faeces were collected from both animals at time intervals up to 170 hours post dose for Group 1 and up to 120 hours for Group 2. Cage debris/rinse was collected after each faecal sample collection. A cage wash/wipe was conducted following the final sample collection.

The animals were fasted for approximately 16 hours (Group 1) and 14 hours (Group 2) prior to treatment. Food was returned to the Group 1 animal at approximately 8 hours post-dose and offered to the Group 2 animals at approximately 4 hours post-dose. Water was not withheld. Body weights were recorded prior to treatment. Clinical signs were recorded during the study.

Main study:

In a (August) 2002 study, the rates and routes of excretion of radioactivity following dermal administration to five non-naïve male rhesus monkeys were investigated. Group assignments are given in Table A6_2-4.

A single dermal application of Calypso SC 480 containing ^{14}C-thiacloprid (actual dose 133 µg at 5.53 µg/cm^2) to shaved back skin (4 cm × 6 cm) of each animal (dose volume 100 µl). The area was demarcated with a Duoderm patch to isolate the dosing area. Each application site was then covered with an aluminium protective device and secured with bandages. During dosing the animals were chemically restrained (ketamine HCl) and then placed in a primate chair for an 8-hour period. After 8 hours, the protective dressings were removed and the application site washed with soapy water and dried. The skin was then swabbed with isopropyl alcohol and washed with soapy water.

A urine pan/screen wash/wipe was conducted at 4 and 8 hours post-dose and a chair wash/wipe was conducted at 8 hours post-dose after each animal was removed from the primate chair. Urine, faeces, and cage debris/rinse were collected at specified time intervals up to 144 hours post dose.

The animals were fasted for approximately 11-12 hours prior to treatment. Food was offered at approximately 4.5-5 hours post-dose. Water was not withheld. Body weights were recorded prior to treatment. Clinical signs were recorded during the study.

4 RESULTS AND DISCUSSION

Exploratory study:

There were no treatment-related clinical signs or dermal effects observed during the study. The results of the intravenous and dermal investigations are presented in Tables A6_2-2 and A6_2-3.

Following intravenous dosing, the recoveries were 66.63% in urine, 5.34% in faeces and 19.6% in cage wash/debris (attributed to excretion in urine). Excretion was rapid with approximately 65% of the dose within 8 hours of dosing. Approximately 7.6% of the radioactivity remained in the animal at 170 hours post dosing.

Following dermal administration, the recoveries were 1.17% in urine, 0.16% in faeces and 0.08% in cage debris/rinse. Approximately 98% of the radioactivity was washed from the skin at 8 hours or associated with the protective coverings. Tape stripping did not remove any additional radioactivity from the skin. This suggested that any skin residues would be systemically available or bound to the deeper layers of the skin.

Section A6.2
Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (2)
Dermal absorption

Main study:

There were no treatment-related clinical signs or dermal effects observed during the study. The results of the investigations are presented in Tables A6_2-5 and A6_2-6. Most of the radioactivity was washed from the surface of the skin.

The mean total recovery of the residual activity was 93.60% of the dose at the 8-hour sampling point. The mean total from excreta and the samples associated with excretion was 3.15%. The overall mean total recovery for radioactivity was 96.75%.

5 CONCLUSION

5.1	**Conclusion**	**Exploratory study:** The total recoveries were 92.37% and 99.47% for intravenous and dermal administration, respectively. A dermal absorption of 1.55% of the applied dose was determined. **Main study:** Dermal absorption normalised to 100% total recovery is 3.26% (3.15/96.75 × 100). The elimination time profile indicates that most of the absorbed material was excreted within the first 24 hours. A summary of the dermal absorption data is presented in Table A6_2-7.	X
5.1.1	Reliability	[redacted]	X

Section A6.2
Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (2)

Dermal absorption

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/06/2006
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*

Section A6.2 **Annex Point IIA6.2**	**Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (2)** Dermal absorption

Remarks

TableA6_2-1: Exploratory study: Dose administration

Route	Animal weight	Dose volume	Residue in catheter or micropipet		Dose administered		
			µCi	µg	µCi	µCi/kg	µg
i.v.[a]	3.2 kg	1.0 mL (1.089 g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Dermal[b]	2.7 kg	0.100 mL	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[a] the concentration of the radioactivity was 24.4 µCi/g and the concentration of thiacloprid was 0.216 mg/g.
[b] the concentration of the radioactivity was 251 µCi/mL and the concentration of thiacloprid was 2.24 mg/mL.

TableA6_2-2: Exploratory study: Elimination of radioactivity following intravenous (i.v.) and dermal dosing

Time (hours)	Percentage of the dose	
	Group 1 (234 µg/26.4 µCi)[a] (i.v. dosing)	Group 2 (222 µg/24.8µCi) (dermal dosing/9.25µg/cm^2)
Urine		
0-4	[redacted]	[redacted]
4-8	[redacted]	[redacted]
8-12	[redacted]	[redacted]
12-24	[redacted]	[redacted]
24-48	[redacted]	[redacted]
48-72	[redacted]	[redacted]
72-96	[redacted]	[redacted]
96-120	[redacted]	[redacted]
120-144	[redacted]	[redacted]
144-168	[redacted]	[redacted]
Subtotal	[redacted]	[redacted]
Faeces		
0-4	[redacted]	NS
4-8	[redacted]	[redacted]
8-12	[redacted]	[redacted]
12-24	[redacted]	[redacted]
24-48	[redacted]	[redacted]
48-72	[redacted]	[redacted]
72-96	[redacted]	[redacted]
96-120	[redacted]	[redacted]
120-144	[redacted]	[redacted]
144-168	[redacted]	[redacted]
Subtotal	[redacted]	[redacted]
Cage debris/rinse		
0-4	[redacted]	[redacted]
4-8	[redacted]	[redacted]
8-12	[redacted]	[redacted]
12-24	[redacted]	[redacted]
24-48	[redacted]	[redacted]
48-72	[redacted]	[redacted]
72-96	[redacted]	[redacted]
96-120	[redacted]	[redacted]
120-144	[redacted]	[redacted]
144-168	[redacted]	[redacted]
Subtotal	[redacted]	[redacted]

[a] Target dose. [b] Includes 0.03% recovered from 168-170 hour urine sample. [c] NS = no sample. [d] NA = not applicable.

Table A6_2-3 Exploratory study: Total recoveries

Sample	Percentage of the dose	
	Group 1 (234 µg) (i.v. dosing)	Group 2 (222 µg) (dermal dosing)
Elimination		
Urine		
Faeces		
Cage debris/rinse		
Chair wipe, urine pan wash/wipe		
Cage wash		
Subtotal		
Patch		
Protective device		
First four soapy swabs[b]		
Remaining soapy swabs		
Dry swabs		
Tape strips		
Alcohol swabs		
Subtotal		
Overall total		

[a] not applicable;
[b] a combination of the radioactivity from the soapy water extracts and the actual swabs.

Table A6_2-4: Main study: Dose administration (dermal absorption in vivo, monkeys)

Animal weight	Dose volume	Residue in micropipet		Dose administered				
		µCi	µg	µCi	µCi/kg	µg	µg/cm^2	µg/kg
4.5 kg	0.100 mL							
4.7 kg	0.100 mL							
5.1 kg	0.100 mL							
4.2 kg	0.100 mL							
6.1 kg	0.100 mL							
Mean								

[a] the concentration of the radioactivity was 150 µCi/mL and the concentration of thiacloprid was 1.34 mg/mL.

Table A6_2-5: Main study: Elimination of radioactivity following dermal dosing (mean values/n=5)

Time (hours)	Percent dose (%)		
	Urine	Faeces	Cage debris/rinse
0-4			
4-8			
8-12			
12-24			
24-48			
48-72			
72-96			
96-120			
120-144			

[a] range

Table A6_2-6: Main study: Total recoveries (mean values/n=5)

Sample	Percent of dose
Elimination	
Urine	
Faeces	
Cage debris/rinse	
Urine pan wash/wipe (4 hours)	
Chair wipe, urine pan wash/wipe (8 hours)	
Cage wash	
Cage wipe	
Subtotal	
Residual	
Patch	
Protective device	
First four soapy swabs[a]	
Remaining soapy swabs	
Dry swabs	
Alcohol swabs	
Subtotal	
Overall total	

[a] a combination of the radioactivity from the soapy water extracts and the actual swabs.

Table A6_2-7: Summary of the concentrations tested and percent absorbed

Test system (number)	Dose volume	Dose	Concentration tested	Dermal absorption (% of dose applied)
Monkey (n = 1)	0.100 mL			
Monkey (n = 5)	0.100 mL			

Section A6.2
Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (1)
Dermal absorption

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]2002): Application for approval for the use of YRC 2894 Calypso on various crops [redacted] Report No. MR-439/02, date: 2002-10-21. *Addendum I to PPP-Monograph; Chapter: B.6.12 Dermal Adsorption - Dermal penetration – b) in vivo data*	
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 428	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	X

3 MATERIALS AND METHODS

This study was performed to determine the dermal absorption of ^{14}C-YRC 2894 SC (i.e. ^{14}C-thiacloprid) when administered dermally to male rhesus monkeys.

The summarised results and protocol of an on going 2002 *in vivo* study have been submitted to support the proposed uses of thiacloprid (i.e. a draft report only). The test facility was the [redacted] This study involves 5 non-naive male monkeys (2-4 years of age) and a dose level of approximately 6 µg/cm^2 which reflects human exposure to in-use dilutions.

The animals were subjected to a pre-health assessment involving physical examinations, haematology and serum chemistry.

The test material was applied to shaved back skin for 8 hours using Duoderm patch material placed around the dosing site (4 x 6 cm) and a protective aluminium dome (animals in a restraint chair). A dose level of approximately 6 µg/cm^2 was applied to the test site. After 8 hours, the protective covering were removed and the application sites washed with a series of soap/water cotton-tipped swabs and cotton swabs dipped into isopropyl alcohol until the radioactivity in the last swab was less than 50,000 DPM.

During the study, urine and faeces were collected over 0-120 hours and frozen until analysed. Cage debris/cage rinse, cage wash, chair wash/wipe, chair/urine pan and screen pan wash/wipe were evaluated for radioactivity (LSC). The animals were sacrificed at the termination of this study.

Section A6.2 **Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (1)**

Annex Point IIA6.2 Dermal absorption

4 RESULTS AND DISCUSSION

Based on the summarised results, the total mean recovery of radioactivity was 97% of the applied dose. The great majority of this radioactivity (over 92%) was recovered in skin wash. Dermal penetration based on radioactivity in urine, faeces, pan, chair and cage wash and cage debris was 3.15% of the applied dose.

5 CONCLUSION

5.1 Conclusion

These results demonstrate that the *in-vivo* dermal absorption of the in-use dilution across primate skin is low (3%). The amount of thiacloprid placed on the skin was approximately 6 µg/cm^2 for 8 hours. X

[redacted]

5.1.1 Reliability [redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (1)

Dermal absorption

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/06/2006
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 A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (2)

Whole-body autoradiography in the rat

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1996): YRC 2894: general rat metabolism study. Part A: distribution of the total radioactivity in the rat determined by conventional whole-body autoradiography and radioluminography [redacted] Report No. PF4145, date: 1996-06-26.

PPP-Monograph: B.6.1 Absorption distribution excretion and metabolism (toxicokinetics). B.6.1.1Absorption, distribution and excretion. B.6.1.1.1 Whole-body autoradiography in the rat

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

Not applicable: conventional whole-body autoradiography using X-ray film and by the quantitative methodology of radioluminography

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1996 study, male Wistar rats (1/time point) were gavaged with single doses of ^{14}C-methylene radiolabelled YRC 2894 (thiacloprid) (in 0.5% Tragacanth) at 5.0 mg/kg bw. An additional rat was administered a single intravenous dose of 1.0 mg/kg bw. The distribution of radioactivity was investigated at 5 minutes (i.v. administration) and at 1, 4, 8, 24 and 48 hours (gavaged animals) by whole-body autoradiography (WBA). Tissue radioactivity levels were quantified using radioluminography. X

4 RESULTS AND DISCUSSION

Results indicate the rapid absorption and excretion of YRC 2894 (Table A6.1). Levels of radioactivity in all tissues examined were highest at one hour following oral administration. Levels decreased in all tissues and were low at the final time point of 48 hours. The highest concentration at each time point was noted in the preputial gland, a finding attributed to contamination with urine at sacrifice. Loss of urine can occur due to anaesthesia or muscle rigidity at sacrifice. This urine can contaminate the preputial gland because of the retrograde position of the penis. This assumption is supported by the fact that at 48 hours post administration, the concentration in the preputial gland was considerably lower and elimination of radioactivity was close to completion (i.e. low levels of radioactivity in the urine). High levels in the preputial gland (5 mins after i.v administration indicates very rapid excretion) and kidneys are consistent with rapid urinary excretion of X

thiacloprid (YRC 2894). Comparatively low levels in, and rapid elimination from fat is consistent with the polar character of YRC 2894 and indicates that accumulation in adipose tissue is unlikely. In the later stages of the study (24-48 h), autoradiographs indicate a comparatively high level of radioactivity associated with the connective tissue of the skin and aorta wall and in glandular organs. Autoradiography also revealed relatively high levels of radioactivity associated with the fur and nasal mucosa at later time points, suggesting some excretion in sweat and mucus. Findings from the intravenously dosed animal indicate a rapid and relatively even distribution in tissues.

5 CONCLUSION

5.1 Conclusion

After single oral administration of 5 mg [^{14}C] thiacloprid/kg bw to rats, the absorption and excretion of the radioactivity was rapid (after 48 hours very low levels of radioactivity in all tissues). Excretion took place mainly via urine, sweat and mucus. Relative high absorption was found in the connective tissue of the skin and aorta wall and in glandular organs.

Findings from the intravenously dosed animal (1.0 mg/kg bw) indicate a rapid and relatively even distribution in tissues.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (2)

Whole-body autoradiography in the 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	31.05.2006
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_2-1 Distribution of YRC 2894 (thiacloprid) (radioluminography)

Tissue	Concentration (µg/g equivalent)					
	1h	4h	8h	24h	48h	5 min (iv)
Blood	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bone	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bone marrow	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Brain	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Brown fat	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Liver	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Muscle	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Preputial gland	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Renal cortex	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Spleen	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Testes	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Thyroid	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (3)

Absorption, distribution and excretion in the rat

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998): [Methylene-^{14}C] YRC 2894: general rat metabolism study Part B: toxicokinetics and metabolism in the rat [redacted] Report No. PF4331, date: 1998-02-05.

