



**Report Title: Percutaneous Teratology Study in the Rabbit**

**Test Type:** Reproduction Toxicity

**Conducting Laboratory and Location:** International Research and Development Corporation

**Test Substance(s):** T-0184.01

**Species:** Rabbit

**# of Animals:** 80 mature virgin New Zealand White Rabbits; 20 per group

**Test Conditions:** 0, 10, and 30% OP in isopropanol/water (1/1) applied dermally (2ml/kg) for 4 hours on days 7-18 of gestation.

**Results:** No teratogenic or fetogenic effects. At high dose pronounced maternal toxicity believed to be due to severe skin irritation evidenced by one death, four spontaneous abortions and a severe body weight loss. At low dose, only effect was severe irritation (no maternal toxicity).

**Study #:** 191-259

**Report Date:** 5/4/79

**Accession #:** 31622

BIOLOGICAL SAFETY TEST SUMMARY REVIEW

Test Material: Octopirox

Division: TG

TSIN: T0184.01

Div. Req. #: TGSE 1525

Type Study: Percutaneous Teratology

Div. Toxicologist: G. M. Benke

Test Facility: IR&DC

Report No: 191-259

Date Report Written: 05-04-79

Date Rec'd by Operations Section: 05-07-79

\*\*\*\*\*

(1) This report has been reviewed and found in agreement with the protocol and to comply with all applicable Federal Regulations:

HSD Operations Section Monitor: H. A. Damm Date: 11/14/85

(2) This report has been reviewed for specific scientific content:

HSD Scientific Review By: Gregory A. L. Alden Date: 1/3/85  
Joseph C. L. Alden (P&G)

(3) This report has been reviewed for scientific quality and is summarized below. Where applicable, the retention limits for specimens to be returned to the P&G Archive are given.

*No evidence of alterations in the numbers of fetuses or litters with malformations was produced by T0184.01 in this study. Pronounced maternal toxicity was produced at the high treatment dose. No teratogenic effects were observed in this test.*

Retention Limits (month & year)	
Slides:	_____
Blocks:	_____
Wet Tissues:	_____
Teratology:	_____

Divisional Toxicologist: J. E. Weaver Date: 2/10/86  
J. E. Weaver

CIRCULATE BACK TO HSD SCIENTIFIC MONITOR, THEN TO HSD LIAISON

This report is approved for microfilming and entry into P&G Toxicology Files

HSD Scientific Reviewer: C. L. Alden Date: 2/11/86

Corporate HSD Liaison: C. L. Alden Date: 2/11/86

Return to HSD Operations Section, Room 2S179, MVL

Entered into Safety Data System by M. L. Brown Date: 2-14-86

Microfilming Completed by \_\_\_\_\_ Date: \_\_\_\_\_

International Research and Development Corporation

SPONSOR: The Procter and Gamble Company  
COMPOUND: T-0184.01  
SUBJECT: Percutaneous Teratology Study  
in the Rabbit

COPY SENT TO  
*L.M. Benke*  
(D. Main)  
DATE: *5/9/79*

RECEIVED BY  
MAY 7 1979  
G.I. DEPARTMENT

*Edwin I. Goldenthal*

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Date: May 4, 1979

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I. SYNOPSIS

Pregnant New Zealand White rabbits were used to evaluate the teratogenic potential of T-0184.01 in this study. The compound was administered dermally from days 7 through 18 of gestation at dosage levels of 200 and 600 mg/kg/day (concentrations of 10% and 30% weight/volume T-0184.01: vehicle respectively) at a rate of 2 ml/kg/day. One control group received the vehicle, 50:50 isopropanol to water, on a comparable regimen at 2 ml/kg/day. The environmental control group received no treatment.

During gestation, the females were observed for mortality and clinical signs of toxicity. Cesarean sections were performed on gestation day 28 and the number of viable and nonviable fetuses, early and late resorptions, corpora lutea and total implantations were recorded. The fetuses were weighed, sexed and examined for external, soft tissue and skeletal abnormalities.

Mean maternal body weight gains, all cesarean section data and all fetal morphological data were comparable in the environmental control group and the vehicle control group. Reddened application sites were noted in two rabbits in the vehicle control group.

T-0184.01, when applied dermally, caused severe skin irritations in both treatment groups. An increase in staining and matting of the anogenital region was also noted in the 600-mg/kg/day dosage group when compared to the control groups.

One rabbit in the 600-mg/kg/day dosage group died prior to its scheduled sacrifice date. The cause of death was not determined at necropsy. Four rabbits in the 600-mg/kg/day dosage group aborted prior to sacrifice.

Mean maternal body weight gains in the 200-mg/kg/day dosage group were comparable to the control groups.

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A severe body weight loss was noted in the 600-mg/kg/day dosage group during treatment and continued until late in gestation.

There were no biologically meaningful differences in the mean number of post implantation losses, corpora lutea or in the male to female sex ratio for either of the T-0184.01 treated groups when compared to both control groups. A decrease in the mean number of implantations was noted in both treatment groups when compared to the control groups. However, ovulation and implantation occurred prior to the initiation of compound administration; and therefore, this decrease is not considered to be treatment related. There was a decrease in the mean number of live fetuses in the 600-mg/kg/day dosage group when compared to the control groups. This decrease may be due to the decrease in implantations in this group, as postimplantation losses were comparable to the control groups. A reduction in mean fetal body weights was noted in the 600-mg/kg/day dosage group when compared to the control groups.

There were no biologically meaningful differences in the number of fetuses or litters with malformations or variations between the T-0184.01 treated groups and the control groups.

A dosage level of 600 mg/kg/day produced severe maternal toxicity as was evident by an increase in the incidence of abortion, maternal body weight loss and a reduction in fetal body weights in this group. This dosage level did not produce a teratogenic effect in rabbits.

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II. COMPOUND

The compound was received from the Procter and Gamble Company, Cincinnati, Ohio as shown below:

<u>Date Received</u>	<u>Label</u>	<u>Description</u>
August 7, 1978	Sample Code TSIN 0184.01 Type of Test Teratology Study Wt. (Inc. Lid, Cont, Label) 1193.20 Submitter Code WT 1433-92 Div. Request Doc. #1525 Storage Conditions/Hazard Room Temp Date Rec'd 7/26/78 Expiration Date 7/83	White fluffy somewhat chunky powder
August 16, 1978	Sample Code TSIN 0184.01 Type of Test Teratology Wt. (Inc. Lid, Cont, Label) 609.10 grams Submitter Code WT 1433-92 Div. Request Doc. #1525 Storage Conditions/Hazard Ambient Conditions Date Rec'd 8/10/78 Expiration Date 7/80	White fluffy somewhat chunky powder
September 11, 1978	Sample Code TO 184.01 Type of Test Teratology (Rabbit) Wt. (Inc. Lid, Cont, Label) 355 g Submitter Code WT 1433-160 Div. Request Doc. 152 Storage Conditions/Hazard Room Temp in Dark Date Rec'd 9/7/78 Expiration Date 9/7/79	White powder
September 11, 1978	Sample Code TO 184.02 <sup>a</sup> Type of Test Teratology (Rabbit) Wt. (Inc. Lid, Cont, Label) 624 g Submitter Code WT 1433-160 Div. Request Doc. 152 Storage Conditions/Hazard Room Temp in Dark Date Rec'd 9/7/78 Expiration Date 9/7/79	White powder

<sup>a</sup>Compound TO 184.02 was used for the last five days of treatment (9/22/78 - 9/26/78). However, throughout the report the test material will be referred to as T-0184.01.

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III. METHODS AND PROCEDURES

A. ANIMALS:

Eighty sexually mature virgin New Zealand White rabbits (obtained from Langshaw Farms, Augusta, Michigan) were used in this teratology study. The rabbits were individually housed in hanging wire-mesh cages in a controlled environment to maintain a temperature range of  $22 \pm 2^{\circ}\text{C}$ , a relative humidity range of 40% to 60%, a 12 hour light/12 hour dark photo-cycle and 10 to 15 fresh air changes per hour.

All rabbits were ear tagged and assigned a unique identification number. These rabbits were approximately 6 months old at the time of study initiation. The rabbits were inseminated on or after their 18th day in this laboratory. All of the rabbits received Triple Sulfa Oral<sup>b</sup> in their drinking water for a total of 10 consecutive days during the acclimation period. The concentration of Sulfa in their water was 80 ml/5 gallons for the first 5 days and 18 ml/5 gallons for the last 5 days. Purina<sup>®</sup> Rabbit Chow<sup>®</sup> and tap water were available ad libitum.

Insemination was initiated on August 21, 1978 and the last cesarean section was performed on October 6, 1978.

B. INSEMINATION PROCEDURES:

Five proven male rabbits of the same strain were selected to serve as semen donors. Semen from one male was used to inseminate 4 females on a given day (1 female from each dosage level).

Semen was collected by means of an artificial vagina<sup>c</sup>. The gelatinous plug was removed from the ejaculate and the semen was immediately evaluated for motility. Semen with a 50% or greater motility

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<sup>b</sup>Triple Sulfa Oral is a product of Quality Plus Products Co., Inc., Fort Dodge, Iowa.

<sup>c</sup>Artificial vagina and insemination pipette for rabbits, obtained from the Holborn Surgical Instrument Co., Ltd., London, England.

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was diluted with 4.0 ml of 0.9% Sodium Chloride for Injection U.S.P. at 35°C. The minimum concentration of motile sperm in the dilutions used for insemination was  $12 \times 10^6$ /ml. One-fourth to 1/2 ml of dilute semen was introduced into the anterior vagina of each female with an insemination pipette<sup>c</sup>. Ovulation was induced with 100 units of A.P.L.<sup>d</sup> (human chorionic gonadotropin) administered intravenously via the marginal ear vein within one hour of insemination.

The day of insemination was designated day 0 of gestation. Insemination procedures were performed on 10 separate days. Two females from each dosage level were inseminated per day.

