Section A 6.4 Subchronic Toxicity

Annex Point IIA 6.4 6.4.1 Subchronic oral toxicity in the Beagle dog

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]. (2004): Technical Grade Dichlofluanid (Euparen) – A 90-Day Subchronic Toxicity Feeding Study in the Beagle Dog. [redacted], Report No. [redacted] (unpublished)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. U.S. EPA Health Effects Test Guidelines, OPPTS 870.3150 (1998) 2. OECD Guidelines for Testing of Chemicals, Section 4, Guideline 409, September, 1998. 3. JMAFF, Ref. No. 12 Nousan No. 8147, November 2000
2.2	**GLP**	Yes
2.3	**Deviations**	No

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 crystalline powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Expiration date Oct 2, 2004
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	Five months, 6.9-8.8 kg
3.2.6	Number of animals per group	4 per sex and dose group
3.2.7	Control animals	Yes

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3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	*Ad libitum*
3.3.3	Post-exposure period	None
3.3.4	**Oral**	
3.3.4.1	Type	Dietary
3.3.4.2	Concentration	Nominal concentrations: 0, 400, 680, 1000 ppm Actual concentrations: 0, 383, 650, 924 ppm Equivalent to: 0, 13, 24, 35 mg/kg body weight/day (males) 0, 13, 20, 34 mg/kg body weight/day (females)
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes At least once daily for signs of toxicity. Detailed clinical observations for clinical signs of toxicity were performed on all animals at study initiation and on a weekly basis thereafter.
3.4.1.2	Mortality	Yes Twice daily for moribundity and mortality, except for once on weekends and holidays.
3.4.2	Body weight	Yes Individual body weights were measured weekly throughout the study. Body weights were also taken immediately prior to necropsy to allow for calculation of organ to body weight ratios.
3.4.3	Food consumption	Yes Feed intake was determined daily throughout the study.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes Following the acclimation period and prior to initiation of dosing, ophthalmic exams were conducted on all animals. Ophthalmic exams were also conducted on all animals just prior to termination of the study.
3.4.6	Haematology	Yes Once prior to administration of the test substance, on all animals. Following initiation of dosing, approximately every four weeks, on all animals. Parameters: blood cell morphology, mean corpuscular haemoglobin (MCH), erythrocytes (RBC), mean corpuscular haemoglobin concentration (MCHC), haematocrit (HCt), haemoglobin (Hgb), platelets (PLTS), leukocytes (WBC, total & differential), reticulocytes

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		(Retic), prothrombin time (PT), mean corpuscular volume (MCV), activated partial thromboplastin time (APTT)
3.4.7	Clinical Chemistry	Yes Once prior to administration of the test substance, on all animals. Following initiation of dosing, approximately every four weeks, on all animals. Parameters: alanine aminotransferase (ALT), albumin (Alb), A/G ratio, alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (T-Bili), bile acids, blood urea nitrogen (BUN), calcium (Calc), chloride (Cl), total cholesterol (Chol), creatinine (Creat), creatinine phosphokinase (CK), gamma-glutamyltranspeptidase (GGT), globulin (Glob), glucose (Gluc), lactic dehydrogenase (LDH), phosphate (Phos), potassium (K), total protein (T-Prot), sodium (Na), triglycerides (Trig), thyroid stimulating hormone (TSH), thyroxine (T4), tri-iodo-thyronine (T3), uric acid (Uric-A)
3.4.8	Urinalysis	Yes Once prior to administration of the test substance, on all animals. Following initiation of dosing, approximately every four weeks, on all animals. Parameters: appearance, microscopic exam of sediment, specific gravity, pH, ketones, glucose, urobilinogen, occult blood, nitrites, bilirubin, urine volume 24 hour, leukocytes, urine creatinine (24 hour)
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, all animals. Organs: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary, spleen, testicle, thymus, thyroid, uterus
3.5.2	Gross and histopathology	Yes, all animals. Organs: adrenal, aorta, bone (rib/CCJCT, sternum, marrow), brain (cerebellum, cerebrum – midbrain, medulla/pons), caecum, cervix, colon, duodenum, epididymis, eye, Fallopian tube (oviduct), gall bladder, heart, ileum, jejunum, kidney, larynx, liver, lung, lymph node (mesenteric, retropharyngeal), mammary gland, muscle, nasopharynx, nerve (optic, sciatic), oesophagus, ovary, pancreas, parathyroid, pituitary, prostrate, rectum, salivary gland, skin, spinal cord (cervical, thoracic, lumbar), stomach, spleen, testicle, thymus, thyroid, trachea, urinary bladder, uterus, vagina Gross pathology was performed on all animals; histopathology was conducted on control and high-dose group.
3.5.3	Other examinations	–
3.5.4	Statistics	Statistical significance was determined at $p<0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p<0.001$ was used. All tests were two-tailed, except for gross and histopathological lesion evaluations that were one-tailed. Continuous data was analyzed by Bartlett's test for homogeneity. If the data was homogeneous, an ANOVA was performed followed by Dunnett's t-test on parameters showing a significant effect by ANOVA. If the data were non-homogeneous, a Kruskal-Wallis ANOVA was

