Section A 6.5 Chronic Toxicity

Annex Point IIA 6.5 6.5 Chronic toxicity studies in the Beagle dog

Official use only

1 REFERENCE

1.1	**References**	[redacted] 1992, Technical grade dichlofluanid (Euparen® VM 90): oral dosing chronic toxicity studies in the Beagle dog, [redacted], Report No. [redacted], 1992-09-17 (unpublished); Addendum to Original Report No. [redacted]., 1996-06-06 (unpublished); Supplemental submission to Report No. [redacted]., 1997-09-15 (unpublished) [redacted], 2004, Re: [redacted]; Technical grade dichlofluanid (Euparen VM 90): oral dosing chronic toxicity studies in the beagle dog; Report No. [redacted] – Determination of NOEL/NOAEL. [redacted] 2004-03-03 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	Bayer Chemicals AG
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes

The study was conducted in accordance with:

1. OECD-Guideline 452,
2. US-EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, series 83-1
3. US-EPA (TSCA), Health Effects Testing Guidelines, 40 CFR Section 798.3320
4. Japan, Ministry of Agriculture, Forestry and Fisheries, Guidance of Toxicology Study Data for Application of Agriculture Chemical Registration, 59 NohSan No. 4200

2.2 GLP Yes

2.3 Deviations Yes

Deviations from OECD-Guideline 452:

- Careful clinical observations: only once a week (daily is recommended in the OECD-guideline 452)
- Accessory sexual organs: seminal vesicles and vagina were not collected.

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3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The active ingredient concentration of the dichlofluanid technical was determined prior to initiation of dosing and periodically during the course of the studies to verify stability under freezer storage conditions. The concentration of dichlofluanid was verified monthly during the studies.
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle dog
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	First and second study: Age: approximately 6 months (males and females) First study: Average weight [kg] in the first week of treatment: Males: Control: 7.2 Low dose group: 8.1 Mid dose group: 7.2 High dose group: 7.7 Females: Control: 7.6 Low dose group: 7.0 Mid dose group: 7.5 High dose group: 7.5 Second study: Average weight [kg] at day 0: Males: Control: 8.74 Dose group: 8.02 Females: Control: 7.53 Dose group: 7.61
3.2.6	Number of animals per group	First and second study: 4 per sex per group
3.2.7	Control animals	First and second study: Yes

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3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	First and second study: One year
3.3.2	Frequency of exposure	Daily
3.3.3	Post-exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Gelatine capsule
3.3.4.2	Concentration	First study: 0, 2.5, 12.5 or 62.5 mg/kg bw. Due to excessive toxicity, the original high-dose level of 62.5 mg/kg bw was subsequently reduced to 37.5 mg/kg bw after a treatment period of approximately three month. Second study: 0 or 1.25 mg/kg bw
3.3.4.3	Vehicle	—
3.3.4.4	Controls	First study: Gelatine capsule filled with magnesium oxide at a concentration equivalent to that received by the highest dose tested. Second study: Controls were not treated.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes Animals were inspected twice a day (once a day at weekends and on bank holidays). Detailed examination of individual animals was performed once a week.
3.4.1.2	Mortality	Yes Animals were inspected twice a day (once a day at weekends and on bank holidays).
3.4.2	Body weight	Yes Body weights were recorded once a week and were also measured before autopsy.
3.4.3	Food consumption	Yes Feed intake was determined daily until end of study.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes Prior to initiation of dosing and prior to sacrifice, ophthalmic exams were conducted on all animals.

