Section A6.2/06 **Percutaneous absorption (*in vivo* test)**

Annex Point IIA-VI.6.2 **Rat**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines: OECD guideline no. 424 (1997); US-EPA FIFRA, 40 CFR 158, subdivision F, series 85-3; Japan MAFF, 59 NohSan notification no. 4200. No deviations from test guidelines. Description of method: dermal, non-occlusive exposure with radiolabelled test substance, 3 dose groups of 16 male rats each, 96 hours exposure, with interim kills of 4 rats per group after 1, 10 and 24 hours exposure, mass balance to determine direct and indirect absorption.	
5.2	**Results and discussion**	The greatest direct dermal absorption of etofenprox into the systemic circulation amounted to 6.57% of the applied dose and occurred 96 hours after the start of dermal exposure. Indirect absorption, localized in the skin initially, accounted for up to 30.3% of the applied dose. The maximum total dermal absorption (direct + indirect) amounted to 33.0% of the applied dose. The extent of direct dermal absorption was not markedly influenced by the applied dose, but increased with increasing dermal contact time. Indirect absorption into the skin was highest at the highest dose level.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A6_2_06-1. Summary of mass balance and dermal absorption data.

Group ($\mu g/cm^2$)	Necropsy time (hrs)	Total recovery ± SD (% applied dose)	Dermal absorption ± SD (% applied dose)	
			Direct + indirect	Direct
5	1	92.6±2.27	4.59±0.61	<0.005
	10	95.1±2.28	14.1±5.72	0.58±0.25
	24	95.1±23.7	12.3±2.32	1.91±0.12
	96	96.9±3.22	14.5±6.00	5.07±1.15
50	1	87.3±5.65	7.10±4.21	0.03±0.05
	10	105±5.19	16.4±5.02	0.78±0.16
	24	101±6.54	20.3±9.38	2.19±0.31
	96	98.4±4.87	16.0±3.93	6.10±1.84
250	1	103±6.47	8.52±6.30	<0.005
	10	132±8.42	30.0±1.92	0.74±0.20
	24	134±6.06	32.4±6.09	2.14±0.21
	96	120±7.89	33.0±11.3	6.57±0.35
250 (normalized data)	1	-	8.41	<0.005
	10	-	22.8	0.57
	24	-	24.2	1.60
	96	-	27.5	5.51

Table A6_2_06-2. Summary of radioactivity in matrices contributing to the calculation of direct dermal absorption.

Group ($\mu g/cm^2$)	Necropsy time (hrs)	Group mean radioactivity (% applied dose) ± SD in:						Direct[a] absorption
		blood	carcass	cage wash	cage wipe	urine	feces	
5	1	ND	ND	ND	ND	<0.005	ND	<0.005
	10	ND	0.28±0.33	0.04±0.01	ND	0.26±0.07	ND	0.58±0.25
	24	ND	0.84±0.08	0.09±0.07	ND	0.50±0.15	0.49±0.22	1.91±0.12
	96	ND	0.42±0.49	0.13±0.16	ND	1.08±0.17	3.44±0.45	5.07±1.15
50	1	ND	0.02±0.05	ND	ND	<0.005	NS	0.03±0.05
	10	0.02±0.01	0.52±0.09	0.03±0.02	0.03±0.01	0.18±0.08	ND	0.78±0.16
	24	0.02±0.00	1.20±0.18	0.07±0.04	0.07±0.03	0.48±0.25	0.35±0.13	2.19±0.31
	96	ND	0.85±0.32	0.17±0.13	0.16±0.05	1.02±0.25	3.89±1.13	6.10±1.84
250	1	ND	ND	<0.005	ND	<0.005	ND	<0.005
	10	0.02±0.00	0.50±0.11	0.05±0.03	0.03±0.04	0.14±0.11	ND	0.74±0.20
	24	0.02±0.01	1.29±0.22	0.06±0.02	0.05±0.03	0.40±0.17	0.31±0.07	2.14±0.21
	96	<0.005	0.87±0.21	0.12±0.06	0.16±0.05	1.10±0.24	4.32±0.36	6.57±0.35

[a] total direct absorption (% applied dose) = sum of blood, carcass, cage wash, cage wipe, urine and feces; ND not detectable; NS no sample

Table A6_2_06-3. Summary of radioactivity in application site skin and in other matrices contributing to mass balance.

Group ($\mu g/cm^2$)	Necropsy time (hrs)	Group mean radioactivity (% applied dose) ± SD in:				Indirect[b] absorption
		filter papers	enclosure	skin wash	skin[a]	
5	1	0.08±0.17	0.32±0.03	87.6±1.77	4.59±0.61	4.59±0.61
	10	0.11±0.08	0.28±0.10	80.6±7.29	13.5±5.77	13.5±5.77
	24	0.11±0.08	0.47±0.14	82.2±23.8	10.4±2.40	10.4±2.40
	96	0.23±0.22	0.68±0.29	81.5±6.69	9.44±5.57	9.44±5.57
50	1	0.03±0.01	0.23±0.11	79.9±9.44	7.07±4.15	7.07±4.15
	10	0.05±0.01	0.52±0.32	88.3±9.79	15.6±5.03	15.6±5.03
	24	0.19±0.14	0.81±0.39	79.6±10.5	18.1±9.32	18.1±9.32
	96	0.14±0.05	0.86±0.22	81.6±6.89	9.85±2.82	9.85±2.82
250	1	0.48±0.61	0.32±0.10	94.1±10.6	8.52±6.29	8.52±6.29
	10	0.30±0.42	0.55±0.13	101±6.8	29.2±1.88	29.2±1.88
	24	0.20±0.13	1.11±0.62	100±4.51	30.3±5.96	30.3±5.96
	96	2.19±3.79	0.87±0.38	83.8±10.9	26.4±11.4	26.4±11.4

[a] application site skin after washing; [b] total indirect absorption (% applied dose) = amount remaining in skin at application site after skin washing; ND not detectable; NS no sample

Table A6_2_06-4. Summary of the time course of urinary and fecal elimination of radioactivity - animals killed at 96 hrs.

Group ($\mu g/cm^2$)	**Collection interval (hours post-application)**	**Group mean radioactivity (% applied dose) ± SD in:**	
		urine	**feces**
5	0 - 24	0.45 ± 0.08	0.50 ± 0.15
	24 - 48	0.30 ± 0.05	1.23 ± 0.11
	48 - 72	0.19 ± 0.06	0.98 ± 0.17
	72 - 96	0.13 ± 0.06	0.73 ± 0.26
50	0 - 24	0.38 ±0.10	0.30 ± 0.12
	24 - 48	0.27 ±0.07	1.46 ± 0.37
	48 - 72	0.22 ± 0.09	1.16 ± 0.45
	72 - 96	0.15 ± 0.07	0.97 ± 0.35
250	0 - 24	0.35 ± 0.06	0.39 ± 0.20
	24 - 48	0.32 ± 0.10	1.43 ± 0.21
	48 - 72	0.24 ± 0.07	1.25 ± 0.15
	72 - 96	0.19 ± 0.06	1.24 ± 0.12

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** typing error: not OECD guideline 424 but 427 was meant (confirmed by letter at 2005/06/30) **3.1.3. Description** Not specified: The exact specification is not considered as absolutely indispensable for dermal uptake studies. **3.3.6. Exposure period** The substance was applied at maximum for 10h then washed off. Animals were sacrificed and analysed at 1, 10, 24 and 96h.

Conclusion	**5.2. Results and Discussion**: After discussion of the data with the applicant the following interpretation of the data for use within the exposure assessment was provided by the applicant and agreed to by the CA: The time course of urinary and faecal elimination of etofenprox in the animals killed at 96 hours demonstrates reducing overall excretion after 48 hours. Therefore, in line with the guidance on dermal absorption provided by the European Commission document Sanco/222/2000 rev. 6 (November 27, 2002), it was considered prudent to include a proportion of the indirect absorption in the direct dermal absorption value. Direct dermal absorption is not markedly influenced by applied doses in the range 5 to 250 µg/cm^2. Total direct dermal absorption at 96 hours is 5.07, 6.10 and 6.57% applied dose for doses of 5, 50 and 250 µg/cm^2, respectively (table A6.2.06.2). On the other hand, overall indirect dermal absorption at 96 hours does increase with applied doses from 50 to 250 µg/cm^2, from ~9.65% applied dose (mean of 5 and 50 µg/cm^2) to 26.4% applied dose at 250 µg/cm^2 (table A6.2.06.3.). Nevertheless, the high dose of 250 µg/cm^2 used in this study was intended to be in excess of any in-use human exposure levels. Therefore, it is considered appropriate to calculate a proportion of indirect dermal absorption to add to the direct absorption. This conclusion was based on two observations: Reduced urinary and faecal elimination of etofenprox at the last collection period of 72 to 96 hours (table A6.2.06.4). The static levels of etofenprox in the skin (i.e. indirect absorption) from 10 hours to 96 hours, suggesting very limited mobilisation into the general circulation. At 250 µg/cm^2, the levels in the skin remained approximately constant at 10, 24 and 96 hours (29.2, 30.3 and 26.4% applied dose, respectively; table A6.2.06.3.). When these data are normalised to account for recoveries of 103 - 134%, the skin retention values are 22.2, 22.6 and 22.0% applied dose, respectively. We concur that the highest concentration of 250 µg/cm^2 is the most applicable for human risk assessment based on conservative risk assessment principles, and a value for the relevant proportion of indirect absorption can be obtained by consideration of the disappearance from skin from 10 to 96 hours. Since there was virtually no disappearance from skin during this time in the 250 µg/cm^2 group, the calculated disappearance in the 50 µg/cm^2 group (15.6% at 10 hours to 9.85% at 96 hours) can be used and applied to the 250 µg/cm^2 group to give a "worst case" value. This provides a value of 36.9% disappearance (from 10 - 96 hours at 50 µg/cm^2) of skin localised etofenprox. Applying this to the higher indirect absorption value (22.6% - normalised value) of the 250 µg/cm^2 group gives a proportion of 8.33% of applied dose to be added to the (normalized) direct absorption of 5.51%, which amounts to <u>13,8% of total dermal absorption</u>. This low uptake rate is furthermore supported by the following considerations: In vivo and in vitro experiments have demonstrated that in general there is an inverse relation between concentration (area dose) and percentage of absorption (see "Guidance document for dermal absorption", rev. 6. 2002). Therefore the worst case scenario is normally the most diluted solution and not automatically the highest concentration. Furthermore the secondary exposure rates are in the range of the lowest dose in this study (5µg/cm^2). Now the total uptake rate at the lowest dose is with 14,5% nearly identical to the proposed 13,8%. Furthermore the limited dermal uptake and mobilisation into the blood stream seems plausible for us, since the log Po/w is with 6.9 far above 4, which means that it is reasonable to assume that the a.s. is somewhat trapped in the epidermis (see also Appendix IV of TGD).
Reliability	1

Acceptability	Acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3.2 Repeated dose toxicity

Annex Point IIA-VI.6.3 **Dermal**
Rabbit, 28-day

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2000): A 28-day repeated dose dermal toxicity study in rabbits with technical MTI-500; [redacted] VA 20172-0474, USA; unpublished report no. 011077-1 (June 28, 2000). Dates of work: October 04, 1999 - December 22, 1999	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
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 92/69/EEC, method B.9; OECD guideline no. 410 (1981); US-EPA OPPTS 870.3200	
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. 21049	
3.1.2	Specification	Deviating from specification given in section 2 as follows	X
3.1.2.1	Description	Clear to straw colour liquid	
3.1.2.2	Purity	99.18%	
3.1.2.3	Stability	No information in the report	
3.2	**Test Animals**		
3.2.1	Species	Rabbits	
3.2.2	Strain	New Zealand White	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	12 - 13 week old / weight range 2.44 - 3.08kg	
3.2.6	Number of animals per group	10 males and 10 females per group	

Section A6.3.2	**Repeated dose toxicity**
Annex Point IIA-VI.6.3	**Dermal** **Rabbit, 28-day**

3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	**Dermal**
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	7 days per week
3.3.3	Postexposure period	2 additional groups (water control and highest dose) were kept for 14 days after the end of exposure.
3.3.4	**Dermal**	
3.3.4.1	Area covered	About 10 % of body surface
3.3.4.2	Occlusion	Semiocclusive
3.3.4.3	Vehicle	None (the test substance was applied as a liquid, unformulated, but maintained at approximately 38°C before application)
3.3.4.4	Dose applied	0 (water only), 400, 650 and 1000mg/kg bw/day.
3.3.4.5	Total volume applied	Depends on body weight of each animal
3.3.4.6	Duration of exposure	6 h/day
3.3.4.7	Removal of test substance	Dilute soap solution followed by water
3.3.4.8	Controls	Water only
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes Recorded daily after exposure.
3.4.1.2	Mortality	Yes Checked twice daily
3.4.2	Body weight	Yes. Recorded pre-dose, weekly throughout the treatment and recovery periods, and at necropsy.
3.4.3	Food consumption	Yes Generally recorded for 6 days/wee during the treatment and recovery periods.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes All animals were given an ophthalmoscopic examination pre-dose and towards the end of the treatment and recovery periods.

Section A6.3.2	**Repeated dose toxicity**
Annex Point IIA-VI.6.3	**Dermal** **Rabbit, 28-day**

3.4.6	Haematology	Yes Haematology analysis was performed on blood samples withdrawn from food-deprived animals at scheduled necropsies. number of animals: all animals Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, prothrombin time, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration.
3.4.7	Clinical Chemisty	Yes Clinical chemistry determination were performed on blood samples withdrawn from food-deprived animals at scheduled necropsies. number of animals: all animals Parameters: blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total protein, albumin, glucose, sodium, potassium, calcium, chloride, inorganic phosphorus, creatine kinase, total cholesterol, gamma-glutamyltransferase, globulin, albumin/globulin ratio.
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenal glands, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart, pituitary, thyroid.
3.5.2	Gross and histopathology	Yes All animals from the control and the highest dose groups (0 and 1000 mg/kg bw/day). The tissues from all animals treated at 0 or 1000mg/kg bw/day and killed at the end of treatment or at the end of the recovery period, and application site skin and gross lesions from all study animals, were processed and examined by light microscopy. organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, uterus, female mammary gland, prostate, urinary bladder, gall bladder, lymph nodes, skeletal muscle with peripheral nerve, bone marrow, normal and treated skin, eyes, entire bone femur, nose, testes, epididymides, seminal vesicles, ovaries, cervix, vagina
3.5.3	Other examinations	Not applicable
3.5.4	Statistics	Where appropriate, data were analysed statistically using Bartlett's test followed by Dunn's summed-rank test or unpaired t-test with Welch's correction for non-parametric data. Parametric data were subsequently analysed using Dunnett's test.
3.6	**Further remarks**	

Section A6.3.2 **Repeated dose toxicity**
Annex Point IIA-VI.6.3 **Dermal**
Rabbit, 28-day

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	The incidence of mild erythema of the application site skin in the treated groups was slightly higher than in the controls throughout the study. Occasionally, moderate erythema occurred in the treated groups, but the mean total erythema scores (Table A6_3_2-1) were not generally dose-related. Scabbing, desquamation or exfoliation, edema, fissuring and thickening of the application sites also tended to be slightly more prevalent in the treated groups during the last 3 weeks of treatment.
4.1.2	Mortality	There were no deaths or treatment-related clinical signs at any dose level.
4.2	**Body weight gain**	No effects.
4.3	**Food consumption and compound intake**	No effects.
4.4	**Ophtalmoscopic examination**	There were no treatment-related ocular findings at any dose level.
4.5	**Blood analysis**	
4.5.1	Haematology and clinical chemistry	There were no treatment-related effects on the haematological and clinical chemistry profiles in week 4 at any dose level. Although the group mean prothrombin time of males at 1000mg/kg bw/day (6.1sec) was marginally, but significantly ($p < 0.05$), shorter than the controls (6.5sec), the minimal difference is considered not to be an adverse effect. The proportion of lymphocytes in the 1000mg/kg bw/day male group (57.7%) was also significantly ($p < 0.05$) lower than the control group (68.3%), but in the absence of effects on the total and other white cell populations, the difference is considered not to be toxicologically relevant. There were no other statistically significant differences in week 4 between the etofenprox -treated groups and the controls with respect to haematological and clinical chemistry profiles.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No statistically significant effects on the absolute and relative organ weights at any dose level.

Section A6.3.2 **Repeated dose toxicity**
Annex Point IIA-VI.6.3 **Dermal**
Rabbit, 28-day

4.6.2	Gross and histopathology	There were no treatment-related gross necropsy findings at any dose level. Treatment-related histopathological alterations were confined to the application site skin, in which an increased incidence, severity and/or extent of epidermal hyperplasia, chronic dermal infiltration and dermal heterophil infiltrates occurred in the males at all dose levels (Table A6_03_2-2). Females in all etofenprox -treated groups showed increased incidences of diffuse epidermal hyperplasia. The treatment-related lesions were graded minimal to mild in severity. Recovery group animals at 1000mg/kg bw/day showed evidence of resolution of the skin lesions since all skin lesions were graded as minimal and were less extensive than at 4 weeks (Table A6_03_2-2). However, the incidences of focal epidermal hyperplasia and heterophil infiltration in males and focal chronic inflammation in females remained slightly raised. There were no systemic treatment-related histopathological alterations in animals treated at 1000mg/kg bw/day.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test guidelines: 92/69/EEC, method B.9; OECD guideline no. 410 (1981); US-EPA OPPTS 870.3200. No deviations from test guidelines. Description of method: rabbit, dermal exposure for 28 days, 6 h/day, water control group and 3 dose groups , 10 males and 10 females/group, 2 additional groups (water control and highest dose) kept for a postexposure period of 14 days, observations included body weight, food consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, organ weight and full *post mortem* examination.	
5.2	**Results and discussion**	A no-observed-adverse-effect-level (NOAEL) for systemic effects was established as > 1000mg/kg bw/day, based on the absence of systemic effects at this dose level. Minor local skin irritation occurred at all dose levels of etofenprox, but there was evidence of lesion resolution after 14 days off-dose.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL	not determined	
5.3.2	NO(A)EL	> 1000mg/kg bw/day	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6_03_2-1. Mean total application site skin erythema score/week/rabbit.

Dose level (ppm)	Sex	Week 1	Week 2	Week 3	Week 4
0	Male	0.15	0.10	0	0
400		1.6	1.4	1.0	0.7
650		1.9	1.5	1.4	0.7
1000		3.1	0.6	0.35	0.6
0	Female	1.1	0	0	0.05
400		4.8	2.3	1.5	0.7
650		5.2	1.5	0.50	0.9
1000		5.2	1.6	0.45	1.1

Table A6_03_2-2. Selected histopathological alterations in animals killed after 4 weeks.

Organ:	Males treated at (mg/kg bw/day):				Females treated at (mg/kg bw/day):			
- histopathological alteration	**0**	**400**	**650**	**1000**	**0**	**400**	**650**	**1000**
No. animals examined (28-day kill)	10	10	9	10	10	10	10	10
Application site skin, dermis:								
- foreign body	1	0	5	0	2	0	1	3
- focal haemorrhage	0	0	0	0	2	2	1	0
- diffuse heterophil infiltrate	0	0	2	7	4	1	0	1
- focal heterophil infiltrate	1	4	4	0	4	4	6	3
- diffuse chronic inflammation	0	0	2	8	5	3	2	3
- focal chronic inflammation	5	7	5	2	3	7	5	6
- focal granulomatous inflammation	0	0	2	0	0	0	1	3
Application site skin, epidermis:								
- diffuse hyperplasia	0	6	9	9	5	9	8	10
- focal hyperplasia	1	3	0	0	3	0	1	0
No. animals examined (recovery)	10	0	0	10	10	0	0	10
Application site skin, dermis:								
- foreign body	1	-	-	2	1	-	-	1
- focal haemorrhage	0	-	-	0	1	-	-	0
- diffuse heterophil infiltrate	0	-	-	0	1	-	-	0
- focal heterophil infiltrate	0	-	-	3	1	-	-	2
- diffuse chronic inflammation	0	-	-	0	3	-	-	0
- focal chronic inflammation	4	-	-	4	4	-	-	8
- focal granulomatous inflammation	1	-	-	2	1	-	-	1
Application site skin, epidermis:								
- diffuse hyperplasia	1	-	-	1	5	-	-	1
- focal hyperplasia	2	-	-	6	3	-	-	4

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. Within the present study the specification does not deviate to these indications.
Conclusion	**5.2. Results and Discussion** "The fact that dermal findings were not dose related is probably due to the fact that the test material was applied undiluted to all three dose level. Since dermal findings are generally more dependent on concentration than on the amount applied it would be expected that the dermal findings would be similar at all the dose levels .It is also possible that the dosing procedure itself, i.e. daily wrapping and bandaging for 6 hours contributed to the dermal observations in the rabbits…" (p26 of original report). As indicated the dermal 28 day NOAEL of > 1000 mg/kg bw is only related to systemic toxicity. A NOAEL for dermal effects cannot be established based on this study. Local dermal effects resulting from high dose (400 mg/kg bw day) and long term exposure could be relevant for the risk assessment.
Reliability	1
Acceptability	acceptable
Remarks	**Table A6_03_2-1** The dose levels correspond to the unit [mg/kg bw/day] and not to [ppm]
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1.1 Subchronic toxicity (rodent)

Annex Point IIA-VI.6.4

Oral

Rat, dietary exposure

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1983a): Assessment of the toxicity of MTI-500 in rats during dietary administration for 13 weeks; [redacted] unpublished report no. MTC 56/821067 (July 07, 1983), re-issued amended pages December 18, 1985. Dates of work: June 29, 1982 - September, 1982 (in life phase)	
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 The method exceeds the requirements of 88/302/EEC (B.26)	X
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Deviations: none.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Etofenprox	
3.1.1	Lot/Batch number	Batch no. ST-101	
3.1.2	Specification	Deviating from specification given in section 2 as follows	X
	3.1.2.1 Description	Brown crystalline solid	
	3.1.2.2 Purity	96%	
	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-derived rats (CD strain)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	5 - 6 week old, weight range 127 – 214g	
3.2.6	Number of animals per group	20 males and 20 females per group	
3.2.7	Control animals	Yes	

Section A6.4.1.1 Subchronic toxicity (rodent)

Annex Point IIA-VI.6.4 **Oral**

Rat, dietary exposure

3.3	**Administration/ Exposure**	**Oral**
3.3.1	Duration of treatment	13 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Dietary admixture
3.3.4.2	Concentration	- constant nominal dietary concentrations of 0, 50, 300, 1800 and 10800ppm. - mean achieved dose levels were 0, 3.3, 20, 120 and 734mg/kg bw/day (males) and 0, 3.8, 23, 142 and 820mg/kg bw/day (females).
3.3.4.3	Vehicle	Corn oil
3.3.4.4	Concentration in vehicle	Not reported
3.3.4.5	Total volume applied	Not reported
3.3.4.6	Controls	Untreated diet containing a comparable volume of corn oil as the top dose group.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. Detailed clinical examinations were performed daily for the first 4 weeks and weekly thereafter.
3.4.1.2	Mortality	Yes. Morbidity/mortality checks were performed once or twice daily
3.4.2	Body weight	Yes. Body weights were recorded pre-dose, weekly throughout the study, and at necropsy.
3.4.3	Food consumption	Yes. Food consumption was determined weekly by measurement.
3.4.4	Water consumption	Yes. Water consumption was determined weekly by visual appraisal. In addition, the daily water consumption of all animals treated at 0 or 10800ppm was measured accurately during weeks 4 and 10 and for all groups in week 11.
3.4.5	Ophthalmoscopic examination	Yes. Ophthalmoscopic examinations were performed on all animals treated at 0 or 10800ppm pre-dose and in weeks 5 and 13.

