Section A6.8.1.1 **Teratogenicity Study**
Annex Point IIA-VI.6.8.1 **Rat**
Oral, gavage

comparable ages to those of the controls. None of the group mean values in the treated groups was significantly different ($p > 0.05$) from the controls. There were no treatment-related macroscopic findings at necropsy at any dose level in the F_0 parental females and surplus F1 weanlings.

4.4 Other effects

There were no clinical signs during the F1 post-weaning development phase that were related to treatment of the F_0 parental females. F1 body weight gains of both sexes during the pre-mating phase at all dose levels were unaffected by parental treatment. However, the weight gain of females derived from maternal animals treated at 5000mg/kg bw/day was 7% lower than the control value during gestation, but the difference was not statistically significant ($p > 0.05$). The effect did not persist during lactation, and there were no treatment-related effects on weight gain at the lower dose levels. The food consumption of all F1 treated groups was unaffected by parental treatment. There were no treatment-related effects at any dose level on the mean age of vaginal patency or on the behavioural development of the F1 progeny after 6 weeks of age. In all groups derived from etofenprox treated females the mean age of vaginal patency, and measurements of mobility, rearing activity, inquisivity, motor coordination and passive avoidance were not significantly different ($p > 0.05$) from control values. There were no treatment-related effects at any dose level on the reproductive capacity of F1 progeny derived from treated F_0 females. Thus, mating performance as assessed by median pre-coital time and stage of oestrous at mating, pregnancy incidence, duration of gestation and litter parameters were generally comparable to, and not significantly different ($p > 0.05$) from control values. Necropsy of the F1 adult progeny and the F2 weanlings did not reveal any treatment-related gross lesions at any dose level.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test method complies with 88/302/EEC, Part B

The deviation from the method does not influence the integrity or validity of the study.

Description of method: rat, oral gavage of mated females, from day 6 to day 17 of gestation, 35 females per group, 3 dose levels, 1 control group (vehicle, i.e. aqueous methylcellulose), 21-24 females killed on day 20 of gestation to investigate possible effects on embryo-fetal development , remaining 11 - 14 females/group were allowed to litter normally and rear their young. Observations included body weight, food consumption, mortality, clinical signs, post mortem examination of all sacrificed animals (including foetuses) , developmental/behavioural examinations of pups from 6 weeks of age, reproductive capability of pups from approximately 84 days of age.

5.2 Results and discussion

A no-observed-effect-level (NOEL) for all effects on parental females was established as 12.5mg/kg bw/day, based on the occurrence of minor clinical signs at 250mg/kg bw/day. However, the no-observed-adverse-effect-level (NOAEL) is considered to be 250mg/kg bw/day, based on slightly reduced weight gain during gestation at 5000mg/kg bw/day.

Section A6.8.1.1 **Teratogenicity Study**

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Oral, gavage

An NOEL for reproductive effects in treated females was established as 5000mg/kg bw/day, the highest dose level employed, based on the absence of effects at this dose level.

An NOEL for embryofetal and post-natal developmental effects including reproductive capacity was established as 250mg/kg bw/day, based on the occurrence of minimally reduced weight gain during gestation in F1 generation progeny at 5000mg/kg bw/day.

5.3 Conclusion

5.3.1	LO(A)EL maternal toxic effects	Not reported
5.3.2	NO(A)EL maternal toxic effects	NOEL, parental, all effects: 12.5mg/kg bw/day NOAEL, parental, all effects: 250mg/kg bw/day NOEL, parental, reproductive effects: 5000mg/kg bw/day
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Not reported
5.3.4	NO(A)EL embryotoxic / teratogenic effects	250mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Table A6_8_1_1-1. Summary of litter parameters - F_0 generation - day 20 sacrifice.

Parameter	**Group mean value at (mg/kg bw/day):**			
	0	**12.5**	**250**	**5000**
No. females paired for day 20 kill	24	22	21	23
No. pregnant - day 20 sacrifice	20	20	20	20
No. corpora lutea	12.7	12.6	12.3	12.8
No. implantation sites	11.6	10.6	10.6	11.1
Pre-implantation loss (%)	7.4	14.7	13.7	12.5
No. resorptions (mean/litter): - early - late - total	 0.5 0.1 0.6	 0.4 0.3 0.6	 0.5 0.1 0.6	 0.6 0.2 0.7
Post-implantation loss (%)	4.7	5.9	5.0	6.8
No. live fetuses (mean/litter)	11.0	9.9	10.1	10.4
Total no. fetuses	220	189	201	208
Sex ratio (% males)	50.6	53.1	49.0	41.8
Litter weight (g)	38.51	34.78	35.93	36.60
M + F fetal weight (g)	3.50	3.49	3.58	3.54

Table A6_8_1_1-2. Incidences of fetal malformations and anomalies - day 20 sacrifice.

Group (mg/kg bw/day)	Fetal incidence of:								
	Malformations			Anomalies[e]					
				Visceral			Skeletal		
	Examined	No.	Mean (%)	Examined	No.	Mean (%)	Examined	No.	Mean (%)
0	220	1[a]	0.4	108	5	4.6	111	17	15.5
12.5	189	1[b]	0.5	97	5	4.0	91	13	14.0
250	201	1[c]	0.5	100	4	4.3	100	8	7.4
5000	208	3[d]	1.6	98	12	13.4	107	13	10.7

[a] fetus with partial absence of inferior vena cava, sinistral rotation of heart and interventricular septal defect, absent intermediate lung lobe and distortion of right adrenal; [b] fetus with palate irregularities, agnathia and distortion of other cranio-facial structures and bilateral open eye; [c] fetus with interventricular septal defect; [d] one fetus with unilateral microphthalmia, one fetus with hydrocephaly and absent innominate artery, one fetus with right microphthalmia and left anophthalmia; [e] excluding fetuses with malformations

Table A6_8_1_1-3. Summary of litter parameters - F_0 generation - littering groups.

Parameter	Group mean value at (mg/kg bw/day):			
	0	12.5	250	5000
No. females paired (for littering)	11	13	14	12
No. pregnant - littering	11	12	14	12
Duration of gestation (days)[a]	21.7	21.7	21.9	21.8
No. pups born (mean/litter)	11.5	11.3	10.9	11.2
No. live pups at birth (mean/litter)	11.2	11.3	10.9	11.2
No. pups on day 4 pre-cull	10.8	11.2	10.7	11.1
Cumulative loss (%) - birth - day 4	5.7	1.3	1.6	0.6
No. pups on day 12 (mean/litter)	7.9	8.0	8.0	8.0
No. pups on day 21 (mean/litter)	7.9	8.0	8.0	8.0
Cumulative loss (%) - day 4 - day 21	0.0	0.0	0.0	0.0
Total no. of progeny	126	136	153	134
Sex ratio (% males)	46.2	54.5	54.2	54.6
Litter weight (g):				
- birth	67.3	68.6	66.7	67.4
- day 21	377.0	366.7	396.9	390.1
M + F fetal weight (g):				
- birth	6.0	6.1	6.2	6.1
- day 4	9.3	9.2	9.4	9.4
- day 8	16.9	16.8	17.2	16.8
- day 21	47.7	45.9	49.6	48.8

[a] mean duration for both day 20 kill and littering groups

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1.2. Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. ST-103 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 96,3% slightly lower than in the 5 batch analysis. Therefore the specification does not relevantly deviate to these indications.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.1.2 Teratogenicity Study

Annex Point IIA-VI.6.8.1

Rabbit

Oral, gavage

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2000): Rabbit developmental toxicity study with Etofenprox: [redacted] unpublished report no. 6648-146 (September 13, 2000). Dates of work: May 17, 2000 - June 15, 2000.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. OECD guideline no. 414 (1999) - exceeds 88/302/EEC; US-EPA OPPTS 870.3700; Japan MAFF, 59 NohSan 4200 (1985)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	Batch no. 21088	
3.1.2	Specification	Deviating from specification given in section 2 as follows	X
3.1.2.1	Description	Pale yellow crystal	
3.1.2.2	Purity	96.68%	
3.1.2.3	Stability	No information provided in the report.	
3.2	**Test Animals**		
3.2.1	Species	rabbit	
3.2.2	Strain	(Hra:(NZW)SPF strain	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	5 month old, body weight 2.88 - 4.68kg	
3.2.6	Number of animals per group	22 females per group	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Females mated prior to study start.	

Section A6.8.1.2 — Teratogenicity Study

Annex Point IIA-VI.6.8.1

Rabbit

Oral, gavage

3.3	**Administration/ Exposure**	*Oral*
3.3.1	Duration of exposure	Day 6 through 28 of gestation (day 0 = day following confirmed mating)
3.3.2	Postexposure period	None
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Nominal dose levels of 0, 30, 100 and 300mg/kg bw/day
3.3.5	Vehicle	Aqueous methylcellulose
3.3.6	Concentration in vehicle	Not reported
3.3.7	Total volume applied	Not reported
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Mortality	Yes. Mortality checks were performed twice daily
3.4.2	Body weight	Yes. Body weights were recorded on days 0 and 4, at 3-day intervals from day 6 to day 27 of gestation, and on the day of necropsy.
3.4.3	Food consumption	Yes. Food consumption was recorded from day 4 of gestation until necropsy.
3.4.4	Clinical signs	Yes. Clinical signs were recorded approximately one hour after treatment. All animals were given a full clinical examination on 10 occasions.
3.4.5	Examination of uterine content	The uterus was excised, weighed, and examined for the number and position of implantation sites, live and dead fetuses, early and late resorptions and abnormalities of the placentae and amniotic sacs. The ovaries were examined for the number of corpora lutea. Gross lesions and the uterus were preserved.
3.4.6	Examination of foetuses	Live fetuses were weighed, examined for external and oral cavity abnormalities, identified and then killed. A mid-coronal slice was made in the head of each fetus to evaluate the cranial organs. The thoracic and abdominal organs were examined fresh using Staples' technique and the sex recorded by examination of the internal reproductive organs. Fetal visceral abnormalities were preserved where possible. The fetal carcasses were eviscerated and processed for skeletal evaluation, including degree of ossification, using alizarin red S staining. Findings were classified as either variations or malformations. Variations were defined as structural deviations considered not to have an effect on body conformity or well-being. Malformations were defined as developmental deviations that were gross structural changes, incompatible with life or which may affect the quality of life.
3.5	**Further remarks**	- The stability and homogeneity of the 30 and 300mg/mL formulations of etofenprox were assessed on the first formulations to be prepared. All formulations were analysed for achieved concentration on 4 occasions.

Section A6.8.1.2 **Teratogenicity Study**

Annex Point IIA-VI.6.8.1 **Rabbit**

Oral, gavage

- Animals dying or aborting during the study and animals killed in a moribund condition were subjected to necropsy and examined for gross lesions. The uterus and ovaries were examined for implantations and corpora lutea, respectively. The extent of development of the implantation sites was determined to indicate early or late resorbing fetuses, dead fetuses or normally developing fetuses.

- Animals sacrificed at the end of the study were examined for gross abnormalities of the cervical, thoracic and abdominal viscera.

3.6 Statistics

Where appropriate, data were analysed by one-way ANOVA or ANCOVA. Levene's test was used to analyse the homogeneity of variances. The incidences of some skeletal variations were analysed using the Cochrane-Armitage test for linear trend followed by Fisher-Irwin exact test.

Section A6.8.1.2
Annex Point IIA-VI.6.8.1

Teratogenicity Study
Rabbit
Oral, gavage

4 RESULTS AND DISCUSSION

4.1 Analytics

Formulations containing 30 or 300mg/mL etofenprox were stable at 38 - 42°C for at least 10 days, at which time the 30 and 300mg/mL formulations contained 101.4 and 98.6% of the day 0 concentrations, respectively. Homogeneity of the 30 and 300mg/mL formulations was acceptable, with RSD values of 4.24 and 2.13%, respectively, for 12 analyses/concentration. Analysis of all formulations for achieved concentration on 4 occasions demonstrated the achieved concentrations were within the range 99.1 to 109.8% nominal concentrations.

4.2 Maternal toxic Effects

X

Mortalities:

One, one and four females treated at 30, 100 or 300mg/kg bw/day, respectively, died or were killed prematurely. One animal treated at 30mg/kg bw/day aborted, one animal at 100mg/kg bw/day died on day 16 and 2 animals at 300mg/kg bw/day aborted. A further animal at 300mg/kg bw/day died during abortion on day 26 of gestation and the other animal at 300mg/kg bw/day was killed in a moribund condition. Treatment-related clinical signs were confined to a higher incidence of thinness and few or no feces at 300mg/kg bw/day.

Clinical signs (see T able A6_8_1_2-1):

Females treated at 300mg/kg bw/day showed weight loss from day 6 to 9 and from day 21 - 29, resulting in an overall group mean weight loss of 37.4g during the treatment period. Thus, at termination on day 29, the group mean body weight was 9.0% lower than the control value. The weight gains during treatment at lower dose levels were not affected by treatment and were not significantly different ($p > 0.05$) from the control gain. The mean food consumption at 300mg/kg bw/day was reduced throughout the treatment period and the overall food consumption during treatment was significantly ($p < 0.05$) lower than the control consumption by 18.9%. The food consumption during treatment at lower dose levels was not affected by treatment and was not significantly different ($p > 0.05$) from the control consumption.

Gross findings:

Treatment-related gross findings at necropsy in maternal animals were confined to gastrointestinal distention and mucosal irritation in animals at 300mg/kg bw/day that died or were killed prematurely. There were no treatment-related gross lesions at necropsy in the animals that survived to day 29.

Effects on litter (see Table A6_8_1_2-2):

All animals that died or were killed prematurely were pregnant. There were no effects of treatment at any dose level on pregnancy incidence, gravid uterus weight, number of corpora lutea, pre-implantation loss, number of live fetuses and sex ratio. There was, however, a slight increase ($p > 0.05$) in post-implantation loss at 300mg/kg bw/day due to an increased incidence of litters with resorptions. The mean number of live fetuses at 300mg/kg bw/day was comparable to the control group

Section A6.8.1.2 **Teratogenicity Study**
Annex Point IIA-VI.6.8.1 **Rabbit**
Oral, gavage

due to a higher number of implantations at 300mg/kg bw/day. The covariate-adjusted mean fetal weights at 300mg/kg bw/day were significantly ($p < 0.01$) reduced, but the effect was not apparent at lower dose levels. Therefore, it is considered that reduced fetal weight at 300mg/kg bw/day was a consequence of maternal toxicity.

4.3 Teratogenic / embryotoxic effects

See Tables A6_8_1_2-3 and A6_8_1_2-4.

The nature and incidence of fetal malformations did not indicate an effect of treatment at any dose level.

There were no external variations in any experimental group. Soft tissue variations were those commonly seen in rabbits of this strain and the incidences were comparable in all treatment and control groups. All skeletal variations were of the type commonly occurring in rabbits of this strain. Three skeletal variations occurred at significantly higher fetal incidences in one or more treated groups compared with the controls. Unossified 5th sternebra was significantly higher ($p < 0.05$) at 30 and 100mg/kg bw/day, but not at 300mg/kg bw/day. Since there was no dose-relationship and all incidences were within the historical control range of 4.5 - 17%, the differences are considered not to be related to treatment with etofenprox. The incidences of unossified talus and 13th full rib were significantly ($p < 0.05$) higher at 300mg/kg bw/day. The increased incidence of unossified talus is considered to be a consequence of the observed intrauterine growth retardation. The incidence of 13th full rib was slightly higher than the maximum historical control incidence of 42%, but since the difference was numerically small and the litter incidence at 300mg/kg bw/day (88%) was comparable to the control group litter incidence (86%), it is considered not to be treatment-related. The incidences of all other individual skeletal variations in the treated groups were not significantly different ($p > 0.05$) from the controls.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test guidelines: OECD guideline no. 414 (1999) - exceeds 88/302/EEC; US-EPA OPPTS 870.3700; Japan MAFF, 59 NohSan 4200 (1985).

No deviations from the test guidelines.

Description of method: rabbit, oral gavage of mated females, from day 6 to day 28 of gestation, 22 females per group, 3 dose levels, 1 control group (vehicle, i.e. aqueous methylcellulose), all surviving animals sacrificed on Day 29. Observations included body weight, food consumption, mortality, clinical signs, *post mortem* examination of all sacrificed animals (including foetuses).

5.2 Results and discussion

A no-observed-effect-level (NOEL) for maternal animals was established as 100mg/kg bw/day, based on the occurrence of reduced weight gain and food consumption at 300mg/kg bw/day. X

An NOEL for teratogenicity was established as >300mg/kg bw/day, based on no excess incidences of malformations at this dose level. An NOEL for general embryofetal toxicity was established as 100mg/kg bw/day, based on the occurrence of slightly increased post-implantation

Section A6.8.1.2 **Teratogenicity Study**
Annex Point IIA-VI.6.8.1 **Rabbit**
Oral, gavage

loss and intrauterine growth retardation at 300mg/kg bw/day.

5.3 Conclusion

5.3.1	LO(A)EL maternal toxic effects	Not reported
5.3.2	NOEL maternal toxic effects	100mg/kg bw/day
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Not reported
5.3.4	NOEL / teratogenic effects	>300mg/kg bw/day
5.3.5	NOEL / general embryofetal toxicity	100mg/kg bw/day
5.3.6	Reliability	1
5.3.7	Deficiencies	No

Table A6_8_1_2-1. Summary of maternal body weight and food consumption data.

Parameter	Group mean value at (mg/kg bw/day):			
	0	30	100	300
Body weight (kg) on:				
- day 0	3.68	3.71	3.65	3.68
- day 6	3.85	3.85	3.82	3.81
- day 29	4.10	4.11	4.04	3.70**
Weight change (g) day 6 - 29	248	311	214	- 37.4**
Food consumption (g/day):				
- days 4 - 6	165.7	171.2	172.4	170.8
- days 6 - 29	152.3	155.7	146.5	123.5*

* $p < 0.05$; ** $p < 0.01$

Table A6_8_1_2-2. Summary of litter parameters.

Parameter	Group mean value at (mg/kg bw/day):			
	0	**30**	**100**	**300**
No. mated	22	22	22	22
No. pregnant	21	20	21	21
No. aborted	0	1	0	3
No. dying	0	0	1	2
No. with live litter on day 29	21	19	20	17
Gravid uterus weight (g)	472.6	522.4	503.2	436.7
No. corpora lutea	9.7	10.1	10.3	10.4
No. implantation sites	8.1	8.7	9.0	9.3
Pre-implantation loss (%)	16.1	12.9	14.5	10.9
Resorptions (%mean/litter):				
- early	0.2	0.1	0.3	0.6
- late	0.2	0.1	0.1	0.4
- total	0.4	0.2	0.4	1.0
Total no. dead fetuses	0	0	0	0
Post-implantation loss (%mean/litter)	4.3	1.5	3.8	10.1
Total no. live fetuses	162	163	172	141
No. live fetuses (mean/litter)	7.7	8.6	8.6	8.3
Sex ratio (% males)	58	52	46	45
Fetal weight[a] (g):				
- male	41.32	43.37	42.47	35.92**
- female	41.62	43.13	41.16	35.11**
- mean (M + F)	41.70	43.45	41.94	35.66**

[a] covariate adjusted; ** $p < 0.01$

Table A6_8_1_2-3. Summary of external, visceral and skeletal malformations.

Parameter	Incidence (%) at (mg/kg bw/day):			
	0	**30**	**100**	**300**
No. litters evaluated	21	19	20	17
No. fetuses evaluated	162	163	172	141
Incidence of external malformations				
- litter	0	0	5.0	0
- fetal	0	0	0.6[a]	0
Incidence of visceral malformations				
- litter	0	0	5.0	0
- fetal	0	0	0.6[b]	0
Incidence of skeletal malformations				
- litter	4.8	5.3	15.0	0
- fetal	0.6[c]	0.6[d]	1.7[e]	0

[a] one fetus with open eye; [b] one fetus with heart and/or great vessel malformation and malpositioned kidney; [c] one fetus withvertebral anomaly with/without rib anomaly; [d] one fetus with fused skull bones; [e] one fetus with fused skull bones, one fetus with major fusion of sternebrae, one fetus with forked/fused ribs

Table A6_8_1_2-4. Incidence of external, visceral and skeletal variations.