C. ORGANIZATION OF TEST GROUPS AND TREATMENT:

The inseminated females were randomly assigned by a computer-generated program according to body weight to four groups consisting of one environmental control group, one vehicle control group and two treatment groups of 20 rabbits each. These rabbits were assigned to groups concurrently with the rabbits from study #191-230. The environmental control group for study #191-230 was used as a common control group for both studies.

T-0184.01 was administered dermally as a single daily dose on days 7 through 18 of gestation at dosage levels of 200 and 600 mg/kg/day (concentrations of 10% and 30% weight/volume T-0184.01: vehicle respectively) at a rate of 2 ml/kg/day. The vehicle control group received the vehicle, 50:50 reagent grade isopropanol:distilled water on a comparable regimen at 2ml/kg/day. The backs of the females were clipped before applying the test material, and at 3 day intervals

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<sup>c</sup>Artificial vagina and insemination pipette for rabbits, obtained from the Holborn Surgical Instrument Co., Ltd., London, England.  
<sup>d</sup>A.P.L. is a registered trademark for Chorionic Gonadotropin of Ayerst Laboratories, Inc., New York, N.Y.

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**F. FETAL MORPHOLOGICAL OBSERVATIONS:**

All fetuses were individually weighed, tagged and examined for external abnormalities, including the palate and eyes. Each fetus was dissected, internally sexed and examined for visceral abnormalities, including the brain by a mid-coronal slice. The heart was dissected by a modification of the method described by R. E. Staples<sup>1</sup>. The eviscerated, skinned fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson<sup>2</sup> for subsequent skeletal examination.

**G. STATISTICAL ANALYSES:**

Any statistical analyses that may become necessary will be done by the sponsor.

IV. RESULTS

A. MATERNAL OBSERVATIONS:

Nasal discharge, scabbing, hair loss, soft stool and diarrhea were occasionally noted in all groups and are not considered to be treatment related. Reddening at the application site was noted in two rabbits in the vehicle control group. Reddening, edema, swelling, cracking, subcutaneous hemorrhaging, sloughing of the dermal layer and loss of skin elasticity at the site of compound application were noted in the 200- and 600-mg/kg/day dosage groups. An increase in staining and matting of the anogenital region was also noted in the 600-mg/kg/day dosage group when compared to the control groups.

One rabbit in the 600-mg/kg/day dosage group died prior to its scheduled sacrifice date. The cause of death was not determined at necropsy. Four rabbits in the 600-mg/kg/day dosage group aborted. Three of these rabbits aborted prior to their scheduled sacrifice date and one aborted on its scheduled day of sacrifice. Survival was 100% in the control groups and the 200-mg/kg/day dosage group.

Maternal body weights are presented in Table 1. Mean maternal body weight gains in the environmental control group, the vehicle control group and the 200-mg/kg/day dosage group were comparable. A body weight loss was noted during the treatment period and continued until near the end of the gestation period in the 600-mg/kg/day dosage group when compared to the control groups. This resulted in a slight mean maternal body weight loss over the entire gestation period in the group.

Temperature, light/dark cycle and air exchanges per hour in the rabbit rooms during the study were maintained within the specified limits. The mean relative humidity exceeded the range by approximately 14% during the study.

**B. CESAREAN SECTION OBSERVATIONS:**

A summary of maternal and fetal observations is presented in Table 2 and individual values are presented in Table 3. The mean number of viable fetuses, postimplantation losses, implantations, corpora lutea, the male to female sex ratio and mean fetal body weights were comparable in the environmental control group and the vehicle control group. There were no biologically meaningful differences in the mean number of postimplantation losses, corpora lutea or in the male to female sex ratio for either T-0184.01 treated group when compared to the control groups. There was a slight decrease in the mean number of implantations in both treated groups when compared to the control groups. However, ovulation and implantation occurred prior to the initiation of compound administration and this decrease is not considered to be treatment-related. There was a slight decrease in the mean number of live fetuses in the 600-mg/kg/day dosage group when compared to the control groups. This decrease may be due to the decrease in implantations in this group as postimplantation losses were comparable to the control groups. Mean fetal body weights were moderately reduced in the 600-mg/kg/day dosage group when compared to the control groups.

**C. FETAL MORPHOLOGICAL OBSERVATIONS:**

Summarized findings of all external, soft tissue and skeletal fetal examinations are presented in Table 4 and individually listed in Table 5. The findings are listed as malformations or developmental and genetic variations. There was a slight increase in the number of fetuses and the number of litters with malformations in the environmental control group when compared to the vehicle control group. This is considered to be random occurrence. There were no biologically meaningful differences in the number of fetuses or litters with malformations or variation between the T-0184.01 treated groups and the control groups.

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References

1. Stroles, R. E. (1974), Detection of Visceral Alterations in Mammalian Diseases, Teratology, 9: A37-A38.
2. Dawson, A. B. (1926), A Note on the Staining of the Skeleton of Cleared Specimens with Alizarin Red S, Stain Technol., 1: pp 123-124.

## T-0184.01: Percutaneous Teratology Study in the Rabbit

TABLE 1. Individual Body Weights, Grams

Group, Can No.	Day of Gestation									
	0	3	7	10	13	16	19	22	25	28
<u>0 mg/kg/day (Environmental Control):</u>										
37152 <sup>a</sup>	3182	3340	3392	3188	3626	3635	3778	3725	3987	4020
37163 <sup>a</sup>	3092	3308	3361	3586	3644	3623	3667	3729	3720	3790
37164 <sup>a</sup>	3095	3461	3472	3502	3601	3667	3667	3978	-028	4017
37183	3094	3299	3444	3417	3470	3562	3645	3662	3614	3705
37166	3386	3460	3539	3558	3609	3662	3681	3692	3724	3727
37147	3205	3814	3842	3956	3910	4007	4062	4123	4133	4193
37168 <sup>b</sup>	3292	3457	3482	3606	3705	3723	3711	3768	3757	3791
37169	3593	3696	3777	3941	3944	4054	4023	4116	-044	4064
37170	3236	3336	3445	3305	3611	3691	3636	3671	3703	3734
37171 <sup>a</sup>	3142	3184	3274	3367	3460	3468	3479	3533	3570	3601
37172	3344	3537	3716	4005	4135	4242	4179	4059	4269	4311
37173	3517	3600	3620	3720	3721	3804	3866	3805	3933	3941
37174	3013	3127	3200	3228	3301	3356	3454	3509	3657	3751
37175	3091	3075	3146	3235	3276	3200	3212	3119	3118	3478
37176	3667	3742	3906	4113	4203	4262	4103	4263	4350	4330
37177	3592	3642	3696	3738	3777	3823	3691	3671	3899	3806
37178 <sup>a</sup>	3180	3202	3242	3326	3325	3373	3479	3572	3520	3661
37179	3209	3263	3128	3276	3468	3557	3596	3656	3692	3713
37180	3053	3100	3206	3209	3352	.	3490	353	3589	3596
37181	3049	3163	3281	3303	3372	3468	3470	3467	3566	3753
Mean	3334	3422	3511	3579	3650	3724	3722	3763	3821	3663
<u>0 mg/kg/day (Vehicle Control):</u>										
37322	4536	4607	3520	3552	3477	3611	3686	3721	3780	3699
37321 <sup>a</sup>	2994	2759	2542	2655	2671	3062	3217	3207	3270	3347
37324 <sup>a</sup>	3102	3348	2998	2690	2889	2854	3146	3227	3236	3305
37325	3382	3696	3595	3672	3739	3977	3990	3995	4202	4081
37326	3602	3826	3643	3551	3612	3647	3611	3757	3753	3864
37327	3517	3620	3697	3696	3732	3621	3952	4046	4137	4145
37328 <sup>a</sup>	3256	3496	3429	3544	3679	3920	3769	3937	3964	4057
37329	3523	3607	3628	3723	3806	4020	3961	4056	-100	4146
37330	3808	3723	3790	3713	3819	3964	3994	4145	4154	4200
37331 <sup>a</sup>	3762	3601	3704	3700	3831	3770	3759	3830	3964	4011
37332 <sup>a</sup>	3000	3150	3279	3291	3320	3293	3397	3358	3512	3613
37333	3515	3572	3527	3751	3846	3906	4096	3926	4045	3890
37334	3222	3274	3378	3428	3435	3414	3523	3570	3618	3656
37335	3314	3441	3458	3448	3442	3435	3461	3671	3728	3829
37336	3462	3838	3947	3979	4005	4060	3886	4006	4137	4177
37337	3214	3310	3402	3528	3661	3727	3518	3875	3996	4030
37338	3406	3598	3692	3696	3796	3804	3871	3951	3936	3956
37339 <sup>a</sup>	3534	3525	3761	3787	3858	3963	3953	4125	4089	3983
37340	3895	4056	4115	4022	4235	4196	4365	4434	4449	4604
37341	3272	3328	3581	3519	3722	3809	3935	4030	4003	4068
Mean	3392	3677	3641	3663	3732	3820	3840	3942	4001	4023

<sup>a</sup>Nonpregnant, not used in calculation of mean.

T-0184-01:

## Percutaneous Teratology Study in the Rabbit

TABLE 1. Cont.

