Section A 6.5/6.7 Chronic Toxicity/Carcinogenicity

Annex Point IIA 6.5/6.7 6.5/6.7 Combined chronic toxicity/carcinogenicity study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1993, KUE 13032 C (c.n. dichlofluanid) - Study on chronic toxicity and carcinogenicity in Wistar rats (administration in the feed over 105 weeks), [redacted], Report No. [redacted], 1993-06-15 (unpublished) [redacted], 1994, KUE 13032 C (c.n. dichlofluanid) - Study on chronic toxicity and carcinogenicity in Wistar rats (administration in the feed over 105 weeks), Amendment to Report No. [redacted], Report No. [redacted], 1994-09-19 (unpublished)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 the OECD-Guideline 453 and the recommendations contained in EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, series 83.5
2.2	**GLP**	Yes
2.3	**Deviations**	Yes Satellite groups: the OECD-guideline 453 recommended the examination of 20 animals per sex in the control and high dose group. In this study, 10 additional animals in every dose group were examined. Haematology and urinalysis: only 10 animals per sex per group instead of 20 animals were examined. The recommended sampling time at 3 month was not performed.

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]

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3.1.2.3	Stability	The homogeneity and stability of the test substance in food were analysed throughout the period of use (feeding period of approx. 7 or 3 days) using sample mixes. The results showed the test substance to be homogeneously distributed and stable at the concentrations used throughout the period of use. The test substance content of the food was generally checked at regular intervals throughout the study (about every 6 ± 1 weeks). This was done by analysing samples of the food mixes used.
3.2	**Test Animals**	
3.2.1	Species	Wistar rats
3.2.2	Strain	BOR:WISW (SPF-CpB)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	Age: 5- 6 weeks (males and females) Weight: males: mean 97 g (74 – 116 g); females: mean 88 g (70 – 104 g)
3.2.6	Number of animals per group	60 animals group/sex
3.2.6.1	at interim sacrifice	10 animals/group/sex
3.2.6.2	at terminal sacrifice	50 animals/group/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	105 weeks
3.3.2	Interim sacrifice(s)	after 53 weeks
3.3.3	Final sacrifice	after 105 weeks
3.3.4	Frequency of exposure	Daily
3.3.5	Post-exposure period	None.
3.3.6	Type	In food
3.3.7	Concentration	Food 0, 180, 900 and 4500 ppm (intake of test substance: 0, 9.4, 54.4, and 301.3 mg/kg bw/day for males and 0, 13.5, 73.1, and 420.7 mg/kg bw/day for females) Food consumption per day ad libitum, except during the urine collection period.
3.3.8	Vehicle	—
3.3.9	Concentration in vehicle	—
3.3.10	Total volume applied	—

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3.3.11	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Body weight	Yes Before treatment commenced, than once a week up to and including week 13 and than every two weeks up to and including week 103. Body weights were also measured before autopsies after 53 and 105 weeks of treatment.
3.4.2	Food consumption	Yes Weekly in the first 13 weeks of treatment, and every 4 weeks until end of study.
3.4.3	Water consumption	No
3.4.4	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.
3.4.5	Macroscopic investigations	Palpable masses. Due to an increase in aggressive behaviour, the animals were only palpated once every three weeks, rather than once a week, after week 77.
3.4.6	Ophthalmoscopic examination	Yes 3 or 4 days before the study commenced on 20 animals each group, than after 53 weeks of treatment and before final autopsy after 103 weeks of treatment on 20 male and 20 female animals from control and high dose treatment group.
3.4.7	Haematology	Yes Number of animals: 10 animals/sex/group Time points: After 26/27, 53/54, 79/80 and 104/105 weeks of treatment Parameters: haematocrit, haemoglobin concentration, erythrocyte count, red cell morphology, total and differential leukocyte count, reticulocyte count, platelet count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV). Other: —