Section A6.4.1.1 **Annex Point IIA-VI.6.4**	**Subchronic toxicity (rodent)** **Oral** **Rat, dietary exposure**
3.4.6 Haematology	Yes Hematology was performed on 10 animals/sex/group during weeks 6 and 12-14. Hematology was also performed pre-dose on 10 animals/sex designated as health check animals. Blood samples were withdrawn from food-deprived animals, except for the pre-dose investigations. Further blood samples were taken from 10 non-food-deprived males treated at 0 or 10800ppm in week 13 and prothrombin and activated partial thromboplastin times measured, to investigate a treatment-related increase in prothrombin times of males at 10800ppm. A third blood sample was taken from 10 food-deprived males treated at 0 or 10800ppm in week 14 and thrombotest, prothrombin and activated partial thromboplastin times measured, to further investigate the effect. Parameters: packed cell volume, haemoglobin concentration, red cell count, reticulocyte count, mean corpuscular haemoglobin concentration, mean cell volume, mean corpuscular haemoglobin, total white cell count, differential WBC count, platelet count. During weeks 12 and 14, a thrombotest was performed. During weeks 13 and 14, prothrombin time and activated partial thromboplastin time were estimated.
3.4.7 Clinical Chemistry	Yes. Blood chemistry was performed on 10 animals/sex/group during weeks 6 and 12-14. Blood chemistry analyses were also performed pre-dose on 10 animals/sex designated as health check animals. Blood samples were withdrawn from food-deprived animals, except for the pre-dose investigations and for clotting times in week 13. Parameters: glucose, total protein, albumin, globulin, urea nitrogen, creatine, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, total lactic dehydrogenase, totalbilirubin, sodium, potassium, calcium, inorganic phosphorus, chloride, cholesterol, tri-iodothyronine, thyronine.
3.4.8 Urinalysis	Yes Urine samples were collected overnight during week 6 and week 12, from 10 animals/sex/group. Urine was collected from water-deprived animals. Parameters: volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, haemoglobin.

Section A6.4.1.1 **Subchronic toxicity (rodent)**

Annex Point IIA-VI.6.4 **Oral**

Rat, dietary exposure

3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes All animals killed at schedule sacrifice. The weight of major organs of individual rats dying during the study were recorded at the discretion of the pathologist. Organs: adrenals, brain, heart, liver, kidneys, ovaries, pituitary, spleen, testes, thymus, thyroids, uterus.
3.5.2	Gross and histopathology	Yes. All animals killed at schedule sacrifice. Organs and tissues: adrenals, brain, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, mid-colon, oesophagus, ovaries, pancreas, pituitary, prostate, salivary glands, skeletal muscle, spleen, sternum, stomach, testes, thymus, thyroids, trachea, urinary bladder, uterus. Two additional sections of frozen liver stained with PAS or oil red O were also examined microscopically.
3.5.3	Statistics	Where applicable, data were analysed statistically by ANOVA followed by Student's t-test and Williams' test, or Bartletts' test for equality of variance followed by one-way ANOVA or the Kruskal-Wallis ranking test, or Fisher's exact probability test and Mantel's test, or ANCOVA. Inter-group comparisons were performed using Williams' test for a dose-related response.
3.6	**Further remarks**	- The stability and homogeneity of the diets were confirmed prior to the start of the study. The achieved concentrations of all diets from the first and last diet batches were determined by analysis. - Pre-treatment health check: ten males and ten females were selected for health check purposes at the time of allocation of animals to groups. These rats were then killed and a macroscopic examination was carried out.

Section A6.4.1.1 **Subchronic toxicity (rodent)**
Annex Point IIA-VI.6.4 **Oral**
Rat, dietary exposure

4 RESULTS AND DISCUSSION

4.1 Analytics

The stability of diet formulations containing 20 or 20000ppm etofenprox maintained at room temperature was shown to be acceptable after 18 days storage, at which time means of 100.5 and 104.5% starting concentrations remained. Homogeneity of mixing was acceptable since standard deviations for 6 analyses per concentration were 0.393 and 369 for 20 and 20000ppm, respectively. Achieved concentrations in weeks 1 and 13 were also acceptable with all diet formulations, on both occasions, being within the range 96 - 113% nominal concentrations.

4.2 Observations

4.2.1 Clinical signs

There were no treatment-related clinical signs in either sex at any dose level.

4.2.2 Mortality

One control male died during study week 13, shortly after blood sampling. Necropsy revealed frothy blood in a number of major vessels. All other study animals survived to the scheduled termination date.

4.3 Body weight gain

There was a treatment-related decrease of 15.8 and 10.1% in the overall weight gain of males and females treated 10800ppm, respectively. A similar but slightly less marked (8.0%) effect in females at 1800ppm. The effect was statistically significant only at 10800ppm ($p < 0.001$ and $p < 0.05$ in males and females, respectively) (Table A6_4_1_1_01-1).

4.4 Food and water consumption and compound intake

- Food consumption was not affected by treatment at any dose level (Table A6_4_1_1_01-1). Although the food consumption of the female groups treated at 50 or 10800ppm was significantly ($p < 0.05$) lower than the control consumption, the difference amounted to 4.8% and is considered to be within the range of normal variation of food consumption.

- The water consumption of the animals treated at 10800ppm was reduced by 10.8- 18.4% in males and by 6.9 – 11.1% in females. The water consumption at lower dose levels was comparable to the controls in week 11. There were no treatment-related ocular lesions in the animals treated at 10800ppm.

4.5 Ophtalmoscopic examination

There were no treatment-related ocular lesions in the animals treated at 10800ppm.

4.6 Blood analysis

Section A6.4.1.1 **Subchronic toxicity (rodent)**

Annex Point IIA-VI.6.4 **Oral**

Rat, dietary exposure

4.6.1	Haematology	See Table A6_4_1_1_01-2. There were no treatment-related effects at any dose level on red blood cell parameters or white blood cell total and differential counts. Although some group mean values attained statistical significance, all individual values, with the exception of one male at 50ppm and a female at 1800ppm, were within the range of laboratory normal expected values. Slightly higher group mean platelet counts were evident in both sexes at 10800ppm on both sampling occasions. The differences from the controls were of a small magnitude (9.3 - 17.4% increase) but were statistically significant ($p < 0.01$) in both sexes in week 6 and in females in week 12. Since there was no clear corroborative evidence, the differences are considered not to be of toxicological relevance. Males treated at 10800ppm, but not females, showed significantly ($p < 0.01$) prolonged clotting times in week 12. Male groups at lower dose levels were not affected. Further investigations also showed statistically significant ($p < 0.001$) prolongation of the PT and APTT in males at 10800ppm. The results suggest that both the intrinsic and extrinsic systems were affected. Pooling of blood plasma from control and high dose males gave an intermediate PT value of 11.5 seconds, indicating that the prolonged PT was not a consequence of a circulating anticoagulant. Therefore, it was concluded that etofenprox was affecting the synthesis of a number of clotting factors, indicative of liver dysfunction.
4.6.2	Clinical chemistry	See Table A6_4_1_1_01-3. Thyroxine activities were reduced in males treated at 1800 and 10800ppm at both sampling intervals. The effect was not apparent in males at lower dose levels or in the females at any dose level. There was no clear effect on T_3 activity at any dose level although the females at 10800ppm showed a significantly ($p < 0.05$) higher activity in week 6 only. Cholesterol concentrations were significantly ($p < 0.01$) elevated in males at 10800ppm in weeks 6 and 12 and in males at 1800ppm ($p < 0.05$) and females at 10800ppm ($p < 0.01$) in week 12 only. The cholesterol concentrations of lower dose levels were unaffected by treatment. GPT, GOT and LDH activities of males at 1800ppm and 10800ppm were increased in week 6 ($p < 0.05$, $p < 0.01$ or $p > 0.05$), but the values were comparable to the controls in week 12. GPT, GOT and LDH activities were unaffected by treatment at all dose levels in the females. All other clinical chemistry parameters were unaffected by treatment at all dose levels.
4.6.3	Urinalysis	There was no effect of treatment at any dose level on the quantitative and qualitative characteristics of urine.
4.7	**Sacrifice and pathology**	

Section A6.4.1.1 **Subchronic toxicity (rodent)**
Annex Point IIA-VI.6.4 **Oral**
Rat, dietary exposure

4.7.1	Organ weights	See Table A6_4_1_1_01-4. The males treated at 1800 or 10800ppm showed increased absolute and adjusted thyroid weights. The adjusted weights were significantly ($p < 0.05$ or 0.01) elevated in comparison to the controls. The absolute and adjusted thyroid weights of all treated female groups and the male groups at 50 or 300ppm were not significantly ($p > 0.05$) different from the controls. The adjusted liver and adrenal weights of both sexes at 10800ppm were significantly ($p < 0.01$) increased by up to 35.3% and 17.5%, respectively, in comparison with the control. Females at 1800ppm showed a similar, but less marked, effect in the liver which was also statistically significant ($p < 0.01$). There were no other treatment-related effects on organ weights at any dose level.
4.7.2	Gross and histopathology	- Macroscopic findings Treatment-related gross lesions at necropsy were confined to enlarged liver in 4 animals of each sex at 10800ppm and in 2 males at 1800ppm. All other gross lesions were those commonly seen in animals of the strain employed and their distribution between the groups did not indicate an effect of treatment. - Histopatholgy: see Table A6_4_1_1_01-5. Treatment-related histopathological findings occurred in the liver and thyroid gland. Minimal centrilobular enlargement of hepatocytes occurred in one female treated at 1800ppm and in 9 females treated at 10800ppm. The effect was not evident in males at any dose level, despite the documented increase in the adjusted liver weights of males at 10800ppm. Oil red O and PAS staining of liver sections revealed no treatment-related alterations. Minimal to moderate numbers of microfollicles occurred in the thyroid glands of both treated and control animals, but the incidences were increased in both sexes treated at 1800 and 10800ppm. All other histopathological alterations were considered to be spontaneous in origin and of no toxicological consequence. Specifically, there were no treatment-related histopathological alterations in the adrenal gland to account for the increased weight at 10800ppm.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines not specified in report but method exceeds the requirements of 88/302/EEC (B.26). No deviations from the test guideline. Description of method: rat, dietary exposure for 13 weeks, 4 dose levels plus one control group, observations included body weight, food and water consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, urinanalysis, organ weight and full *post mortem* examination.

Section A6.4.1.1 **Subchronic toxicity (rodent)**

Annex Point IIA-VI.6.4 **Oral**

Rat, dietary exposure

5.2	**Results and discussion**	A no-observed-adverse-effect-level (NOAEL) was established as 300ppm, equivalent to dose levels of 20 and 23mg/kg bw/day in males and females, respectively, based on the occurrence of reduced weight gain (females only), reduced circulating thyroxine activity and clinical signs of liver dysfunction (males only), and hepatocyte enlargement (females only), at 1800ppm, and additionally at 10800ppm reduced body weight gain and water consumption, prolonged clotting time (males only), and an increased incidence of microfollicles in the thyroid gland.
5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NOAEL	20 and 23mg/kg bw/day in males and females, respectively
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6_4_1_1_01-1. Group mean total food consumption and overall body weight gain.

Dose level (ppm)	Sex	Mean total food consumption (g/animal for weeks 1 - 13)	Overall mean weight gain (g)
0	Male	2339	323
50		2324	320
300		2289	307
1800		2345	323
10800		2265	272***
0	Female	1659	138
50		1578*	132
300		1634	139
1800		1638	127
10800		1579*	124*

* $p < 0.05$; *** $p < 0.001$

Table A6_4_1_1_01-2. Summary of group mean blood clotting indices.

Dose level (ppm)	Sex	Week 12	Week 13		Week 14		
		TT (sec)	PT (sec)	APTT (sec)	TT (sec)	PT (sec)	APTT (sec)
0	Male	26.2	11.5	18.7	25.8	11.4	20.7
50		26.2	-	-	-	-	-
300		26.7	-	-	-	-	-
1800		27.1	-	-	-	-	-
10800		39.2**	11.8	25.5***	33.9***	13.4***	35.3***
0	Female	24.6	-	-	-	-	-
50		24.1	-	-	-	-	-
300		23.1	-	-	-	-	-
1800		24.9	-	-	-	-	-
10800		22.8*	-	-	-	-	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; TT thrombotest; PT prothrombin time; APTT activated partial thromboplastin time; - not investigated

Table A6_4_1_1_01-3. Selected group mean clinical chemistry values.

Dose level (ppm)	Sex	Week of sampling	GPT (mU/mL)	GOT (mU/mL)	LDH (mU/mL)	Chol (mg/dL)	T_3 (ng/dL)	T_4 (µg/dL)
0	Male	6	27	56	598	49	42	7.2
50			28	64	804	51	49	7.3
300			29	66	872	51	45	7.8
1800			35*	74**	1036	51	45	6.3
10800			42**	84**	1675**	60**	45	5.7**
0	Female		20	56	584	51	47	5.3
50			19	54	468	58	48	4.8
300			24	55	505	53	47	6.1
1800			22	52	498	53	48	5.6
10800			20	55	471	56	60*	6.1
0	Male	12	29	59	262	37	46	5.2
50			23	63	417	39	52	4.8
300			34	73	379	41	55	4.9
1800			31	55	233	44*	51	4.3*
10800			34*	53	282	52**	51	3.9**
0	Female		23	51	188	39	53	3.0
50			17*	49	239	49	51	2.8
300			21*	52	231	44	54	2.8
1800			18*	50	221	42	57	3.0
10800			17**	53	268	58**	56	3.0

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

Table A6_4_1_1_01-4. Selected group mean absolute and adjusted organ weights.

Dose level (ppm)	Sex	Liver weight (g)		Thyroid weight (mg)		Adrenals weight (mg)	
		absolute	adjusted[a]	absolute	adjusted[a]	absolute	adjusted[a]
0	Male	22.8	22.2	23	22	58	57
50		23.4	22.8	24	24	55	54
300		22.7	23.0	24	24	59	60
1800		24.3	23.4	27	27*	59	57
10800		27.0	28.8**	28	29**	65	67**
0	Female	12.2	11.9	21	21	77	76
50		11.7	11.7	21	21	79	79
300		12.4	12.2	20	20	74	73
1800		12.8	13.0**	22	22	82	82
10800		15.7	16.1**	24	24	87	88**

[a] statistically adjusted using body weight as covariate; * $p < 0.05$; ** $p < 0.01$

Table A6_4_1_1_01-5. Treatment-related histopathological findings.

Dose level (ppm)	Sex	Incidence of hepatocyte enlargement	Incidence of thyroid microfollicles
0	Male	0 / 19	10 / 19
50		0 / 20	11 / 20
300		0 / 20	5 / 20
1800		0 / 20	19 / 20
10800		0 / 20	18 / 20
0	Female	0 / 20	0 / 20
50		0 / 20	0 / 20
300		0 / 20	0 / 20
1800		1 / 20	2 / 20
10800		9 / 20	9 / 20

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** It is not clear in which regard the study exceeds the requirements of the test guidelines. Eventually the full histopathology is meant; according to OECD 408or B26 histopathology is just required for the high dose and the control groups and should only be extended to the other groups for the effects observed in the high dose group. **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-101 contained the same main impurities as later production batches (e.g. 5 batch analysis) at comparable percentages. The concentration of etofenprox is with 96% lower than in the 5 batch analysis. Therefore the specification within in the present study 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 A6.4.1.2
Annex Point IIA-VI.6.4

Subchronic toxicity (non-rodent)

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

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

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

Detailed justification: A study with exposure of the dog for 52 weeks is available (see section A6_05_2)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Evaluation of applicant's justification	The justification is acceptable according to TGD on data requirements, chapter 2A, point 6.4.2.:"The subchronic toxicity study in the second animal species [can be waived], if there is a one year toxicity study in the second animal species available"
Conclusion	Agree with applicant
Remarks	-
	COMMENTS FROM...
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.4.3.1 Subchronic toxicity (rodent)

Annex Point IIA-VI.6.4 **Inhalation**

Aerosol, 90-day inhalation, rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985): Ethofenprox (MTI-500) 90-day inhalation study in rats; [redacted] unpublished report no. MTC 81/841257 (August 23, 1985). Dates of work: June 25, 1984 (first exposure) - experimental completion date not specified	
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**	No Methods used complies with OECD guideline no. 413 (1981)	
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. 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%	
3.1.2.3	Stability	No information in the report	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Wistar rat, Crl:COBS WI BR strain	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 - 7 week old Wistar rats/ weight range 170 - 277g	
3.2.6	Number of animals per group	15 males and 15 females per group	
3.2.7	Control animals	Yes	

Section A6.4.3.1 Subchronic toxicity (rodent)

Annex Point IIA-VI.6.4

Inhalation

Aerosol, 90-day inhalation, rat

3.3	**Administration/ Exposure**	**Inhalation**
3.3.1	Duration of treatment	13 weeks
3.3.2	Frequency of exposure	6 days per week, 6 hours per day
3.3.3	Postexposure period	No
3.3.4	**Inhalation**	
3.3.4.1	Concentrations	Target concentrations : 0 (air only), 0 (vehicle), 0.04, 0.20 and 1.0 mg/L Analytical concentrations: 0, 0, 0.042, 0.21 and 1.01mg/L (see Table A6_04_3_1-1).
3.3.4.2	Particle size	The particle size distribution of the atmospheres was determined gravimetrically, using a 3-stage impinger with hexane/propanol/ethyl acetate trapping fluid, once during each exposure period for all treated groups. Particles were separated by equivalent aerodynamic diameters into > 5.5, 2.0 - 5.5 and < 2.0µm. about 90% of particles < 5.5µm (see Table A6_04_3_1-1).
3.3.4.3	Type or preparation of particles	Aerosol in air of 9:1w/w etofenprox and acetone, created by a stainless steel jet atomiser connected, via a glass elutriation column, to a 500L steel and glass exposure chamber.
3.3.4.4	Type of exposure	Whole body
3.3.4.5	Vehicle	Acetone
3.3.4.6	Concentration in vehicle	Aerosol in air of 9:1w/w etofenprox and acetone analytical concentrations of 0 (air control), 0 (acetone control), 0.042, 0.21 and 1.01mg/L
3.3.4.7	Duration of exposure	6 hours per day
3.3.4.8	Controls	Air control group and vehicle (acetone) control group
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, twice daily when not in the exposure chambers
3.4.1.2	Mortality	Yes, twice daily when not in the exposure chambers
3.4.2	Body weight	Yes. Body weights were recorded pre-dose, before exposure on day 1, at weekly intervals thereafter and at necropsy.
3.4.3	Food consumption	Yes, determined weekly
3.4.4	Water consumption	Yes, determined weekly
3.4.5	Ophthalmoscopic examination	Yes. Ophthalmoscopic examinations were performed on 5 animals/sex/group pre-exposure and during week 13.