1st part (a separate study summary was dedicated to the identification of the metabolites)

PPP-Monograph: B.6.1 Absorption distribution excretion and metabolism (toxicokinetics). B.6.1.1 Absorption, distribution and excretion. B.6.1.1.2 Absorption, distribution and excretion in the rat (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 Pesticide Assessment Guidelines; Subdivision F, § 85-1; General rat metabolism (November 1982)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1998 study, Wistar rats (5/sex/group) were gavaged with ^{14}C-methylene labelled YRC 2894 (thiacloprid) (in 0.5% Tragacanth) as detailed below (Table A6_2-1). An additional group of five males was administered a single intravenous injection of YRC 2894 at 0.5 mg/kg bw. Faeces, urine, plasma and expired carbon dioxide were collected for up to 48 hours when the animals were sacrificed. X

A commercial computer programme (TOPFIT) was used to analyse the plasma curves and calculate the pharmacokinetic parameters presented in Table A6_2-2. Linear standard compartment models (1-4 compartments) were used for the computations.

4 RESULTS AND DISCUSSION

Plasma levels of radioactivity indicate the rapid absorption of YRC 2894 following oral administration (Table A6_2-2). Maximum plasma concentrations were achieved after 1-1.5 hours at the low dose level and 3-4 hours at the high dose level. Maximum concentrations were similar in males and females of the low dose and repeat dose groups. The maximum plasma concentration in females of the high dose groups was approximately 1.75 times that of the males. A comparison of the plasma curves suggested that absorption was slower and possibly incomplete at X

high dose levels.

In most of the tests, the elimination of the radioactivity from plasma could be approximated by a combination of two exponential terms, from which elimination half-lives were calculated. The terminal half-lives varied between approximately 7 and 80 hours. The final elimination phases took place at very low concentration levels, hence their contribution to the AUC was marginal.

The large volume of distribution (Vd) suggests the rapid distribution of YRC 2894 and/or its metabolites into tissues. The relatively small mean residence times (MRT) indicate that redistribution into plasma prior to excretion was also rapid. The report author states that the high MRT and $t_{1/2}$ values for repeat-dosed females are due to an unsatisfactory mathematical model of the plasma curve. This assertion is supported by the comparable clearance (Cl) values for both sexes. The total clearance (average = 2.1 ml/min) had the same order of magnitude as the glomerular filtration rate of the rat.

Radioactivity was excreted primarily in urine (53.0-68.1%) and largely during the first 24 hours following oral administration (Table A6_2-3). Significant faecal excretion of radioactivity was also seen in most groups (24.7-39.1%), with the exception of high dose females (9.1%). This finding and the relatively high levels of radioactivity associated with the gastrointestinal tract in this group indicate delayed faecal excretion. The lower excretion rate in high dose animals may be due to the slower absorption process. Faecal radioactivity in intravenously dosed animals was comparable to gavaged animals, suggesting significant biliary excretion of thiacloprid (YRC 2894). Excretion of radiolabelled carbon dioxide in expired air was found to be minimal (≤0.005% of the administered dose).

Tissue residues at 48 hours post-dosing were minimal in low dose and repeat-dosed animals, accounting for 0.41-0.66% of administered radioactivity (Table A6_2-4). Residues were slightly higher in high dose males (1.63%), particularly in the liver and kidney. Total residues were significantly higher in high dose females (26.6%), largely due to gastrointestinal residues. Findings indicate the delayed absorption or excretion of YRC 2894 in this group.

5 CONCLUSION

5.1 Conclusion

Plasma levels of radioactivity indicated that radioactivity was absorbed rapidly following oral administration (max. concentration at 1-4 hours).

Rapid distribution of thiacloprid and its metabolites into tissues.

Elimination half-lives were between 7 and 80 hours, excretion was significant at low dose levels and took place via urine and to a less extent defecation.

After 48 hours post-dosing tissues residues were minimal (highest concentration in liver and kidney). Females presented delayed absorption and excretion.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (3)

Absorption, distribution and excretion in the 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	31.05.2006
Materials and Methods	[redacted]

Section A6.2 **Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (3)**

Annex Point IIA6.2

Absorption, distribution and excretion in the rat

Results and discussion

[redacted]

Conclusion

Reliability

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (3)

Absorption, distribution and excretion in the rat

Acceptability	Acceptable
Remarks	None
	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_2-1 Study design

Dose Level	Route	Samples	Sex
1 mg/kg bw	Gavage	Urine, faeces, plasma, expired CO_2	M
1 mg/kg bw	Intravenous	Urine, faeces, plasma	M/F
1 mg/kg bw	Gavage	Urine, faeces, plasma	M/F
1 mg/kg bw (unlabelled) x 14 + 1 mg/kg bw	Gavage	Urine, faeces, plasma	M/F
100 mg/kg bw	Gavage	Urine, faeces, plasma	M/F

Table A6_2-2 Plasma levels of 14C-methylene labelled thiacloprid (YRC 2894)

Time Point	Mean plasma levels of YRC 2894 (g/kg equivalent)							
	1 mg/kg (iv)		1 mg/kg		1 mg/kg Repeat		100 mg/kg	
	M	F	M	F	M	F	M	F
5 min	[redacted]							
10 min	[redacted]							
20 min	[redacted]							
40 min	[redacted]							
1 h	[redacted]							
1.5 h	[redacted]							
2 h	[redacted]							
3 h	[redacted]							
4 h	[redacted]							
6 h	[redacted]							
8 h	[redacted]							
24 h	[redacted]							
32 h	[redacted]							
48 h	[redacted]							
Kinetic Parameters								
Vd (l/kg)	[redacted]							
Cl (ml/min)	[redacted]							
$t_{1/2}$ (h)[a]	[redacted]							
AUC (µg/ml.h)	[redacted]							
MRT (h)	[redacted]							

Key: a) Terminal half-lives, mostly based on two exponential terms. b) The report considered this result to be artificial because the unsatisfactory mathematical description of the plasma curve required three elimination terms.

Table A6_2-3 Excretion of radioactivity (% of administered dose)

Sample	Time	1 mg/kg (iv)		1 mg/kg			100 mg/kg		1 mg/kg Repeat	
		M	F	M	F	M	M	F	M	F
Urine	4h	[redacted]								
	8h	[redacted]								
	24h	[redacted]								
	48h	[redacted]								
Faeces	24h	[redacted]								
	48h	[redacted]								
Expired air	4h	[redacted]								
	8h	[redacted]								
	24h	[redacted]								
	48h	[redacted]								
Total excreted		[redacted]								
Carcass		[redacted]								
GI tract		[redacted]								
Total Recovery		[redacted]								

Table A6_2-4 Residual radioactivity 48 hours after dosing

Tissue	Residues at 48 hours (µg/g equivalent)							
	1 mg/kg (iv)		1 mg/kg		100 mg/kg		1 mg/kg Repeat	
	M	F	M	F	M	F	M	F
RBC	[redacted]							
Plasma	[redacted]							
Spleen	[redacted]							
GI tract	[redacted]							
Liver	[redacted]							
Kidney	[redacted]							
Fat	[redacted]							
Gonads	[redacted]							
Uterus	[redacted]							
Muscle	[redacted]							
Bone	[redacted]							
Heart	[redacted]							
Lung	[redacted]							
Brain	[redacted]							
Skin	[redacted]							
Carcass	[redacted]							

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (4)

Absorption, distribution and excretion in the rat

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1997): [Thiazolidine-4,5-^{14}C]YRC 2894: absorption, distribution, excretion and metabolism in the rat [redacted] Report No. PF4299, date: 1997-12-08, *revised 1998-03-05, revised 1998-06-29.*

1st part (a separate study summary was dedicated to the identification of the metabolites)

PPP-Monograph: B.6.1 Absorption distribution excretion and metabolism (toxicokinetics). B.6.1.1 Absorption, distribution and excretion. B.6.1.1.2 Absorption, distribution and excretion in the rat (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

US-EPA Pesticide Assessment Guidelines Subdivision F, § 85-1 General rat metabolism (November 1982)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1997 study, groups of Wistar rats (5/sex) were gavaged with ^{14}C-thiazolidine labelled YRC 2894 (thiacloprid) in 0.5% Tragacanth as detailed in Table A6_2-1. Plasma, urine, faeces and expired carbon dioxide were collected for up to 48 hours when animals were terminated.

4 RESULTS AND DISCUSSION

Plasma radioactivity levels indicate the rapid absorption of YRC 2894 (Table A6_2-2). Maximum plasma levels were attained at 2-3 hours (low dose level) and at 4 hours (high dose level). Plasma radioactivity levels also indicate the rapid distribution and elimination of YRC 2894 at the low dose level. Slower rates of absorption and excretion were seen at the high dose level, with significant plasma levels of radioactivity at 48 hours. The large volumes of distribution (Vd) suggest the rapid distribution of YRC 2894 into tissues. The relatively small mean residence times (MRT) indicate that redistribution into plasma prior to excretion was also rapid. X

Radioactivity was excreted primarily in the urine (60.2-82.9%) and largely during the first 24 hours following oral administration (Table

A6_2-3). Faecal excretion was also significant (13.3-18.6%). Excretion of radiolabelled carbon dioxide in expired air was found to be minimal (0.86%).

Total tissue residues at 48 hours in the low dose group accounted for 1.6-3.3% of the administered radioactivity (Table A6_2-4). Residues were highest in the liver in all low dose groups. In the high dose group, residues accounted for 12.4% of the administered radioactivity, largely due to high gastrointestinal tract residues. Findings at this dose level indicate delayed absorption or faecal excretion in this group.

^{14}C-thiazolidine labelled YRC 2894 was found to be rapidly and extensively absorbed in the rat. No significant differences in the extent or route of excretion were noted between dose groups or sexes. Findings in high dose group males are consistent with delayed absorption.

Plasma levels of radioactivity were comparable for ^{14}C-thiazolidine and ^{14}C-methylene labelled YRC 2894. The clearance values were slightly greater for ^{14}C-methylene labelled YRC 2894. While the AUC values were markedly greater for the ^{14}C-thiazolidine labelled YRC 2894. The AUC value for the ^{14}C-thiazolidine labelled YRC 2894 was approximately 170 times greater than the value obtained for the ^{14}C-methylene labelled YRC 2894 at the 100 mg/kg bw dose level. Excretion of radioactivity in urine was slightly greater and consequently lower in faeces for ^{14}C-thiazolidine labelled YRC 2894. Residues of ^{14}C-thiazolidine labelled YRC 2894 were found to be higher than residues of ^{14}C-methylene labelled YRC 2894.

5 CONCLUSION

5.1 Conclusion

Plasma levels of radioactivity indicated that radioactivity was absorbed rapidly following oral administration (max. concentration at 2-3 hours). Slightly slower rates of absorption (max. concentration at 4h) and excretion were seen at the high dose level.

Rapid distribution and elimination of thiacloprid and its metabolites into tissues at low dose levels. Excretion took place via urine and to a less extent defecation, amount in expired air was minimal.

After 48 hours post-dosing tissues residues were < 3.3 % (highest concentration in liver and gastrointestinal tract). Residues of ^{14}C-thiazolidine labelled YRC 2894 were found to be higher than residues of ^{14}C-methylene labelled YRC 2894.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (4)

Absorption, distribution and excretion in the 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	01.06.2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (4)

Absorption, distribution and excretion in the rat

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_2-1 Study design

Dose Level	Route	Sample	Sex
1 mg/kg bw	Gavage	Urine, faeces, expired CO_2	M
1 mg/kg bw	Gavage	Urine, faeces, plasma	M/F
100 mg/kg bw	Gavage	Urine, faeces, plasma	M

Table A6_2-2 Plasma levels of 14C-thiazolidine labelled YRC 2894

Time Point	Mean plasma levels (µg/kg equivalent)		
	1 mg/kg		100 mg/kg
	M	F	M
5 min	[redacted]	[redacted]	[redacted]
10 min	[redacted]	[redacted]	[redacted]
20 min	[redacted]	[redacted]	[redacted]
40 min	[redacted]	[redacted]	[redacted]
1 h	[redacted]	[redacted]	[redacted]
1.5 h	[redacted]	[redacted]	[redacted]
2 h	[redacted]	[redacted]	[redacted]
3 h	[redacted]	[redacted]	[redacted]
4 h	[redacted]	[redacted]	[redacted]
6 h	[redacted]	[redacted]	[redacted]
8 h	[redacted]	[redacted]	[redacted]
24 h	[redacted]	[redacted]	[redacted]
32 h	[redacted]	[redacted]	[redacted]
48 h	[redacted]	[redacted]	[redacted]
Kinetic Parameters			
Vd (l/kg)	[redacted]	[redacted]	[redacted]
Cl (ml/min.kg)	[redacted]	[redacted]	[redacted]
$t_{1/2}$ (h)[a & b]	[redacted]	[redacted]	[redacted]
AUC (µg/ml.h)	[redacted]	[redacted]	[redacted]
MRT (h)	[redacted]	[redacted]	[redacted]

Key: a) Based on the first elimination phase.
b) The terminal elimination half-lives ranged from approximately 10-44 hours.

Table A6_2-3 Excretion of radioactivity (% of administered dose)

Sample	Time	1 mg/kg bw			100 mg/kg
		M	F	M	M
Urine	4h	[redacted]	[redacted]	[redacted]	[redacted]
	8h	[redacted]	[redacted]	[redacted]	[redacted]
	24h	[redacted]	[redacted]	[redacted]	[redacted]
	48h	[redacted]	[redacted]	[redacted]	[redacted]
Faeces	24h	[redacted]	[redacted]	[redacted]	[redacted]
	48h	[redacted]	[redacted]	[redacted]	[redacted]
Expired air	48h	[redacted]	[redacted]	[redacted]	[redacted]
Total excreted		[redacted]	[redacted]	[redacted]	[redacted]
Carcass		[redacted]	[redacted]	[redacted]	[redacted]
GI tract		[redacted]	[redacted]	[redacted]	[redacted]
Total Recovery		[redacted]	[redacted]	[redacted]	[redacted]

Table A6_2-4 Residual radioactivity 48 hours after dosing

Tissue	Residues at 48 hours (µg/g equivalent)		
	1 mg/kg		100 mg/kg
	M	F	M
RBC	[redacted]	[redacted]	[redacted]
Plasma	[redacted]	[redacted]	[redacted]
Spleen	[redacted]	[redacted]	[redacted]
GI tract	[redacted]	[redacted]	[redacted]
Liver	[redacted]	[redacted]	[redacted]
Kidney	[redacted]	[redacted]	[redacted]
Fat	[redacted]	[redacted]	[redacted]
Adrenals	[redacted]	[redacted]	[redacted]
Gonads	[redacted]	[redacted]	[redacted]
Uterus	[redacted]	[redacted]	[redacted]
Muscle	[redacted]	[redacted]	[redacted]
Bone	[redacted]	[redacted]	[redacted]
Heart	[redacted]	[redacted]	[redacted]
Lung	[redacted]	[redacted]	[redacted]
Brain	[redacted]	[redacted]	[redacted]
Thyroid	[redacted]	[redacted]	[redacted]
Skin	[redacted]	[redacted]	[redacted]
Carcass	[redacted]	[redacted]	[redacted]

Section A6.2
Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (5)
Metabolites analysis

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998): [Methylene-14C] YRC 2894: general rat metabolism study Part B: toxicokinetics and metabolism in the rat [redacted] Report No. PF4331, date: 1998-02-05.

