Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8 6.8.2 Two-generation reproduction study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1991, KUE 13032 c (c. n. Dichlofluanid) – Two-generation study on rats, [redacted], Report No. [redacted], 1991-09-02 (unpublished)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 performed in accordance with the recommendations of the 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 The following deviations from the current OECD-Guideline 416 occurred: 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 interval had a factor of 5 between the dosages. Organ weights: Determinations of the following organ weights were not performed: P and F1 parental generation: brain, pituitary, thyroid, adrenals, uterus, epididymes, 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 epididymis, 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. An additional stability test of the 900 ppm and 4500 ppm mixes covering the lengthier period of use in the case of isolated females during the gestation and lactation periods was performed after conclusion of the study. The active ingredient levels in the 0 ppm, 900 ppm and 4500 ppm food mixes (following blending and after storage of the mix for seven days in the animal room) were determined by analysis after the study had progressed for three weeks, and once during each three-month study interval according to a randomising list. The active ingredient levels in the 180 ppm food mixes were determined (following blending and after storage of the mix for three days in the animal room) after the study had progressed for three weeks, once during each six-week study interval according to a randomising list, at the end of the study, and at additional times.
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	Males and females No siblings were presented in the animals used. However, it was necessary to mate siblings in the 4500 ppm F1B dose group, because of the small number of remaining animals.
3.2.5	Age/weight at study initiation	<u>Males:</u> Weight: 98 – 148 g Age: 6 - 7 weeks <u>Females:</u> Weight: 91 – 129 g Age: 6 - 7 weeks
3.2.6	Number of animals per group	30 animals per sex per group each generation (P and F1B) <u>Remark:</u> Due to the high rate of mortality in the F1B pups of the 4500 group during lactation and after weaning, only two male and four female F1B animals from this group were available for further treatment
3.2.7	Mating	Premating period in F0 generation until first mating: 73 days First mating period in F0 generation: 19 days Gestation – lactation (F1A pups) – waiting period: 65 days Second mating period F0 generation: 19 days

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		Gestation – lactation (F1B pups) until approx. 105 days old: 127 days First mating period of F1B generation 19 days Gestation – lactation (F2A pups) – waiting period 58 days Second mating period of F1B generation 19 days Gestation period – lactation (F2B pups) up to 3 weeks including sacrifice 43 days
3.2.8	Duration of mating	19 days
3.2.9	Deviations from standard protocol	Yes, Second mating of parent and F1 generations and standardisation of litter size.
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	73 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, 180, 900 or 4500 ppm <u>P generation:</u> Mean intake of test-substance during the pre-mating period: 0, 15.7, 86.5 and 591.1 mg/kg bw for males and 0, 17.3, 111.7 and 779.4 mg/kg bw for females. <u>F1B generation:</u> mean intake of test-substance during the pre-mating period: 0, 19.7, 130.8 and 725.0 mg/kg bw for males and 0, 21.2, 145.0 and 689.2 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	Blank formulation.
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.

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3.4.2	Body weight	Yes All animals were weighted at the start of the study. Male animals: weighted at weekly intervals to the point of necropsy. Female animals: weighted at weekly intervals to the point insemination was established, than they were weighted 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 weighted 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 Food intakes of all animals were determined weekly prior to insemination, or three times per week (only the 180 ppm dose group). 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 three additional days per week (only 180 ppm dose group). Water consumption: No.
3.4.4	Oestrus cycle	Yes Only unfertilised females. Following the mating period, the status of menstrual cycle in unfertilised females was determined over a period of 12 days.
3.4.5	Sperm parameters	Not performed.
3.4.6	Offspring	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, ovaries.
3.4.8	Histopathology P and F1	Yes, liver, spleen, kidneys, brain, pituitary, adrenals, vagina, uterus, ovaries, mammary gland with skin, testes, epididymes, coagulation glands, seminal vesicles, prostate, entire intestines, cranial domes and all organs with macroscopic changes.
3.4.9	Histopathology F1 not selected for mating, F2	Yes, liver, spleen, kidneys, brain, pituitary, adrenals, vagina, uterus, ovaries, mammary gland with skin, testes, epididymes, coagulation glands, seminal vesicles, prostate, entire intestines, cranial domes and all organs with macroscopic changes.
3.5	**Further remarks**	—