Section A6.4.3.1 **Annex Point IIA-VI.6.4**		**Subchronic toxicity (rodent)** **Inhalation** **Aerosol, 90-day inhalation, rat**	
3.4.6	Haematology	Yes. Performed on 5 animals/sex/group pre-dose and during week 5, and on all animals during week 12. Blood samples were collected after overnight deprivation of food. Parameters: packed cell volume, haemoglobin, red cell count, mean corpuscular haemoglobin concentration, mean corpuscular volume, total white cell count, differential count, cell morphology, platelet count, thrombotest.	
3.4.7	Clinical Chemistry	Yes. Performed on 5 animals/sex/group pre-dose and during week 5, and on all animals during week 12. Blood samples were collected after overnight deprivation of food. Parameters: glucose, total protein, albumin, albumin/globulin ratio, urea nitrogen, creatinine, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, total bilirubin, sodium, potassium, calcium, inorganic phosphorus, chloride, cholesterol, tri-iodothyronine (T3) and thyroxine (T4) concentrations.	
3.4.8	Urinalysis	No	X
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes Organs: liver, kidneys, adrenals, testes, ovaries, spleen, brain, heart, lungs and thyroid	
3.5.2	Gross and histopathology	Yes All tissues from the animals treated at 0 (air) or 1.01mg/L, and major tissues and organs including gross lesions from all animals of all groups, were examined microscopically. Organs and tissues examined from all groups: trachea, lungs and bronchi, heart, stomach, duodenum, jejunum, ileum, kidney, testes, ovaries, liver, spleen, pancreas, thyroid, parathyroid, adrenals, brain, pituitary, all gross lesions. Organs and tissues examined from control and high dose groups: nasal passages (tongue, pharynx, larynx), aorta, oesophagus, caecum, colon, rectum, urinary bladder, epididymides, seminal vesicles, prostate, uterus, salivary glands, lymph nodes, eyes, sciatic nerve, sternum, spinal cord, muscle (tigh), skin, mammary gland, femur, remaining head.	
3.5.3	Statistics	Where appropriate, data were subjected to statistical analysis. Body weight, food and water consumption were analysed using Student's t-test. Haematology and clinical chemistry data were analysed using Bartlett's test followed by the Kruskal-Wallis test or ANOVA. ANOVA was followed by Williams' test and the Kruskal-Wallis test by a non-parametric equivalent of Williams' test. Organ weight data was analysed using ANCOVA using body weight as the covariate.	

Section A6.4.3.1 **Subchronic toxicity (rodent)**

Annex Point IIA-VI.6.4 **Inhalation**

Aerosol, 90-day inhalation, rat

3.6	**Further remark**	The nominal exposure concentrations were calculated from the difference in weight of test article at the start and end of exposure and total air-flow and were 0.14, 0.64 and 3.85mg/L, in order of ascending dose level. The analytically-determined atmosphere concentrations were in good agreement with the target concentrations (Table A6_4_3_1-1). The differences between nominal and actual atmosphere concentrations were due to compound deposition in the elutriation chamber and pipe-work leading to the exposure chambers. The results of the multiple homogeneity analyses indicated a homogeneous distribution of etofenprox within the chambers. The data derived from multiple ports at the same time interval indicated that the variation was no greater than the variation in values determined from the same sampling port at different times. The range of chamber concentrations, expressed as a percentage of the mean chamber concentration, was 14.3, 13.6 and 21.3%, in order of ascending concentration. Particle size distribution analysis indicated that 90.1, 90.3 and 90.9% of particles had equivalent aerodynamic diameters of less than 5.5µm, in order of ascending concentration. Therefore, a large proportion of particles at all concentrations was respirable.
	4 RESULTS AND DISCUSSION	
4.1	**Observations**	
4.1.1	Clinical signs	There were no adverse reactions during exposure at any dose level. However, scab formation, predominantly on the skin of the back of the ears, occurred during the early part of the exposure period in animals of both sexes at 1.01mg/L and in females at 0.21mg/L. Scab formation was considered to result from compound deposition on the fur, resulting in excessive grooming overnight. The effect was accompanied by local hair loss in some animals treated at 1.01mg/L.
4.1.2	Mortality	No mortalities at any dose level.
4.2	**Body weight gain**	No effects. Therefore, the group mean terminal body weights of the male treated groups, in order of ascending concentration, were 100.9, 97.0 and 102.7% of the vehicle control group. The corresponding values for the female treated groups were 104.6, 101.8 and 104.9%.

Section A6.4.3.1	Subchronic toxicity (rodent)	
Annex Point IIA-VI.6.4	Inhalation Aerosol, 90-day inhalation, rat	
4.3 **Food consumption and water consumption**	See Table A6_04_3_1-2. The overall mean food consumption of the female group treated at 1.01mg/L was slightly, but significantly ($p < 0.05$), greater than that of the air control group during the latter part of the exposure period. However, the effect is considered not to be of toxicological relevance. The food consumption of all other female groups and of all male groups was not affected by treatment. The overall mean water consumption of all etofenprox -treated groups was slightly greater than both control groups, but none of the values was statistically significant ($p > 0.05$). In the absence of histomorphological effects in the kidneys, the finding is considered not to represent an adverse effect.	
4.4 **Ophtalmoscopic examination**	No ocular abnormalities were detected in any of the animals examined after at least 12 weeks of exposure.	
4.5 **Blood analysis**		
4.5.1 Haematology	See Table A6_04_3_1-3. There were no treatment-related adverse haematological findings at any dose level. Differences between treated and control groups in red and white blood cell parameters that achieved statistical significance ($p < 0.05$ or 0.01) in weeks 5 and 12 were minimal and considered not to have any toxicological relevance since all values were considered to be within normal limits.	
4.5.2 Clinical chemistry	See Table A6_04_3_1-3 There were statistically significant differences ($p < 0.05$ or 0.01) between treated and control groups in weeks 5 and/or 12 for a number of parameters, glucose, total protein, albumin, creatinine, A/G ratio, GPT and GOT activities, chloride and cholesterol. Since the differences were minor, none was considered to be toxicologically relevant or an adverse effect. There was no effect of treatment at any dose level on the concentrations of the circulating thyroid hormones, T3 and T4.	
4.5.3 Urinalysis	Not performed	X
4.6 **Sacrifice and pathology**		
4.6.1 Organ weights	See Table A6_04_3_1-4. Treatment-related, significant ($p < 0.05$ or 0.01) increases in the weights of the thyroid (both sexes at 1.01mg/L), liver (both sexes at 1.01mg/L, females at 0.21mg/L) and kidneys (both sexes at 1.01 and 0.21mg/L) (). Adrenal weights were also increased in both sexes at 1.01mg/L, but statistical significance was apparent only in the females. Although the weights of the heart and lungs in males at 1.01 and 0.21mg/L and ovary weights at 1.01mg/L were significantly higher than the control groups, there were no correlating histopathological findings, and the differences in weights were considered to be incidental to treatment.	

Section A6.4.3.1 **Subchronic toxicity (rodent)**
Annex Point IIA-VI.6.4 **Inhalation**
Aerosol, 90-day inhalation, rat

4.6.2	Gross and histopathology	Treatment-related histopathological alterations were identified in the thyroid, liver and adrenals. Minimal enlargement of centrilobular hepatocytes occurred in 4 of 10 males and 4 of 10 females at 1.01mg/L. This change was not evident in any of the other treated or control groups. Minimally increased numbers of microfollicles in 4 of 10 males, and increased height of the follicular epithelium in 2 of 10 males occurred at 1.01mg/L. This change was not evident in other male groups or in any of the female treated groups. Minimally increased adrenal cortical width occurred in 3 of 10 females at 0.21mg/L and in 4 of 10 females at 1.01mg/L, compared with a control female incidence of 1 in 10. The change did not occur in the other female group or in any of the male treated groups. All other histopathological findings were considered to be unrelated to treatment or of no toxicological relevance.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test method complies with OECD guideline no. 413 (1981) No deviations from test guidelines. Description of method: rat, inhalation exposure for 13 weeks days, 6 days/week, 6 h/day, aerosol of a 9:1 solution of etofenprox in acetone, air control group, acetone control group, observations included body weight, food and water consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, organ weight and full *post mortem* examination.
5.2	**Results and discussion**	A no-observed-effect-level (NOEL) for all effects was established as 0.042mg/L, based on the occurrence of increased liver and kidney weights and histopathological alterations in the adrenals of females at 0.21mg/L, and histopathological alterations in liver, thyroid and adrenals at 1.01mg/L.
5.3	**Conclusion**	
5.3.1	LO(A)EL	0.21mg/L
5.3.2	NOEL	0.042mg/L
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6_4_3_1-1. Summary of analytically-determined test atmosphere characteristics.

Test atmosphere characteristic	Value at target concentration (mg/L) of:		
	0.04	**0.2**	**1.0**
Mean daily chamber concentration (mg/L)	0.042	0.21	1.01
Range (mg/L)	0.024 - 0.080	0.14 - 0.28	0.76 - 1.23
Homogeneity range of values (mg/L)	0.032 - 0.037	0.20 - 0.23	0.81 - 1.01[a]
Homogeneity range (% mean value)	14.3	13.6	21.3
Particle size distribution (% < 5.5µm)	90.1	90.3	90.9

[a] one value of 0.63mg/L omitted due to loose sampling port

Table A6_4_3_1-2. Summary of group mean cumulative food and water consumption.

Parameters	Week	Males treated at (mg/L):					Females treated at (mg/L):				
		0 (air)	0	0.042	0.21	1.01	0 (air)	0	0.042	0.21	1.01
Food (g/animal)	- 1	174	172	171	167	169	134	134	135	131	132
	1	372	367	368	356	363	276	276	281	272	271
	5	1149	1112	1118	1088	1137	815	826	835	822	829
	9	1931	1856	1882	1857	1939	1347	1374	1387	1384	1408
	13	2657	2566	2606	2574	2686	1849	1886	1898	1910	1935*
Water (g/animal)	- 1	227	214	230	212	220	220	227	215	206	212
	1	423	407	440	406	420	399	401	402	381	395
	5	1339	1350	1439	1393	1420	1221	1240	1257	1253	1279
	9	2232	2295	2447	2465	2472	2038	2071	2095	2125	2179
	13	3163	3277	3502	3595	3585	2873	2918	2947	3036	3070

* $p < 0.05$ (*vs.* air control group)

Table A6_4_3_1-3. Summary of haematological and clinical chemistry findings achieving statistical analysis.

Week / parameter	Males treated at (mg/L):					Females treated at (mg/L):				
	0 (air)	0	0.042	0.21	1.01	0 (air)	0	0.042	0.21	1.01
Week 5:										
Hb (g/dL)	16.6	15.9	16.0	16.1	15.6**	15.0	14.7	14.8	14.8	15.5*
RBC (10^6/mm^3)	8.2	7.8	7.6	8.0	7.5**	7.2	7.1	7.2	7.3	7.4
Platelets (10^3/mm^3)	732	788	774	842	864**	654	742	646	684	736
PCV (%)	53	50	51	52	51	48	48	48	49	50*
Week 12:										
RBC (10^6/mm^3)	8.8	8.6	8.5	8.5	8.4*	7.4	7.2	7.4	7.6	7.1
MCHC (%)	31.6	31.8	31.3	30.8**	30.7**	30.5	30.8	31.0	30.6	30.3
MCV (fL)	60	60	62*	62*	62**	66	66	65	65	68
Neutrophil (10^3/mm^3)	1.19	1.21	1.51	2.44*	2.04*	1.22	1.26	1.03	1.43	1.01
Eosinophil (10^3/mm^3)	0.00	0.00	0.18*	0.21*	0.08*	0.02	0.07	0.10	0.20*	0.10*
Monocyte (10^3/mm^3)	0.01	0.01	0.09	0.10	0.18*	0.05	0.06	0.04	0.09	0.09
Week 5:										
Glucose (mg/dL)	124	155	127	131	92*	108	102	101	120	126
Albumin (g/dL)	3.8	3.7	3.8	3.8	3.9*	4.0	4.1	4.1	4.0	4.0
Creatinine (mg/dL)	0.5	0.6**	0.6**	0.6**	0.6**	0.6	0.6	0.6	0.5	0.5
A/G ratio	1.16	1.19	1.20	1.22	1.25	1.40	1.63	1.45	1.53	1.36**
Week 12:										
Glucose (mg/dL)	158	138	150	137*	136*	102	113	120	127	99
Total protein (g/dL)	7.2	7.1	7.2	7.1	6.7**	7.0	7.3	7.1	7.1	7.4*
Creatinine (mg/dL)	0.6	0.6	0.6	0.7**	0.7**	0.8	0.8	1.0	0.8	0.8
A/G ratio	1.15	1.30	1.18	1.13	1.37**	1.31	1.22	1.30	1.25	1.21
GPT (mU/mL)	27	26	30	26	27	23	22	19*	18*	17**
GOT (mU/mL)	48	49	48	49	46	51	46	45	44*	44*
Chloride (mEq/dL)	101	99	102	100	101	100	103*	102*	102*	102**
Cholesterol (mg/dL)	54	58	60	56	53	59	60	60	65	72*

* $p < 0.05$; ** $p < 0.01$ (*vs.* air control group)

Table A6_4_3_1-4. Summary of organ weight findings

Organ	Sex	Group mean weight at (mg/L):				
		0 (air)	**0**	**0.042**	**0.21**	**1.01**
Thyroid (mg)	Male	23	20	22	23	31**
Heart (g)		1.36	1.40	1.36	1.45*	1.44**
Lungs (g)		1.57	1.59	1.59	1.67*	1.63*
Liver (g)		21.31	20.40	20.53	20.81	24.00**
Adrenals (mg)		63	64	62	63	69
Kidneys (g)		3.22	3.25	3.30	3.38**	3.59**
Thyroid (mg)	Female	18	16	19	16	20**
Heart (g)		0.92	0.94	0.98	0.96	0.97
Lungs (g)		1.20	1.22	1.20	1.30	1.26
Liver (g)		10.56	11.10	10.98	11.91**	12.81**
Adrenals (mg)		83	81	82	89	95*
Kidneys (g)		1.94	1.92	1.97	2.09*	2.14**
Ovaries (mg)		91	88	100	113*	101*

* $p < 0.05$; ** $p < 0.01$ (*vs.* air control group)

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. **3.4.8. and 4.5.3. Urine Analysis** not performed, but not mandatory according to OECD guideline 413
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.5.1/01 Chronic toxicity (rodent)

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1986a): Ethofenprox (MTI-500) potential tumorigenic and toxic effects in prolonged dietary administration to rats; [redacted] unpublished report no. MTC 59/85581 (January 24, 1986). Dates of work: January 04, 1983 - February 14, 1985.	
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 88/302/EEC	X
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. 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-derived rats (CD strain)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 - 7 week old , group mean bodyweight ranges 248 - 252g for males and 171 - 175g for females.	
3.2.6	Number of animals per group	- Main groups: 50 animals/sex/group - Satellite groups (at each dose level, intented for blood and urinanalysis at intervals and for interim kill after 26 or after 56 weeks): 10 animals/sex/group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.5.1/01 Chronic toxicity (rodent)

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

3.3.1	Duration of treatment	At least 106 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	- Constant nominal concentrations: 0, 30, 100, 700 and 4900ppm - Overall mean achieved dose levels: 0, 1.1, 3.7, 25.5 and 186.7mg/kg b.w/day (males) and 0, 1.4, 4.8, 34.3 and 249.1mg/kg b.w/day (females)
3.3.4.3	Vehicle	Corn oil
3.3.4.4	Concentration in vehicle	Not reported
3.3.4.5	Total volume applied	Not reported
3.3.4.6	Controls	Untreated diet containing a comparable volume of corn oil.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. The animals were observed once daily for clinical signs of toxicity for the first 4 weeks and weekly thereafter. The weekly clinical examinations included a palpation for tissue masses.
3.4.1.2	Mortality	Yes, checked twice daily. Animals found dead and those killed in extremis were subjected to necropsy as soon as possible after death.
3.4.2	Body weight	Yes, recorded weekly throughout the study.
3.4.3	Food consumption	Yes, recorded weekly throughout the study.
3.4.4	Water consumption	Yes, monitored by visual inspection throughout the study and accurate measurement was performed for the groups treated at 0 or 4900ppm in weeks 5, 12 and 23.
3.4.5	Ophthalmoscopic examination	Yes. Ophthalmic examinations were performed on all animals treated at 0 or 4900ppm pre-dose, in weeks 14, 26, 52 and 78, and at termination.
3.4.6	Haematology	Yes. Blood samples were collected for haematology from 10 animals/sex/group in weeks 15/16, 24/25, 50/51, 76/77, and 102/103. Blood samples were taken from food-deprived animals. Parameters: packed cell volume, haemoglobin concentration, red cell count, reticulocyte count, mean corpuscular haemoglobin, mean cell volume, total white cell count, differential WBC count, platelet count, thrombotest.

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

3.4.7	Clinical Chemistry	Yes. Blood samples were collected for clinical chemistry from 10 animals/sex/group in weeks 15/16, 24/25, 50/51, 76/77, and 102/103. Blood samples were taken from food-deprived animals. Parameters: glucose, total protein, albumin, globulin, urea nitrogen, creatinine, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, total lactic dehydrogenase, total bilirubin, sodium, potassium, calcium, inorganic phosphorus, chloride, cholesterol, tri-iodothyromine, thyroxine.
3.4.8	Urinalysis	Yes. Urine samples were collected for urinalysis from 10 animals/sex/group in weeks 15/16, 24/25, 50/51, 76/77, and 102/103. Urine was collected during food and water deprivation. Parameters: volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, haemoglobin.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. All animals. Organs: adrenals, brain, heart, liver, kidneys, lungs, ovaries, pituitary, spleen, testes, thymus, thyroids, uterus.
3.5.2	Gross and histopathology	Yes. All animals Organs and tissues: adrenals, brain, eyes, gastro-intestinal tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon and rectum), harderian gland, head, heart, kidneys, liver, lungs, lymph nodes, mammary gland, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal column, spleen, testes with epididymides, thymus, thyroids, trachea, tongue, trachea, urinary bladder, uterus.
3.5.3	Other	Two additional frozen sections of liver were stained with oil red O and PAS and an additional section of kidney was stained with PAS or oil red O.
3.5.4	Statistics	Where applicable, data were analysed statistically by Bartletts' test for equality of variance followed by one-way ANOVA or the Kruskal-Wallis ranking test. ANOVA was followed by Student's t-test and Williams' test. The Kruskal-Wallis test was followed by non-parametric equivalents of the t-test and Williams' test. Where appropriate, ANCOVA was used in place of ANOVA. Mortality was analysed using log-rank methods. Selected tumours were analysed according to IARC recommendations. Trend tests were also applied based on nominal dose levels.

Section A6.5.1/01 **Chronic toxicity (rodent)**
Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

3.6	**Further remarks**	- The stability and homogeneity of diet formulations containing 20 or 20000ppm were confirmed by analysis prior to start of the study. Achieved concentrations in all diets were determined by analysis on 10 occasions during the study. - Pre-treatment health check: ten males and ten females were selected for health check purposes at the time of allocation of animals to groups. Pre-treatment routine haematology was carried out. These rats were then killed and a macroscopic examination was performed.	
		4 RESULTS AND DISCUSSION	
4.1	**Analysis of diet**	Analysis of diet samples demonstrated adequate homogeneity, stability and achieved concentration of etofenprox in diet. Mean values of the homogeneity ranged from 100.5 to 106% and 96.5 to 101% of the theoretical concentrations of 20 and 20000ppm, respectively. Stability analysis revealed etofenprox was stable in diet at concentrations of 20 or 20000ppm at ambient temperature for 18 days, at which time 103.5 and 104% nominal concentrations remained. The mean achieved concentrations of all diets analysed on 10 occasions ranged from 91.0 to 116% of nominal concentrations, although on one occasion the 30ppm diet was 130.7% nominal concentration.	
4.2	**Observations**		
4.2.1	Clinical signs	The nature and incidences of clinical findings, including palpable tissue masses, did not indicate an effect of treatment at any dose level.	
4.2.2	Mortality	The pattern of mortality did not indicate an effect of treatment at any dose level. There was no evidence of an effect of treatment on the distribution of the multifarious factors contributing to the pre-terminal deaths that occurred during the study. Survival incidences in the main study groups at termination were 46, 48, 46, 58 and 54% in males, and 50, 34, 40, 32 and 52% in females, in order of ascending dose level (control groups first). Therefore, it is considered that etofenprox did not influence survival at any dose level.	X
4.3	**Body weight gain**	See Table A6_5_1-1. There was a treatment-related decrease in the mean body weight gain of males and females at 4900ppm throughout the treatment period. The effect was more severe in the females, and at termination, the overall weight gain of the males and females had been reduced by 24.2 and 34.0%, respectively. There was no effect of treatment on weight gain at lower dose levels, although males at 700ppm, in common with both sexes at 4900ppm, showed overall weight loss from week 78 to termination.	X

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

4.4	**Food consumption and compound intake**	See Table A6_5_1-2. The overall food consumption was reduced in males treated at 4900ppm, mainly resulting from lower consumption from week 79 until termination, although statistical significance was not attained. There was no effect of treatment on male food consumption at lower dose levels or overall female food consumption at any dose level; although females at 4900ppm showed significantly ($p < 0.01$) lower consumption in weeks 53 - 78. The effect on food consumption at 4900ppm was accompanied by marginally reduced water consumption.
4.5	**Ophtalmoscopic examination**	There were no treatment-related ophthalmoscopic findings in either sex treated at 4900ppm at any of the examinations.
4.6	**Blood analysis**	
4.6.1	Haematology	See Table A6_5_1-3. There was a treatment-related slight increase in the clotting times of males at 4900ppm, relative to the controls, in weeks 16 and 25, which were statistically significant ($p < 0.05$ or 0.01). Significantly higher clotting times were also recorded in males at 700ppm in week 25 and in all male groups in weeks 51 and 77. However, the individual values at all dose levels in weeks 51 and 77 are considered to be within the normal range of variation. Therefore, an unequivocal treatment-related effect on clotting time is considered to have occurred in males at 4900ppm in weeks 16 and 25. There was no effect of treatment on clotting times in females at any dose level. Although there were indications of a slight anaemia, characterised by reduced haemoglobin concentration and red blood cell count, in males at 4900ppm in week 25, the effect was not apparent at subsequent investigations and did not occur in females at any dose level. Therefore, the minor haematological changes in males at 4900ppm are considered to be of equivocal toxicological relevance. Other minor inter-group differences in haematological values comprising platelet counts up to 16.6% lower in all female treated groups in week 16 and in all male groups in weeks 25 and 77, and lower numbers of circulating lymphocytes in males at 700 or 4900ppm in week 25. Since these findings were inconsistent they are considered not to be treatment-related.
4.6.2	Clinical chemistry	Throughout the course of the study, there were no treatment-related effects on plasma clinical chemistry at any dose level. Although many parameters showed statistically significant differences between the control and treated groups, the differences were either minimal or showed no dose-relationship. T_3 and T_4 activities were similar to control values in all treatment groups, although females at 4900ppm in week 25 showed significantly ($p < 0.01$) lower T_3 activity (33.3% lower than control value).

