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An Embryotoxicity Study of the Fungicide Tridemorph and Its Commercial Formulation Calixin

J. MERKLE, V. SCHULZ, AND H.P. GELBKE
Department of Toxicology of BASF Aktiengesellschaft, D-6700 Ludwigshafen, Federal Republic of Germany

ABSTRACT Tridemorph (*N*-tridecyl-2,6-dimethylmorpholine), the active ingredient of the commercially formulated fungicide Calixin, is a teratogen in rats and mice. The no-effect level for embryotoxic effects was 27.5 mg/kg for mice and 20.6 mg/kg for rats.

By contrast, when Calixin, which contains 83% tridemorph, was administered orally at dose levels of 0.156, 0.722, and 3.909 mg/kg, no embryotoxic effects were observed in two strains of rats. Our extensive investigations, carried out under exposure conditions resembling as closely as possible those reported in another study, did not reproduce the previous findings of teratogenicity of Calixin.

Calixin is a fungicide mainly used for the control of cereal mildew (Erysiphe graminis), with both protective and curative properties in cucumbers, beans, peas, potatoes, tobacco, bananas, coffee, hevea, and tea. Its active ingredient is tridemorph (*N*-tridecyl-2,6-dimethylmorpholine). Both compounds are liquids. Calixin, containing 75% tridemorph, was recently tested for embryotoxic effects and caused teratogenic effects in rats in doses as low as 0.6 mg/kg (Schtenberg et al., '81). These investigators administered the compound by gavage in doses up to 65 mg/kg from the 7th to the 15th or from the 1st to the 20th day of gestation. Hemorrhages and hydronephrosis in the lower doses and cleft palate and micrognathia in the highest one were the most common findings in the fetuses. According to Schtenberg et al. ('81), hemorrhages induced by morpholine derivatives are characteristic findings.

Preliminary observations in our laboratory indicated that much higher doses were needed to cause teratogenic effects. Therefore, we decided to embark on a more thorough investigation of the teratogenic potential of Calixin in two different strains of rats.

In this study, we report the observations made following administration of both Calixin as well as its active ingredient tridemorph to the two species of pregnant rodents and describe the manifestations of embryotoxicity. One of the aims of these investigations was to reproduce the adverse effects described in rats by duplicating as closely as possible the exposure conditions reported by Schtenberg et al ('81). In accordance with Schtenberg, only the lower dosages, up to 3.91 mg/kg were tested.

MATERIALS AND METHODS

In accordance with internationally valid guidelines, we used the active ingredient tridemorph (*N*-tridecyl-2,6-dimethylmorpholine — technical grade) and to reproduce the investigations of Schtenberg et al. ('81) we used the specific commercial product Calixin, containing about 83% tridemorph [Tridemorph, Calixin, BASF Aktiengesellschaft, Ludwigshafen, Federal Republic of Germany (FRG)].

Animals

NMRI mice and Sprague-Dawley rats of known age, and purchased from a commercial supplier (Wiga, 8741 Sulzfeld, FRG) were used for the investigations with tridemorph. Wistar rats (Ivanovas, 7964 Kisslegg/Allgäu, FRG) and Sprague-Dawley rats (Wiga, 8741 Sulzfeld, FRG) were used for the experiemnts with Calixin. The mice were housed in groups of five/cage in rooms without air-conditioning, whereas the rats were kept in groups of two/cage in air-conditioned rooms (temperature 22 ± 2°C at a relative humidity of 55 ± 5%) and with a light/dark cycle of 12 hours

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each. The animals were provided with tap water and laboratory chow ad libitum.

Mating

Mice were mated with fertile males of the same strain between 8.00 and 10.00 hours. If a vaginal plug was detected in the females they were considered to be fertilized. Rats were mated with fertile males of the same strain from 16.00 hours until 7.30 hours on the following day. Vaginal smears were prepared in the morning. If sperm were detected the animals were considered to be fertilized. The day of fertilization in both mice and rats was designated day 0. The pregnant animals were randomly distributed among the test groups.