4 RESULTS AND DISCUSSION

4.1 Effects

4.1.1 Parent males (P)

Appearance, behaviour and mortality:

In the 4500 ppm group, all animals exhibited accelerated growth and a white discoloration of the teeth starting with week five, as well as bloody noses starting with week two. Isolated male P animals in the 4500 ppm group displayed temporary emaciation and weight loss as well as piloerection.

Body weight: The body weight gains of the male animals were reduced at 4500 ppm.

Food consumption: The food intakes of male P animals were increased at 900 ppm and above.

Organ weight: The relative liver weight in the male animals lay above the control to a statistically significant extent at 180 ppm and above. No toxicological significance is attributed to this increase in the 180 ppm and 900 ppm groups, because of the absence of a dose relationship up to 900 ppm and the smallness of the deviation from control (4.5 % and 4.3 %, respectively). The relative liver weight in male 4500 ppm group lay markedly (15.4%) above the control figure. The relative kidney weight in male animals was elevated to a statistically significant extent at 900 ppm and above. The relative testicle weight in the 4500 ppm group lay above the control to a statistically significant extent.

Pathology: In the 4500 ppm group animals exhibited a white discoloration of the cranium. Histopathological examinations revealed to thickened craniums at 900 ppm and above.

No histopathological correlations for the organ weight differences were found.

4.1.2 Parent females (P)

Appearance, behaviour and mortality:

In the 4500 ppm group, all animals exhibited accelerated growth and a white discoloration of the teeth starting with week five, as well as bloody noses starting with week two. No test substance-related mortalities occurred.

Body weight: The body weight gains of the female animals were reduced at 900 ppm and above.

Food consumption: Increased food intake in the 4500 ppm dose group.

Organ weights: The absolute spleen weight in the 4500 ppm group females was reduced to a statistically significant extent. The relative kidney weight in female animals was elevated to a statistically significant extent at 900 ppm and above.

Pathology: In the 4500 ppm group animals exhibited a white discoloration of the cranium. Histopathological examinations revealed to thickened craniums at 4500 ppm.

Haemosiderosis was commonly determined the spleen in the 4500 ppm group.

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4.1.3	F1 males	Appearance, behaviour and mortality: In the 4500 ppm group all surviving F1B animals exhibited accelerated growth of the teeth, as well as bloody noses. Male F1B animals in the 180 ppm and 900 ppm groups died during the first week following introduction to the study in isolated cases, and male F1B animals in the 4500 ppm group commonly. Pups exhibiting laboured breathing (F1A & F1B generations, 900 ppm) and/or cyanotic coloration (F1A generation), and cold/thin pups (F1B 900 ppm; F1A, 4500 ppm) were increasingly seen at 900 ppm and above. Body weight: The body weight gains of the male animals were reduced at 4500 ppm. A reduced body weight development of the pups during lactation occurred in some cases after dosing of 900 ppm and above. Food consumption: The food intakes in male and female F1B animals of the 4500 ppm group lay below that in the control group during the initial weeks of treatment; they were higher than the control thereafter. Organ weight: The relative kidney weight was elevated at 900 ppm and above, and the relative spleen weight in animals at 4500 ppm. Pathology: In the 4500 ppm group animals exhibited a white discoloration of the cranium. Offspring examination: Reduced birth weight at 4500 ppm. The viability index (F1A generation, 900 ppm; F1B generation, 4500 ppm), lactation index (F1B generation, 900 ppm; F1A generation, 4500 ppm) were reduced at 900 ppm and above in some cases. Skeletal deviations: Vertebral body retardation was observed in one male pub of the 900 ppm group.
4.1.4	F1 females	Appearance, behaviour and mortality: In the 4500 ppm group all surviving F1B animals exhibited accelerated growth of the teeth, as well as bloody noses. Isolated female F1B animals in all groups treated with dichlofluanid exhibited piloerection during the initial weeks following introduction to the study. At 4500 ppm female F1B animals died commonly. Body weight: The body weight gains of the female animals were reduced at 900 ppm and above. Food consumption: The food intakes in female F1B animals of the 4500 ppm group lay below that in the control group during the initial weeks of treatment; they were comparable to the control thereafter. Organ weights: The absolute kidney weight in the female lay above the control at 900 ppm and above (not statistically significant at 4500 ppm) The relative kidney weight was elevated animals at 900 ppm and above. Pathology: In the 4500 ppm group animals exhibited a white discoloration of the cranium. Histopathological examinations revealed to an increased incidence of non-ossified zones in the cranial area at 900 ppm. Offspring examination: Reduced birth weight at 4500 ppm. The viability index (F1A generation, 900 ppm; F1B generation, 4500 ppm), lactation index (F1B generation, 900 ppm; F1A generation, 4500 ppm) were reduced at 900 ppm and above in some cases. Skeletal deviations: Vertebral body retardation was observed in two female pups of the 900 ppm group.