## Individual Body Weights, Grams

Group, Dam No.	Day of Gestation									
	0	3	7	10	13	16	19	22	25	28
<b>200 mg/kg/day:</b>										
17342	3170	3212	3297	3294	3419	3483	3404	3437	3505	3495
17343	3376	3453	3499	3486	3693	3749	3818	3870	3834	3748
17344 <sup>a</sup>	3364	3462	3646	3658	3531	3597	3486	3570	3452	3558
17345 <sup>a</sup>	3829	4080	4065	-124	4047	4091	4151	+260	-093	3915
17346	3771	3830	3931	3974	3899	+100	4051	+229	+255	4353
17347 <sup>a</sup>	3214	3397	3472	3568	3540	3663	3663	3625	3482	3325
17348	3536	3566	3640	3651	3597	3694	3650	3640	3707	3739
17349 <sup>a</sup>	3430	3480	3569	3675	3616	3633	3714	3750	3517	3684
17350 <sup>a</sup>	3881	3738	3899	4000	4106	4190	4266	4328	3955	3863
17351	3575	3636	2954	3151	3520	3664	3638	3637	3567	3715
17352 <sup>a</sup>	3026	3199	3209	3427	3386	3409	3491	3463	3578	3728
17353 <sup>a</sup>	3243	3321	3370	3500	3606	3516	3627	3560	3842	3970
17354 <sup>a</sup>	3714	3836	3900	4044	3971	4002	3881	3955	4038	4099
17355	3892	3926	3923	4084	4144	4215	4174	4343	4554	4537
17356	3482	3567	3595	3593	3695	3695	3525	3800	3916	3860
17357	3130	3153	3208	3324	3395	3484	3327	3601	3754	3796
17358	3941	3906	3904	3750	3826	3757	3798	3799	3779	3701
17359	3185	3232	3310	3356	3345	3479	3519	3522	3799	3774
17360	4301	4421	4447	-221	4424	4534	+576	6,29	4576	4545
17361	3653	3527	3785	3318	3668	3698	3708	4010	3848	4011
Mean	3601	3626	3622	3712	3805	3774	3904	3926	3940	
<b>60 mg/kg/day:</b>										
17362	3223	3465	3315	3643	3594	3308	3307	3164	3269	3356
17363	3208	3412	3379	3642	3523	3442	3467	3464	3593	3403
17364 <sup>a</sup>	3227	3617	3595	3373	3587	3420	3336	3519	3586	3390
17365 <sup>a</sup>	3479	3598	3541	3639	3618	3706	3470	3310	3286	3403
17366 <sup>a</sup>	3543	3677	3759	3700	3820	3807	3833	3946	3903	3774
17367 <sup>a</sup>	3475	3392	3378	3264	3136	3216	3281	3338	3240	3091
17368	3758	3702	3793	3809	3618	3811	3687	3790	3726	3745
17369	3667	3734	3818	3587	3559	3480	3348	3244	3036	2766 <sup>b</sup>
17370 <sup>a</sup>	3340	3156	3158	3035	3142	3051	3160	3094	2994	2805
17371	3442	3458	3573	3671	3574	3741	3792	3644	3452	3345
17372	3765	3869	4020	4171	4249	4249	3951	3834	4072	3786
17373	3206	3327	3441	3422	3355	3397	3174	3069	3069	3069
17374	3237	3339	3403	3489	3390	3574	3523	3222	3112 <sup>c</sup>	
17375 <sup>a</sup>	3203	3243	3271	3403	3515	3422	3314	3083	3071	
17376 <sup>a</sup>	3102	3161	3219	3229	3132	3215	3096	3005	3064	3045
17377	3439	3460	3561	3652	3615	3694	3451	3742	3630	3350
17378 <sup>a</sup>	3038	3097	3280	3202	3279	3260	3225	3329	3144	3049
17379	4225	4144	4315	3780	3534	3261	2929	0144, gestation day 21		
17380	3640	3752	3829	3621	3489	3506	3494	3425	3425	3425
									3425	3425
17381	3411	3480	3580	3356	3374	3446	3594	3692	3465	3445
Mean	3510	3596	3662	3654	3591	3573	3494	3481	3480	3490

<sup>a</sup> Non-gravid, not used in calculation of mean.<sup>b</sup> Aborted, gestation day 28<sup>c</sup> Aborted, gestation day 27

191-259

- Not available

## T-0184-D1. Percutaneous Toxicology Study in the Rabbit

TABLE 2. Summary of Maternal and Fetal Observations at Cesarean Section

	Environmental Control (0 mg/kg/day)	Vehicle Control (0 mg/kg/day)	T-0184-D1 (mg/kg/day)	
			100	600
No. of animals on study:	20	20	20	20
No. of animals that died:	0	0	0	1
No. nongravid:	0	0	0	0
No. gravid:	0	0	0	1
No. of animals examined at cesarean section:	20	20	20	19
No. nongravid:	6	5	8	8
No. gravid:	14	14	12	11
No. aborted:	0	0	0	4
No. of dams with resorptions only:	2	2	2	2
No. of dams with live fetuses:	12	12	10	5
No. of live fetuses/dam:	5.6	5.9	5.7	4.3
No. of post implantation losses/dam:	1.4	0.8	0.4	0.9
No. of implantations/dam:	7.1	6.7	6.1	5.1
No. of corpora lutea/dam:	9.8	10.2	9.5	10.3
Sex ratio - MALE:FEMALE:	41:38	50:33	38:30	14:15
Mean Fetus body weight (g):	35.3	36.4	37.4	30.2

T-0184.01:

## Percutaneous Teratology Study in the Rabbit

TABLE 3. Cesarean Section Data for Individual Females

Group, Dam No.	Fetuses		Resorptions		Post- Implan- tation Loss	Im- plan- tations	Corpora Lutea	Sex Distribution		Mean Male Body Wt. (g)	Mean Female Body Wt. (g)	Mean Combined Body Wt. (g)
	Live	Dead	Late	Early				Male	Female			
<u>0 ug/kg/day (Environmental Control):</u>												
37162	Nonpregnant											
37163	Nonpregnant											
37164	Nonpregnant											
37165	7	0	0	0	0	7	9	3	4	30.4	30.8	30.7
37166	8	0	1	0	1	9	9	3	3	38.6	37.3	37.8
37167	5	0	0	0	0	5	10	2	3	33.6	34.2	34.9
37168	Nonpregnant											
37169	7	0	0	0	0	7	8	6	1	34.0	34.3	34.0
37170	0	0	0	1	1	1	1	-	-	-	-	-
37171	Nonpregnant											
37172	0	0	0	6	6	6	6	-	-	-	-	-
37173	10	0	0	0	0	10	19	5	5	36.6	33.9	35.2
37174	6	0	0	1	1	7	7	3	3	42.8	37.8	39.8
37175	4	0	0	0	0	4	13	2	4	32.7	29.2	29.7
37176	1	0	0	3	3	4	12	1	0	47.2	-	46.6
37177	8	0	0	1	1	9	-	6	2	6.4	37.8	35.8
37178	Nonpregnant											
37179	7	0	0	0	0	7	9	4	3	34.3	33.4	34.0
37180	7	0	0	0	0	7	9	4	3	27.4	33.1	30.1
37181	7	0	0	2	2	9	21	2	3	36.9	35.2	35.7
Total	79	0	1	19	20	99	137	62	38			
Mean	5.6	0.0	0.1	1.4	1.4	7.1	9.8			35.8	33.8	35.3
<u>2 ug/kg/day (Vehicle Control):</u>												
37322	6	0	0	2	2	8	10	4	2	37.0	37.0	37.0
37323	Nonpregnant											
37324	Nonpregnant											
37325	3	0	0	0	0	3	8	4	4	60.2	41.0	49.6
37326	0	0	0	2	2	2	7	-	-	-	-	-
37327	7	0	0	0	0	7	8	1	6	40.5	34.2	35.1
37328	Nonpregnant											
37329	4	0	0	3	3	7	8	4	0	43.3	-	43.3
37330	4	0	0	0	0	4	11	2	0	35.0	-	35.0
37331	Nonpregnant											
37332	Nonpregnant											
37333	6	0	0	0	0	6	7	4	2	40.3	36.3	38.9
37334	0	0	0	1	1	1	2	-	-	-	-	-
37335	8	0	0	0	0	8	14	5	3	31.5	29.5	30.4
37336	1	0	0	0	0	1	13	3	0	36.3	-	36.4
37337	9	0	0	0	0	9	10	5	6	34.0	29.9	32.2
37338	6	0	0	1	1	7	16	4	4	40.5	37.5	39.0
37339	Nonpregnant											
37340	11	0	2	0	2	13	15	5	5	36.6	33.6	34.9
37341	9	0	0	0	0	9	14	7	7	32.5	34.6	33.0
Total	63	0	2	9	11	94	143	50	33			
Mean	5.9	0.0	0.1	0.6	0.8	6.7	10.2			37.3	34.8	36.4

- Not applicable

I-0184.01:

## Percutaneous Toxicology Study in the Rabbit

TABLE J. Cont. Cesarean Section Data for Individual Females

Group, Dam No.	Fetuses		Resorptions		Post Implan- tation Loss	Im- plante- tions	Corpora Lutea	Sex Distribution		Mean Male Body Wt. (g)	Mean Female Body Wt. (g)	Mean Combined Body Wt. (g)
	Live	Dead	Late	Early				Male	Female			
<u>200 mg/kg/day:</u>												
37342	0	0	0	1	1	1	8	-	-	-	-	-
37343	7	9	0	0	0	7	9	5	2	36.2	35.0	34.4
37344	Nongravid											
37345	Nongravid											
37346	5	0	0	1	1	6	10	3	2	40.0	34.7	37.9
37347	Nongravid											
37348	0	0	0	1	1	1	6	-	-	-	-	-
37349	Nongravid											
37350	Nongravid											
37351	3	0	0	0	0	3	9	0	3	40.0	34.7	46.2
37352	Nongravid											
37353	Nongravid											
37354	Nongravid											
37355	12	0	0	0	0	12	12	8	4	32.2	33.7	32.7
37356	8	0	0	0	0	8	8	5	3	37.6	40.6	39.7
37357	5	0	0	0	0	5	9	2	3	21.2	34.4	31.3
37358	9	0	0	1	1	10	10	6	3	31.0	34.0	32.0
37359	7	0	0	1	1	3	11	1	1	30.1	30.1	30.1
37360	10	0	0	0	0	10	10	5	5	31.5	32.3	31.5
37361	7	0	0	0	0	7	9	3	4	40.8	36.1	39.2
Total	58	0	0	5	5	73	104	38	30			
Mean	5.7	0.0	0.0	0.4	0.4	6.1	9.5			36.5	37.5	37.4
<u>600 mg/kg/day:</u>												
37362	8	0	0	0	0	8	22	3	5	23.7	28.0	26.4
37363	0	0	0	1	1	1	4	-	-	-	-	-
37364	Nongravid											
37365	Nongravid											
37366	Nongravid											
37367	Nongravid											
37368	6	0	0	0	0	6	9	2	4	29.6	29.0	29.2
37369	Aborted - gestation day 28											
37370	Nongravid											
37371	0	0	0	4	4	4	7	-	-	-	-	-
37372	8	0	0	0	0	8	16	3	5	31.9	32.1	32.6
37373	Aborted - gestation day 22											
37374	Aborted - gestation day 27											
37375	Nongravid											
37376	Nongravid											
37377	5	0	0	0	0	5	13	3	2	26.4	24.6	25.7
37378	Nongravid											
37379	Died - gestation day 21 - gravid											
37380	Aborted - gestation day 23											
37381	1	0	0	1	1	4	11	3	0	38.2	-	38.2
Total	30	0	0	6	6	36	72	14	16			
Mean	4.3	0.0	0.0	0.9	0.9	5.1	10.3			30.0	28.7	30.4

\*Corpora Lutea - regressing, too small to count.

