Section A6.7 Carcinogenicity

Annex Point IIA6.7 6.7 Study for oncogenicity in B6C3F$_1$ mice

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1994, KUE 13032 C (c.n. dichlofluanid)- Study for oncogenicity in B6C3F$_1$ mice (administration in the feed over 2 years), [redacted], Report No. [redacted], 1993-11-16 (unpublished) and [redacted], 1994, KUE 13032 C (c.n. dichlofluanid)- Study for oncogenicity in B6C3F$_1$ mice (administration in the feed over 2 years) Amendment to Report No. 22679, [redacted] Report No. [redacted], 1994-09-19 (unpublished)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 451 and the recommendations contained in EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, series 83.2.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes In comparison to the OECD-Guideline 451 the following deviations could be ascertained: - Careful clinical observations: only once a week instead of a daily examination. - Haematological examination: the recommended blood collection at 18 months 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.

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3.1.2.1	Description	White powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The homogeneity and stability of the test substance in the feed were analysed before the start of the study. During the study period, test article purity in the feed was checked at regular intervals (approx. 6 weeks). This was performed by analysing samples taken from the feed used. Mixtures proved to be stable in the concentration range used and throughout the period of use as well as being homogeneously distributed.
3.2	**Test Animals**	
3.2.1	Species	Mice
3.2.2	Strain	B6C3F$_1$ (SPF-bred)
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 20 g (16 – 25 g); females: mean 17 g (14 – 21 g)
3.2.6	Number of animals per group	60 per 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	2 years (743 days)
3.3.2	Interim sacrifice(s)	After 53 weeks
3.3.3	Final sacrifice	After 105/106 weeks
3.3.4	Frequency of exposure	Daily
3.3.5	Post-exposure period	None.
		Oral
3.3.6	Type	In food
3.3.7	Concentration	Food: 0, 200, 1000, or 5000 ppm (approx. intake of test substance: 0, 50.1, 260, or 1400 mg/kg bw/day for males and 0, 63.7, 315, and 1900 mg/kg bw/day for females) Food consumption per day ad libitum.
3.3.8	Vehicle	—
3.3.9	Concentration in vehicle	—

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3.3.10	Total volume applied	—
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 106. Body weights were also measured before autopsies in week 53 and week 105/106.
3.4.2	Food consumption	Yes Weekly from the commencement of the study until termination of the 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). A detailed examination of individual animals was performed once a week..
3.4.5	Macroscopic investigations	Palpable masses
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes Number of animals: 10 animals/sex/group Time points: after week 51, 52 or 53 and week 104 or 105 of treatment. Parameters: haematocrit, haemoglobin concentration, erythrocyte count, leukocyte count, thrombocyte count, differential blood count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV). Other: A differential blood count was performed on those animals killed as moribund. No assessment of the differential blood count of these particular animals was performed due to their low number and their distribution over the individual treatment groups
3.4.8	Clinical Chemistry	Yes

		Number of animals:	10 animals/sex/group
		Time points:	after week 51, 52 or 53 and week 104 or 105 of treatment.
		Parameters:	sodium, potassium, calcium, chloride, phosphate, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase.
		Other	—
3.4.9	Urinalysis	No	
3.4.10	Pathology	Yes	
3.4.11	Organ Weights	Yes	
		from:	10 animals/sex/group at interim sacrifice, all surviving animals at terminal sacrifice
		Organs:	brain, testes, liver, lung, spleen, kidneys, adrenals, ovaries
			Other: —
3.4.12	Histopathology	Yes	
		from:	all dose groups
		from:	10 animals/sex/group at interim sacrifice
			all surviving animals at terminal sacrifice
			and all animals that died intercurrently.
		Organs:	brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, extraorbital lachrymal glands stomach, small and large intestines, liver, gall bladder, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, ovaries with ovarian tubes, testes, uterus, ureter, urethra, vagina, prostate, epididymis, urinary bladder, lymph nodes, peripheral nerve, bone marrow, skin, eyes with eye lids and optical nerves, nasal turbinates, femur with knee joint, Harderian glands, head, Zymbal's gland, musculature (femoral), seminal vesicles, sternum, tongue, mammary region, tissues with significant macroscopic changes.
3.4.13	Other examinations	Determination of fluoride in bones and teeth was performed after 53 weeks on the animals scheduled for interim autopsy and at study termination.	