Parameter	**Incidence (%) at (mg/kg bw/day):**			
	0	**30**	**100**	**300**
No. litters evaluated	21	19	20	17
No. fetuses evaluated	162	163	172	141
Incidence of external variations - litter - fetal	 0 0	 0 0	 0 0	 0 0
Incidence of visceral variations - litter - fetal	 76 23	 63 18	 85 20	 59 16
Incidence of skeletal malformations - litter - fetal	 100 80	 100 79	 100 76	 100 88
Fetal incidence of unossified talus	0	0	2.3	4.3*
Fetal incidence of unossified 5th sternebra	4.3	12*	16*	9.2
Fetal incidence of 13th full rib	40	42	33	56*

* $p < 0.05$

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1.2. Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. 21088 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 96,68% slightly lower than in the 5 batch analysis. Therefore the specification does not relevantly deviate to these indications.
Conclusion	**4.2. Maternal toxic effects** and **5.2. Results and discussion** The NOEL for maternal animals can be established as 100mg/kg bw/day based on the occurrence of reduced weight gain (-9%) and reduced food consumption (-18,9%) and unscheduled deaths (4 of 22) at 300 mg/kg bw/day.
Reliability	1
Acceptability	Agree with applicant's version
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA-VI.6.8.2 **Rat, oral**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985d): Effect of Ethofenprox (MTI-500) on multiple generations of the rat; [redacted] unpublished report no. MTC 67/85706 (December 20, 1986; re-issued amended pages January 07, 1985). Dates of work: July 12, 1983 - November 06, 1984.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Not specified in report but the method conformed to 88/302/EEC, Part B	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Deviations: histopathology on reproductive tracts from F_0 generation animals not performed; pituitary and cervix not examined in F1b animals.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	Batch no. ST-103	
3.1.2	Specification	Deviating from specification given in section 2 as follows	X
3.1.2.1	Description	Pale yellowish crystalline solid	
3.1.2.2	Purity	96.3%	
3.1.2.3	Stability	No information in the report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley (CrL:COBS CD (SD) BR strain	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 week old, 106 - 148g body weight	
3.2.6	Number of animals per group	F_0 (i.e. P) generation: 28 animals / sex / group The F_0 generation was mated twice to provide F1a and F1b generations. Groups of 24 animals/sex/group, selected from the F1b litters, were mated twice to provide F2a and F2b generations. Selected F2b progeny (1 male and 1 female pup per litter) were reared to maturity.	
3.2.7	Mating	The F_0 generation animals of both sexes were paired for a 20-day mating period during which time daily vaginal smears were taken to detect mating and to detect anomalies of the oestrous cycle. The day sperm or a	

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		plug were detected was considered day 0 of gestation. Mated females were allowed to rear their young to day 21 *post partum*, at which time 24 weanlings/sex were selected to form the F1a generation. A further one weanling/sex/litter were selected for necropsy, organ weight analysis and full tissue preservation for possible full histological evaluation. Excess weanlings were subjected to necropsy and gross *post mortem* examination and then discarded. Approximately 10 days after weaning of the F1a generation, the F_0 generation animals were re-mated employing a different pairing regime and mating unmated females with previously successful males and *vice versa*. The F1b generation was selected on day 21 *post partum* as for the F1a generation. The procedure was repeated for the F1b generation to produce the F2a and F2b generations.
3.2.8	Duration of mating	20 days
3.2.9	Deviations from standard protocol	Second mating of parent and F1 generations (see point 3.2.7)
3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral Dietary admixture
3.3.1	Animal assignment to dosage groups	F_0 generation – first mating: 28 females paired in each group; second mating: 28 females paired in each group, except at 100 ppm (26 females paired). F1B generation: – first and second matings: 24 females paired in each group except high dose group (23).
3.3.2	Duration of exposure before mating	10 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	The diets were fed continuously to the F_0 generation for 25 weeks (from 6 weeks of age to weaning of the F1b generation), continuously to the F1 generation for 28 weeks (from weaning to weaning of the F2b generation) and continuously to the F2 generation for at least 13 weeks from weaning.
		Oral
3.3.4	Type	admixture in the diet
3.3.5	Concentration	- Nominal concentrations of 0, 100, 700 and 4900ppm. - Achieved dietary concentrations were determined by analysis at approximately 10-week intervals during the study. The stability and homogeneity of mixing had been established in previous studies. The mean achieved dose levels were calculated only for the pre-mating phase of each generation and, therefore, do not take account of the increase in food consumption during lactation. Achieved intakes of test substance are presented in Table A6_08_2-1.
3.3.6	Vehicle	Corn oil
3.3.7	Concentration in vehicle	No information in the report

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3.3.8	Total volume applied	No information in the report
3.3.9	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes. All parental animals of all generations were observed regularly to detect clinical signs.
3.4.2	Body weight	Yes. Parental animals were weighed weekly throughout the study, including gestation and lactation, but except during the mating period when females were weighed at 2-day intervals. Body weights of the F1a, F1b, F2a and F2b animals were recorded at birth, 4, 8, 12 and 21 days *post partum*.
3.4.3	Food/water consumption	Food consumption was measured weekly throughout the pre-mating phases. Water consumption was measured in F1a animals during weeks 11 and 12, in F1b animals during weeks 5, 6, 13 and 14, and in F2b animals during weeks 7 and 8.
3.4.4	Oestrus cycle	Vaginal smears were taken during the mating period to enable the number of animals that mated on specific days to be determined. This information was used to: - detect whether or not pregnancy was interrupted after mating - detect marked anomalies of the oestrus cycle - determine the median pre-coital time for the group.
3.4.5	Sperm parameters	Testes, prostates and seminal vesicles of males that failed to induce pregnancy were weighed and preserved in 10% formalin to permit histological examination.
3.4.6	Offspring	Number and sex of pups, weight gain, physical or behavioural abnormalities, mortality
3.4.7	Organ weights P and F1	Weighed prior to fixation: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, prostate with seminal vesicles, spleen, testes with epididymides, thymus, uterus with vagina. Weighed after fixation: pituitary, thyroids.
3.4.8	Histopathology P and F1	In the first instance histopathological examination was restricted to F1B adults in the controls and 4900 ppm groups adrenal glands, bone marrow (sternum), bone (femur), brain, caecum, duodenum, eyes, ileum, lymph nodes, heart, kidneys, liver, lungs, ovaries, pancreas, pituitary, prostate with seminal vesicles, salivary gland, skeletal muscle, spleen, stomach, testes with epididymides, thymus, thyroids, urinary bladder, uterus with vagina. Examination was then extented to other adult animals as follows: - F0 generation: kidneys of controls and at 4900 ppm - F1B generation: kidneys at 100 and 700 ppm, liver and thyroids at 700 ppm

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3.4.9	Histopathology F1 not selected for mating, F2	F2B generation: thyroids of female at all dosages
3.5	**Further remarks**	Statistical analyses were performed using the litter as the sampling unit. Non-parametric Kruskal-Wallis and Jonckheere's tests were used to analyse litter size and weight, pup mortality and weight. Organ weights were analysed by ANCOVA using body weight as the covariate. Treatment means were compared to control means using Williams' test.

4 RESULTS AND DISCUSSION

4.1 Effects

4.1.1	Clinical signs	Treatment-related clinical signs were confined to occasional red/brown staining of the tray paper under some cages of F1a, F1b and F2b animals at 700 or 4900ppm. The effect was not apparent at 100ppm.
4.1.2	Mortality	There were 6 deaths in F_0 and F1b parental animals. A male F0 animal treated at 100ppm died in week 10, and 2 female F_0 generation animals from the same group died or were killed in week 23. The female deaths were associated with dystocia, but since there were no F_0 deaths at higher dose levels, all F_0 deaths were considered incidental to treatment. A male F1b animal at 700ppm died in week 8, a male F1b animal at 4900ppm was killed in a moribund condition in week 17 and a female F1b animal at 4900ppm died in week 14. Based on renal changes observed at necropsy in the 4900ppm animals, death may have been related to treatment. Similar renal findings were not evident in the animal at 700ppm that died.
4.1.3	Body weight	See Table A6_8_2-2. There was no effect of treatment at any dose level on the weight gains of F_0 and F2b males, but the overall weight gain of F1b males at 4900ppm was slightly reduced by 5.7% in week 14. Lower dose levels were not affected. The pre-mating weight gains of F_0 and F1b females were slightly reduced at 4900ppm, as was the overall weight gain of F1a females at 4900ppm, but lower dose levels were not affected. There was no consistent effect of treatment at any dose level in any generation on the weight gains during gestation and lactation.
4.1.4	Food and water consumption	There was no effect of treatment at any dose level in any generation on food consumption during the pre-mating or post-weaning growth periods. The water consumption of both sexes treated at 4900ppm in the F1a, F1b and F2b generations was increased by up to 52.4% relative to the controls. The effect was more marked in the F1 litters than in the F2b animals. No effect on water consumption was apparent at lower dose levels.
4.1.5	Mating performance	See Table A6_8_2-3. The mating performance of males and females, as assessed by pregnancy incidence and median pre-coital time, was not affected by treatment at any dose level in either generation at either pairing. The duration of gestation was also unaffected by treatment.

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4.1.6 Litter paramaters

* Litter size (Table A6_8_2-4)

No treatment-related effects occurred at any dose level in any of the 4 matings on litter size at birth and pup mortality to day 12 *post partum*. However, pup mortality at 4900ppm in all 4 litters was slightly, but not significantly ($p > 0.05$), higher than the controls from day 12 to day 21 *post partum*. Two instances of total litter loss occurred at 4900ppm during the latter half of lactation. Cumulative litter loss was elevated only in the F1a litters but was not statistically significant ($p > 0.05$). Pup mortality at lower dose levels was comparable to the control values.

* Pup weight and sex ratio (Table A6_8_2-5)

The pup weights at birth of all generations at all dose levels were comparable to the controls, but pup weights in all generations were reduced at 4900ppm from day 4 *post partum*. The effect generally gained statistical significance ($p < 0.05$ or 0.01) from day 8 and persisted until weaning on day 21. Pup weights at lower dose levels were unaffected by treatment. There was no effect at any dose level on sex ratio. Clinical signs in neonates were confined to some pups at 4900ppm that showed body tremors, distended abdomen and abnormal gait during late lactation, some of which subsequently died.

4.1.7 Gross findings at necropsy

See Table A6_8_2-6.

There were no treatment-related gross findings at necropsy at any dose level in the F_0 generation parental animals. However, at 4900ppm many of the F1a, F1b, and F2b adults, and weanlings from most litters, showed enlarged, swollen or misshapen kidneys, the frequency of which indicated a clear treatment-related effect. The effect was not apparent at lower dose levels. A small number of weanlings from F1a and F2a litters treated at 700 or 4900ppm also showed ocular defects or subcutaneous haemorrhage.

4.1.8 Organ weight

See Table A6_8_2-7.

There was a treatment-related, statistically significant ($p < 0.05$ or 0.01) increase in the group mean kidney weights of all generations at 4900ppm, except F_0 females. The effect was apparent in both adult and weanling animals of both sexes, and also extended to F2b adult females at 700ppm. Kidney weights of the other generations at 700ppm and of all generations at 100ppm were not affected by treatment. There was a treatment-related, statistically significant ($p < 0.01$) increase in the group mean liver weights of all generations at 4900ppm with adult and weanling animals of both sexes affected. The effect extended to weanling animals of the F1a, F2b and male F1b generations treated at 700ppm. Liver weights were unaffected by treatment at 100ppm. Thyroid weights were significantly ($p < 0.05$ or 0.01) elevated in adult animals of both sexes at 4900ppm in all generations, except for F1b adult females. The effect was not apparent in weanling animals. Although the thyroid weights of all treated groups of F1b females were significantly ($p < 0.05$ or 0.01) higher than the controls, the differences were considered to be due to unusually low control thyroid weights. Significantly lower thyroid weights in weanlings of some generations are considered to be a consequence of unusually high control values. Other treatment-related effects on organ weights were confined to slightly, but significantly ($p < 0.05$ or 0.01) increased heart weights in weanlings of all generations and in F2b adult females at 4900ppm. Heart

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weights were generally not affected at lower dose levels, although those of F1a female weanlings at 100 or 700ppm were also significantly ($p < 0.05$ or 0.01) higher than the controls. There were no treatment-related effects on the weights of the reproductive organs in either sex at any dose level.

4.1.9 Histopathology

See Table A6_8_2-8.

Treatment-related histopathological alterations occurred in the kidneys, liver and thyroid glands of F1b generation adult animals. Cystic collecting ducts occurred in a large proportion of these animals at 4900ppm and were frequently associated with focal medullary fibrosis, mineral deposits, vascular congestion or haemorrhage in the medulla and acute inflammatory cell infiltration in the ducts. The occurrence of cortical scarring in 14 males and 11 females, and increased incidences of basophilic tubules (males) and dilated cortical tubules (females) at 4900ppm were considered to be sequelae to the medullary changes. Epithelial hyperplasia in the collecting ducts of one male and 2 females at 4900ppm was also considered to be treatment-related. A marginally higher incidence of glomerulonephritis at 4900ppm is considered not to be treatment-related. With the exception of one female at 700ppm that showed cystic collecting ducts in both the renal medulla and cortex, the foregoing renal alterations were not evident at 700ppm. No treatment-related renal alterations occurred in the F_0 generation adults. Treatment-related alterations in the liver occurred at 4900ppm only, and comprised minimal hepatocyte enlargement in many of the F1b animals examined. The occurrence of minimal hepatocyte vacuolation in males at 700 and 4900ppm is considered not to be related to treatment. Thyroid alterations were confined to 6 males that showed slightly increased height of the columnar epithelium. There were no treatment-related histopathological alterations in the other tissues examined in F1b animals at 4900ppm, including male and female reproductive organs (prostate, seminal vesicles, testes epididymides, ovaries, uterus and vagina).

4.2 Other

Achieved concentrations of etofenprox in all formulations prepared on 10 occasions were within the range 91 - 112% nominal concentrations, with the exception of two 100ppm samples that were 81% nominal concentration.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test guidelines not specified in report but the method conformed to 88/302/EEC, Part B except for:

Deviations: histopathology on reproductive tracts from F_0 generation animals not performed; pituitary and cervix not examined in F1b animals.

Description of method: rat, 2-generations reproductive study with second mating of parent and F1 generations, dietary admixture, dose levels of 100, 700 and 4900 ppm, control group. Examinations included mortality, clinical signs, food and water consumption, body weight, mating performance, litter parameters, organ weights, gross findings at necropsy, histopathology.

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5.2 Results and discussion

A no-observed-effect-level (NOEL) for all effects in F_0 generation parental animals was established as 700ppm, equivalent to minimum dose levels of 37 and 44mg/kg bw/day, in males and females, respectively, based on the occurrence of reduced weight gain and increased kidney, liver and thyroid weights at 4900ppm.

A NOEL for reproductive effects in F_0 and F1b parental generations was established as 700ppm, equivalent to minimum dose levels of 37 and 44mg/kg bw/day in males and females, respectively, based on minimally increased pup mortality and reduced pre-weaning weight gain at 4900ppm.

A NOEL for effects on pup development was established as 100ppm, equivalent to minimum dose levels of 4.3 and 5.6mg/kg bw/day in males and females, respectively, based on the occurrence of increased kidney and liver weights, and a single occurrence of histopathological alterations in the kidney at 700ppm, and additionally increased water consumption, pre-weaning tremors and abnormal gait, increased heart weights, and histopathological alterations in the kidneys, liver and thyroid at 4900ppm.

5.3 Conclusion

5.3.1	LO(A)EL	Not reported
5.3.1.1	Parent males	
5.3.1.2	Parent females	
5.3.1.3	F1 males	
5.3.1.4	F1 females	
5.3.1.5	F2 males	
5.3.1.6	F2 females	
5.3.2	NOEL	
5.3.2.1	Parent males	37 mg/kg bw/day
5.3.2.2	Parent females	44 mg/kg bw/day
5.3.2.3	F1 males	5.5 mg/kg bw/day
5.3.2.4	F1 females	6.5 mg/kg bw/day
5.3.2.5	F2 males	4.3 mg/kg bw/day
5.3.2.6	F2 females	5.6 mg/kg bw/day
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6_8_2-1. Achieved intakes of test substance (mg/kg bw/day)

Approx. age (wks)	Generation	Males treated at (ppm):			Females treated at (ppm):		
		100	700	4900	100	700	4900
5	F1a	14.1	102	744	14.3	104	753
	F1b	13.7	97	702	13.4	95	686
6	F2b	12.9	90	670	13.2	92	670
7	F_0	10.6	74	509	10.0	71	481
14	F1b	5.5	37	279	6.5	48	316
16	F_0	5.0	37	246	6.6	44	343
16	F1a	5.1	35	267	5.9	44	309
19	F2b	4.3	30	225	5.6	40	302

Table A6_8_2-2. Summary of body weights.

Generation	Time (weeks)	Group mean body weight (g) of:							
		Males treated at (ppm):				Females treated at (ppm):			
		0	100	700	4900	0	100	700	4900
F_0	0	193	195	193	194	150	150	149	150
	10	507	508	508	514	292	289	288	273
	25	638	651	646	655	351	356	345	348
F1a	4	103	103	104	93	92	92	94	84
	16	519	519	532	507	295	295	293	273
F1b	4	101	110	107	95	92	94	94	85
	14	504	511	520	475	273	278	271	257
	30/31	674	692	713	655	337	350	344	336
F2b	4	113	115	112	106	101	101	101	94
	19	565	553	559	559	307	305	306	291
F_0 1st mating	D0 gestation	-	-	-	-	294	289	289	272
	D20 gestation	-	-	-	-	407	403	397	388
F_0 2nd mating	D0 gestation	-	-	-	-	324	323	323	304
	D20 gestation	-	-	-	-	442	434	432	420
F1b 1st mating	D0 gestation	-	-	-	-	273	278	276	258
	D20 gestation	-	-	-	-	384	389	376	368
F1b 2nd mating	D0 gestation	-	-	-	-	313	312	309	299
	D20 gestation	-	-	-	-	419	422	419	403

Table A6_8_2-3. Summary of mating performance - F_0 and F1b generations.

Parameter	Generation / mating	Group mean value at (ppm):			
		0	100	700	4900
No. females paired	F_0 / 1st	28	28	28	28
Pregnancy incidence (%)		96	89	96	100
Median pre-coital time (days)		3.0	2.0	2.5	3.0
Duration of gestation (days)		21.7	21.8	21.7	21.8
No. females paired	F_0 / 2nd	28	26	28	28
Pregnancy incidence (%)		96	92	96	100
Median pre-coital time (days)		3.0	2.0	3.0	3.0
Duration of gestation (days)		21.8	22.0	21.9	21.7
No. females paired	F1b / 1st	24	24	24	23
Pregnancy incidence (%)		88	88	88	100
Median pre-coital time (days)		2.5	3.0	2.0	2.0
Duration of gestation (days)		22.0	22.2	21.9	21.8
No. females paired	F1b / 2nd	24	24	24	23
Pregnancy incidence (%)		88	88	96	100
Median pre-coital time (days)		3.0	2.5	3.0	3.0
Duration of gestation (days)		21.9	21.7	21.8	21.5

Table A6_8_2-4. Summary of pre-weaning survival.

Parameter	Generation / mating	Group mean value at (ppm):			
		0	100	700	4900
No. pregnant	F_0 / 1st	27	25	27	27[b]
Total litter size at birth		13.5	13.4	12.4	13.6
Live litter size at birth		13.4	13.2	12.4	13.3
Litter size on day 12		12.8	12.5	11.9	12.7
Litter size on day 21		12.7	12.4	11.8	11.7
Cumulative litter loss (%)[a]		5.4	7.5	4.6	13.9
No. pregnant	F_0 / 2nd	27	24[c]	27	28
Total litter size at birth		14.0	11.7*	12.7	14.1
Live litter size at birth		13.6	11.1**	12.4	14.1
Litter size on day 12		13.3	10.8**	11.9	13.5
Litter size on day 21		13.3	10.8**	11.9	13.1
Cumulative litter loss (%)[a]		4.9	6.7	5.8	7.1
No. pregnant	F1b / 1st	20[b]	21	21	21[d]
Total litter size at birth		13.2	12.9	11.5	12.7
Live litter size at birth		12.9	12.2	11.4	12.6
Litter size on day 12		12.5	11.9	11.3	12.3
Litter size on day 21		12.5	11.9	11.2	11.8
Cumulative litter loss (%)[a]		5.1	8.1	2.1	7.5
No. pregnant	F1b / 2nd	21	20[b]	23	22[b]
Total litter size at birth		12.4	14.0	13.2	13.5
Live litter size at birth		12.1	13.8	13.0	13.3
Litter size on day 12		11.8	13.4	12.3	13.0
Litter size on day 21		11.7	13.3	12.3	12.6
Cumulative litter loss (%)[a]		6.2	4.8	6.2	6.2

[a] birth - day 21; [b] excluding animal with total litter loss; [c] excluding one animal killed prior to weaning; [d] excluding 2 animals with total litter loss; * $p < 0.05$; ** $p < 0.01$

Table A6_8_2-5. Summary of mean pup weights and sex ratios.

Parameter	Generation	Group mean value at (ppm):			
		0	100	700	4900
Mean pup weight at birth (g)	F1a	5.7	5.8	5.8	5.6
Mean pup weight day 12 (g)		20.9	20.8	22.1	19.1**
Mean pup weight day 21 (g)		40.4	39.8	42.2	38.4
Sex ratio at birth (%M)		50.2	51.0	48.4	50.2
Sex ratio - day 21 (%M)		51.6	51.6	48.9	50.2
Mean pup weight at birth (g)	F1b	5.8	6.2*	6.0*	5.6
Mean pup weight day 12 (g)		21.5	24.3**	23.2	19.4**
Mean pup weight day 21 (g)		40.7	46.5**	43.8	37.4*
Sex ratio at birth (%M)		47.0	50.5	46.5	49.7
Sex ratio - day 21 (%M)		47.0	50.7	47.1	49.1
Mean pup weight at birth (g)	F2a	5.6	5.7	5.7	5.5
Mean pup weight day 12 (g)		21.8	23.3	24.1	19.9*
Mean pup weight day 21 (g)		43.3	45.9	46.8	39.5*
Sex ratio at birth (%M)		51.5	50.9	52.0	47.8
Sex ratio - day 21 (%M)		50.4	50.1	51.0	50.2
Mean pup weight at birth (g)	F2b	5.7	5.7	5.6	5.6
Mean pup weight day 12 (g)		23.5	22.0	23.0	20.2**
Mean pup weight day 21 (g)		46.5	43.6	45.7	41.0**
Sex ratio at birth (%M)		52.8	44.3	46.9	47.8
Sex ratio - day 21 (%M)		54.2	45.5	47.3	47.1

Table A6_8_2-6. Incidence of selected clinical or necropsy findings in weanlings.

Generation	Observation	Incidence in:							
		1st litters (a) treated at (ppm):				2nd litters (b) treated at (ppm):			
		0	100	700	4900	0	100	700	4900
F1	No. examined	293	268	271	293	318	230	275	320
	Kidney lesions [a]	12	0	0	217	0	0	0	196
	Ocular lesions	0	0	2[c]	3[c]	0	0	0	0
	Haemorrhage	0	1[b]	1	1	0	0	0	0
F2	No. examined	250	250	234	253	205	229	240	234
	Kidney lesions	0	0	0	157	0	0	0	108
	Ocular lesions	0	1[c]	0	0	0	0	0	0
	Haemorrhage	0	0	0	3	0	0	0	0

[a] excludes renal pelvic dilatation; [b] associated with traumatic injury to cranium; [c] ocular lesions were small eye, lenticular opacity, dark eye or intraocular haemorrhage

Table A6_8_2-7. Selected organ weights in adult and weanling animals.