- not applicable

191-259

## T-0184.01: Percutaneous Teratology Study in the Rabbit

TABLE 4. Summary of the Incidence of Malformations and of Developmental and Genetic Variations

	Environmental	Vehicle	T-0184.01 (mg/kg/day)	
	Control (0 mg/kg/day)	Control (0 mg/kg/day)	100	400
No. of litters examined:	12	12	10	5
No. of fetuses examined externally:	79	83	68	30
No. of fetuses examined skeletally:	79	83	68	30
No. of fetuses examined for soft tissue:	79	83	68	30
<u>No. of Fetuses (No. of Litters)</u>				
<u>Malformations Observed:</u>				
Scoliosis with/without associated rib anomalies:	5 (4)		5 (3)	3 (1)
Fused centra:		1 (1)		
Short or absent tail:	2 (1)		2 (2)	2 (1)
Fused skull bones:	1 (1)		1 (1)	
Atlanto-occipital defect:			1 (1)	
Small kidney, thread like ureter:				2 (2)
Sternum fused:				1 (1)
Total No. of Fetuses (Litters) with Malformations:	6 (4)	1 (1)	9 (3)	4 (2)
<u>Variations - Developmental and Genetic Observed:</u>				
17 presacral vertebrae:	12 (7)	12 (6)	8 (6)	8 (3)
15 presacral vertebrae:			1 (1)	
8 cervical vertebrae with 8th cervical rib:			1 (1)	
13th full rib(s):	29 (8)	35 (11)	19 (8)	10 (3)
13th rudimentary rib(s):	13 (7)	6 (4)	12 (7)	3 (2)
Sternum 9 and/or #6 unossified:	18 (7)	16 (9)	11 (4)	9 (3)
7th sternum between sternum #5 and #6:			1 (1)	
Sternum misaligned:		1 (1)	1 (1)	2 (1)
Reduced ossification of the skull:				1 (1)
Accessory skull bones:		1 (1)	1 (1)	
Bent thyroid arch(es):	2 (2)	14 (6)	5 (3)	1 (1)
Talus unossified:	1 (1)	1 (1)	1 (1)	2 (1)
Major vessel variation:	3 (3)	3 (3)	6 (3)	1 (1)
Posterior process of squamosal bent:		1 (1)		

## T-0184.01: Percutaneous Teratology Study in the Rabbit

TABLE 5. Individual Fetal Malformation Data

Dose Number	Male Number	Dosage Level (mg/kg/day)	Fetal Malformations
37166	26691	0-EC	8 fetuses - no malformations
37326	26691	0-VC	Resorptions only
37346	26691	200	3 fetuses - no malformations; #1-scoliosis, fused ribs and interrupted ossification of 1 rib; #4-scoliosis and short tail
37366	26691	600	Nongravid
37162	30912	0-EC	Nongravid
37165	30912	0-EC	6 fetuses - no malformations; #5-scoliosis, fused and forked ribs
37169	30912	0-EC	7 fetuses - no malformations
37171	30912	0-EC	6 fetuses - no malformations
37179	30912	0-EC	7 fetuses - no malformations
37180	30912	0-EC	7 fetuses - no malformations
37322	30912	0-VC	6 fetuses - no malformations
37325	30912	0-VC	8 fetuses - no malformations
37329	30912	0-VC	4 fetuses - no malformations
37334	30912	0-VC	Resorptions only
37339	30912	0-VC	Nongravid
37340	30912	0-VC	11 fetuses - no malformations
37342	30912	100	Resorptions only
37345	30912	200	Nongravid
37349	30912	200	Nongravid
37354	30912	200	Nongravid
37359	30912	200	2 fetuses - no malformations
37361	30912	200	10 fetuses - no malformations
37362	30912	600	8 fetuses - no malformations
37365	30912	600	Nongravid
37369	30912	600	Aborted
37374	30912	600	Aborted
37379	30912	600	Died - gravid
37380	30912	600	Aborted
37164	30913	0-EC	Nongravid
37171	30913	0-EC	Nongravid
37175	30913	0-EC	1 fetus - no malformations
37178	30913	0-EC	Nongravid
37181	30913	0-EC	5 fetuses - no malformations; #5-scoliosis and 1 rib absent; #7-fused frontals, scoliosis and 1 forked rib
37324	30913	0-VC	Nongravid
37331	30913	0-VC	Nongravid
37335	30913	0-VC	3 fetuses - no malformations
37338	30913	0-VC	7 fetuses - no malformations; #3-fused centers
37341	30913	0-VC	9 fetuses - no malformations
37344	30913	200	Nongravid
37351	30913	200	3 fetuses - no malformations
37356	30913	200	3 fetuses - no malformations
37358	30913	200	9 fetuses - no malformations
37361	30913	200	7 fetuses - no malformations
37364	30913	600	Nongravid
37371	30913	600	Resorptions only
37376	30913	600	Nongravid

EC - Environmental Control  
VC - Vehicle Control

Y-0184.01.

## Parental Toxicology Study in the Rabbit

TABLE 5. Cont.

## Individual Fetal Malformation Data

Dam Number	Male Number	Doseage Level (ug/kg/day)	Fetal Malformations
37378	30913	600	Nongravid
37381	30913	600	0 fetuses - no malformations; #1-small kidney, thread-like ureter and scoliosis and fused ribs; #2-small kidney, thread-like ureter, scoliosis and fused ribs and short, thread-like tail; #6-short, thread-like tail and scoliosis and fused ribs
37263	30916	0-EC	Nongravid
37270	30916	0-EC	Resorptions only
37172	30916	0-EC	Resorptions only
37177	30916	0-EC	8 fetuses - no malformations
37323	30916	0-VC	Nongravid
37330	30916	0-VC	4 fetuses - no malformations
37332	30916	0-VC	Nongravid
37337	30916	0-VC	9 fetuses - no malformations
37343	30916	200	1 fetus - no malformations; #1-scoliosis; #2-fused skull bones; #3-scoliosis and fused ribs; #4-scoliosis; #6-scoliosis and malformed rib; #7-atloido-occipital defect
37350	30916	200	Nongravid
37352	30916	200	Nongravid
37357	30916	200	4 fetuses - no malformations; #4-scoliosis, malformed rib and tail absent with thread-like tissue at site
37367	30916	600	Resorptions only
37370	30916	600	Nongravid
37371	30916	600	3 fetuses - no malformations
37377	30916	600	4 fetuses - no malformations; #1-fused sternbrae
37167	30918	0-EC	3 fetuses - no malformations
37168	30918	0-EC	Nongravid
37173	30918	0-EC	5 fetuses - no malformations; #5-short tail thread-like at base; 3rc sacral arch malformed, 4th sacral and all caudal vertebrae absent, #7-short tail thread-like at base
37175	30916	0-EC	3 fetuses - no malformations; #5-scoliosis and 1 rib absent
37327	30918	0-VC	7 fetuses - no malformations
37328	30918	0-VC	Nongravid
37333	30918	0-VC	6 fetuses - no malformations
37335	30918	0-VC	8 fetuses - no malformations
37347	30918	200	Nongravid
37349	30918	200	Resorptions only
37353	30918	200	Nongravid
37355	30913	200	12 fetuses - no malformations
37367	30918	600	Nongravid
37368	30918	600	4 fetuses - no malformations
37373	30918	600	Aborted
37375	30918	600	Nongravid

EC - Environmental Control  
VC - Vehicle Control

INTERDEPARTMENTAL CORRESPONDENCE

FROM G. M. Benke DATE 4/27/79  
TO File Memo RETENTION LIMIT Non-Discretionary  
SUBJECT STATISTICAL ANALYSIS OF ATTENTION  
TGSE 1525: OCTOPRIX TOPICAL  
TERATOLOGY IN THE RABBIT

The attached letter by R. D. Bruce and E. C. Drago and computer printouts summarize statistical data from tables 2 and 4 of IRBC Study 191-259, a topical teratology study of T-0184.01.

Statistical analysis of table 2 did not reveal any significant effects in No. of live fetuses, post-implantation losses, implants, or corpora lutea. There was a statistically significant decrease in average pup weight ( $p < .021$ ). This was not unexpected since the test material produced maternal toxicity (2 abortions).

Statistical analyses (Chi square) of both number of fetuses and number of litters with malformations did not show any significant increases among tested and control groups.