2nd part

PPP-Monograph: B.6.1 Absorption distribution excretion and metabolism (toxicokinetics). B.6.1.2 Metabolism. B.6.1.2.1 ^{14}C-methylene labelled YRC 2894

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

US-EPA Pesticide Assessment Guidelines, Subdivision F, § 85-1, General rat metabolism (November 1982)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

The metabolism of ^{14}C-methylene labelled YRC 2894 (thiacloprid) was investigated in a 1998 study. Faecal and urine samples analysed were collected as part of the study reported before (*cf.* [redacted] 1998, 1st part). Metabolites were identified by HPLC, GC-MS, LC-MS and 1H-NMR.

4 RESULTS AND DISCUSSION

A total of 14 metabolites were identified, accounting for 57.8-78.7 of the administered radioactivity (Table A6_2-1). Small amounts (0.9-6.4%) of unchanged parent were also identified. With the exception of high dose females, the major metabolite in all groups was identified as M07 (11.5-34.2%). The major metabolite in high dose females was found to be (M12 + M13), accounting for 13.2% of the administered radioactivity. The major faecal metabolites were found to be M01 and unchanged parent. The amount of M11 was found to be significantly higher in top dose males (13.4%) than in top dose females (1.4%). Both M11 and M16 were found in higher amounts in male excreta while M06 and M07 were found in higher amounts in females.

The main metabolic transformation was the oxidative cleavage of the parent molecule to yield 6-chloronicotinic acid (M03) which then

Section A6.2 **Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (5)**

Annex Point IIA6.2 Metabolites analysis

reacted with glycine to form a hippuric acid type conjugate (M07). Other metabolic reactions included oxidation of the N-nitrile group to form amide derivatives (M11 & M17), opening of the thiazolidine ring by oxidation to give sulfoxide compounds (M08 & M10) and the replacement of the chlorine atom attached to the pyridine ring of chloronicotinic acid by mercaptoacetic acid (M06). In the high dose groups, the levels of unchanged parent compound were increased while the levels of M07 decreased. This suggested that the metabolic rate was reduced at high dose levels.

5 CONCLUSION

5.1 Conclusion

The samples of another study for absorption-distribution-excretion were analysed to identify the metabolites formed after oral administration of thiaclorpid in the rat. A total of 14 different metabolites were detected. The metabolic rate was reduced at high dose levels.

X

[redacted]

5.1.1 Reliability

[redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (5)

Metabolites analysis

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01.06.2006
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_2-1 Metabolites of ^{14}C-methylene YRC 2894

Metabolite		Identified metabolites (% of administered radioactivity)							
		1 mg/kg iv		1 mg/kg		1 mg/kg Repeat		100 mg/kg	
		M	F	M	F	M	F	M	F
M06	U								
	F								
M03	U								
M07	U								
M08	U								
	F								
M09	U								
	F								
M10	U								
	F								
M11	U								
	F								
M12 + M13	U								
	F								
M14	U								
	F								
M15	U								
	F								
M16	U								
	F								
M01	U								
	F								
YRC 2894	U								
	F								
M17	U								
	F								
Identified	U								
	F								
Total									

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (6)

Metabolites analysis

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1997): [Thiazolidine-4,5-^{14}C]YRC 2894: absorption, distribution, excretion and metabolism in the rat [redacted] Report No. PF4299, date: 1997-12-08, *revised 1998-03-05, revised 1998-06-29.*

2nd part (conducted in 1998).

PPP-Monograph: B.6.1 Absorption distribution excretion and metabolism (toxicokinetics). B.6.1.2 Metabolism. B.6.1.2.2 ^{14}C-thiazolidene labelled YRC 2894

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 Pesticide Assessment Guidelines, Subdivision F, § 85-1, General rat metabolism (November 1982)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

The metabolism of ^{14}C-methylene labelled YRC 2894 (thiacloprid) was investigated in a 1998 study. Faecal and urine samples analysed were collected as part of the study reported before (*cf.* [redacted], 1997, 1st part). Metabolites were identified by HPLC, GC-MS, LC-MS and ^{1}H-NMR.

4 RESULTS AND DISCUSSION

YRC 2894 (thiacloprid) was found to be extensively metabolised. A total of 17 metabolites accounting for 55.3-64.3% of the administered radioactivity were identified (Table A6_2-1). The major metabolites were found to be M19 (11.4%) in low dose males, M22 (22.2%) in low dose females and M01 (10.3%) in high dose males. The major faecal metabolites were found to be M01 and unchanged parent. X

The metabolic transformations included oxidative cleavage of the methylene bridge, hydroxylation and conjugation of the thiazolidine ring followed by further oxidation to the ketone, hydroxylation of the cyanamide moiety, opening of the thiazolidine ring at two positions followed by further oxidation to the carboxylic acid and methylation of the sulphur atom. The formation of an oxazole ring (M16) was explained by re-closure of the ring in the metabolite M26. The report

Section A6.2 **Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (6)**

Annex Point IIA6.2 Metabolites analysis

stated that this reaction had been observed artificially for isolated M26.

5 CONCLUSION

5.1 Conclusion

The samples of another study for absorption-distribution-excretion were analysed to identify the metabolites formed after oral administration of thiacloprid in the rat. A total of 17 different metabolites were detected. X

[redacted]

5.1.1 Reliability [redacted]

Section A6.2

Annex Point IIA6.2

Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study (6)

Metabolites analysis

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	01.06.2006
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_2-1 Metabolites of ^{14}C-thiazolidine YRC 2894

Metabolite	% administered radioactivity		
	1 mg/kg		100 mg/kg
	M	F	M

M18	**U**	[redacted]	[redacted]	[redacted]
M19	**U**	[redacted]	[redacted]	[redacted]
	F	[redacted]	[redacted]	[redacted]
M20	**U**	[redacted]	[redacted]	[redacted]
M21	**U**	[redacted]	[redacted]	[redacted]
M22	**U**	[redacted]	[redacted]	[redacted]
M23	**U**	[redacted]	[redacted]	[redacted]
M08	**U**	[redacted]	[redacted]	[redacted]
M24	**U**	[redacted]	[redacted]	[redacted]
M25	**U**	[redacted]	[redacted]	[redacted]
M10	**U**	[redacted]	[redacted]	[redacted]
	F*	[redacted]	[redacted]	[redacted]
M11	**U**	[redacted]	[redacted]	[redacted]
M26	**U**	[redacted]	[redacted]	[redacted]
M12	**U**	[redacted]	[redacted]	[redacted]
M27	**U**	[redacted]	[redacted]	[redacted]
M16	**U**	[redacted]	[redacted]	[redacted]
	F	[redacted]	[redacted]	[redacted]
M01	**U**	[redacted]	[redacted]	[redacted]
	F	[redacted]	[redacted]	[redacted]
YRC 2894	**U**	[redacted]	[redacted]	[redacted]
	F	[redacted]	[redacted]	[redacted]
Total	U	[redacted]	[redacted]	[redacted]
	F	[redacted]	[redacted]	[redacted]
Total Identified		[redacted]	[redacted]	[redacted]

* *sum of M10 and M11*

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (1)**

Annex Point IIA6.3 Rat, 14 days

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1995b *[Monograph: 1995a]*): YRC 2894 - Pilot toxicity study on rats [redacted] Report No. 23861, date: 1995-03-22.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.1 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 (pilot study);

Mainly in compliance with OECD guideline 407 (1981)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 pilot study, Wistar rats (3/sex/dose) were administered YRC 2894 (thiacloprid) (purity: [redacted]) by gavage at dose levels of 0, 5, 10, 20, 60 or 120 mg/kg bw/day over a period of 14 days. The test material was administered in demineralised water with 2% v/v Cremophor EL.

Clinical observations, body weight and group food and water intakes were recorded at appropriate time points. Clinical pathology (haematology, serum biochemistry) was performed at the end of treatment. Blood glucose and urine were evaluated on day 9. The following investigations were also carried out: a) enzyme induction in liver tissue, b) thyroid hormones levels in the blood, c) cell proliferation in selected organs using PCNA techniques (top dose group). In addition, immunotoxicological examinations were also performed: spleen, lymph node and bone marrow cell counts, macrophage activity and mitogen stimulation in spleen and lymph nodes, Ig determinations in serum and FACScan analysis of spleen and lymph node cells (cell populations stained with surface markers/fluorescence evaluation techniques). An extensive list of organs were weighed and subjected to gross pathological and histopathological examination at necropsy.

4 RESULTS AND DISCUSSION

No animals died during the study. Clinical observations included reduced reactivity in males at 120 mg/kg bw/day, and a reduced production of faeces in females at 60 mg/kg bw/day and above. Mean body weight gains and food intakes were reduced in both sexes at 60 mg/kg bw/day and above. X

There were no treatment-related effects on the red blood cell parameters

but the mean leukocyte count was reduced in both sexes at 120 mg/kg bw/day. There were no treatment-related effects on thyroid hormone levels (TSH, T3 & T4). The main biochemical changes detected in serum are presented in Table A6_3_1-1.

Increased levels of ASAT, ALAT and AP were noted in females at 120 mg/kg bw/day. Examination of the liver tissue provided evidence of enzyme induction in both sexes at 60 mg/kg bw/day and above (Table A6_3_1-2). Males also displayed marginal enzyme induction at 20 mg/kg bw/day.

The immunotoxicological investigations revealed that the cell count in the spleen of males was reduced at 120 mg/kg bw/day but this may be a chance effect due to large individual variations. Increased macrophage activation and other changes in cell counts were reported but they may also be related to variation and small sample size. The cell proliferation assays revealed increased cell proliferation in the perivenular region of the liver in females at 120 mg/kg bw/day. A reduction in cell proliferation, particularly in the renal medulla, and a lesser extent in the renal cortex, was reported in females at 120 mg/kg bw/day.

The mean thymus weights (absolute) were reduced at dose levels > 60 mg/kg bw/day. The mean relative liver weights of males and females were increased at dose levels > 60 mg/kg bw/day. While mean absolute liver weight were increased in both sexes at 120 mg/kg bw/day (*cf.* Table A6_3_1-3). These liver weight changes correlated histopathologically with a slightly untypical structure of the hepatocellular cytoplasm. Microscopy also revealed an increased mitotic rate in the thyroids of male rats at 120 mg/kg bw/day.

A NOAEL of 20 mg/kg bw/day was determined for this study based on reduced body weight gain, reduced food intake and clinical signs at the next highest dose level.

5 CONCLUSION

5.1 Conclusion

The NOAEL in this study was 20 mg/kg bw based on impaired body weight development and clinical signs at doses of ≥ 60 mg/kg bw.

[redacted]

5.1.1 Reliability [redacted]

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (1)**

Annex Point IIA6.3 Rat, 14 days

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27/06/2006
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_3_1-1 Biochemical changes detected in serum (mean values)

Dose (mg/kg bw/day)	0	5	10	20	60	120
Males						
ASAT (U/l)						
ALAT (U/l)						
AP (U/l)						
CHOL (mmol/l)						
Females						
ASAT (U/l)						
ALAT (U/l)						
AP (U/l)						
CHOL (mmol/l)						

Table A6_3_1-2 Liver enzyme and triglyceride determinations (mean values)

Dose (mg/kg bw/day)	0	5	10	20	60	120
Males						
P 450 (nmol/g)						
O-DEM (mU/g)						
N-DEM (mU/g)						
TRIGL (mcmol/g)						
ECOD (nmol/g/min)						
EROD (nmol/g/min)						
ALD (nmol/g/min)						
EH (nmol/g/min)						
[redacted]						
GLU-T (nmol/g/min)						
Females						
P 450 (nmol/g)						
O-DEM (mU/g)						
N-DEM (mU/g)						
TRIGL (mcmol/g)						
ECOD (nmol/g/min)						
EROD (nmol/g/min)						
ALD (nmol/g/min)						
EH (nmol/g/min)						
GS-T (nmol/g/min)						
UDPGT (nmol/g/min)						

Key: a) O-demethylase (O-DEM). b) N-demethylase (N-DEM). c) 7-ethoxycoumarin deethylase (ECOD). d) 7-ethoxyresorufin deethylase (EROD). e) aldrin epoxidase (ALD). f) epoxide hydrolase (EH). g) glutathione-S-transferase (GS-T). h) UDP-glucuronyl transferase (UDPGT). i) Triglycerides (TRIGL).

Table A6_3_1-3 Mean liver weight (absolute and relative) in male and female rats

Dose (mg/kg bw/day)	0	5	10	20	60	120
Males						
Body weight (g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Absolute (mg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Relative (mg/100g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females						
Body weight	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Absolute (mg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Relative (mg/100g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Section A6.3.1 Short-term repeated dose toxicity (28 days) – oral (2)

Annex Point IIA6.3 Rat, 14 days

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1996c): YRC 2894 - Study for subacute oral toxicity in rats (Feeding study over 2 weeks) [redacted] Report No.: 25720, date: 1996-12-09. Amendment Report No. 25720A, date: 1999-02-22. *PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.1 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; OECD guideline 407
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In 1994 study, Wistar rats (5/sex/dose) were fed diet containing 0, 25, 100, 500 or 2000 ppm YRC 2894 (thiacloprid) (purity: [redacted]) for 14 days. The objective of this study was to investigate the effects of the test material on the liver and thyroid.

Clinical signs of toxicity, body weight changes, food consumption and water intakes were recorded at suitable time points. Clinical chemistry tests were carried out after 7 days and at the end of treatment. All the animals were examined macroscopically at termination. The liver and thyroid were removed and weighed, and subjected to gross and histopathological examinations. Liver samples were taken for enzyme determinations.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equivalent to 0, 2.5, 11.2, 49.2 and 187.6 mg/kg bw/day in males, and 0, 2.3, 9.8, 49.5 and 187.2 mg/kg bw/day in females, at dose levels of 0, 25, 100, 500 and 2000 ppm, respectively.