Absolute organ weight	Generation	Group mean value in:							
		Males at (ppm):				Females at (ppm):			
		0	100	700	4900	0	100	700	4900
Kidneys (g)[a]	F_0	4.847	4.776	4.790	5.266**	2.865	2.887	2.825	2.987
Thyroids (mg)	(adult)	27.7	27.0	30.6	33.7**	23.4	22.4	24.5	27.0*
Liver (g)[a]		26.55	25.97	25.97	30.97**	19.47	19.15	19.32	23.07**
Kidneys (g)[a b]	F1b	4.596	4.808	4.832	5.588**	2.80	2.98	2.95	3.52**
Thyroids (mg)[b]	(adult)	27.3	31.4	30.3	34.5**	26.2	25.7	24.9	27.4
Liver (g)[a]		27.93	27.81	27.32	32.40**	18.49	18.88	18.82	22.50**
Kidneys (g)[a]	F2b	4.11	3.96	4.15	4.70**	2.187	2.319	2.344*	2.897**
Thyroids (mg)	(adult)	34.5	34.2	36.3	39.1**	14.8	18.0*	18.0*	18.8**
Liver (g)[a]		22.36	23.20	23.27	27.93**	11.15	11.26	11.52	13.81**
Kidneys (g)[a]	F1a	0.64	0.64	0.66	1.08**	0.62	0.63	0.66	1.07**
Thyroids (mg)	(weanling)	5.1	5.0	5.0	4.8	5.2	4.8	5.3	5.0
Liver (g)[a]		2.26	2.37	2.49**	2.82**	2.19	2.20	2.38**	2.75**
Kidneys (g)	F1b	0.659	0.745	0.670	0.969**	0.647	0.748	0.667	0.985**
Thyroids (mg)[a]	(weanling)	8.4	7.2	7.8	7.0*	5.4	6.1*	6.1*	7.0**
Liver (g)[a]		2.42	2.46	2.56*	2.91**	2.34	2.44	2.44	2.81**
Kidneys (g)	F2a	0.677	0.738	0.746	0.982**	0.662	0.690	0.704	0.957**
Thyroids (mg)[a]	(weanling)	5.3	5.4	5.5	5.2	5.6	5.3	5.5	5.3
Liver (g)[a]		2.49	2.60	2.59	2.88**	2.32	2.31	2.41	2.75**
Kidneys (g)	F2b	0.734	0.712	0.738	0.955**	0.697	0.684	0.743	0.944**
Thyroids (mg)	(weanling)	9.5	6.7**	6.6**	5.9**	8.0	6.7**	6.7**	6.5**
Liver (g)[a]		2.55	2.64	2.74**	3.06**	2.44	2.51	2.63**	2.82**

[a] covariate adjusted to body weight; [b] log transformed data; * $p < 0.05$; ** $p < 0.01$

Table A6_8_2-8. Selected histopathological alterations in F1b adult animals.

Organ and findings	Group mean value in:							
	Males at (ppm):				Females at (ppm):			
	0	**100**	**700**	**4900**	**0**	**100**	**700**	**4900**
No. examined (kidneys)	24	24	23	23	24	24	24	23
- cystic collecting ducts:								
-- medulla	0	0	0	17	0	0	1	19
-- cortex / medulla	0	0	0	5	0	0	0	4
- focal fibrosis[a]	0	0	0	9	0	0	0	17
- vascular congestion / haemorrhage[a]	0	0	0	4	0	0	0	5
- mineral deposits[a]	0	0	0	3	0	0	0	17
- acute inflammatory cells in ducts	0	0	0	7	0	0	0	4
- cortical scarring	0	0	0	14	0	0	0	11
- epithelial hyperplasia	0	0	0	1	0	0	0	2
- dilated tubules[b]	0	0	0	3	2	2	1	7
- glomerulonephritis[c]	2	1	3	5	1	0	0	3
No. examined (liver)	24	0	23	23	24	0	24	23
- hepatocyte enlargement[d]	0	-	0	18	0	-	0	9
- hepatocyte vacuolation								
-- moderate	1	-	1	1	0	-	0	0
-- minimal	2	-	5	4	0	-	1	0
No. examined (thyroids):	24	0	23	23	24	0	24	23
- increased height of columnar epithelium	0	-	0	6	0	-	0	0

[a] medullary; [b] cortical; [c] early progressive; [d] minimal

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** Corresponds to 67/548/EC Annex V method B35 or OECD method 416. **3.1.2. Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. ST-103 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 96,3% slightly lower than in the 5 batch analysis. Therefore the specification does not relevantly deviate to these indications.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.9 Developmental Neurotoxicity

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2003): Etofenprox developmental neurotoxicity study in the rat by oral (dietary) administration; [redacted] unpublished report no. MTU 215/032731 (August 07, 2003) Dates of work: August 28, 2002 – June 26, 2003	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes No applicable EU guideline. The study design was based on US-EPA Health Effects Test Guideline OPPTS 870.6300 (1998) and was approved by US-EPA prior to initiation of the study	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	Batch no. 87137	
3.1.2	Specification	Deviating from specification given in section 2 as follows	X
3.1.2.1	Description	white solid (amber liquid when molten)	
3.1.2.2	Purity	99.2%	
3.1.2.3	Stability	Expiration date December 2003	
3.2	**Reference Substance (positive control)**	Not applicable	

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3.3	**Test Animals**	
3.3.1	Species	Rat
3.3.2	Strain	Crl:CD(SD)BR IGS strain
3.3.3	Source	[redacted]
3.3.4	Sex	Female
3.3.5	Rearing conditions	In compliance with the US-EPA guidance (OPPTS 870.6300)
3.3.6	Age/weight at study initiation	10 - 12 weeks old / weight range 199 - 361g
3.3.7	Number of animals per group	F0 females 24 females per treatment level and 24 females for untreated control, of which 10 animals per group subjected to a detailed semi-quantitative observation battery of in-the-hand and standard arena observations, (on days 12 and 18 of gestation and days 4, 11 and 21 of lactation). F1 generation Litters were culled, by a process of random selection, to 4 progeny of each sex, where possible, on day 4 of lactation. Up to 3 F1 progeny/sex/litter (10 - 13 progeny/sex/group) were allocated, by random selection on day 4, to one of 6 further functional investigations, or for necropsy and brain investigations at 21 days of age, or for CNS/PNS histopathology at 63 - 67 days of age
3.3.8	Control animals	Yes (untreated)
3.4	**Administration**	admixture to the diet
3.4.1	Exposure	F0 females: diet given *ad libitum,* from Day 6 of gestation to Day 21 of lactation F1 generation: offspring were potentially exposed to etofenprox via the mother *in-utero* and during lactation. In addition, offspring were directly exposed towards the end of the lactation period as they weaned from suckling to the test diets (but offspring were not exposed to treated diet after Day 21 of lactation of the F0 females).
3.4.2	Dose Levels	Constant nominal concentrations: 0, 250, 700, 2100 ppm Mean achieved dose levels: 0, 28.4, 79.2 and 238mg/kg bw/day
3.4.3	Vehicle	Corn oil
3.4.4	Total volume applied	< 0.175% corn oil in the formulated diets (250, 700 and 2100 ppm)
3.4.5	Postexposure period	F0 females: not applicable (sacrifice on Day 21 of lactation, i.e. last day of treatment) F1 offspring: - 10 males and 10 females per group were sacrificed on last day of treatment (i.e. interim sacrifice, on same day as F0 females) → no postexposure period - remaining offspring: sacrifice at *ca.* Day 65 → postexposure period of *ca.* 44 days

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3.4.6	Anticholinergic substances used	not applicable
3.4.7	Controls	Diet with corn oil
3.5	**Examinations**	
3.5.1	Observation schedule	see Figure A6_09_03_1. Note: all observations, weighings, necropsy examinations and histopathological examination and measurement procedures on maternal animals and progeny were performed without knowledge of the treatment group.
3.5.2	Observations on F0 females	Food consumption: recorded for Days 0-2, 3-5, 6-9, 10-13, 14-16, 17-19 of gestation and for Days 1-3, 4-6, 7-10, 11-13, 14-16, 17-20 of lactation and calculated as daily consumption values. Body weight: recorded on Days 0, 3, 6, 10, 14, 17 and 20 of gestation, then daily until parturition, as well as on Days 1, 4, 7, 11, 14, 17 and 21 of lactation. General condition and clinical signs: daily during acclimation period, at least twice a day during allocation to the study. Full physical examination on Days 0, 7, 13 and 20 of gestation and on the dams that littered on Days 1, 7, 14 and 21 of lactation. Observations outside the home cage: on a sample of 10 animals per group, on Days 12 and 18 of gestation, then on Days 4, 11 and 21 of lactation 1. In the hand observations: ease of removal from cage, autonomic functions, salivation, lacrimation, piloerection, exophthalmos, pupil closure reflex, fur condition, reactivity to handling. 2. Standard arena observations: palpebral closure, posture, gait, tremors, twitches, convulsions, activity, rearing behaviour, grooming, urination and defaecation. *Post mortem* examination - Macroscopic pathology Sacrifice at Day 21 of lactation. The number of implantation sites was recorded (except 17 females) in parental females, and the brains of maternal animals were removed and weighed, and was retained in fixative together with all grossly abnormal tissues. The mammary tissue of females with total litter loss during weaning was examined and also retained.

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3.5.3	Observations on F1 progeny	All F1 progeny were examined *ca.* 24 hours after birth and numbers of live and dead progeny, body weights, sexes and clinical signs recorded. Subsequently, all litters were examined at least once daily during lactation until day 21 and clinical signs were recorded daily until 28 days of age. Twice daily morbidity/mortality checks were also performed. Body weights were recorded on days 4, 7, 11, 14, 17, 21 and 28 *post partum*. Offspring selected for further study were given a full physical examination weekly until termination. Unallocated progeny were retained until termination. Body weights of selected F1 progeny were recorded weekly until termination and sexual maturation was assessed by recording the start and completion of balano-preputial separation or the occurrence of vaginal opening. Neuropathology Up to 3 F1 progeny/sex/litter (10 - 13 progeny/sex/group) were allocated, by random selection on day 4, to one of 6 further functional investigations, or for necropsy and brain investigations at 21 days of age, or for CNS/PNS histopathology at 63 - 67 days of age. The following validated techniques were employed: 1. Quantitative motor activity was measured at 13, 17, 22 and 28 days of age using an automated monitoring system measuring ambulatory and rearing activity. Data were collected during 10 successive 6-minute intervals. 2. Functional observation battery (FOB) on days 4, 11, 21, 35, 45 and 60. On day 4, the speed of the surface righting reflex was determined semi-quantitatively, and animals were evaluated individually in a perspex arena for one minute for activity, maximum distance traveled and maximum pivoting angle. Any other abnormal locomotor of behavioural observations were also recorded. On day 11, ambulatory, rearing and grooming activity was measured and the presence or absence of urination noted. Abnormalities in coordination, physical condition and behaviour were also noted. On days 21, 35, 45 and 60, a detailed series of in-the-hand and standard arena observations was performed. Where applicable, a semi-quantitative grading system was used. 3. Auditory startle response habituation was measured on an automated system recording startle amplitude and latency to peak response during 5 consecutive blocks of 10 trials. The startle stimulus comprised a 40-millisec burst of white noise at *ca.* 105dB at 12-sec intervals. 4. Auditory startle response pre-pulse inhibition was measured on an automated system recording startle amplitude and latency to peak response for 10 trials with a pre-pulse of sound immediately before the startle stimulus, and the same without a pre-pulse of sound, presented in pseudo-random order. The startle stimulus comprised a 50-millisec burst of white noise at *ca.* 118dB and the pre-pulse was a 50-millisec burst of white noise at *ca.* 85dB preceding the startle stimulus by 150 millisec. The inter-trial interval was 10, 12, 14 or 16 sec selected from a Latin square.

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5. Learning & memory at 23/24 days of age were evaluated in a Morris water-filled swimming maze using a series of 3 trials on each of 4 successive days for each animal. A 90-sec period was allowed to complete the task, and the time (latency) to reach a submerged and non-visible fixed platform was recorded, together with the number of pool sectors crossed. A different starting point was used for each trial. Failure to complete the task was recorded as 90 sec latency.

6. Learning & memory at 58/59 days of age were evaluated as for 23/24 days of age.

Post mortem examination - Necropsy at 21 days of age
Ten progeny/sex/group.

- Brain pathology: the brains were transected from the spinal cord above the first cervical spinal nerve, weighed, measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum, and at the widest part of the cerebral hemispheres and then preserved together with any grossly abnormal tissues. The preserved tissues of progeny from females treated at 0 or 2100ppm were processed and examined by light microscopy. The areas examined were the olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, cerebrum, tectum, tegmentum, medulla oblongata, cerebellum and pons. In addition, morphometric measurement of the thicknesses of the neocortex, corpus callosum, hippocampus and the folia of the cerebellum (pyramis) was performed.

Neuropathology - Necropsy at 63-67 days of age
Up to 10 progeny/sex/group.

- Brain pathology: the brains were transected from the spinal cord above the first cervical spinal nerve, weighed, measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum, and the at the widest part of the cerebral hemispheres and then preserved. The areas of brain examined and brain morphometric measurements were as for day 21 progeny.

- Histopathological examination of CNS/PNS tissues

- Dorsal root fibres and ganglia (cervical and lumbar), eyes, optic and sciatic nerves, skeletal muscle, spinal cord, tibial nerves (at knee joint and calf muscle branch) and ventral root fibres (cervical and lumbar), together with any grossly abnormal tissues and the carcass, were also preserved in appropriate fixative. The preserved tissues of progeny from females treated at 0 or 2100ppm were processed, in paraffin wax or resin (sciatic and tibial nerves) and examined by light microscopy. Spinal cord (transverse and longitudinal sections at the cervical and lumbar swellings), dorsal and ventral root fibres (longitudinal sections at the cervical and lumbar levels), dorsal root ganglia (cervical and lumbar levels), eyes, optic nerve, skeletal muscle (transverse section), and the sciatic and tibial nerves (longitudinal and transverse sections) were also examined.

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3.5.4	Clinical Chemistry	No
3.6	Reproductive indices	Reproductive indices were calculated for gestation, post-implantation survival, live births, viability and lactation. Statistical analyses were performed, where appropriate, using frequency analysis if 75% of data across all groups were the same value. Treatment groups were compared by a Mantel test for trend in proportions and by Fisher's exact test for each group against the control. If Bartlett's test for homogeneity of variance was not significant at 1%, then parametric analysis was applied. If significant, data transformations were tried, before using non-parametric tests. Brain morphometry data were analysed using Student's t-test. Brain weights were analysed using Bartlett's test, followed by either Behrens-Fisher or Dunnett's test.

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4 RESULTS AND DISCUSSION

4.1 F0 females

Mortality / clinical signs

No maternal deaths or treatment-related clinical findings at any dose level.

Functional observation battery (FOB)

Treatment-related maternal findings in the detailed FOB were confined to a consistently higher rearing activity in females at 2100ppm, which was most pronounced (increased by 125% relative to the control - $p < 0.05$) on day 18 of gestation. There were no maternal FOB findings at 250 and 700ppm

Body weight

Females treated at 700 or 2100ppm showed a slight, transient decrease in weight gain from days 6 - 10 of gestation, during which time weight gain was significantly ($p < 0.05$ or 0.01) reduced by 14% in both groups. Thereafter, weight gains were comparable to the control gain and the group mean body weights at the end of gestation were comparable to the controls in all treated groups. There were no adverse effects on weight gain in any group during lactation.

Food consumption

Unaffected by treatment at all dose levels

Macroscopic pathology

No treatment-related maternal gross necropsy findings or effects on absolute and relative brain weights at any dose level.

Reproductive indices

There was no effect of treatment at any dose level on the duration of gestation, being within the expected range of 22 - 23 days for all animals of all treatment groups. There were no effects of treatment at any dose level on littering performance, implantation number, post-implantation survival index, litter size at birth, sex ratio, and offspring survival up to day 14 of lactation (Table A6_9_3-1). At 2100ppm, a treatment-related marginal increase in pre-weaning mortality occurred between days 14 and 21, during which time 5.7% of progeny died compared with 0.6% of the control progeny. However, overall pup mortality to weaning was comparable in all treated and control groups. Pre-weaning survival between days 14 and 21 was unaffected by treatment at 250 and 700ppm.

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4.2 F1 progeny

Mortality

There was no effect of treatment at any dose level on the post-weaning survival of the F1 progeny.

Clinical signs

Treatment-related adverse clinical signs occurred in a small number of F1 progeny of females treated at 700 and 2100ppm (Table A6_9_3-2). The effects at 2100ppm comprised increased incidences of dark or opaque and/or enlarged, prominent eyes, and a subcutaneous haemorrhagic lesion manifested as apparent cuts, reddening or bruising of the tail and/or paws. Further veterinary examination of 6 progeny at 2100ppm and one at 700ppm confirmed the presence of intraocular haemorrhage. Although there were isolated occurrences of similar lesions in control progeny, the increased litter and progeny incidences suggested an effect of treatment. The effect at 700ppm was confined to a slightly increased incidence of the eye lesion only. The incidences at 250ppm were comparable to the control incidences.

Body weight

The group mean day 1 body weights and subsequent weight gains to day 63 of F1 progeny of both sexes were unaffected by treatment at all dose levels, since none was significantly different ($p > 0.05$) from the control values (Table A6_9_3-3).

Sexual development

No effect of treatment at any dose level on the sexual development of F1 progeny as assessed by the age and body weight at vaginal opening or balano-preputial separation.

Behavioural development

There was no effect of treatment at any dose level on the behavioural development of offspring during the maternal treatment period, but thereafter there was an indication that treatment at 2100ppm had influenced the auditory startle response of female offspring. The amplitude of the response was generally higher, and habituation, as measured by the decrease in amplitude of the response, was reduced in these animals at day 23/24 of age. The effect on amplitude was more marked by day 58 of age (Table A6_9_3-4). Female progeny at lower dose levels were not affected. Males at 700 and 2100ppm also showed a shorter latency to peak response at 58 days of age, but in isolation, the differences were considered to be of equivocal toxicological relevance. Pre-pulse inhibition of the auditory startle response was unaffected by treatment at all dose levels in 23/24 day old progeny. At 58 days of age, male progeny only at 2100ppm, showed a greater degree of pre-pulse inhibition (36.6%) than the controls (18.1%), but the difference was not statistically significant ($p > 0.05$). The mean latency to peak response with pre-pulse in these animals was significantly ($p < 0.05$) longer (17.4msec) than the control value (12.4msec).

In male 58 day old progeny at 2100ppm, cage floor (low beam) activity was significantly ($p < 0.05$) lower than the controls (Table A6_9_3-5). However, the toxicological relevance of the finding in male offspring is equivocal because there were no conclusive similar effects at earlier testing intervals and no histopathological alterations in the nervous system at 2100ppm. Activity levels of male progeny at lower dose levels, and females of all treatment groups, were comparable to control levels.

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There were no effects of treatment at any dose level on the observations and semi-quantitative measurements made in-the-hand or in the standard arena on selected F1 progeny up to 60 days of age, *viz.* surface righting, distance travelled, maximum pivoting angle, rearing activity, grooming behaviour, physical condition, locomotor coordination, posture/gait, autonomic function, reactivity to handling and pupil closure reflex on day 35. Similarly, there was no effect of treatment at any dose level on learning and memory at any age tested.

Pathology – Necropsy at 21 days of age

There were no treatment-related gross findings at necropsy in F1 progeny killed prematurely, found dead, culled or killed on schedule at 21 days, other than confirmation of ocular defects in 2 progeny of each sex at 2100ppm. The absolute and relative brain weights and brain external dimensions of all treated groups and the thicknesses of the neocortex, hippocampus, corpus callosum and cerebellum at 2100ppm of 21 day old progeny of both sexes were unaffected by treatment. All values were not significantly different from the controls, except for female relative brain weight at 700ppm that was significantly ($p < 0.05$) higher. There were no treatment-related histopathological alterations in the brains of 21 day old progeny at 2100ppm.

Pathology – Necropsy at 63-67 days of age

Treatment-related necropsy findings in 63 - 67 day old progeny were confined to a higher proportion of males in all treatment groups with unilateral or bilateral renal pelvic dilatation (Table A6_9_3-6). Histopathological examination of kidneys from control and 2100ppm progeny revealed cortical tubular dilatation and hydronephrosis at similar incidences in both groups. Female progeny were not affected. X

The absolute and relative brain weights and brain external dimensions of all treated groups and the thicknesses of the neocortex, corpus callosum, hippocampus and cerebellum at 2100ppm of 65 day old progeny of both sexes were unaffected by treatment. Although the mean thickness of the hippocampus in males at 2100ppm was significantly ($p < 0.05$) greater than the controls by 9% and that of the females significantly ($p < 0.05$) less than the controls by 9%, neither was considered to be treatment-related. In the males, the difference was due in part to an unusually thin hippocampal region in one male control. In the females, a comparison of the contemporary control mean value with recent historical control data suggested the contemporary control was slightly greater than would be expected. There were no treatment-related histopathological alterations in the tissues of the central and peripheral nervous systems in 65 day old offspring derived from females treated at 2100ppm.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test guideline: No applicable EU guideline. The study design was based on US-EPA Health Effects Test Guideline OPPTS 870.6300 (1998) and was approved by US-EPA prior to initiation of the study

Deviations: not applicable

Description of method: treatment of the F0 dams by diet admixture, from Day 6 of gestation to Day 21 of lactation. Exposure of F1 offspring *in-utero* and via maternal milk up to weaning. Observations for F0 females covered mortality, clinical signs, functional observation battery, body weight, food consumption, *post mortem* macroscopic pathology (sacrifice at Day 21 of lactation) and reproductive indices. Observations for F1 offspring covered mortality, clinical signs, body weight, sexual development, behavioural development, brain pathology (for the pups selected for interim sacrifice at Day 21 of lactation), brain pathology and other tissues histopathology (for the pups sacrificed at study termination, i.e. at age of 63-67 days).

5.2 Results and discussion

Etofenprox does not produce selective developmental neurotoxicity in F1 progeny at dose levels that produce slight maternal toxicity. However, slightly impaired pre-weaning survival and a low incidence of subcutaneous haemorrhagic lesions occur in progeny at 238mg/kg/ bw/day, and low incidences of ocular lesions at ≥ 79mg/kg bw/day. Since the ocular lesions were generally associated with intraocular haemorrhage, they may share a common aetiology with the subcutaneous lesions. An overall NOEL was established as 28.4mg/kg bw/day. Functional developmental effects with a possible relationship to treatment were confined to higher mean auditory startle response amplitudes in female offspring and a clustering of differences in motor activity and latency to peak startle response in males at 238mg/kg bw/day. However, histomorphological development of the central and peripheral nerve tissues were unaffected by treatment with etofenprox at 238mg/kg bw/day, the highest dose level employed.

