Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 6.8.2 Two-generation reproduction study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1992, KUE 13032 c (c.n.: Dichlofluanid) – Supplementary two-generation study on rats, [redacted], Report No. [redacted], 1992-12-11 (unpublished)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**	Yes The methods used in this study followed the recommendations of OECD-Guideline 416 and the recommendations contained in EPA, Pesticide Assessment Guidelines, Subdivision F, series 83.4.
2.2	**GLP**	Yes
2.3	**Deviations**	Yes

Deviations from the current OECD-Guideline 416:

Dosage: For the dietary studies the OECD-Guideline 416 recommends a dose interval that should not exceed a factor of 3. In this study, the chosen dose intervals had have a factor of 5 between dosages.

Organ weights:

Determinations of the following organ weights were not performed:

P and F1 parental generation:

brain, pituitary, thyroid, adrenals, uterus, epididymides, prostate, seminal vesicles with coagulating glands and their fluids (as one unit).

The recommended examinations of organs (brain, spleen, thymus) from pups of each litter both from the F1 and F2 generation were not performed

The following observations are missing:

- Determination of the oestrus cycle and sperm parameters (total number of testicular spermatids and cauda epididymal sperm, sperm morphology and motility).
- Number of corpora lutea
- Some parameters of physical development of the F1 offspring (e.g. functional investigations like motor activity, sensory function, reflex ontogeny).
- Lack of gross necropsy and histopathological examination of at least one randomly selected pup/sex/litter from both the F1 and F2 generation.
- Full histopathology of the vagina, uterus with cervix, and ovaries (optional for the P animals), one testis, one epididymidis, seminal vesicles, prostate and coagulating gland for all high dose and control P and F1 animals selected for mating.

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 stability of the active ingredient throughout the period of use, and the homogeneous distribution of the active ingredient in the food mix were verified before the study was initiated (90 ppm) or (900 ppm) in the course of the prior two-generation study. The active ingredient levels in the food mixes were determined by analysis – after blending and following storage of the blends in the animal room for periods of 12 days (900 ppm) or two days (90 ppm) – at the beginning of treatment, then once during each three month (0 ppm, 900 ppm) or six week (90 ppm) study interval at a time determined by a randomising list, and finally at the end of the study.
3.2	**Test Animals**	Rat
3.2.1	Species	Wistar rats
3.2.2	Strain	Bor: WISW (SPF Cpb)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females No siblings were presented in the animals used.
3.2.5	Age/weight at study initiation	<u>Males:</u> Weight: 96 – 136 g, age: 6 - 7 weeks <u>Females:</u> Weight: 97 – 123 g, age: 6 - 7 weeks
3.2.6	Number of animals per group	30 animals per sex per group each generation (P and F1B).
3.2.7	Mating	Premating period in F0 generation until first mating: 77 days First mating period in F0 generation: 21 days Gestation – lactation (F1A pubs) – waiting period: at least 57days Second mating period F0 generation: 21 days Gestation – lactation (F1B pubs) until approx. 16 weeks old: 140 days First mating period of F1B generation 21 days Gestation – lactation (F2A pubs) – waiting period at least 65 days Second mating period of F1B generation 21 days Gestation period – lactation (F2B pubs) up to 3 weeks including sacrifice 43 days
3.2.8	Duration of mating	3 weeks
3.2.9	Deviations from standard protocol	Second mating of parent or F1 generations, standardisation of litter size

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3.2.10	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	See table A6_8_2-1. below.
3.3.2	Duration of exposure before mating	77 days
3.3.3	Duration of exposure in general P, F1, F2 males, females	From beginning of the study until sacrifice of parent, F1 and F2-generation.
3.3.4	Type	In food
3.3.5	Concentration	0, 90 and 900 ppm P generation: mean intake of test-substance during the pre-mating period: 0, 7.33 and 72.0 mg/kg bw for males and 0, 8.11and 79.94 mg/kg bw for females. F1B generation: mean intake of test-substance during the pre-mating period: 0, 9.44 and 102.30 mg/kg bw for males and 0, 11.20 and 117.54 mg/kg bw for females. Food consumption per day ad libitum.
3.3.6	Vehicle	—
3.3.7	Concentration in vehicle	—
3.3.8	Total volume applied	—
3.3.9	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes General clinical observation: twice daily (once daily on weekends and public holidays). Careful clinical observation: once a week.
3.4.2	Body weight	Yes All animals were weighed at the start of the study. Male animals: weighed at weekly intervals to the point of necropsy. Female animals: weighed at weekly intervals to the point insemination was established, than they were weighed on gestation days 0, 7, 14 and 20 and on days 0, 4, 7, 14 and 21 after birth of the pups. The female animals were again weighed at weekly intervals after the F1A/F2A pups had been weaned, or all pups of a litter had died. Male and female P and F1B animals were weighed on (or one day before) the date of necropsy