Treatment

Tridemorph was dissolved in olive oil. The doses administered to mice by gavage were 27.5, 81.7, and 245.1 mg/kg body weight (corresponding to 1/90, 1/30, and 1/10, respectively, of the LD_{50}) and doses administered by the same route to rats were 20.6, 60.2, and 189.2 mg/kg (corresponding to 1/45, 1/15, and 1/5, respectively, of the LD_{50}). These doses were administered to rats in a volume of 5 ml/kg and to mice in a volume of 2 ml/kg body weight. For control purposes, animals received an equal volume of olive oil by gavage or remained untreated. All animals were treated from the 6th to 15th day of gestation.

Calixin was administered to rats by gavage as an aqueous emulsion to which one drop of Tween 60 had been added. The dose volume was 5 ml/kg body weight. The dosage administered were 0.156, 0.722, and 3.909 mg/kg body weight. One group of animals remained entirely untreated. Another group of animals was given distilled water to which one drop of Tween 60 had been added; this group will hereafter be referred to as the Tween 60 group. Following the protocol reported by Schtenberg et al. ('81) the animals were treated from the 1st to the 19th or from the 7th to the 15th day of gestation.

During the dosing period rats and mice received with the test compound once daily in the morning. The volume to be administered was based on the weight of the animals on the first day of treatment (Calixin) or on that on day 0 (tridemorph) and was not changed as pregnancy progressed. This procedure is in accordance with the Environmental Protection Agency (EPA) Guidelines (Environmental Protection Agency, '78).

Fetuses of rats or mice removed by cesarean section on day 20 or 18 of gestation were examined for external findings, weighed, and fixed either in 95% alcohol in preparation for clearing and alizarin red S staining for skeletal visualization (Dawson, '26) or in Bouin's fluid in preparation for study of internal organs by the razor blade sectioning technique (Wilson and Warkany, '65). The findings obtained in the fetuses were classified into anomalies, variations, and retardations.

Statistical evaluation

The litter was the experimental unit for the evaluation.

Calixin

1. Body weight and body weight gain as well as weight, length, and placental weight of viable fetuses were evaluated by means of analyses of trend according to Williams ('71, '72).
2. Conception rate, litters with anomalous fetuses, and litters that showed fetuses with variations and retardations were analyzed with the exact test according to Fisher ('73).
3. Implantations, live and dead embryos per pregnant animal, anomalies, variations, and retardations as a percentage of viable fetuses per litter were evaluated by means of the asymptotic U-test according to Mann-Whitney ('70).

Tridemorph

The results were evaluated by means of analysis of variance and subsequent t-test or by means of an $r \times c$ contingency and subsequent 2×2 contingency test.

RESULTS

Calixin

Treatment from the 7th to the 15th or from the 1st to 19th day of gestation: Wistar rats (Tables 1, 2, and 5); Sprague-Dawley rats (Tables 3–5). The oral administration of 0.156, 0.722, and 3.909 mg/kg body weight (=0.13, 0.6, and 3.25 mg, respectively, of technical grade purity-active ingredient) to pregnant rats failed to produce either clinical signs of toxicity or anomalies in the offspring. The increased weight of the fetuses or placentas in the two highest dose groups showed no real dose relationship and was interpreted as not compound related. All the findings in the

TABLE 1. Wistar rats: Treatment 7th–15th day p.c.