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5.3	**Conclusion**		
5.3.1	NOEL	Overall NOEL = 250 ppm (≡ 28.4mg/kg bw/day). Basis: Etofenprox does not produce selective developmental neurotoxicity at dose levels that produce slight maternal toxicity. However, slightly impaired pre-weaning survival during the latter part of lactation and a low incidence of subcutaneous haemorrhagic lesions occur at 2100ppm, and low incidences of ocular lesions at 700 and 2100ppm. An NOAEL for functional development of offspring was established as 700ppm, equivalent to a mean dose level of 79mg/kg bw/day, based on higher mean auditory startle response amplitudes in female offspring and a clustering of differences in motor activity and latency to peak startle response in males at 2100ppm. An NOEL for histomorphological development of central and peripheral nerve tissues was established as > 2100ppm, equivalent to an average dose level of 238mg/kg bw/day, based on the absence of histomorphological alterations at the highest dose level employed.	X
5.3.2	Reliability	1	
5.3.3	Deficiencies	No	

Figure A6_9_3-1. Treatment and observations schedule

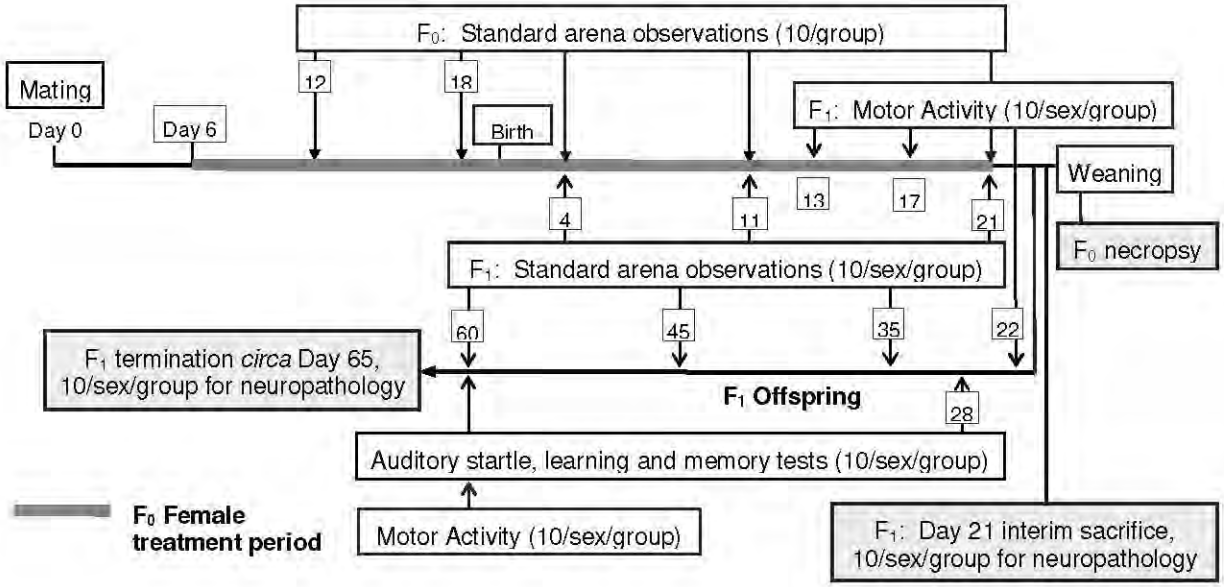

Table A6_9_3-1: Summary of litter data and survival

Parameter	Value in group treated at (ppm):			
	0	250	700	2100
No. mated / no. pregnant	24 / 24	24 / 24	24 / 24	24 / 24
No. with live litters	24	24	24	24
No. with total litter loss	1	0	1	0
No. with weaned progeny	23	24	23	24
Mean no. implantations	16.0	16.1	15.7	16.6
Mean total litter size on day 1	15.2	15.5	14.8	15.3
Mean live litter size on day:				
1	14.6	15.3	14.7	15.2
4 (pre-cull)	14.2	14.6	14.0	15.2
4 (post-cull)	8.0	7.9	8.0	8.0
7	7.7	7.9	8.0	8.0
14	7.5	7.8	7.9	8.0
21	7.4	7.8	7.9	7.5
28	6.5	6.8	6.9	6.5
Gestation index (%)	100	100	100	100
Post-implantation survival index (%)	93.9	95.7	92.2	92.4
Live birth index (%)	96.6	98.4	99.2	99.2
Viability index (%)	97.1	95.6	95.9	99.8
Lactation index (%) on day 21	92.9	97.9	98.4	93.8
Sex ratio (% males) on day:				
1	49.7	52.1	45.3	48.2
4 (pre-cull)	49.1	52.5	44.9	48.2
4 (post-cull)	50.5	50.5	46.7	50.0
21	52.1	49.0	47.0	49.7

Table A6_9_3-2: Summary of treatment-related clinical signs in F1 progeny

Observation	**No. progeny (litters) affected at (ppm):**			
	0	**250**	**700**	**2100**
One eye large/prominent and dark	0	0	1 (1)	8 (6)
One eye large/prominent	0	1[a] (1)	1[a] (1)	1 (1)
One eye large and opaque	1 (1)	1[a] (1)	0	0
One or both eyes opaque	0	0	3[a] (1)	1 (1)
One or both eyes dark	0	0	0	2 (2)
				1[a] (1)
Cut/bleeding on tail	0	0	0	2 (2)
Reddened/swollen/bruised areas on tail	1 (1)	0	3 (1)	4 (3)
Cut/bleeding on toes/paws	0	0	1 (1)	2 (2)
Swollen/bruised/reddened paw(s)	1 (1)	1 (1)	1 (1)	5 (4)

[a] first recorded post-weaning

Table A6_9_3-3: Summary of group mean body weights - F1 progeny.

Parameter	**Value in group treated at (ppm):**			
	0	**250**	**700**	**2100**
Group mean body weight (males) on day:				
1	6.5	6.6	6.5	6.3
4 (pre-cull)	8.8	9.0	8.9	8.6
4 (post-cull)	8.9	9.0	9.0	8.6
7	14.8	14.9	14.4	13.8
14	32.2	32.5	31.3	30.7
28	85.4	88.2	84.1	82.8
63	358	374	359	363
Group mean body weight (females) on day:				
1	6.1	6.1	6.1	6.0
4 (pre-cull)	8.4	8.5	8.4	8.2
4 (post-cull)	8.4	8.6	8.4	8.3
7	13.8	14.1	13.8	13.2
14	30.8	31.5	30.2	29.3
28	78.9	80.6	77.3	75.7
63	227	233	226	229

Table A6_9_3-4: Auditory startle response - peak startle amplitude, habituation and latency to peak response.

Group (ppm)	Parameter	Age (days) and sex	Group mean value in trial numbers:				
			1 - 10	11 - 20	21 - 30	31 - 40	41 - 50
0	Startle amplitude	23/24 males	198.3	163.7	142.3	152.2	147.9
250			206.2	160.8	164.2	164.1	150.8
700			170.7	175.5	168.3	159.5	142.6
2100			192.5	186.1	177.0	168.0	153.3
0		23/24 females	164.2	148.9	131.0	121.9	128.7
250			149.5	126.5	123.4	120.1	121.5
700			166.8	136.8	142.9	141.0	141.4
2100			186.5	181.6	174.0	168.9*	179.6
0		58 males	383.4	317.9	311.1	294.8	292.0
250			325.7	307.6	282.9	316.1	338.2
700			403.6	330.6	316.6	311.0	310.6
2100			492.4	413.6	440.5	385.4	471.2*
0		58 females	310.9	235.3	239.3	241.4	257.8
250			335.5	259.5	299.7	273.9	246.0
700			332.9	280.6	280.0	298.4	304.9
2100			408.4	381.8*	342.8*	352.5*	372.4**
0	Latency (msec)	23/24 males	19.1	19.4	23.0	21.7	23.0
250			24.2	25.3	26.4	24.7	26.6
700			23.0	23.1	23.4	24.7	24.2
2100			18.8	21.1	21.0	23.1	20.8
0		23/24 females	22.2	22.2	20.6	21.5	23.4
250			20.5	22.2	19.6	21.7	21.7
700			22.6	21.4	22.2	22.6	23.6
2100			22.1	22.0	22.4	23.0	22.6
0		58 males	21.1	16.4	20.2	18.4	17.6
250			20.8	17.9	13.5	15.3	13.3
700			14.8*	13.6	14.3	13.6*	15.6
2100			13.9*	15.8	15.5	12.0*	13.2
0		58 females	18.1	16.7	17.1	15.6	16.5
250			16.0	15.5	14.2	15.7	16.7
700			16.4	16.0	15.6	16.5	16.9
2100			15.1	15.2	15.1	13.3	14.4

* $p < 0.05$; ** $p < 0.01$

Table A6_9_3-5: Summary of motor activity (quantitative assessment) - F1 progeny.

Age at testing / beam location	Sex	Group mean total[a] activity (beam breaks) at (ppm):			
		0	250	700	2100
13 days:	Male				
high beam		0.8	0.3	1.4	0.2
low beam		375.9	261.0	266.3	435.2
17 days:					
high beam		21.3	71.9	39.1	30.9
low beam		235.2	513.5	340.7	517.9
22 days:					
high beam		53.5	27.8	19.4	36.2
low beam		248.9	169.2	129.0	171.0
58 days:					
high beam		281.0	298.0	270.5	218.4
low beam		1094.4	1082.5	1034.4	822.6*
13 days:	Female				
high beam		1.2	0.6	1.0	1.1
low beam		249.7	225.5	202.9	315.6
17 days:					
high beam		29.5	49.1	44.4	41.4
low beam		474.6	407.2	384.7	531.7
22 days:					
high beam		34.6	43.1	45.7	31.7
low beam		180.2	229.6	240.1	195.6
58 days:					
high beam		282.4	275.8	224.3	262.6
low beam		1207.3	1534.9	1098.4	1360.6

[a] mean number of beam breaks during the 10 x 6-minute testing blocks; * $p < 0.05$

Table A6_9_3-6: Incidence of renal pelvic dilatation in male 63 - 67 day old F1 progeny.

Observation	Incidence at (ppm):			
	0	250	700	2100
No. examined	76	80	74	79
No. animals with pelvic dilatation	9	21	17	24
No. litters affected	7	12	14	15
No. animals with bilateral pelvic dilatation	1	1	0	7

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09.08.2005
Materials and methods	**2.1. Guideline study** OECD draft method 426 (developmental neurotoxicity study) is a follow up of the US guideline **3.1.2. Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. ST-103 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 96,3% slightly lower than in the 5 batch analysis. Therefore the specification does not relevantly deviate to these indications.
Conclusion	**4.1. F1 Progeny, Pathology- necropsy at 63-76 days of age** The renal pelvic dilatation at 63 -67 days of age in a higher portion of male offspring at all dose levels are considered as minor findings since: Females were not affected and no other histopathological renal findings were significantly different between control and high dose group. Furthermore considering the whole range of doses tested, the dose dependency remains questionable for us: The doses 0/28/79/238 mg/kg bw with 76/80/74/79 animals, respectively results in 9/21/17/24 animals affected and 7/12/14/15 litters affected with unilateral or bilateral pelvic dilatation. With bilateral pelvic dilatations some dose dependency could be claimed since in the different dose groups 1/1/0/7 animals were affected. In case the last mentioned effect should be taken into consideration it is covered by the overall NOAEL of 28.4 mg/kg bw. **5.3.1. NOAEL** The proposed histological NOEL of > 238mg/kg bw/day is considered to be a NOAEL, since the renal pelvic dilatation (at 63 -67 days of age in a higher portion of male offspring at all dose levels) is considered to be a minor finding.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	

Acceptability

Remarks

Section A6.10 Mechanistic study (rodent)

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Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2003a): 4-week dietary investigative study on thyroid function and hepatic microsomal enzyme induction with MTI-500 in rats: [redacted] unpublished report no. 6648-156 (August 22, 2003). Dates of work: April 02, 2003 - August 22, 2003.
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Not applicable
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 AIM OF THE STUDY

The study was performed to investigate the effects of oral administration of etofenprox on the induction of specific hepatic microsomal enzymes and their influence on pituitary-thyroid homeostasis and thyroid morphology and cytology. The results were used to elucidate the etiology of slightly enhanced thyroid adenoma formation in a rat combined chronic toxicity / carcinogenicity study (Green *et al.*, 1986a, unpublished report no. MTC 59/85581, section A6.5.1/01).

4 MATERIALS AND METHODS

4.1	**Test material**	Etofenprox	
4.1.1	Lot/Batch number	Batch no. 87031	
4.1.2	Specification	Deviating from specification given in section 2 as follows	X
4.1.2.1	Description	Off-white to light yellowish solid	
4.1.2.2	Purity	99%	
4.1.2.3	Stability	No information in the report	
4.2	**Test Animals**		
4.2.1	Species	Rat	
4.2.2	Strain	Sprague-Dawley-derived rats (Crl:CD(SD)IGS BR strain)	
4.2.3	Source	[redacted]	

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4.2.4	Sex	Male and female
4.2.5	Age/weight at study initiation	6 - 7 week old, weight range 145 - 283g
4.2.6	Number of animals per group	*20* animals/sex/group, split as follows: - 10 animals/sex/group were treated for 14 ± 1 days, of which 5 animals/sex/group were killed at the end of the treatment period and the remaining 5 animals/sex/group were killed after a 4-week treatment-free recovery period. - 10 animals/sex/group were treated for 28 ± 1 days, of which 5 animals/sex/group were killed at the end of the treatment period and the remaining 5 animals/sex/group were killed after a 4-week treatment-free recovery period.
4.2.7	Control animals	Yes
4.3	**Administration/ Exposure**	Oral
4.3.1	Duration of treatment	14 ± 1 days or 28 ± 1 days, depending on the group
4.3.2	Frequency of exposure	Daily
4.3.3	Postexposure period	4 weeks
4.3.4	**Oral**	
4.3.4.1	Type	Dietary admixture
4.3.4.2	Concentration	- Constant nominal concentrations: 0, 1250, 5000 and 20000ppm - Overall mean achieved dose levels: see Table A6_10-1.
4.3.4.3	Vehicle	Corn oil
4.3.4.4	Concentration in vehicle	Not reported
4.3.4.5	Total volume applied	Not reported
4.3.4.6	Controls	Untreated diet containing a comparable volume of corn oil.
4.3.4.7	Diet analysis	The diet formulations containing 1250 or 20000ppm prepared in week 1 were analysed for stability after storage at room temperature for 8 and 15 days, and for homogeneity. Achieved concentrations in all formulations were determined in week 1 and 4.
4.4	**Examinations**	
4.4.1	Observations	
4.4.1.1	Clinical signs	Yes, recorded daily.
4.4.1.2	Mortality	Yes, checked twice daily.
4.4.2	Body weight	Yes. Body weights were determined pre-dose, on day 1 and weekly thereafter and at necropsy.
4.4.3	Food consumption	Yes. Food consumption was determined weekly.

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4.4.4	Cell proliferation measurements	Seventy-two ± 8 hours before being killed, up to 5 animals/sex/group were surgically implanted with an osmotic pump pre-loaded with 2mL bromodeoxyuridine (BrdU) at a concentration of 20mg/mL for cell proliferation measurements. Pumps were filled on the day of implantation and maintained at 37°C until implantation. Implantation was performed under anesthesia by aseptic insertion under the skin of the dorsum. A necropsy was performed on all animals that died during the surgical implantation procedure.
4.4.5	Haematology	No.
4.4.6	Clinical Chemistry	Yes. Blood samples were collected without anticoagulant from all animals at scheduled necropsy following overnight food deprivation. The serum was separated and analysed for thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) concentrations.
4.5	**Sacrifice and pathology**	
4.5.1	Organ Weights	Yes. All animals. Liver and thyroid/parathyroid.
4.5.2	Histopathology	Yes. All animals treated at 0 or 20000ppm and all decedents Organs: liver, left thyroid and duodenum.
4.5.3	Analysis of microsomal fractions	The remaining liver was used for the preparation of the microsomal fraction and subsequent determination of microsomal protein concentration and UDPGT activity using both the 4-methylumbelliferone (4-MUGT, Collier, et al., 2000; Ullrich and Bock, 1984) and p-nitrophenol-glucuronosyltransferase methods (p-NPGT, Winsnes, 1969; Burchell and Coughtrie, 1989). Protein concentration in hepatic microsomal fractions were determined by UV/Vis spectroscopy using a Bio-Rad DC protein assay based on the Lowry (1951) method as reported by Peterson (1979). All microsomal protein and enzyme standards were analysed in duplicate and samples were analysed in triplicate.

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4.5.4	Cell proliferation	Samples of liver, thyroid and duodenum from animals treated at 0 or 20000ppm were subjected to cell proliferation analysis by the avidin-biotin peroxidase method for the detection of antigen-antibody complex. Cell proliferation data were analysed statistically using Students t-test at the 95% level. Other data were analysed using Levene's test for variance homogeneity. One-way ANOVA was used to analyse homogeneous or transformed data. If significant, Dunnett's multiple comparison t-test was performed for 2-way pairwise comparisons at the 5% level.
4.5.5	Analysis of thyroid peroxidase extracts	The right thyroid from all animals in each sex/group were pooled, deep-frozen and then analysed for thyroid microsomal peroxidase activity (TP).
4.5.6	Bromodeoxyuridine immunochemistry	Unstained slides of liver, thyroid and duodenum BrdU incorporated into S-phase cells was localised by a chromagen and counter-stained with haematoxylin. The slides were first examined under low magnification and BrdU labelling was quantified under high power by examination of at least 3500 hepatocytes in 10 random fields/animal and 500 thyroid follicular cells/animal. Systemic delivery of BrdU was confirmed in duodenum. The labelling indices were expressed as the percentage of labelled cells.

5 RESULTS AND DISCUSSION

5.1	**Analytics**	Stability, homogeneity and achieved concentrations in the formulations were acceptable. The 1250 and 20000ppm formulations were stable for at least 15 days at which time means of 92.0 and 92.3% initial concentrations remained, respectively. Mean values of the homogeneity analyses were within the range 93.2 - 94.4% nominal concentration, and achieved concentration analyses in weeks 1 and 4 for all formulations were within the range 89.2 - 94.4% nominal concentration.
5.2	**Observations**	
5.2.1	Clinical signs	No treatment-related clinical signs were evident at any dose level.
5.2.2	Mortality	Five animals died during the osmotic pump implantation procedure, one control female, 2 males and one female at 1250ppm and one male at 20000ppm. No clear reason for death was evident, but the death of a control animal and the absence of any dose-relationship in the treated groups suggested the deaths were incidental to treatment with etofenprox.

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5.3	**Body weight gain**	See Table A6_10-2. Body weight gain in both sexes at 20000ppm was reduced at 14 and 28 days of treatment, such that group mean body weights were significantly ($p < 0.05$) lower than the controls by 6.8% at 14 days and by 12.4 and 7% in males and females at 28 days (Table 5.5.4.1). The group mean body weight of females at 5000ppm was also significantly ($p < 0.05$) lower than the control value by 7.4% at 28 days. The group mean body weights and weight gains in recovery animals were not significantly ($p > 0.05$) different from control values.
5.4	**Food consumption**	The food consumption of females at 20000ppm was reduced relative to the controls throughout the treatment period. The differences were significant ($p < 0.05$) during the first 2 weeks of treatment and amounted to a decrease of up to 21.7%. The food consumption of females at 5000ppm was also significantly reduced during the first week of treatment only. Food consumption at other dose levels, and at all dose levels during the treatment-free period, was not significantly different from control values.
5.5	**Hormone analysis**	Serum TSH activity was significantly ($p < 0.05$) increased in both sexes after 2 weeks treatment at 20000ppm, and in females at 5000 and 20000ppm after 4 weeks treatment. Furthermore, the activity at all dose levels, in both sexes, at both sampling intervals, was between 58.7 and 211% higher than the respective control value. Based on the consistency of these differences, the data suggest a treatment-related increase in serum TSH activity in both sexes at all dose levels. The effect in females was dose-related, and was fully reversible within 4 weeks in both sexes (see Table A6_10-3). There was no effect of 2 or 4 weeks of treatment at any dose level in either sex on serum T3 activity. All values were not significantly ($p > 0.05$) different from control values (see Table A6_10-4). Serum T4 concentration was significantly ($p < 0.05$) lower than the control value by 34.4% in males treated for 2 weeks at 20000ppm, and also slightly lower (23.3%; $p > 0.05$) after 4 weeks treatment. However, the effect was not apparent at lower dose levels or in females at any dose level after 2 or 4 weeks treatment. The effect in males was fully reversible during the treatment-free period (see Table A6_10-5).

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5.6	**Microsomal enzyme analysis**	Treatment-related effects on microsomal protein yield were confined to a significant ($p < 0.01$) increase in males treated for 4 weeks at 20000ppm. The effect was fully reversible within 4 weeks. There were no effects in males after 2 weeks treatment and no effects in females at 20000ppm after 2 or 4 weeks (see Table A6_10-6). Hepatic microsomal 4-MUGT activity was significantly ($p < 0.01$) increased by up to 81% in both sexes treated for 2 weeks at 20000ppm, and in males at 5000ppm. The effects showed some degree of reversibility, but enzyme activities remained significantly ($p < 0.05$ or 0.01) higher after a 4-week treatment-free period. There was no apparent effect on hepatic microsomal 4-MUGT activity after 4 weeks treatment (see Table A6_10-7). Hepatic microsomal UDPGT activity using p-nitrophenol as acceptor substrate was significantly ($p < 0.01$) increased by up to 92.1% in both sexes treated for 2 weeks at 20000ppm. Activities were also increased in both sexes treated at 5000ppm for 2 weeks, but neither was statistically significant ($p > 0.05$). However, based on the consistency of the effect at the end of the recovery period, the reviewer considers the increased activities to be an effect of treatment. The effects at 5000 and 20000ppm showed no evidence of reversibility during the 4-week treatment-free period. There was no apparent effect on hepatic microsomal UDPGT activity at any dose level after 4 weeks treatment (see Table A6_10-8).
5.7	**Thyroid peroxidase activity**	See Table A6_10-9. Females treated for 2 weeks at 20000ppm showed higher TP activity than the control group but the difference was not statistically significant ($p > 0.05$). There were no other differences between treated and control groups after 2 weeks treatment. All treated groups of both sexes showed lower TP activity than the controls after 4 weeks treatment, and remained slightly depressed in males after the 4-week treatment-free period. However, none of the differences achieved statistical significance and therefore an effect of treatment is equivocal. Female controls treated for 4 weeks with a subsequent recovery period showed unusually low TP activities making interpretation difficult.
5.8	**Bromodeoxyuridine analysis**	There were no statistically significant increases in hepatic or thyroid cell proliferation rates, as measured by the BrdU labelling indices, in either sex at any dose and treatment regime (see Table A6_10-10).Hepatic labelling incidences were comparable to control values in all groups and in both sexes. However, the thyroid labelling indices in males treated at 20000ppm for 2 or 4 weeks were greater than control values by up to 72.7%, suggesting mildly increased cell proliferation. The thyroid labelling indices in these animals at the end of the 4 week recovery period were lower than control values, suggesting a rapid return to normal proliferative status on withdrawal of exposure to etofenprox. The significantly lower thyroid labelling index in females treated for 2 weeks is considered incidental to treatment with etofenprox because the value was within the control range.