*G. M. Benke*

G. M. Benke

amb

cc: TGSE 1525  
R. D. Bruce  
 N. A. Derner

RECEIVED BY

MAY 1 1979

H. A. Derner



ECHO CHECK OF H8442 CONTROL C.404

3/29/79  
2

0 200 400

VEN POSF WFSRNSF 200 H8442 VS ENVIRONMENT H8442 VS ENVIRON

5 APRIL 1974 (M) - PRECUT ANEMUS TEMATGLON - RABBIT

NO. LIVE FETUSES POST IMPLANT LOSS/IMPLANTS

CONCORD LUTEA

AVERAGE PIG WT.

0 0 0 1

94 1 5

94 1 5 STATISTICAL ANALYSIS SUMMARY SUBSTANTIAL 1-2 1 2 2 2 0

See Summary  
on final pages

TU144.01-PERCUITANEMUS TERATORGY-HABBIT  
 RESIDUES 1- 51 4 GROUPS WITH SIZES 20 20 20 20

3/29/79

	NO. OF FETUSES	HOST TUMPLANT LUSS	IMPLANTS	COMMON LUTES	AVERAGE PUP WT.
VEHICLE 37322	0.	0.	0.	10.	37.0
VEHICLE 37323	0.	0.	0.	0.	0.
VEHICLE 37324	0.	0.	0.	0.	0.
VEHICLE 37325	4.	1.	1.	8.	30.6
VEHICLE 37326	0.	0.	0.	7.	0.
VEHICLE 37327	7.	0.	0.	5.	30.1
VEHICLE 37328	0.	0.	0.	0.	0.
VEHICLE 37329	4.	3.	7.	6.	30.3
VEHICLE 37330	4.	1.	4.	11.	30.0
VEHICLE 37331	0.	0.	0.	0.	0.
VEHICLE 37332	0.	0.	0.	0.	0.
VEHICLE 37333	1.	0.	0.	7.	30.4
VEHICLE 37334	0.	1.	1.	2.	0.
VEHICLE 37335	0.	0.	0.	14.	30.4
VEHICLE 37336	5.	0.	3.	13.	30.4
VEHICLE 37337	0.	0.	0.	11.	30.2
VEHICLE 37338	0.	0.	0.	16.	30.0
VEHICLE 37339	0.	0.	0.	0.	0.
VEHICLE 37340	11.	0.	13.	15.	30.4
VEHICLE 37341	4.	0.	4.	14.	30.0
GRAND AVERAGES	5.7	0.8	4.7	10.2	30.3
STAT DATA ERRORS	0.	0.3	0.9	1.0	1.05
200 MG/KG 37342	0.	1.	1.	0.	0.
200 MG/KG 37343	7.	0.	7.	4.	30.4
200 MG/KG 37344	0.	0.	0.	0.	0.
200 MG/KG 37345	0.	0.	0.	0.	0.
200 MG/KG 37346	5.	1.	6.	10.	37.9
200 MG/KG 37347	0.	0.	0.	0.	0.
200 MG/KG 37348	1.	1.	1.	0.	0.
200 MG/KG 37349	0.	0.	0.	0.	0.
200 MG/KG 37350	0.	0.	0.	0.	0.
200 MG/KG 37351	0.	0.	0.	0.	0.
200 MG/KG 37352	0.	0.	0.	0.	0.
200 MG/KG 37353	0.	0.	0.	0.	0.
200 MG/KG 37354	0.	0.	0.	0.	0.
200 MG/KG 37355	12.	1.	12.	12.	32.7
200 MG/KG 37356	0.	0.	0.	0.	0.
200 MG/KG 37357	5.	0.	5.	4.	31.3
200 MG/KG 37358	4.	1.	10.	10.	32.0
200 MG/KG 37359	7.	1.	3.	11.	30.1
200 MG/KG 37360	10.	0.	10.	10.	31.5
200 MG/KG 37361	7.	0.	7.	9.	30.2
GRAND AVERAGES	5.7	0.8	6.1	4.5	31.4
STAT DATA ERRORS	1.1	0.1	1.0	0.5	2.06
400 MG/KG 37362	0.	0.	0.	12.	20.4
400 MG/KG 37363	0.	1.	1.	4.	0.
400 MG/KG 37364	0.	0.	0.	0.	0.
400 MG/KG 37365	0.	0.	0.	0.	0.
400 MG/KG 37366	0.	0.	0.	0.	0.
400 MG/KG 37367	0.	0.	0.	0.	0.
400 MG/KG 37368	0.	0.	0.	4.	20.2
400 MG/KG 37369	0.	0.	0.	0.	0.
400 MG/KG 37370	0.	0.	0.	0.	0.
400 MG/KG 37371	0.	0.	0.	7.	0.

T018401-PPFCUTANENUS TERATOLOGY-RABBIT

3/29/79

		NO. LIVE FETUSES	POST IMPLANT LOSS	EMPHANES A	COMPOSA LITER	AVERAGE POP. WT.
600 MG/KG	37372	0	0	0	10	32.0
600 MG/KG	37373	-	-	-	-	-
600 MG/KG	37374	-	-	-	-	-
600 MG/KG	37375	-	-	-	-	-
600 MG/KG	37376	-	-	-	-	-
600 MG/KG	37377	0	0	0	13	25.7
600 MG/KG	37378	-	-	-	-	-
600 MG/KG	37379	-	-	-	-	-
600 MG/KG	37380	-	-	-	-	-
600 MG/KG	37381	7	1	4	11	34.2
GRUP AVERAGE		4.4	0.6	5.1	10.3	30.67
STANDARD ERRORS		1.3	0.6	0.9	1.5	2.24
ENVIRONMENT	37152	-	-	-	-	-
ENVIRONMENT	37153	-	-	-	-	-
ENVIRONMENT	37154	-	-	-	-	-
ENVIRONMENT	37155	7	0	7	9	30.7
ENVIRONMENT	37156	0	1	0	9	31.8
ENVIRONMENT	37157	5	1	5	10	34.0
ENVIRONMENT	37158	-	-	-	-	-
ENVIRONMENT	37159	1	0	1	0	34.0
ENVIRONMENT	37160	1	1	1	2	-
ENVIRONMENT	37171	-	-	-	-	-
ENVIRONMENT	37172	-	-	-	-	-
ENVIRONMENT	37173	10	0	10	10	30.2
ENVIRONMENT	37174	0	0	7	7	29.9
ENVIRONMENT	37175	0	0	0	13	29.7
ENVIRONMENT	37176	1	0	0	12	29.8
ENVIRONMENT	37177	0	1	0	12	30.8
ENVIRONMENT	37178	-	-	-	-	-
ENVIRONMENT	37179	7	1	7	0	34.0
ENVIRONMENT	37180	7	1	7	9	30.1
ENVIRONMENT	37181	7	2	9	21	30.7
GRUP AVERAGE		5.6	1.4	7.1	9.8	31.30
STANDARD ERRORS		0.9	0.7	0.6	1.1	1.30



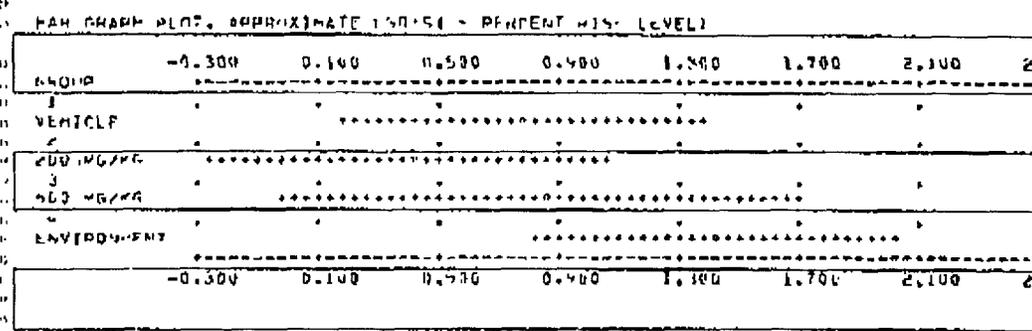
TC194.01-RECURRENT TREATMENT-RABBIT - POST IMPLANT LOSS 3/29/79

SUMMARY							
AVERAGE =	0.4936	STD. DEV. =	1.5467	C.V. =	176.626		
MEAN MEAN LSD =	1.3-33						
SOURCE							
GROUP	NO	AVERAGE	STD. ERROR	GRP. STD. DEV	SIGN. DIFF.	T	PROB
VEHICLE	14	0.2557	0.4286	1.056			
2ND W/VEH	7	0.4167	0.4508	0.5147	1.2674	-1.14	0.268
4TH W/VEH	7	0.1571	0.6033	1.4034	1.4913	0.12	0.911
ENVIRONMENT	14	1.5284	0.4286	2.4718	1.2177	0.90	0.383

SOURCE	DF	SS	F	PROB
TOTAL	47	159.494013		
MEAN	1	17.531406		
GROUPS	3	0.404448	2.101849	0.144
RESIDUAL	43	141.558159	2.747887	

CONTRAST NO.	CONTRAST	F	PROB	T	PROB	F(1,43)	PROB
1	1.412344	0.100521	0.043	0.836		0.429	0.516
2	-0.701172	0.331	0.567	-1.47	0.157		
3	1.00	1.00	0.32	1.00			
4	-1.571429	1.923006	0.596	0.444	-0.60	0.516	

\* DENOTES SIGNIFICANCE AT 5% LEVEL. T-TESTS USE WELCH'S APPROXIMATION.  
 STATISTIC FOR COMPARISONS WITH CONTROL = STUDENT'S T  
 PARTITION CRITERION = 24.33 (PROB = 0.000) (TESTS ANOVA ASSUMPTIONS)



DISTRIBUTION-FREE MULTIPLE COMPARISONS

GROUP	N	MEAN	4A < SUM	WAVE	AVE	DIFF-COINT	SIGN	DIFF
VEHICLE	14	0.0	142.0	24.76	0.0	9.44		
200 MG/KG	12	0.0	259.0	21.63	-2.14	9.51		
600 MG/KG	7	0.0	145.0	23.77	-0.44	11.19		
ENVIRONMENT	14	0.5	361.0	25.79	1.32	9.14		

KRUSKAL-WALLIS STATISTIC = 0.77 (DF = 3) PROB = 0.857

TEST FOR TIE BASED ON RANKS = 4.26 (DF = 2) PROB = 0.121

ADJUSTED STATISTIC = 0.22 (DF = 1) PROB = 0.637

INDICATES SIGNIFICANCE AT .05 LEVEL IF GROUPS ARE OF EQUAL SIZE THEN DIFFERENCES INDICATED BY \* MAY ALSO BE CONSIDERED SIGNIFICANT. COMPARISONS ARE BASED ON AN ANALYSIS OF THE LOGS.