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

4.7 Urinalysis

See Table A6_5_1-4.

Urinalysis performed in week 50, but not at other sampling intervals, revealed increased volumes of a more dilute urine, relative to the controls, in males at 700 or 4900ppm. Since the differences were transient and not apparent in males at lower dose levels or in females at any dose level, they are considered to be of doubtful toxicological significance. Urinary protein concentrations in both sexes at 4900ppm were sporadically higher than control values and significantly ($p < 0.05$ or 0.01) higher in males at week 24 and females at week 15. However, no differences were apparent at week 103 and differences earlier in the treatment period are considered to reflect inherent variation in the parameter as urinary concentrations increase with age. There were no treatment-related effects at any dose level on the other urinary constituents examined.

4.8 Sacrifice and pathology

4.8.1 Organ weights

See Table A6_5_1-5.

Male and female animals treated at 4900ppm killed after 26, 52 and at least 106 weeks of treatment showed increased mean absolute liver weights which were up to 30.7% higher than the control weights and statistically significant ($p < 0.05$ or 0.01) when adjusted using body weight as the covariate. Liver weights at lower dose levels were not affected by treatment. The mean thyroid weights of males at 4900ppm killed after 26 weeks treatment and at 700 and 4900ppm killed after 106 weeks of treatment were increased by up to 35.1% and were significantly ($p < 0.05$ or 0.01) higher than the controls. The thyroid weights of males at lower dose levels and females at all dose levels were not affected by treatment. The lung weights of males and females at 4900ppm were also significantly ($p < 0.05$ or 0.01) increased after 26 weeks treatment, but not after 52 and 106 weeks treatment. Slightly increased kidney weights were evident in females at 4900ppm after 26 weeks of treatment and in males at 700 and 4900ppm after 52 weeks treatment, but no differences from the control groups were evident at 106 weeks. Since lung and kidney weights at 106 weeks were unaffected by treatment at all dose levels and there were no correlating histopathological alterations, the higher organ weights at the interim kills are considered not to be of toxicological relevance. Although other statistically significant differences between treated and control group organ weights were apparent, all are considered to reflect the differences in terminal body weights.

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

4.8.2 Gross and histopathology

- Macrospocopic findings: see Table A6_5_1-6.

Necropsy revealed treatment-related macroscopic lesions in the liver (enlargement or swelling), lungs (pale foci) and thyroid gland of animals treated at 4900ppm. The nature and incidences of all other gross lesions, mainly attributable to senility, early neoplastic change, environment and changes secondary to chronic disease were within the expected background range and are considered unrelated to treatment.

- Histopathology: see Tables A6_5_1-7 and A6_5_1-8.

Treatment-related, non-neoplastic histopathological alterations occurred in the liver and thyroid gland. Centrilobular hepatocyte enlargement occurred in 5 of 27 males and 8 of 26 females treated at 4900ppm and killed after 106 weeks. A further decedent male from this group also showed hepatocyte enlargement extending to mid-zonal hepatocytes. This alteration also occurred in 9 of 10 males and 3 of 10 females at 4900ppm killed after 26 weeks treatment, but it was not present in any animal at this dose level killed after 52 weeks treatment. Centrilobular hepatocyte enlargement did not occur in any of the control animals or in the animals treated with etofenprox at lower dose levels. Foci or areas of eosinophilic hepatocytes, sometimes associated with vacuolated hepatocytes, occurred at higher incidences in both sexes at 4900ppm and in males at 700ppm killed after 106 weeks. The alteration is considered to be treatment-induced. Foci of basophilic hepatocytes occurred at higher incidence in all male treated groups killed after 106 weeks, but since the incidences were not dose-related, the toxicological significance of the occurrence is equivocal.

Vacuolated centrilobular hepatocytes occurred at higher incidence in the females treated at 700 and both sexes at 4900ppm killed after 106 weeks. At 4900ppm, the change was associated with a higher incidence of lipid in hepatocytes. Males at 4900ppm also showed a higher incidence of pericholangitis than the controls. Since these alterations are common spontaneous geriatric changes, the higher incidences are considered not to be treatment-related. Examination of PAS-stained liver sections revealed no treatment-related differences between the treated and control groups.

There was a treatment-related increase in the incidence of cystic follicles in the thyroid gland of females at 4900ppm killed after 106 weeks or dying prematurely, but not in females at this dose level killed after 26 or 52 weeks treatment. There was also a higher incidence of increased height of the thyroid epithelial cells in females at 4900ppm killed after 26 weeks, but not in the groups killed after 52 or 106 weeks of treatment. Although the incidence of thyroid cystic follicles in males of all treated groups was higher than the control incidence after 106 weeks treatment, they were within the historical control range and considered unrelated to treatment with etofenprox.

All other non-neoplastic histopathological alterations are considered to be spontaneous in origin, frequently geriatric changes, and unrelated to treatment with etofenprox

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

4.8.2	Gross and histopathology	Treatment-related neoplastic alterations were confined to the thyroid gland of females treated at 4900ppm and scheduled to be killed after 106 weeks treatment. There was no statistically significant treatment-related effect on the incidences of follicular cell carcinoma in either males or females. In males, there was a statistically significant trend with dose ($p = 0.009$) for the combined incidences of follicular cell adenoma and carcinoma, but the pair-wise comparison between the control group and the 4900ppm group was not statistically significant ($p = 0.08$). Therefore, in the absence of a treatment-related statistically significant effect at 4900ppm, the significant trend test is considered not to be of toxicological importance. In the females, the combined incidences of adenoma and carcinoma showed a statistically significant positive trend ($p < 0.001$) and a significantly ($p = 0.005$) increased incidence at 4900ppm. The effect in females at 4900ppm was due to an increased incidence of benign follicular cell adenomas, since only a single carcinoma occurred at this dose level. All other neoplastic alterations were of the type, and occurred at incidences, expected for the strain used. Therefore, there was no indication of a carcinogenic effect at dose levels up to 4900ppm.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test guidelines: 88/302/EEC No deviations from test guideline. Description of method: rat, dietary exposure for at least 106 weeks, 4 dose levels plus one control group, satellite groups at each dose levels, with interim kill after 26 or 52 weeks of treatment, observations included body weight, food and water consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, urinanalysis, organ weight and full *post mortem* examination.	
5.2	**Results and discussion**	A no-observed-effect-level (NOEL) for all effects was established as 100ppm, equivalent to dose levels of 3.7 and 4.8mg/kg bw/day in males and females, respectively, based on an increased incidence of eosinophilic hepatocytes in males at 700ppm, and additionally at 4900ppm, reduced food consumption and weight gain, increased liver, kidney and thyroid weights, hepatocyte enlargement, prolonged blood clotting times (males only), and in females only, an increased incidence of benign thyroid follicular cell adenomas. A NOEL for carcinogenic effects was established as 4900ppm, the highest dose level employed, equivalent to dose levels of 187 and 249mg/kg bw/day in males and females, respectively, based on the absence of frank carcinogenic effects at this dose level.	X
5.3	**Conclusion**		
5.3.1	LO(A)EL	Not reported	

Section A6.5.1/01 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **110-week combined oncogenicity and chronic study, rat**

5.3.2	NOEL	- all effects: 3.7 and 4.8mg/kg bw/day in males and females, respectively. - carcinogenic effects: 187 and 249mg/kg bw/day in males and females, respectively.	X
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Table A6_5_1-1. Summary of group mean body weight gain data.

Period of treatment (weeks)	Group mean body weight gain (g) in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
0 - 26	377	387	381	359	359	173	171	172	163	155**
26 - 52	111	112	108	108	96*	79	74	76	72	65*
52 - 78	52	71	57	66	44	54	46	62	59	26**
78 - 110	88	38	15	-45	-44	69	47	35	52	-3
0 - 110	554	546	591	503	420	374	313	362	324	247

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

Table A6_5_1-2. Summary of group mean food consumption data.

Period of treatment (weeks)	Group mean food consumption (g/animal) in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
1 - 26	4333	4324	4321	4106	4298	3180	3056	3113	3064	3098
27 - 52	4350	4382	4324	4258	4342	3414	3262	3379	3306	3305
53 - 78	4534	4551	4472	4411	4401	3689	3496	3618	3564	3433**
79 - 104	4792	4792	4612	4553	4531	4045	3885	3866	3942	3814
105 - 110	1174	1174	1195	1047	980	1007	906	1003	938	942
1 - 110	19182	19182	19320	19413	16812	15398	14313	14537	14176	15216

** $p < 0.01$

Table A6_5_1-3. Group mean thrombotest times, with ranges where relevant.

Dose level (ppm)	Sex	Group mean thrombotest time (sec) in week:				
		16	25	51	77	102
0	Male	33 (28 - 40)	30 (27 - 33)	24 (22 - 26)	26 (20 - 30)	26 (23 - 31)
30		31 (29 - 34)	29 (25 - 32)	28* (22 - 32)	28* (24 - 31)	27 (23 - 30)
100		33 (30 - 36)	31 (29 - 35)	26* (23 - 29)	30** (26 - 36)	25 (20 - 29)
700		35 (26 - 47)	34** (28 - 37)	26* (22 - 28)	30** (29 - 32)	25 (22 - 28)
4900		38* (32 - 47)	38** (33 - 43)	27** (24 - 31)	31** (26 - 36)	25 (21 - 29)
0	Female	24	22	23	24	23
30		22	22	24	23	23
100		25	22	23	24	22
700		26*	23	23	23	23
4900		25*	22	22	23	22

* $p < 0.05$; ** $p < 0.01$; (range)

Table A6_5_1-4. Selected group mean urinalysis data.

Week of test and parameter (units)	Group mean value in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
Week 15										
- volume (mL)	6.3	5.3	6.6	5.9	5.8	3.1	4.0	3.7	3.1	3.6
- SG	1044	1045	1043	1044	1046	1047	1041	1044	1046	1043
- protein (mg/dL)	57	69	42	62	71	4	0	0	8	21**
Week 24										
- volume (mL)	4.1	2.9	5.0	4.6	5.5	2.9	3.8	3.1	2.7	3.7
- SG	1050	1052	1046	1050	1046	1050	1042	1047	1048	1046
- protein (mg/dL)	27	40	26	38	63*	11	1	1	6	13
Week 50										
- volume (mL)	4.7	5.3	4.9	6.4	7.1*	3.8	2.8	3.5	3.0	3.5
- SG	1047	1047	1047	1038*	1038*	1038	1043	1036	1043	1034
- protein (mg/dL)	67	151	81	154	201	214	108	108	253	97
Week 76										
- volume (mL)	8.2	8.1	7.4	6.0	6.5	7.9	5.9	6.1	6.0	5.6
- SG	1039	1043	1040	1041	1045	1031	1041	1033	1036	1034
- protein (mg/dL)	243	206	170	116	485	66	196	168	172	122
Week 103										
- volume (mL)	11.1	10.4	11.0	11.8	8.6	9.4	10.3	9.6	9.4	10.9
- SG	1037	1036	1037	1037	1036	1030	1029	1030	1033	1027
- protein (mg/dL)	350	322	636	174	243	170	310	285	613	181

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

Table A6_5_1-5. Selected group mean organ weight data.

Week of test and organ weight (g)	Group mean value in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
Week 26										
- liver	25.1	24.6	23.8	22.2	28.2**	12.6	13.1	11.7	10.8	15.6**
- kidneys	4.77	4.44	4.69	4.68	4.86	2.58	2.77	2.65	2.45	2.91*
- lungs	2.05	2.07	2.28	1.91	2.46**	1.54	1.68	1.59	1.52	1.74*
- thyroid (mg)	27	26	27	28	35*	20	18	20	21	21
Week 52										
- liver	24.4	25.9	25.9	24.9	31.9**	14.3	14.8	14.6	15.0	16.6*
- kidneys	4.19	4.86	4.60	4.99**	5.05**	3.38	2.94	3.01	3.02	3.14
- lungs	1.99	2.14	2.07	2.03	1.99	1.45	1.52	1.54	1.53	1.60
- thyroid (mg)	31	30	35	36	35	25	32	26	25	27
Week 106										
- liver	25.5	25.4	25.9	26.2	29.2**	19.2	18.2	19.1	20.0	21.9**
- kidneys	6.52	6.64	6.80	6.70	6.76	4.04	3.99	3.78	3.88	3.80
- lungs	2.51	2.24	2.24	2.27	2.31	1.81	1.67	1.62	1.77	1.75
- thyroid (mg)	37	41	40	46*	50**	39	32	34	36	41

* $p < 0.05$ (data adjusted using body weight as covariate); ** $p < 0.01$ (data adjusted using body weight as covariate)

Table A6_5_1-6. Incidences of selected gross lesions at necropsy.

Type	Sex	Organ and lesion	Incidence of lesion at (ppm):				
			0	30	100	700	4900
Incidental deaths	Male	Number examined	27	26	27	21	23
		Liver enlargement	3	2	5	1	4
		Liver swelling	6	7	9	4	5
		Lungs - pale foci	9	5	7	4	9
		Thyroid enlarged	1	0	0	0	0
	Female	Number examined	25	33	30	34	24
		Liver enlargement	1	1	1	1	1
		Liver swelling	12	8	8	12	7
		Lungs - pale foci	4	6	7	10	9
		Thyroid enlarged	0	0	0	0	1
Termination (week 106)	Male	Number examined	23	24	23	29	27
		Liver enlargement	1	1	0	1	4
		Liver swelling	4	7	4	10	9
		Lungs - pale foci	4	8	12	12	19
		Thyroid enlarged	0	0	5	4	3
	Female	Number examined	25	17	20	16	26
		Liver enlargement	1	0	0	3	2
		Liver swelling	5	3	6	3	6
		Lungs - pale foci	5	6	4	5	10
		Thyroid enlarged	2	0	1	0	4

Table A6_5_1-7. Incidences of selected histopathological alterations.

Necropsy	Sex	Organ and lesion	Incidence of lesion at (ppm):				
			0	**30**	**100**	**700**	**4900**
Incidental deaths	Male	Number examined	27	26	27	21	23
		Hepatocyte enlargement	0	0	0	0	1
		Eosinophilic hepatocytes	1	0	2	2	6
		Basophilic hepatocytes	1	3	2	3	3
		Pericholangitis	5	6	6	6	9
		Thyroid cystic follicles	3	3	2	2	2
	Female	Number examined	25	33	30	34	24
		Hepatocyte enlargement	0	1	0	0	0
		Eosinophilic hepatocytes	2	2	1	5	10
		Basophilic hepatocytes	8	12	17	17	14
		Pericholangitis	3	1	5	7	3
		Thyroid cystic follicles	0	1	3	5	6
Week 26 (scheduled)	Male	Number examined	10	10	10	10	10
		Hepatocyte enlargement	0	0	0	0	9
		Eosinophilic hepatocytes	0	0	0	0	0
		Basophilic hepatocytes	0	0	0	0	0
		Pericholangitis	0	0	0	1	2
		Thyroid cystic follicles	0	0	0	0	0
		Increased height of thyroid follicular epithelium	0	0	0	0	0
	Female	Number examined	10	10	10	10	10
		Hepatocyte enlargement	0	0	0	0	3
		Eosinophilic hepatocytes	0	0	0	0	0
		Basophilic hepatocytes	0	0	0	0	0
		Pericholangitis	0	0	0	1	0
		Thyroid cystic follicles	0	0	0	0	0
		Increased height of thyroid follicular epithelium	0	0	1	0	5

Table A6_5_1-7 (continued). Incidences of selected histopathological alterations.

Necropsy	Sex	Organ and lesion	Incidence of lesion at (ppm):				
			0	**30**	**100**	**700**	**4900**
Week 52 (scheduled)	Male	Number examined	10	10	9	9	10
		Hepatocyte enlargement	0	0	0	0	0
		Eosinophilic hepatocytes	0	0	0	0	0
		Basophilic hepatocytes	0	0	0	0	0
		Pericholangitis	0	0	1	1	2
		Increased height of thyroid follicular epithelium	0	0	1	0	0
	Female	Number examined	10	10	10	10	9
		Hepatocyte enlargement	0	0	0	0	0
		Eosinophilic hepatocytes	0	0	0	0	0
		Basophilic hepatocytes	0	0	0	0	0
		Pericholangitis	1	1	2	0	0
		Increased height of thyroid follicular epithelium	0	0	0	0	0
Week 106 (scheduled)	Male	Number examined	23	24	23	29	27
		Hepatocyte enlargement	0	0	0	0	5
		Eosinophilic hepatocytes	3	11	9	15	16
		Basophilic hepatocytes	4	17	13	25	10
		Pericholangitis	9	5	6	8	19
		Thyroid cystic follicles	0	5	6	6	5
		Increased height of thyroid follicular epithelium	0	0	0	0	0
	Female	Number examined	25	17	20	16	26
		Hepatocyte enlargement	0	0	0	0	8
		Eosinophilic hepatocytes	4	3	5	2	12
		Basophilic hepatocytes	16	11	15	9	16
		Pericholangitis	1	3	2	2	5
		Thyroid cystic follicles	4	0	4	1	7
		Increased height of thyroid follicular epithelium	0	0	0	0	0

Table A6_5_1-8. Incidences of selected neoplastic histopathological alterations in all animals scheduled to be killed after 106 weeks.

Sex	Thyroid gland alteration	Incidence at (ppm):				
		0	30	100	700	4900
Male	No. animals examined	50	50	50	50	50
	Follicular cell carcinoma	0	0	1	3	2
	Follicular cell adenoma	6	6	4	5	11
	Follicular cell adenoma and/or carcinoma	6	6	5	8	13
Female	No. animals examined	50	50	50	50	50
	Follicular cell carcinoma	0	0	0	2	1
	Follicular cell adenoma	0	3	2	0	9
	Follicular cell adenoma and/or carcinoma	0	3	2	2	9*

*p = 0.005

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** Annex V method B33 "Combined chronic/ carcinogenicity test" **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	**5.3.2. NOAEL** The data representation, discussion and derivation of the overall NO(A)EL for this study is consistent and can be agreed to. It is recognised that there is a probability of thyroid tumour development at high doses of 186,7 (m) and 249 (f) mg/kg bw/day (50x above the NOAEL of 3,7(m) and 4,8 (f) mg/kg bw/day). However this is mechanistically of minor relevance (seeA6.10 mechanistic study and remarks below).
Reliability	1
Acceptability	acceptable.
Remarks	**4.2.2. Mortality** till week 78 below 50% for each group and at termination no difference between control and treated groups. **4.3. Body weight gain** was not significantly reduced in low dose groups. Body weight changes after week 78 are less relevant (geriatric effects). **4.8.2; 5.2; 5.3.2:** Neoplastic alterations of thyroid: The p-value of 0,08 calculated for the pair-wise comparison of males from control and high dose group indicates non- significance for an α of 5%. However 5% is a cut off value, useful for decisions but not necessarily for biological interpretation. Therefore in the light of the significant trend test for males, the significant though benign effect with females and the thyroid organ weight, marcroscopic and histological alterations it is prudent to assume that at the high doses of 187 (male) or 249 (female) mg/kg bw there is some probability of thyroid tumour development, whereby the incidence for males is borderline to statistical significance. However, the genotoxicity tests are negative, the neoplastic effect is seen only at very high doses, the mechanistic study indicates that the effect is mediated by an indirect, non-genotoxic mechanism with a clear NOAEL for the primary effect on the liver of 81.2mg/kg bw/day and the effect is due to different T4 kinetics less relevant for humans. Therefore the neoplastic alternations of the thyroid are of minor importance. The overall NOAEL of 3,7 and 4,8 mg/kg bw for this study remains acceptable.
	COMMENTS FROM...
Date	

Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.5.1/02 Chronic toxicity (rodent)

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1986b): Ethofenprox (MTI-500) potential tumorigenic and toxic effects in prolonged dietary administration to mice; [redacted] unpublished report no. MTC 58/85582 (January 06, 1986). Dates of work: January 05, 1983 - January 31, 1985.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted] Mitsui Chemcals 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 Method conformed to 88/302/EEC.	X
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. 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	Mouse	
3.2.2	Strain	Swiss mice (CD1 strain)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	6 week old , group mean bodyweight ranges 29 - 31g for males and 21 - 22g for females	
3.2.6	Number of animals per group	- Main groups: 52 animals/sex/group - Satellite groups (at each dose level, intented for blood and urinanalysis at intervals and for interim kill after 26 or after 56 weeks): 24 animals/sex/group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.5.1/02 Chronic toxicity (rodent)

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

3.3.1	Duration of treatment	At least 104 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Concentration	- Constant nominal concentrations: 0, 30, 100, 700 and 4900ppm - Overall mean achieved dose levels: 0, 3.1, 10.4, 75.2 and 546.9mg/kg b.w/day (males) and 0, 3.6, 11.7, 80.9 and 615.5mg/kg b.w/day (females)
3.3.4.3	Vehicle	Corn oil
3.3.4.4	Concentration in vehicle	Not reported
3.3.4.5	Total volume applied	Not reported
3.3.4.6	Controls	Untreated diet containing a comparable volume of corn oil.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. The animals were observed once daily for clinical signs of toxicity for the first 4 weeks and weekly thereafter. The weekly clinical examinations included a palpation for tissue masses.
3.4.1.2	Mortality	Yes, checked twice daily. Animals found dead and those killed in extremis were subjected to necropsy as soon as possible after death.
3.4.2	Body weight	Yes, recorded weekly throughout the study.
3.4.3	Food consumption	Yes, recorded weekly throughout the study.
3.4.4	Water consumption	Yes, monitored by visual inspection throughout the study and accurate measurement was performed for the groups treated at 0 or 4900ppm in weeks 5, and for all main study groups during weeks 12 and 23.
3.4.5	Ophthalmoscopic examination	Yes. Ophthalmic examinations were performed on all animals treated at 0 or 4900ppm pre-dose and in weeks 14, 26, 52, 78 and 100.
3.4.6	Haematology	Yes. Blood samples were collected for haematology from 10 animals/sex/group in weeks 14 - 16, 24 - 26, 50/52, and from main group animals in weeks 76 - 78 and 101 - 103. Parameters: packed cell volume, haemoglobin concentration, red cell count, reticulocyte count, mean corpuscular haemoglobin, mean cell volume, total white cell count, differential WBC count, platelet count.