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3.4.6	Haematology	Yes Number of animals: all animals Time points: prior to study initiation and at approximately 3, 6, 9 and 12 (terminal sampling) months on study. Parameters: haematocrit, haemoglobin concentration, erythrocyte count, red cell morphology, total and differential leukocyte count, platelet count, reticulocytes, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV). Remark: Additional bleedings were conducted at 14, 15 and 19 weeks on the high dose animals of the first study, and did not necessarily involve all study animals or all clinical pathology parameters.
3.4.7	Clinical Chemistry	Yes Number of animals: all animals Time points: prior to study initiation and at approximately 3, 6, 9 and 12 (terminal sampling) months on study. Parameters: sodium, potassium, calcium, chloride, phosphate, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, globulin, albumin, creatinine kinase, lactic dehydrogenase, gamma-glutamyl transpeptidase, cholinesterase (see remark), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lipids, T_3 and T_4 (see remark) Remark: Additional bleedings were conducted at 14, 15 and 19 weeks on the high dose animals of the first study, and did not necessarily involve all study animals or all clinical pathology parameters. The hormones triiodothyronine (T_3) and thyroxine (T_4) were evaluated from week 19 until terminal sampling in the first study and in the entire second study. Cholinesterase determinations were performed only in the second study (see 3.5.3)
3.4.8	Urinalysis	Yes Number of animals: all animals Time points: prior to study initiation and at approximately 3, 6, 9 and 12 (terminal sampling) months on study. Parameters: specific gravity, pH, protein, glucose, blood, urobilinogen, bilirubin, ketone bodies.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, ovaries, brain, heart, lungs, spleen, testes, pituitary gland, thyroid
3.5.2	Gross and histopathology	Yes Necropsies were conducted on all animals. All collected organs were examined histopathologically.

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Examined organs:
brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, large intestines, liver, gall bladder, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, mammary gland, prostate, urinary bladder, lymph nodes, peripheral nerve, bone marrow, skin, eyes and optical nerves, eyelids, epididymis, extraorbital lacrimal glands, femur, femorotibial joint (only second study), ribs (costochondral junction), larynx, musculature, sternum and all gross lesions with a border of normal tissue.

3.5.3 Other examinations — Cholinesterase determinations were performed only in the second study. Dichlofluanid is not considered a cholinesterase inhibitor; however the second study shared the control with a separate one-year dog study which tested a recognised cholinesterase inhibitor.

3.5.4 Statistics

First study:
With the exception of organ weights, group means were evaluated initially by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range test.
Organ weights an ANOVA was performed initially, followed by a Student's t-test.

Second study:
For body weight and food consumption a Student's t-test was performed.
A Mann-Whitney U test was used to statistically evaluate clinical pathology and organ weight data.

3.6 Further remarks —

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs — Clinical signs attributable to the compound were not indicated in either sex up to and including a dose of 2.5 mg/kg. Compound-related observations included red gingivae in 12.5 mg/kg males. Excessive salivation, rough coat, decreased activity, dehydration, inflammation of the ears, and red gingivae being noted in 37.5 mg/kg males and/or females.

4.1.2 Mortality — Early deaths included one high-level female dog which was sacrificed due to moribundity after approximately three month exposure to 62.5 mg/kg bw of the test substance. Following reduction the dose to 37.5 mg/kg, no further death or early sacrifices occurred.

4.2 Body weight gain — No declines in body weight gain were observed in either sex up to and including a dose of 2.5 mg/kg bw. Body weight gain in 12.5 mg/kg males and 37.5 mg/kg males and females generally lagged behind controls and was considered compound-related.

4.3 Food consumption and compound intake — Consistent and statistically significant declines in feed consumption were observed beginning at weeks 4 and 13 in 37.5 mg/kg bw dosed females and males, respectively.

Upon reducing the dose at week 14 (62.5 to 37.5 mg/kg bw), feed consumption remained generally depressed in the female through week 27 but had recovered or was approaching recovery by week 20 in the male. A compound-related effect was not suggested in either sex up to