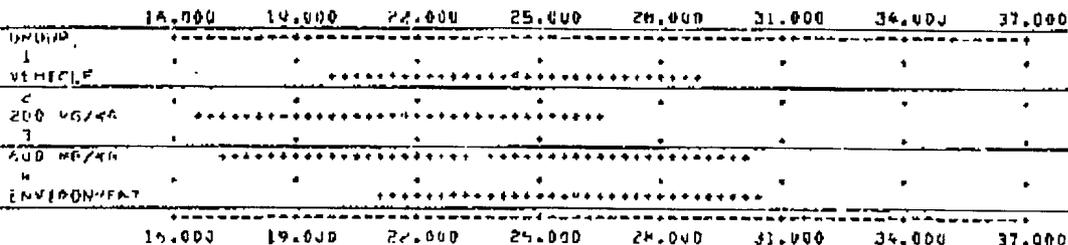
CONTRAST NO.	COEFF	STD. ERROR	T	PROB.	U	Z(U)	PROB.
1	-100.3	107.1	-0.94	0.347			
2	-4.2	4.9	-0.86	0.391	69.5	-0.82	0.404
3	-2.2	5.7	-0.39	0.694	44.0	-0.39	0.743

DISTRIBUTION-FREE PAIR-WISE COMPARISONS WITH CONTROL GROUP

GROUP	R OF SUM	U	Z(U)	PROB.	PROB.	U	PROB.	PROB.
200 MG/KG	151.0	73.0	-0.62	0.535	0.435	0.0	0.286	0.286
600 MG/KG	75.0	44.0	-0.66	0.507	0.440	0.143	0.143	0.143
ENVIRONMENT	361.0	183.5	0.26	0.793	0.500	0.143	0.143	0.143

RECOMMENDATION-EXTENSIVE TIES ON ONE OR MORE PAIRINGS - CONSIDER USE OF T(UT) TO DETERMINE SIGNIFICANCE

FOR GRAPH PLOT, APPROXIMATE RANKS AT PERCENT RISK LEVEL



COLLEGE

AVERAGE =	4.4735	10.0000	2.9400	0.0000	48.474
MINIMUM =	1.0000	0.0000	0.0000	0.0000	0.0000

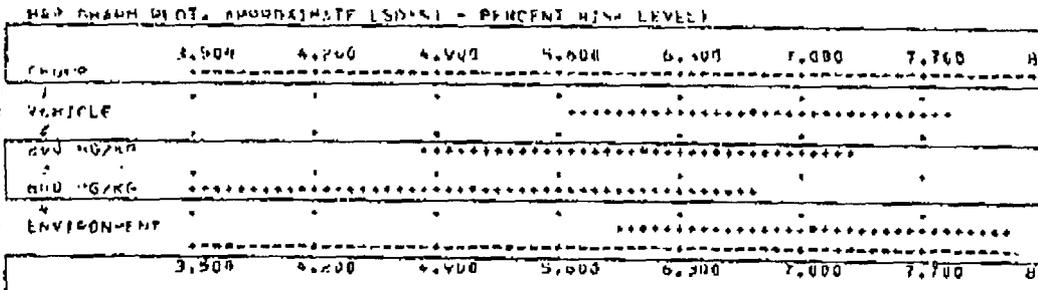
  

GROUP	DF	AVERAGE	STU. ERROR	STD. DEV	SIG. DIFF.	T	PROB
VEHICLE	14	5.7143	1.7982	3.2440			
200 MG/KG	12	4.4444	1.0822	2.0000	2.2714	-0.54	0.594
400 MG/KG	7	5.1429	1.1208	2.4000	2.7904	-1.14	0.262
ENVIRONMENT	14	7.0714	0.7402	2.0000	2.2783	0.32	0.753

SOURCE	DF	SS	MS	F	PROB
TOTAL	47	2307.4943			
MEAN	1	1900.5017			
GROUPS	3	19.9202	6.643274	0.745	0.531
RESIDUAL	43	387.0724	8.999358		

CONTRAST	DF	SS	MS	F	PROB	T	PROB	F(0.05)	P(0.05)
CONTRAST 10.	1	10.0000	10.0000	1.120	0.275			0.002	0.777
VEHICLE RESPONSE	1	10.0000	10.0000	1.120	0.275				
CONTRAST 10.	2	10.0000	5.0000	0.560	0.405	0.64	0.405		
200 MG/KG VS ENVIRONMENT	1	1.0000	1.0000	0.112	0.735				
CONTRAST 10.	3	10.0000	3.3333	0.373	0.820	1.34	0.170		
400 MG/KG VS ENVIRONMENT	1	1.0000	1.0000	0.112	0.735				

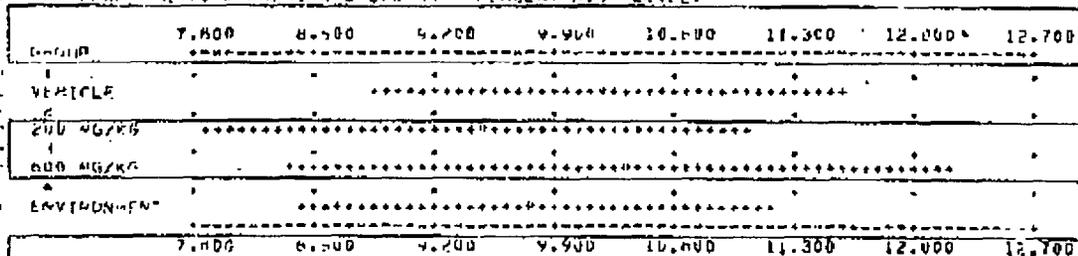
\* DEFINES SIGNIFICANCE AT 5% LEVEL  
 STATISTIC FOR COMPARISONS WITH CONTINUOUS VARIABLES  
 ASSUMES NORMAL DISTRIBUTION OF RESIDUALS AND ANOVA ASSUMPTIONS



MID E									
AVERAGE		9.9130	TD.DF.VE	3.0150	C.V.=	36.47%			
MORM.MEAN I.S.D.		3.1043							
MID E I									
GROUP	NO	AVERAGE	STD.ERROR	GRP	STD.DEV	STD.DIFF.	T	PROB	
VEHICLE	14	1.2107	0.2662	3.0000					
200 MG/KG	13	4.6565	1.0400	1.5075	2.9417	-0.67	0.511		
400 MG/KG	7	1.2457	1.3003	3.9041	3.3798	0.04	0.970		
ENVIRONMENT	14	4.7457	0.9406	4.2072	2.7596	-0.21	0.843		
SAMPLE	DF	SS	MS	F	PROB	T	PROB	F(LOF)	PROB
TOTAL	46	5173.9914							
MEAN	1	4521.34375							
GROUPS	3	6.782089	1.594812	0.122	0.947				
RESIDUAL	42	546.865443	13.02060						
CONTRAST									
CONTRAST NO.	DF	SS	MS	F	PROB	T	PROB	F(LOF)	PROB
VEH VEH RESPONSE	1	70.45611	70.45611	0.020	0.889			0.321	0.574
CONTRAST NO.	2	0.13169	0.075546	0.052	0.821	-0.27	0.790		
200 MG/KG VS ENVIRON	1	1.00	1.00	0.084	0.767	0.27	0.746		
CONTRAST NO.	3	0.500000	0.166666	0.084	0.767	0.27	0.746		
400 MG/KG VS ENVIRON	1	1.00	1.00						

\* DENOTES SIGNIFICANCE AT 5% LEVEL. T-TESTS USE WELCH'S APPROXIMATION.  
 STATISTIC FOR COMPARISON WITH CONTROL STUDENT'S T  
 WELCH'S T-TESTS AND ANOVA ASSUMPTIONS

SEE GRAPH PLOT, APPROXIMATE L.S.D. @ 5 PERCENT SIG. LEVEL







TD1N4-01-PERCUTANEOUS TERATOLOGY-94881T  
 STATISTICAL ANALYSIS SUMMARY

3/29/79

NO. LIVE FETUSES	VEHICLE				ENVIRONM ENT		OTHER TESTS		PROB.
	AVG	5.9	5.7	4.3	5.6	VEH DOSE RESPONSE	200 MG/KG VS ENVIRON		
	SD	3.3	3.4	3.4	3.1	200 MG/KG VS ENVIRON	0.400		
	N	14	12	7	14	600 MG/KG VS ENVIRON	0.397		
	PROB.		0.297	0.306	0.276				
POST IMPLANT LOSS	AVG	0.4	0.4	0.9	1.4	VEH DOSE RESPONSE	0.834		
	SD	1.1	0.5	1.5	2.5	200 MG/KG VS ENVIRON	0.434		
	N	14	12	7	14	600 MG/KG VS ENVIRON	0.143		
	PROB.		0.595	0.971	0.034				
IMPLANTS	AVG	6.7	6.1	5.1	7.1	VEH DOSE RESPONSE	0.275		
	SD	3.2	3.6	2.5	4.3	200 MG/KG VS ENVIRON	0.405		
	N	14	12	7	14	600 MG/KG VS ENVIRON	0.370		
	PROB.		0.594	0.262	0.153				
CHORDA	AVG	1.2	0.5	11.3	4.8	VEH DOSE RESPONSE	0.444		
LUTFA	SD	3.4	1.5	4.0	4.2	200 MG/KG VS ENVIRON	0.430		
	N	14	11	7	14	600 MG/KG VS ENVIRON	0.630		
	PROB.		0.727	1.000	0.567				
AVERAGE PUP WT.	AVG	36.3	37.4	30.4*	35.3	VEH DOSE RESPONSE	0.0219		
	SD	3.6	6.5	5.1	4.7	200 MG/KG VS ENVIRON	0.337		
	N	12	10	5	12	600 MG/KG VS ENVIRON	0.076		
	PROB.		0.626	0.832	0.609				