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4.1.5	F2 males	Appearance, behaviour and mortality: Cold/thin pups (F2A generation, 900 ppm; F2B generation, 4500 ppm) were increasingly seen at 900 ppm and above. Body weight: The body weight gains of the female animals were reduced at 900 ppm and above. Reduced birth weight at 4500 ppm and increased incidence of vertebral body retardation. The lactation index (F2A generation, 900 ppm; F2B generation, 4500 ppm) were reduced at 900 ppm and above in some cases.
4.1.6	F2 females	Clinical signs: Cold/thin pups (F2A generation, 900 ppm; F2B generation, 4500 ppm) were increasingly seen at 900 ppm and above. Body weight: The body weight gains of the female animals were reduced at 900 ppm and above. Offspring examination: Reduced birth weight at 4500 ppm and increased incidence of vertebral body retardation. The lactation index (F2A generation, 900 ppm; F2B generation, 4500 ppm) were reduced at 900 ppm and above in some cases.
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.

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 Slight deviations occurred and were described in 2.3 (see above).

5.2 Results and discussion

The appearance and behaviour of all P animals and all surviving F1B exhibited accelerated growth and a white discoloration (P only) of the teeth, as well as bloody noses at 4500 ppm. Isolated male P animals in the 4500 ppm group displayed temporary emaciation and weight loss as well as piloerection. Isolated female F1B animals in all groups treated with dichlofluanid exhibited piloerection during the initial weeks following introduction to the study. Pups exhibiting laboured breathing and/or cyanotic coloration, and cold/thin pups were increasingly seen at 900 ppm and above. The disturbance in general condition of the F1B animals following weaning can result from the very high food and active ingredient intakes (based on the body weight) during this growth-intense period; however, a reduced survival ability due to an effect during lactation also cannot be excluded.

Mortalities occurred in male and female F1B animals in the 4500 ppm group. Pathological examination of these animals afforded no evidence for test substance-related damage.

The body weight gains of the female animals were reduced at 900 ppm and above and those of the male rats at 4500 ppm.

A reduced body weight development of the pups during lactation occurred in some cases after dosing of 900 ppm and above.

The food intakes of male P animals were increased at 900 ppm and above and those of female P animals at 4500 ppm. The food intakes in male and female F1B animals of the 4500 ppm group lay below that in the control group during the initial weeks of treatment; they were comparable to (F1B females) or higher (F1B males) than the control