X

No deaths occurred during the study. Constipation was evident on day 1 in 1/5 females at 500 ppm and 3/5 males and 4/5 females at 2000 ppm. Significant reductions in mean body weight were seen in both sexes at 2000 ppm and small reductions were seen in females at 500 ppm. The mean body weight values are presented in Table A6_3_1-1. Food consumption was significantly reduced during week 1 at 2000 ppm. Water intake was increased in 2000 ppm females during week 2.

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (2)**

Annex Point IIA6.3 Rat, 14 days

4 RESULTS AND DISCUSSION

The main clinical chemistry changes are presented in Table A6_3_1-2 . On day 7, cholesterol levels were significantly increased in males at 100 ppm and above and in females at 2000 ppm. After 14 days of treatment, cholesterol levels were increased in both sexes at the top dose only. There was a significant increase in thyroid stimulating hormone (TSH) in females on day 14 at 2000 ppm. No treatment-related effects were seen on the levels of triiodothyronine (T3) and thyroxine (T4) or the thyroxin-binding capacity (TBC).

Liver enzymes (7-ethoxycoumarin deethylase (ECOD), aldrin epoxidase (ALD), epoxide hydrolase (EH), glutathione-S-transferase (GS-T), UDP-glucuronyl transferase (UDPGT)) were induced in both sexes at 500 ppm and above.

Gross necropsy revealed liver distinct lobulation in males (4/5) and females (2/5) at 2000 ppm and in females at 25 (1/5), 100 (1/5) and 500 ppm (1/5). There was an increase in the relative weights of liver (males and females) and thyroids (females) at 2000 ppm. Microscopic examination revealed hepatocellar hypertrophy in both sexes at dose levels >500 ppm. This hypertrophy correlated with slight cytoplasmic changes. The thyroids of males exhibited an increased mitotic rate at dose levels >500 ppm and hypertrophy of the follicular epithelium at 2000 ppm.

Based on body weight effects in females and thyroid effects in males at the next highest dose, a NOAEL of 100 ppm (equivalent to 11.2 and 9.8 mg/kg bw in males and females, respectively) was determined for this study.

5 CONCLUSION

5.1 Conclusion

The NOAEL of 100 ppm (equivalent to 11.2 mg/kg bw in males and to 9.8 mg/kg bw in females) is based on body weight effects in females and morphological changes in the thyroids (hypertrohic follicular epithelium) at doses of ≥ 500 ppm.

[redacted].

5.1.1 Reliability

[redacted]

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (2)**

Annex Point IIA6.3 Rat, 14 days

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/06/2006
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_3_1-1 Mean body weights (g) of male and female rats

Dose (ppm)	0	25	100	500	2000
Males					
Day 0	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 7	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 14	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females					
Day 0	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 7	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Day 14	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: $p \leq 0.05$, ** $p \leq 0.01$ (U test)

Table A6_3_1-2 Mean clinical chemistry and hormonal changes

Dose (ppm)	0	25	100	500	2000
Males-Day 7.					
GGT (U/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cholesterol (mmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bile acid (mcmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TSH (mcg/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Males-Day 14.					
GGT (U/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cholesterol (mmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bile acid (mcmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TSH (mcg/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females-Day 7.					
GGT (U/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cholesterol (mmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Protein (g/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Albumon (g/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TSH (mcg/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females-Day 14.					
GGT (U/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cholesterol (mmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bile acid (mcmol/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
TSH (mcg/l)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: $p \leq 0.05$, ** $p \leq 0.01$

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (3)**

Annex Point IIA6.3 Mouse, 3 weeks

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1 REFERENCE

1.1 Reference

[redacted] 1994): YRC 2894 - Pilot study on subacute toxicity in B6C3F1 mice (Administration in feed over 3 weeks) [redacted] Report No. 23450, date: 1994-11-04.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.2 Mice (Study1)

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;

in general agreement with OECD guideline 407

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 pilot study, B6C3F1 mice (3/sex/dose) were administered YRC 2894 (thiacloprid) ([redacted] purity) in diet at dose levels of 0, 100, 1000 or 10,000 ppm for up to 3 weeks.

Clinical observations, body weight and food and water intake were recorded at suitable time points. A macroscopic examination was performed at necropsy and selected organs (liver and kidneys) were removed and weighed.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The daily intakes were equivalent to 0, 30.1, 367.8 and 4141.0 mg/kg bw/day in males, and 0, 63.9, 559.3 and 5785.1 mg/kg bw/day in females, at 0, 100, 1000 and 10,000 ppm, respectively.

4 RESULTS AND DISCUSSION

No animals died during the study or showed clinical signs of toxicity. Body weight gain was reduced in males at 10,000 ppm. At this dose level food intake was reduced and, therefore, feed efficiency decreased in males. Food intake was reduced in females at 1000 ppm and above. Water intake was reduced in females at 10,000 ppm.

Gross necropsy revealed enlarged livers in 2/3 males at 10,000 ppm. Liver weights (absolute and relative) were increased in both sexes at 1000 ppm and above. Although liver enzyme activities were not determined in this study, the liver weight increases are regarded as a consequence of liver enzyme induction, which has been demonstrated for mice in other

X

studies.

Based on reduced food intake in females and increased liver weight in both sexes at 1000 ppm (liver enzyme activities were not determined), a NOAEL of 100 ppm was determined for this study (equivalent to 30.1 and 63.9 mg/kg bw in males and females, respectively). This non-GLP study was conducted in general agreement with OECD guidelines (No 407, 1981).

5 CONCLUSION

5.1 Conclusion

A dose of 100 ppm (equivalent to 30.1 mg/kg bw in males) is regarded as the NOAEL based on effects on body weight development and feed intake and liver weight increase.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (3)**

Annex Point IIA6.3 Mouse, 3 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30/06/2006
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 A6.3.1 **Short-term repeated dose toxicity (28 days) – oral (4)**

Annex Point IIA6.3 Mouse, 14 days

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1 REFERENCE

1.1 Reference

[redacted] (1997a). YRC 2894 - Study for subacute oral toxicity in mice (Feeding study over 2 weeks) [redacted] Report No. 26017, date: 1997-20-25.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.2 Mice (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 407

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In 1994 study, B6C3F1/Bom mice (5/sex/dose) were fed diet containing 0, 50, 200, 2000 or 10000 ppm YRC 2894 (thiacloprid) (purity: [redacted]) for 14 days. The objective of this study was to investigate the effects of the test material on the liver.

Clinical signs of toxicity, body weight changes, food consumption and water intakes were recorded at suitable time points. Haematological and biochemical investigations were carried out at the end of the study. All the animals received a macroscopic examination at necropsy. The liver was removed, weighed and subjected to microscopic examination. Liver samples were taken for enzyme determinations.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equivalent to 0, 21.6, 84.3, 765.1 and 4143.2 mg/kg bw/day in males, and 0, 29.8, 113.2, 1201.2 and 5449.8 mg/kg bw/day in females at dose levels of 0, 50, 200, 2000 and 10000 ppm, respectively.

4 RESULTS AND DISCUSSION

No animals died during the study or showed clinical signs of toxicity. Body weights and food intakes were not affected by treatment. Water intakes were reduced in males at 2000 ppm and above. X

In top dose males, there was a significant reduction in the mean cholesterol value and a significant increase in the mean protein value. Whereas in females, there was a significant reduction in mean albumen, cholesterol and bilirubin values at the top dose level. Dose-dependent

enzyme induction was evident in the liver of both sexes at dose levels ≥ 2000 ppm with some marginal effects at 200 ppm (Table A6_3_1-1).

No gross findings were seen at necropsy. Mean liver weight (absolute and relative) was increased in both sexes at dose levels ≥ 2000 ppm (Table A6_3_1-2). Microscopy revealed hypertrophy of the centrilobular hepatocytes in males at 200 ppm and above and in females at 2000 ppm and above. The lipid content in hepatocytes was increased in both sexes at 2000 ppm and above.

Based on the liver effects (weight, lipid content and enzyme induction) at 2000 ppm, a NOAEL of 200 ppm was determined for this study (equivalent to 84.3 and 113.2 mg/kg bw/day in males and females, respectively).

5 CONCLUSION

5.1 Conclusion

The NOAEL of 200 ppm (equivalent to 84.3 mg/kg bw in males) is based on stronger liver effects (increased liver weight and an increased lipid content in hepatocytes) at 2000 ppm. X

[redacted]

5.1.1 Reliability [redacted]

Section A6.3.1 Short-term repeated dose toxicity (28 days) – oral (4)

Annex Point IIA6.3 Mouse, 14 days

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30/06/2006
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_3_1-1 Liver enzyme determinations

Dose	0	50	200	2000	10000
Males					
ECOD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
EROD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ALD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
EH*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
GS-T**	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
UDPGT*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females					
ECOD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
EROD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ALD*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
EH*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
GS-T**	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
UDPGT*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: * = nmol/g/min, ** = µmol/g/min

Table A6_3_1-2 Absolute and relative liver weight (mean values)

Dose	0	50	200	2000	10000
Males					
Body weight (g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Absolute (mg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Relative (mg/100 g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Females					
Body weight (g)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Absolute (mg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Relative (mg/100 g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: * $p \leq 0.05$, ** $p \leq 0.01$

Section A6.3.2 Short-term repeated dose toxicity (28 days) – dermal

Annex Point IIA6.3 Rat, 28 days

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1997b): YRC 2894 - Study for subacute dermal toxicity in rats (four-week treatment and two-week recovery period) [redacted] Report No. 25959, date: 1997-02-07.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.2 Other routes. B.6.3.2.2 Dermal exposure

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 410; US-EPA FIFRA § 82-2; Directive 88/302/EEC method B.9

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1995 study, Wistar rats (5/sex/dose) were dermally administered YRC 2894 (thiacloprid) (purity: [redacted] at levels of 0, 100, 300 and 1000 mg/kg bw/day by dermal application. Satellite groups (5/sex/dose) were administered 0 and 1000 mg/kg bw/day followed by a 2 week recovery period. The animals were exposed for 6 hours per day and received 22 applications over a period of 28 days. After exposure, the dressings were removed and the application sites were cleaned with soap and water.

Dose selection was based on a previous dose ranging study using 0 and 1000 mg/kg bw/day (T7060074). The effects reported in this study included lower food intake, white blood cell changes, increased cholesterol levels, reduced triglyceride levels, increased liver weight, lower thymus weights and dark-red spleens.

Clinical observations, body weight and food intake were recorded at suitable time points. The treated skin site were examined daily and any reactions were scored on the Draize scale. All the animals were given a macroscopic examination at necropsy. Blood was samples were taken for haematological and biochemical determinations. The following organs were removed and weighed: brain, heart, lungs, liver, spleen, kidneys, adrenals, thymus and testes. Histopathology of retained organs and tissues was carried out.

No animals died during the study or showed clinical signs of toxicity. There were no treatment-related effects on body weight. No local skin X

reactions were observed at the treatment sites. In females, there was a transient decrease in food intake at 1000 mg/kg bw/day.

The haematological and clinical chemistry did not reveal any treatment-related changes.

There were no treatment-related macroscopic findings at necropsy. The mean liver weights (absolute and relative) were increased in both sexes at 1000 mg/kg bw/day (Table A6_3_1-2). At the end of the recovery period the liver weights of the 1000 mg/kg bw/day group were comparable to the control values.

4 RESULTS AND DISCUSSION

Microscopy revealed centrilobular hypertrophy in combination with a more homogeneously structured cytoplasm was seen in male livers at dose levels ≥ 300 mg/kg bw/day and in females at 1000 mg/kg bw/day. These findings are considered to be a consequence of liver enzyme induction and usually indicate an adaptive response. At the end of the recovery period these changes were still evident in 2/5 males but not in females. The thyroids of males and females displayed a follicular cell hypertrophy at 1000 mg/kg bw. This effect was reversible in females but not completely reversible in males within the 2-week recovery period (1/5 males still exhibited the effect). There were no treatment-related microscopic skin findings at the treatment sites.

Based on the liver and thyroid effects at the next highest dose, NOAELs of 100 and 300 mg/kg bw/day were determined for systemic toxicity in males and females, respectively. The NOAEL for skin reactions was established at 1000 mg/kg bw/day.

5 CONCLUSION

5.1 Conclusion

The NOAEL for skin reactions was established at 1000 mg/kg bw, and for systemic effects at 100 and 300 mg/kg bw for males and females respectively based on liver (centrolobular hypertrophy) and thyroid effects (follicular cell hypertrophy) at 1000 mg/kg bw.

[redacted]

5.1.1 Reliability [redacted]

Section A6.3.2 Short-term repeated dose toxicity (28 days) – dermal

Annex Point IIA6.3 Rat, 28 days

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/07/2006
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_3_1-2 Absolute and relative organ weights (mean values)

Dose (mg/kg bw/day)	0	100	300	1000
Males (main study)				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs[a] liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel[b] liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Females (main study)				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Males (recovery group)				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Females (recovery group)				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]

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

Section A6.3.3 **Short-term repeated dose toxicity (28 d)–inhalation (1)**

Annex Point IIA6.3 Rat, 5 days x 6 hours

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1995). YRC 2894 - Pilot study on subacute inhalation toxicity in rats (Exposure: 5 x 6 hours [redacted] Report No. 24248, date: 1995-08-21.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.2 Other routes. B.6.3.2.1 Inhalation exposure (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;

Pilot study as far as possible according to OECD guidelines 403, 412; US-EPA FIFRA § 82-4; Directive 88/302/EEC method B.8

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 pilot study, Wistar rats (10/sex/dose) were exposed (nose only/dynamic chamber) to YRC 2894 (thiacloprid) (dust purity: [redacted]) at analysed concentrations of 0, 1.97, 19 or 205 mg/m³ air for six hours per day on five consecutive days. The exposure period was followed by a recovery period of approximately 3 days or two weeks.

Clinical observations, body weight and food intake were recorded at suitable time points. Reflexes and the rectal temperatures were evaluated during the course of the study. Fifty per cent of the animals were sacrificed on the third post-exposure day. The remaining animals were sacrificed at the end of the 2-week post-exposure observation period. All the animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, kidneys, liver, thymus, thyroid, heart, lung, and spleen. Liver samples were taken for enzyme determinations. At each sacrifice blood was sampled for basic haematological and biochemical determinations. Histopathology was not performed in this study.

The MMADs (GSDs) of the particles were 3.3 µm (2.3), 2.9 µm (2.1) or 3.3 µm (1.8) at 1.97, 19 or 205 mg/m³ air, respectively. Between 44-55% of aerosol mass was less than 3 µm.