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

3.4.7	Clinical Chemistry	Yes. Blood samples were collected for clinical chemistry from 10 animals/sex/group in weeks 14 - 16, 24 - 26, 50/52, and from main group animals in weeks 76 - 78 and 101 - 103. Parameters: glucose, total protein, albumin, globulin, urea nitrogen, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, cholesterol.
3.4.8	Urinalysis	Yes. Urine samples were collected for urinalysis from 10 animals/sex/group in weeks 14 - 16, 24 - 26, 50/52, and from main group animals in weeks 76 - 78 and 101 - 103. Urine samples were collected during food and water deprivation. Parameters: volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, haemoglobin.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. All animals surviving to scheduled sacrifice. Organs: adrenals, brain, heart, liver, kidneys, lungs, ovaries, pituitary, spleen, testes, thymus, thyroids, uterus.
3.5.2	Gross and histopathology	Yes. All animals Organs and tissues: adrenals, brain, eyes, gall bladder, gastro-intestinal tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon and rectum), harderian gland, head, heart, kidneys, liver, lungs, lymph nodes, mammary gland, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal column, spleen, sternum, testes with epididymides, thymus, thyroids, tongue, trachea, urinary bladder, uterus. Two additional frozen sections of liver were stained with oil red O and PAS and an additional section of kidney was stained with PAS or oil red O.
3.5.3	Statistics	Where applicable, data were analysed statistically by Bartletts' test for equality of variance followed by one-way ANOVA or the Kruskal-Wallis ranking test. ANOVA was followed by Student's t-test and Williams' test. The Kruskal-Wallis test was followed by non-parametric equivalents of the t-test and Williams' test. Where appropriate, ANCOVA was used in place of ANOVA. Mortality was analysed using log-rank methods. Selected tumours were analysed using the time-to-tumour method according to IARC (1980) recommendations. Trend tests were also applied based on nominal dose levels.

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

3.6	**Further remark**	- The stability and homogeneity of diet formulations containing 20 or 20000ppm were confirmed by analysis prior to start of the study. Achieved concentrations in all diets were determined by analysis in week 1 and subsequently at 3-monthly intervals. - Pre-treatment health check: ten males and ten females were selected for health check purposes at the time of allocation of animals to groups. these mice were then killed and a macroscopic examination was carried out.	
		4 RESULTS AND DISCUSSION	
4.1	**Analysis of diet**	Analysis of diet samples demonstrated adequate homogeneity, stability and achieved concentration of etofenprox in diet. Mean values of the homogeneity ranged from 100.5 to 106% and 96.5 to 101% of the theoretical concentrations of 20 and 20000ppm, respectively. Stability analysis revealed etofenprox was stable in diet at concentrations of 20 or 20000ppm at ambient temperature for 18 days, at which time 100.5 and 104.5% nominal concentrations remained. The mean achieved concentrations of all diets analysed on 9 occasions ranged from 90.7 to 120% of nominal concentrations, although on one occasion the 30ppm diet was 133% nominal concentration.	
4.2	**Observations**		
4.2.1	Clinical signs	There were no treatment-related clinical signs of toxicity at any dose level in either sex, other than a few male mice at 4900ppm that showed slight pallor of the extremities.	
4.2.2	Mortality	There was a treatment-related increase in male mortality at 4900ppm, but not in the females at 4900ppm or in either sex at lower dose levels. The effect in males at 4900ppm is considered to be related to the increased incidence of renal lesions. Survival incidences at termination were 46, 44, 27, 35 and 19% (males) and 54, 54, 48, 44 and 54% (females), in order of ascending dose level starting with controls.	X
4.3	**Body weight gain**	See Table A6_5_2-1. The body weight gains of both sexes treated at 4900ppm were significantly ($p < 0.05$ or 0.01) reduced during the first 52 weeks of treatment leading to reductions in overall weight gain of 27.8 and 13.8% in males and females, respectively. There was no effect of treatment on body weight gain at lower dose levels, although males at 700ppm showed significantly ($p < 0.01$) reduced overall weight gain due to group mean weight loss in weeks 78 - 107.	X

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

4.4	**Food consumption and compound intake**	See Table A6_5_2-1. There was no effect of treatment on food consumption in either sex at any dose level. Although the food consumption in weeks 1 - 26 for all male treated groups was significantly ($p < 0.05$) higher than control consumption by 2 - 8%, the food consumption in subsequent 26-week blocks and the overall consumption during weeks 1 - 107 of the male treated groups were not significantly different ($p > 0.05$) from the control values. Female food consumption at all dose levels was comparable to, and not significantly ($p > 0.05$) different from, control consumption throughout the study. Water consumption was significantly ($p < 0.01$ or 0.001) increased by up to 51.7% at 4900ppm during weeks 12 and 23 in males and in weeks 5, 12 and 23 in females. The water consumption at lower dose levels was unaffected by treatment, although males at 700ppm showed significantly ($p < 0.01$) higher water consumption in week 23 which is considered to be due to higher food consumption.
4.5	**Ophtalmoscopic examination**	There were no treatment-related ophthalmological findings in either sex treated at 4900ppm at any of the examinations.
4.6	**Blood analysis**	
4.6.1	Haematology	See Table A6_5_2-2. Males treated at 4900ppm showed evidence of slight anaemia during the first 52 weeks of study characterised by significantly ($p < 0.05$ or 0.01) reduced haemoglobin concentration (Hb), red blood cell counts (RBC) and mean cell haemoglobin concentration (MCHC) and raised mean cell volume (MCV). MCHC and MCV were also significantly ($p < 0.05$ or 0.01) affected in males at 700ppm, as was MCHC in males at 100ppm in weeks 26 and 52. There was no evidence of anaemia in females at any dose level or in males at 30ppm. The effects on red blood cell parameters were not evident subsequently in weeks 78 and 101. Other treatment-related effects on haematological profiles were confined to significantly ($p < 0.05$ or 0.01) increased platelets counts in females at 4900ppm in weeks 78 and 101 which were up to 61.5% higher than control values.
4.6.2	Clinical chemistry	There were no effects on the clinical chemistry profiles at any dose level or sampling interval that were considered to be related to treatment. There was a number of statistically significant ($p < 0.05$ or 0.01) differences between the treated and control groups in the week 16, 26 and 50 investigations, but all individual values were considered to be within the normal range of variation. The differences comprised slightly lower glucose concentrations in all female treated groups in week 16, marginally increased total protein and globulin concentrations in males at 4900ppm in week 26, increased urea concentrations in males at 700 and 4900ppm and decreased urea concentrations in females at 100, 700 and 4900ppm in week 50. Subsequent clinical investigations showed significantly higher urea concentrations in males at 100, 700 and 4900ppm in week 76, and higher alkaline phosphatase and GOT activities in males at 4900ppm in week 103. These values were considered to represent normal variation for animals of this age.

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

4.7	**Urinalysis**	Treatment-related effects on urine parameters were confined to a larger volume of more dilute urine in male treated groups in week 52 and reduced urine specific gravity in males at 4900ppm in weeks 77 and 102. There were no other qualitative or quantitative differences between the control and treated groups that were considered to be treatment-related.
4.8	**Sacrifice and pathology**	
4.8.1	Organ weights	See Table A6_5_1-3. Liver weights, adjusted using body weight as covariate, of both sexes at 4900ppm were increased in week 26 and at termination, but not at 52 weeks. The effect was statistically significant ($p < 0.05$) in males at 26 weeks and in females at termination ($p < 0.01$). Lower dose groups were not affected at any sampling interval. The only other statistically significant inter-group difference in organ weights was spleen weight in males treated at 4900ppm in week 52. Females at 4900ppm and both sexes at lower dose groups were not affected. The spleen weights of both sexes at all other sampling intervals were not significantly different ($p > 0.05$) from the controls.

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

4.8.2 Gross and histopathology

- Macrospocopic findings: see Table A6_5_2-4.

Treatment-related macroscopic findings at necropsy were confined to the kidneys. Renal cortical scarring occurred at higher incidences in both sexes at 4900ppm. The effect was also apparent in males at 4900ppm killed after 26 or 52 weeks of treatment (6 occurrences compared with an incidence of 0 in control males). Pallor of the kidneys occurred at higher incidence in both sexes treated at 100, 700 or 4900ppm, and masses or unilateral enlargement occurred at higher incidences in males at 4900ppm. The nature and incidence of all other gross findings at necropsy were within background ranges and were considered to be incidental to treatment with etofenprox.

- Histopathology: see Table A6_5_2-5.

Treatment-related non-neoplastic histopathological alterations at week 52, at termination and in incidental deaths were confined to the kidneys since there were no histopathological correlates for the increased liver weight at 4900ppm. The renal change was predominantly a tubular lesion of groups of basophilic and dilated tubules, sometimes associated with focal tubular loss. An increased incidence and/or severity of the lesion occurred in both sexes at 4900ppm in week 52, and in both sexes at 100, 700 and 4900ppm at termination and/or in incidental deaths. Grades 1 / 2 were generally minimal severity with few tubules showing the lesion. Grades 3 / 4 / 5 were more severe, and involved the cortex, medulla and papilla. Grade 3 was classified as moderate severity involving large groups of tubules. Grades 4 / 5 were more marked and involved the entire renal parenchyma, and resulted in, or contributed to, death in some animals. Dilated/cystic Bowman's capsules, dilated medullary tubules, focal loss of tubules, prominent interstitial papillary tissue and papillary mineralization were associated with the primary renal change and showed a similar pattern of increasing incidence with dose level. Cortical cysts lined by columnar epithelium also showed an increased incidence in animals treated at 4900ppm. Treatment-related renal lesions were not apparent at 30ppm, although 3 males at 30ppm showed grade 2 tubular lesions, a severity grade observed in untreated mice in previous studies. There were no other treatment-related non-neoplastic histopathological alterations at any dose level.

There were no treatment-related effects in either sex at any dose level on the incidence of any tumour type or on the total number of tumour-bearing animals. However, 2 terminal kill males and one male decedent at 4900ppm, and one decedent male at 700ppm, showed a renal tumour, 2 of which were benign at 4900ppm. Renal tumours did not occur in any female group or in the other male treated or control groups. The laboratory historical control incidence of renal cortical tumours is 0.0 - 2.0%, suggesting the incidence at 4900ppm slightly exceeds the historical control incidence. Although the trend test for renal cortical adenoma + carcinoma was statistically significant ($p = 0.001$) for groups 1 - 5, the comparison of incidences between the control and 4900ppm groups was not statistically significant ($p = 0.08$). The trend test for groups 1 - 4 was marginally significant ($p = 0.046$) and there was no statistical significance between the control and 700ppm group ($p = 0.47$). Since only a single malignant renal tumour occurred in each of the groups treated at 700 and 4900ppm, it is concluded that there was no evidence of carcinogenic potential.

Section A6.5.1/02 **Chronic toxicity (rodent)**

Annex Point IIA-VI.6.5 **108-week combined oncogenicity and chronic study, mouse**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines not specified in report but method conformed to 88/302/EEC. No deviations from test guideline 88/302/EEC. Description of method: mouse, dietary exposure for at least 104 weeks, 4 dose levels plus one control group, satellite groups at each dose levels, with interim kill after 26 or 52 weeks of treatment, observations included body weight, food and water consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, urinanalysis, organ weight and full *post mortem* examination.
5.2	**Results and discussion**	A no-observed-effect-level (NOEL) for all effects was established as 30ppm, equivalent to dose levels of 3.1 and 3.6mg/kg bw/day in males and females, respectively, based on an increased incidence of dilated / basophilic renal cortical tubules at dose levels ≥ 100ppm, and additionally at 4900ppm, increased mortality in males, reduced weight gain, minor haematological changes and increased liver weight. A NOEL for carcinogenic effects was established as 4900ppm, the highest dose level employed, equivalent to dose levels of 546.9 and 615.5mg/kg bw/day in males and females, respectively, based on no excess tumours at this dose level.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported
5.3.2	NOEL	- all effects: 3.1 and 3.6mg/kg bw/day in males and females, respectively. - carcinogenic effects: 546.9 and 615.5mg/kg bw/day in males and females, respectively.
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6_5_2-1. Summary of group mean body weight gain, food and water consumption.

Parameter:	**Group mean weight gain (g), total food (g/animal) and water (mL/day) consumption in:**									
Interval	**Males treated at (ppm):**					**Females treated at (ppm):**				
	0	**30**	**100**	**700**	**4900**	**0**	**30**	**100**	**700**	**4900**
Food:										
- week 1 - 26	757	816*	803*	798*	769*	716	739	724	722	727
- week 27 - 52	849	888	869	904	879	820	829	796	790	778
- week 53 - 78	787	814	862	849	827	750	766	740	728	727
- week 79 - 107	1040	1022	1076	979	953	790	887	794	843	806
- week 1 - 107	3431	3506	3683	3404	3655	2879	3157	2861	2955	2955
Water:										
- week 5	5.8	-	-	-	6.3	6.1	-	-	-	6.8**
- week 12	6.1	6.3	6.4	6.7	8.5***	6.5	6.5	6.7	6.4	7.9***
- week 23	6.0	5.7	6.3	7.0**	9.1***	6.6	6.5	6.8	6.5	8.2***
Weight gain:										
- week 0 - 26	14.6	14.4	13.9	13.4	10.4**	9.3	11.2	9.6	10.2	8.3*
- week 26 - 52	1.9	2.1	2.2	2.3	0.5*	2.4	2.4	2.8	2.3	1.3*
- week 52 - 78	0.5	1.0	1.6	0.0	-0.9	1.8	2.0	1.3	1.9	1.3
- week 78 - 107	-2.0	-3.7	-1.3	-3.5	-0.3	0.4	-1.8	-1.1	-3.5	-0.8
- week 0 - 107	17.6	14.4	13.0	8.9**	12.7*	12.4	13.0	11.6	14.8	10.7

Table A6_5_2-2. Selected group mean haematological parameters at representative intervals.

Interval:	**Group mean haematological parameters in:**									
- Parameter (unit)	**Males treated at (ppm):**					**Females treated at (ppm):**				
	0	**30**	**100**	**700**	**4900**	**0**	**30**	**100**	**700**	**4900**
Week 15:										
- PCV (%)	48	49	48	48	47	47	47	49	48	46
- Hb (g/dL)	14.0	14.2	14.0	13.6	12.9**	13.8	13.9	14.1	13.9	13.1*
- RBC (10^6/mm^3)	7.5	7.5	7.3	7.1	7.0*	7.0	7.2	7.4	7.3	6.6
- MCHC (%)	29.4	29.1	28.9	28.3*	27.8**	29.4	29.7	28.9	28.9	28.7
- MCV (fL)	64	65	66	68*	66*	67	65	66	66	69
Week 26:										
- PCV (%)	45	44	46	47	44	46	47	47	47	44
- Hb (g/dL)	14.4	14.1	13.7	14.3	12.8**	13.8	14.3	14.5	14.5	13.4
- RBC (10^6/mm^3)	7.6	7.2	7.0	7.5	6.8*	7.2	7.3	7.5	7.1	6.5
- MCHC (%)	31.7	32.1	29.8*	30.2*	28.9**	30.0	30.5	30.8	30.6	30.3
- MCV (fL)	60	61	67*	63*	65**	65	65	63	67	68
Week 52:										
- PCV (%)	46	43	50	46	42*	45	45	46	48	46
- Hb (g/dL)	15.3	14.3	15.5	13.5	12.7**	14.1	14.4	14.6	15.3	14.5
- RBC (10^6/mm^3)	7.8	7.2	8.0	6.6	6.3**	6.9	7.0	7.4	7.9	7.2
- MCHC (%)	33.5	33.3	31.0*	29.2**	30.3**	31.9	32.1	31.8	31.5	31.9
- MCV (fL)	59	61	63	74**	68**	65	65	63	62	64
Week 101:										
- PCV (%)	45	44	43	48	43	41	40	41	39	40
- Hb (g/dL)	13.0	13.1	12.8	13.5	12.4	12.0	12.1	12.2	11.4	11.4
- RBC (10^6/mm^3)	7.6	7.6	7.2	7.9	6.7	6.7	6.9	6.6	6.4	6.2
- MCHC (%)	29.0	30.0	29.5	28.3	28.4	28.6	30.0	29.4	28.9	28.5*
- MCV (fL)	59	58	60	61	66**	67	59	65	62	65*

Table A6_5_2-3. Selected group mean organ weights.

Interval: - organ (units)	Group mean organ weights in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
Week 26:										
- liver[a] (g)	2.09	2.02	2.17	2.11	2.43*	1.55	1.59	1.69	1.72	1.63
- spleen (g)	0.132	0.114	0.103	0.142	0.141	0.121	0.120	0.119	0.116	0.122
Week 52:										
- liver[a] (g)	2.27	2.35	2.48	2.40	2.35	2.09	1.93	1.81	1.81	1.85
- spleen (g)	0.121	0.134	0.123	0.135	0.160*	0.158	0.130	0.108	0.111	0.141
Termination:										
- liver[a] (g)	2.87	2.83	2.94	2.80	3.16	1.83	1.90	1.99	1.87	2.01**
- spleen (g)	0.138	0.169	0.127	0.117	0.105	0.157	0.223	0.239	0.232	0.190

[a] weights adjusted using body weight as covariate before statistical analysis; * $p < 0.05$; ** $p < 0.01$

Table A6_5_2-4. Incidence of selected macroscopic findings in kidneys at necropsy - animals dying during the study or killed after at least 104 weeks.

Organ: - finding	Number of occurrences in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
Kidneys:										
- mass(es)	0	0	0	1	4	1	1	2	0	1
- cortical scarring	12	5	9	10	23	7	8	4	6	19
- enlargement	1	3	3	4	7	0	1	4	1	0
- pale colour	7	8	12	15	23	6	8	12	10	13

Table A6_5_2-5. Selected non-neoplastic histopathological alterations in the kidneys.

Interval: - kidney lesion	Incidence in:									
	Males treated at (ppm):					Females treated at (ppm):				
	0	30	100	700	4900	0	30	100	700	4900
Week 26 - no. examined	10	10	10	10	10	10	10	10	10	10
Dilated/basophilic tubules[a]:										
- grade 1	0	0	0	0	0	1	0	0	0	0
- grade 2	0	0	0	0	2	0	0	0	0	1
- grade 3	0	0	0	0	0	0	0	0	0	0
- grade 4	0	0	0	0	0	0	0	0	0	0
- grade 5	0	0	0	0	0	0	0	0	0	0
Week 52 - no. examined	13	8	13	13	12	12	13	14	13	13
Dilated/basophilic tubules[a]:										
- grade 1	0	0	0	0	2	1	0	0	3	3
- grade 2	0	0	0	0	0	0	0	0	0	0
- grade 3	0	0	0	0	2	0	0	0	0	1
- grade 4	0	0	0	0	1	0	0	0	0	0
- grade 5	0	0	0	0	0	0	0	0	0	0

Table A6_5_2-5 (continued). Selected non-neoplastic histopathological alterations in the kidneys.

