose selection was originally based on a previous study in which rats were dosed by gavage with 100 mg/kg bw/day on gestation days 18-19. This study did not show treatment-related dystocia but did show that 100 mg/kg bw/day was extremely toxic during the terminal period of pregnancy.

Clinical observations, body weight and food intake were recorded at appropriate time points. The number of live and stillborn pups was recorded. A gross necropsy was performed on dams sacrificed moribund or found dead on days gestation days 18-21.

The analysed concentrations of the dosing solutions were at least 95% of the target concentration. Twelve animals were found dead and 4 animals were sacrificed moribund during gestation days 20-24: 1/27 (sacrificed moribund), 0/9, 7/29 (2 sacrificed moribund) and 8/25 (1

sacrificed moribund) at 0, 17.5, 35 and 60 mg/kg bw/day, respectively. Clinical signs of toxicity were seen at dose levels ≥35 mg/kg bw/day and included hypoactivity, chromorhinorrhea and clear vaginal discharge.

4 RESULTS AND DISCUSSION

The mean body weights of the 35 and 60 mg/kg bw/day groups were significantly lower than the control group during the dosing period (14% lower on gestation day 21 for both groups). There was a significant dose-related reduction in mean body weight gain at dose levels ≥35 mg/kg bw/day. The difference in weight gain from gestation day 18-21 was 41.3 g, 13.3 g, -18.3 g and –24.9 g at 0, 17.5, 35 and 60 mg/kg bw/day, respectively. Significant reductions in food intake were seen on gestation days 18-21 at all dose levels: 36-54%, 86-91% and 87-94% at 0, 17.5, 35 and 60 mg/kg bw/day, respectively.

The reproductive parameters are presented in Table A6_10-1. A dose-related increase in the incidence of stillbirths is evident at 35 mg/kg bw/day and above. When the low group size and cannibalisation are taken into consideration, it is possible that a slight increase in stillbirths may be occurring at 17.5 mg/kg bw/day.

Marked toxicity was observed at all dose levels and included death, clinical signs, body weight changes and reduced food intake at 35 mg/kg bw/day and above. A marked reduction in food intake was also observed at 17.5 mg/kg bw/day. Therefore, a NOEL was not determined for general toxicity. Dystocia was not observed, but there was a clear increase in the incidence of stillbirths at dose levels ≥35 mg/kg bw/day. Therefore, a NOAEL of 17.5 mg/kg bw/day was determined for the reproductive effects seen in this study.

5 CONCLUSION

5.1 Conclusion

Dystocia was not observed after short-term treatment so that apparently a longer duration of treatment is needed for this phenomenon. Marked toxicity was observed in pregnant rats which thus appear more sensitive than nonpregnant animals. No NOEL was established for general toxicity whereas the reproductive NOEL was 17.5 mg/kg bw.

[redacted]

5.1.1 Reliability

[redacted]

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (6)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15/09/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1 A summary of the reproductive data

Dose (mg/kg bw/day)	0	17.5	35	60
No. of animals.				
No. pregnant.				
No. of litters.				
Mean litter size.				
Total No. of pups.				
No. of live births.				
No. of stillborn pups (% of total pups).				
No. cannibalised[a].				
Mean No. of viable pups.				
Live birth index (mean).				

Key: a) The cannibalised pups could not be classified because the lung were not available for the floatation test.
b) 1 litter had 3 stillbirths + 1 partially cannibalised pup and 1 litter had 2 stillbirths + 1 partially cannibalised pup.

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (7)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](1998): Further examination of the increased occurrence of dystocia and stillbirths observed in a reproductive bioassay with an experimental cyanamide (YRC 2894)[redacted]Report No. 108360, date: 1998-08-31. [redacted]1998b *[Monograph: 1998a]*): YRC 2894 - Determination of aromatase activity in ovary tissue of a modified 1-generation study in Sprague Dawley rats[redacted]Report No.: 27718, date: 1998-07-27. *PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 5)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes; OECD guideline 416; US-EPA FIFRA §83-4; US-EPA TSCA 40 CFR Sect. 798.4700
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

Further examination of the increased incidence of dystocia and stillbirths observed in the reproductive bioassays.

In a modified 1-generation study, Sprague-Dawley rats were administered diet containing 0 or 800 ppm YRC 2894 (thiacloprid) (purity:[redacted] for 10 weeks before mating and during pregnancy. All animals were 6-8 weeks of age when exposure to YRC 2894 was initiated. Groups of pregnant female rats (10-16/dose) were sacrificed following: i) a 9 ± 1 week premating period; ii) a premating + mating/pregnancy + gestation phase concluding with sacrifice on gestation day 18 or 21 (day 0 = sperm positive); and iii) a post-partum phase with sacrifice on post-partum day 2 (day 0 = delivery). The oestrus cycle was monitored by daily vaginal cytology. Only rats, which showed at least 2 consecutive 4-day oestrus cycles, were used in this study. Breeding males and virgin females were approximately 17 weeks of age.