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3.4.8	Clinical Chemistry		Yes
		Number of animals:	10 animals/sex/group
		Time points:	After 26/27, 53/54, 79/80 and 104/105 weeks of treatment
		Parameters:	sodium, potassium, calcium, chloride, phosphate, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lipids.
		Other	—
3.4.9	Urinalysis	Yes	
		Number of animals:	10 animals/sex/group
		Time points:	After 26/27, 53/54, 79/80 and 104/105 weeks of treatment
		Parameters:	Appearance, volume, density, osmolality, specific gravity, pH, protein, glucose, blood, urobilinogen, bilirubin, ketone bodies, microscopic sediment examination (bacteria, epithelial cells, leucocytes, erythrocytes, amorphous salt, triplephosphate).
		Other	—
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		from:	10 animals/sex/group at interim sacrifice, all surviving animals at terminal sacrifice
		Organs:	liver, kidneys, adrenals, testes, ovaries, brain, heart.
			Other —
3.4.11	Histopathology	Yes	
		from:	all dose groups
		from:	10 animals/sex/group at interim sacrifice
			all surviving animals at terminal sacrifice
		Organs:	brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, ureter, urethra, vagina, female mammary gland, prostate, urinary bladder, lymph nodes, peripheral nerve, bone marrow, skin, eyes with eye lids and optical nerves, cranium, epididymis, extraorbital lachrymal glands, femur, Harderian glands, head, larynx, musculature (femoral), seminal vesicles, sternum, tongue, Zymbal's gland, tissues with significant macroscopic changes.

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		Other —
3.4.12	Other examinations	Determination of fluoride in bones and teeth was performed after 53 weeks on the animals scheduled for interim autopsy and after 105 weeks on the first 10 animals from each group, which had randomly selected for the ophthalmological investigations.
3.5	**Statistics**	Body weight, medical laboratory tests, food consumption, organ weight: "U test" of H.B. Mann and D. R. Whitney, Ann. Math. Stat. 18, 50, 1947 or F. Wilcoxon, Biometrics 1, 80, 1945 at significance levels of α = 5 % and α = 1 %. Survival: WILCOXON-test (BRESLOW test, Breslow, N. E., Biometrika 1, 579, 1979) Clinical pathology: Fisher's exact probability test at significance levels of α = 5 % and α = 1 % and 0.1 %. Cochran-Armitage trend test at significance levels of α = 5 % and α = 1 % and 0.1 %. Fluoride calculations: Means, standard deviation and the median were calculated on a routine basis. Groups were compared at the confidence level of 95 % (p = 0.05). Box test (F-test) Post-hoc comparison of pairs of treatment groups (one and two-sided) is carried out in accordance with the modified Tukey-Kramer significance test (Games and Howell)
3.6	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	No effects up to and including doses of 900 ppm. The body weights of male and female animals in the 4500 ppm dose group were significantly lower than those of the control animals.
4.2	**Food consumption**	Mean food consumption per day was comparable in all groups. The mean food intake per animal/kg bw calculated in the 4500 ppm dose group is higher if the lower body weights of the animals in this group are taken into account.
4.3	**Clinical signs**	At the weekly inspection, skin changes were observed on the noses of the animals in the 4500 ppm dose group. Histopathological investigations did not identify any related findings. The incisors of the animals in this group also had to cut frequently. This was thought to be due to a hardening on the teeth caused by fluoride deposition, which meant that there was less wear on the teeth. Both effects were considered to be treatment-related. Mortality was not affected.
4.4	**Macroscopic investigations**	No evidence of treatment-related effects could be deduced from the frequency, localisation and time of appearance of palpable tissue masses.
4.5	**Ophthalmoscopic examination**	No effects.