4 RESULTS AND DISCUSSION

No deaths occurred during the study. Clinical signs were observed at the top dose and included ungroomed pelt, piloerection, reduced X

motility, tremor, bradypnea, laboured breathing pattern, flaccid appearance, mydriasis and emaciation. Based on the respiratory effects, the report concluded that the test aerosol had a minor potential to act as an upper respiratory tract irritant. All animals showed normal reflexes. An increase in grip strength was observed in both males and females at 19 mg/m³ and above on day 4 but this finding was not confirmed by grip measurements made three days later. Rectal temperatures were significantly reduced at 205 mg/m³.

Significant reductions in body weight were observed in both sexes at 205 mg/m³ on day 4 (last exposure day) and day 7 (3rd post-exposure day). Thereafter, the body weights of the control and treatment groups were similar. Food intake was reduced in both sexes at 205 mg/m³ on day 4 (last exposure day).

At the highest dose, clotting time (hepatoquick) was decreased, protein levels were increased in males, and hepatic N-/O-demethylases, cytochrome P450, and liver triglycerides were significantly increased on day 7. Bile-acid levels and to a minor extent glutamate dehydrogenase activities were decreased, and hepatic N-/O-demethylases were statistically significantly increased in females on day 7. No biochemical changes were evident at the terminal sacrifice.

Gross necropsy revealed dark spleens at 19 mg/m³ (4 females) and 205 mg/m³ (4 females) at the interim necropsy. Dark spleens were not observed in any animals at the terminal necropsy. At 205 mg/m³, liver size and weight were increased, and thymus size and weight were reduced in males at the interim sacrifice. There were no treatment-related macroscopic findings or organ weight changes in any animals at the terminal sacrifice.

Based on the liver effects at the top dose, a NOAEL of 19 mg/m³ was determined for both sexes in this study.

5 CONCLUSION

5.1 Conclusion

A NOAEC could be established at 19 mg/m³ air based on liver weight effects at 205 mg/m³ air.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.3.3 **Short-term repeated dose toxicity (28 d)–inhalation (1)**

Annex Point IIA6.3 Rat, 5 days x 6 hours

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/07/2006
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 A6.3.3 Short-term repeated dose toxicity (28 d)–inhalation (2)

Annex Point IIA6.3 Rat, 4 weeks x 5 days x 6 hours

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1 REFERENCE

1.1 Reference [redacted]1998): YRC 2894 - Subacute inhalation toxicity on rats (Exposure 5 x 6 hour/week for 4 weeks)[redacted]Report No. 27689, date: 1998-07-20.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.2 Other routes. B.6.3.2.1 Inhalation exposure (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 403, 412; US-EPA FIFRA § 82-4; Directive 88/302/EEC method B.8

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1997 study, Wistar rats (10/sex/dose) were exposed (nose only/dynamic chamber) to YRC 2894 (thiacloprid) (dust purity: [redacted]) at analysed concentrations of 0, 2.0, 18.2 or 143.4 mg/m³ air for six hours per day on 5 days per week for 4 weeks. The highest dose group was initially exposed to a target concentration of 200 mg/m³. However, due to marked respiratory distress and reduced body weight gain the target concentration was reduced from the 2nd week.

Clinical observations, body weight and food intake were recorded at suitable time points. Reflexes and the rectal temperatures were evaluated during the course of the study. Ophthalmologic examinations were performed prior to the start of the study and towards the end. Urinalysis was also performed near to the end of the study. All the animals were given a macroscopic examination at necropsy. Selected organs were removed and weighed, and blood was sampled for haematological and biochemical determinations. Histopathology of retained organs and tissues was performed in this study.

For all treatment groups, the MMAD of the particles was approximately 2.9 µm with a GSD of 1.8. Between 51-55% of aerosol mass was less than 3 µm.

4 RESULTS AND DISCUSSION

No deaths occurred during the study. Clinical signs were observed at the top dose and included bradypnea, reduced motility, tremor, laboured breathing pattern, piloerection, ungroomed hair-coat, atony, rales,

salivation, mydriasis, miosis and vocalisation. These signs were considered to be a consequence of respiratory distress rather than as central nervous effects. Reduced body weights (days 4-11) and hypothermia were evident in both sexes at the top dose level.

The red blood cell parameters were not affected by treatment. There were significant increases in the levels of phosphate, glucose, cholesterol, bile acids and calcium and increased alkaline phosphatase activity in top dose females. Glucose and phosphate levels were significantly increased in top dose males. The results of the liver determinations are present in Table A6_3_3-1.Liver enzyme induction was detected at 143.4 mg/m³ air which was more pronounced in females than in males. A marginal effect in some enzymes was also seen at 18.2 mg/m³. Changes indicative of an effect on the thyroid were not seen during this study.

No treatment-related effects were revealed by the opthalmological examinations. Urinalysis did not reveal any treatment-related effects.

There were no treatment-related macroscopic findings at necropsy. The main organ weight changes are presented Table A6_3_3-2. The increased lung weights in males lack a dose-response relationship and there was no compound-induced effect on female lung weight. Therefore, the male lung weight changes were considered to be incidental. The thyroid weights of males were slightly increased at 18.2 mg/m³ 143.4 mg/m³ (not dose dependent).

The microscopic examination revealed minimal to slight hypertrophy of hepatocytes >18.2 mg/m³ air in males and at 143.4 mg/m³ in females. Slight hypertrophy was observed in the thyroidal follicular epithelium of two males at 143.4 mg/m³. Microscopy did not detect any treatment-related findings in the respiratory tract.

Based on clinical signs, reduced body weight and the liver and thyroid effects at the next highest dose, a NOAEL of 18.2 mg/m³ was determined for this study.

5 CONCLUSION

5.1 Conclusion

A NOAEC could be established at 18.2 mg/m³ air based on liver weight and thyroid effects at 143.4 mg/m³ air.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.3.3 Short-term repeated dose toxicity (28 d)–inhalation (2)

Annex Point IIA6.3 Rat, 4 weeks x 5 days x 6 hours

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04/07/2006
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_3_3-1 Liver enzyme and triglyceride determinations

Dose (mg/m³)	0	2	18.2	143.4/200[a]
Males				
N-DEM (mU/g).	[redacted]	[redacted]	[redacted]	[redacted]
O-DEM (mU/g).	[redacted]	[redacted]	[redacted]	[redacted]
P450 (nmol/g).	[redacted]	[redacted]	[redacted]	[redacted]
Trigl (mcmol/g).	[redacted]	[redacted]	[redacted]	[redacted]
Females				
N-DEM (mU/g).	[redacted]	[redacted]	[redacted]	[redacted]
O-DEM (mU/g).	[redacted]	[redacted]	[redacted]	[redacted]
P450 (nmol/g).	[redacted]	[redacted]	[redacted]	[redacted]
Trigl (mcmol/g).	[redacted]	[redacted]	[redacted]	[redacted]

Key: a) Exposure level reduced in 2nd week. b) * $p \leq 0.05$, ** $p \leq 0.01$.

Table A6_3_3-2 Absolute and relative organ weights (mean values)

Dose (mg/m³)	0	2.0	18.2	143.4/200[a]
Males				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs[b] liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel[c] liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs lung wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel lung wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Rel lung wt (mg/100g)[d].	[redacted]	[redacted]	[redacted]	[redacted]
Abs thyroid wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel thyroid wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Females				
Body wt (g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs liver wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel liver wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]
Abs lung wt (mg).	[redacted]	[redacted]	[redacted]	[redacted]
Rel lung wt (mg/100g).	[redacted]	[redacted]	[redacted]	[redacted]

Section A6.4.1 **Subchronic Toxicity – oral (1)**

Annex Point IIA6.4 Rat, 13 weeks

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1 REFERENCE

1.1 Reference

[redacted] 1997): YRC 2894 - Investigations of subchronic toxicity in Wistar rats (Feeding study over 12 weeks with a subsequent recovery period over 5 weeks) [redacted] Report No. 26239, date: 1997-05-06.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.1 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 407

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1994 study, Wistar rats (10/sex/dose) were administered YRC 2894 (thiacloprid) (purity: [redacted]) in diet at concentrations of 0, 25, 100, 400 or 1600 ppm for up to 13 weeks. In addition, two recovery groups (10/sex/dose) received 0 or 1600 ppm for the same period of time and were observed for an additional 5 weeks.

Clinical observations, body weight and group food and water intakes were recorded at appropriate time points. Ophthalmologic examinations were performed prior to the start of the study on all animals and in week 12 on the main control and top dose groups. Haematology, biochemistry (including thyroid parameters) and urinalysis were performed in week 3 and 11 for the main groups and week 17 for the recovery groups.

Immunotoxicological investigations were performed at the end of treatment using cardiac blood, mesenteric lymph nodes, femur with bone marrow and half of the spleen of 5 animals per group were sampled and immediately chilled. The following parameters were determined: cell counts, FACScan analyses (spleen stained with surface markers for B-cells, T-helper cells, lymphocytes and antigen presenting cells/fluorescence evaluation techniques), macrophage activity after PMA (4,3-phorbol-12-myristate-13-acetate) stimulation, mitogen stimulation (lipopolysaccharide) and antibody levels. Liver samples were taken at necropsy for enzyme and triglyceride determinations. Liver and kidney samples were also taken to determine whether the test

material had an effect on cell proliferation (immunohistochemical demonstration of proliferating cell nuclear antigen). An extensive list of organs were weighed and subjected to gross pathological and histopathological examination at necropsy. The liver, lungs, kidneys and thyroid glands were examined microscopically at all dose levels. Other organs and tissues were examined in the control and top dose groups only.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were 0, 1.9, 7.3, 28.6 and 123.2 mg/kg bw/day in males, and 0, 2.0, 7.6, 35.6 and 160.6 mg/kg bw/day in females at dose levels of 0, 25, 100, 400 or 1600 ppm, respectively.

4 RESULTS AND DISCUSSION

No animals died during the study or showed clinical signs of toxicity. Mean body weights and body weight gains were reduced in both sexes at 1600 ppm. At the end of the treatment period, males body weights were approximately 84% of the control value and females were approximately 86% of the control value. During the recovery period, the body weights of the 1600 ppm animals were lower than control values but the difference diminished with time. Food intakes were not affected by treatment but water intake was reduced in males at 1600 ppm. No treatment-related ophthalmic findings were seen during this study.

The mean clotting times in females were significantly reduced at 1600 ppm (3 and 12 weeks). The biochemical changes are presented in Table A6_4_1-1. A significant increase in the mean creatinine value was also seen in males at 1600 ppm. The small but significant increase in mean protein concentration in 100 ppm females at week 3 was not seen at week 12. The report considered this finding to be toxicologically irrelevant because it was small, not dose-related and within historical control values. Other isolated biochemical changes were considered to be incidental and unrelated to treatment. No significant changes in cholesterol or protein levels were detected at the end of the recovery period.

The results of the thyroid hormone valuations are presented in Table A6_4_1-2. There were no significant treatment-related effects on females at any dose level. At week 3, T3 concentrations were significantly increased in males at all dose levels. Whereas the T3 concentration at week 12 was only significantly increased at 1600 ppm. T4 concentrations were increased in males at 400 ppm and above at week 3 only. The thyroxine-binding capacity (TBC) was significantly increased in 1600 ppm males at 3 weeks, at all dose levels at week 12 and, in 400 and 1600 ppm females at week 3. The report considered these TBC findings to be incidental and not of biological significance because they were small, not dose-related, and the individual values lay within the reference range for animals of this age (apart from 100 ppm males at week 12). In addition there were no effects on thyroid weight or morphology at these low dose levels and no thyroid effects were seen in other rat studies at these dose levels. The report considered the small effects on thyroid hormone levels to be secondary to the stronger liver enzyme induction, especially UDP-glucuronyl transferase. No significant changes in the hormone levels were detected at the end of the

recovery period.

The results of the liver enzyme and triglyceride determinations are presented in Table A6_4_1-3. A liver enzyme induction was detected at a dose level of 400 ppm (cytochrome P450, NDE, ODE) and more pronounced after 1600 ppm in both sexes (cytochrome P450, NDE, ODE, ECOD, ALD, EROD). There was no evidence of enzyme induction at the end of the recovery period. Urinalysis revealed an increase in sodium and calcium in 1600 ppm males at weeks 3, 11/12 or 17. The results of the gross and histological examinations, the organ weight determinations and cell proliferation investigations provided no indication of kidney damage.

The results of the immunotoxicological tests showed that there was no effect on cell counts or cell size distribution of spleen and lymph node cells. There was a slight increase in macrophage activation at 1600 ppm and a slight increase in males of lipopolysaccharide (LPS) stimulated cells (mitogenic stimulation) in the spleen at 1600 ppm. Antibody levels (IgA, IgG and IgM) were not affected by treatment. There was no evidence of an effect on cell proliferation in the liver or kidney evaluations.

No gross treatment-related findings were detected at necropsy. Relative liver weights were increased at 400 ppm in males and at 1600 ppm in both sexes. Thyroid weights were increased in males at the highest dose. The microscopic examination revealed hepatocellular hypertrophy associated with cytoplasmic changes probable due to enzyme induction. These liver changes were seen all animals at 1600 ppm and in 9/10 males and 2/10 females at 400 ppm. At the end of the recovery period, only 3/10 males had minimal hepatocellular hypertrophy.

Based on the clinical chemistry changes and liver effects at 400 ppm, a NOAEL of 100 ppm was determined for this study (equivalent to 7.3 and 7.6 mg/kg bw in males and females, respectively).