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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 $\alpha = 5$ % and $\alpha = 1$ % (two-tailed). Survival: The survival curves were evaluated using the BMDP routine 1L. Subsequently comparison: WILCOXON Test (BRESLOW Test, Breslow, N.E., Biometrica 51, 579, 1979), with weighing according to the group size proportional to the time of the event. Clinical pathology: Fisher's exact probability test (two-tailed) at significance levels of $\alpha = 5$ %, $\alpha = 1$ % and $\alpha = 0.1$ %. Cochran-Armitage trend test (two-tailed) at significance levels of $\alpha = 5$ %, $\alpha = 1$ % and $\alpha = 0.1$ %. Clinical signs (findings): Fisher's exact test (two-tailed) at significance levels of $\alpha = 5$ %, and $\alpha = 1$ % (Sachs, L., Angewandte Statistik, 6th Ed., Springer-Verlag, 1984) 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) If a difference from the above F-test is noted: 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 200 ppm for males and up to including 1000 ppm for females. Body weight gain among the treated males was retarded from 1000 ppm, compared to the control groups; this finding was observed in the females from 5000 ppm.
4.2	**Food consumption**	Mean daily feed consumption per animal was comparable to that of the controls for all treatment groups. Due to retardation in body weight gain, the values for mean feed consumption per kg body weight were higher for animals in the 5000 ppm dose group.
4.3	**Water consumption**	—

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4.4	**Clinical signs**	Daily inspection of the animals revealed loss of hair from 1000 ppm, with rough coats and poor general condition observed following 5000 ppm. The incisors of the animals in the 5000 ppm group also had to be 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.
4.5	**Macroscopic investigations**	No treatment-related effects.
4.6	**Ophthalmoscopic examination**	—
4.7	**Haematology**	No evidence of treatment-related changes to the red or white blood count, or to the blood coagulation rate or haematopoietic organs and tissues following dosing of 200 ppm. The erythrocyte count was lowered following 5000 ppm, with lower haemoglobin and haematocrit values among the males; at the end of the study changes (generally slight) in the morphology of the erythrocytes were increased among the males from 1000 ppm; this was also observed among the females from 5000 ppm. The thrombocyte count in both sexes after 5000 ppm was higher than that of the controls. At the end of the study, the leucocyte count was higher among the males following 1000 ppm, and among the females following 5000 ppm. These changes could result from fluoride intoxication (possibly due to interaction of the metal cations with difference enzymes, and/or an effect on iron metabolism). A direct cytotoxic effect by the test article on the formed part of the blood is not assumed from these changes, which were very largely slight in degree.
4.8	**Clinical Chemistry**	The protein and albumin content in the blood was lower in both sexes following 5000 ppm. This – as well as higher liver weights following 5000 ppm – could indicate that liver functions (e. g., protein synthesis) were affected. However, histopathological examinations revealed no liver lesions. Further tests revealed an increase in alkaline phosphatase activity in both sexes from 1000 ppm: this is probably due to increased fluoride uptake. Examination of the electrolytes in the plasma showed slightly higher sodium and potassium levels at both examination dates for the females following 5000 ppm, and slightly higher potassium levels in the males from 1000 ppm at study termination. These changes were possibly due to subjection to high fluoride levels, which resulted in kidney lesions (see also section 4.11 and 4.12 below) in both sexes following a dose of 5000 ppm.
4.9	**Urinalysis**	—