Interval: **- kidney lesion**	**Incidence in:**									
	Males treated at (ppm):					**Females treated at (ppm):**				
	0	**30**	**100**	**700**	**4900**	**0**	**30**	**100**	**700**	**4900**
Termination - no. examined	24	23	14	18	10	28	28	25	23	28
Dilated/basophilic tubules[a]:										
- grade 1	5	3	3	6	3	3	0	2	3	6
- grade 2	0	1	2	1	0	0	0	0	1	4
- grade 3	0	0	0	0	3	0	0	0	0	5
- grade 4	0	0	0	0	0	0	0	0	0	0
- grade 5	0	0	0	0	0	0	0	0	0	0
- dilated/cystic Bowman's capsule	1	3	2	8	2	2	2	3	4	9
- dilated medullary tubules	1	0	0	4	3	0	0	0	0	5
- focal loss of tubules	0	1	0	0	2	2	2	1	4	11
- prominent interstitial papillary tissue	0	0	0	0	2	0	0	0	0	8
- papillary mineralisation	0	0	0	0	1	0	0	0	0	6
- corticomedullary scarring	0	1	0	0	1	3	0	0	1	10
- cortical cyst(s)[b]	3	3	4	5	2	2	1	1	5	8
Unscheduled - no. examined	28	29	38	33	42	24	24	27	29	24
Dilated/basophilic tubules[a]:										
- grade 1	2	5	3	3	2	0	0	3	3	1
- grade 2	0	2	2	5	4	1	1	1	0	2
- grade 3	0	0	1	1	8	0	0	0	2	1
- grade 4	0	0	1	1	6	0	0	1	0	1
- grade 5	0	0	0	0	4	0	0	0	0	0
- dilated/cystic Bowman's capsule	0	2	1	2	12	1	1	1	2	2
- dilated medullary tubules	0	1	2	3	15	1	1	1	1	1
- focal loss of tubules	0	0	2	1	16	0	0	1	0	1
- prominent interstitial papillary tissue	0	0	0	0	9	0	0	1	0	10
- papillary mineralisation	0	0	0	0	2	0	0	0	0	1
- corticomedullary scarring	0	0	0	0	0	0	0	0	0	0
- cortical cyst(s)[b]	8	4	5	5	19	2	4	4	7	7

[a] cortical tubules [b] cyst(s) lined by columner epithelium

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** Annex V method B33 "Combined chronic/ carcinogenicity test" **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	**4.2.2. Mortality** Survival in all groups is > 50% till week 78 **4.3. Body weight gain** was not significantly reduced in low dose groups
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.5.2 Chronic toxicity (non rodent)

Annex Point IIA-VI.6.5 **52-week, dog**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985b): Ethofenprox (MTI-500) toxicity to dogs by repeated dietary administration for 52 weeks followed by a recovery period of 8 weeks; [redacted] unpublished report no. MTC 71/85234 (October 25, 1985). Dates of work: September 06, 1983 - November 01, 1984.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
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 Method conformed to 88/302/EEC.	
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. 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	Dog	
3.2.2	Strain	Ppurebred beagle dogs	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	22 - 27 weeks old / 7.2 - 9.8kg body weight	

Section A6.5.2 Chronic toxicity (non rodent)

Annex Point IIA-VI.6.5 **52-week, dog**

3.2.6	Number of animals per group	- main groups (treated for 52 weeks and then sacrificed): 4 males and 4 females per group - recovery groups (control and high dose level; treated for 52 weeks but then maintained untreated under observation for 8 weeks after the cessation of treatment, before necropsy): 2 males and 2 females per group.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	52 weeks
3.3.2	Frequency of exposure	Daily
3.3.3	Postexposure period	- none for main groups - 8 weeks for the two recovery groups (control and high dose)
3.3.4	**Oral**	
3.3.4.1	Type	in food
3.3.4.2	Concentration	- constant nominal concentrations: 0, 100, 1000 and 10000ppm - overall mean achieved dose levels were 0, 3.46, 33.4 and 352mg/kg bw/day (males) and 0, 3.17, 32.2 and 339mg/kg bw/day (females).
3.3.4.3	Vehicle	Corn oil
3.3.4.4	Concentration in vehicle	Not reported
3.3.4.5	Total volume applied	Not reported
3.3.4.6	Controls	Untreated diet containing a comparable volume of corn oil.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes. The animals were observed frequently throughout each working day for overt signs of toxicity.
3.4.1.2	Mortality	Yes, checked daily.
3.4.2	Body weight	Yes. Body weights were recorded pre-dose, weekly throughout the study and at necropsy.
3.4.3	Food consumption	Yes. Individual food consumption was recorded daily.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes. Ophthalmological examinations were performed on all animals pre-test and during weeks 6, 12, 25 and 51 using an indirect ophthalmoscope.

Section A6.5.2 **Chronic toxicity (non rodent)**

Annex Point IIA-VI.6.5 **52-week, dog**

3.4.6	Haematology	Yes. Performed on all animals (food-deprived) pre-test and during weeks 6, 12, 25 and 51 of treatment and in week 8 of the recovery period. Parameters: packed cell volume, red cell count, mean corpuscular haemoglobin concentration, mean corpuscular volume, total white cell count, platelet count, erythrocyte sedimentation rate, reticulocyte count, differential WBC count, prothrombin time, activated partial thromboplastin time.
3.4.7	Clinical Chemistry	Yes. Performed on all animals (food-deprived) pre-test and during weeks 6, 12, 25 and 51 of treatment and in week 8 of the recovery period. Parameters: total protein, albumin, globulin, urea, creatinine, sodium, potassium, calcium, chloride, inorganic phosphorus, cholesterol, glucose, alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase, total bilirubin, gamma-glutamyltransferase, creatine phosphokinase, ornithine carbamoyltransferase activity.
3.4.8	Urinalysis	Yes. Performed on all animals (food-deprived) pre-test and during weeks 6, 12, 25 and 51 of treatment and in week 8 of the recovery period. Parameters: volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, haem. pigments.
3.4.9	Analytics	The homogeneity and stability of 100 and 10000ppm formulations were confirmed by analysis prior to the start of the study. Achieved concentrations in all diets were confirmed in weeks 1, 13, 26 and 52.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes. Organs: adrenals, brain, heart, liver, kidneys, lungs, pancreas, pituitary, spleen, testes, ovaries, thymus, thyroids, uterus, prostate.
3.5.2	Gross and histopathology	Yes. All animals Organs and tissues: , adrenals, aorta, brain, caecum, colon, duodenum, eyes and optic nerves, femur and articular surface, gall bladder, , heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes, mammary gland, oesophagus, pancreas, pituitary, rectum, salivary glands, sciatic nerve, , skeletal muscle, skin, spinal cord, sternum, spleen, stomach, testes, ovaries, thymus, , thyroids, tongue, trachea, urinary bladder, uterus, prostate, vagina.

Section A6.5.2 **Chronic toxicity (non rodent)**

Annex Point IIA-VI.6.5 **52-week, dog**

3.5.3	Statistics	Where applicable, data were analysed statistically by ANOVA followed by Student's t-test and Williams' test, or Bartletts' test for equality of variance followed by one-way ANOVA or the Kruskal-Wallis ranking test, or Fisher's exact probability test and Mantel's test, or ANCOVA. Inter-group comparisons were performed using Williams' test for a dose-related response.
3.6	**Further remarks**	Additional frozen samples of liver and kidneys were stained with oil red O.

4 RESULTS AND DISCUSSION

4.1	**Analytics**	The homogeneity and stability of etofenprox diet formulations at concentrations of 100 and 10000ppm were shown to be satisfactory. After 18 days storage at ambient room temperature, means of 91.7% and 92.6% starting concentrations remained. The relative standard deviations at 100 and 10000ppm for 6 or 12 homogeneity analyses were 6.22 and 1.24, respectively. The achieved concentration of etofenprox in the experimental diets was satisfactory at all 4 sampling intervals. All diets at all sampling intervals were within the range 90.5 - 104% nominal concentrations.
4.2	**Observations**	
4.2.1	Clinical signs	There were no treatment-related clinical signs at any dose level during the study.
4.2.2	Mortality	There were no deaths at any dose level during the study.
4.3	**Body weight gain**	Body weight gain throughout the study was unaffected by treatment at all dose levels
4.4	**Food consumption and compound intake**	Food consumption throughout the study was unaffected by treatment at all dose levels. Overall mean achieved dose levels were 0, 3.46, 33.4 and 352mg/kg bw/day (males) and 0, 3.17, 32.2 and 339mg/kg bw/day (females).
4.5	**Ophtalmoscopic examination**	There were no treatment-related ophthalmological findings at any of the examination intervals.
4.6	**Blood analysis**	
4.6.1	Haematology	See Table A6_05_2-1. A trend towards lower packed cell volume, haemoglobin concentration and red blood cell counts relative to the controls occurred in weeks 6, 25 and 51 in both sexes treated at 10000ppm. However, in many instances statistical significance was not evident and all individual values were within the range of expected values. All other group mean haematological parameters in the etofenprox -treated groups were comparable to the control values.

Section A6.5.2 **Chronic toxicity (non rodent)**

Annex Point IIA-VI.6.5 **52-week, dog**

4.6.2	Clinical chemistry	See Table A6_05_2-2. Total protein and albumin serum concentrations were slightly but significantly ($p < 0.05$ or 0.01) decreased from week 6 of treatment in both males and females at 10000ppm. Serum cholesterol concentrations were also decreased in these animals. The effect was statistically significant ($p < 0.05$ or 0.01) at all intervals except in females in week 51 ($p > 0.05$). Serum alkaline phosphatase (SAP) activities in both sexes at 10000ppm were increased at all sampling intervals from week 6, with statistical significance ($p < 0.05$ or 0.01) achieved in males at weeks 12, 25 and 51 and in females for weeks 6 and 51. Protein, albumin and cholesterol concentrations at 10000ppm were not significantly different ($p > 0.05$) from the controls at the end of the recovery period and SAP activities had returned to levels below those of the controls. Therefore, there was evidence of recovery. All other group mean serum clinical chemistry parameters in the etofenprox -treated groups were comparable to the control values.
4.6.3	Urinalysis	There were no treatment-related effects at any dose level on the cellular or chemical constituents of urine.
4.7	**Sacrifice and pathology**	
4.7.1	Organ weights	See Table A6_05_2-3. The absolute and/or relative weights of the liver, kidneys, lungs and pancreas were increased in both sexes at 10000ppm, some of which achieved statistical significance ($p < 0.05$ or 0.01). However, since all kidney, lung and pancreas weights were within the normal range of variation and there were no biochemical or histopathological alterations in these organs, the differences from the control values are considered not to be toxicologically relevant. The organ weights of the recovery group animals at 10000ppm were within the normal range of variation and none showed a statistically significant ($p > 0.05$) variation from the controls. The organ weights of the groups treated at lower dose levels were unaffected by treatment.
4.7.2	Gross and histopathology	Treatment-related gross findings at necropsy were confined to 4 animals treated at 10000ppm and killed at 52 weeks that showed accentuated lobular markings of the liver. There were no treatment-related gross findings at necropsy in the recovery group animals treated at 10000ppm. Treatment-related histopathological alterations were confined to 2 females treated at 10000ppm and killed at 52 weeks. These animals showed minimal swelling of centrilobular hepatocytes, which correlated with the gross finding at necropsy of accentuated lobular pattern of the liver. All other animals treated at 10000ppm showed no treatment-related histopathological alterations in the liver. The histopathological appearance of all other tissues examined at 10000ppm and all tissues at lower dose levels were unaffected by treatment. The histopathological alterations in the liver of some females killed at 52 weeks were not evident in animals of either sex treated at 10000ppm and killed at the end of the 8-week recovery period.

Section A6.5.2 **Chronic toxicity (non rodent)**

Annex Point IIA-VI.6.5 **52-week, dog**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guidelines not specified in report but method conformed to 88/302/EEC. No deviations from 88/302/EEC. Description of method: beagle dog, dietary exposure, daily exposure for 52 weeks, 2 additional groups to observe recovery after 8 weeks untreated, 3 dose levels and 1 control group, observations included body weight, food consumption, mortality, clinical signs, ophthalmology, haematology, blood chemistry, urinanalysis, organ weight and full *post mortem* examination.
5.2	**Results and discussion**	A no-observed-effect-level (NOEL) was established as 1000ppm, equivalent to dose levels of 33.4 and 32.2mg/kg bw/day in males and females, respectively, based on the occurrence of evidence of reversible, minimal liver dysfunction and increased liver weight in both sexes and histopathological alterations in the liver of some females at 10000ppm.
5.3	**Conclusion**	
5.3.1	LO(A)EL	Not reported
5.3.2	NOEL	33.4 and 32.2mg/kg bw/day in males and females, respectively.
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Table A6_05_2-1. Selected group mean haematological parameters.

Week of study	Dose level (ppm)	PCV (%)		Hb (g/dL)		RBC (10^6/mm^3)		MCHC (%)	
		Male	Female	Male	Female	Male	Female	Male	Female
Pre-dose	0	42	43	13.2	13.2	5.6	5.6	31.7	31.0
	100	43	43	13.3	13.3	5.8	5.8	31.4	31.2
	1000	42	42	13.2	13.3	5.6	5.5	31.8	32.1**
	10000	41	43	12.9	13.7	5.4	5.6	31.7	31.8**
6	0	46	48	14.7	15.2	5.9	5.9	32.1	31.8
	100	43	42*	13.8	13.7	5.7	5.6	31.9	32.5
	1000	43	45	13.9	14.7	5.6	6.0	32.5	32.6
	10000	41*	45	13.3*	14.0	5.5	5.7	32.3	31.3
12	0	40	39	13.4	12.9	5.4	5.3	34.1	33.6
	100	43	40	14.4	13.5	5.7	5.3	33.6	34.0
	1000	44	40	14.7*	13.5	5.6	5.4	34.0	33.5
	10000	42	45**	14.1	14.6*	5.4	5.7	33.6	32.6
25	0	46	47	13.4	13.8	5.6	5.7	29.4	29.7
	100	44	48	13.2	14.0	5.6	5.9	29.9	29.4
	1000	45	47	13.3	13.9	5.8	5.8	29.7	29.6
	10000	40	44	12.0	13.2	4.9*	5.4	30.3	29.9
51	0	46	46	16.0	15.8	6.7	6.5	34.6	34.5
	100	44	47	15.6	16.3	6.6	6.7	35.3	34.5
	1000	42	42	15.6	15.2	6.3	6.0	37.3**	36.1*
	10000	40*	40*	14.7	15.3	5.7**	5.7	37.1**	38.2*
60	0	46	45	16.2	15.6	6.9	6.1	35.5	34.6
	1000	46	50	16.0	17.3	6.3	6.7	34.8	35.0

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

Table A6_05_2-2. Selected group mean serum clinical chemistry parameters.

Week of study	Dose level (ppm)	Protein (g/dL)		Albumin (g/dL)		SAP (mU/mL)		Cholesterol (mg/dL)	
		Male	Female	Male	Female	Male	Female	Male	Female
Pre-dose	0	5.5	5.4	3.0	3.1	248	258	162	140
	100	5.6	5.5	3.2	3.2	228	267	148	149
	1000	5.8	5.2	3.0	3.1	196	284	154	147
	10000	5.5	5.4	3.0	3.1	231	239	129*	142
6	0	5.7	5.7	3.3	3.5	213	208	171	152
	100	5.8	6.0*	3.4	3.4	182	212	157	143
	1000	5.9	5.7	3.3	3.5	199	246	159	160
	10000	5.2*	4.8**	2.9**	2.8**	279	267*	121**	112**
12	0	5.7	5.5	3.1	3.1	201	219	151	139
	100	5.6	5.8	3.1	3.1	173	196	146	141
	1000	5.3	5.5	2.9	3.1	194	241	140	142
	10000	5.2*	5.0*	2.6**	2.6**	334**	249	107**	109*
25	0	6.0	6.1	3.2	3.2	147	151	140	162
	100	6.0	6.2	3.2	3.3	129	110	119	126
	1000	5.8	5.8	3.0	3.2	143	160	123	150
	10000	5.2**	5.4**	2.6**	2.7**	345**	217	96**	111*
51	0	6.2	6.0	3.2	3.2	117	132	139	149
	100	6.0	6.1	3.0*	3.2	108	90	117	125
	1000	6.2	5.9	3.0*	3.1	173	179	139	168
	10000	5.3**	5.6**	2.6**	2.7**	322*	264*	100**	140
60	0	6.7	6.6	3.5	3.4	188	153	160	202
	1000	6.4	6.2	3.3	3.4	151	111**	132	117

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

Table A6_05_2-3. Selected group mean absolute and relative organ weights.

Sex	Dose level (ppm)	Liver		Kidneys		Pancreas		Lungs	
		(g)	(% bw)	(g)	(% bw)	(g)	(% bw)	(g)	(% bw)
Male	0	314.0	2.70	51.4	0.44	24.5	0.21	90.8	0.78
	100	365.7	3.04	55.3	0.46	27.0	0.23	106.5*	0.89
	1000	388.7	2.90	61.6	0.46	28.9	0.22	109.2	0.82
	10000	461.3**	3.92**	59.9	0.51	30.7**a	0.26	105.0*	0.90
Female	0	323.5	2.67	52.0	0.43	25.5	0.21	90.8	0.76
	100	340.0	2.62	56.4	0.44	28.6	0.22	106.2	0.83
	1000	345.0	3.01	53.7	0.47	28.4	0.25	88.0	0.77
	10000	509.7**	4.04**	63.5*	0.50	34.9**a	0.28	106.1	0.85
Both sexes	0	314.9	2.58	51.6	0.42	28.4	0.23	101.7	0.83
(recovery)	10000	348.2	3.05	56.9	0.50	33.5	0.29	104.7	0.92

* $p < 0.05$; ** $p < 0.01$; a statistically significant only when male and female data combined

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 A6.6.1 Genotoxicity *in vitro*

Annex Point IIA-VI.6.6 **Gene mutation in bacteria**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985): Reverse mutation in *Salmonella typhimurium*, test substance MTI-500; [redacted] unpublished report no. 162001-M-06185 (August 22, 1985). Dates of work: May 16, 1985 – June 21, 1985.	
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 79/831/EEC, Annex V, Part B; OECD guideline no. 471 (1981)	
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. 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	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	*S. typhimurium*: TA 98, TA 100, TA 1535, TA 1537 and TA 1538	X
3.2.2	Deficiencies / Proficiencies	Histidine-auxotrophic strains	
3.2.3	Metabolic activation system	S9 mix. The S9 fraction for metabolic activation was obtained from the livers of male SD strain rats pre-treated with phenobarbitone and betanaphthoflavone.	
3.2.4	Positive control	Concurrent strain-specific mutagens were applied to all strains as positive controls in both experiments (Table A6_6_1-1).	X
3.3	**Administration / Exposure; Application of test substance**		

Section A6.6.1 **Genotoxicity *in vitro***
Annex Point IIA-VI.6.6 **Gene mutation in bacteria**

3.3.1	Concentrations	0 (untreated), 0 (solvent, i.e. dimethylsulphoxide), 200, 400, 800, 1600 and 3200µg/plate with and without metabolic activation. Note: The highest dose level used in the main assays was determined in a preliminary toxicity test in which all 5 tester strains were exposed to 0 (untreated), 0 (solvent), 100, 316, 1000, 3160 and 10000 µ g/plate etofenprox, with and without metabolic activation.
3.3.2	Way of application	Spread on to the surface of a minimal medium agar plate, allowed to solidify prior to incubation (solvent: dimethylsulphoxide)
3.3.3	Pre-incubation time	72 hours at 37°C
3.3.4	Other	- Additional plates were prepared to confirm the sterility of the test substance formulations and the S9 mix, and dilutions of the bacterial cultures were plated on to nutrient agar to establish bacterial concentrations. - Assay acceptance criteria: Not specified in report, but report indicates that the assay system was functioning correctly based on positive responses to the positive control substances. - Criteria for a positive response: The assay would be considered positive, without statistical analysis, if for any strain there was an increase of at least twice the solvent control value for mean number of revertants in at least 2 consecutive concentrations or at the last non-toxic concentration. In addition, there must be evidence of a dose-response relationship showing increasing numbers of revertants with increasing dose and the effect must be reproducible.
3.4	**Examinations**	
3.4.1	Number of cells evaluated	After incubation, the numbers of revertant colonies were enumerated by manual counting.
3.5	**Statistics**	Statistical analysis of the results was performed by regression analysis.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	In both independent assays, etofenprox did not induce a dose-related increase in the incidences of revertant colonies in any strain at any dose level either in the presence or absence of metabolic activation (Table A6_6_1-2). In contrast, the positive control substances produced marked increases in the incidences of revertant colonies in all strains both with and without metabolic activation.
4.2	**Cytotoxicity**	No.

Section A6.6.1 Genotoxicity *in vitro*

Annex Point IIA-VI.6.6 **Gene mutation in bacteria**

4.3	**Sterility**	The sterility of the S9 mix and the test substance formulations was confirmed by the absence of colonies on additional agar plates. The estimated bacterial titres for each strain in each assay are shown in Table A6_6_1-3.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test method: 79/831/EEC, Annex V, Part B; OECD guideline no. 471 (1981) Deviations: none Description of method: *in vitro* bacterial reverse mutation assay in S. *typhimurium* , with and without S9 metabolic activation, solvent and positive controls (i.e. concurrent strain-specific mutagens).
5.2	**Results and discussion**	Etofenprox and/or metabolites does not induce gene mutations in the strains of *S. typhimurium* used in the study at doses up to 3200µg/plate, the maximum concentration at which test substance precipitation did not occur.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6_6_1-1. Positive control substances and dose levels.

Strain	Without metabolic activation (µg/plate):		With metabolic activation (µg/plate):	
TA 98	2-nitrofluorene (2-NF)	2.0	2-aminoanthracene (2AA)	1.0
TA 100	Sodium azide (SA)	1.0	2-aminoanthracene	1.0
TA 1535	Sodium azide	1.0	2-aminoanthracene	1.0
TA 1537	9-aminoacridine (9-AA)	50	2-aminoanthracene	1.0
TA 1538	2-nitrofluorene	2.0	2-aminoanthracene	1.0

Table A6_6_1-2. Mean number of revertant colonies/plate.