3.4.3	Food/water consumption	Food consumption: yes Except during the mating periods, the food intakes of all animals prior to establishment of insemination were determined four times per week. After insemination had been established, the food intakes of the female animals were determined on gestation days 0, 7, 14 and 20; and on days 0, 4, 7, 14 and 21 after birth of the pups, as well as on up to four additional days per week. Water consumption: no.
3.4.4	Oestrus cycle	Yes 12 days after mating period (menstrual cycle in unfertilised females).
3.4.5	Sperm parameters	No.
3.4.6	Offspring	Yes, Number and sex of pups Stillbirths Live births Presence of gross anomalies Weight gain Physical or behavioural abnormalities Viability index Lactation index
3.4.7	Organ weights P and F1	Yes, Liver, spleen, kidney, testes, and ovaries.
3.4.8	Histopathology P and F1	Cranial domes and all organs with macroscopic changes. In addition, all fixed organs from all parent animals which died intercurrently or were sacrificed in moribund condition, from male parents when both females mated with them did not become gravid, and from female parents which did not become gravid after either of the two matings were examined for histopathology
3.4.9	Histopathology F1 not selected for mating, F2	Pups: all fixed organs exhibiting macroscopic changes were histopathologically examined
3.5	**Further remarks**	F1B and F2B pups which died at birth, those which died up to postpartum day 4, and those which were sacrificed on postpartum day 4 in the course of litter reduction were examined for skeletal system deviations using the Dawson technique as described in Stain Techn. 1, 123-124 (1926).
		4 RESULTS AND DISCUSSION
4.1	**Effects**	
4.1.1	Parent males	Clinical signs: no treatment-related effects. Mortality: no treatment-related effects. Body weight gain: in comparison to the control group, the weight gains of males at 900 ppm were reduced. Food consumption: reduced intakes of food in the 900 ppm group. Organ weights: no treatment-related effects. Gross pathology: no treatment-related effects. Histopathology: no treatment-related effects.

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4.1.2	Parent females	Clinical signs: no treatment-related effects. Mortality: no treatment-related effects. Body weight gain: no effects. Food consumption: reduced intakes of food in the 900 ppm dose group. Organ weights: no treatment-related effects. Gross pathology: no treatment-related effects. Histopathology: no treatment-related effects. Effects on reproduction (insemination index, fertility index, gestation index, gestation period): none
4.1.3	F1 males	Clinical signs: no treatment-related effects. Mortality: at 900 ppm isolated F1B animals died or were sacrificed during the first after study begin. Pathological examination of these animals afforded no evidence for test substance-related damage. Body weight gain: no effects. Food consumption: no effects. Organ weights: no treatment-related effects. Gross pathology: no treatment-related effects. Histopathology: no treatment-related effects. Offspring examination: no treatment-related effects. The pup weight showed no adverse effects up to postpartum seven at levels up to and including 90 ppm. The pup weight at 900 ppm lay below those in the control group on postpartum day 21 (F1B).
4.1.4	F1 females	Clinical signs: no treatment-related effects. Mortality: at 900 ppm one female F1B animal died. Pathological examination of these animals afforded no evidence for test substance-related damage. Body weight gain: in comparison to control group, the weight gains of females at 900 ppm were reduced. Food consumption: reduced food intake in the 900 ppm dose group. Organ weights: liver and kidney weights were elevated at 90 ppm and above. However, since no significant histopathological findings were made in the liver or kidneys at levels up to and including 4500 ppm in the prior two-generation study with dichlofluanid ([redacted], KUE 13032 c (c. n. dichlofluanid) – Two-generation study on rats, [redacted], Report No. [redacted], 1991-09-02), the organ weight differences were not assessed as organ damage. Gross pathology: no treatment-related effects. Histopathology: no treatment-related effects. Effects on reproduction (insemination index, fertility index, gestation index, gestation period): none Offspring examination: no treatment-related effects. The pup weight underwent no adverse effects up to postpartum seven at levels up to and including 90 ppm. The pup weight at 900 ppm lay below those in the control group from postpartum day 14 onward (F1B).

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4.1.5	F2 males	Offspring examination: no treatment-related effects. The pup weight underwent no adverse effects up to postpartum seven at levels up to and including 90 ppm. The body weight at 900 ppm lay below those in the control group from postpartum day 14 onward (F2B) and on postpartum day 21 (F2A).
4.1.6	F2 females	Offspring examination: no treatment-related effects. The pup weight underwent no adverse effects up to postpartum seven at levels up to and including 90 ppm. The body weight at 900 ppm lay below those in the control group from postpartum day 14 onward (F2A, F2B) and on postpartum day 21 (F2A).
4.2	**Other**	—

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Dichlofluanid was examined for possible effects on reproduction in a two-generation study on Wistar rats with two litters per generation. This study represents a supplement to a prior two-generation study ([redacted], KUE 13032 c (c. n. dichlofluanid) – Two-generation study on rats, [redacted]. Report No. [redacted], 1991-09-02) with dichlofluanid, in which elevated mortality in the F1B pups following weaning could not be excluded at and above the low (180 ppm) dose.