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		and including a dose of 2.5 mg/kg bw.
4.4	**Ophthalmoscopic examination**	No significant findings.
4.5	**Blood analysis**	
4.5.1	Haematology	A non- statistical anaemia in 37.5 mg/kg males starting at six month continuing to study termination was thought to be test substance-related.
4.5.2	Clinical chemistry	A test substance induced increase of liver enzymes (alanine-amino-transferase and/or aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl-transpeptidase) in 12.5 and 37.5 mg/kg bw males and females occurred. Additionally, thyroid hormone alterations were observed in 2.5 mg/kg bw dose group males (T_4) and 37.5 mg/kg bw males and females ($T_3 + T_4$). The change in thyroid hormone levels is based on a single outlier that was measured in a single animal at a single time point. There was no dose-response relationship for this effect and there were no macroscopic or microscopic findings associated with it. In addition, the hormone levels fall within their respective normal range of values (expert judgement: [redacted], 2004).
		Increased cholesterol and/or triglyceride levels due to the test substance were detected in 12.5 and 37.5 mg/kg males and females.
		A substance-related increase of the renal parameters (blood urea nitrogen and/or creatinine) occurred in the 12.5 mg/kg males and 37.5 mg/kg males and females.
4.5.3	Urinalysis	All alterations in the urinalysis parameters were considered unrelated to the test compound.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Decreased absolute and relative thyroid and testicular weights in 37.5 mg/kg males.
4.6.2	Gross and histopathology	Histopathological effects attributed to the test substance were:
		A chronic nephropathy characterised by proximal tubule nephrosis was found in 12.5 mg/kg bw dosed females and in 37.5 mg/kg bw males and females.
		Liver changes in 37.5 mg/kg males and females, including hepatocellular degeneration, haemosiderin pigment accumulation, and biliary hyperplasia with periportal inflammation.
		Thyroid follicular cell degeneration in 37.5 mg/kg males and females and in one 12.5 mg/kg female.
		Hyperplasia of the pituitary gland's basophils in 37.5 mg/kg males and females.
		Testicular degeneration in 37.5 mg/kg males.
		Thymic atrophy in 37.5 mg/kg males.
4.7	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The studies were conducted in accordance with the OECD-Guideline 452, US-EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision

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6.5 Chronic toxicity studies in the Beagle dog

F, series 83-1, US-EPA (TSCA), Health Effects Testing Guidelines, 40 CFR Section 798.3320 and Japan, Ministry of Agriculture, Forestry and Fisheries, guidance of Toxicology Study Data for Application of Agriculture Chemical Registration, 59 NohSan No. 4200.

The objective of the study was to determine the majority of chronic effects and to determine dose-response relationships. In the first study dichlofluanid was administered in a range of 2.5 to 62.5(37.5) mg/kg bw. A supplemental study was performed using dose levels of 0 or 1.25 mg dichlofluanid/kg bw/day. This second study was conducted to include an additional dose level at which no effects would occur. This was felt to be necessary based on the initial assumption that a treatment-related effect occurred at 2.5 mg/kg in males. However, closer inspection of these findings led to the considered interpretation that this finding was neither treatment-related nor of any toxicological significance ([redacted], 2004).

5.2 Results and discussion

First study:

Under the study conditions described, dichlofluanid caused changes in body weight gain, feed consumption, clinical chemistry parameters, organ weights and histopathology.

Clinical signs attributable to the compound were not seen up to and including a dose of 2.5 mg/kg. Compound-related observations included red gingivae in 12.5 mg/kg males. Excessive salivation, rough coat, decreased activity, dehydration, inflammation of the ears, and red gingivae were noted in 37.5 mg/kg males and/or females.

An increase of liver enzymes occurred in 12.5 and 37.5 mg/kg bw males and females. Additionally, thyroid hormone alterations were observed in 2.5 mg/kg bw dose group males and 37.5 mg/kg bw males and females. The elevated T_4 levels in 2.5 mg/kg males are considered to be incidental and of no toxicological relevance. This assessment is based inter alia on the lack of a dose-response relationship and the absence of macro- or microscopical findings correlated with this effect (Berthold, 2004).

Absolute and relative thyroid and testicular weights were decreased in 37.5 mg/kg males.