SYMBOLS APPEARING BY GROUP AVERAGES/MEDIANS INDICATE DIFFERENCES FROM VEHICLE  
 \* . . . \*\* denote significance by normal distn. methods with P<.05, .01, .001 respectively

*R. N. Bane*  
 3/29/79

## CALCIFICATIONS

	VEHICLE	28UMG/10	80UMG/10	ENVIRON
NUMBER OF FETUSES	4/ 14	3/ 13	4/ 14	4/ 3
PERCENT	28.57	23.08	28.57	13.33
CHI-SQ VS VEHICLE	0.0	2.401	0.724	0.304
PROB.	(1.000)	(0.122)	(0.391)	(0.584)
CHI-SQ VS ENVIRON	0.414	5.665	0.075	0.0
PROB.	(0.521)	(0.018)	(1.000)	(1.000)
NUMBER OF LITTERS	4/ 12	3/ 12	3/ 11	2/ 5
PERCENT	33.33	25.00	27.27	40.00
CHI-SQ VS VEHICLE	0.0	1.011	0.000	5.091
PROB.	(1.000)	(0.317)	(1.000)	(0.022)
CHI-SQ VS ENVIRON	0.007	0.144	0.034	0.0
PROB.	(1.000)	(0.702)	(1.000)	(1.000)

\*=SIGNIFICANTLY DIFFERENT FROM VEHICLE (P<.05)

\*=SIGNIFICANTLY DIFFERENT FROM ENVIRON (P<.05)

CONTINUITY CORRECTION OF 0.5 USED. TAU-TAILED PROBABILITIES.  
FISHER EXACT METHOD USED WHEN MINIMUM EXPECTED VALUE <1.

No Significant Differences (P<.05)

R.W. Bruce

3/29/79



International Research  
and Development Corporation

MATTAWAN, MICHIGAN U.S.A. 49071 TELEPHONE (616) 668-3346

October 9, 1978

Mr. Hal A. Derner  
The Procter and Gamble Company  
Research and Development Department  
Miami Valley Laboratories  
P. O. Box 39175  
Cincinnati, Ohio 45247

Dear Hal:

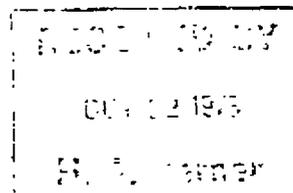
Originally the study involving four compounds (T-0310-02, T-0311-02, T-0312-02, TO 184.01) was priced at \$78,000 and given the IRDC study number 191-230. However, due to the issuing of two protocols, two IRDC study numbers have been issued and the charge proportionally adjusted. Dr. Muller's protocol, IRDC 191-230, with three compounds (T-0310-02, T-0311-02, T-0312-02) will total \$53,500. Dr. Benke's protocol, IRDC 191-259, with one compound and an additional vehicle control group will total \$24,500.

If I can be of additional assistance, please do not hesitate to contact me.

Sincerely,

D. Clifford Jessup, Ph.D.  
Associate Director of Research

DCJ/cp



TC184.01

From: N. A. DEWNER

9/22/78

TO: G. M. BENKE & D. Müller

Subject: Costing of IR + DC Teratology Study

IR + DC has provided the detail cost of your teratology study on the basis of numbers of groups, splitting the common control, as follows:

D. Müller <sup>Study</sup> (297-230)

1/2 no treatment

1 vehicle control

6 treatment

7 1/2

} \$ 53,500

G. Benke <sup>Study</sup> (297-259)

1/2 no treatment

1 vehicle control

2 treatment

3 1/2

} \$ 24,500

N. A. Dewner



THE PROCTER & GAMBLE COMPANY

File  
TERA  
T0184

WINTON HILL TECHNICAL CENTER

5110 CENTER HILL ROAD CINCINNATI, OHIO 45224

September 12, 1978

Dean Rodwell, M.S.  
IR&DC  
Mattawan, Michigan 49071

Dear Dean:

This will serve to document the message I left with your secretary today relative to retained samples of T0184.01. It will be okay to retain 5 ml samples (10 and 30%) in tightly sealed glass or plastic containers. These should be kept frozen until picked up by myself or D. Müller during our next visit. Sample containers should be as small as possible to minimize evaporation.

Sincerely yours,

THE PROCTER & GAMBLE COMPANY

*G. M. Benke*

G. M. Benke, Sc.D.

amb

cc: Toxicology Files (TGSE 1525)  
H. A. Derner ✓

RECEIVED BY  
SEP 14 1978  
H. A. Derner

THE PROCTER & GAMBLE COMPANY

WINDY HILL TECHNICAL CENTER

4140 CENTER HILL ROAD CINCINNATI, OHIO 45224

September 5, 1978

Dean Rodwell, M.S.  
International Research & Development Corp.  
Mattawan, Michigan 49071

Dear Dean:

Thank you for your efforts spent yesterday during my visit to assure a smooth, but comprehensive, evaluation of the methods and procedures used in the teratology study. As per our discussion, I would like to review, for the record, any needed actions or protocol changes.

1. Relative humidity - in our opinion the high rh values are unlikely to affect the outcome of the study. However, the possibility of interactions with any of a number of disease forms makes it important that a) attempts to reduce the rh to at least 60% be made, and documented, and b) daily or twice-daily rh determinations be made and recorded and made a part of the study report. I would appreciate being made aware of the progress of efforts to lower the rh values.
2. Sample retention - only an amount of T0184.01 sufficient to fill a 20 ml glass vial will be needed, instead of the originally requested 25g. Also, 20 ml aliquots of each dilution (not 25 ml) will be needed.
3. Please make it part of the study and report record that the coccidiosis seen in some rabbits upon arrival was cured prior to the start of the study.

Sincerely,

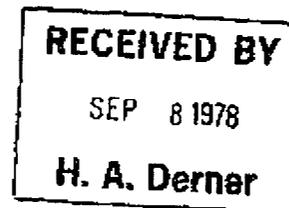
THE PROCTER & GAMBLE COMPANY

*G. M. Benke*

G. M. Benke, Sc.D.

amb

cc: Toxicology File 1524, 1525  
D. Miller  
A. McDermott  
H. A. Derner  
G. G. Cloyd





THE PROCTER & GAMBLE COMPANY

WINTON HILL TECHNICAL CENTER

610 CENTER HILL ROAD CINCINNATI, OHIO 45224

September 7, 1978

Dean Rodwell, M.S.  
IRDC  
MatLawan, Michigan 49071

Dear Dean:

As per your telephone request today I am sending to you an additional sample of test material T0184.01 for the teratology study. This was taken from the same lot of material as the previous 2 lots, and it is the last amount of material from this lot. I am also sending some T0184.02, which is a new lot from the supplier and is to be used only if you run out of T0184.01.

Sincerely,

THE PROCTER & GAMBLE COMPANY

G. M. Benke, Sc.D.  
Principal Investigator

amb

cc: H. A. Derner ✓  
Toxicology Files/TCSE 1525

Encl.



# THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175  
CINCINNATI, OHIO 45247

August 1, 1978

Dr. D. C. Jessup  
International Research and  
Development Department  
Mattawan, Michigan 49071

Dear Dr. Jessup:

This is to authorize you to carry out an Acute Percutaneous Teratology Study according to the attached Protocol and in conformance with the stipulations of the Laboratory Services Agreement dated April 1, 1978.

The test material is a solid identified as T0184.01.

The Principal Investigator for this work is Dr. G. M. Benke. Matters involving the scientific aspects of the work can be handled directly with the Principal Investigator and any unused samples are to be returned to the Principal Investigator at the following address:

Dr. G. M. Benke  
The Procter & Gamble Company  
Winton Hill Technical Center  
Room 2S08  
6110 Center Hill  
Cincinnati, Ohio 45224  
Telephone No. (513) 977-5275

As you are aware, this is the study that is to be run together with Dr. Muller's study on compounds T0310.02, T0311.02 and T0312.02.

Three copies of the final report are to be sent to the attention of:

Mr. H. A. Derner  
The Procter & Gamble Company  
Research and Development Department  
Miami Valley Laboratories  
P. O. Box 39175  
Cincinnati, Ohio 45247

TGSE  
1525  
Op. Sect.  
File

C.R.C.  
9/6/79

THE PROCTER & GAMBLE COMPANY

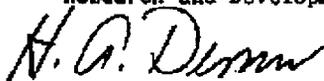
Dr. D. C. Jessup, IR&DC  
August 1, 1978  
Page 2

Please include the following information in a letter sent to my attention:

- 1) We would like you to state that you agree to do the work required by the protocol and, to make sure that the correct protocol will be used, indicate the number and issue of the protocol that we sent. Please indicate the protocol number and issue in the final report.
- 2) We would like you to give your best estimate of when the study will begin.
- 3) We would like to know the study number if one has been assigned.
- 4) We would like to know what the cost will be.
- 5) We would like to know your estimated report date. Usually we specify a date when a report is needed. It is very important that we know if you can meet this date.