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4.6	**Haematology**	No effects.	
4.7	**Clinical Chemistry**	The medical laboratory tests on blood samples in weeks 26/27, 53/54, 79/80 and 104/105 showed a slight and sometimes significant decrease in the plasma activity of alanine-aminotransferase in both sexes at doses of 4500 ppm. Tests on the other enzymes, substrates and electrolytes revealed no evidence of treatment related effects.	
4.8	**Urinalysis**	No effects.	
4.9	**Pathology**	Interim autopsy after 53 weeks: a change in the colour and consistency of the cranium (whitish, hardened) was recorded in animals of both sexes in the 4500 ppm dose group.	
		Terminal autopsy: a change in the colour of the cranium (whitish) was recorded in male and females at 4500 ppm.	X
4.10	**Organ Weights**	Liver: Determination of the relative organ weights showed the weights of both male and female livers to be (sometimes significantly) higher at and above doses of 900 ppm. Because the histopathological investigations provided no evidence of liver damage, there may have been marginal functional effects, such increases in the rate of metabolism, at doses of 4500 ppm.	X
		Kidneys: At the interim autopsy, an increase in the relative weight of the kidneys of females in the 4500 ppm dose group was recorded. The final autopsy showed an increase in the absolute and/or relative weight of the kidneys of all treated males and of treated females at and above doses of 900 ppm.	X
4.11	**Histopathology**	Cranium: Histopathological investigations revealed an increase of osteosclerosis and lamellar growth patterns on the cranial surfaces at and above doses of 180 ppm.	
		Kidneys: Histopathological examination of the kidneys provided no evidence of morphological changes.	
		Forestomach: Histopathological investigations revealed acanthosis and hyperkeratosis of the forestomach of animals in the 4500 ppm dose group due to local irritation caused by the test substance.	
		Thyroid: A higher number of follicular adenomas of the thyroid were recorded in male and female animals of the 4500 ppm dose group. This result is probably due to effect of the test substance on the thyroid feedback mechanism and is not considered as evidence of a primary carcinogenic effect.	
4.12	**Other examinations**	A dose-related, significant increase in the fluoride concentrations determined in the bones (femoral) and teeth (incisors) was recorded at and above doses of 180 ppm.	X
4.13	**Time to tumours**	—	
4.14	**Other**	—	

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The objective of the study was to determine the no effect level of the Dichlofluanid when administered continuously in food, and to identify target organs and any carcinogenic effects.

The study was conducted in accordance with the OECD-Guideline 453 and the recommendations contained in EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, series 83.5.

5.2 Results and discussion

At the weekly inspection, skin changes were observed on the noses of the animals in the 4500 ppm dose group. The incisors of the animals in this group also had to cut frequently.

The body weights of male and female animals in the 4500 ppm dose group were significantly lower than those of the control animals.

A dose-related, significant increase in the fluoride concentrations determined in the bones (femoral) and teeth (incisors) was recorded at and above doses of 180 ppm. Histopathological investigations revealed an increase of osteosclerosis and lamellar growth patterns on the cranial surfaces at and above doses of 180 ppm.

Acanthosis and hyperkeratosis of the forestomach of animals were observed in the 4500 ppm dose group due to local irritation caused by the test substance.

At doses of 4500 ppm, a higher incidence of neoplastic changes was observed in the thyroids of males and females. There were probably due to effects on the thyroid feedback mechanism.

Under the study conditions described, Dichlofluanid caused changes characteristic of fluorosis at doses of 180 ppm.

5.3 Conclusion

No “no-effect dose” (with regard to fluoride deposition) was attained in this study. The no-effect dose was therefore estimated on the basis of the fluoride concentrations determined in the femoral bones at the end of the study (non GLP). On the basis of this estimate, a no-effect dose would probably be approximately achieved with a 154.5 ppm food mix for males and a 36.7 ppm food mix for females.

LOEL: Signs of fluorosis at 180 ppm (approx. 9.4 mg/kg bw/day for males and 13.5 mg/kg bw/day for females).

Extrapolated NEL: 154.5 ppm (7.73 mg/kg bw/day) for males and 36.7 ppm (1.84 mg/kg bw/day) for females.

5.3.1 Reliability 2

5.3.2 Deficiencies No

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	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14/10/04
Materials and Methods	As described above [IUCLID 5.4 4/11]
Results and discussion	A statistically significant dose-dependent increase in the fluoride content of the teeth (3 x, 12 x and 39 x and 3 x, 11 x and 54 x; at the low, middle and high doses, in males and females respectively) and bone (2.5 x, 7.3 x and 32 x and 2 x, 5 x and 16 x; at the low, middle and high doses, in males and females respectively) was observed in all treated animals. A statistically significant increase in cranial osteosclerosis was also observed in all treated males (34/50, 34/50 and 49/50 compared to11/50 in controls) and in high dose females (46/50 compared to 13/50 in controls). Increases in relative liver and kidney weights were observed. However, these changes are not considered toxicologically significant, as no histopathological abnormalities were observed. It should be noted that no non-neoplastic histopathological abnormalities were noted in the thyroid.
Conclusion	It was not possible to identify a NOAEL from this study, as fluorosis of the teeth and bones was observed at all dose levels. LOEL: Signs of fluorosis at 180 ppm (approx. 9.4 mg/kg/day for males and 13.5 mg/kg/day for females).
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA generally agrees with the applicant's summary and conclusions. However, additional information regarding the magnitude of fluorosis and associated cranial changes has been included. The UK CA does not consider it appropriate to extrapolate a NOAEL and this value has been omitted. The carcinogenicity findings are discussed in section 6.7.
	COMMENTS FROM ...
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_5/7-1.A Results of clinical chemistry haematology and urinalysis