		thereafter.	
		Differences in organ weights which were found in spleen, liver, kidney and testicle are not assessed as organ damage since toxicologically significant histopathological correlatives for these organ weight differences were absent.	
		In the 4500 ppm group animals exhibited a white discoloration of the cranium. Histopathological examinations revealed to thickened craniums at 4500 ppm. The observed histopathological changes speak for an effect on the bone metabolism and presumably result from the elevated fluorine intake due to the test substance.	
		Vertebral body retardation was observed in three F1B pups (5.6%) in the 900 ppm group. However since the pups came from the same litter, and the percentages of F2B pups exhibiting vertebral body retardation were comparable to the control group figure at levels up to and including 900 ppm, the occurrence of vertebral body retardation in the 900 ppm F1B group is assessed as incidental rather than treatment-related. In the 4500 ppm group the birth weights of the F1A and F2A pups were reduced and the fraction of F2B pups exhibiting vertebral body retardation increased. In addition, a treatment-related fertilisation delay of the F1B animals in the second mating could not be excluded in this group. The survival rate of the pups (viability index and lactation index) underwent no treatment –related effect in the 180 ppm group. The viability index (F1A generation, 900 ppm; F1B generation, 4500 ppm), lactation index (F1B & F2A generations, 900 ppm; F1A & F2B generations, 4500 ppm) were reduced at 900 ppm and above in some cases.	
		The low dose of dichlofluanid (180 ppm) was thus tolerated by the P generation without adverse effects under the described conditions. Treatment-related mortality in male animals following introduction to the study could not be excluded in the F1B generation at levels of 180 ppm and above. Since mortality in male F1B animals nonetheless underwent no dose-related effect at levels up to and including 900 ppm, and the disturbance in general condition of the female animals in the 180 ppm and 900 ppm groups were only observed transiently (three weeks at maximum), a supplementary study involving doses of 0, 90 and 900 ppm was performed to determine whether the disturbance in general condition and mortality observed in the F1B animals at 180 ppm and above following introduction to the study need be assessed as treatment effects.	X
5.3	**Conclusion**	It was thus impossible to establish a no-effect level for general tolerance by the parent animals or postnatal development due to the disturbance in general condition of the F1B animals following weaning at dichlofluanid doses of 180 ppm and above in the diet.	
5.3.1	LO(A)EL		X
5.3.1.1	Parent males	Disturbance of general condition, LOEL = 180 ppm	
5.3.1.2	Parent females	Disturbance of general condition, LOEL = 180 ppm	
5.3.1.3	F1 males	Disturbance of general condition, LOEL = 180 ppm	
5.3.1.4	F1 females	Disturbance of general condition, LOEL = 180 ppm	
5.3.1.5	F2 males	Disturbance of general condition, LOEL = 180 ppm	
5.3.1.6	F2 females	Disturbance of general condition, LOEL = 180 ppm	
5.3.2	NO(A)EL	Due to results of study, no no-effect levels could be established.	

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5.3.2.1	Parent males	—
5.3.2.2	Parent females	—
5.3.2.3	F1 males	—
5.3.2.4	F1 females	—
5.3.2.5	F2 males	—
5.3.2.6	F2 females	—
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	17/08/05
Materials and Methods	As described above [IUCLID 5.8.1 1/3]
Results and discussion	As described above
Conclusion	**5.2** The UK CA does not consider any of the effects reported at 180 ppm to be of toxicological significance. This is based on the lack of dose response, transient nature and also the lack of similar findings in the follow-up study (Holzum, 1992). **5.3** Overall the UK CA considers parental and pup NOAELs to be 180 ppm.
Reliability	2
Acceptability	Acceptable
Remarks	
	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 180 ppm**	**Medium Dose 900 ppm**	**High Dose 4500 ppm**
Parents	**m**	30	30	30	30
1st mating	**f**	30	30	30	30
F_{1A}-pups	**m + f**	295	254	289	274
Parents	**m**	30	28	30	30
2nd mating	**f**	30	28	30	30
F_{1B}-pups	**m + f**	262	245	243	216
F_{1B}	**m**	30	30	29	2
1st mating	**f**	30	30	29	4
F_{2A}-pups	**m + f**	280	311	296	46
F_{1B}	**m**	30	30	29	2
2nd mating	**f**	30	30	29	4
F_{2B}-pups	**m + f**	296	303	297	41