Histopathological findings attributed to the test substance were: chronic nephropathy in 12.5 mg/kg females and in 37.5 mg/kg bw males and females. Liver changes were observed in 37.5 mg/kg males and females. Thyroid follicular cell degeneration was seen in 37.5 mg/kg males and females and in one 12.5 mg/kg female. Hyperplasia of the pituitary gland's basophils occurred in 37.5 mg/kg males and females. Testicular degeneration and thymus atrophy were noted in 37.5 mg/kg males.

Second study:

No compound-related effects were noted.

5.3 Conclusion

For the beagle dog dosed orally with technical grade dichlofluanid for one year, a systemic NOAEL of 2.5 mg/kg bw /day was established.

5.3.1 LO(A)EL

LOAEL: 12.5 mg/kg bw/day, based on reduced body weight gain and altered clinical chemistry parameters.

5.3.2 NO(A)EL

NOAEL: 2.5 mg/kg bw/day

5.3.3 Other

—

5.3.4 Reliability

1

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5.3.5	Deficiencies	No

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Annex Point IIA 6.5 6.5 Chronic toxicity studies in the Beagle dog

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26/10/04
Materials and Methods	As described above [IUCLID 5.4 9/11]
Results and discussion	*Clinical Chemistry* A number of changes in clinical chemistry parameters were observed. However, as no dose response or temporal relationship could be discerned, these changes are not considered to be of toxicological significance. *Histopathology* The most prominent effect was minimal-moderate chronic nephropathy, observed in animals administered dichlofluanid at doses of 12.5 mg/kg/day and above (7/8 and 8/8 at 12.5 and 37.5 mg/kg/day, compared to 0/8 in controls).
Conclusion	It was possible to identify a NOAEL from these studies, as chronic nephropathy was observed at doses of 12.5 mg/kg/day and above. NO(A)EL: 2.5 mg/kg/day
Reliability	1
Acceptability	Acceptable
Remarks	The UK CA generally agrees with the applicant's summary and conclusions. However, additional information regarding clinical chemistry parameters and histopathological changes in the kidney have been included, as these have been used to determine the NOAEL.

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

Due to results of second study, tables not needed

Table A6_5-1.A Results of clinical chemistry, haematology and urinalysis (first study)

Clinical chemistry	Sex	Unit	Control 0 mg/kg bw	Low dose 2.5 mg/kg bw	Medium dose 12.5 mg/kg bw	High dose 62.5/37.5[#] mg/kg bw
Alanine-aminotransferase		U/l	**14, 19, 27, 40, 53 weeks after start of treatment**			
	male		—	—	↑ (at week 27 only)	↑
	female		—	—	↑ (at week 40 only)	↑
Aspartat-aminotransferase		U/l	**14, 19, 27, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	↑ (at week 14 only)	↑
Alkaline-phosphatase		U/l	**14, 19, 27, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	—	↑
γ-glutamyl-transpeptidase		U/l	**14, 19, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	↑ (at week 53 only)	↑
Cholesterol		mg/dl	**14, 19, 27, 40, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	↑	↑
Triglyceride		mg/dl	**14, 19, 27, 40 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	—	↑

[#]62.5 mg/kg bw was administered for approximately 14 weeks before being reduced to 37.5 mg/kg bw for the remainder of the study.

↓ Decrease

↑ Increase

— not different from control

Table A6_5-1.A Results of clinical chemistry, haematology and urinalysis (first study, continued)

Clinical chemistry	Sex	Unit	Control 0 mg/kg bw	Low dose 2.5 mg/kg bw	Medium dose 12.5 mg/kg bw	High dose 62.5/37.5[#] mg/kg bw
Creatinine		mg/dl	**14, 19, 27, 40, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	—	↑
Blood urea nitrogen		mg/dl	**27, 40, 53 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	—	—
T3		ng/ml	**19, 27, 40, 53 weeks after start of treatment**			
	male		—	—	—	↓
	female		—	—	—	↓
T4		µg/dl	**19, 27, 40, 53 weeks after start of treatment**			
	male		—	↑	—	↓
	female		—	—	—	↓

[#]62.5 mg/kg bw was administered for approximately 14 weeks before being reduced to 37.5 mg/kg bw for the remainder of the study.