Clinical chemistry	Sex	Unit	Control	Low dose 180 ppm	Medium dose 900 ppm	High dose 4500 ppm
Alanine-aminotransferase		U/l	**27 weeks after start of treatment**			
	male		—	—	—	↓*
	female		—	—	—	↓
			54 weeks after start of treatment			
	male		—	—	—	↓**
	female		—	—	—	↓*
			80 weeks after start of treatment			
	male		—	—	—	↓
	female		—	—	—	↓
			105 weeks after start of treatment			
	male		—	—	—	↓
	female		—	—	—	↓**

↓ decrease

— not different from control

* difference against control $p \leq 0,05$ significant

** difference against control $p \leq 0,01$ significant

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in rats (main groups)

Parameter	Control data				Low dose 180 ppm		Medium dose 900 ppm		High dose 4500 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined			50	50	50	50	50	50	50	50		
Mortality			12%	22%	18%	26%	30%	22%	16%	20%	-	-
Clinical signs			—	—	—	—	—	—	Skin changes on the noses, incisors had to cut frequently.		+	+
Body weight gain			—	—	—	—	—	—	↓*	↓*	+	+
Food consumption			—	—	—	—	—	—	—	—	-	-
Clinical chemistry			**Effects described in table A 6_5/7-2.A above**									
Haematology			—	—	—	—	—	—	—	—	-	-
Urinalysis			—	—	—	—	—	—	—	—	-	-
Number of animals examined			44	39	41	37	35	39	42	40		
Overall tumour incidence:			25	33	30	23	28	35	29	24	-	-
No. of animals with neoplasms			16	27	24	19	18	26	25	18	-	-
No. of animals with benign neoplasms			9	27	14	16	16	20	18	12	-	-
No. of animals with malignant neoplasms			5	0	9	2	0	3	5	3	-	-
No. of animals with benign and malignant neoplasms			2	0	1	1	2	3	2	3	-	-
No. of animals with metastasis-ing neoplasms			1	0	0	1	0	1	0	0	-	-

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

↑ increase

↓ decrease

— not different from control * difference against control $p \leq 0{,}05$ significant

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in rats (main groups), continued

Parameter	Control data				Low dose 180 ppm		Medium dose 900 ppm		High dose 4500 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ: forestomach												
Non-neoplastic changes: hyperkeratosis/ acanthosis			2/49	0/50	3/50	2/50	3/50	0/50	21/49	12/48	+	+
Organ: thyroid												
Follicular cell adenoma, benign			1/50	0/50	0/50	0/50	0/50	0/50	5/49	4/50	+	+
Follicular cell cacinoma, malignant			0/50	0/50	0/50	0/50	0/50	1/50	1/49	1/50	+	+
Non-neoplastic changes: focal follicular growth anomaly			1/50	0/50	1/50	0/50	2/50	2/50	3/49	4/50	+	+
Other: bone												
Gross pathology			—	—	—	—	—	—	Change in colour (whitish)		+	+
Microscopic pathology			—	—	Increase of osteosclerosis and lamellar growth pattern on the cranial surfaces						+	+
Fluoride content			—	—	↑**	↑**	↑**	↑**	↑**	↑**	+	+
Other: teeth												
Fluoride content			—	—	↑**	↑**	↑**	↑**	↑**	↑**	+	+

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

↑ increase

↓ decrease

— not different from control

* difference against control $p \leq 0,05$ significant

** difference against control $p \leq 0,01$ significant