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		performed followed by the Mann-Whitney U-test to identify statistical significance between groups. □ Frequency data that were examined statistically, were initially analyzed by a Chi-Square procedure. If there was statistical significance using the Chi-square test, each treatment group was compared to the control group using a Fisher's Exact test.
3.6	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No compound-related clinical signs.
4.1.2	Mortality	No deaths occurred during the study.
4.2	**Body weight gain**	No compound-related effect on body weight.
4.3	**Food consumption and compound intake**	No compound-related effect on food consumption.
4.4	**Ophthalmoscopic examination**	No abnormal ophthalmic findings.
4.5	**Blood analysis**	
4.5.1	Haematology	There were non-statistical decreases in the high-dose group male red blood cell count (RBC), haemoglobin (Hgb) and haematocrit (Hct) values at days 64 and 86. These changes were not considered to be compound-related since these values were not statistically significant from controls, the changes were still relatively small and of questionable biological significance, and/or all but one of the values were within the historical control ranges.
4.5.2	Clinical chemistry	Clinical chemistry changes that were considered to be compound-related were limited to high-dose males and females: 1) minimal statistically increased urea nitrogen, 2) minimal statistically increased creatinine values in females, 3) statistically increased alanine aminotransferase values in females, and 4) a non-statistical increase in ALT values in males.
4.5.3	Urinalysis	No compound-related effects on urinalysis.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No compound-related effects on organ weights.
4.6.2	Gross and histopathology	No compound-related gross pathology findings. The only compound-related microscopic findings were minimal liver changes (hepatocellular vacuolization, individual cell necrosis, pigmentation, and inflammation were noted in high-dose group males and females). Most of these minimal liver changes occurred in the periportal region of the liver. Vacuolization, necrosis and/or pigmentation were limited to an occasional individual cell. In general, the minimal inflammatory responses were random but sometimes associated with the biliary system.
4.7	**Other**	–

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Technical grade dichlofluanid was administered via the diet to 4 male and 4 female purebred Beagle dogs, *Canis familiaris*, per dose group for 13 weeks. The test compound was administered at nominal doses levels of 0 (control group), 400, 680, and 1000 ppm (actual doses were 0, 383, 650, and 924 ppm). During the study, the animals were evaluated for the effect of the test compound on body weight, food consumption, clinical signs, the eyes, clinical chemistry, and haematology. Gross necropsy evaluations were performed on all adults. Histopathological evaluation of selected tissues was conducted on the control and high-dose groups.

The study was conducted in accordance with: U.S. EPA Health Effects Test Guidelines, OPPTS 870.3150 (1998, OECD Guidelines for Testing of Chemicals, Section 4, Guideline 409, September, 1998, and JMAFF, Ref. No. 12 Nousan No. 8147, November 2000

5.2 Results and discussion

The following summarizes the findings from this study:

1. There were no deaths during the study.
2. There were no compound-related clinical signs.
3. There were no abnormal ophthalmic findings.
4. There was no compound-related effect on body weight.
5. There was no compound-related effect on food consumption.
6. Clinical chemistry changes that were considered to be compound-related were limited to high-dose males and females: 1) minimal statistically increased urea nitrogen, 2) minimal statistically increased creatinine values in females, 3) statistically increased alanine aminotransferase values in females, and 4) a non-statistical increase in ALT values in males.
7. There were compound-related haematological findings.
8. There were no compound-related effects on urinalysis.
9. There were no compound-related gross pathology findings.
10. There were no compound-related effects on organ weights.
11. The only compound-related microscopic findings were minimal liver changes (hepatocellular vacuolization, individual cell necrosis, pigmentation, and inflammation were noted in high-dose group males and females).

5.3 Conclusion

The NOEL for this study was 650 ppm, based on liver effects and clinical chemistry findings at the 924 ppm dose level.

5.3.1	LO(A)EL	
5.3.2	NO(A)EL	
5.3.3	Other	–
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12/10/04*
Materials and Methods	As described above [IUCLID 5.4 6/11]
Results and discussion	As described above
Conclusion	LO(A)EL: 34-35 mg/kg /day NO(A)EL: 20-24 mg/kg /day
Reliability	1
Acceptability	Acceptable
Remarks	The UK CA agrees with applicant's summary and conclusions.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4-1. Results of clinical chemistry, haematology and urinalysis

Parameter changed	Unit	Controls 0 ppm	Low dose 383 ppm	Medium dose 650 ppm	High dose 924 ppm
Males					
BUN	mg/dL	—	—	—	↑*
ALT	U/L	—	—	—	↑#
Females					
BUN	mg/dL	—	—	—	↑*
Creatinine	mg/dL	—	—	—	↑*
ALT	U/L	—	—	—	↑*

* $p < 0.05$
# not statistically significant

Table A6_4-2. Results of repeated dose toxicity study

Parameter	Controls 0 ppm		Low dose 383 ppm		Medium dose 650 ppm		High dose 924 ppm		Dose-response +/–	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	4	4	4	4	4	4	4	4		
Mortality	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	–	–
Clinical chemistry										
BUN	—	—	—	—	—	—	↑	↑	+	+
Creatinine	—	—	—	—	—	—	—	↑	–	+
ALT	—	—	—	—	—	—	—	↑	–	+
Liver										
Micropathology										
Vacuolization	0/4	0/4	n.e.*	n.e.	0/4	1/4	1/4	3/4	+	+
Individual cell necrosis	0/4	0/4	n.e.	n.e.	0/4	1/4	1/4	3/4	+	+
Chronic inflammation	0/4	0/4	n.e.	n.e.	0/4	0/4	1/4	1/4	+	+

*n.e. = not examined