	Untreated	Tween 60	Calixin parameters investigated		
			0.156 mg/kg	0.722 mg/kg	3.909 mg/kg
No. of pregnant animals	26	26	27	26	27
Body weight gain during pregnancy (g) ± SD	98.19 ± 10.38	97.15 ± 11.20	98.67 ± 10.67	94.65 ± 10.50	95.93 ± 8.70
Implantations (mean) ± SD	10.42 ± 1.53	10.23 ± 1.27	10.11 ± 1.69	9.77 ± 1.61	9.70 ± 1.54
% resorptions/animal ± SD	7.0 ± 14.24	3.44 ± 6.09	2.12 ± 4.05	6.07 ± 10.50	4.14 ± 6.39
No. live fetuses/ no. fetuses per litter	253/9.73	257/9.88	267/9.89	239/9.19	251/9.30
Weight of the fetuses (g) ± SD	3.25 ± 0.18	3.39 ± 0.18	3.33 ± 0.22	3.41 ± 0.22[1]	3.45 ± 0.15[1]
Weight of the placentas (g) ± SD	0.52 ± 0.06	0.51 ± 0.04	0.52 ± 0.05	0.56 ± 0.07[2]	0.52 ± 0.05[2]
n fetuses with anomalies/in					
n litters	10/9	13/9	14/9	6/6	12/5
%/litter	5.09	5.27	5.39	2.58	4.57
n fetuses with variations/in					
n litters	1/1	2/1	4/4	2/2	3/3
%/litter	0.55	0.70	1.36	0.91	1.21
n fetuses with retardations/in					
n litters	16/11	19/8	14/11	15/11	19/10
%/litter	6.67	7.11	5.18	6.19	6.89

Abbreviations: n, number; SD, standard deviations.
[1]Significance 95% related to untreated group.
[2]Significance 95% related to Tween 60 group.

TABLE 2. Wistar rats: Treatment 1st–19th day p.c.

			Calixin parameters investigated		
	Untreated	Tween 60	0.156 mg/kg	0.722 mg/kg	3.909 mg/kg
No. of pregnant animals	24	23	21	21	23
Body weight gain during pregnancy (g) ± SD	95.96 ± 13.59	93.30 ± 11.59	95.43 ± 11.29	87.14 ± 17.98	89.70 ± 12.25
Implantations (mean) ± SD	9.75 ± 1.57	10.35 ± 2.39	10.48 ± 1.91	8.67 ± 3.18[1]	9.96 ± 1.49
% Resorptions/animal ± SD	7.80 ± 12.46	3.37 ± 6.19	5.66 ± 8.23	8.84 ± 13.57	4.59 ± 7.94
No. live fetuses/ no. fetuses per litter	218/9.08	230/10.00	207/9.86	170/8.10	218/9.48
Weight of the fetuses (g) ± SD	3.37 ± 0.22	3.24 ± 0.18	3.35 ± 0.18	3.38 ± 0.23[1]	3.42 ± 0.26[2]
Weight of the placentas (g) ± SD	0.59 ± 0.05	0.56 ± 0.04	0.56 ± 0.05	0.62 ± 0.12[1]	0.60 ± 0.06[1]
n fetuses with anomalies/in					
n litters	2/2	6/3	2/2	4/2	7/7
%/litter	0.94	2.72	1.01	1.98	3.39
n fetuses with variations/in					
n litters	—	1/1	2/2	2/2	4/3
%/litter	—	0.33	1.05	0.83	2.41
n fetuses with retardations/in					
n litters	12/8	18/10	10/5	10/9	17/9
%/litter	6.05	8.15	4.30	7.65	7.05

Abbreviations in Table 1.
[1]Significance 95% related to Tween 60 group.
[2]Significance 99% related to Tween 60 group.

TABLE 3. Sprague-Dawley rats: Treatment 7th–15th day p.c.