The methods used in this study were in accordance with the OECD-Guideline 416 and the recommendations contained in EPA, Pesticide Assessment Guidelines, Subdivision F, series 83.4. Deviations occurred due to the updated OECD-Guideline 416 and were described in 2.3 (see above).

5.2 Results and discussion

The appearance and behaviour of the parent animals underwent no treatment-related effect at levels up to and including 900 ppm. Isolated F1B animals in the 900 ppm dose group died or were sacrificed in moribund condition. Pathological examination did not reveal any evidence for test-substance-related damage.

The body weight development and food intakes of the parent animals were adversely affected in some cases at 900 ppm.

No treatment-related findings were made at necropsy or subsequent histopathological examination of the parent animals at levels up to and including 900 ppm. The elevated liver and kidney weights of female F1B animals at 90 ppm and above were not assessed as organ damage.

The reproduction parameters: insemination index, fertilisation performance, fertility index, gestation index, gestation period, total number of pups born, number of dead pups at birth (live birth index), percentages of male and female pups, litter size at birth and pup survival rate (viability and lactation indices) underwent no treatment-related effect at levels up to and including 900 ppm.

No test substance-related clinical or gross pathological findings were observed in the pups. The skeletal development in the pups up to postpartum day four was unaffected at levels up to and including 900 ppm.

The pup weights from postpartum day 14 on were adversely affected at 900 ppm.

A dichlofluanid dose of 90 ppm in the diet was thus tolerated without adverse effects on the parent animals or the reproduction under the described conditions.

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5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.1.1	Parent males	Reduced weight gains, 900 ppm (= 72.0 mg/kg bw)
5.3.1.2	Parent females	Reduced food intake, 900 ppm (= 79.94 mg/kg bw)
5.3.1.3	F1 males	Increased mortality in parents, reduced pup weight, 900 ppm (= 102.3 mg/kg bw)
5.3.1.4	F1 females	Reduced weight gains, reduced food intake, reduced pup weight (= 117.54 mg/kg bw)
5.3.1.5	F2 males	Reduced pup weight, 900 ppm
5.3.1.6	F2 females	Reduced pup weight, 900 ppm
5.3.2	NO(A)EL	
5.3.2.1	Parent males	90 ppm (= 7.33 mg/kg bw)
5.3.2.2	Parent females	90 ppm (= 8.11 mg/kg bw)
5.3.2.3	F1 males	90 ppm (= 9.44 mg/kg bw)
5.3.2.4	F1 females	90 ppm (= 11.20 mg/kg bw)
5.3.2.5	F2 males	90 ppm
5.3.2.6	F2 females	90 ppm
5.3.3	Reliability	2
5.3.4	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	1/11/04
Materials and Methods	As described above [IUCLID 5.8.1 2/3]
Results and discussion	As described above
Conclusion	As described above
Reliability	2
Acceptability	Acceptable
Remarks	The UK CA agrees with the applicant's summary and conclusions.
	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_8_2-1. Table for animal assignment for mating

		Number of animals		
		Controls 0 ppm	**Low Dose 90 ppm**	**High Dose 4500 ppm**
Parents 1st mating	**m**	29	30	30
	f	30	30	30
F_{1A}-pubs	**m + f**	296	280	245
Parents 2nd mating	**m**	29	29	30
	f	30	30	30
F_{1B}-pubs	**m + f**	274	271	267
F_{1B} 1st mating	**m**	30	30	30
	f	30	30	30
F_{2A}-pubs	**m + f**	321	336	307
F_{1B} 2nd mating	**m**	30	30	30
	f	30	30	30
F_{2B}-pubs	**m + f**	285	314	336

Table A6_8_2-2. Table for reproductive toxicity study

Parameter			Control		Low dose 90 ppm		Medium dose 900 ppm		Dose response +/-	
		Genera-tion	m	f	m	f	m	f	m	f
Mortality	Incidence	**P**	1	0	1	1	0	0	-	-
		F_{1B}	0	0	0	0	4	1	+	-
		F_2								
Food consumption		**P**	—	—	—	—	↓	↓	+	+
		F_{1B}	—	—	—	—	—	↓	-	+
		F_2								
Body weight gain		**P**	—	—	—	—	↓	—	+	-
		F_{1B}	—	—	—	—	—	↓	-	+
		F_2								
Pub weight gain		**F_{1A}**	—	—	—	—	—	—	-	-
		F_{1B}	—	—	—	—	↓	↓	+	+
		F_{2A}	—	—	—	—	↓	↓	+	+
		F_{2B}	—	—	—	—	↓	↓	+	+

↑ increase

↓ decrease

— not different from control