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5.9 Sacrifice and pathology

5.9.1 Organ weights

At necropsy, there were no treatment-related gross lesions in any of the treated groups, but there was a treatment-related and dose-related increase in the absolute and relative liver weights of males treated for 2 or 4 weeks at 5000
or 20000ppm (Table A6_10-11). Although not all values were statistically significant, there was a clear dose-relationship. A similar effect was apparent in females treated for 2 or 4 weeks at 20000ppm. Liver weights showed a substantial degree of recovery in these groups after the 4-week treatment-free period, at which time neither the absolute nor relative weights were significantly ($p > 0.05$) different from control values.

The absolute and relative thyroid weights of both sexes treated for 2 or 4 weeks at 20000ppm were between 21.9 and 66.0% higher than control values, but none of the values was statistically significant (Table A6_10-12). At the lower dose levels of 1250 and 5000ppm, absolute and relative weights were 15.7 - 31.2% and 9.0 - 40.4% higher than control values, respectively. The reviewer considers the differences at 20000ppm to be biologically relevant and probably related to treatment because of the consistency and magnitude of the differences. At lower dose levels, the differences were generally less marked and there was no consistency between the dose levels.

5.9.2 Histopathology

Treatment-related effects occurred in the liver of both sexes treated at 20000ppm. The effects comprised increased numbers of multinucleated hepatocytes after 2 and 4 weeks treatment which persisted to the end of the treatment-free periods, and centrilobular hepatocyte hypertrophy in both sexes after 2 weeks and in females only after 4 weeks (Table A6_10-13). In females, the latter effect persisted throughout the treatment-free periods. There were no treatment-related histopathological effects in the thyroid of animals treated at 20000ppm.

6 APPLICANT'S SUMMARY AND CONCLUSION

6.1 Materials and methods

Test guidelines: not applicable (investigative study).

Description of method: investigative study to study the etiology of the increased incidence of female rat thyroid follicular cell adenomas based on the observation that etofenprox had been shown to produce increased liver weight and hepatic hypertrophy in the rat after short-term [redacted] 1983a; [redacted] 1985 – sections A6_4_1/01 and A6_4_3_1) and long-term administration [redacted] 1986a, section A6_5_1/01). Specifically, this study investigated the hypothesis that etofenprox produces a primary effect on the liver of microsomal enzyme induction, ultimately leading to a secondary effect of increased thyroid follicular cell adenomas mediated by a physiological homeostatic mechanism.

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6.2	**Results and discussion**	The liver was identified as the primary target organ. Short-term oral administration of etofenprox produced a primary effect on liver characterised by increased microsomal protein content in males, increased UDPGT activity and liver weight, and hepatic hypertrophy in both sexes. Treatment-induced effects identified that were considered to be secondary to hepatic microsomal enzyme induction comprised a reversible and transient depression of circulating T4 hormone in males, a reversible increase in serum TSH concentrations in both sexes, mild thyroid follicular cell proliferation in males, and increased thyroid weight in both sexes. The secondary effects are considered to represent a series of events, each of which was a physiological consequence of the previous event. There was equivocal evidence for a treatment-related increase in thyroid weights and depression of thyroid peroxidase activity in both sexes. A no-observed-effect-level (NOEL) for the primary effect on the liver was established as 1250ppm in both sexes, equivalent to dose levels of 81.2 and 90.2mg/kg bw/day in males and females, respectively, based on the occurrence of increased hepatic UDPGT activity at 5000ppm.
6.3	**Conclusion**	
6.3.1	NOEL	For primary effects on liver: 81.2 and 90.2mg/kg bw/day in males and females, respectively
6.3.2	Reliability	1
6.3.3	Deficiencies	No

Table A6_10-1. Mean achieved dose levels

Nominal diet concentration (ppm)	Mean achieved dose levels (mg/kg bw/day) in:			
	Males		Females	
	Minimum	Maximum	Minimum	Maximum
1250	81.2	106	90.2	110.3
5000	316	422	380	432
20000	1332	1868	1520	1723

Table A6_10-2. Summary of group mean body weight.

Day of test	Group mean body weight (g) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
1	249	248	250	244	170	171	166	165
8	304	306	309	289	193	194	186	180*
14	336	337	337	313*	208	213	200	194*
22	383	373	374	342	233	230	214*	218
28	402	393	393	352*	242	243	224*	225*
28 (recovery groups only)	392	406	401	379	229	246	228	218
42	433	435	439	411	252	261	244	237
56	469	459	464	435	274	274	261	251

* $p < 0.05$

Table A6_10-3. Summary of group mean serum TSH concentration in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean TSH concentration (ng/mL) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	0.123	0.320[a]	0.318	0.537*	0.057[b]	0.116[b]	0.177	0.354*
4 weeks / without recovery	0.218	0.551	0.346	0.530	0.063	0.130	0.202*	0.369*
2 weeks / with recovery	0.436	0.240	0.285	0.124	0.105	0.147	0.123	0.106
4 weeks / with recovery	0.306	0.424	0.270	0.211	0.093	0.098	0.094	0.090

[a] mean of 3 animals only, excluded from statistical analysis; [b] mean of 4 animals; * $p < 0.05$

Table A6_10-4. Summary of group mean serum T3 concentration in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean T3 concentration (ng/dL) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	92.16	83.87[a]	82.82	92.80[b]	98.25[b]	112.58[b]	104.94	102.55[b]
4 weeks / without recovery	92.04	92.68	110.02	99.24	105.94	114.02	105.36	116.30
2 weeks / with recovery	72.16	88.94	94.80	86.04	87.34	76.66	89.28	83.00
4 weeks / with recovery	72.02	83.84	85.46	79.52	103.92	90.00	98.02	97.12

[a] mean of 3 animals only, excluded from statistical analysis; [b] mean of 4 animals

Table A6_10-5. Summary of group mean serum T4 concentration in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean T4 concentration (µg/dL) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	6.1	5.3[a]	4.8	4.0*	3.4[b]	4.6[b]	3.7	3.0[b]
4 weeks / without recovery	6.0	5.8	5.6	4.6	3.6	4.6	3.9	3.6
2 weeks / with recovery	3.5	3.4	4.3	4.3	2.6	2.7	3.0	2.8
4 weeks / with recovery	4.4	5.2	5.2	4.9	3.2	2.8	3.0	3.9

[a] mean of 3 animals only, excluded from statistical analysis; [b] mean of 4 animals; * $p < 0.05$

Table A6_10-6. Summary of group mean hepatic microsomal protein concentration in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean microsomal protein (mg/g liver) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	45.1	49.7[a]	49.2	51.8[b]	30.8[b]	32.4[b]	32.7	36.3
4 weeks / without recovery	44.5	45.7	47.8	59.9**	36.4	37.2	39.0	37.0
2 weeks / with recovery	39.7	38.3	47.8*	46.0	30.7	36.3	34.3	42.4*
4 weeks / with recovery	39.3	38.6	37.2	40.7	43.6	47.6	47.0	49.9

[a] mean of 3 animals; [b] mean of 4 animals; * $p < 0.05$; ** $p < 0.01$

Table A6_10-7. Summary of group mean hepatic microsomal 4-MUGT activity in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean 4-MUGT activity (nmol/mg protein/min) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	9.94	9.99[a]	13.5**	18.0**[b]	12.3[b]	13.1[b]	14.1	19.0**
4 weeks / without recovery	7.42	7.15	8.72	7.14	12.1	13.9	12.1	14.7
2 weeks / with recovery	9.73	9.63	12.2*	15.9**	9.97	9.91	12.4*	14.5**
4 weeks / with recovery	8.76	8.94	9.74	10.7	9.33	9.74	9.41	10.4

[a] mean of 3 animals only, excluded from statistical analysis; [b] mean of 4 animals; * $p < 0.05$; ** $p < 0.01$

Table A6_10-8. Summary of group mean hepatic microsomal p-NPGT activity in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	Group mean p-NPGT activity (nmol/mg protein/min) in:							
	Males treated at (ppm):				Females treated at (ppm):			
	0	1250	5000	20000	0	1250	5000	20000
2 weeks / without recovery	13.9	12.6[a]	18.6	26.7**[b]	12.9[b]	17.7[b]	19.8	32.5**
4 weeks / without recovery	21.8	20.7	19.0	19.4	13.1	14.0	13.6	15.8
2 weeks / with recovery	18.6	24.2	26.6*	34.7**	12.2	13.3	17.8	24.5**
4 weeks / with recovery	13.5	16.2	15.6	20.3	12.3	11.1	9.8	13.3

[a] mean of 3 animals only, excluded from statistical analysis; [b] mean of 4 animals; * $p < 0.05$; ** $p < 0.01$

Table A6_10-9. Summary of group mean thyroid peroxisomal peroxidase activity in groups of 5 animals/sex.

No. weeks treatment / ± recovery period of 4 weeks	**Group mean TP activity (optical density/min/mg protein) in:**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**1250**	**5000**	**20000**	**0**	**1250**	**5000**	**20000**
2 weeks / without recovery	1.04	0.84	1.10	1.05	1.01	0.83	1.12	1.71
4 weeks / without recovery	1.25	0.78	0.79	0.20	1.25	0.75	0.74	0.88
2 weeks / with recovery	1.27	0.64	0.85	0.90	1.01	0.73	0.92	0.98
4 weeks / with recovery	0.99	0.44	0.82	0.68	0.35	0.85	0.95	0.76

Table A6_10-10. Summary of group mean hepatic and thyroid BrdU labelling indices.

No. weeks treatment / ± recovery period of 4 weeks	**Group mean BrdU labelling index (%) in:**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**1250**	**5000**	**20000**	**0**	**1250**	**5000**	**20000**
Liver								
2 weeks / without recovery	0.25	-	-	0.10	0.86	-	-	0.75
4 weeks / without recovery	0.26	-	-	0.23	0.58	-	-	0.31
2 weeks / with recovery	0.22	-	-	0.34	0.47	-	-	0.66
4 weeks / with recovery	0.30	-	-	0.29	0.32	-	-	0.57
Thyroid								
2 weeks / without recovery	7.2	-	-	10.0	9.5	-	-	5.7*
4 weeks / without recovery	4.4	-	-	7.6	6.8	-	-	4.1
2 weeks / with recovery	3.7	-	-	2.2*	3.4	-	-	4.8
4 weeks / with recovery	4.0	-	-	2.4	2.6	-	-	1.7

- not evaluated; * $p < 0.05$

Table A6_10-11. Summary of group mean liver weight data.

No. weeks treatment / ± recovery period of 4 weeks	**Group mean value in:**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**1250**	**5000**	**20000**	**0**	**1250**	**5000**	**20000**
Absolute liver weight (g)								
2 weeks / without recovery	9.86	10.73	12.69*	13.58*	6.26	6.77	6.87	8.20*
4 weeks / without recovery	10.76	12.13	12.64	13.95	7.35	7.33	7.12	9.09*
2 weeks / with recovery	10.69	11.54	11.85	11.17	6.75	7.37	7.11	6.90
4 weeks / with recovery	11.57	11.54	10.88	11.52	7.38	7.69	6.84	7.06
Relative liver weight (% body weight)								
2 weeks / without recovery	3.22	3.45*	3.95*	4.65*	3.32	3.54	3.80	4.64*
4 weeks / without recovery	2.92	3.36	3.55	4.49*	3.28	3.35	3.61*	4.35*
2 weeks / with recovery	2.72	2.78	2.86	2.88	2.90	2.96	3.12	3.10
4 weeks / with recovery	2.63	2.68	2.51	2.83	2.95	3.06	2.91	3.09

* $p < 0.05$

Table A6_10-12. Summary of group mean thyroid weight data.

No. weeks treatment / ± recovery period of 4 weeks	**Group mean value in:**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**1250**	**5000**	**20000**	**0**	**1250**	**5000**	**20000**
Absolute thyroid weight (mg)								
2 weeks / without recovery	16.2	20.2	22.8	25.6	14.1	18.5	16.3	17.3
4 weeks / without recovery	20.2	25.2	21.3	25.6	17.8	20.6	19.4	21.7
2 weeks / with recovery	20.2	22.0	22.5	24.2	20.5	16.2	19.8	20.5
4 weeks / with recovery	27.5	23.0	22.6	25.3	16.7	19.6	19.3	21.0
Relative thyroid weight (% body weight)								
2 weeks / without recovery	0.0053	0.0065	0.0071	0.0088	0.0075	0.0096	0.0090	0.0098
4 weeks / without recovery	0.0055	0.0071	0.0060	0.0083	0.0080	0.0094	0.0099	0.0104
2 weeks / with recovery	0.0052	0.0053	0.0054	0.0063	0.0088	0.0065*	0.0087	0.0092
4 weeks / with recovery	0.0063	0.0054	0.0053	0.0062	0.0067	0.0078	0.0082	0.0093

* $p < 0.05$

Table A6_10-13. Summary of microscopic pathology findings in the liver of survivors.

Observation - Group	**No. with observation / no. examined in:**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**1250**	**5000**	**20000**	**0**	**1250**	**5000**	**20000**
Centrilobular hypertrophy:								
- 2 weeks / without recovery	0 / 4	-	-	2 / 4	0 / 4	-	-	4 / 4
- 4 weeks / without recovery	1 / 5	-	-	0 / 5	0 / 5	-	-	5 / 5
- 2 weeks / with recovery	1 / 5	-	-	1 / 5	3 / 5	-	-	4 / 5
- 4 weeks / with recovery	0 / 5	-	-	0 / 5	0 / 5	-	-	5 / 5
↑ multinucleated hepatocytes:								
- 2 weeks / without recovery	0 / 4	-	-	2 / 4	0 / 4	-	-	4 / 4
- 4 weeks / without recovery	0 / 5	-	-	1 / 5	0 / 5	-	-	5 / 5
- 2 weeks / with recovery	0 / 5	-	-	1 / 5	0 / 5	-	-	4 / 5
- 4 weeks / with recovery	0 / 5	-	-	0 / 5	0 / 5	-	-	4 / 5

- not examined

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1.2. Specification** According to document A3 the physical state changes from white crystals to amber liquid with decreasing purity from 99,8 % to 99,3%. Within the 5 batch analysis a purity between 97,2 % and 99,6% is indicated. 87031 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is within the range of the 5 batch analysis. Therefore the specification does not relevantly deviate to these indications.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12.1
Annex Point IIA-VI.6.9

Medical surveillance data on manufacturing plant personnel

Official use only

1 REFERENCE

1.1	**Reference**	Yamazaki, Y. (1992): Health report; Industrial Hygiene Section, Ohmuta Factory, Mitsui Toatsu Chemicals, Inc., Fukuoka 836, Japan; unpublished report no. not specified (April 17, 1992). Personnel monitoring period: January 1987 - March 1992.

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Etofenprox
3.2	**Persons exposed**	Staff who worked on the production line in a triple shift pattern during the period 1987 - 1992
3.2.1	Sex	Male
3.2.2	Age/weight	No information in report.
3.2.3	Known Diseases	*No information in report.*
3.2.4	Number of persons	21
3.3	**Examinations**	The staff were examined annually for blood biochemistry (GOT, GPT, ϒ-GPT, ALP, TTT, total cholesterol, neutral fat, blood glucose, urea nitrogen and uric acid) and also had an X-ray and ECG recorded. Twice yearly examinations were performed for the following parameters: Height, weight, vision, hearing Blood pressure Hematology (RBC, Hb, Ht and WBC) Urinalysis (glucose, protein and occult blood) Other medical features (subjective and objective symptoms, lifestyle, family history, past history) The normal range of values, against which the measured values were compared, is shown in Table A6_12_1-1.

4 RESULTS

4.1	**Results of examinations**	Although several different abnormal values were obtained from the 21 operators, there was no consistent pattern suggestive of an effect due to exposure to etofenprox. Individual values falling outside the normal ranges are summarised in Table A6_12_1-2.

Section A6.12.1
Annex Point IIA-VI.6.9

Medical surveillance data on manufacturing plant personnel

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The Ohmuta factory of Mitsui Toatsu Chemicals, Inc. was producing [redacted]nnum etofenprox technical during the period 1987 - 1992. The production line was operated by 21 male staff who worked in a triple shift pattern. The report documents the health assessments made on the production operatives.

5.2 Conclusion

There is no pattern of abnormalities in production operatives that suggest adverse health effects due to exposure to etofenprox.

Table A6_12_1-1. Normal range values for medical surveillance parameters.

Parameter (units not specified)	Normal range	
	Male	Female
Blood pressure (diastolic)	< 90	< 90
Blood pressure (systolic)	95 - 159	95 - 159
Urine (glucose)	-	-
Urine (protein)	-	-
Urine (occult blood)	-	-
RBC	370 - 570	350 - 550
Hb	13 - 18	12 - 16
Ht	39 - 53	35 - 49
WBC	3100 - 10000	3100 - 10000
GOT	< 40	< 40
GPT	< 55	< 55
ϒ-GPT	< 99	< 99
ALP	80 - 260	80 - 260
TTT	< 6	< 6
Total cholesterol	120 - 260	120 - 260
Neutral fat	50 - 150	50 - 150
Blood glucose	< 120	< 120
Uric acid	< 7.9	< 6.9
Urea nitrogen	< 21.9	< 21.9

Table A6_12_1-2. Summary of abnormal values in production line staff - etofenprox (January 1987 - March 1992).

ID	Age / sex	Exposure	Abnormal findings (and dates)
A	43 / M	01.87 - 03.92	Disturbance of vertebral disc (09.88 - 03.90) Neutral fat: 198mg/dL (09.90)
B	41 / M	01.87 - 03.92	NAD
C	49 / M	07.87 - 03.92	Disturbance of conjunctiva (11.91 - 03.92)
D	21 / M	04.89 - 03.92	ALP: 263IU/L (11.89) Treated for keratitis (05.87 and 11.91)
E	47 / M	11.87 - 03.92	WBC: 12200/mm^3 (11.89) WBC: 10500/mm^3 (09.90)
F	47 / M	07.87 - 03.92	Treated for duodenal ulcer (05.88 - 05.90) Treated for duodenal ulcer (05.91 - 03.92)
G	48 / M	07.87 - 03.92	Treated for neuralgia (11.88) ϒ-GPT 110IU/L; GPT 67IU/L; neutral fat:307mg/dL (11.89) Migraine (05.90) GOT 46IU/L; GPT 83IU/L; neutral fat 235mg/dL; migraine (11.90) Migraine (05.91) ϒ-GPT 107IU/L; GPT 58IU/L; neutral fat:228mg/dL; migraine (11.91) Migraine (03.92)
H	44 / M	02.88 - 03.92	Treated for duodenal ulcer (11.90 - 03.92)
I	41 / M	01.87 - 03.92	NAD
J	40 / M	10.87 - 03.92	NAD
K	39 / M	10.88 - 03.92	Blood pressure: 138 / 98 (05.88) ALP 69IU/L; neutral fat 206mg/dL; uric acid 8.1mg/dL; blood pressure 158 / 96 (11.89) ALP 71IU/L; neutral fat 274mg/dL; uric acid 8.1mg/dL; blood pressure 150 / 96 (11.90) Blood pressure: 154 / 100 (05.91) ALP 71IU/L; neutral fat 274mg/dL; uric acid 8.1mg/dL (11.91) Treated for gout (03.92)
L	43 / M	01.87 - 03.92	NAD
M	45 / M	01.87 - 03.92	Blood pressure: 150 / 102, treated for hypertension (05.88, 11.91, 03.92) Blood pressure: 142 / 98 - 158 / 108 (11.88 - 11.91) GPT 65IU/L; neutral fat 265mg/dL (11.89) GOT 45IU/L; GPT 60IU/L (11.90)
N	41 / M	01.87 - 03.92	Total cholesterol: 271mg/dL (11.89) Total cholesterol: 271mg/dL; neutral fat 174mg/dL (11.90) Neutral fat: 164mg/dL (11.91)
O	42 / M	01.87 - 03.92	Treated for cholelithiasis (05.88) Treated for allergic rhinitis (05.89) Neutral fat: 188mg/dL (11.89) Neutral fat: 193mg/dL (11.90)
P	37 / M	07.87 - 03.92	NAD
Q	35 / M	01.87 - 03.92	NAD
R	49 / M	10.87 - 03.92	Under diabetic management and treated for hypertension from 11.88. Blood pressure: 156 / 96 (11.88) Blood pressure: 150 / 106 (05.89) Blood pressure: 134 / 98; neutral fat 179mg/dL; blood glucose 127mg/dL (11.89) Blood pressure: 160 / 100; neutral fat 202mg/dL; blood glucose 176mg/dL (11.90) Blood glucose 194mg/dL (11.91)
S	19 / M	04.91 - 03.92	NAD
T	42 / M	01.87 - 03.92	Urinary glucose positive (11.89, 11.90, 05.91)
U	24 / M	04.88 - 11.89	NAD

NAD all examinations no abnormalities detected

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	-
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.13 Toxic effects on livestock and pets
Annex Point IIIA-VI.2

JUSTIFICATION FOR NON-SUBMISSION OF DATA — **Official use only**

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [X] **Other justification [ ]**

Detailed justification:

Licking

Because of the low acute oral toxicity of etofenprox (NOAEL = 2000 mg/kg bw [redacted] 2003a, section A 6.1.1/01), its solubility in water (saliva) (solubility = 0.0225 mg/L, Kunz 2000, section A 3.5/01) and its low concentration in wood (63 mg / m^2, highest amount based on intended uses), toxic effects on livestock and pets from licking treated wood are not to be expected. X

Example:

Applying a safety factor of 100 to the oral NOAEL leads to an acute toxicity trigger value of 20 mg/kg body weight. A 600 kg cow would have to extract 12000 mg etofenprox to exceed this value, corresponding to190 m^2 treated wood (assuming 0.063 g a.s./ m^2). A dog of 8 kg body weight would need to extract 160 mg or 2.5 m^2 treated wood. Assuming that etofenprox has the same solubility in saliva and water, this would correspond to 7000 L saliva.