At the sacrifice times rats were asphyxiated in a CO_2 chamber and terminated by exsanguination via cardiac puncture. Plasma, for determination of circulating oxytocin levels, was collected on gestation day 21; serum was collected at all other time points. The standard

clinical chemical parameters were assayed.

Hepatic microsomal enzyme assays were conducted in perfused liver on cytochrome P450, N-demethylase and p-nitroanisole O-demethylase. Reduced glutathione was measured in liver and in uterus. Uterine and cervical prostaglandin E_2 and $F_{2\alpha}$ content were measured.

Circulating luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were determined using a radioimmunoassay. All other blood hormone determinations were made using commercially available kits, which were used as sold or slightly modified. The specific hormones measured were estradiol, progesterone, corticosterone, T4, T3, TSH, oxytocin and prolactin after the premating phase (week 9), on gestation days 18 and 21, and at 2 days post-partum. In addition, estrogen and progesterone receptor levels were determined in the cytosolic and nuclear fractions of the uterus.

At each sacrifice liver, uterus, ovaries, mammary gland, adrenals, pituitary and cervices were weighed, and tissue specimens collected for biochemical and/or histopathological analysis. Hypothalamus was collected but not weighed. Histopathological examinations of the liver, adrenals, uterus, ovaries, mammary gland, cervix, pituitary and hypothalamus-related tissue were carried out.

The concentration, stability and homogeneity of the test material in the diet were acceptable. At 800 ppm, the mean daily intakes were equivalent to 54.0 mg/kg bw in males and 60.4 mg/kg bw in females.

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (7)

4 RESULTS AND DISCUSSION

One female was found dead on day 50 and two pregnant females were sacrificed due to prolonged or incomplete parturition. One of the pregnant females showed only slight indications that parturition had been initiated and did not deliver. The second female successfully delivered several pups but did not complete the delivery process (2 live and 2 dead pups were found in the uterus at necropsy). In both cases, the animals were given at least 24 hours to complete the process. No clinical signs were observed during the study. Significant reductions in mean body weight gain were noted at 800 ppm during the premating and gestation period. X

The hepatic enzyme activities in the treated animals were elevated at all time points (Table A6_10-1).

The hormone and cholesterol changes are presented in Table A6_10-2. Estradiol levels were significantly increased at the end of the premating period and on lactation day 2. Corticosterone levels were significantly raised at all time points. The elevated LH and progesterone levels were possibly related to the fact that some of the animals on lactation day had not delivered and were hormonally comparable with pregnant animals. No changes were detected in FSH, T4, T3, TSH, oxytocin or prolactin levels. Prostaglandin and reduced glutathione (GSH) levels were not affected by treatment.

The uterine oestrogen and progesterone receptor concentrations were not affected by treatment.

Liver weight was increased in the treated animals at necropsy (Table A6_10-3). Microscopy revealed centrilobular hepatocytomegaly (LM) and proliferation of the smooth endoplasmic reticulum (EM) in the liver.

In addition to the above determinations, the retained liver and ovary samples were retrospectively examined for aromatase activity. The results of the determinations in ovarian tissue are presented in Table A6_10-4. Serum oestradiol levels are presented in Table A6_10-5.

No aromatase induction was detected in the ovaries at the end of the premating phase and during gestation. Ovarian aromatase activity, however, was increased at 800 ppm on day 2 of lactation. In particular, levels were especially high in animals that had dystocia and did not deliver. These animals were not comparable to the control animals that had delivered and were endocrinologically in lactation. Thus, this increase in aromatase activity in the ovaries was not related to YRC 2894 administration.

Increased hepatic aromatase activity was detected at 800 ppm (7.5 pmol/g/min and 14.7 pmol/g/min at 0 and 800 ppm, respectively).

The above data suggest that the adverse YRC 2894-induced effect on steroid levels results from an effect on the liver and that the underlying mechanism involves hepatic enzyme induction.

5 CONCLUSION

5.1 Conclusion

This study confirmed an effect of YRC 2894 on steroid levels, especially on estradiol, by an action on the liver at a dose of 800 ppm, for which in other studies the underlying mechanism, an enzyme resp.

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (7)

aromatase induction, was detected. Studies on kinetics in pregnant vs. non-pregnant rats.