	Untreated	Tween 60	Calixin parameters investigated		
			0.156 mg/kg	0.722 mg/kg	3.909 mg/kg
No. of pregnant animals	25	22	23	23	24
Body weight gain during pregnancy (g) ± SD	140.92 ± 20.04	134.50 ± 22.36	129.70 ± 33.93	140.87 ± 26.02	144.38 ± 25.70
Implantations (mean) ± SD	13.04 ± 2.42	13.59 ± 2.34	12.26 ± 2.97	13.35 ± 2.60	12.46 ± 2.96
% resorptions/animal ± SD	2.40 ± 5.06	4.01 ± 4.31	3.28 ± 4.60	4.08 ± 4.26	2.95 ± 4.94
No. live fetuses/ no. fetuses per litter	318/12.72	288/13.09	273/11.87	294/12.78	291/12.13
Weight of the fetuses (g) ± SD	3.67 ± 0.19	3.66 ± 0.29	3.67 ± 0.30	3.84 ± 0.22[1,3]	3.89 ± 0.27[2,4]
Weight of the placentas (g) ± SD	0.56 ± 0.06	0.56 ± 0.06	0.59 ± 0.11	0.59 ± 0.06	0.59 ± 0.06
n fetuses with anomalies/in					
n litters	8/5	4/4	3/2	1/1	11/6
%/litter	2.46	1.78	1.60	0.31	6.26
n fetuses with variations/in					
n litters	2/2	1/1	1/1	1/1	1/1
	0.57	0.35	0.36	0.30	0.32
n fetuses with retardations/in					
n litters	44/16	46/14	35/15	23/12	28/14
%/litter	13.80	15.57	13.80	8.48	9.74

Abbreviations as in Table 1.
[1]Significance 95% related to untreated group.
[2]Significance 99% related to untreated group.
[3]Significance 95% related to Tween 60 group.
[4]Significance 99% related to Tween 60 group.

TABLE 4. Sprague-Dawley rats: Treatment 1st–19th day p.c.

			Calixin parameters investigated		
	Untreated	Tween 60	0.156 mg/kg	0.722 mg/kg	3.909 mg/kg
No. of pregnant animals	24	25	24	25	25
Body weight gain during pregnancy (g) ± SD	133.21 ± 17.07	138.32 ± 23.36	145.50 ± 16.12	133.36 ± 14.91	134.20 ± 18.51
Implantations (mean) ± SD	13.0 ± 2.52	12.56 ± 2.99	13.46 ± 1.72	12.84 ± 2.43	13.08 ± 2.10
% resorptions/animal ± SD	5.46 ± 9.09	3.45 ± 7.51	3.81 ± 7.38	8.45 ± 14.88	5.16 ± 5.29
No. live fetuses/ no. fetuses per litter	297/12.38	305/12.20	311/12.96	293/11.72	310/12.40
Weight of the fetuses (g) ± SD	3.63 ± 0.13	3.73 ± 0.21	3.68 ± 0.21	3.73 ± 0.21	3.86 ± 0.19[1,2]
Weight of the placentas (g) ± SD	0.60 ± 0.07	0.58 ± 0.06	0.58 ± 0.06	0.61 ± 0.08	0.58 ± 0.06
n fetuses with anomalies/in					
n litters	10/8	8/5	12/9	4/4	8/8
%/litter	4.40	2.40	3.92	1.45	2.92
n fetuses with variations/in					
n litters	10/8	5/5	10/10	11/8	6/5
%/litter	4.40	1.54	3.10	3.56	1.99
n fetuses with retardations/in					
n litters	30/13	26/14	42/20	19/13	18/9
%/litter	11.39	8.36	13.69	5.90	5.96

Abbreviations as in Table 1.
[1]Significance 99% related to untreated group.
[2]Significance 95% related to Tween 60 group.

TABLE 5. Anomalies

	Control untreat.	Tween 60	Calixin 0.156 mg/kg	Calixin 0.722 mg/kg	Calixin 3.909 mg/kg
	Wistar rats: 7th–15th day p.c./1st–19th day p.c.				
Cleft thoracic vertebral centra	3/1	3/—	6/1	2/—	2/3
Cleft lumbar vertebral centra	—/—	—/—	1/—	—/—	—/—
Partial exencephaly	—/—	—/—	—/—	—/—	1/—
Wavy ribs unilaterally	5/—	7/3	5/—	—/3	3/3
Wavy ribs bilaterally	2/1	4/1	3/1	4/1	6/1
Anasarca	—/—	—/—	—/—	1/—	—/—
Kyphosis	—/—	—/—	—/—	1/—	—/—
Microphthalmia	—/—	—/2	—/—	—/—	—/—
	Sprague-Dawley rats: 7th–15th day p.c./1st–19th day p.c.				
Cleft thoracic vertebral centra	2/7	—/3	2/5	1/3	6/5
Cleft lumbar vertebral centra	—/1	—/1	1/—	—/—	2/—
Wavy ribs unilaterally	2/3	1/2	—/2	—/1	3/1
Wavy ribs bilaterally	4/—	1/3	—/3	—/—	2/1
Fetus with several anomalies	—/—	—/—	—/1	—/—	—/1
Aplasia of thoracic vertebral centra	—/—	—/—	—/1	—/—	—/—
Hydroureter	—/—	2/—	1/—	—/—	—/—
Shortened rib	—/—	—/—	—/—	—/—	1/—