5 CONCLUSION

5.1 Conclusion

The NOAEL of 100 ppm (equivalent to 7.3 mg/kg bw in males) was based on stronger liver effects and also thyroid effects at 400 ppm and higher doses.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.4.1 Subchronic Toxicity – oral (1)

Annex Point IIA6.4 Rat, 13 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/07/2006
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_4_1-1 Clinical chemistry changes (mean values)

	Week 3		Week 12		Week 17 (recovery group)	
Dose (ppm)	Cholesterol (mmol/l)	Protein (g/l)	Cholesterol (mmol/l)	Protein (g/l)	Cholesterol (mmol/l)	Protein (g/l)
Males						
0	[redacted]					
25	[redacted]					
100	[redacted]					
400	[redacted]					
1600	[redacted]					
Females						
0	[redacted]					
25	[redacted]					
100	[redacted]					
400	[redacted]					
1600	[redacted]					

Key: * $p \leq 0.05$, ** $p \leq 0.01$

Table A6_4_1-2 Thyroid hormones in the blood (mean values)

	Week 3			Week 12			Week 17 (Recovery group)		
Dose (ppm)	T3[1]	T4[2]	TBC[3]	T3[1]	T4[2]	TBC[3]	T3[1]	T4[2]	TBC[3]
5.2 Males									
0	[redacted]								
25	[redacted]								
100	[redacted]								
400	[redacted]								
1600	[redacted]								
5.3 Females									
0	[redacted]								
25	[redacted]								
100	[redacted]								
400	[redacted]								
1600	[redacted]								

Key: 1) Triiodothyronine (nmol/l). 2) Thyroxine (nmol/l). 3) Thyroxine binding capacity. 4) * $p \leq 0.05$, ** $p \leq 0.01$

Table A6_4_1-3 Liver enzyme and triglyceride determinations (mean values)

Dose (ppm)	N-DEM	O-DEM	P-450	TRIGL	ECOD	EROD	ALD	EH	GS-T	UDPGT
Males week 13										
0	[redacted]									
25										
100										
400										
1600										
Females week 13										
0										
25										
100										
400										
1600										
Males week 17 (recovery group)										
0										
1600										
Females week 17 (recovery group)										
0										
1600										

Key: 1) N-DEM = N-demthylase (mU/g). 2) O-DEM = O-demthylase (mU/g). 3) P-450 = Cytochrome P-450 (nmol/g). 4) TRIGL = Triglyceride levels (µmol/g). 5) ECOD = 7-Ethoxycoumarin deethylase (nmol/g/min). 6) EROD = 7-Ethoxyresorufin deethylase (nmol/g/min). 7) ALD = Aldrin epoxidase (nmol/g/min). 8) EH = Epoxide hydrolase (nmol/g/min). 9) GS-T = Glutathione-S-transferase (µmol/g/min). 10) UDPGT = UDP-glucuronyl-transferase (nmol/g/min). 11) * $p \leq 0.05$, ** $p \leq 0.01$

Section A6.4.1 Subchronic Toxicity – oral (2)

Annex Point IIA6.4 Mouse, 14 weeks

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1 REFERENCE

1.1 Reference

[redacted] 1995). YRC 2894 - Subchronic range-finding study for a two-year study in B6C3F1 mice (Administration in feed over about 14 weeks) [redacted] Report No. 23834, date: 1995-03-14.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.2 Mice (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 408; US-EPA FIFRA § 82-1; Directive 88/302/EEC method B.7

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1999 range-finding study, B6C3F1 mice (10/sex/dose) were fed diet containing 0, 50, 250, 1250 or 6250 ppm YRC 2894 (thiacloprid) (purity: [redacted]) for approximately 14 weeks.

Clinical signs of toxicity, body weight changes, food consumption and water intakes were recorded at suitable time points. Haematological and biochemical investigations were carried out in weeks 12 and 13. All the animals received a macroscopic examination at necropsy. The following organs were removed and weighed: brain, liver, heart, spleen, kidneys, adrenals and gonads. Liver samples were taken for enzyme determinations. Sections of liver, kidney, pituitary gland, thyroid gland and organs or tissues exhibiting gross changes from all animals and dosage groups were examined microscopically. In addition, the testes and epididymides from animals dosed with 0 and 6250 ppm were examined.

The concentration, stability and homogeneity of the test material in the diet were acceptable. The mean daily intakes were equivalent to 0, 19.9, 102.6, 542.4 and 2819.9 mg/kg bw/day in males, and 0, 27.2, 139.1, 704.3 and 3351.0 mg/kg bw/day in females at dose levels of 0, 50, 250, 1250 and 6250 ppm, respectively.

There were no treatment-related deaths or clinical signs of toxicity during the study. The deaths that occurred prior to the scheduled sacrifice are given in Table A6_4_1-1. Seven of these deaths are believed to be due blood sampling from the retro-orbital venous plexus. X

4 RESULTS AND DISCUSSION

A significant reduction in mean body weight (14%) was observed in males at 6250 ppm. An increase in the mean food intake was observed in males at 1250 ppm and above (8-12%). A slight reduction in food efficiency was evident at these dose levels. The mean water intake was reduced in males at 6250 ppm.

There were no treatment-related haematological changes. The main clinical chemistry changes are presented in Table A6_4_1-2. The significant reductions in protein levels in females at dose levels ≤1250 ppm were not considered biologically relevant because the individual values are within the normal range and control values are relatively high.

Liver enzyme induction occurred in both sexes at 1250 and 6250 ppm, with marginal effects at 250 ppm (Table A6_4_1-3).

Gross examination did not reveal any findings in the animals that died prematurely or were killed at the scheduled sacrifice. The main organ weight changes are presented in Table A6_4_1-4.

The mean relative liver weights were increased in both sexes at 1250 ppm and above. The microscopic examination revealed hepatocellular hypertrophy in males at 250 ppm and in both sexes at 1250 ppm and above. The mean relative adrenal weights were slightly (not statistically significant) increased in females at 1250 ppm and above. Microscopically this correlated with a dose-related increase in the severity of (fatty) vacuolation of the female adrenal X-zone at 50 ppm and above leading to hypertrophy of this zone (Table A6_4_1-5). The ovaries appeared to have lower numbers of old corpora lutea at 1250 ppm and above. The interstitial glands of the ovaries appeared to be activated at 1250 ppm and above. These glands are derived mostly from atretic follicles and respond to gonadotrophin stimulation but their function is unknown ([redacted], 1991). There was a reduction or loss of sex-specific vacuolation in the proximal tubules of kidneys in males at 1250 ppm and above.

The X-zone is located between the zona reticularis and the adrenal medulla (function unclear). The presence of this extra adrenocortical zone appears to be dependent on age and reproductive status. Based on histology and ultrastructural criteria, the X-zone has been described in mice, voles, red squirrels, shrews, rabbits and the cat. [redacted] (1986) appears to suggest that histologically similar tissue may be present in the foetal zone of the human adrenal gland. The human foetal cortex disappears during the first six months of the neonatal period ([redacted], 1988). The mouse X-zone involutes in males at puberty and persists in females until the first pregnancy. In non-pregnant females, it degenerates during adulthood depending on the genetic background of the animal [redacted], 1983). In Swiss mice, it has been shown that the formation and degradation of this zone are influenced by pituitary and gonadal function [redacted] 1986).

Based on the liver effects (enzyme induction, weight and morphology) at the next highest dose, a NOAEL of 50 ppm was determined for male rats in this study (equivalent to 19.9 mg/kg bw/day). Because of the

effects on the adrenal X-zone in females, a NOEL was not established in female mice.

5 CONCLUSION

5.1 Conclusion

The NOAEL of 50 ppm (equivalent to 19.9 mg/kg bw in males) was based on stronger liver effects (weight, enzyme induction, morphology) at 250 ppm. Due to slight effects on the adrenal X zone in females, no NOEL was established in female mice.

[redacted]

5.1.1 Reliability [redacted]

Section A6.4.1 Subchronic Toxicity – oral (2)

Annex Point IIA6.4 Mouse, 14 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/07/2006
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_4_1-1 The number of deaths which occurred prior to the scheduled sacrifice

Dose	Males	Females
0		
50		
250		
1250		
6250		

found dead, b) killed in moribund state.

Table A6_4_1-2 Biochemical changes

Parameter	0 ppm	50 ppm	250 ppm	1250 ppm	6250 ppm
Males					
AP (U/l)					
Cholesterol (mmol/l)					
Triglycerides (mmol/l)					
Bilirubin (µmol/l)					
Females					
Cholesterol (mmol/l)					
Bilirubin (µmol/l)					
Protein (g/l)					
Albumin (g/l)					

Key: a) * $p \leq 0.05$, ** $p \leq 0.01$. b) AP = alkaline phophatase.

Table A6_4_1-3 Enzyme determinations in liver homogenates (mean values)

Dose (ppm)	N-DEM (mU/g)		P450 (nmol/g)	
	Males	Females	Males	Females
0				
50				
250				
1250				
6250				

$p \leq 0.05$, ** $p \leq 0.01$

Table A6_4_1-4 Absolute and relative organ weights (mean values)

Parameter/dose	0 ppm	50 ppm	250 ppm	1250 ppm	6250 ppm
Males					
Body weight (g).					
Abs[b] liver wt (mg).					
Rel[c] liver wt.					
Abs kidney wt (mg).					
Rel kidney wt (mg).					
Females					
Body weight (g).					
Abs liver wt (mg).					
Rel liver wt.					
Abs adrenal wt (mg).					
Rel adrenal wt (mg).					
Abs kidney wt (mg).					
Rel kidney wt (mg).					
Abs heart wt (mg).					
Rel heart wt (mg).					

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

Table A6_4_1-5 The severity and incidence of vacuolation/hypertrophy in the adrenal X-zone of female mice

Dose (ppm)	0	50	250	1250	6250
No. animals					
Grade 1 (minimal)					
Grade 2 (slight)					
Grade 3 (moderate)					
Grade 4 (marked)					
Grade 5 (massive)					
Incidence					
Mean grade					

Section A6.4.1 Subchronic Toxicity – oral (3)

Annex Point IIA6.4 Dog, 10 weeks

Official use only

1 REFERENCE

1.1 Reference

[redacted]1998a): YRC 2894 - Subacute toxicity in Beagle dogs (Dose range finding study by feed admixture over at least 10 weeks)[redacted]Report No. 27177, date: 1998-02-05, *revised 1999-02-11.*

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.3 Dogs (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 409

2.2 GLP [redacted] X

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In 1994 study, Beagle dogs (2/sex/dose) were fed diet containing YRC 2894 (thiacloprid) (purity: [redacted]) at 0, 100, 300 and 1000 ppm for up to 10 weeks. Since no clinical signs and no other effects were seen in the animals, the highest dose was increased from 1000 to 2500 ppm in a step-wise manner (1250 ppm on day 19, 1600 ppm on day 26 and 2500 ppm on day 38). An additional group of 2 males and 2 females were also added to the study on day 38 and received diet containing 2500 ppm for 4 weeks.

Clinical observations, body weight and food intake were recorded at appropriate time points. Water intake was also monitored. Reflex reactions, pulse rate and body temperatures were recorded prior to the study and in weeks 4 and 9. Laboratory investigations (haematology and biochemistry) were performed prior to the start of the study and in weeks 2, 4, 5, 6 and 9. Urine was collected (6 hours) prior to the start of the study and in weeks 2, 4, 5 and 9. All animals were sacrificed at the end of the study and received a gross examination. Selected organs were removed and weighed (brain, heart, liver, lungs, spleen, adrenals, kidneys, pancreas, thyroid, pituitary, testes, prostate gland, uterus, thymus, ovaries). The organs were fixed and subjected to a microscopic examination. Liver samples were taken and used for enzyme determinations.

The concentration, stability and homogeneity of the test material in the diet were acceptable. Dose levels in study week 9 were equivalent to average doses of 0, 3.3, 9.6, 80.0 and 65.7 (satellite group) mg/kg

bw/day for both sexes.

4 RESULTS AND DISCUSSION

No deaths occurred during the study. There were no clinical signs of toxicity or treatment-related effects on reflex responses, pulse rates or body temperature. Slight reductions in feed intake were seen in both sexes after the dose had been increased to 1600-2500 ppm (weeks 4-8). Reductions in food intake and body weight gain were observed at 2500 ppm in females of the satellite group. X

There were no treatment-related effects on the haematological parameters. At 2500 ppm (both groups), there were some slight increases in urea and creatinine concentrations. A slight increase in ALT activity was seen in 2500 ppm males in the satellite group, which was partly reversible during the study period. Thyroxine (T4) levels were slightly reduced and triiodothyronine (T3) levels and the thyroxin-binding capacity (TBC) were slightly increased in 2500 ppm females in the satellite group. Marginal increases in the activities of the liver enzymes, EROD, EH and GS-T (females only) were seen at 2500 ppm. The report considered these mild enzyme changes to be responsible for the changes in thyroid parameters (T3, T4 & TBC). Urinalysis did not reveal any treatment-related changes.

No gross findings were seen at necropsy. Prostate weights (absolute and relative) were increased at 2500 ppm (both groups). The microscopic examination revealed slight cytoplasmic changes in liver cells of one female in the 1000-2500 group and one male and one female in the 2500 ppm satellite group. Based on the effects on body weight, food intake, and the liver at the top dose level, a NOAEL of 300 ppm was determined for this study (equivalent to 9.6 mg/kg bw/day for both sexes).

5 CONCLUSION

5.1 Conclusion

The NOAEL of 300 ppm (equivalent to 9.6 mg/kg bw) is based on effects on feed intake, body weight and on the liver at 1000 - 2500 ppm.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.4.1 Subchronic Toxicity – oral (3)

Annex Point IIA6.4 Dog, 10 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05/07/2006
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 A6.4.1 **Subchronic Toxicity – oral (4)**

Annex Point IIA6.4 Dog, 15 weeks

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998): YRC 2894 -Subchronic toxicity study in Beagle dogs (Feeding study for about 15 weeks [redacted] Report No. 27464, date: 1998-05-08.

PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.3 Dogs (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 409

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1995 study, Beagle dogs (4/sex/dose) were fed diet containing YRC 2894 (thiacloprid) (purity: [redacted]) at 0, 250, 1000 or 2000 ppm for 15 weeks. The high dose group was initially treated with 4000 from day 1 to 4. This dose level caused severe vomiting, refusal to feed, body weight reductions and slight tremor. Therefore, after a period without treatment from day 5 to day 14, this group was treated with a dose of 2000 ppm from day 15 onwards. This period was compensated for by a 2-week extension of the study.

Clinical observations, body weight and food intake were recorded at suitable time points. Body temperature, pulse rate and reflex reactions were evaluated prior to the start of the study and in weeks 7 and 15. During the same weeks, ECGs and ophthalmologic investigations were performed. Laboratory investigations (haematology, biochemistry and urinalysis) were performed prior to the start of the study and in weeks 2, 7 and 15. All animals were sacrificed at the end of the study and received a gross examination. Selected organs were removed and weighed (brain, heart, liver, lungs, spleen, adrenals, kidneys, pancreas, thyroid, pituitary, testes, prostate gland, uterus, thymus and ovaries). An extensive list of organs and tissues were examined microscopically. Liver samples were taken and used for enzyme determinations.

The concentration, stability and homogeneity of the test material in the diet were acceptable. Daily intakes were equivalent to 0, 8.5, 34.9 and 68.0 mg/kg bw/day in males and 0, 8.9, 34.7 and 65.3 mg/kg bw/day in females, at 0, 250, 1000 and 2000 ppm, respectively.