Applying the endpoints for toxicity to humans (AOEL = ARfD = 0.2 mg/kg bw/day, see Doc. II-A) and comparing it to the required volume of saliva to extract the a.s. leads to a similar conclusion, since 5000 L saliva would be required for cows or 70 L for dogs. X

Skin contact

Based on the low acute dermal toxicity of etofenprox (NOAEL = 2000 mg/kg, [redacted] 2003b section A 6.1.2/01), its low dermal penetration (< 13 %, [redacted] 1999, section A 6.2/06), the fact that the a.i. is not a skin irritant [redacted] 1985a, section A 6.1.4.s) and not a skin sensitizer [redacted] 1985, section A 6.1.5) and taking further the low concentrations of etofenprox in wood (see above), toxic effects on livestock and pets from dermal contact with treated wood are not to be expected.

Exposure via feeding stuff

Exposure of animals to etofenprox in feeding stuff from its use as wood preservative can only be incidental and can only be indirect via contaminated water or plants grown on contaminated soil. Considering the low toxicity and the low predicted environmental concentrations of etofenprox in soil (see Doc II-B), exposure of livestock to etofenprox via feeding stuff from its use as wood preservative is negligible.

Conclusion:

Based on the low probability of exposure to toxic levels and animal welfare considerations a separate exposure study was not conducted.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Evaluation of applicant's justification	Licking An additional more conservative way of calculation is as follows: According to document A.5. the highest concentration of a.s in wood should be 2,5g/ m^3 (pressure method for class 3 wood). The solubility in water is 0.0225 mg/l, there are no values for salvia. To archive the human ADI value of 0,03 mg/kg day a cow of 600 kg needs to lick with 800 litres of salvia per day at least 7,2 dm^3 (= litres) of wood or 0,72 m^2 of wood-surface (in case just the upper 1 cm of wood is considered to be accessible to liberation) .
Conclusion	Recalculation of the figures leads to different values. However the values are still very low. In any case this is subject to risk characterisation. The justification is sufficient and acceptable. No further testing is necessary.
Remarks	-
	COMMENTS FROM...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA-VII.7.6.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Van der Gaauw A. (2001): ^{14}C-Etofenprox: hydrolysis at three different pH values; RCC Ltd, 4452 Itingen, Switzerland; unpublished report no. 731158 (September 17, 2001). Dates of experimental work: November 28, 2000 – April 03, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: EEC C.7 (1992) OECD 111 (1981) EPA OPPTS 835.2110	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	x
3.1.1	Lot/Batch number	MR-9301	
3.1.2	Specification	As given in section 2	x
3.1.3	Description	White crystal	
3.1.4	Purity	99.7%	
3.1.5	Radiolabelling	(A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/20 - Specific activity: 3.24 MBq/mg - Radiochemical purity: 98.9% (determined in the appl. solution) (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/31 - Specific activity: 4.20 MBq/mg) - Radiochemical purity: 98.9% (determined in the appl. solution)	
3.1.6	Further relevant properties	Solubility in water: 22.5 µg/L at 20 ± 0.5°C Vapour pressure: 8.13 x 10^{-7} Pa at 25°C	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	not applicable	
3.3	**Test solution**	A detailed description of the preparation of the test solution and of the replications and experimental conditions is given in tabular form (see enclosed tables A7_1_1_1_1_01-1 and A7_1_1_1_1_01-2, respectively)	
3.4	**Testing procedure**		

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA-VII.7.6.2.1

3.4.1	Test system	Incubation was in the dark for avoiding photolytic effects, oxygen was removed in an ultrasonic bath, the incubation vessels were gas tight. Due to the low water solubility of the test item a very low amount of radioactivity was applied in the first experiment, which was recovered with a high variability. Therefore a second experiment was set up using bigger incubation volumes (50 ml). A detailed description of the test system is given in tabular form (see enclosed table A7_1_1_1_1_01-3)
3.4.2	Temperature	50°C.
3.4.3	pH	Experiment 1: 4.52 to 4.52; 7.67 to 7.75; 9.05 to 9.02 4.56 to 4.63; 7.62 to 7.69; 9.04 to 9.02 Experiment 2: 3.98 to 4.16; 6.99 to 7.13; 9.01 to 9.17
3.4.4	Duration of the test	0 to 120 hours
3.4.5	Number of replicates	2 + 1 in two experiments
3.4.6	Sampling	The whole amount of sample was transferred to a separation funnel and portioned with ethyl acetate (3x). After evaporation of the organic solvent, the residue was dissolved in acetonitrile and analysed.
3.4.7	Analytical methods	Radioactivity was measured with a Packard liquid scintillation counter (TRI-CARB 2500TR) using INSTA-GEL II Plus. Sampling aliquots of 0.5 ml were diluted to 4 ml, 5 ml aliquots to 10 ml. For TLC analysis samples were concentrated, TLC with toluene solvent an pre-coated (5 cm x 20 cm) silca gel 60 F 254 (Merck) using a CAMAG Linomat for application. Detection: Berthold Automatic TLC-Linear Analyser or a Fuji BAS 1000 phosphor imager Detection limits (LOD 2x background): 0.36 µg/l for LSC Quantification limit (LOQ 3x background): 0.55 µg/l for LSC and 0.013 for TLC
3.5	**Preliminary test**	Yes Test item was stable, therefore only a preliminary test was required.
		4 RESULTS
4.1	**Concentration and hydrolysis values**	In tabular form (see enclosed table A7_1_1_1_1-4) Recoveries of the total radioactivity during the 5-day period were within the range of 57 to 128 % of the applied radioactivity. This was considered acceptable given the low solubility of the test item. Low recoveries were attributed to adsorption of the test item to the glass surface
4.2	**Hydrolysis rate constant (k_h)**	The test item was stable at 50°C for 120 hours
4.3	**Dissipation time**	The test item was stable at 50°C for 120 hours
4.4	**Concentration – time data**	The results are summarised in table A7_1_1_1_1-7. The test item was stable.
4.5	**Specification of the transformation products**	The test item was stable at 50°C for 120 hours

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA-VII.7.6.2.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Guidelines: - OECD Guideline 111 (May 12, 1981)
- Directive 92/69/EEC part C.7 (1992)

The hydrolytic stability of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC 276/31; radiochemical purity 98.9%; specific activity: 4.20 MBq/mg) and [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC 277/20; radiochemical purity: 98.9%, specific activity: 3.24 MBq/mg) was investigated in aqueous buffer at three relevant pH values.

Buffer solutions of pH 4 (0.713 g citric acid; 0.156 g sodium chloride and 0.165 g sodium hydroxide), pH 7 (0.209 potassium dihydrogen phosphate and 0.318 g disodium hydrogen phosphate) and pH 9 (0.144 g potassium dihydrogen phosphate and 1.020 g disodium tetraborate decahydrate) were prepared with purified water. Buffer solutions were sterilised by autoclaving for about 30 minutes.

The application solution was prepared in acetonitrile based on a 1+1 mixture of both radiolabelled etofenprox. In a preliminary test, an aliquot of 20 mL of the aqueous solutions was transferred into each sterilised test vessel. Thereafter, a volume of 200 µL of the application solution was added to the test vessels to obtain concentrations of ^{14}C-etofenprox of 2.700, 2.225 and 3.010 µg/L at pH 4, 7 and 9, respectively. The incubations vessels (2.5 cm inner diameter and 4.5 cm height) were made of glass with foil-lined screw caps.

Due to the low recoveries obtained from the first experiment, a second experiment using a larger sample volume was performed in order to test the working up procedure and to confirm the results of the first experiment. Aliquots of 50 mL of the aqueous solutions were separately transferred to sterilised glass flasks and a volume of 500 µL of the application solution was added, to obtain concentrations of ^{14}C-etofenprox of 1.969, 2.459 and 2.597 µg/L at pH 4, 7 and 9, respectively.

A total of 8 individual samples of 20 mL and 4 individual samples of 50 mL, for each pH value, were incubated in a water bath in the dark at 50 ± 0.1°C under agitation, for the first and second experiment, respectively. Duplicate samples were taken and analysed by TLC immediately after treatment and after 2.4 hours, 24 and 120 hours of incubation. Then, their pH was measured.

5.2 Results and discussion

In the first experiment, a large variation of the recovery of radioactivity was observed for all samples during the 5 day incubation period. The second experiment showed that after 5 days of incubation the recovered radioactivity was higher than 95% of the initial amount applied.

No degradation of etofenprox, i.e. less than 10% hydrolysis, was observed during the 5-day incubation period at each of the three pH values investigated.

5.2.1 k_H The test item was stable at 50°C for 120 hours

5.2.2 DT_{50} The test item was stable at 50°C for 120 hours

5.2.3 r^2 The test item was stable at 50°C for 120 hours

5.3 Conclusion

The results showed that [^{14}C]-etofenprox is hydrolytically stable and that no degradation was observed in buffer solutions at pH 4, 7 and 9 for at least 5 days at 50°C in the dark. Therefore, it can be considered that [^{14}C]-etofenprox is stable in water under environmentally relevant

Section A7.1.1.1.1/01 Hydrolysis as a function of pH and identification of breakdown products

Annex Point IIA-VII.7.6.2.1

acidic, neutral and alkaline conditions.

5.3.1 Reliability 1

5.3.2 Deficiencies None

Table A7_1_1_1_1_01-1: Type and composition of buffer solutions (specify kind of water if necessary).

pH	Type of buffer (final molarity)	Composition
4*	Citrate (0.0005 to 0.0021 M)	0.713 g citric acid (monohydrate), 0.156 g sodium chloride and 0.165 g sodium hydroxide were diluted to 2 litres with purified water and autoclaved (30 min at 121°C)
7*	Phosphate (0.0005 to 0.0021 M)	0.209 g potassium dihydrogen phosphate and 0.318 g disodium hydrogen phosphate were diluted to 2 litres with purified water and autoclaved (30 min at 121°C)
9*	Borate (0.0005 to 0.0021 M)	0.144 g potassium dihydrogen phosphate and 1.020 g disodium tetraborate decahydrate were diluted to 2 litres with purified water and autoclaved (30 min at 121°C)

* the pH of the solutions was adjusted using 0.1 M NaOH or HCl.

Table A7_1_1_1_1_01-2: Description of test solution.

Criteria	Details
Purity of water	purified, sterile water.
Preparation of test medium	Buffer solutions were used (see above)
Test concentrations (mg a.i./L)	pH 4: 11853 dpm/20 ml; 0.0027 mg/l pH 7: 9769 dpm/20 ml; 0.0022 mg/l pH 9: 13211 dpm/20 ml; 0.0030 mg/l
Temperature	50 °C
Controls	Analysis before incubation
Identity and concentration of co-solvent	Acetone stock solution contained 6.732 MBq/5 ml propyl label and 7.029 MBq benzyl label. The application solution was prepared by adding 0.04 ml stock solution to a flask, after evaporation of the acetone 105 ml acetonitrile were added. 0.2 ml acetonitrile solution were added to 20 ml buffer (1 % co-solvent acetonitrile) *(% v/v).*
Replicates	8 in experiment 1 (duplicate analysis at each pH) 4 in experiment 2

Table A7_1_1_1_1_01-3: Description of test system.

Compound	Details
Glassware	Experiment: round glass vials, capped, 2.5 cm i.d., 4.5 cm high, 20 ml solution Experiment 2: Pulvis glass flasks with 50 ml solution
Other equipment	Water bath, pH electrode
Method of sterilization	Autoclaved 30 minutes at 121 °C.

Table A7_1_1_1_1_01-4: Hydrolysis of test compound, transformation products and reference substance, expressed as percentage of initial concentrations, at pH 4, pH 7 and pH 9 (Experiment 1).

pH = 4 **Compound**	**Sampling times (*hours*)**			
	0	**2.4**	**24**	**120**
Parent compound	98.5%	99.6%	99.2%	98.9%
M1 (adsorbed parent)	1.5%	0.4%	0.8%	1.1%
Reference compound	-	-	-	-
Volatiles *(if measured)*	-	-	-	-
Total % recovery	100.0%	100.0%	100.0%	100.0%

pH = 7 **Compound**	**Sampling times (*hours*)**			
	0	**2.4**	**24**	**120**
Parent compound	94.7%	100.0%	100.0%	99.5%
M1 (adsorbed parent)	5.4%	0.0%	0.0%	0.5%
Reference compound	-	-	-	-
Volatiles *(if measured)*	-	-	-	-
Total % recovery	100.1%	100.0%	100.0%	100.0%

pH = 9 **Compound**	**Sampling times (*hours*)**			
	0	**2.4**	**24**	**120**
Parent compound	90.1%	97.3%	98.5%	97.1%
M1 (adsorbed parent)	9.9%	2.7%	1.5%	2.9%
Reference compound	-	-	-	-
Volatiles *(if measured)*	-	-	-	-
Total % recovery	100.0%	100.0%	100.0%	100.0%

Table A7_1_1_1_1_01-5: Dissipation times of parent compound, transformation products and reference compound at pH 4, pH 7 and pH 9.

Compound	**pH 4**		**pH 7**		**pH 9**	
	DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**	**DT_{50}**	**DT_{90}**
Parent compound	stable		stable		stable	
M1	n.a.		n.a.		n.a.	
Reference compound	-		-		-	

n.a. = not applicable, M1 is believed to be adsorbed parent.

Table A7_1_1_1_1_01-6: Specification and amount of transformation products.

CAS-Number	***CAS and/or IUPAC* Chemical Name(s)**	**Amount [%] of parent compound measured at**		
		pH 4	**pH 7**	**pH 9**
	not applicable			

Table A7_1_1_1_1_01-7: Hydrolysis of test compound expressed as concentration of parent (in µg/l), at pH 4, pH 7 and pH 9.

pH = 4 **Parent compound**	**Sampling times (*hours*)**			
	0	**2.4**	**24**	**120**
Experiment 1A and B	2.700	2.677	2.659	2.700
Experiment 2	2.617	2.700	2.700	2.640
Mean	2.659	2.689	2.680	2.670

pH = 7 **Compound**	**Sampling times (*hours*)**			
	0	**2.4**	**24**	**120**
Experiment 1A and B	2.019	2.225	2.225	2.204
Experiment 2	2.193	2.225	2.225	2.225
Mean	2.106	2.225	2.225	2.215

pH = 9 **Compound**	**Sampling times (*hours*)**			
	0	***2.4***	***24***	***120***
Experiment 1A and B	2.620	2.942	2.921	2.874
Experiment 2	2.804	2.915	3.010	2.973
Mean	2.712	2.928	2.965	2.923

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** The specification for this batch is given in section 3 (RCC Study No. 751803).
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	Van der Gaauw A. (2003): Aqueous photolysis of [^{14}C]-Etofenprox under laboratory conditions and determination of quantum yield; RCC Ltd., Itingen, Switzerland; unpublished report No. 755526 (May 07, 2003) Dates of experimental work: April 25, 2000 – January 04, 2001	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: Directives 95/36/EEC and 94/37/EEC SETAC Guideline (March 1995) OECD Guidance Document (97)21 EPA OPPTS 835.2210 Japan MAFF Guideline, 16	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	MTI-500 (Etofenprox)	x
3.1.1	Lot/Batch number	MR-9301	
3.1.2	Specification	As given in section 2 Deviating from specification given in section 2 as follows	x
3.1.3	Purity	99.5%	
3.1.4	Radiolabelling	(A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/20 - Specific activity: 1.22 GBq/mmol (= 3.24 MBq/mg) - Radiochemical purity: 100% (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/31 - Specific activity: 1.58 GBq/mmol (= 4.20 MBq/mg) - Radiochemical purity: 100%	

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

3.1.5	UV/VIS absorption spectra and absorbance value	UV/VIS absorption spectra: similar at pH values from 1 to 12; absorption maximum at 273 nm. Absorption (A) data of a 61.4 mg/L solution of etofenprox in acetonitrile/methanol, 1 cm pathlength: 290 nm: A = 0.0176 300 nm: A = 0.0007 310 nm: A = 0.0007 320 nm: A = 0.0005, no absorption > 330 nm
3.1.6	Further relevant properties	Solubility in water: 22.5 µg/L at 20 ± 0.5°C Vapour pressure: 8.13 x 10^{-7} Pa at 25°C Hydrolytic stability: hydrolytically stable at pH 4, 7 and 9 Oxidising properties: not oxidising pKa value: Etofenprox has no sites which can either be protonated or dissociate at pH 3 to 10
3.2	**Reference substances**	Yes: Pentachlorophenol, 2,4-Dichlorophenol, 3,4-Dichloroaniline (quantum yields of these test substances were determined for system validation)
3.3	**Test solution**	A detailed description of the preparation of the test solution including controls, test material concentrations, buffer solutions, number of replicates and experimental conditions is given in tabular form (see enclosed table A7_1_1_1_2_01-1).
3.4	**Testing procedure**	
3.4.1	Test system	A detailed description of the test system, including kind of tubes and other equipment used, sterilisation method is given in tabular form (see enclosed table A7_1_1_1_2_01-2).
3.4.2	Properties of light source	A detailed description of the artificial light source is given in tabular form (see enclosed table A7_1_1_1_2_01-2).
3.4.3	Determination of irradiance	The absolute light intensity was measured with actinometer solutions containing uranyl nitrate (0.02 mol/L) and oxalic acid (0.1 mol/L). Oxalic acid concentration was measured by titration with potassium permanganate 0.02 mol/L).
3.4.4	Temperature	25 ± 1°C
3.4.5	pH	Buffer solution: pH 7.1 Pond water: pH 8.1
3.4.6	Duration of the test	15 days continuous irradiation
3.4.7	Number of replicates	2 illuminated and 1 control (incubation in the dark) replicates per sampling interval and test system (buffer solution, pond water)
3.4.8	Sampling	Irradiated buffer solution samples: after 0, 1, 1.8, 4.6, 11.7 and 15 days Irradiated pond water samples: after 0, 1, 1.8, 4.6, 6.7, 13.5 and 15 days Control samples: after 1.8, 4.6, 6.7, 11.7 and 15 days

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

3.4.9	Analytical methods	Radiocarbon determinations procedure (LSC): Packard Liquid Scintillation Counter equipped with DPM and luminescence options (TRI-CARB TR-2500 or 2550 TR/LL); scintillation mixture: INSTA-GEL II Plus (Packard Instruments Comp., USA). - Detection limit (LOD 2x background): 0.19 µg/l - Quantification limit (LOQ 3x background): 0.28 µg/l Thin-Layer Chromatography (TLC): For TLC analysis samples were concentrated, TLC with toluene solvent on precoated (5 cm x 20 cm) silica gel 60 F 254 (Merck) using a CAMAG Linomat for application. Detection: non labelled reference items by UV (254 nm) visualisation; radioactive test substance by phosphor imaging (Fuji BAS 1000) - Quantification limit (LOQ): 0.053 µg/l Solvent systems: SS 4: toluene/ethyl acetate (19:1; v/v) SS 5: toluene SS 7: toluene/ethyl acetate/ acetic acid (90:10:1) SS 9: chloroform/ methanol acetic acid (95:5:1) SS 11: toluene/ ethyl acetate/ acetic acid (99:1) High-Performance Liquid Chromatography (HPLC): HPLC (YMC-Pack ODS AS column, 150 mm x 3 mm, 3µm) with UV (L-4000, Merck-Hitachi, at 276 nm) and Radio (Flo-One\Beta A-500 cell, Packard) detection was used to confirm the results of TLC. H_3PO_4/Acetonitrile gradient at 0.43 ml/min.
3.5	**Transformation products**	Yes
3.5.1	Method of analysis for transformation products	Transformation products were measured by TLC and confirmed by HPLC, using the methods described under 3.4.9

4 RESULTS

4.1	**Screening test**	Not performed
4.2	**Actinometer data**	A uranyl acetate / oxalic acid actinometer was used; the conversion of oxalic acid was measured by titration with 0.02 M potassium permanganate. Volume needed before irradiation: 20.3 ml; Volume needed after irradiation: 16.6 ml. This corresponds to $3.878*10^{20}$ photo reacted actinometer molecules. Quantum yield actinometer: $\Phi = 0.56$ The number of photons absorbed by the actinometer was $6.93*10^{20}$ in 20 minutes over a surface of 28.3 cm^2. The number of photons absorbed by the test solutions corrected for test geometry and absorption was $5.23*10^{15}$ photons in 0.047 days with a surface of 4 cm^2.

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

4.3 Controls

Yes, incubation in the dark: when incubated under the same conditions in the dark, etofenprox was shown to be stable in both the buffer solution (pH 7) and the natural pond water. The radioactivity in the dark control samples was characterised as percentage of applied radioactivity (%) and as µg parent equivalents/L (ppb): x

Test system		Sampling Times (days)				
		1.8	4.6	6.7	11.7	15 **
Buffer solution	%	95.9	100.0	100.0	95.2	96.2
	ppb	5.02	5.24	5.27	4.99	5.07
Pond water	%	96.4	97.2	96.1	94.0	90.6
	ppb	5.05	5.09	5.07	4.92	4.78

** 15-day sample showed low recovery due to adsorption to glass

4.4 Photolysis data

4.4.1 Concentration values

Radioactivity in the irradiated samples characterised as µg/L parent equivalents (ppb).

Buffer solution (pH 7):

Replicate	Sampling Times (days)					
	0.0	1.0	1.8	4.6	11.7	15.0
A	5.24	4.39	3.95	2.08	0.91	0.50
B	5.24	4.16	4.07	2.97	1.21	0.50
Mean	*5.25*	*4.27*	*4.01*	*2.52*	*1.06*	*0.50*

Pond water:

Replicate	Sampling Times (days)						
	0.0	1.0	1.8	4.6	6.7	13.5	15.0
A	5.24	4.70	4.26	3.22	1.88	1.92	1.57
B	5.24	4.57	4.38	3.54	2.69	2.15	1.87
Mean	*5.24*	*4.64*	*4.32*	*3.38*	*2.29*	*2.04*	*1.72*

4.4.2 Mass balance

Mass balance of etofenprox expressed as % of applied radioactivity. Test vessels were rinsed with ethyl acetate to gain test material that was adsorbed to the glass surface.