TABLE 6. NMRI mice: Treatment 6th–15th day p.c.

	Tridemorph parameters investigated Test group				
	1 Contr. untreat.	2 Contr. oil	3 27.5 mg/kg	4 Contr. untreat.	5 Contr. oil
No. pregnant animals	23	27	26	23	22
Body weight gain during pregnancy (g) ± SD	24.04 ± 4.56	25.39 ± 2.87	25.31 ± 3.60	24.89 ± 3.81	22.41 ± 5.85
Implantations (mean) ± SD	11.13 ± 2.70	11.59 ± 1.62	12.12 ± 1.51	11.70 ± 1.99	10.82 ± 2.36
% resorptions/animal ± SD	10.79 ± 11.85	5.44 ± 7.70	5.46 ± 8.80	6.78 ± 6.53	10.08 ± 21.14
No. live fetuses/no. fetuses per litter	227/9.87	296/10.96	298/11.46	250/10.87	215/9.77
Weight of the fetuses (g)	1.27 ± 0.13	1.26 ± 0.12	1.26 ± 0.12	1.20 ± 0.15	1.27 ± 0.15[1]
Weight of the placentas (g)	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02[2]
n fetuses with anomalies/in					
n litters	1/1	2/2	9/8	4/4	6/4
%/litter	0.29	0.62	2.98	1.60	2.74
n fetuses with variations/in					
n litters	63/19	67/23	71/20	74/19	57/19
% litter	29.35	23.22	22.76	29.78	25/97
n fetuses with retardations/in					
n litters	5/4	13/8	8/3	9/7	6/4
% litter	1.98	4.41	2.34	3.57	2.62

Abbreviations as in Table 1.
[1]5 + 6 related to test group 4 significance 99%.
[2]5 related to test group 4 significance 95%.

TABLE 6. NMRI mice: Treatment 6th–15th day p.c. (continued)

	Tridemorph parameters investigated Test group			
	6 81.7 mg/kg	7 Contr. untreat.	8 Contr. oil	9 245.1 mg/kg
No. pregnant animals	26	24	28	29
Body weight gain during pregnancy (g) ± SD	21.82 ± 5.75	21.86 ± 4.43	23.99 ± 5.85	21.19 ± 4.19
Implantations (mean) ± SD	11.46 ± 1.58	11.33 ± 1.46	11.50 ± 2.53	11.45 ± 2.35
% resorptions/animal ± SD	14.38 ± 26.86	8.66 ± 8.17	10.81 ± 18.71	8.06 ± 9.86
No. live fetuses/no. fetuses per litter	259/9.96	248/10.33	285/10.18	305/10.52
Weight of the fetuses (g)	1.17 ± 0.11[1]	1.16 ± 0.13	1.28 ± 0.13[3]	1.13 ± 0.17[4]
Weight of the placentas (g)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01[5]
n fetuses with anomalies/in				
n littters	5/3	1/1	—/—	127/23
%/litter	2.29	0.46	—	39.15
n fetuses with variations/in				
n litters	58/19	52/20	67/23	78/26
%/litter	23.51	21.53	24.49	26.76
n fetuses with retardations/in				
n litters	13/6	11/6	5/4	19/7
%/litter	4.97	3.95	2.48	6.33

Abbreviations as in Table 1.
[1]5 + 6 related to test group 4 significance 99%.
[2]5 related to test group 4 significance 95%.
[3]8 related to test group 7 significance 99%.
[4]9 related to test group 7 significance 95%.
[5]9 related to test group 7 significance 99%.

