Section A6.10 Mechanistic Study

Annex Point IIA6.10 6.10 Subchronic study in rats to ascertain the dose-time-effect relationship in the effect on the thyroid

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1981, Subchronic study to ascertain the dose-time-effect relationship in the effect on the thyroid (feeding study over 9 weeks), [redacted], Report No. [redacted], 1981-10-27 (unpublished) [redacted], 1981, Effect of subchronic KUE 13032c (Euparen® active ingredient) administration on the thyroid function in male rats, [redacted] (= Report No [redacted]), [redacted], Pharma Report No. [redacted], 1981-03-11 (unpublished)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**	No No guidelines available.
2.2	**GLP**	No GLP was not compulsory at the time the study was performed.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Dichlofluanid
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	—
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	—
3.2	**Test Animals**	
3.2.1	Species	Rats
3.2.2	Strain	Wistar TNO/W.74 (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	Age: 9 –10 weeks Mean weight: 125.2 g

Section A6.10 Mechanistic Study

Annex Point IIA6.10 6.10 Subchronic study in rats to ascertain the dose-time-effect relationship in the effect on the thyroid

3.2.6	Number of animals per group	80 per dose at study begin. At day 7, 21, and 63 ten animals each for gross pathology and for testing the function of the thyroid were employed.	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	Nine weeks	
3.3.2	Frequency of exposure	Daily	
3.3.3	Post-exposure period	None.	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	Food: 0, 150, 500, 1500 or 4500 ppm (= 0, 11.93, 39.30, 120.90, 355.08 mg/kg bw/day) Food consumption per day ad libitum.	X
3.3.4.3	Vehicle	—	
3.3.4.4	Concentration in vehicle	—	
3.3.4.5	Total volume applied	—	
3.3.4.6	Controls	Plain diet.	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, daily.	
3.4.1.2	Mortality	Yes, daily.	
3.4.2	Body weight	Yes, weekly.	
3.4.3	Food consumption	Yes, weekly.	
3.4.4	Water consumption	No.	
3.4.5	Ophthalmoscopic examination	No.	
3.4.6	Haematology	No.	
3.4.7	Clinical Chemistry	Yes, Number of animals: all animals Time points: Parameters: Calcium, inorganic phosphate, total thyroxin (T_4) For further examinations see: [redacted], 1981, Effect of subchronic [redacted] (Euparen® active ingredient) administration on the thyroid function in male rats [redacted] (= Report No. XXXX), [redacted], Pharma Report No. [redacted], 1981-03-11 (unpublished)	

Section A6.10 Mechanistic Study

Annex Point IIA6.10 6.10 Subchronic study in rats to ascertain the dose-time-effect relationship in the effect on the thyroid

3.4.8	Urinalysis	No.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, Organs: liver, thyroid, adrenals
3.5.2	Gross and histopathology	Yes, All dose groups for gross pathology: 10 animals/group at day 7 and 21 of treatment; 30 animals per group at day 63 of treatment. Histopathology: 50 animals from control and high dose group Organs: liver, adrenals, thyroid, skeletal muscle and all grossly apparent alterations
3.5.3	Other examinations	—
3.5.4	Statistics	The calculations made were: arithmetic group means, standard deviation, upper and lower confidence limits at confidence limits at the confidence level of 95% and 99%. The test groups' values for the doses investigated were compared with the controls with the Mann, Whitney and Wilcoxon significance test (U test) at the significance level $\alpha = 5\%$ and $\alpha = 1\%$.
3.6	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	No effects.
4.1.2	Mortality	No effects.
4.2	**Body weight gain**	During the entire study period the weight gains of the 4500 ppm dose group animals was slightly (about 5%) retarded, but only significantly up to the third week ($p< 0.01$). At the same time the body weights after 1500 ppm were also significantly lower.
4.3	**Food consumption and compound intake**	Only the high dose group (4500 ppm) showed a slightly slower feed intake (5%) than the control groups.
4.4	**Ophthalmoscopic examination**	—
4.5	**Blood analysis**	
4.5.1	Haematology	—
4.5.2	Clinical chemistry	Isolated significant deviations in calcium levels occurred after a dichlofluanid feeding period of seven days, but they were not distributed in relation to dose. All single values were also within the norm for animals of this age, and there were therefore toxicologically without significance. After 21 and 63 study days slightly lower T_4 concentrations were noted in all dose groups, which were statistically significant at 1500 ppm and 4500 ppm after 21 days. The inorganic phosphate level was only significantly lower in the high dose group (4500 ppm) at day 21 of treatment. The significantly lower (150 and 500 ppm groups) and higher (1500 ppm group) Ca levels after 21 study days