Dose (µg/plate)	Metabolic activation	Mean number of revertant colonies/plate in:				
		TA1535[a]	TA1537	TA1538	TA98	TA100[a]
		1st assay				
0 (untreated)	–	30	16	28	32	162
0 (vehicle)		31	15	25	38	159
200		29	17	20	28	141
400		26	15	22	27	134
800		35	18	25	26	130
1600		34	14	27	34	141
3200		29	16	19	43	144
0 (untreated)	+	20	27	37	48	168
0 (vehicle)		18	29	32	51	137
200		13	15	33	32	137
400		17	18	32	36	143
800		19	19	29	32	149
1600		21	20	29	40	137
3200		16	18	23	40	135
PC	–	506	59	263	316	727
PC	+	216	81	855	774	1634
		2nd assay				
0 (untreated)	–	17	12	26	31	111
0 (vehicle)		22	17	21	27	109
200		23	11	27	33	101
400		24	13	18	32	96
800		16	9	19	31	87
1600		13	11	24	28	75
3200		15	13	26	28	92
0 (untreated)	+	11	18	34	48	114
0 (vehicle)		16	19	33	45	107
200		12	16	39	33	104
400		10	13	31	38	107
800		13	20	27	51	110
1600		12	25	35	49	98
3200		12	18	28	36	94
PC	–	575	125	270	241	698
PC	+	176	97	960	1088	1188

PC positive control; [a] results for strains TA 1535 and TA 100 were obtained in repeat assays since the numbers of revertant colonies on control plates were unacceptably high in the absence of metabolic activation.

Table A6_6_1-3. Estimated mean bacterial titres

Strain	Mean bacterial titre ($x10^8$) in:	
	1st assay	2nd assay
TA1535	2.84	2.21
TA1537	3.60	3.06
TA1538	5.26	4.82
TA98	4.37	3.38
TA100	2.44	2.71

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. **3.2.1. Organism/cell type** Instead of the TA102 the TA 1538 strain was used. These strains are not equivalent with regard to the detection of oxidising mutagens and cross-linking agents. The sensitivity of TA1538 is more similar to TA98. However the test was carried out before also TA 102, WP2uvrA or WP2 uvra (pKM101) were defined by the OECD guideline 471. The data package for genotoxicity and for carcinogenicity available for etofenprox does not indicate genotoxic concern. Therefore there this deviation to the actual OECD guideline is considered acceptable. **3.2.4. Positive control** According to the OECD guideline 471 2-aminoanthracene should not be used as the sole indicator of the efficacy of the S9 mix. If 2-aminoanthracene is used, each batch of S9 should also be characterised with a mutagen that requires metabolic activation by microsomal enzymes, e.g. benzo(a)pyrene, dimethylbenzanthracene. Within the original study report it is stated that the single S9 batch used for this study was characterised also with benzo(a)pyrene.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	

Acceptability	
Remarks	

Section A6.6.2 Genotoxicity *in vitro*

Annex Point IIA-VI.6.6 **Cytogenicity in mammalian cells**

Cultured human peripheral lymphocytes

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1985a): In vitro assessment of the clastogenic activity of MTI-500, Ethofenprox, in cultured human peripheral lymphocytes; [redacted] unpublished report no. 85/MT0017/430 (July 17, 1985). Dates of work: May 07, 1985 – June 27, 1985	
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 Test method not specified in report but method complies with 92/69/EEC (B.11)	X
2.2	**GLP**	No	
2.3	**Deviations**	Yes S9 metabolic activation included for less than duration of cell cycle and only one harvest time employed; no independent repeat experiment.	X
		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	**Study Type**	In Vitro mammalian chromosome aberration test	
3.2.1	Organism/cell type	Human peripheral lymphocytes (source: non-smoking male, no current medication) were established in complete culture medium containing small inocula of whole blood and phytohaemagglutinin.	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	S9 mix. The S9 fraction was obtained from male CD strain rat liver induced with a single intraperitoneal dose of 500mg/kg Aroclor 1254.	

Section A6.6.2
Annex Point IIA-VI.6.6

Genotoxicity *in vitro*
Cytogenicity in mammalian cells
Cultured human peripheral lymphocytes

3.2.4	Positive control	Ethylmethanesulphonate (EMS - 400µg/mL) applied for 24 hours was the positive control material without activation and cyclophosphamide (CP - 6µg/mL) applied for 24 hours was the positive control material both with and without metabolic activation.	
3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	0 (solvent, i.e. dimethylsulphoxide, control), 6.25, 12.5, 25.0 and 50µg/mL, with and without S9 metabolic activation. Note: The dose levels used in the main study were determined in a preliminary toxicity test in which duplicate culture of human lymphocytes were exposed *in vitro* to etofenprox at concentrations of 4, 20, 100, 500 and 2500µg/mL for 24 hours without S9 metabolic activation, and for 2 hours with S9 metabolic activation followed by a further 22 hours exposure without S9. Since it was not possible to accurately determine the maximum concentration for use in the main study (i.e. one producing ca. 50% inhibition of mitotic activity), an additional dose level was included in the main study.	X
3.3.2	Way of application	Incorporation in the culture medium, in triplicate (solvent: dimethylsulphoxide). All treatments were applied on the same day, at a volume of 25µL to produce a final culture volume of 4.5mL.	
3.3.3	Pre-incubation time	24 hours without metabolic activation and for 2 hours with metabolic activation followed by a further 22 hours exposure without *S9*	
3.3.4	Incubation	The cultures were incubated at 37°C for 2 hours with shaking, after which the cultures were washed, re-suspended in a final volume of 10mL and incubated at 37°C for the remainder of the 24-hour exposure period. Three hours prior to harvesting, the cultures were treated with 0.4µg/mL colcemide to arrest cells in metaphase. The cultures were left to incubate for a further 3 hours after which the cell suspensions were centrifuged and re-suspended in hypotonic KCl for 10 minutes to permit swelling.	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	The cells were fixed, spread on to glass slides (4 per culture), air-dried and stained with Giemsa. Where possible, 100 well-spread metaphase figures containing 46 centromeres per replicate in each group, including the solvent and positive controls, were scored. The slides were examined blind for chromosome number and specific and non-specific structural chromatid and chromosome aberrations. In addition, approximately 1000 cells/culture were scored and the mitotic index calculated as the percentage of cells in metaphase."	

Section A6.6.2
Annex Point IIA-VI.6.6

Genotoxicity *in vitro*
Cytogenicity in mammalian cells
Cultured human peripheral lymphocytes

3.5	**Statistics**	The data were evaluated statistically using the Fisher exact probability test. Data from replicate cultures, with or without metabolic activation were compared. If significant differences were apparent, the data from cultures with and without activation were analysed separately. If there were no significant differences, the data were combined for statistical analysis. The assay acceptability and evaluation criteria for the study were not specified in the report.
		4 RESULTS AND DISCUSSION
4.1	**Preliminary note**	5 of the 6 cultures treated at 50µg/mL had sufficient metaphases for scoring, but the 6th culture pellet was lost during processing. Therefore, cultures treated at 6.25µg/mL were excluded from the analysis, and cultures treated at 12.5, 25 and 50µg/mL were evaluated.
4.2	**Genotoxicity**	
4.2.1	Miotic indices	See Table A6_6_2-1. The mitotic indices with and without metabolic activation showed some variation but when the data were combined, the mean mitotic indices were 8.8, 4.1 and 1.2% at 12.5, 25 and 50µg/mL etofenprox, respectively, compared to a solvent control value of 6.2%. Therefore, a treatment- and dose-related decrease in mitotic activity was demonstrated.

Section A6.6.2
Annex Point IIA-VI.6.6

Genotoxicity *in vitro*
Cytogenicity in mammalian cells
Cultured human peripheral lymphocytes

4.2.2	Chromosome aberration	See Table A6_6_2-2. There were no statistically significant differences ($p > 0.05$) in the incidences of aberrant cells (including and excluding gaps) within dose levels of etofenprox treated with and without metabolic activation. Therefore, the data within dose levels were combined for statistical comparison with the solvent control group. Since the positive control substance, cyclophosphamide, showed a significantly higher ($p < 0.001$) incidence of aberrant cells with metabolic activation than without, the data were not combined for analysis. The reported incidences including gaps of aberrant lymphocytes in etofenprox treated groups were 1.8% (range 0 – 6%), 2.2% (range 1 – 4%) and 3.4% (range 1 – 9.8%), in order of ascending dose level, compared with a solvent control incidence of 2.2% (range 1 – 4%). The reported incidences excluding gaps in etofenprox treated groups were 1.5, 1.3 and 1.6%, in order of ascending dose level, compared with a solvent control incidence of 1.7%. None of the incidences, either including or excluding gaps, was significantly different ($p > 0.05$) from the control incidence. The control and treated incidences of aberrant cells with and without gaps calculated by the reviewer were similar to the reported incidences for all dose levels of etofenprox. In contrast to the etofenprox -treated cultures, the positive control substances, CP with metabolic activation and EMS without metabolic activation, produced markedly increased and statistically significant ($p < 0.001$) incidences of aberrant cells, including and excluding gaps. The incidence of aberrant cells was not significantly increased ($p > 0.05$) by CP without metabolic activation. These data confirmed the efficacy of the metabolic activation system employed and the sensitivity of the test system to a direct-acting clastogen.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test guidelines not ot specified in report but method complies with 92/69/EEC (B.11) Deviations: S9 metabolic activation included for less than duration of cell cycle and only one harvest time employed; no independent repeat experiment. Description of method: in vitro clastogenic activity test in human peripheral lymphocytes, with and without S9 metabolic activation, solvent control.	
5.2	**Results and discussion**	Etofenprox and/or metabolites do not induce structural or numerical chromosomal aberrations in human peripheral lymphocytes at dose levels up to those producing marked cytotoxicity.	X
5.3	**Conclusion**		
5.3.1	Reliability	1	X
5.3.2	Deficiencies	No	

Table A6_6_2-1. Mitotic indices.

Dose level etofenprox (µg/mL)	Metabolic activation	No. lymphocytes examined	Mitotic index (%)	
			Mean of cultures	Group mean
0 (solvent)	–	3060	6.9	6.2
	+	3361	5.5	
12.5	–	3121	10.3	8.8
	+	3123	7.3	
25	–	3188	2.8	4.1
	+	3057	5.3	
50	–	3143	1.4	1.2
	+	3071	1.0	
6 (CP)	–	3446	7.9	5.3
	+	3120	2.6	
400 (EMS)	–	3160	1.3	1.3
	+	NA	NA	

NA not applicable

Table A6_6_2-2. Incidence of chromatid and chromosome aberrations.

Parameter	DMSO		etofenprox						CP		EMS	
	0µg/mL		12.5µg/mL		25µg/mL		50µg/mL		6µg/mL		400µg/mL	
	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Total no. cells scored	300	300	300	300	300	300	300	141	300	300	300	NA
Aberrant cells (%)	1.67	2.67	1.33	2.33	2.67	2.00	3.00	4.26[a]	3.00	18.00	20.67	NA
Aberrant cells less gaps (%)	1.33	2.00	1.00	2.00	2.00	1.00	1.00	2.94	1.67	14.00	16.67	NA
Total no. cells with SSG	1	3	1	3	2	3	6	2	5	17	19	NA
Total no. cells with DSG	0	0	0	0	0	0	0	0	0	4	1	NA
Total no. cells with SSB	0	2	0	5	2	1	1	3	1	8	20	NA
Total no. cells with DSB	0	0	0	0	0	0	0	0	0	2	6	NA
Total no. cells with E	0	0	0	0	0	0	0	0	0	18	21	NA
Total no. cells with F	2	6	3	4	4	2	2	2	3	24	31	NA
Total no. cells with OA	2[b]	0	0	0	0	0	0	0	1[b]	0	0	NA
	Group totals reported (combined data for - / + S9)											
Total no. cells scored	600		600		600		441		300	300	300	NA
Mean mitotic index (%)	6.2		8.8		4.1		1.2		7.9	2.6	1.3	NA
Total no. aberrant cells	13		11		13		15		9	54	62	NA
Total aberrant cells (%)	2.2		1.8		2.2		3.4		3.0	18.0**	20.7*	NA
No. aberrant cells less gaps	10		9		8		7		5	42	50	NA
Total aberrant cells less gaps (%)	1.7		1.5		1.3		1.6		1.7	14.3**	16.7*	NA

[a] 2 replicates only – one replicate with incidence of 2.00% and one replicate with 41 scorable metaphases 9.8%; [b] endoreduplication; SSG single strand gap;

DSG double strand gap; SSB single strand break; DSB double strand break; E exchange; F fragment; OA other aberration; * significantly higher than solvent control value ($p < 0.001$); ** significantly higher than solvent control value and significantly higher than value without metabolic activation ($p < 0.001$)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27.05.2005
Materials and methods	**2.1. Guideline study** The methods corresponds to EC method B10 and OECD method 473 **2.3. Deviations** The deviations listed under 2.3. are considered relevant (see paragraph 23 and 25 of OECD method 473) **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. **3.3.1. Concentrations** Additional intermediate dose levels were chosen compared to the levels of the preliminary test
Conclusion	**5.2. Results and discussion** The negative result is considered to be of limited reliability due to the deviations indicated.
Reliability	2
Acceptability	The data are considered nevertheless acceptable although – see conclusion above.
Remarks	-
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.3/01 — Genotoxicity *in vitro*

Annex Point IIA-VI.6.6 — **Gene mutation in mammalian cells**
Chinese hamster V79 cells

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1985a): Gene mutation in Chinese hamster V79 cells: test substance MTI-500; [redacted] unpublished report no. 162002-M-06985 (August 22, 1985). Dates of work: June 05, 1985 – July 29, 1985	
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 79/831/EEC, Annex V, Part B	
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. 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	**Study Type**	In vitro mammalian cell gene mutation test	
3.2.1	Organism/cell type	Chinese hamster lung fibroblasts (V79, HGPRT$^+$, cell line V79 4(H), mycoplasma-free, source [redacted]	
3.2.2	Deficiencies / Proficiencies	Cells sensitive to the lethal effects of 6-thioguanine	
3.2.3	Metabolic activation system	S9 mix. The S9 fraction for metabolic activation was obtained from the livers of male SD strain rats previously treated with phenobarbitone and betanaphthaflavone.	
3.2.4	Positive control	Concurrent positive control substances, ethylmethane-sulphonate (EMS – 5 and 10mM) without S9 and dimethylnitrosamine (DMN – 5 and 10mM) with S9 were also assayed.	

Section A6.6.3/01
Annex Point IIA-VI.6.6

Genotoxicity *in vitro*
Gene mutation in mammalian cells
Chinese hamster V79 cells

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	0 (solvent control), 9.75, 19.5, 39.0, 78.0 and 156µg/mL with and without metabolic activation. Note: Preliminary solubility and cytotoxicity tests to select dose levels were performed on etofenprox dissolved in DMSO in single cultures of V79 cells. Nine doubling concentrations of etofenprox from 0.609µg/mL to the highest soluble concentration in culture medium of 156µg/mL, plus 1% vehicle control, with and without S9 activation, were evaluated during an exposure period of 3 hours. Following incubation for 6 days, the colonies were stained and counted. Cytotoxicity was expressed as percentage survival relative to the solvent control.
3.3.2	Way of application	dissolved in medium (solvent: dimethylsulphoxide)
3.3.3	Pre-incubation time	3 hours at 37°C
3.3.4	Incubation	After exposure, the cells were washed, complete medium added and returned to the incubator. The following day, the cultures were trypsinised and an aliquot diluted to determine cell viability. The cells were re-plated at a general density of 1.0 x 10^6 viable cells and sub-cultured 2 days later. Six days after treatment, the cultures were trypsinised, re-suspended and counted by microscopy. Approximately 1 x 10^5 cells were plated on to each of 5 petri dishes and 3 hours later a final concentration of 7.5µg/mL 6-thioguanine (6-TG) was added for selection of $HGPRT^-$ mutant cells. Approximately 200 cells were also plated on to each of 3 petri dishes for the estimation of plating efficiency. An adequate number of cells was re-plated to maintain the treated cultures for the 2nd expression time at 9 days, at which time plates were prepared to determine mutant numbers and plating efficiency. Plates for determination of mutant frequency were incubated at 37°C for 11 - 15 days to ensure adequate colony size.
3.4	**Examinations**	
3.4.1	Number of cells evaluated	The colonies were fixed, stained and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of colonies in the cloning efficiency dishes.
3.5	**Statistics**	Mutant frequencies were subjected to ANOVA using transformed mutant frequencies followed by step-wise fitting of 3 factors to the data, detection of differences between independent assays, expression time and dose.

Section A6.6.3/01
Annex Point IIA-VI.6.6

Genotoxicity *in vitro*
Gene mutation in mammalian cells
Chinese hamster V79 cells

3.6	**Other**	- Assay acceptance criteria: not specified in report, but both solvent and positive control groups produced results indicating the correct functioning of the test system. - Evaluation criteria: The assay is considered positive if a dose-dependent, statistically significant and reproducible increase in mutant frequency occurs, in at least 2 dose levels. The mutant frequency should be at least 5 times the highest solvent control value.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	See Table A6_6_3-2, A6_6_3-3 and A6_6_3-4. The mutation frequencies in both independent assays after 6 and 9 days expression, with and without metabolic activation, did not indicate an effect of treatment with etofenprox at the range of concentrations assayed. There were no 5-fold or more increases in mutation frequency at any concentration of etofenprox and none of the incidences was significantly different ($p > 0.05$) from the appropriate solvent control group. Mutation frequencies with and without metabolic activation were generally higher in the 2nd assay than in the first assay, producing a statistically significant ($p < 0.001$ without S9, $p < 0.05$ with S9) effect of experiment number. Both EMS without S9 and DMN with S9, at both concentrations employed, produced very marked increases in mutation frequency in the range 8.6 – 98.3 times solvent control incidences.
4.2	**Cytotoxicity**	See Table A6_6_3-1. In both main assays, etofenprox was mildly cytotoxic at the highest concentration of 156µg/mL, as indicated by relative survival incidences in the range 70 – 91% ().

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guideline: 79/831/EEC, Annex V, Part B Deviations: none Description of method: *in vitro* gene mutation in Chinese hamster V79 cells, with and without S9 metabolic activation, solvent control .
5.2	**Results and discussion**	Etofenprox and/or metabolites is not mutagenic in the V79-HPRT forward mutation assay.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6_6_3-1. Relative survival.

Dose level (µg/mL)	Without S9		With S9	
	Mean plate count	Survival (% control)	Mean plate count	Survival (% control)
	1st assay			
0 (solvent)	233.8	100	229.8	100
9.75	264.7	113	247.3	108
19.5	237.3	102	228.3	99
39.0	249.3	107	232.3	101
78.0	144.0	62	236.7	103
156	165.0	71	161.7	70
EMS – 5mM	159.7	68	NA	NA
EMS – 10mM	78.0	33	NA	NA
DMN – 5mM	NA	NA	52.7	23
DMN – 10mM	NA	NA	20.7	9
	2nd assay			
0 (solvent)	189.5	100	237.2	100
9.75	215.3	114	217.3	92
19.5	201.7	106	224.0	94
39.0	238.0	126	226.3	95
78.0	222.3	117	223.7	94
156	172.0	91	182.3	77
EMS – 5mM	131.3	69	NA	NA
EMS – 10mM	80.7	43	NA	NA
DMN – 5mM	NA	NA	128.7	54
DMN – 10mM	NA	NA	88.7	37

NA not applicable;

Table A6_6_3-2. Relative plating efficiency and mean mutation count – 1st assay.

Dose level (µg/mL)	Without S9		With S9	
	Mean PE (% control)	Mean no. mutant colonies/plate	Mean PE (% control)	Mean no. mutant colonies/plate
	6 days expression			
0 (solvent)	100	3.3	100	2.4
9.75	104	5.6	98	6.2
19.5	103	3.0	-	-
39.0	99	3.2	105	3.0
78.0	105	4.0	100	2.6
156	92	3.7	90	2.0
EMS – 5mM	107	53.4	NA	NA
EMS – 10mM	51	92.8	NA	NA
DMN – 5mM	NA	NA	41	49.4
DMN – 10mM	NA	NA	57	133.8
	9 days expression			
0 (solvent)	100	3.7	100	3.9
9.75	92	2.6	98	6.2
19.5	92	2.2	95	6.0
39.0	84	4.8	104	2.8
78.0	87	5.8	94	4.8
156	82	4.6	87	3.2
EMS – 5mM	83	53.8	NA	NA
EMS – 10mM	69	105.6	NA	NA
DMN – 5mM	NA	NA	82	60.0
DMN – 10mM	NA	NA	50	79.0

NA not applicable; PE plating efficiency; - one culture failed to plate on expression day 6

Table A6_6_3-3. Relative plating efficiency and mean mutation – 2nd assay.