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4.10	**Pathology**	Spontaneous deaths/animals killed as moribund: no treatment-related effects. Interim necropsy: changes in the colour and consistency of the roof of the skull were observed in all animals of both sexes at necropsy, following 5000 ppm (7 males and 9 females). Terminal necropsy: the following gross pathological findings were noted at 5000 ppm: colour changes to the roof of the skull (white) for all animals in this dose group (42 males and 40 females), thickening of the bone in the knee region (7 males and 22 females), nodes in the mucous membrane of the duodenum (10 males and 18 females), kidney changes (rough surface induration among 14 males and 5 females, deformation in 3 males). Thickening of the bone in the knee region was observed in 9 of 44 females at 1000 ppm.
4.11	**Organ Weights**	At interim necropsy after 53 weeks and at terminal necropsy after 2 years, the absolute organ weights determined for the brain, lungs, spleen, adrenals and testes were statistically significantly lower in some cases compared to the controls, particularly following 5000 ppm – but also in other dose groups in individual instances. Slightly higher differences to the controls were determined in some of these organs when standardised to a body weight of 100 g (relative organ weight). Liver: Liver weights were slightly increased (approximately 15 to 18 % relative increase) in both sexes after interim necropsy, following 5000 ppm; this increase was significant at terminal necropsy (approximately 44 to 47 % relative increase). Kidneys: Following 5000 ppm, lower kidney weights were recorded at interim necropsy among the females, and at terminal necropsy among both sexes. Statistically significantly different values to the control groups were determined in isolated cases in the lower dose groups and/or for other organs (spleen, lung, brain, ovaries, testes). These differences are of no toxicological significance, as the differences were slight and/or there was no recognisable relationship to the dose level or the duration of the treatment.

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4.12	**Histopathology**	Bone: A change in colour and consistency was recorded in animals in the 5000 ppm dose group sacrificed at the interim autopsy. Increased changes in the colour and consistency of the roof of the skull, as well as increased bone thickening in the knee region were observed at terminal necropsy after 5000 ppm. Histopathological investigations revealed bone thickening in the roof of the skull and the turbinates of both sexes following 5000 ppm, as well as thickening of the femur among the females in this dose group. Teeth: A significantly greater number of animals with alveolitis (dental) were recorded among the males in the 5000 and the 1000 ppm dose groups, and among females in the 5000 ppm group. Alveolitis (dental) was also observed among some males in the 200 ppm dose group. This is thought to be secondary result of the reduced tooth wear. Kidneys: Histopathological examination of the kidneys revealed a significantly higher incidence of basophilic tubuli of the cortex – characterised by reduced cell volume and an increase in basophiles in the males and females of the 5000 ppm dose group. Stomach/Duodenum: Histopathological investigations revealed more frequent dysplasia of the stomach mucosa in the males following 5000 ppm, as well as increased number of animals of bot sexes with atypical dysplasia of the duodenal mucosa. There was no evidence of changes to the gastrointestinal tract up to and including 1000 ppm.
4.13	**Other examinations**	On both examination dates a dose-related, significant increase in the fluoride concentrations determined in the bones (femoral) and teeth (incisors) was recorded at and above doses of 200 ppm.
4.14	**Time to tumours**	—
4.15	**Other**	—

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The objective of the study was to determine the no effect level of 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 451 and the recommendations contained in EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, series 83.2.
5.2	**Results and discussion**	Under the study conditions described, administration of dichlofluanid doses from and including 200 ppm led to fluoride deposition in the bones and teeth. Fluoride deposition from 200 ppm, and the correlating individual bone changes (thickening of bone), are not discussed as lesions. Subjection of the organism to high fluoride doses following 5000 ppm also resulted in conspicuous somatic changes characterised by fluorosis and nephrotoxic effects. There was an increased incidence of dysplasia of the duodenal mucosa in both sexes following 5000 ppm, additionally of the stomach mucosa in the males. Both changes are considered to result from local irritation following chronic administration of high doses of dichlofluanid. A higher incidence of neoplastic changes in organs and tissues was not determined.