Table A6_8_2-2. Table for reproductive toxicity study

Parameter			Control		Low dose 180 ppm		Medium dose 900 ppm		High dose 4500 ppm		Dose-response +/-	
		Generation	m	f	m	f	m	f	m	f	m	f
Mortality	Incidence	**P**	-	-	-	2	-	2	-	1	-	-
		F_{1B}	-	1	3	2	3	1	30	17	+?	+?
		F_2										
Food consumption		**P**	—	—	—	—	↑	—	↑	↑	+	+
		F_{1B}	—	—	—	—	—	—	↑	—	-	-
		F_2										
Body weight gain		**P**	—	—	—	—	—	↓	↓	↓	+	+
		F_{1B}	—	—	—	—	—	↓	↓	↓	+	+
		F_2										
Clinical Observations	Incidence											
Teeth: accelerated growth/colour change		**P**	-	-	-	-	-	-	30	30	+	+
Accelerated growth		**F_{1B}**	-	-	-	-	-	-	2	4	+	+
		F_2										
Bloody nose		**P**	-	-	-	-	-	-	30	30	+	+
		F_{1B}	-	-	-	-	1	1	4	4	+	+
		F_2										
General condition: Piloerection		**P**	1	-	-	-	-	1	2	1	+	-
		F_{1B}	1	1	1	3	-	5	1	4	-	+
		F_2										
Thin		**P**	-	-	-	-	1	1	3	-	+	-
		F_{1B}	2	1	1	0	2	1	3	4	-	+
		F_2										
Weight loss		**P**	1	-	-	4	2	3	4	5	+	-
		F_{1B}	1	2	-	-	1	-	4	1	+	-
		F_2										

↑ increase

↓ decrease

— not different from control

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

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

Table A6_8_2-2. Table for reproductive toxicity study, continued

Parameter		Generation	Control m	Control f	Low dose 180 ppm m	Low dose 180 ppm f	Medium dose 900 ppm m	Medium dose 900 ppm f	High dose 4500 ppm m	High dose 4500 ppm f	Dose-response +/- m	Dose-response +/- f
Clinical findings in pups	Incidence											
Labored breathing		F_{1A}	6	2	2	2	9	9	24	14	+	+
		F_{1B}	2	2	1	1	7	9	12	21	+	+
		F_{2A}	1	1	4	3	6	1	0	0	-	-
		F_{2B}	5	0	1	1	0	0	0	0	-	-
Cold		F_{1A}	2	0	0	1	5	8	25	33	+	+
		F_{1B}	2	2	1	6	6	5	24	18	+	+
		F_{2A}	3	2	0	0	16	9	0	1	+	+
		F_{2B}	1	0	0	0	2	2	0	1	-	-
Thin		F_{1A}	6	7	0	1	9	7	42	30	+	+
		F_{1B}	5	6	7	4	23	22	27	55	+	+
		F_{2A}	21	18	10	11	51	52	16	14	+	+
		F_{2B}	14	8	3	9	2	6	13	10	+	+
Colour change		F_{1A}	1	1	3	1	4	11	28	12	+	+
		F_{1B}	4	1	2	0	1	5	3	4	-	-
		F_{2A}	5	1	5	4	5	2	0	0	-	-
		F_{2B}	5	1	3	1	3	2	0	0	-	-
Organ weights												
Liver		P	—	—	↑*	—	↑*	—	↑**	—	+	-
		F_{1B}	—	—	—	—	—	—	—	—	-	-
		F_2										
Kidney		P	—	—	—	—	↑*	↑*	↑**	↑**	+	+
		F_{1B}	—	—	—	—	↑**	↑**	↑*	↑*	+	+
		F_2										

↑ increase

↓ decrease

— not different from control

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

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

Table A6_8_2-2. Table for reproductive toxicity study, continued

Parameter			Control		Low dose 180 ppm		Medium dose 900 ppm		High dose 4500 ppm		Dose-response +/-	
		Genera-tion	m	f	m	f	m	f	m	f	m	f
Organ weights												
Testes		**P**	—		—		—		↑**		+	
		F_{1B}	—		—		—		—		-	
		F_2										
Pathology	Incidence											
Cranium: white discoloration		**P**	0	0	0	0	0	0	24	20	+	+
		F_{1B}	0	0	0	0	0	0	2	1	+	+
		F_2										
Histopathologic examination	Incidence											
Cranium: incomplete ossification		**P**	0	6	0	2	1	1	0	7	-	-
		F_{1B}	4	2	2	0	6	9	0	0		
		F_2										
Cranium: thickened		**P**	1	0	4	5	8	4	19	24	+	+
		F_{1B}	0	0	1	1	0	0	0	0		
		F_2										