[redacted]

5.1.1 Reliability [redacted]

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (7)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1 Liver enzyme determinations (nmol/min/mg protein)

Dose (ppm)	0	800
Cytochrome P450		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]
N-demethylase		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]
O-demethylase		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]

Key: a) not determined for 2 dystocic animals. b) * $p \leq 0.05$

Table A6_10-2 Circulating hormone and cholesterol levels

Dose (ppm)	0	800
Estradiol (pg/ml)		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2[a]	[redacted]	[redacted]
Progesterone (ng/ml)		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]
LH (ng/ml)		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]
Corticosterone (ng/ml)		
After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]
Cholesterol (mg/dl)		

After 9 ± 1 weeks	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]

Key: a) including the 2 dystocic animals. b) * $p \leq 0.05$

Table A6_10-3 Absolute and relative liver weight determinations

Dose (ppm)	0	800	0	800
	Absolute (g)		Relative (g/100g)	
After 9 ± 1 weeks	[redacted]	[redacted]	[redacted]	[redacted]
Gestation day 18	[redacted]	[redacted]	[redacted]	[redacted]
Gestation day 21	[redacted]	[redacted]	[redacted]	[redacted]
Lactation day 2	[redacted]	[redacted]	[redacted]	[redacted]

Key: a) 2 dystocic animals omitted. b) * $p \leq 0.05$

Table A6_10-4 Mean aromatase activity in ovarian tissue

Dose	Pre-mating	Gestation day 18	Lactation day 2
(ppm)	(pmol/g/min)	(pmol/g/min)	(pmol/g/min)
0	[redacted]	[redacted]	[redacted]
800	[redacted]	[redacted]	[redacted]

Key: a) Range. b) The two animals with the highest activities (26.9 and 48.9 pmol/g/min did not deliver).
c) The mean = 20.2 pmol/g/min when the two animals that did not deliver are omitted.

Table A6_10-5 Mean serum oestradiol (approximate values from bar chart)

Dose	Pre-mating	Gestation day 18	Lactation day 2
(ppm)	(pg/ml)	(pg/ml)	(pg/ml)
0	[redacted]	[redacted]	[redacted]
800	[redacted]	[redacted]	[redacted]

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (8)

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1 REFERENCE

1.1	**Reference**	[redacted] 1998): YRC 2894 - Special study for subacute oral toxicity in rats (toxicokinetics in pregnant and non-pregnant rats) [redacted] Report No. 27657, date: 1998-07-14. *PPP-Monograph Chapter: B.6.8 Other toxicological studies. B.6.8.4 Supplementary investigations into the reproductive findings in rats (Study 6)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; No guideline available
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

Pharmacokinetics of YRC 2894 in pregnant and non-pregnant rats.

This study (non-GLP/no guidelines) was performed to determine if differences exist in the pharmacokinetics between pregnant and non-pregnant rats that might explain differences in the toxicity between the groups and possibly the dystocia.

Females Sprague-Dawley rats were administered diet containing YRC 2894 (thiacloprid) (purity; [redacted]) during pre-mating and gestation. Eight pregnant rats and 12 non-pregnant rats received 1000 ppm while 5 pregnant and 5 non-pregnant rats served as controls.

Clinical observations and body weights were recorded at appropriate time points. Blood samples were taken from the orbital plexus of anaesthetised animals on gestation days 0, 7, 14 and 21, and from non-pregnant females at comparable time-points. The number of live and stillborn pups born and the duration of gestation were recorded. The rats were sacrificed after giving birth.

Homogeneity and stability of the test substance were known from a previous study ([redacted],1998; DP 69590). The concentration in the feed mixture was not analysed. No food intake data were included in the report.

No compound-related deaths occurred during the study. There were no treatment-related clinical signs of toxicity or body weight changes. The mean gestation length and the number of stillborn pups were similar in control and treated groups.

The results of the plasma determinations are presented graphically in

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (8)

Figure 5.8. In non-pregnant rats a concentration of approximately 60 nmol/mL was determined whereas in pregnant rats levels showed a tendency to increase during the gestation until the delivery from 60 nmol/ml up to 80 nmol/ml, the highest concentration prior to birth.

4 RESULTS AND DISCUSSION

The plasma levels of YRC 2894 increased during gestation and reached a peak at the end of the gestation period. This higher systemic exposure in pregnant animals might explain the observed effects on birth at the end of the gestation period. However, other data for non-pregnant animals shows a good match for pregnant animals (Figure A6_10-1).