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5.3	**Conclusion**	No "no-effect dose" (with regard to fluoride deposition) was attained in this study. There was no evidence that the test substance had a carcinogenic effect at doses up to and including 5000 ppm.
5.3.1	Reliability	1
5.3.2	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	29/10/04
Materials and Methods	As described above [IUCLID 2/3]
Results and discussion	As described above
Conclusion	As described above
Reliability	1
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicant's summary and conclusions. The repeated-dose findings are discussed in section 6.5.
	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_7-1. Table for Clinical Chemistry, Haematology and Urinalysis

Haematology	Sex	Unit	Control	Low dose 200 ppm	Medium dose 1000 ppm	High dose 5000 ppm
Erythrocyte count		10^{12}/l	**52 weeks after start of treatment**			
	male		—	—	—	↓**
	female		—	—	—	↓
			104 weeks after start of treatment			
	male		—	—	↓*	↓**
	female		—	—	—	↓
Haemoglobin		g/l	**52 weeks after start of treatment**			
	male		—	—	—	↓
	female		—	—	—	↓**
			104 weeks after start of treatment			
	male		—	—	—	↓**
	female		—	↓*	↓	↓
Hematocrit		l/l	**52 weeks after start of treatment**			
	male		—	—	—	—
	female		—	—	—	—
			104 weeks after start of treatment			
	male		—	—	↓*	↓**
	female		—	—	—	↓
MCHC (mean cell haemoglobin con-centration)		g/l erythrocytes	**52 weeks after start of treatment**			
	male		—	—	—	—
	female		—	—	—	↓**
			104 weeks after start of treatment			
	male		—	—	↓*	↓**
	female		—	—	↓**	↓**

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_7-1. Table for Clinical Chemistry, Haematology and Urinalysis (continued)

Haematology	Sex	Unit	Control	Low dose 200 ppm	Medium dose 1000 ppm	High dose 5000 ppm
Leucocyte count		10^9/l	**52 weeks after start of treatment**			
	male		—	—	—	↑
	female		—	—	—	↑
			104 weeks after start of treatment			
	male		—	↑*	↑*	↑**
	female		—	—	↑*	↑**
Thrombocyte count		10^9/l	**52 weeks after start of treatment**			
	male		—	—	—	↑**
	female		—	—	—	↑**
			104 weeks after start of treatment			
	male		—	—	—	↑**
	female		—	—	—	↑**
Erythrocyte morphology		—	**52 weeks after start of treatment**			
	male		—	—	—	—
	female		—	—	—	—
			104 weeks after start of treatment			
	male		—	—	Slight morphological changes (anisocytotic, poikilocytotic and/or polychromatic erythrocytes)	
	female		—	—	—	Slight morphological changes (anisocytotic, poikilocytotic and/or polychromatic erythrocytes)

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_7-1. Table for Clinical Chemistry, Haematology and Urinalysis (continued)

Clinical Chemistry	Sex	Unit	Control	Low dose 200 ppm	Medium dose 1000 ppm	High dose 5000 ppm
Protein		g/l	**52 weeks after start of treatment**			
	male		—	—	—	↓**
	female		—	—	—	↓**
			104 weeks after start of treatment			
	male		—	—	—	↓**
	female		—	—	—	↓**
Albumin		g/l	**52 weeks after start of treatment**			
	male		—	—	—	↓**
	female		—	—	—	↓*
			104 weeks after start of treatment			
	male		—	—	—	↓*
	female		—	—	—	↓**
Sodium		mmol/l	**52 weeks after start of treatment**			
	male		—	—	—	—
	female		—	—	—	↑**
			104 weeks after start of treatment			
	male		—	—	↑	—
	female		—	—	—	↑**

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_7-1. Table for Clinical Chemistry, Haematology and Urinalysis (continued)