were unrelated to dose and differed absolutely to a very slight degree (below 5%) from controls. At the end of study the Ca level was slightly (5%) but significantly higher after dosing with 1500 ppm and 4500 ppm. The determinations of calcium and inorganic phosphate provided no indications at any time of effects on the parathyroids in the dose groups up to 4500 ppm.

4.5.3 Urinalysis —

4.6 Sacrifice and pathology

4.6.1 Organ weights

The liver weights of the 4500 ppm dose group after 21 days of treatment were slightly (about 10%) but significantly lower, and significantly higher at study termination. The lower liver weights were considered as a consequence of the significantly lower body weights. The higher liver weights obtained at the end of the study might be interpreted as substance-induced, taken in combination with the results of previous studies of dichlofluanid.

In all dose groups the thyroid weights were higher after seven study days. These differences from the control were, with the exception of the 500 ppm group, statistically significant ($p < 0.01$). After 21 study days, only slight differences in the thyroid weights, compared with the control, were obtained for all dose groups. At the end of the study the thyroids were significantly heavier in the 500 ppm dose group and significantly lighter after dosing with 1500 ppm.

4.6.2 Gross and histopathology

No effects.

4.7 Other —

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

To establish the dose-time-effect relationship of the thyroid effect of dichlofluanid, male rats administered this active substance with their feed in concentrations of 0, 150, 500, 1500 and 4500 ppm for seven, 21 or 63 days. The animals were inspected daily and any changes and symptoms were recorded. The animals were weighed weekly. The feed consumption was found by weighing the uneaten feed.

The clinical laboratory examinations were conducted on day 7, 21 and 63 after starting of feeding with ten rats each dose. The blood was taken from animals narcotised with ether from the retro-orbital venus plexus (Nöller, H.G., Klein. Wschr. 33, 770, 1955). The total thyroxine (T_4) was determined with the Merckotest®Emit® –tfg Thyroxine manual, the anorganic substrates calcium and phosphate were quantified by flame photometry and Gomorri's method (Gomorri, G., J. Lab. Clin. Med. 27, 955, 1942), respectively. To test functioning of the thyroid ten animals per group were taken after 7, 21, and 63 study days. The iodine accumulation in the thyroid (iodide phase) was ascertained by means of the radioiodine test. The concentrations of the thyroid hormones triiodothyronine (T_3) and tetraiodothyronine (T_4) in the serum were determined by radioimmunology (hormone phase). These thyroid function examinations were referred apart from the current study ([redacted], 1981, Effect of subchronic [redacted] (Euparen® active ingredient) administration on the thyroid function in

Section A6.10 Mechanistic Study

Annex Point IIA6.10 6.10 Subchronic study in rats to ascertain the dose-time-effect relationship in the effect on the thyroid

male rats, [redacted], Pharma Report No. [redacted], 1981-03-11).

After 7 and 21 days of feeding, ten animals from each group were narcotised with ether and sacrificed by exsanguination as were the 30 surviving animals per group after 63 study days. The animals were then dissected and grossly examined. The following organs from all animals were weighed: thyroid, liver and adrenals. For histopathology the following organ material was fixed in 10% formaldehyde solution: liver, adrenals, thyroid, skeletal muscle, and all grossly apparent alterations. Approximately 5 µm-thick Paraplast sections were produced from the fixed organs and stained with haemalum-eosin. 50 animals each from the control and the high dose group (4500 ppm) were examined.

5.2 Results and discussion

Under the conditions described above doses up to 500 ppm were tolerated without any effect.