TABLE 7. Anomalies NMRI mice: Treatment 6th–15th day p.c.

	Tridemorph test group								
	1 Contr. untreat.	2 Contr. oil	3 27.5 mg/kg	4 Contr. untreat.	5 Contr. oil	6 81.7 mg/kg	7 Contr. untreat.	8 Contr. oil	9 245.1 mg/kg
Cleft palate	1	1	5	3	5	3	1	—	127
Brachygnathia inferior	—	1	—	—	1	—	—	—	—
Scoliosis	—	—	—	—	—	—	—	—	1
Cleft thoracic vertebral centrum	1	—	—	—	—	—	—	—	—
Fused ribs	—	—	—	—	—	2	—	—	—
Kinky tail	—	1	4	1	—	—	—	—	—
Cleft thoracic vertebral centrum	—	—	—	—	—	1	—	—	—

fetuses did not reveal any relation to the dose as regards the frequency of their incidence. Moreover, all findings correspond to changes that occur spontaneously in the strain of rats examined.

Tridemorph

Mice (Tables 6, 7). The oral administration of 27.5, 81.7, and 245.1 mg/kg body weight to pregnant mice failed to produce clinical signs of toxicity. A reduction in the fetal weight in doses of 81.7 and 245.1 mg/kg did not have any influence on ossification of the skeleton. A reduction in the placental weight was only found in the highest dose group. The highest dose of 245.1 mg/kg caused teratogenic effects. Cleft palates were the main anomalies observed. The other findings, such as variations and retardations, correspond to changes that occur spontaneously in the strain of mice used. At dose levels of 81.7 and 27.5 mg/kg, the anomalies, variations, and retardations observed showed no compound-related effect either in type or incidence.

Rats (Tables 8, 9).. The highest dose of 189.2 mg/kg caused severe toxicity in dams, and administration of the test substance was discontinued after the fifth dose. The obvious clinical signs of toxicity (apathy, accelerated respiration, and loss of weight) caused mortality in about 60%. The animals died between day 10 and 13 of gestation. The dams that survived were sacrificed on the 20th day of gestation. This maternally toxic dose had an embyrolethal and teratogenic effect. Most of the anomalies affected the head (cleft palates), the vertebral column (cleft vertebral centra), the kidneys (dilated renal pelvis with hydroureter), and the extremities (syndactyly and oligodactyly). In addition to a reduction in the weight of the fetuses, which was also manifest in a retardation of ossification, the weight of the placentas was increased. Variations (fused sternal bones) were evident to an increased extent.

A dose of 60.2 mg/kg, however, was tolerated by the rats without visible adverse symptoms. This dose, too, was found to cause teratogenic effects. Brachygnathia and cleft vertebral centra occurred, in addition to cleft palates (Table 9). The slightly reduced fetal weight did not affect ossification. In addition, structural variations were increased at this dose level.

The deviations in fetal morphology observed after the administration of 20.6 mg/kg were in the range of the variations that

TABLE 8. Sprague-Dawley rats: Treatment 6th–15th day p.c.