5 CONCLUSION

5.1 Conclusion

It was demonstrated that the plasma levels of YRC 2894 increased during the gestation and reached highest concentration at the end of the gestation period which might explain why a higher toxicity with secondary unspecific effects on birth was observed in pregnant rats at the end of the gestation period.

[redacted]

5.1.1 Reliability [redacted]

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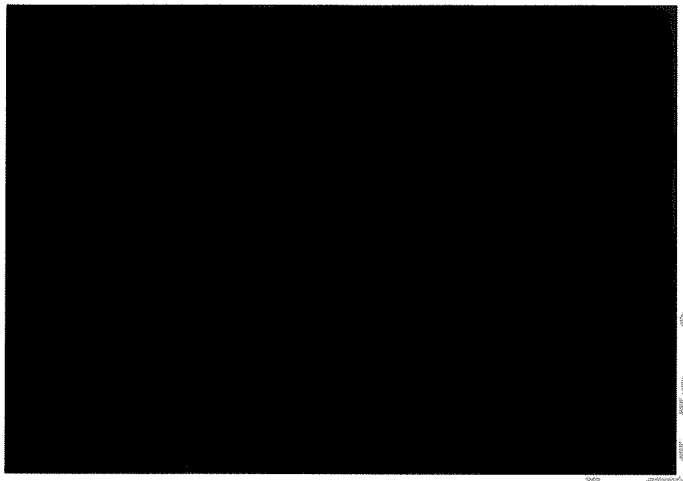
Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (8)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Figure A6_10-1. Plasma levels of YRC 2894 (thiacloprid) in pregnant vs. non-pregnant rats



Student t-Test:
p > 0.050:
p <= 0.050: *
p <= 0.010: **

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (9)

Official use only

1 REFERENCE

1.1	Reference	[redacted]2003): YRC 2894, YRC 2894-sulfonic acid amide and YRC 2894-sulfonic acid sodium salt: Determination of Liver Effects in Female Rats after a 7 Day Administration in the Diet[redacted]eport No. T1073146, date: 2003-11-26. *Addendum III to PPP-Monograph. Appendix II. Determination of liver effects in female rats(evaluation of hepatic enzyme activity)*
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

The following investigations were performed to determine the effects in rat liver following the repeated oral administration of YRC 2894 (thiacloprid) and the metabolites YRC 2894-sulfonic acid Na-salt (M30) and YRC 2894-sulfonic acid amide (M34).

Female Wistar rats (5/group) were administered 0 (control), 1000 ppm YRC 2894, 1000 ppm YRC 2894-sulfonic acid amide or 1000 ppm YRC 2894-sulfonic acid Na-salt in diet for 7 days. Dose selection was based on preliminary range finding studies.

Clinical observations were performed daily. Body weight and food intake were determined weekly.

The animals were necropsied (no gross pathology), the livers were removed and weighed and enzyme determinations in liver tissue were performed. The activities of the cytochrome P450-dependent monooxygenases (ECOD, EROD & ALD), epoxide hydrolase (EH) and the conjugation enzymes (GS-T & GLU-T) were measured. In addition, hepatic aromatase activity was determined.

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Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (9)

4 RESULTS AND DISCUSSION

Analysis confirmed the concentration, stability and homogeneity of the test substances.

There were no deaths or treatment-related clinical signs during the study. Following administration of YRC 2894 (thiacloprid), the mean body weight gain of Group 2 rats was significantly reduced (-37%). The mean body weights of Groups 3 & 4 were not affected by treatment. Food consumption was reduced in all treatment groups as compared to the controls; the reductions were 21.4%, 19.6% and 12.9% for YRC 2894, YRC 2894-sulfonic acid Na-salt (M30) and YRC 2894-sulfonic acid amide (M34), respectively.

The body weight and liver weight data are presented in the Table A6_10-4.

There were significant increases in the liver weights of the Group 2 rats only, i.e. those treated with YRC 2894. This increase in liver weight is most likely due to enzyme induction.

The results of phase I and phase II enzyme determinations are presented in Table 6_10-3 and the determinations for aromatase activity are presented in Table 6_10-4 .

YRC 2894 (thiacloprid) caused a strong induction of most of the enzymes investigated; ECOD (5.2-fold), ALD (3.0-fold), EH (4.6-fold), GS-T (2.5-fold) and GLU-T (2.8-fold). There was no effect on EROD activity. The metabolites YRC 2894-sulfonic acid Na-salt and YRC 2894-sulfonic acid amide did not induce any of the liver enzymes investigated.