↑ increase

↓ decrease

— not different from control

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

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

Table A6_8_2-2. Table for reproductive toxicity study, continued

Parameter			Control	Low dose 180 ppm	Medium dose 900 ppm	High dose 4500 ppm	Dose-response +/-
P-generation, first mating							
Reproductive Performance							
Mating index	%		100	100	100	100	-
Fertility index	%		93.3	80.0	83.3	83.3	-
Duration of pregnancy	Mean (d)		22.3	22.2	22.2	22.2	-
Live birth index	%		98.0	97.6	99.7	100*	-
Gestation index	%		100	100	100	100	-
Litter size	Mean		10.3	10.8	11.5*	11.0	-
Pup weight	Mean (g)		5.8	5.8	5.5*	5.3**	+
Sex ratio	Male/female (%/%)		41.5/58.5	48.0/52.0	46.2/53.8	51.5/48.5	-
Viability index	%		98.3	98.0	93.8**	81.4**	+
Lactation index	%		86.6	98.9**	95.2**	58.9**	+
P-generation, second mating							
Reproductive Performance							
Mating index	%		96.7	100	100	96.6	-
Fertility index	%		82.8	82.1	82.1	82.1	-
Duration of pregnancy	Mean (d)		22.2	22.1	22.1	22.2	-
Live birth index	%		98.9	97.1	100	99.5	-
Gestation index	%		100	100	100	100	-
Litter size	Mean		10.8	10.3	10.6	9.3	-
Litter weight	Mean						
Pup weight	Mean (g)		5.5	5.8**	5.6	5.4	-
Sex ratio	Male/female (%/%)		55.2/44.8	48.3/51.7	49.8/50.2	51.6/48.4	-
Viability index	%		98.1	99.6	95.9	85.1**	+
Lactation index	%		91.2	91.9	73.2**	32.8**	+

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

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

Table A6_8_2-2. Table for reproductive toxicity study, continued

Parameter			Control	Low dose 180 ppm	Medium dose 900 ppm	High dose 4500 ppm	Dose-response +/-
F_{1B}-generation, first mating							
Reproductive Performance							
Mating index	%		96.7	100	100	100	-
Fertility index	%		89.7	96.7	93.1	100	-
Duration of pregnancy	Mean (d)		22.3	22.3	22.3	22.0	-
Live birth index	%		98.9	98.4	99.7	100	-
Gestation index	%		100	100	100	100	-
Litter size	Mean		10.7	10.6	10.9	11.5	-
Pup weight	Mean (g)		5.7	5.9	5.7	5.3*	
Sex ratio	Male/female (%/%)		51.6/48.4	49.7/50.3	50.2/49.8	65.2/34.8	-
Viability index	%		90.6	96.4**	84.4*	87.0	
Lactation index	%		71.5	94.2**	58.2**	35.5**	+
F_{1B}-generation, second mating							
Reproductive Performance							
Mating index	%		100	100	100	100	-
Fertility index	%		86.7	96.7	93.1	100	-
Duration of pregnancy	Mean (d)		22.4	22.3	22.3	22.0	-
Live birth index	%		99.0	98.0	98.3	100	-
Gestation index	%		100	100	100	100	-
Litter size	Mean		11.3	10.2	10.8	10.3	-
Pup weight	Mean (g)		5.5	5.7*	5.6	5.4	-
Sex ratio	Male/female (%/%)		49.5/50.5	46.8/53.2	48.0/52.0	48.8/51.2	-
Viability index	%		89.4	95.0*	95.2*	95.1	
Lactation index	%		84.2	94.5**	93.0**	63.3*	+

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

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