			Tridemorph parameters investigated		
	Untreated	Olive oil	20.6 mg/kg	60.2 mg/kg	189.2 mg/kg[1]
No. pregnant animals	21	27	24	30	29/13[2]
Body weight gain during pregnancy (g) ± SD	137.43 ± 15.48	128.26 ± 17.68	133.75 ± 17.68	122.20 ± 23.46	115.62 ± 34.46
Implantations (mean) ± SD	13.86 ± 2.35	13.33 ± 3.03	13.50 ± 2.99	13.27 ± 2.64	14.54 ± 2.07
% resorptions/animal ± SD	6.88 ± 7.82	7.47 ± 7.11	7.92 ± 14.76	9.33 ± 18.98	14.19 ± 11.74
No. live fetuses/no. fetuses per litter	272/12.95	332/12.30	296/12.33	370/12.33	163/12.54
Weight of the fetuses (g) ± SD	3.78 ± 0.29	3.76 ± 0.30	3.79 ± 0.33	3.71 ± 0.33[3]	3.39 ± 0.47[3]
Weight of the placentas (g) ± SD	0.52 ± 0.07	0.54 ± 0.07[3]	0.55 ± 0.08[3]	0.54 ± 0.07[3]	0.64 ± 0.16[3]
n fetuses with anomalies/in					
n litters	11/ 8	12/10	12/9	212/28	86/12
%/litter	4.10	3.32	4.06	27.29	53.76
n fetuses with variations/in					
n litters	2/2	3/3	3/3	33/13	15/4
%/litter	0.68	0.90	2.03	9.37	10.08
n fetuses with retardations/in					
n litters	98/21	111/25	88/23	70/27	76/13
%/litter	34.65	32.36	30.72	18.85	52.02

Abbreviations as in Table 1.
[1]Treatment 6th–10th day p.c.
[2]Only 13 animals could be sacrificed on day 20 of gestation.
[3]Significance 99% related to untreated group.

TABLE 9. Anomalies Sprague-Dawley rats: 6th–15th day p.c.

			Tridemorph parameters investigated		
	Untreated	Olive oil	20.6 mg/kg	60.2 mg/kg	189.2 mg/kg[1]
Anasarca	—	—	1	—	3
Brachygnathia inferior	—	—	1	96	2
Syndactyly	—	—	—	—	13
Oligodactyly	—	—	—	—	2
Pseudoankylosis	—	—	—	—	3
Cleft thoracic vertebral centrum	11	10	9	24	28
Cleft thoracic vertebral centra	—	—	2	12	14
Cleft thoracic lumbar centrum	1	—	1	—	—
Aplasia of sacral vertebrae	—	—	1	—	—
Aplasia of coccygeal vertebrae	—	2	1	—	—
Vertebral arches fused	—	—	—	2	—
Kinky tail	—	—	—	—	6
Atresia ani	—	—	1	—	—
Dilated renal pelvis and hydroureter	—	—	—	—	3
Cleft palate	—	—	—	191	54

[1]Treatment 6th–10th day p.c.

occur spontaneously in Sprague-Dawley rats. The statistically significant increase in the placental weight after the administration of olive oil and of 20.6 and 60.2 mg/kg of tridemorph is believed to have no adverse biological effects.

Hematoma, hydrocephalies or edema, which Schtenberg et al. ('81) regarded as typical of the effect of Calixin, could not be detected either after the administration of tridemorph or after the administration of Calixin.

DISCUSSION

Tridemorph, which is the active ingredient of the commerically produced fungicide Calixin and contributes 83% to the formulation, caused embryotoxic including teratogenic effects in mice and rats. The latter species proved to be more sensitive. In mice there was no effect at a dose level of 81.7 mg/kg while there was massive teratogenicity at 245.1 mg/kg body weight. In rats the teratogenic effects appeared at 60.2 mg/kg body weight. The no-effect level for other embryotoxic effects (e.g., fetal weight, retardations) was 27.5 mg/kg for mice and 20.6 mg/kg for rats.

According to Schtenberg et al. ('81), Calixin had teratogenic effects at doses as low as 0.6 mg/kg. This dose, calculated on the basis of the active ingredient tridemorph, is equivalent to 0.45 mg/kg. This amounts to approximately 1/50 of the no-effect dose for embryotoxicity in the Wistar and Sprague-Dawley rats used in the present study. On the other hand, the teratogenic effect observed in rats by Schtenberg et al. ('81) after the administration of 65 mg/kg as the highest dose of Calixin is approximately the same as the effect that we found after the administration of 60.2 mg/kg commercial-grade-purity tridemorph.

In our studies with Calixin, neither teratogenic effects nor the specific fetal lesions described by Schtenberg et al. ('81), which were designated by the authors as typical (hemodynamic distrubances) of the morpholine derivates, could be detected. We did not find hemorrhages at any of the doses of tridemorph administered.

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