4 RESULTS AND DISCUSSION

There were no deaths, clinical signs or effects on food intake or body weight following the introduction of the amended dosing regimen. Body temperature, pulse rate and the reflex reactions were not affected by treatment. The ECGs and ophthalmologic investigations did not reveal any treatment-related effects. The lack of clinieal signs at the chosen dose levels was not the consequence of poor absorption. Blood samples were taken 0, 2, 4, 6 and 24 hours after feeding at week 14. Peak plasma values as measured 4 to 6 hours after administration were approximately 2, 6, and 14 µg/ml at 250, 1000 and 2000 ppm, respectively. Compared with the administered doses the blood levels are regarded as very high, thus, indicating a high absorption rate.

There were no treatment-related effects on the haematological parameters. At 2000 ppm, there were slight increases in mean transaminase activities at some time-points (mainly at 2 weeks), which resolved during the study period. T4 levels were slightly decreased and TBC slightly increased at 1000 and 2000 ppm. Evidence of a slight liver enzyme induction (N-demethylase, UDP Glu-T) was observed at 2000 ppm. Urinalysis did not reveal any treatment-related changes.

There were no treatment-related macroscopic findings at necropsy. The main organ weight changes are presented in Table A6_4_1-1. The historical control data provided by the applicant are presented in Table A6_4_1-2.

The increased liver weight seen in all treatment groups did not exhibit a dose-response relationship. There were no microscopic findings in the liver and the report states that all the weight values are within the historical control values. Thus, the apparent inereases in liver weight may be due or partly due to the low control values. A dose-related increase in mean testicular weight (relative) is evident but all the values are within the historical control values. The mean prostate weights (absolute and relative) were increased at 1000 ppm and above. Microscopy revealed slight to moderate hypertrophy of the prostate in all the dogs at these dose levels.

At 2000 ppm, there was a slightly increased incidence of spermatocytic degeneration in the testes (2/4 dogs) and/or in the epididymides (4/4 dogs compared to one control dog). The interstitial testicular cells (Leydig cells) also appeared to be slightly more prominent in three dogs at this dose level. However, such findings are known to show a wide variation with respect to severity and incidence in young dogs.

Based on the prostate effects at the next highest dose, a NOAEL of 250 ppm was determined for males in this study (equivalent to 8.5 mg/kg bw/day). A NOAEL of 2000 ppm was determined for female dogs (equivalent to 65.3 mg/kg bw/day in females).

5 CONCLUSION

5.1 Conclusion

A NOAEL of 250 ppm (equivalent to 8.5 mg/kg bw in males and to 8.9 mg/kg bw in females) was established in this study, based on liver and prostate effects at 1000 ppm.

[redacted]

5.1.1 Reliability [redacted]

Section A6.4.1 Subchronic Toxicity – oral (4)

Annex Point IIA6.4 Dog, 15 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	07/07/2006
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_4_1-1 Absolute and relative organ weights (mean values)

Parameter/dose	0 ppm	250 ppm	1000 ppm	2000 ppm
Males				
Mean body weight (kg)	[redacted]			
Abs[b] liver wt (g).				
Rel[c] liver wt (g/kg).				
Abs testes wt (g).				
Rel testes wt (g/kg)				
Abs prostate wt (g).				
Rel prostate wt (g/kg).				
Females				
Mean body weight (kg)				
Abs liver wt (g).				
Rel liver wt (g/kg).				
Abs ovarian wt (g)				
Rel ovarian wt (g/kg).				
Abs uterine wt (g)				
Rel uterine wt (g/kg).				

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

Table A6_4_1-2 Historical control data for liver, prostate and testes weight

Organ	Mean ± SD (range)
Absolute liver weight.[a]	[redacted]
Relative liver weight.[a]	
Absolute testes weight.	
Relative testes weight.	
Absolute prostate weight.	
Relative prostate weight.	

Key: a) range for male dogs.

Section A6.4.2 Annex Point IIA6.4	Subchronic Dermal Toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [ ]	
Detailed justification:	A subchronic dermal study (90 days) using the active substance was not performed. Instead, a subacute dermal toxicity study in rats was conducted. This study revealed effects that were identical to those seen in the respective oral studies. These effects were limited to hepatocellular and thyroid follicular hypertrophy. Based on these findings, a route-specific toxicity of thiacloprid via the dermal route can be excluded and the risk assessment for dermal exposure can be performed using route-to-route extrapolation.	
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	07/07/2006	
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 A6.4.3 Annex Point IIA6.4	Subchronic Inhalation Toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [X]	**Technically not feasible** [ ] **Scientifically unjustified** [X] **Other justification** [ ]	
Detailed justification:	A subchronic inhalation study (90 days) using the active substance was not performed. Instead, a subacute inhalation study with thiacloprid in rats was conducted. The effects seen in this study were comparable to that observed in the repeated-dose oral studies in rodents. Liver enlargement and thyroid effects were seen at the highest concentration of thiacloprid in the test atmosphere. Thus, there is no indication for route-specific toxicity of thiacloprid. Conducting a subchronic inhalation study in addition to the subchronic oral study is therefore not justified since route-to-route extrapolation is possible. Furthermore, both the vapour pressure of thiacloprid and the concentration of the active substance in the treatment solutions are too low to expect appreciable exposure via inhalation. The highest exposure via inhalation to thiacloprid is expected for manual spray applications of the water-based guide recipe. The respective exposure is estimated to be 10.64×10^{-4} mg/kg/day (*cf.* Doc. II-B, Section 3.2.3). This exposure leaves a margin of safety of 1156 when compared to the lowest overall NOAEL of 1.23 mg/kg/day. Thus, the requirement for a subchronic inhalation study in rodents can also be waived based on exposure considerations.	
Undertaking of intended data submission [ ]	–	

Section A6.4.3 Annex Point IIA6.4	Subchronic Inhalation Toxicity
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	07/07/2006
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 A6.5/6.7

Annex Point IIA6.5/6.7

Chronic Toxicity – oral and Carcinogenicity study (1)

Rat, 2 years

Official use only

1 REFERENCE

1.1 Reference

[redacted] 1998): YRC 2894 - Combined chronic toxicity/carcinogenicity study in wistar rats - Dietary administration over 2 years [redacted] Report No. 27480, date: 1998-05-14.

PPP-Monograph Chapter: B.6.5 Chronic toxicity and carcinogenicity. B.6.5.1 Chronic toxicity and carcinogenicity 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;

OECD guideline 453; US-EPA FIFRA, § 83-5; Directive 88/302/EEC method B.33

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

YRC 2894 (thiacloprid), batch no. 290894, purity: [redacted] was administered via the diet to Wistar rats (50 males and 50 females per dose), in doses of 0, 25, 50, 500 and 1000 ppm over a period of up to 107 weeks. In addition, 10 rats per dose and sex, respectively, were treated likewise with YRC 2894 and sacrificed after a treatment period of about 1 year. Also determinations of liver enzymes were conducted for the chronic part of this study. Average daily test substance intake was - in ascending order of dose - 1.2, 2.5, 25.2 and 51.7 mg/kg body weight per day in male rats, and 1.6, 3.3, 33.5 and 69.1 mg/kg bw per day in females, respectively.

4 RESULTS AND DISCUSSION

The neoplastic findings are presented in Table A6_7-1. A summary of the tumour incidence and the number of animals with tumours are presented in Table A6_7-2.

The increased incidences of uterine adenocarcinomas at dose levels ≥ 500 ppm and reduced incidence of galactocele in the mammary glands in 1000 ppm females may be caused by alterations in hormone metabolism resulting from the liver effects. Mechanistic studies indicate that the induction of the microsomal liver enzymes by YRC 2894 (thiacloprid) also included aromatase, an enzyme involved in estradiol synthesis. This results in increased plasma estradiol levels and continuous stimulation of the uterine endometrium, which may explain the increased incidence of uterine adenocarcinomas in old and acyclic

rats.

In males, there are small increases in the number of animals with neoplasms, those with more than one neoplasm, and the number of benign neoplasms, which can be explained by the increased incidence of thyroid follicular cell adenoma. The single follicullar cell adenoma seen at 50 ppm was considered to be within the historical control data for this strain of rat. In females, there is an increased incidence in the number of animals with metastasising malignant neoplasms, which is caused by the higher uterine adenocarcinoma incidence. The combined uterine adenoma and adenocarcinoma incidences at 25 and 50 ppm are lower than the control incidence.

Based on liver enzyme induction, NOAELs of 25 and 50 ppm were determined for the non-neoplastic effects in males and females, respectively (equivalent to 1.23 and 3.3 mg/kg bw/day in males and females, respectively). A NOAEL of 50 ppm was determined for oncogenicity in both sexes based on the increased incidences of uterine adenocarcinoma in females and thyroid follicular cell adenoma in males.

5 CONCLUSION

5.1 Conclusion

The NOAEL of this study was 25 ppm (equivalent to 1.23 mg/kg bw) in males and 50 ppm (equivalent to 3.3 mg/kg bw) in females. A strong microsomal enzyme induction was observed at doses of ≥ 50 ppm in males and of ≥ 500 ppm in females. As a consequence of this liver enzyme induction, effects on the thyroid were seen. A TSH increase was measured in 1000 ppm animals at most time-points and an increased incidence of follicular cell adenomas in 500 and 1000 ppm males and very slightly in 1000 ppm females was seen. As a further consequence of the liver enzyme induction which included also an induction of aromatase, a key enzyme in estradiol synthesis, an increased incidence of uterine tumours was seen at 500 and 1000 ppm. The mechanism of this high dose phenomenon was clarified.

[redacted]

5.1.1 Reliability [redacted]

Section A6.5/6.7 — Chronic Toxicity – oral and Carcinogenicity study (1)

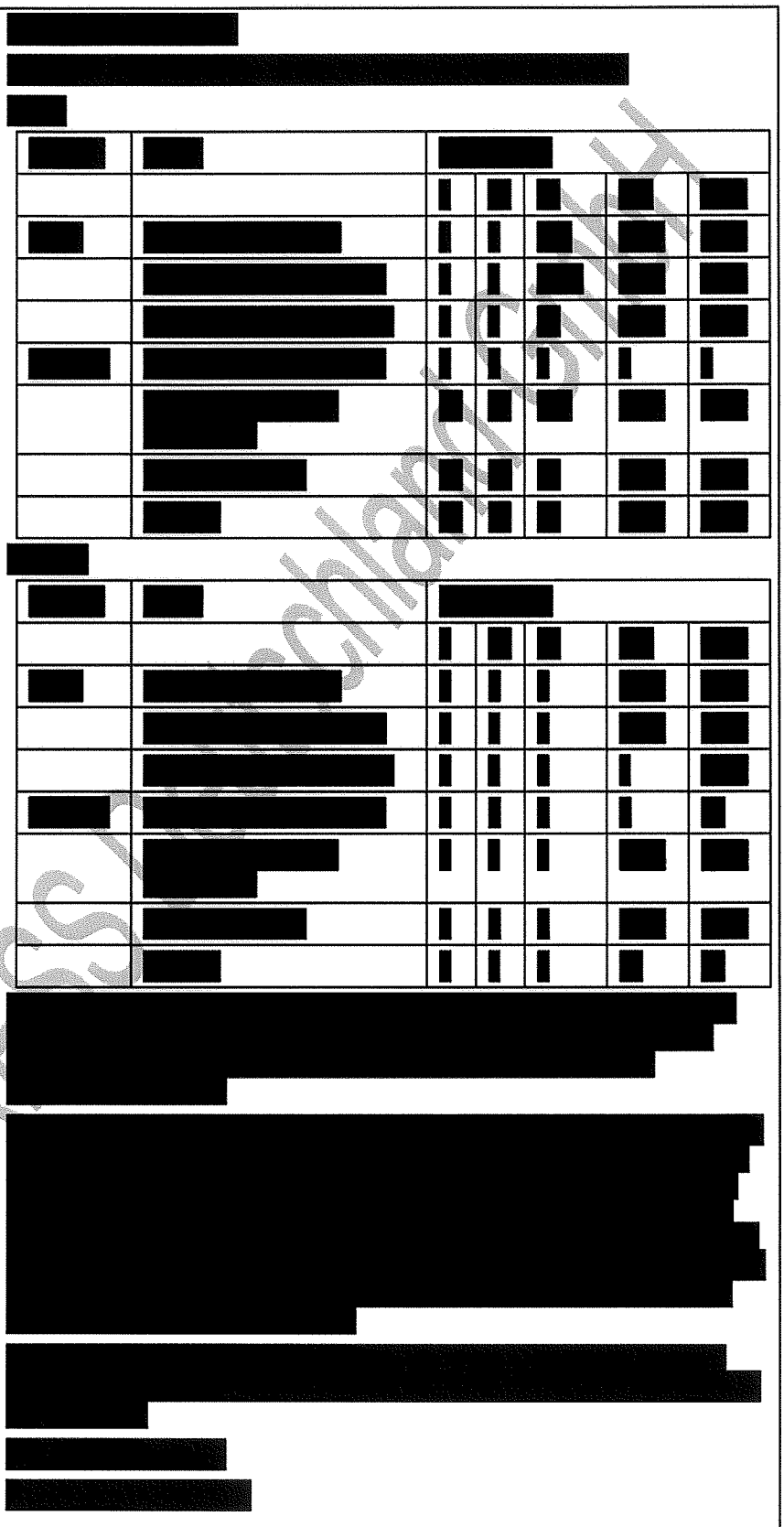
Annex Point IIA6.5/6.7 — Rat, 2 years

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	02/08/2006
Materials and Methods	[redacted]

Section A6.5/6.7 **Chronic Toxicity – oral and Carcinogenicity study (1)**

Annex Point IIA6.5/6.7 Rat, 2 years

Results and discussion



Section A6.5/6.7 Chronic Toxicity – oral and Carcinogenicity study (1)

Annex Point IIA6.5/6.7

Rat, 2 years

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_7-1 Neoplastic findings for all animals scheduled for terminal necropsy

Dose (ppm)	0	25	50	500	1000	0	25	50	500	1000
	Males					Females				
UTERUS n										
stromal polyp (b)										
glandular polyp (b)										
adenoma (b)										
adenocarcinoma (m)										
squamous cell carcinoma (m)										
mixed Muellerian tumor (m)										
adenosquamous carc. (m)										
stromal sarcoma (m)										
schwannoma (m)										
granular cell tumor (b)										
THYROID GLAND n										
C-cell adenoma (b)										
follicular cell adenoma (b)										
C-cell carcinoma (m)										

m = malignant, b = benign * $p \leq 0.05$, ** $p \leq 0.01$

Table A6_7-2 The incidence of tumors and tumor bearing animals

Dose (ppm)	0	25	50	500	1000	0	25	50	500	1000
	Males					Females				
No. of animals with neoplasms	■	■	■	■	■	■	■	■	■	■
No. of animals with more than one primary neoplasm	■	▮	▮	■	■	■	■	■	■	■
No. of animals with metastases	▮	▮	▮	▮	▮	▮	▮	▮	■	■
No. of primary neoplasms	■	■	■	■	■	■	■	■	■	■
No. of benign neoplasms	■	■	■	■	■	■	■	■	■	■
No. of malignant neoplasms	■	▮	▮	■	▮	■	▮	■	■	■

Section A6.5 Chronic Toxicity – oral (2)

Annex Point IIA6.5 Dog, 52 weeks

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]1998b): YRC 2894 - Chronic toxicity study in Beagle dogs (52 week feeding study)[redacted]Report No. 27563, date: 1998-06-22. *PPP-Monograph Chapter: B.6.3 Short-term toxicity. B.6.3.1 Oral studies. B.6.3.1.3 Dogs (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 452
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1996/7 study, Beagle dogs (4/sex/dose) were fed diet containing YRC 2894 (thiacloprid) (purity: [redacted]) at 0, 40, 100, 250 or 1000 ppm for 52 weeks. In addition, three groups of dogs (3/sex/dose) were fed diet containing the test material at 0, 100 or 1000 ppm for 26 weeks. The dogs were 27-32 weeks old at week-1 and weighed between 7 to 11 kg.