Buffer solution (pH 7); mean overall recovery = 89.4%

Replicate	Sampling Times (days)					
	0.0	1.0	1.8	4.6	11.7	15.0
A	60.5	101.3	94.5	102.6	76.8	101.8
B	83.1	93.7	103.0	75.2	79.4	100.3
Mean	*71.8*	*97.5*	*98.8*	*88.9*	*78.1*	*101.1*

Pond water: mean overall recovery = 86.0%

Replicate	Sampling Times (days)						
	0.0	1.0	1.8	4.6	6.7	13.5	15.0
A	84.7	100.7	100.9	100.6	43.5	77.9	103.7
B	45.2	104.5	108.2	88.1	93.2	79.1	74.1
Mean	*65.0*	*102.6*	*104.6*	*94.4*	*68.4*	*78.5*	*88.9*

4.4.3 k^c_p

Measured photolysis rate constant k^c_p for the test substance.

Buffer (pH 7): - 0.148

Pond water: - 0.087

4.4.4 Kinetic order

First order (samples with recoveries < 75% were not used for the calculation of kinetics)

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

4.4.5 k^c_p / k^a_p

The used actinometer does not include a determination of the photolysis rate constant, since constant absorption is assumed.

4.4.6 Reaction quantum yield (ϕ^c_E)

The quantum yield of etofenprox was determined to be

Φ = 0.248 in buffer solution (pH 7) and

Φ = 0.147 in natural pond water.

4.4.7 k_{pE}

The direct photolysis sunlight rate constant of the test substance in water bodies in the environment is not given in the report, but can be easily calculated by assuming 1st order kinetics and using the equation $t_{1/2} = \ln 2 / k_{pE}$

Theoretical photolysis rate constant (days^{-1}) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	- 0.075	- 0.089	- 0.050	- 0.032
Latitude 40° N	- 0.062	- 0.083	- 0.034	- 0.016
Latitude 50° N	- 0.047	- 0.073	- 0.018	- 0.005

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

4.4.8 Half-life ($t_{1/2E}$)

Estimation of half-lives of etofenprox in an aquatic environment at different latitudes, on the basis of the quantum yield value that was determined in the buffer solution (software used: GCSOLAR v.1.2, EPA)

Theoretical lifetime (days) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	9.2	7.8	13.8	21.8
Latitude 40° N	11.2	8.4	20.6	44.2
Latitude 50° N	14.9	9.5	38.4	131.0

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

4.5 Specification of the transformation products

see enclosed table A7_1_1_1_2_01-3

Section A7.1.1.1.2/01 Annex Point IIA-VII.7.6.2.2 Phototransformation in water including identity of transformation products

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test guidelines: - Directives 95/36/EEC and 94/37/EEC
- SETAC Guideline (March 1995)
- OECD Guidance Document (97)21
- EPA OPPTS 835.2210
- Japan MAFF Guideline, 16

x

The photolytic degradation of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC 276/31; radiochemical purity 100%; specific activity: 4.20 MBq/mg) and [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC 277/20; radiochemical purity: 100%, specific activity: 3.24 MBq/mg) under artificial sunlight and the quantum yield of photodegradation according to the ECETOC method was determined in sterile buffer solution at pH 7 and natural pond water.

The buffer solution (0.01 M phosphate) was prepared using ultra pure water. The natural water was collected from a pond system (Ormalingen BL/Switzerland) at a depth of 10-20 cm below the surface and then, passed through a 0.2 mm sieve. Before use, one liter of buffer solution and pond water were sterilised by autoclaving.

The application solution was prepared in acetonitrile based on a 1+1 mixture of both radiolabelled etofenprox. Aliquots of 22 mL of both aqueous solutions were transferred to each individual test vessel (25 mL). Thereafter, duplicate aliquots of 400 µL of the application solution were added to the test vessels to obtain a concentration of ^{14}C-etofenprox of 0.288 mg/L. Each incubation vessel (cylindrical of 2.26 x 11 cm for inner diameter and height, respectively) consisted of Duran glass covered with quartz glass plates screwed onto the top.

All vessels were maintained at 25 ±1 °C using a refrigerated circulator and exposed to continuous light for 15 days. Samples were exposed to artificial sunlight from a 1.8 kW xenon irradiation source (Suntest CPS, Heraeus®), equipped with an UV filter system to remove wavelengths below 290 nm. A mean light intensity of 17.2 W/m^2 within the visual light spectrum (300 nm to 400 nm) was used. Corresponding control samples were maintained under the same conditions but in the dark.

The gas mixture leaving each vessel was passed through a series of traps, for any liberated $^{14}CO_2$ and organic volatile.

Duplicate samples were taken for analysis after 0, 1, 1.8, 4.6, 11.7 and 15 days of continuous irradiation for the buffer solution and after 0, 1, 1.8, 4.6, 6.7, 13.5 and 15 days for the pond water. Dark control samples were taken from both test systems on days 1.8, 4.6, 6.7, 11.7 and 15 of incubation. At each interval, the samples were radiochemically quantified by LSC and analysed by HPLC and TLC. Trapping solutions for CO_2 (2N NaOH) and for organic volatiles (ethylene glycol) were exchanged at each sampling time and the trapped radioactivity was determined.

Additionally, the quantum yield [Φ] of disappearance of ^{14}C-etofenprox was determined with the ECETOC method using the uranyl acetate/ oxalic axid actinometer. These values were used to calculate the environmental lifetimes in pure water at different latitudes and seasons, using the GCSOLAR Vers. 1.20 (1999) computer program.

Section A7.1.1.1.2/01 Phototransformation in water including identity of transformation products

Annex Point IIA-VII.7.6.2.2

5.2 Results and discussion

5.2.1 k^c_p

Measured photolysis rate constant k^c_p for the test substance. x

Buffer (pH 7): 0.148

Pond water: 0.087

5.2.2 K_{pE}

The direct photolysis sunlight rate constant of the test substance in water bodies in the environment is not given in the report, but can be easily calculated by assuming 1st order kinetics and using the equation $t_{1/2} = \ln 2 / k_{pE}$ x

Theoretical photolysis rate constant (days^{-1}) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	0.075	0.089	0.050	0.032
Latitude 40° N	0.062	0.083	0.034	0.016
Latitude 50° N	0.047	0.073	0.018	0.005

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

5.2.3 ϕ^c_E

The quantum yield of etofenprox was determined to be

Φ = 0.248 in buffer solution (pH 7) and

Φ = *0.147 in natural pond water.*

5.2.4 $t_{1/2E}$

Estimation o half-lives of etofenprox in an aquatic environment at different latitudes, on the basis of the quantum yield value that was determined in the buffer solution (software used: GCSOLAR v.1.2, EPA)

Theoretical lifetime (days) at the surface of water *	**Spring**	**Summer**	**Autumn**	**Winter**
Latitude 30° N	9.2	7.8	13.8	21.8
Latitude 40° N	11.2	8.4	20.6	44.2
Latitude 50° N	14.9	9.5	38.4	131.0

* Conditions: pure water close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values

5.3 Conclusion

Validity criteria can be considered as fulfilled.

Etofenprox is rapidly photodegraded under simulated sunlight in both buffer solution at pH 7 and natural pond water. x

The experimental photolytic half-lives were calculated using first-order reaction kinetics to be 4.7 and 7.9 days for buffer solution at pH 7 and pond water, respectively. The control samples incubated under the same conditions in the dark were shown to be stable.

The quantum yield (Φ) of etofenprox was determined to be 0.248 and 0.147 days for buffer solution at pH 7 and pond water, respectively.

Two metabolites, identified as α-CO and PENA, exceeded 10% of the applied radioactivity during the course of the study, and reached their maximum levels after 15 days of irradiation, in sterile buffer and sterile natural water solutions

5.3.1 Reliability 1

5.3.2 Deficiencies No

Table A7_1_1_1_2_01-1: Description of test solution and controls.

Criteria	**Details**
Purity of water	Buffer solution: Baker buffer solution No. 5656 (phosphate, pH 7), diluted to 0.01 mol/L with ultra pure water (ELGA water purifier unit) Natural water: pond water (pH 8), filtered through 0.2 mm sieve - buffer solution and pond water were sterilised (121°C, 30 min) before use
Preparation of test chemical solution	Stock solutions: - ^{14}C-benzyl label: 6.732 MBq/5 ml acetone - ^{14}C-propyl label: 7.029 MBq/5 ml acetone Application solution: Stock solutions (40 µl of each radiolabel) were combined and solvent (acetone) was evaporated; residue was dissolved in 105 ml acetonitrile. Based on the specific activity of 3.685 MBq/mg the concentration of test substance was calculated to be 0.288 mg/L Test solutions: Aliquots of 22 ml of the aqueous solutions (12 flasks/water type) were treated with 400 µl of the application solution. To minimise the adsorption of test item to the glass surface the final content of acetonitrile was increased to 1.8%.
Test concentrations	5.24 µg a.s./L
Temperature	25 ± 1°C
Preparation of a.s. solution	see "Test solutions" above
Controls	Yes, incubations in the dark (one replicate per sampling interval and test medium:)
Identity and concentration of co-solvent	Acetonitrile, 1.8% (v/v)

Table A7_1_1_1_2_01-2: Description of test system.

Criteria	Details
Laboratory equipment	Test vessels: Cylindrical Duran glass vessels (25 ml; inner diameter x height: 2.26 x 11 cm), covered with quartz glass plates screwed onto the top of each vessel Reaction vessel area: 4.0 cm^2 (vessels were put in the centre of the irradiated area UV-VIS spectrometer: Lamba 2, Perkin Elmer. USA Irradiation was top- down; the quartz windows were transparent for all relevant wavelengths.
Test apparatus	Suntest CPS, Original Hanau (Heraeus, Germany)
Properties of artificial light source:	
Nature of light source	Xenon arc lamp, 1.8 kW
Emission wavelength spectrum	300 – 800 nm
Light intensity	17.2 W/m^2
Filters	UV filter to simulate outdoor sunlight (UV-edge 290 nm)
Properties of natural sunlight:	natural sunlight was not used
Latitude	n.a.
Hours of daylight	n.a.
Time of year	n.a.
Light intensity	n.a.
Solar irradiance (L_λ)	n.a.

n.a. = not applicable

Table A7_1_1_1_2_01-3: Specification and amount of transformation products (expressed as percentage of the applied radioactivity) after 15 day incubation in the light.

CAS-No	Common Name	CAS and/or IUPAC Chemical Name	Amount [%] of parent compound measured after 15 days in	
			Buffer solution	Pond water
	Etofenprox		9.4	32.5
	α-CO	2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate	63.6	37.8
	PENA	2-(4-ethoxyphenyl)-2-methylpropyl alcohol	12.0	14.4
	m-PB-acid	3-phenoxybenzoic acid	5.0	3.8
	M1	unknown	1.7	3.3
	M2	unknown	n.d.	2.9
	M3	unknown	4.3	5.3
	M4	unknown	n.d.	n.d.
	M5	unknown	4.0	n.d.

n.d. = not detected

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** The specification for this batch is given in section 3 (RCC Study No. 751803). **4.3 Controls** The controls showed low recoveries at day 12 and 15; though an extra washing step for the test vessels with ethyl acetate was established, showing reasonable recoveries in the buffer and pond water samples. The justification for these low recoveries due to adsorption to glass is therefore satisfactory. Also no radioactivity was detected in the volatile traps.
Conclusion	**5.1 Materials and methods** Test guidelines: Directives 95/36/EEC and 94/37/EEC cannot be considered as test guidelines. The study design is a mixture of the quoted references. Mass balance: The test concentration was below the water solubility limit. After establishing an extra washing step of the test vessels recoveries for the buffer and the pond water could often be raised by more than 50% coming to an overall range from 75 to 108% AR, which is also quite low compared with the recommendations of the quoted guidelines (mass balance for radioactive material should be between 90 and 110 % AR). **5.2.1.und 5.2.2, rate constants** Typing error, see Section 4.4.3 and 4.4.7 for proper values. **5.3 Conclusion** A categorisation of the degradation results like "rapidly/fairly/… photodegradable" is not accepted.
Reliability	1
Acceptability	Acceptable
Remarks	**2.3 Deviations** The content of acetonitrile was raised to 1.8% in the test vessels and was above the recommended 1%. Justification (high adsorption to glass walls) is acceptable.
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Thus, J.L.G., van der Laan-Straathof, J.M.Th. (1992): Determination of the biodegradability of etofenprox in a closed bottle test. Solvay Duphar B.V., Environmental research department, Noordereinde 56, 1243 JJ's-Graveland, The Netherlands; unpublished report no. C.DNL.62.002 (February 28, 1992) Dates of experimental phase: no information in the report.	x
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Ltd.	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 301 D EEC Method C6	
2.2	**GLP**	Yes	
2.3	**Deviations**	Minor deviations: - Ammonium chloride was omitted from the medium to prevent nitrification. - Test duration: 56 days instead of 28 days (but measurements also after 28 days)	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	90S01	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	Low volatility and low water solubility	
3.1.5	Radiolabelled compound	No	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	No	
3.2	**Reference substance**	Sodium acetate	
3.2.1	Initial concentration of reference substance	6.7 mg/litre	

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Secondary (biological) activated sludge. See table 3.3.1.	
3.3.2	Test system	See table 3.3.2	
3.3.3	Test conditions	The test was performed in 42 BOD-bottles (280 ml volume each). These bottles were filled as follows: A: 7 bottles with mineral salt solution B: 7 bottles with mineral salt solution and inoculum C: 7 bottles with mineral salt solution, inoculum and sodium acetate D: 7 bottles with mineral salt solution, inoculum and silica gel E: 7 bottles with mineral salt solution, inoculum and sodium acetate and etofenprox coated on silica gel F: 7 bottles with mineral salt solution, inoculum and etofenprox coated on silica gel *The silical gel was used as a carrier since etofenprox is nearly insoluble in water (details see point 3.3.4).* Although no additional oxygen consumption was expected from the silica gel, controls with silica gel treated with dichloromethane only were included in the test. This technique was described by Nyholm and Seiero ("Biodegradability testing of poorly soluble compounds by means of manometric respirometry", Chemosphere; 21 (12): 1477-1487; 1990) The bottles were completely filled and closed with glass stoppers. They were then incubated in the dark at 20 ± 1°C.	x
3.3.4	Method of preparation of test solution	Sodium acetate: a stock solution of 1.0 g/litre water was prepared. 1.87 ml of this solution was added to the BOD-bottles, giving an initial concentration of 6.7 mg sodium acetate/litre. Etofenprox: the test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silica gel to guarantee a reproducible availability of the compound to the microorganisms. The test substance was first dissolved in dichloromethane (1g/litre). Of this solution, 0.56 ml was added to 2g silica gel (100-200 mesh) weighed in a glass petri dish. The solvent was allowed to evaporate by placing the Petri dish in a ventilated hood for 3 hours and the entire contents were then transferred to the BOD bottle. a stock solution of 1.0 g/litre in dichloromethane was prepared. 0.56 ml of this solution was added to 2 g silica gel and the 2 g silica were added to the BOD-bottles, giving an initial concentration of 2.0 mg etofenprox/litre.	
3.3.5	Initial TS concentration	2.0 mg/litre.	
3.3.6	Duration of test	56 days	
3.3.7	Analytical parameter	Oxygen consumption by the micro-organisms	
3.3.8	Sampling	At time zero (immediately after filling the bottles) the oxygen content and the pH were measured in each of the test condition bottles. The oxygen content was measured after 5, 15, 28 and 56 days in two bottles of every test condition (A to F). The pH was measured at time 0 and after 28 days.	

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Annex Point IIA-VII.7.6.1.1

3.3.9	Intermediates/ degradation products	Not assessed.
3.3.10	Nitrate/nitrite measurement	Not performed.
3.3.11	Controls	Yes, see 3.3.3. Bottles A: control BOD-bottles for the endogenous respiration (comparison to bottles B) Bottles B: control BOD-bottles for the oxygen depletion due to silica gel (comparison to bottles D) and for the biodegradation of the sodium acetate (comparison to bottles C) Bottles C: control BOD-bottles for the toxicity of etofenprox (comparison to bottles E) Bottles D: control BOD-bottles for the biodegradation of etofenprox (comparison to bottles F).
3.3.12	Statistics	Not performed

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Graph	Not available
4.1.2	Degradation	17 % within 28 days for TS
4.1.3	Other observations	
4.1.4	Degradation of TS in abiotic control	Not performed
4.1.5	Degradation of reference substance	72 % within 28 days for sodium acetate
4.1.6	Intermediates/ degradation products	Not assessed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Ready biodegradability of etofenprox has been assessed with the closed bottle test in which the biodegradability of organic compounds in an aerobic environment is determined in a closed bottle. The test compound which provides the sole source of carbon and energy was added to an aqueous mineral salt solution and exposed to relatively low numbers of unadapted micro-organisms for a period of 56 days. To follow the course of biodegradation, the oxygen consumption by the micro-organisms was determined. Sodium acetate was used as a positive control. The biodegradation was calculated as the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand (ThOD). The ThOD are estimated to be 2.59 g O_2/g etofenprox and 0.78 g O_2/g sodium acetate.
5.2	**Results and discussion**	Results are given in tables 5.2a and 5.2b. After 56 days of incubation there is no significant difference between the oxygen content of the bottle with the silica gel with and without etofenprox. Sodium acetate was biodegraded also in the presence of etofenprox. It is

Section A7.1.1.2.1/02 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

		thus concluded that etofenprox is not toxic to the microorganisms. From the results it can be concluded that the inoculum activity is good since 55% of the sodium acetate was degraded within 5 days and 72% within 28 days. This is the maximum degradation that could reasonably be expected for sodium acetate. Unexpectedly, some oxygen depletion due to the silica gel was measured (bottles B-D), but this is of the same order of magnitude as the endogenous respiration. This phenomenon does not influence the results of the tests.	
5.3	**Conclusion**	The active ingredient etofenprox does not meet the "ready biodegradability" criteria (measured BOD within 28 days at least 60% of TOD) as defined in the guidelines.	
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	

Table 3.3.1: Inoculum / Test organism.

Criteria	Details
Nature	secondary (biological) activated sludge
Species	not specified
Strain	not specified
Source	activated sludge plant treating predominantly domestic waste water
Sampling site	RWZI Horstermeer in Nederhorst den Berg, The Netherlands, on August 29, 1991
Laboratory culture	no
Method of cultivation	not applicable
Preparation of inoculum for exposure	not applicable
Pretreatment	The sludge was preconditioned to reduce the endogenous respiration rate. This was done by aerating the sludge (200 mg dry weight (DW)/litre) for one week.
Initial cell concentration	The sludge was diluted to a concentration in the BOD bottles of 2 mg (dry weight)/litre.

Table 3.3.2: Test system.

Criteria	Details
Culturing apparatus	280 ml capacity BOD (biochemical oxygen demand)-bottles (Wertheim, cat. no. 270202) The bottles were completely filled and closed with glass stoppers.
Number of culture flasks/concentration	7
Aeration device	no data
Measuring equipment	The oxygen meter used was a WTW Oxi 96 with WTW EO 96 electrode with an internal magnetic stirrer. The pH meter used was a Philips PW 9420.
Test performed in closed vessels due to significant volatility of TS	no

Table 3.3.3: Test conditions.

Criteria	Details
Composition of medium	1 ml of solutions A, B, C and D were added to 1000 ml distilled water. Solution A contains per 1000 ml distilled water: KH_2PO_4 8.5g K_2HPO_4 21.75g $Na_2HPO_4.2H_2O$ 33.3g The pH was checked and needs to be 7.2 Solution B contains 22.5 g $MgSO_4.7H_2O$ per 1000 ml distilled water. Solution C contains 27.5 g $CaCl_2$ per 1000 ml distilled water. Solution D contains 0.14 g $FeCl_3$ per 1000 ml distilled water.
Additional substrate	The test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silica gel to guarantee a reproducible availability of the compound to the microorganisms.
Test temperature	20 ± 1.5 °C
pH	6.9-7.0
Aeration of dilution water	Not specified
Suspended solids concentration	No assessed
Other relevant criteria	No

Table 5.2 a: Oxygen depletion (mean values of duplicate bottles, calculated from measured values).

O_2 depletion (mg O_2/l)					
Time (days)	Endogenous (A-B)	Silica gel (B-D)	Sodium acetate (B-C)	Sodium acetate + etofenprox (D-E)	Etofenprox (D-F)
5	0.25	0.60	2.90	3.45	0.75
15	0.45	1.20	3.05	4.15	0.85
28	0.50	1.65	3.75	3.90	0.90
56	0.45	2.60	3.75	2.90	-0.20

Table 5.2 b: Biodegradation.