During the entire study period the weight gains of the 4500 ppm dose group animals was slightly (about 5%) retarded, but only significantly up to the third week ($p < 0.01$). At the same time the body weights after 1500 ppm were also significantly lower. Gravimetric, gross pathological and histopathological examinations did not reveal any liver damage in the rats dosed up to 1500 ppm. Animals in the 4500 ppm dose group had higher liver weights than the respective control group at the study termination. However, histopathological examinations did not detect any treatment-related differences. Gross pathology and histopathology in the other organs provided no indication of treatment-related changes in the groups up to 4500 ppm.

The thyroid function was not affected in the groups up to 1500 ppm (see: [redacted], 1981, Effect of subchronic [redacted] (Euparen® active ingredient) administration on the thyroid function in male rats, [redacted], Pharma Report No. [redacted], 1981-03-11). The significant differences of the thyroid function parameters compared with the controls which occurred in the 4500 ppm group point to a slight effect on the thyroid's synthesis and/or incretion phase; a lasting decompensation of the thyroid control system was not noted.

5.3 Conclusion

5.3.1	LO(A)EL	Reduced body weights, 1500 ppm
5.3.2	NO(A)EL	500 ppm (= 39.3 mg/kg bw/day)
5.3.3	Reliability	2
5.3.4	Deficiencies	Yes Homogeneity and stability of the active substance in the feed was not reported.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9/08/06
Materials and Methods	The UK CA notes that the lowest dose was 50 ppm, not 150 ppm as described in the methods section (3.3.4.2) above.
Results and discussion	Repeated dietary administration of dichlofluanid caused a decrease in the blood levels of the thyroid hormones T3 and T4.
Conclusion	These changes are consistent with perturbation of the hypothalamus-pituitary-thyroid (HPT) axis, and would be anticipated from substances perturbing the HPT axis via a number of different mechanisms. However, in isolation, changes in T3 and T4 levels are unable to identify which mechanism may be operating. Therefore, although dichlofluanid decreases blood levels of thyroid hormones, this study does not provide any useful information regarding the mechanism of action.
Reliability	
Acceptability	Acceptable
Remarks	The UK CA has provided additional comment, as the study is relevant for a discussion of the carcinogenic potential of dichlofluanid.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3-1. Results of clinical chemistry in male rats

Parameter changed	Controls 0 ppm			Low dose 150 ppm			Medium dose 500 ppm			Medium dose 1500 ppm			High dose 4500 ppm		
Days after start of treatment	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**
Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Calcium [mmol/l]	—	—	—	↑**	↓*	—	↑*	↓**	—	↑*	↑**	↑**	—	—	↑**
Inorganic Phosphate [mmol/l]	—	—	—	—	—	—	—	—	—	—	—	—	—	↓*	—
T_4 [nmol/l]	—	—	—	—	—	—	—	—	—	—	↓*	—	—	↓*	—

*p < 0.05 in the Mann-Whitney and Wilcoxon U test
**p < 0.01 in the Mann-Whitney and Wilcoxon U test
↑ increase
↓ decrease
— not different from control

Table A6_3-2. Results of repeated dose toxicity study in male rats

Parameter changed	Controls 0 ppm			Low dose 150 ppm			Medium dose 500 ppm			Medium dose 1500 ppm			High dose 4500 ppm			Dose-response +/-
Days after start of treatment	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**	**7**	**21**	**63**	
Number of animals examined	80	60	40	80	60	40	80	60	40	80	60	40	80	60	40	
Body weight	—	—	—	—	—	—	—	—	—	—	↓**	—	↓**	↓**	↓*	+
<u>Organ: liver</u> Organ weight	—	—	—	—	—	—	—	—	—	↑**	—	—	—	↓*	↑*	+
<u>Organ: thyroid</u> Organ weight	—	—	—	↑**	—	—	—	—	↑**	↑**	—	↓**	↑**	—	—	-

*p < 0.05 in the Mann-Whitney and Wilcoxon U test
**p < 0.01 in the Mann-Whitney and Wilcoxon U test
↑ increase
↓ decrease
— not different from control