Clinical Chemistry	Sex	Unit	Control	Low dose 200 ppm	Medium dose 1000 ppm	High dose 5000 ppm
Potassium		mmol/l	**52 weeks after start of treatment**			
	male		—	—	—	—
	female		—	↑*	—	↑*
			104 weeks after start of treatment			
	male		—	—	↑**	↑**
	female		—	—	—	↑**
Alkaline phosphatase		U/l	**52 weeks after start of treatment**			
	male		—	—	↑**	↑**
	female		—		↑**	↑**
			104 weeks after start of treatment			
	male		—	—	↑**	↑**
	female		—	↑*	↑**	↑**

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_7-2. Results of carcinogenicity study (main groups)

Parameter	Control data				Low dose 200 ppm		Medium dose 1000 ppm		High dose 5000 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	50	50
Mortality			10%	8%	4%	16%	4%	12%	16%	20%	-	-
Clinical signs			—	—	—	—	Loss of hair		Loss of hair, rough coat, poor general condition, incisors had to cut frequently.		+	+
Body weight gain			—	—	—	—	↓	—	↓	↓	+	+
Mean food consumption			—	—	—	—	—	—	—	—	-	-
									Due to retardation in body weight gain, values for mean food consumption per kg bw were higher.			
Clinical chemistry	Effects described in Table A6_7-1 (see above).											
Haematology												
Number of animals examined			45	46	48	42	48	44	42	40		
Overall tumour incidence (total number of neoplasms):			25	17	25	20	16	18	11	12	-	-
No. of animals with neoplasms			20	17	21	14	14	13	10	10	-	-
No. of animals with benign neoplasms			13	9	16	5	9	7	7	8	-	-
No. of animals with malignant neoplasms			5	8	3	8	4	3	3	1	-	-

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

↓ decrease

↑ increase

— not different from control

Table A6_7-2. Results of carcinogenicity study (main groups), continued

Parameter	Control data				Low dose 200 ppm		Medium dose 1000 ppm		High dose 5000 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
No. of animals with benign and malignant neoplasms			2	-	2	1	1	3	-	1	-	-
No. of animals with metastasis-ing neoplasms			1	7	1	7	1	3	-	-	-	-
Organ: stomach												
Squamous cell carcinoma, malignant			-	-	-	-	1/50	-	-	-	-	-
Carcinoma malignant			-	-	-	-	-	-	-	1/50	-	-
Non-neoplastic changes: Dysplasia of the mucosa				1/46	-	1/40	1/48	-	10/42	-	+	-
Organ: duodenum												
Adenoma benign			-	-	-	-	-	-	-	1/46	-	-
Adenocarcinoma malignant			-	-	-	-	1/49	-	-	-	-	-
Non-neoplastic changes: Atypical dysplasia of the duodenal mucosa.			-	-	-	-	-	1/44	21/42	19/40	+	+
<u>Other findings:</u> Bone												
Gross pathology:			—	—	—	—	—	Thick-ening in the knee region	Change in colour and consistency of the roof of the skull and increased bone thickening in the knee region		+	+

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

↓ decrease

↑ increase

— not different from control

Table A6_7-2. Results of carcinogenicity study (main groups), continued

Parameter	Control data historical m[a]	Control data historical f[a]	Control data study m[a]	Control data study f[a]	Low dose 200 ppm m[a]	Low dose 200 ppm f[a]	Medium dose 1000 ppm m[a]	Medium dose 1000 ppm f[a]	High dose 5000 ppm m[a]	High dose 5000 ppm f[a]	Dose-response +/- m	Dose-response +/- f
Microscopic pathology			—	—	—	—	—	—	Thickening of the roof of the skull and the turbinates. Only females additionally with thickening of femur.		+	+
Fluoride content			—	—	↑**	↑**	↑**	↑**	↑**	↑**	+	+
<u>Other findings:</u> Teeth												
Microscopic pathology			—	—	Alveolitis	—	Alveolitis	—	Alveolitis		-	+
Fluoride content			—	—	↑**	↑**	↑**	↑**	↑**	↑**	+	+

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

↓ decrease

↑ increase

— not different from control