↓ decrease

↑ increase

— not different from control

Table A6_5-1.A Results of clinical chemistry, haematology and urinalysis (first study, continued)

Haematology	Sex	Unit	Control 0 mg/kg bw	Low dose 2.5 mg/kg bw	Medium dose 12.5 mg/kg bw	high dose 62.5/37.5[#] mg/kg bw
Erythrocyte		millions/mm^3	**27, 40, 53 weeks after treatment**			
	male		—	—	—	↓
Haemoglobin		g/dl	**27, 40, 53 weeks after treatment**			
	male		—	—	—	↓
Packed cell volume		%	**27, 40, 53 weeks after treatment**			
	male		—	—	—	↓

[#]62.5 mg/kg bw was administered for approximately 14 weeks before being reduced to 37.5 mg/kg bw for the remainder of the study.

↓ decrease

— not different from control

Table A6_3-2.B Results of the chronic toxicity study in Beagle dogs (first study)

Parameter	Control 0 mg/kg bw		Low dose 2.5 mg/kg bw		Medium dose 12.5 mg/kg bw		High dose 62.5/37.5[#] mg/kg bw		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	4	4	4	4	4	4	4	4		
Mortality	0/4[a]	0/4[a]	0/4[a]	0/4[a]	0/4[a]	0/4[a]	0/4[a]	1/4[a]	-	-
Clinical signs										
Red gingivae	—	—	—	—	3/4[a]	—	3/4[a]	3/4[a]	+	+
Rough coat	—	—	—	—	—	—	4/4[a]	4/4[a]	+	+
Inflammation of the ears	—	—	—	—	—	—	4/4[a]	—	+	-
Excessive salivation	—	—	—	—	—	—	4/4[a]	3/4[a]	+	+
Decreased activity	—	—	—	—	—	—	3/4[a]	—	+	-
Body weight gain	—	—	—	—	↓	—	↓	↓	+	+
Mean food consumption	—	—	—	—	—	—	↓ (week 14 to 20)	↓ (week 4 to 27)	+	+
Clinical chemistry	Effects described in table A 6_3-2.A above									
Haematology										
Urinalysis										

[#]62.5 mg/kg bw was administered for approximately 14 weeks before being reduced to 37.5 mg/kg bw for the remainder of the study.

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

Table A6_5-2.B Results of the chronic toxicity study in Beagle dogs (first study, continued)

Parameter	Control 0 mg/kg bw		Low dose 2.5 mg/kg bw		Medium dose 12.5 mg/kg bw		High dose 62.5/37.5# mg/kg bw		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Organ: testes										
Organ weight	—	—	—	—	—	—	↓	—	-	-
Microscopic pathology	—	—	—	—	—	—	Testicular degeneration	—	+	-
Organ: thyroid										
Organ weight	—	—	—	—	—	—	↓	—	-	-
Microscopic pathology	—	—	—	—	—	Thyroid follicular cell degeneration	Thyroid follicular cell degeneration		+	+
Organ: liver										
Microscopic pathology	—	—	—	—	—	—	Hepatocellular degeneration, billiary hyperplasia with periportal inflammation, hemosiderin pigment accumulation		+	+
Organ: pituitary gland										
Microscopic pathology	—	—	—	—	—	—	Hyperplasia of the basophiles		+	+
Organ: thymus										
Microscopic pathology	—	—	—	—	—	—	Thymic atrophy	—	+	-
Organ: kidneys										
Microscopic pathology	—	—	—	—	Chronic nephropathy —	Chronic nephropathy +	Chronic nephropathy		+	+

#62.5 mg/kg bw was administered for approximately 14 weeks before being reduced to 37.5 mg/kg bw for the remainder of the study.

↑ increase

↓ decrease

— not different from control