Dose level (µg/mL)	Without S9		With S9	
	Mean PE (% control)	Mean no. mutant colonies/plate	Mean PE (% control)	Mean no. mutant colonies/plate
	6 days expression			
0 (solvent)	100	6.0	100	6.0
9.75	126	10.4	102	3.2
19.5	103	5.0	120	2.8
39.0	133	7.6	108	3.2
78.0	119	5.8	107	4.3
156	106	2.4	95	5.6
EMS – 5mM	104	72.0	NA	NA
EMS – 10mM	100	134.3	NA	NA
DMN – 5mM	NA	NA	63	32.4
DMN – 10mM	NA	NA	83	80.0
	9 days expression			
0 (solvent)	100	7.3	100	4.9
9.75	93	8.2	86	3.2
19.5	79	5.0	85	3.7
39.0	93	4.0	100	5.2
78.0	101	3.0	101	4.2
156	101	4.2	91	6.4
EMS – 5mM	79	54.0	NA	NA
EMS – 10mM	50	123.2	NA	NA
DMN – 5mM	NA	NA	92	43.5
DMN – 10mM	NA	NA	65	78.0

NA not applicable; PE plating efficiency

Table A6_6_3-4. Summary of mutation frequencies.

Dose level (µg/mL)	**Mean mutation frequency/ 10^6 surviving cells**			
	Without S9		**With S9**	
	Day 6	**Day 9**	**Day 6**	**Day 9**
	1st assay			
0 (solvent)	28.55	39.26	20.41	43.53
9.75	46.47	29.89	53.60	70.32
19.5	25.25	25.43	-	70.59
39.0	27.95	60.76	24.26	30.16
78.0	33.01	70.45	22.16	56.66
156	35.10	59.23	18.99	41.11
EMS – 5mM	433.56	689.74	NA	NA
EMS – 10mM	1564.04	1624.62	NA	NA
DMN – 5mM	NA	NA	1015.07	812.64
DMN – 10mM	NA	NA	2007.00	1775.28
	2nd assay			
0 (solvent)	75.79	100.57	69.97	62.95
9.75	104.00	121.48	36.57	47.64
19.5	61.22	87.46	27.14	56.82
39.0	72.27	59.55	34.59	67.10
78.0	61.38	40.91	46.36	53.39
156	28.63	57.14	68.85	90.35
EMS – 5mM	878.05	939.13	NA	NA
EMS – 10mM	1699.37	3375.34	NA	NA
DMN – 5mM	NA	NA	603.73	609.81
DMN – 10mM	NA	NA	1126.76	1549.67

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 A6.6.4 Genotoxicity *in vivo*

Annex Point IIA-VI.6.6.4 **1st *in vivo* mutagenicity study**
Micronucleus test, mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1985b): MTI-500, ethofenprox: assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test; [redacted] unpublished report no. 85/MT0016/406 (July 03, 1985). Dates of work: May 08, 1985 – June 20, 1985.	
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 Test guideline not specified in report but method conforms to 92/69/EEC (B.12).	
2.2	**GLP**	No (UK GLP compliance programme issued formal facility compliance certificate in 1986. Since the study and report were subjected to QA audit with no non-compliance issues of concern, the study report is considered to be acceptable)	
2.3	**Deviations**	Yes Deviations: a group of animals treated at 2000mg/kg was sacrificed at 72 hours post-treatment, in addition to the 24 and 48-hour sacrifices	
		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.	
3.1.2.4	Maximum tolerable dose	2000mg/kg	
3.2	**Test Animals**		
3.2.1	Species	mouse	
3.2.2	Strain	Swiss mice (CD-1 strain)	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	

Section A6.6.4	**Genotoxicity *in vivo***
Annex Point IIA-VI.6.6.4	**1st *in vivo* mutagenicity study**
	Micronucleus test, mouse

3.2.5	Age/weight at study initiation	five to six week of age, body weight range 19.8 – 31.4g	
3.2.6	Number of animals per group	5 groups of 5 animals/sex/dose	
3.2.7	Control animals	- vehicle control: 5 groups of 5 animals/sex - positive control: one group of 5 animals/sex	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	Single dose	
3.3.2	Interval between applications	Not applicable (single dose)	
3.3.3	Postexposure period	24, 48, 72 h after treatment	
		Oral	
3.3.4	Type	Gavage	
3.3.5	Concentration	- groups with 24h postexposure: 0 (vehicle), 80, 400 and 2000mg/kg - groups with 48 and 72 h postexposure: 0 (vehicle) and 2000mg/kg	
3.3.6	Vehicle	0.5% methyl cellulose	
3.3.7	Concentration in vehicle	maximum 133.3 mg/ml	
3.3.8	Total volume applied	*ca.* 15 ml/kg	
3.3.9	Controls	- Vehicle (methylcellulose) - Positive control: 5 animals/sex, treated with a single dose of 30mg/kg chlorambucil in 10% aqueous ethanol	X

Section A6.6.4 **Genotoxicity *in vivo***

Annex Point IIA-VI.6.6.4 **1st *in vivo* mutagenicity study**

Micronucleus test, mouse

3.4	**Examinations**		
3.4.1	Clinical signs	Yes, daily.	
3.4.2	Tissue	bone marrow	
		Number of animals:	all animals or other
		Number of cells:	2000 (1000 polychromatic cells (PCE) or normochromatic cells(NCE)) .
		Time points:	24, 48, 72 h after treatment
		Type of cells	erythrocytes in bone marrow
		Parameters:	polychromatic/normochromatic erythrocytes ratio
			incidences of micronucleated PCE cells (Mn-PCE) and NCE cells (Mn-NCE)
3.5	**Further remarks**	- Body weights were recorded on the day of treatment and at termination.	
		- The data were subjected to statistical analysis using the Mann-Whitney U test. Initially, within-group data from the sexes were compared at the 95% level of confidence. If there was no statistical difference between the sexes, the data were pooled for further analysis. Data from the treated groups at each sampling interval were compared with the vehicle control group.	

4 RESULTS AND DISCUSSION

4.1	**Mortality and clinical signs**	There were no deaths or adverse clinical signs in the main study and all animals were killed on the scheduled date.

Section A6.6.4	**Genotoxicity *in vivo***
Annex Point IIA-VI.6.6.4	**1st *in vivo* mutagenicity study**
	Micronucleus test, mouse

4.2	**Genotoxicity**	No There were no statistically significant ($p > 0.05$) differences in the incidences of micronucleated PCE cells between the sexes in any of the treated or control groups, including the positive controls (Table A6_6_4-1). Therefore, the data from the sexes were combined (Table A6_6_4-2). There were no statistically significant ($p > 0.05$) differences in the incidences of micronucleated PCE cells (sexes combined) between the vehicle control and etofenprox -treated groups at any sacrifice interval. Among the groups sacrificed at 24 hours, the mean incidence of micronucleated PCE cells in the vehicle control group was 0.2 (range 0.0 – 2.0) compared with incidences of 0.8 (range 0.0 – 3.0), 0.6 (range 0.0 – 2.0) and 0.4 (range 0.0 – 2.0) in the MTI500-treated groups, in order of ascending dose level. In contrast, the positive control group showed a significantly higher ($p < 0.001$) incidence of 38.0 (range 24.8 – 54.6), indicating the sensitivity of the test system to a known clastogen. Among animals killed after 48 hours, the mean incidence of micronucleated PCE cells in the vehicle control group was 1.0 (range 0.0 – 3.0) compared with an incidence of 0.8 (range 0.0 – 2.0) in the etofenprox group treated at 2000mg/kg. Among animals killed after 72 hours, the mean incidence of micronucleated PCE cells in the vehicle control group was 1.2 (range 0.0 – 3.9) compared with an incidence of 1.6 (range 0.0 – 3.0) in the etofenprox group treated at 2000mg/kg. The incidences of micronucleated NCE cells were uniformly low in all treated and control groups, indicating the normal status of the test system (Table A6_6_4-1). There was no effect of treatment at any dose level of etofenprox on the PCE/NCE ratio indicating no gross inhibition of cell division (Table A6_6_4-1).	X
4.3	**Other**	<u>Body weight</u> Slight weight loss occurred in some individuals of all treated and control groups with the exception of the vehicle control group killed after 72 hours. No biological significance is inferred for any of the MTI-treated groups since the incidences and degree of weight loss were comparable between MTI-treated groups and the controls. However, all mice treated with chlorambucil lost weight following treatment.	

Section A6.6.4
Annex Point IIA-VI.6.6.4

Genotoxicity *in vivo*
1st *in vivo* mutagenicity study
Micronucleus test, mouse

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Test guideline not specified in report but method conforms to 92/69/EEC (B.12) Deviations: a group of animals treated at 2000mg/kg was sacrificed at 72 hours post-treatment, in addition to the 24 and 48-hour sacrifices Description of method: in vivo mutagenicity test in the mouse (bone marrow erythrocytes), 3 dose levels, 1 positive control and one untreated control, postexposure of 24, 48 and 72 h.
5.2	**Results and discussion**	Etofenprox and/or metabolites does not induce chromosomal or other damage leading to the formation of micronuclei in PCE cells at acute oral (gavage) dose levels of up to 2000mg/kg.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6_6_4-1. Incidences of micronucleated PCE and NCE cells and PCE/NCE ratios – sexes separate.

Parameter	Males treated at (mg/kg):					Females treated at (mg/kg):				
	0	80	400	2000	PC	0	80	400	2000	PC
	24-hour sacrifice									
Total PCE scored	5059	5127	6167	5180	5225	5178	5097	5078	5057	5077
Total Mn-PCE cells	2	6	3	2	207	0	2	4	2	188
Mean Mn-PCE/1000	0.4	1.2	0.4	0.4	39.0	0.0	0.4	0.8	0.4	37.0
Total NCE scored	6532	5414	5078	5355	7004	5423	5086	5896	6216	5678
Total Mn-NCE cells	2	0	4	2	3	2	1	0	1	1
Mean Mn-NCE/1000	0.2	0.0	0.8	0.4	0.3	0.3	0.2	0.0	0.1	0.2
PCE/NCE ratio	0.8	0.9	1.2	1.0	0.7	1.0	1.0	0.9	0.8	0.9
	48-hour sacrifice									
Total PCE scored	5067	-	-	5072	-	5431	-	-	5393	-
Total Mn-PCE cells	4	-	-	4	-	7	-	-	5	-
Mean Mn-PCE/1000	0.8	-	-	0.8	-	1.3	-	-	0.9	-
Total NCE scored	6065	-	-	5182	-	5265	-	-	5202	-
Total Mn-NCE cells	5	-	-	0	-	5	-	-	2	-
Mean Mn-NCE/1000	0.8	-	-	0.0	-	1.0	-	-	0.4	-
PCE/NCE ratio	0.8	-	-	1.0	-	1.0	-	-	1.0	-
	72-hour sacrifice									
Total PCE scored	5088	-	-	5076	-	5074	-	-	5274	-
Total Mn-PCE cells	3	-	-	8	-	9	-	-	8	-
Mean Mn-PCE/1000	0.6	-	-	1.6	-	1.8	-	-	1.5	-
Total NCE scored	5080	-	-	5626	-	5259	-	-	5098	-
Total Mn-NCE cells	0	-	-	5	-	3	-	-	3	-
Mean Mn-NCE/1000	0.0	-	-	0.9	-	0.6	-	-	0.6	-
PCE/NCE ratio	1.0	-	-	0.9	-	1.0	-	-	1.0	-

PC chlorambucil 30mg/kg; - not applicable

Table A6_6_4-2. Incidences of micronucleated PCE and NCE cells and PCE/NCE ratios – sexes combined.

Parameter	Animals treated at (mg/kg):				
	0	80	400	2000	PC
	24-hour sacrifice				
Total PCE scored	10237	10224	11245	10237	10302
Total Mn-PCE cells	2	8	7	4	395
Mean Mn-PCE/1000	0.2	0.8	0.6	0.4	38.0*
Total NCE scored	11995	10500	10974	11571	12682
Total Mn-NCE cells	4	1	4	3	4
Mean Mn-NCE/1000	0.3	0.1	0.4	0.2	0.3
PCE/NCE ratio	0.9	1.0	1.0	0.9	0.8
	48-hour sacrifice				
Total PCE scored	10498	-	-	10465	-
Total Mn-PCE cells	11	-	-	9	-
Mean Mn-PCE/1000	1.0	-	-	0.8	-
Total NCE scored	11330	-	-	10384	-
Total Mn-NCE cells	10	-	-	2	-
Mean Mn-NCE/1000	0.9	-	-	0.2	-
PCE/NCE ratio	0.9	-	-	1.0	-
	72-hour sacrifice				
Total PCE scored	10162	-	-	10350	-
Total Mn-PCE cells	12	-	-	16	-
Mean Mn-PCE/1000	1.2	-	-	1.6	-
Total NCE scored	10339	-	-	10724	-
Total Mn-NCE cells	3	-	-	8	-
Mean Mn-NCE/1000	0.3	-	-	0.7	-
PCE/NCE ratio	1.0	-	-	1.0	-

* significantly different from the vehicle control group ($p < 0.001$); - not applicable

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09.08.2005
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. **3.3.9. Control** Chlorambucil was used as positive control. Other substances are recommended in the "in vivo mammalian erythrocyte micronucleus test" (OECD 474). However Chlorambucil showed a strong positive response. This deviation to the test guideline is considered to be minor.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	**4.2. Genotoxicity** Despite the absence of an effect on the PCE/NCE ratio in the mouse micronucleus study, there is evidence from the tissue distribution study ([redacted]1985a, unpublished report no. [redacted]MTC 68/84610, section IIIA6.2.1) that a low concentration of etofenprox is widely distributed in the bone marrow after administration of 7 doses of 30mg/kg/day. Therefore, the assay is considered a valid assessment of in vivo clastogenic activity.
	COMMENTS FROM...
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.1.1 Teratogenicity Study

Annex Point IIA-VI.6.8.1

Rat

Oral, gavage

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1985b): Effect of Ethofenprox (MTI-500) on pregnancy of the rat with rearing to maturation of the F1 generation; [redacted] unpublished report no. MTC 64/85422 (October 28, 1985). Dates of work: July 06, 1983 - December 21, 1983.	
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 Method conformed to 88/302/EEC, Part B	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes Deviations: additional animals allowed to litter and rear young to maturity for assessment of behavioural development and reproductive capacity. The deviation does not influence the integrity or validity of the study.	
		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	(CrL:COBS CD (SD) BR strain	
3.2.3	Source	[redacted]	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	8 - 9 week old sexually mature, body weight 180 - 220g	
3.2.6	Number of animals per group	35 females per group	

Section A6.8.1.1 Teratogenicity Study

Annex Point IIA-VI.6.8.1

Rat

Oral, gavage

3.2.7	Control animals	Yes
3.2.8	Mating period	Females mated prior to study start
3.3	**Administration/ Exposure**	**Oral**
3.3.1	Duration of exposure	Day 6-17of gestation
3.3.2	Postexposure period	- From Day 17 to Day 20 of gestation for 21 - 24 females/group - From Day 17 of gestation to Day 21 *post partum* for remaining 11 - 14 females/group.
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Nominal dose levels of 0 (vehicle only), 12.5, 250 and 5000mg/kg bw/day
3.3.5	Vehicle	Aqueous methylcellulose
3.3.6	Concentration in vehicle	0, 0.0625, 1.25 and 25.0 % w/v
3.3.7	Total volume applied	20mL/kg etofenprox suspended in aqueous methylcellulose
3.3.8	Controls	Vehicle
3.3.9	Further remark	On day 20 of gestation, 21 - 24 females/group were killed to investigate possible effects on embryo-fetal development. The remaining 11 - 14 females/group were allowed to litter normally and rear their young.
3.4	**Examinations**	
3.4.1	Body weight	Yes, recorded on days 0, 3, 6, 10, 14, 17 and 20 of gestation, and days 0, 7, 14 and 21 *post partum*, where applicable.
3.4.2	Food consumption	Yes, measured throughout gestation and lactation.
3.4.3	Clinical signs	Yes, recorded daily
3.4.4	Examination of uterine content	The uterus was examined for the number and position of live young, the number and distribution of embryonic/fetal deaths, individual fetal weights and gross fetal abnormalities. The ovaries were examined for the number of corpora lutea. Embryonic/fetal deaths were classified as early (placental remnants visible only) or late (both placental and embryonic remnants visible). Uteri or individual uterine horns without visible implantation sites were immersed in ammonium sulphide to aid visualization of implantations. The reproductive tract was retained in fixative for subsequent histopathological examination. Approximately 50% of the fetuses from each litter were preserved in Bouin's fluid for subsequent free-hand razor sectioning and examination for visceral abnormalities (Wilson's technique).
3.4.5	Examination of foetuses	The remaining fetuses were preserved for subsequent macroscopic examination, evisceration and determination of sex prior to processing for skeletal examination by alizarin red S staining. Fetuses with suspected abnormalities were processed by more appropriate techniques for clarification of initial observations. Structural deviations were classified as malformations (rare and/or probably lethal), anomalies

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

(minor differences from the norm occurring relatively frequently) or variations (alternative structures occurring regularly in control fetuses).

3.5 Further remarks

The F_1 progeny of females allowed to litter were examined as soon as possible after the completion of parturition, counted, the sex determined, weighed and examined for external abnormalities. Subsequently, all litters were examined daily to detect dead fetuses, which were subjected to necropsy, where possible. Litters of 9 or more pups were culled by random selection to 8 pups, 4/sex, where possible, on day 4 *post partum*. Culled progeny were examined externally and internally for abnormalities and confirmation of sex. Live pups were also weighed on days 8, 12 and 21. During lactation, all litters were examined at appropriate intervals to determine the age at which the developmental stages of surface righting reflex, pinna unfolding, upper incisor eruption, eye opening, air righting reflex and pupil reflex were achieved.

One pup/sex was selected for further study on day 21 *post partum*, at which time excess pups and F_0 parental females were killed and examined externally and internally for abnormalities. The sex of the pups was confirmed by gonadal inspection. The uterus of one apparently non-pregnant female was immersed in ammonium sulphide solution to reveal evidence of implantation. Selected F1 progeny were maintained untreated and weighed weekly until termination. The food consumption was measured and the animals were regularly observed for signs of abnormal behaviour. The onset of vaginal opening was monitored in all female progeny from day 28. The following developmental/behavioural examinations were performed from 6 weeks of age:

Mobility, rearing activity and inquisivity using the hole-board test
Motor coordination by the inclined plane test
Passive avoidance (one-trial test)

Reproductive capability was assessed from approximately 84 days of age by 1:1 matings for 14 days. During the mating period, females were weighed every 2 days until sperm in a vaginal smear, or a plug, was observed (= day 0 of gestation). Daily vaginal smears were also evaluated to determine marked anomalies of the oestrous cycle. Mated females were weighed on days 0, 7, 10, 14, 17 and 20 of gestation and on days 0, 7, 14 and 21 *post partum*. The F_2 progeny of F_1 parental animals were observed as for the F_1 progeny, except that no developmental milestones were evaluated during lactation. All F_2 progeny and F_1 parental animals were killed on, or shortly after, day 21 *post partum*, and examined for internal and external abnormalities. The sex of the pups was confirmed by gonadal inspection. The uteri of apparently non-pregnant females were immersed in ammonium sulphide solution to reveal evidence of implantation and the testes of males failing to initiate a litter were weighed and preserved for subsequent histopathological examination. Where appropriate, data were analysed using non-parametric techniques (Kruskal-Wallis and Jonckheere's tests) based on the litter as the sample unit. Alternatively, Fisher's exact test was used instead of Kruskal-Wallis where the proportion of tied values exceeded 75%.

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

4 RESULTS AND DISCUSSION

4.1 Analytics

All formulations were analysed for achieved concentration on one occasion during the study achieved concentrations in the formulations were in the range 93.6 - 102.4% nominal concentrations. Formulations of 12.5 and 200mg/mL etofenprox were homogeneous and were stable for at least 72 hours, at which time means of 11.9 and 198mg/mL remained, respectively.

4.2 Maternal toxic Effects

There were no deaths at any dose level in the treated F_0 female parental animals. However, there was a dose-related increase at all dose levels in the incidence of salivation and red-brown staining around the mouth following dosing in F_0 treated females. In addition, most animals treated at 5000mg/kg bw/day showed wet, yellow staining of the fur in the anogenital region towards the end of the treatment period. Weight gain during gestation was marginally reduced at 5000mg/kg bw/day such that the group mean body weight on day 20 of gestation was 3.6% lower than the control value. There were no treatment-related effects on weight gain at lower dose levels, or at any dose level during lactation. Food consumption was unaffected by treatment at all dose levels. There were no treatment-related macroscopic findings at necropsy at any dose level in F_0 females, with the possible exception of a low incidence of minor skin lesions at 5000mg/kg bw/day.

4.3 Teratogenic / embryotoxic effects

See Table A6_8_1_1-1.
There were no treatment-related effects on pregnancy incidence and no statistically significant ($p > 0.05$) differences between the treated and control groups with respect to litter parameters in the F_0 females killed on day 20 of gestation.

See Table A6_8_1_1-2.
There were no treatment-related effects at any dose level on the nature and incidence of malformations, visceral anomalies and skeletal variants. Although the incidence of visceral anomalies at 5000mg/kg bw/day was higher than the control incidence, it was not significantly different ($p > 0.05$). Furthermore, 10 of the 12 anomalies at 5000mg/kg bw/day were isolated haemorrhages, renal pelvic dilatation or displaced testis, all of which were evident in the control group. The other visceral anomaly at 5000mg/kg bw/day was 2 fetuses from one litter with narrow ductus arteriosus.

See Table A6_8_1_1-3.
There were no treatment-related effects at any dose level on the pregnancy incidence, duration of gestation, and litter parameters in the F_0 groups rearing their young. Litter size, pup mortality, litter and pup weights and sex ratio of all treated groups were comparable to the control values.

The developmental landmarks, surface righting reflex, pinna unfolding, upper incisor eruption, presence of startle response, eye opening, pupil reflex and air righting reflex, in all treated groups were achieved at