Clinical observations, body weight and food intake were recorded at appropriate time points. Water intake was monitored. Body temperature, pulse rate and reflex reactions were evaluated prior to the start of the study and in weeks 6, 13,26, 39 and 52. During the same weeks, ECGs and ophthalmologic investigations were performed. Morphological and ultrasonographic investigations of the prostate gland were performed in weeks 8, 17, 26, 35, 44 and 51. Laboratory investigations (haematology, biochemistry and urinalysis) were performed prior to the start of the study and in weeks 6, 13, 39, 44 and 51. Additional blood samples were taken in weeks 5, 12, 25, 38 and 51 for toxicokinetic measurements (prior to and 2, 4, 6 and 24 hours post-feeding). All animals were sacrificed at the end of the study and received a gross examination. Selected organs were removed and weighed (brain, heart, liver, lungs, spleen, adrenals, kidneys, pancreas, thyroid, pituitary, testes, prostate gland, thymus, uterus and ovaries). An extensive list of organs and tissues were examined microscopically. Liver samples were taken and used for enzyme determinations. Samples of the prostate gland were taken for possible hormone analysis.

The dose levels used in this study were based on the 15-week feeding

study and a range finding study (study No T7060010). In the range finding study, dogs were dosed with 95 and 152 mg/kg bw/day for 4 weeks to check if capsule administration might overcome possible palatability problems (equivalent to 2500 and 4000 ppm in the diet, respectively). Determinations of the concentrations of the test material in the plasma on days 1 and 12 revealed higher concentrations on day 12 (approximately 25-30 µg/ml) than after the first administration on day 1 (approximately 5-10 µg/ml). Vomiting, increased salivation, tremor and ptosis were seen at both dose levels with a higher severity at the highest dose. In the highest dose group one of the four animals died. Therefore, this study demonstrated that administration of higher doses by means of capsules is not possible and dietary administration was chosen for this study.

4 RESULTS AND DISCUSSION

The concentration, stability and homogeneity of the test material in the diet were acceptable. Daily intakes were equivalent to 0, 1.42, 3.60, 8.88, 34.42 mg/kg bw/day in males and, 0, 1.39, 3.27, 8.30 and 33.80 mg/kg bw/day in females at 0, 40, 100, 250 and 1000 ppm, respectively.

No deaths occurred during the study. There were no clinical signs of toxicity or treatment-related effects on pulse rate, heart rate or body temperature. The reflex reactions and ECG measurements were not affected by treatment. Body weight was not affected by treatment but there was a slight reduction in food intake in females at 1000 ppm. The ophthalmologic investigations did not show treatment-related changes in any of the animals.

The haematological investigations and urinalysis did not reveal any changes between control and treated animals. A slight decrease of thyroxine levels was seen in the 100 and 1000 ppm males in the animals dosed for 26-weeks, but not in the animals dosed for 52-weeks. There was no evidence of liver enzyme induction at the end of the 52-week treatment period (ECOD, EROD, EH, GS-T and UDPGT were measured).

There were no treatment-related macroscopic findings at necropsy. The main organ weight changes are presented in Table A6_5-1. Historical control data are presented in Table A6_5-2.

An increase in mean liver weight (absolute) was observed in the high dose male dogs at 26 weeks but not at 52 weeks. Microscopic examination revealed hepatocellular cytoplasmic changes in male dogs at 26 weeks but not at 52 weeks. Therefore, the report considered these liver effects to be adaptive rather than an adverse effect. At the interim necropsy, prostate weights and sizes in the 100 and 1000 ppm treatment groups were not different from the controls.

At 52-weeks the prostate weights (absolute and relative) and sizes in the high dose group male animals were slightly increased against control and against the treatment groups 40 to 250 ppm. Additional morphological investigations by sonography of the dog prostates over the entire study period did not indicate compound-induced differences between control and treatment groups. The microscopic examinations did not reveal treatment-related morphological changes in the prostate glands at 26 and 52 weeks. The report states that the prostate weights in

Section A6.5 **Chronic Toxicity – oral (2)**

Annex Point IIA6.5 Dog, 52 weeks

the top dose group were within the individual variation range so that this slight weight change is regarded as incidental and of limited toxicological significance. However, three of the 1000 ppm dogs, one of the 250 ppm dogs and two of the 40 ppm dogs have prostate weights that are noticeably above the cited historical control data. The individual prostate weights and body weights are presented in Table A6_5-3. X

Based on the hepatocellular cytoplasmic effects at the next highest dose, a NOAEL of 250 ppm was determined for males (equivalent to 8.88 mg/kg bw/day). A NOAEL of 1000 ppm was determined for females (equivalent to 33.8 mg/kg bw/day in males).

5 CONCLUSION

5.1 Conclusion

The NOAEL of 250 ppm (equivalent to 8.58 mg/kg bw/day in females) was based on liver effects (hepatocellular cytoplasmic changes) at 1000 ppm.

[redacted]

5.1.1 Reliability

[redacted]

Section A6.5 Chronic Toxicity – oral (2)

Annex Point IIA6.5 Dog, 52 weeks

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25/07/2006
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_5-1 Absolute and relative organ weights for male dogs (mean values)

Parameter/dose	0 ppm	40 ppm	100 ppm	250	1000 ppm
26 weeks					
Body weight (kg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Abs[a] liver wt (g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rel[b] liver wt (g/kg).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Abs prostate wt (g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rel prostate wt (g/kg).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
52 weeks					
Body weight (kg)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Abs[a] liver wt (g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rel[b] liver wt (g/kg).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Abs prostate wt (g).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Rel prostate wt (g/kg).	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Key: a) Abs = absolute. b) Rel = relative. c) Range.

Table A6_5-2 Historical control data provided in the report for the prostate gland (mean values)

Study	Absolute weight	Relative weight
26 weeks	[redacted]	[redacted]
52 weeks	[redacted]	[redacted]

Table A6_5-3 Individual body and prostate weights in dogs at 52-weeks

Dog No	Body weight (kg)	Abs prostate weight (g)
Controls		
1	[redacted]	[redacted]
2	[redacted]	[redacted]
3	[redacted]	[redacted]
4	[redacted]	[redacted]
40 ppm		
5	[redacted]	[redacted]
6	[redacted]	[redacted]
7	[redacted]	[redacted]
8	[redacted]	[redacted]
100 ppm		
9	[redacted]	[redacted]
10	[redacted]	[redacted]
11	[redacted]	[redacted]
12	[redacted]	[redacted]
250 ppm		
13	[redacted]	[redacted]
14	[redacted]	[redacted]
15	[redacted]	[redacted]
16	[redacted]	[redacted]
1000 ppm		
17	[redacted]	[redacted]
18	[redacted]	[redacted]
19	[redacted]	[redacted]
20	[redacted]	[redacted]

Section A6.6.1

Annex Point IIA6.6

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

S. typhimurium, Ames-Test

Official use only

1 REFERENCE

1.1 Reference

Herbold, B. (1995a): YRC 2894 - Salmonella/microsome test plate incorporation and preincubation method. Bayer AG, Report No. 23781, date: 1995-02-21.

PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.1 In vitro assays. B.6.4.1.1 Bacterial reverse mutation assay (plate incorporation and pre-incubation method)

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 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 1994 study, the potential mutagenicity of YRC 2894 (thiacloprid) (purity: [redacted]; dissolved in DMSO) was investigated in S. typhimurium (TA 1535, TA 100, TA 1537 and TA 98). 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). Cultures were pre-incubated for 20 minutes before plating out.

4 RESULTS AND DISCUSSION

Cytotoxicity was noted at the highest concentration. No biologically or statistically significant increase in the number of revertant colonies was seen in any strain at any concentration. Appropriate positive controls (sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene) produced significant increases in revertant colonies. Tests were performed in quadruplicate and results were confirmed in an independently repeated assay.

5 CONCLUSION

5.1 Conclusion YRC 2894 was considered to be non-mutagenic in this assay with and without metabolic activation in the plate incorporation assay as well as in the preincubation modification of the Salmonella/microsome test.

[redacted]

5.1.1 Reliability [redacted]

Section A6.6.1

Annex Point IIA6.6

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

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	14/08/2006
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 A6.6.1
Annex Point IIA6.6

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

S. typhimurium and *E.coli*, Reverse mutation assay

Official use only

1 REFERENCE

1.1 Reference

Otha, K. (1995): YRC 2894 - Reverse mutation assay (Salmonella typhimurium and escherichia coli). Nihon Bayer Agrochem K.K. Report No. RA95011, date: 1995-08-24.

PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.1 In vitro assays, B.6.4.1.2 Bacterial reverse mutation assay

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 (1983); US-EPA FIFRA (1991); MAFF (59 Nohsan No. 4200)

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

In a 1995 study, the potential mutagenicity of YRC 2894 (thiacloprid) (purity: [redacted]; dissolved in DMSO) was investigated in *S. typhimurium* (TA 98, TA 100, TA 1535 and TA 1537) and *E. coli* (WP2 uvrA). Five concentrations of up to 5000 µg/plate were used in the presence and absence of a metabolic activation system (phenobarbital and 5,6-benzoflavone-induced rat liver S9).

4 RESULTS AND DISCUSSION

Precipitation of the test material was seen at the highest concentration used in this study. No biologically or statistically significant increase in the number of revertant colonies was seen at any concentration or in any strain. Appropriate positive controls (2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, 9-aminoacridine and 2-aminoanthracene) produced significant increases in the number of revertant colonies. Tests were performed in triplicate and results were confirmed in an independently repeated assay.

X

5 CONCLUSION

5.1 Conclusion

YRC 2894 was considered to be non-mutagenic in this assay with and without metabolic activation.

[redacted]

5.1.1 Reliability [redacted]

Section A6.6.1

Annex Point IIA6.6

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

S. typhimurium and *E.coli*, 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	14/08/2006
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 A6.6.2
Annex Point IIA6.6

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (1)

Chromosome aberration test

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]1995b): YRC 2894 - In vitro mammalian chromosome aberration test with chinese hamster V79 cells[redacted]Report No. 24516, date: 1995-11-24. *PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.1 In vitro assays. B.6.4.1.4 In vitro mammalian chromosome aberration test*
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; US-EPA FIFRA Subpart F-Genetic Toxicology; Rev. July 1 (1986), PB 84-233295; Directive 92/69/EEC method B.10	X
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	

3 MATERIALS AND METHODS

In a 1995 study, YRC 2894 (thiacloprid) (purity: [redacted]; dissolved in DMSO) was tested for clastogenic potential in Chinese Hamster V79 cells. Cells were exposed for 4 hours to YRC 2894 at concentrations of 75, 300 and 750 µg/ml in the presence and absence of a metabolic activation system (Aroclor 1254-induced rat liver S9). Cells were harvested at 18 and 30 hours, 200 metaphases per concentration were scored for chromosomal aberrations.

4 RESULTS AND DISCUSSION

Cytotoxicity (mitotic index 78.6-80.7% of controls) was seen at the highest concentration used in this assay. No biologically or statistically significant increases in chromosome aberrations were seen at any concentration. Appropriate positive controls (mitomycin C and cyclophosphamide) significantly increased the numbers of chromosomal aberrations. X

5 CONCLUSION

5.1	**Conclusion**	YRC 2894 was not considered to be clastogenic for mammalian cells with and without metabolic activation in vitro. [redacted]	
5.1.1	Reliability	[redacted]	X

Section A6.6.2

Annex Point IIA6.6

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (1)

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	15/08/2006
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.6.2

Annex Point IIA6.6

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (1)

Chromosome aberration test

	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 A6.6.2

Annex Point IIA6.6

Genotoxicity studies - In-vitro cytogenicity study in mammalian cells (2)

UDS-Test

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1996a *[Monograph: 1996b]*): YRC 2894 - Test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro. [redacted] Report No. 25429, date: 1996-09-16. *PPP-Monograph Chapter: B.6.4 Genotoxicity. B.6.4.1 In vitro assays. B.6.4.1.5 Unscheduled DNA synthesis*
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 482; US-EPA FIFRA PB 84-233295; Directive 88/302/EEC method B.18
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

In a 1995 study, YRC 2894 (thiacloprid) (purity: [redacted]; dissolved in DMSO) was tested for the induction of unscheduled DNA synthesis (UDS) in vitro. Freshly prepared cultures of Sprague-Dawley rat hepatocytes were exposed to YRC 2894 at 7 concentrations of up to 500 µg/ml in the presence of 10 µCi/mL ^{3}H-thymidine (13.6 Ci/mmol).

4 RESULTS AND DISCUSSION

Cytotoxicity (45.5% survival) was seen at the highest concentration used in this test. 150 cells per concentration were scored for nuclear and cytoplasmic grains following autoradiography. No statistically significant increase in the number of net nuclear grains was seen at any concentration. A statistically significant increase in the number of net nuclear grains was seen with the positive control (2-acetylaminofluorene).

5 CONCLUSION

5.1	**Conclusion**	YRC 2894 was considered to be negative in the in vitro rat primary hepatocyte unscheduled DNA (UDS) assay.
5.1.1	Reliability	[redacted]