In female rats, liver aromatase activity measured as the formation of tritiated water was readily detectable. Treatment with YRC 2894 approximately doubled aromatase activity in female rat liver (i.e. thiacloprid is a strong inducer of liver aromatase). The metabolites YRC 2894-sulfonic acid Na-salt and YRC 2894-sulfonic acid amide did not induce liver aromatase.

5 CONCLUSION

5.1 Conclusion

YRC 2894 (thiacloprid) produced a strong induction of ECOD (5.2-fold), ALD (3.0-fold), EH (4.6-fold), GS-T (2.5-fold) and GLU-T (2.8-fold). There was no effect on EROD activity. In addition, YRC 2894 was shown to be an inducer of liver aromatase activity. As could be expected, this liver enzyme induction was accompanied by increases in liver weight.

The two more polar metabolites, YRC 2894-sulfonic acid Na-salt (M30) and YRC 2894-sulfonic acid amide (M34) did not produce any effects on the liver enzymes investigated. Neither did they produce any changes in liver weight.

[redacted]

5.1.1 Reliability [redacted]

Section A6.10

Annex Point IIIA, VI, 7

Mechanistic study - any studies necessary to clarify effects reported in toxicity studies (9)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09/10/2006
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_10-1 Dosing regimen

Group	Test substance	Dose (ppm)	[a]Mean daily intakes (mg/kg bw/day)
1	Control	0	0
2	YRC 2894 (thiacloprid)	1000	[redacted]

3	YRC 2894-sulfonic acid amide	1000	[redacted]
4	YRC 2894-sulfonic acid Na-salt	1000	[redacted]

Key: a) Calculated from food intake data.

Table A6_10-2 Mean body weight and liver weight data (absolute and relative values)

Group	Test substance	Dose (ppm)	Body weight (g)	[a]ALW (mg)	[b]RLW (mg/100g bw)
1	Control	0	[redacted]	[redacted]	[redacted]
2	YRC 2894 (thiacloprid)	1000	[redacted]	[redacted]	[redacted]
3	YRC 2894-sulfonic acid amide	1000	[redacted]	[redacted]	[redacted]
4	YRC 2894-sulfonic acid Na-salt	1000	[redacted]	[redacted]	[redacted]

Key: a) ALW = absolute liver weight. b) RLW = relative liver weight. c) Body weight gain (g).

Table A6_10-3 Phase I and phase II enzyme activities (mean values)

Group	Test substance	Dose (ppm)	ECOD	EROD	ALD	EH	GS-T	GLU-T
1	Control	0	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
2	YRC 2894 (thiacloprid)	1000	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
3	YRC 2894-sulfonic acid amide	1000	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
4	YRC 2894-sulfonic acid Na-salt	1000	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: a) ECOD = 7-ethoxycoumarin deethylase (nmol/g min). b) EROD = 7-ethoxyresorufin deethylase (nmol/g min). c) ALD = aldrin epoxidase (nmol/g min). d) EH = epoxide hydrolase (nmol/g min). e) GS-T = glutathione-S-transferase (µmol/g min). f) GLU-T = UDP-glucuronyltransferase (nmol/g min).

Table A6_10-4 Aromatase activity in liver tissue

Group	Test substance	Dose (ppm)	Activity (pmol x min^{-1} x g^{-1})
1	Control	0	[redacted]
2	YRC 2894 (thiacloprid)	1000	[redacted]
3	YRC 2894-sulfonic acid amide	1000	[redacted]
4	YRC 2894-sulfonic acid Na-salt	1000	[redacted]

Section 6.11 **No annex Point**	**Studies on 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:	There were no further studies submitted on toxicokinetics by parenteral routes. They should only be submitted if already existing. In general data on toxicology by parenteral routes are not specifically required under the BPD.	
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	09/10/2006	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 6.12.1 Annex Point IIA 6.9.1	Medical surveillance data on manufacturing plant personnel	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [...]	Other justification [X]	
Detailed justification:	No reports about human findings are available. [redacted]	
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	09/10/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Remarks	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.12.2 Annex Point IIA 6.9.2	**Direct observations, e.g. clinical cases, poisoning incidents, 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:	There have been no reports of adverse health effects in workers exposed to thiacloprid during the experimental investigations. Neither have there been any reports of adverse effects in operators exposed to thiacloprid formulations during field trials.	
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	09/10/2006	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 6.12.3 Annex Point IIA 6.9.3	**Health records, both from industry and any other available sources**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	In a recent literature search there were no health records related to thiacloprid.	
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	09/10/2006	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section 6.12.4 Annex Point IIA 6.9.4	Epidemiological studies on the general population	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	So far, the general population has not been exposed to thiacloprid.	
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	09/10/2006	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		