	Biodegradation (%)			
	5 days	15 days	28 days	56 days
Sodium acetate	55	58	72	72
Etofenprox	14	16	17	-4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2006
Materials and methods	**1.1 Reference:** The correct report number is C.DNL.62.001 **2.3 Deviations:** In addition to the deviations mentioned the following deviations were applied/ocurred: - Instead of an effluent/extract/mixture, activated sludge was used as an inoculum. The inoculum was taken from an actibated sludge plant. - The test compound is practically insoluble in water. For that reason etofenprox was tested in the presence of silicagel to guarantee a reproducible availability of the compound to the microorganisms. - In the protocol it was stated that the bottles were to be placed in a climate room at 20 ± 1°C. In this experiment, howeber, during short times (one day about five hours) the temperature was 20 ± 1.5°C. **3.3.3 Test conditions:** According to OECD guideline 301D at least 10 bottles per run should have been applied instead of only 7.
Conclusion	Agree with the applicant's version.
Reliability	2
Acceptability	Acceptable
Remarks	The test result is consistent with the outcome of a second test on biodegradability (modified sturm test, 32% degradation within 28 days, non-key study).
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Thus, J.L.G., van der Laan-Straathof, J.M.Th. and Keetelaar-Jansen, W.A.J. (1993): Biodegradation of 14C-Etofenprox in an adapted modified Sturm test. Solvay Duphar B.V.,'s-Graveland, The Netherlands; unpublished report no. C.DNL.62.002 (September 10, 1993) Experimental phase :May 28, 1993 – July 20, 1993	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS before 14 May 2000 on existing a.s for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 301 B (1982), EEC Directive 79/831, Annex V, Part C (see table A7_1_1_2_1-1)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 Etofenprox	x
3.1.1	Lot/Batch number	90S01	
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	> 99 %	
3.1.4	Further relevant properties	Water solubility 22.5 µg/L	
3.1.5	Radiolabelled compound	α-^{14}C-MTI-500 (Trebon^{R}) Specific activity: 1.01 MBq/mg Radiochemical purit:y 96.5 %	x
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	Liquid scintillation counting and HPLC	
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test ing procedure**		

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

3.3.1	Inoculum / test species	Not adapted activated sludge (see table A7_1_1_2_1-2)	
3.3.2	Test system	The test was performed in three 2-L glass bottles closed with a plastic screw cap from where a 15 mL plastic tube with holes was suspended. A vial with 5 mL 1 M KOH was placed in this tube to absorb the CO_2. (see table A7_1_1_2_1-3)	
3.3.3	Test conditions	In each bottle, 100 µL of [^{14}C]-etofenprox solution was added to 0.7 L mineral salt medium and 8.8 mL inoculum and exposed under aerobic conditions for a period of 28 days. In addition 100 µL of this solution was added in triplicate to 100 mL of a mixture of acetonitrile/water 1:1 v/v (standard solutions). The flasks were made up to 1000 g with mineral salt medium and incubated on an orbital shaking machine (about 50 rpm) in a room at 20±1°C in the dark for 28 (see table A7_1_1_2_1-4)	
3.3.4	Method of preparation of test solution	A stock solution containing 0.10 mg 14C-etofenprox in acetone was prepared. 0.10 mL of this solution was added to the incubation bottles. 1 mg unlabelled etofenprox / mL was diluted 1:10 with acetonitrile / water 1:1 (v/v). this solution was used as HLPC reference.	
3.3.5	Initial TS concentration	0.0108 mg TS/l	x
3.3.6	Duration of test	29 days	
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	The $^{14}CO_2$ absorption vials were replaced by fresh ones after 7, 14, 21 and 28 days. After 28 days of incubation 5 mL of 0.5 M HCl was added to the test flasks for removing CO_2 or carbonate dissolved in the medium and a fresh CO_2 absorption vial was mounted. On day 29 of the study, the medium, the remaining medium and sludge was filtered over a nylon membrane filter (pore size 0.45 µm). The sludge and the filter were extracted twice with 20 mL acetonitrile by intensive mixing on a tube mixer and ultrasonic bath for 10 minutes. The sludge was separated from the extract by centrifugation for 10 minutes at 3000 g. After this extraction, the filter was removed. The two extracts of each sludge sample were pooled and made up to 45 mL.	
3.3.9	Intermediates/ degradation products	Quantified by HPLC but not identified	
3.3.10	Nitrate/nitrite measurement	Not applicable	
3.3.11	Controls	The sample treatment and analysis method was validated by spiking 50 mL aqueous medium in triplicate with 10 µL of the 14C-etofenprox stock solution. The recovery after freeze drying was 109 % and after HPLC/LSC analyis 108% of nominal.	
3.3.12	Statistics	ng TS = (dpm*Vtot)/(Vinj*sa) with TS is testsubstance 14C dpm is decays per minute (radioactivity unit) Vtot is total volume of sample in mL Vinj is injection volume (0.201mL) s.a. is specific activity to convert dpm to µg (60.06 dpm/ng)	

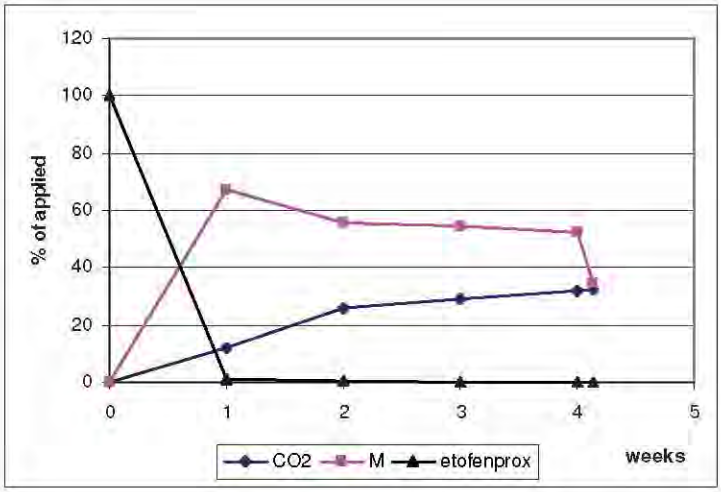
Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

4 RESULTS

4.1 Degradation of test substance

4.1.1 Graph



weeks	CO2	M	etofenprox
0	0	0	100
1	11.9	67.1	1.2
2	25.7	55.7	0.6
3	29.2	54.5	0
4	32	52.2	0
4.14	32.3	34.7	0

M = polar metabolite

4.1.2 Degradation: 99 % degradation at plateau

4.1.3 Other observations: polar intermediates are formed that degrade further to CO_2.

4.1.4 Degradation of TS in abiotic control: Not reported in this study

4.1.5 Degradation of reference substance: Not applicable

4.1.6 Intermediates/ degradation products: See 4.1.1

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The ready biodegradability of [α-^{14}C-benzyl]-etofenprox (radiochemical purity: 96.5%) was determined under aerobic conditions by measuring the $^{14}CO_2$ evolution as well as the loss of parent compound. The inoculum (activated sludge) was obtained from an activated sludge plant treating predominantly domestic waste water. The concentration of the inoculum was 30 mg dry weight/L.

The test was performed in three 2-L glass bottles closed with a plastic screw cap from where a 15 mL plastic tube with holes was suspended. A

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

		vial with 5 mL 1 M KOH was placed in this tube to absorb the CO_2. In each bottle, 100 µL of [^{14}C]-etofenprox solution was added to 0.7 L mineral salt medium and 8.8 mL inoculum and exposed under aerobic conditions for a period of 28 days. In addition 100 µL of this solution was added in triplicate to 100 mL of a mixture of acetonitrile/water 1:1 v/v (standard solutions). The flasks were made up to 1000 g with mineral salt medium and incubated on an orbital shaking machine (about 50 rpm) in a room at 20±1°C in the dark for 28 days. The $^{14}CO_2$ absorption vials were replaced by fresh ones after 7, 14, 21 and 28 days. After 28 days of incubation 5 mL of 0.5 M HCl was added to the test flasks for removing CO_2 or carbonate dissolved in the medium and a fresh CO_2 absorption vial was mounted. On day 29 of the study, the medium, the remaining medium and sludge was filtered over a nylon membrane filter (pore size 0.45 µm). The sludge and the filter were extracted twice with 20 mL acetonitrile by intensive mixing on a tube mixer and ultrasonic bath for 10 minutes. The sludge was separated from the extract by centrifugation for 10 minutes at 3000 g. After this extraction, the filter was removed because it looked clean. The two extracts of each sludge sample were pooled and made up to 45 mL. The pH of the medium was measured at the start and after 28 days of incubation. After 24 hours of incubation, the amount of $^{14}CO_2$ absorbed in the KOH solutions was determined by LSC. At each sampling time 50 mL of the medium of each bottle was taken with a pipette and stored frozen in a sample bottle until analysis. The amount of radioactivity in the medium extracts and in the standard solution was determined by LSC by taking 1 mL aliquots in triplicate. The amount of radioactivity in the extracted sludge samples was determined by combustion analysis. The amount of parent compound, [^{14}C]-etofenprox, and metabolites in the standard solutions, medium extracts and sludge extracts was determined by HPLC/LSC analysis.	
5.2	**Results and discussion**	The amount of [^{14}C]-etofenprox added to the test bottles was 10.8 µg of which 95.8% consists of etofenprox (10.3 µg). The average pH ranged from 7.5 at the start to 6.6 at the end of the experiment. <u>Recovery</u> After 29 days of incubation, the total amount of radioactivity recovered in the CO_2 trap, the medium, in sludge extract and sludge residue was 88.7% of applied radioactivity. <u>Extractable and non-extractable radioactivity in the sludge</u> The amount of extractable radioactivity was 6.7% of applied in the sludge extract and 15% of applied in the sludge residue after 29 days of incubation. <u>Identification of radioactivity</u> The major metabolites detected were CO_2 (32%) and polar metabolites. <u>Biodegradation</u> After one week of incubation, only 1.2% of the added amount of etofenprox could be detected and decreased to 0.6% or less until Day 29. The DT_{50} was determined to be less than 2 days, assuming a first order degradation. Therefore, [^{14}C]-etofenprox can be classified as "readily biodegradable".	x
5.3	**Conclusion**	Etofenprox can be classified as "readily biodegradable".	x
5.3.1	Reliability	1	x

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA-VII.7.6.1.1

5.3.2 Deficiencies No

Table A7_1_1_2_1-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test.

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	inherent
Modified MITI-Test (II)	-	302C	inherent
Modified SCAS-Test	C.12	302A	inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2_1-2: Inoculum / Test organism.

Criteria	Details
Nature	Secondary (biological activated sludge
Species	not specified
Strain	not specified
Source	Activated sludge plant treating predominantly domestic waste water
Sampling site	RWZI Horstermee in nederhorst den Berg, The Netherlands, on June 2, 1993
Laboratory culture	No
Method of cultivation	not specified
Preparation of inoculum for exposure	No pre-treatment, 3.4 mg/mL dray weight
Pretreatment	no adaptation
Initial cell concentration	30 mg/L based on dry weight

Table A7_1_1_2_1-3: Test system.

Criteria	Details
Culturing apparatus	2-litre glass bottle (Scott Duran. dry sterilised at 175°C, closed with plastic screw cap.
Number of culture flasks/concentration	3
Aeration device	orbital shaking
Measuring equipment	Radioactivity measurement with LSC and HPLC
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2_1-4: Test conditions.

Criteria	Details
Composition of medium	1 mL of solutions A, B, C, D, and E were added to 1 L ultrapure water. A: 0.14 g $FeCl_3$ in 1 L distilled water B: 22.5 g $MgSO_4*7H_2O$ in 1 L distilled water C: 27.5 g $CaCl_2$ per 1 L distilled water D: 8.5 g KH_2PO_4 / L 21.75 g K2HPO_4 / L 22.2 g $Na_2HPO_4*2H_2O$ / L 1.7 g NH_4Cl E: 40 g $(NH_4)2SO_4$ per L distilled water
Additional substrate	No
Test temperature	20 ± 1 °C thermostat
pH	Bottle no. start end 1 7.5 6.7 2 7.6 6.6 3 7.5 6.6
Aeration of dilution water	No
Suspended solids concentration	30 mg dry weight / L
Other relevant criteria	orbital shaking, CO2 trapped by 5 mL 1 M KOH in trap inside the incubation bottle

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**3.1 Test material** Information given under this heading refers to the unlabelled reference substance. **3.1.2 Specification** No detailed specification and reference to section 2 was given in the original test report. **3.1.5 Radiolabelled compound** The test was conducted with [alpha-^{14}C-benzyl]-etofenprox, [2-^{14}C-propyl]-etofenprox was not used, though different metabolites can be formed. **3.3.5 Initial TS concentration** The low test concentration was justified in the test report with the low water solubility of etofenprox.
Conclusion	**5.2 Results and discussion** Recovery The mass balance varied from 79% after 1 week to 84% AR. 20% were lost after 1 week of incubation, which was probably caused by adsorption to the sludge. So after 29 days of incubation, the total amount of radioactivity recovered in the CO_2 trap, the medium, in sludge extract and sludge residue was 89% AR. Still 11% AR were missing, which could be due to the formation of other volatile metabolites. **5.3 Conclusion** Etofenprox can be regarded as not readily biodegradable. The pass levels refers to the ultimate degradation of the test substance (60% ThCO_2). The study was conducted with radioactive material. After 4 weeks still 52% AR remained in the medium. This radioactivity was characterised as polar metabolites (etofenprox below detection limit).
Reliability	2
Acceptability	Acceptable
Remarks	The test result is consistent with the outcome of the first test on biodegradability (closed bottle test, 17% degradation within 28 days, key study).
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2.2.2/01 Degradation in Water-Sediment Systems

Annex Point IIIA-XII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	Lewis C.J. (2001): (14C)-MTI-500: Degradation and retention in water-sediment systems. Covance Laboratories Ltd., Harrogate, England; unpublished report no. CLE 719/6-D2142 (January 29, 2001) Experimental phase September 15, 1998 to October 23, 2000 Lewis, C.J. (2001): (14C)-MTI-500: Degradation and retention in water-sediment systems. Amended Final Report 1. Covance Laboratories Ltd., Harrogate, England; unpublished report no. CLE 719/6-D2142 (July 22, 2002) This amendment gives an explanation of the consequences of a mis-labelled reference standard supplied as 4'-OH. The degradation product named Unknown 1 in this study, should have been referred as 4'-OH	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemicals Agro, Inc.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes SETAC (1995) and EC Directive 95/36/EC (1995)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	MTI-500, Etofenprox	x
3.1.1	Lot/Batch number	9604	
3.1.2	Specification	As given in section 2	x
3.1.3	Purity	99.99 %	
3.1.4	Further relevant properties	Solubility in water: 22.5 µg/L	
3.1.5	Radiolabelling	A) [α-^{14}C-benzyl]-etofenprox - Batch: MRH/MTC 277/29 - Specific activity: 366.67 MBq/mmol - Radiochemical purity: >99% (from certificate of analysis) (B) [2-^{14}C-propyl]-etofenprox - Batch: MRH/MTC 276/37 - Specific activity: 576.09 MBq/mmol) - Radiochemical purity: >99% (from CoA)	
3.1.6	TS inhibitory to microorganisms	No	
3.2	**Reference substance**	No	
3.3	**Testing procedure**		

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3.3.1	Sediment	Water and sediment from the Mill stream pond (Dorset, UK) and the Emperor Lake (Derbyshire, UK) were sampled on 7 and 1 July 1998, respectively. The water and sediment samples were passed through a 0.2 and 2 mm sieve, respectively, and dispensed into the incubation vessels on the day (Emperor Lake), or the day after (Mill stream pond). The water and sediment characteristics are listed in Table A7_1_2_2_2_01-1)	x
3.3.2	Test system	Details on laboratory equipment etc. in tabular form (see table A7_1_2_2_2_01-2) Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (dry weight of 16.5 g for Mill stream pond and 24.8 g for Emperor lake) covered with water to a depth of 6 cm (weight of 97.1 and 99.4 g for Mill stream pond and Emperor lake, respectively). Prior to application, the water-sediment units were pre-incubated for 67 days (Mill stream pond, group A) or 74 days (Emperor Lake, group B) in the dark at 20±2°C until equilibration. Moistened CO_2-free air was drawn over the water surface. An additional incubation group consisted of the Emperor lake water-sediment system (group C) acclimatised under a 12 h fluorescent lighting/12 h dark regime.	x
3.3.3	Test conditions	Relevant test conditions in tabular form (see table A7_1_2_2_2_01-3)	
3.3.4	Method of preparation of test solution	Equal amounts (873 kPq) of each radiolabelled test item were pooled, the solvent removed and the residue reconstituted in acetonitrile to produce a 0.35 mg/mL solution. 3.6.1.3 non-labelled test item ware dissolved in 10 ml acetonitrile (0.26 mg/ml).	
3.3.5	Application of test item	After pre-incubation, [^{14}C]-etofenprox was applied at a rate of 32.6 µg/unit, equivalent to a field rate of approximately 200 g a.s./ha and a surface area of the vessels of 15.9 cm^2. The test substance, in acetonitrile (92 µL) was dispensed drop-wise, onto the surface of the water of eight water-sediment units per incubation group.	
3.3.6	Duration of test	Pre-incubation: 67 days Mill Stream (A), 74 days Emperor lake (B) Post application: 99 days	
3.3.7	Temperature / light	20 ± 2 °C / 12 hours light/dark cycles for group C	
3.3.8	Sampling	Single incubation units from each group were removed for analysis at intervals of 0 (immediately after application), 7, 14, 30, 59 and 99 days after application. At each sampling date, the surface water was carefully removed from the sediment and partitioned twice with dichloromethane. The sediment was extracted 3 times with methanol and then with methanol/HCl (95:5, v/v). Sediments for fractionation into fulvic acid, humic acid and humin were extracted with NaOH, and the radioactivity in the humin fraction was determined by combustion followed by LSC. Radioactivity in the surface water's dichloromethane extracts and in the sediment's methanol and methanol/HCl extracts, containing >5% of applied radioactivity was analysed by HPLC and TLC. The trap reagents were collected when the units to which they were attached were removed, and, for units incubated for longer than a month, additionally at 30 and 59 days after the application. Radioactivity in the trapping solutions was quantified by LSC. Finally, the radioactivity associated with the apparatus used in the study was determined by LSC.	
3.3.9	Intermediates/ degradation products	Identified using HPLC, LC-MS or TLC (see below). The radioactivity, containing >5% of applied radioactivity, was analysed	

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		by HPLC for identification of the degradation products.
3.3.10	Analytical methods	HPLC with radioactivity detection, TLC; LC-MS; Liquid scintillation counting. Details see table A7_1_2_2_2_01-4
3.3.11	Statistics	The DT_{50} and DT_{90} values of etofenprox in the two natural water/sediment systems were calculated assuming first order kinetics.
		4 RESULTS.
4.1	**Recovery**	Overall recovery decreased from 96, 98 and 97% of applied radioactivity, initially, to 84, 83 and 93% after 99 days, in incubation groups A, B and C, respectively. Recovery of radioactivity decreased to and remained below 90% in the Mill stream pond system (dark) at 14 days and in the Emperor Lake system (dark) at 99 days. Recovery of radioactivity from the Emperor Lake system, under light/dark cycle, was higher than 90% at all times.
4.2	**Degradation of test substance**	
4.2.1	Mineralisation	After 99 days of incubation, 28, 18 and 19% of the applied radioactivity present in the NaOH traps was shown to be $^{14}CO_2$ in the Mill stream pond, the Emperor Lake incubated in the dark and in the Emperor Lake incubated under a light/dark cycle, respectively. No other volatile products could be detected (≤0.1% of applied radioactivity) in the ethanediol and 2% paraffin in xylene traps.
4.2.2	Test item	The level etofenprox in the surface water amounted initially from 22 to 32% of the applied radioactivity in the three incubated groups. Not more than 1% was detected after 14 days, 30 or 59 days after application in groups A, C and B, respectively. The initial decrease was more rapid in the Mill stream pond system than in the Emperor Lake system and more rapid under a light/dark cycle than in the dark. Levels of etofenprox in the sediment decreased from between 62 and 70% of applied radioactivity, initially, to between 8 and 25% at 99 days, in all groups.
4.2.3	Metabolites	Only one degradate, later identified as 4'-OH (see Amendment no. 1), exceeded 10% of applied radioactivity. It was mainly present in the sediments and reached the maximum levels of 14.4, 16.2 and 21.9% of applied radioactivity, in the whole system, in incubation groups B (day 14), C (day 7) and A (day 7), respectively, and then decreased to ≤10% of applied radioactivity at 30 days. A large number of minor degradation products were present in the water-sediment systems, each accounting for less than 10% of applied radioactivity (structure not identified). Three metabolites were identified as DP, PB-acid (also known as m-PB-acid) and P-acid (also known as EPMP) and reached maximum values of 7.0% (day 30), 2.4% (day 14) and 5.4% (day 30) of the applied radioactivity, respectively, in water and sediment phases of the Emperor Lake system under a light/dark cycle. However, the identity of α-CO (maximum 0.8%) and DE + 4'-OH (maximum 2.5%) was not confirmed by TLC although the metabolic pathway based on the identified compounds proposes these metabolites. Their low accumulation indicates that they are quickly degraded into identified or other unidentified compounds in the water sediment systems. The results are described in more detail in tableA7_1_2_2_2_01-5
4.2.4	Degradation of	Based on the results of the water/sediment study, the degradation rates x

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of etofenprox in the water phases and entire systems were calculated assuming first order kinetic. The DT_{50}- and DT_{90}-values were calculated for the 3 incubation systems. There was a more rapid dissipation of etofenprox under a light/dark cycle (group C) than in the dark (group B).

System	Mill stream pond dark	Emperor Lake dark	Emperor Lake light/dark
Water phase	DT_{50} = 2.1 days DT_{90} = 7.1 days	DT_{50} = 10.4 days DT_{90} = 34.5 days	DT_{50} = 2.1 days DT_{90} = 7.1 days
Entire system	DT_{50} = 6.5 days DT_{90} = 23.8 days	DT_{50} = 20.1 days DT_{90} = 71.0 days	DT_{50} = 7 or 22 days* DT_{90} = 104 or >99 days*

* computed values using the poor fit suggest a DT_{50} of 22 days and DT_{90} of 104 days.

4.2.5 Degradation of 4'-OH metabolite

The degradation rates of the metabolite 4'-OH were calculated only for the total system, because most of the compound was present in the sediment phase

System	Mill stream pond dark	Emperor Lake dark	Emperor Lake light/dark
Entire system	DT_{50} = 29.7days DT_{90} = 97.9 days	DT_{50} = 21.8 days DT_{90} = 59.8 days	DT_{50} = 27.0 days DT_{90} = 87.1 days

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The degradation and metabolism was investigated in two natural water-sediment systems (Mill stream pond and Emperor Lake) using a mixture (1+1) of [2-^{14}C-propyl]-etofenprox (batch no. MRH/MTC276/37; radiochemical purity: >99%) and [α-^{14}C-benzyl]-etofenprox (batch no. MRH/MTC277/29; radiochemical purity: >99%) in the dark over a period of 99 days. In addition the degradation of [^{14}C]-etofenprox was determined in the Emperor Lake water-sediment system under a 12 h dark/12 h light photoperiod.

Water and sediment from the Mill stream pond (Dorset, UK) and the Emperor Lake (Derbyshire, UK) were sampled on 7 and 1 July 1998, respectively. The water and sediment samples were passed through a 0.2 and 2 mm sieve, respectively, and dispensed into the incubation vessels on the day (Emperor Lake), or the day after (Mill stream pond).

Each test vessel (glass cylinders of 4.5 cm diameter) contained a 2.5 cm sediment layer (dry weight of 16.5 g for Mill stream pond and 24.8 g for Emperor lake) covered with water to a depth of 6 cm (weight of 97.1 and 99.4 g for Mill stream pond and Emperor lake, respectively). Prior to application, the water-sediment units were pre-incubated for 67 days (Mill stream pond, group A) or 74 days (Emperor Lake, group B) in the dark at 20±2°C until equilibration. Moistened CO_2-free air was drawn over the water surface. An additional incubation group consisted of the Emperor lake water-sediment system (group C) acclimatised under a 12 h fluorescent lighting/12 h dark regime.

After pre-incubation, [^{14}C]-etofenprox was applied at a rate of 32.6