Sincerely,

THE PROCTER & GAMBLE COMPANY  
Research and Development Department



H. A. Derner  
Professional & Regulatory Services Division

sw

Attachment

cc: G. M. Benke

Test Substance Identification Number T-0184-01  
 Safety Test Request Number 1525  
 Principal Investigator GM Benke 5/2/78  
 (Name)

SECY. NO. 6/11/78  
 SECY. ED. 6/11/78  
 SECY. DIR. 6/11/78  
 PLRS SECY. DIR. 6/11/78

Name of Product or Ingredient (or code designation) Octopirox  
 Brand Notebook Ref. (including Production Code if available) WT1433-92  
 Physical Form Solid Color White Density NK  
 Solubility Up to 30% in 50:50 isopropanol-water Sample Expiration Date 10/5/78  
 stirred 30 minutes.  
 Recommended Storage Conditions 50° - 100° P. Low Humidity  
 Hazards (i.e. flammability, toxic gases) None

Formulated Composition

Component (a)	Nominal Level (% by Wt.)	Acceptable (b) Range	Stock Code No.	Supplier	Lot Number (NB-Ref.)
Octopirox	100%	NK	PDX 263-2	Boechar Lot #E001	WT1433-92

(a) Ingredients will be listed by chemical name. Non-chemical names such as Tergitol 15-S-9 or Yellow Eye DTC #10 may be acceptable but should be provided with the responsible toxicologist. Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-definitive identifications (e.g. Arquad, EC-bazo) are not acceptable.

(b) If information requested is not known then the symbol NK will be entered.

The above information provided by:

J. A. Davis (Signature) 5/2/78  
 (Name) J. A. Davis (Date)



PERCUTANEOUS TERATOLOGY STUDY IN THE RABBIT

Objective

To determine whether T-0184.01 can induce teratogenic effects when applied topically to the skin of rabbits during pregnancy.

Sponsor: The Procter & Gamble Company  
Toilet Goods Division  
6110 Center Hill Road  
Winton Hill Technical Center  
Cincinnati, Ohio 45224

Principal Investigator:

G. M. Benke, Sc.D.  
The Procter & Gamble Company  
Toilet Goods Division  
6110 Center Hill Road  
Winton Hill Technical Center  
Cincinnati, Ohio 45224  
Phone: (513) 977-5275

Scientific Investigator:

Mr. Dean Rodwell  
International Research and Development Corporation  
Mattawan, Michigan 49071.

Experimental Design:

A. Test Materials

The test material will be submitted in numbered, labeled glass jars. The labels will identify the test material and the study. The materials will be stored at room temperature in the dark and will be applied in appropriate concentrations (see C) diluted with isopropanol:water (50:50). Solution will be made fresh for each day of application. The isopropanol:water can be prepared at the start of the study. The test material dilutions are to be prepared fresh daily as follows: Add 60 g of T0184.01 to a tared 200 ml volumetric flask containing a stir bar. To this add a sufficient amount of isopropanol:water (1:1) to bring the level just below the beginning of the flask neck. (IRSDC will supply the reagent-grade isopropanol, and prepare a stock solution, 1:1, in distilled water.) This should be stirred until a clear solution is produced. The stir bar should then be removed and the volume brought to 200 ml with additional vehicle (mix well). This will be the 30% (w/v) solution. The 10% solution will be prepared from this as follows: Add 50 ml of the 30% solution to a flask containing 100 ml isopropanol:water. There will thus be 150 ml of each test solution, more than sufficient to treat 20 3 kg rabbits with 2 ml/kg. The daily preparation and dilution of test material should be performed in the presence of an observer, both of whom should

initial that the procedure was done properly each day. Samples of 25 g of the test material will be taken at the beginning and at the end of the treatment period and sent in plastic or glass vials with appropriate labels to the sponsor for analysis. From each dilution a 25-ml sample will be taken, appropriately labeled (test material, type of study, date of preparation, intended concentration and test group number) and returned to the principal investigator after the study necropsy. Samples will be chosen at random by the sponsor for analysis. Any remaining test material will also be returned to the principal investigator.

Detailed material identification is available at the sponsor under TCSE #1525. This information will not be given to the Scientific Investigator to insure the blind character of this study.

#### B. Animals

- 1) Species: Sexually mature New Zealand Albino Rabbits (supplier to be stated in final protocol).
- 2) Age: 26 months at time of arrival in the test laboratory (minimum).
- 3) Number: 90 females, 10 males (Note that 20 control females to be shared with Dr. Müller's study).
- 4) The purchase order number and date of receipt of the experimental animals from the breeder will be entered into the permanent records.
- 5) All newly arrived animals will be weighed and examined grossly. Animals displaying any unusual or abnormal signs suggestive of impaired health, which, in the opinion of the resident DVM may interfere with the study, will be excluded. The weights and results of the examinations will be permanently recorded. Each animal will be individually identified by ear tags after arrival.
- 6) All remaining animals will be acclimatized to laboratory conditions for 18 days, allowing for the return to sexual reactivity after possible pseudopregnancies in the females. Medication for ear mites and snuffles may be used only during the first 10 days of the acclimatization period.
- 7) After this period the animals will be weighed and examined again. Those females found to be healthy and showing the highest body weight gain during the acclimation period will be employed for the study. The females will be randomly distributed to control and test groups according to weight. The standard randomization procedure used by IRDC may be used. This should become part of the study record. After assignment to test control groups, rabbits will be assigned to numbered cages matching the numbers on the ear tags. The groups will be identified by color codes.

- 8) Eight males (+ 2 extras) will be selected (see Section E) as sperm donors. Each individual male will be randomly assigned to ten females before the females are assigned to the test or control groups. This randomization process will be permanently documented. The males will receive no treatment. If one of the males dies during the course of the study it will be replaced by one of the extra males.

#### C. Test Groups

A total of eighty virgin female New Zealand Albino Rabbits are to be distributed to the test and control groups as follows:

<u>Group</u>	<u>Animals</u>	<u>Treatment</u>
I. No treatment control	20 females	None
II. Vehicle control (isopropanol: water; 50:50)	20 females	Days 7-18 gestation
III. T-0184.01, 10% in isopropanol:water	20 females	
IV. T-0184.01, 30% in isopropanol:water	20 females	

The dose volume for Groups II, III and IV is 2 ml/kg. The no-treatment control (Group I) will be shared with another study conducted by IRDC for P&G.

#### D. Animal Maintenance

- 1) Recommendations contained in HEW Publication # (NIH)F4-23 entitled "Guide for the Care and Use of Laboratory Animals" will be followed.
- 2) The animals are to be housed in individual cages; males and females are to be kept in separate rooms.
- 3) The environmental conditions of the animal rooms will meet the following specifications:
  - a) The light/dark cycle will be 12/12 hours.
  - b) The temperature of the rooms will be between 22 and 25°C.
  - c) The relative humidity will be between 40 and 60%.
  - d) The animal rooms should undergo 10-15 fresh air changes per hour. All air entering the room will be adequately filtered to remove particulates. The type of air filter used will be documented.
  - e) Daily records will be kept on items b) and c) to substantiate protocol adherence. If automatic systems are used, they will be checked daily for proper function. This will be considered sufficient to document adherence to items a) and d).
  - f) The animals will receive Purina Rabbit Chow and tap water ad libitum. For the feed of all animals during the duration of the study one batch of diet will be used.

### E. Insemination

Collect semen from the males by means of an artificial vagina and conduct a sperm test. Select those males showing the highest sperm count and being able to serve the artificial vagina as sperm donors. Keep records on the males in respect to their health, sperm counts and assignment to females. Inseminate two does per day and group with 0.25 ml undiluted semen, after having induced ovulation by injecting 1 mg/kg of pituitary luteinizing hormone (Armour Pharmaceuticals, Kankakee, Illinois) into the marginal ear vein. Continue the program of insemination until all does are pregnant. If insemination has to be repeated use sperm from the same male again. The day of insemination is counted as day 0 of gestation. For more details of this procedure see Appendix I.

### F. Treatment

Before applying the test materials, and at 3-day intervals thereafter, clip a 10 x 15 cm area on the back of the animal with an Oster small animal clipper without damaging the skin. Restrain the animals in the Newmann harness (Newmann, E. A., Lab Animal Care, 13:207-240, 1963) to prevent oral ingestion of test materials. Apply 2 ml/kg of the appropriate concentration of the test materials (see C) from a blunt tipped syringe to the backs of the does on days 7-18 of gestation. Spread the materials evenly by use of a glass rod. After 4 hours remove the animals from the harnesses, rinse them with a spray of water (40°C) and blot them dry with soft towels. The cages will be cleaned with a damp cloth before the animals are returned to remove any test material that might have adhered to them. Weigh the animals every 3 days so that the dose can be maintained at a constant value per kg of body weight. Observe the does daily for mortality and general health. Keep records on all observations. If any mortalities occur, collect the fetuses as described in (G) below as soon as possible and have a gross necropsy to determine the cause of death. All tissues with gross lesions will be preserved in 15 volumes/volume tissue of 10% neutral buffered formalin for possible histopathological evaluation.

### G. Sacrifice

On day 28 of gestation sacrifice the does by injection of 100 mg/kg Nembutal (1 g/kg of 10% solution). Collect the fetuses by Caesarean section. Record the following information for each female.

- a) number of live and dead fetuses
- b) resorptions
- c) implantations
- d) corpora lutea of pregnancy
- e) numbers and descriptions of grossly malformed fetuses (if any)
- f) position of individual fetuses within the uterus

- g) any gross pathological findings in the mothers (in addition to the treated skin area). Tissues showing gross lesions will be taken and preserved in 15 volumes/volume tissue of neutral buffered formalin for possible histopathological evaluation. Whether or not to conduct histopathological evaluations on these tissues and those mentioned in F) will be decided by the Principal Investigator at completion of the study.

#### H. Preparation of Fetuses

Select randomly 1/3 of the fetuses which were alive at the time of sacrifice, clear them with alcoholic KOH, and stain them with Alizarin Red S for study of their skeletons. Follow the procedure described in Appendix II. Fix the remaining 2/3 of the fetuses in Bouin's solution and section them with a razor blade for study of the soft tissues, following the procedure given in Appendix III. Results of these examinations will be recorded for each individual fetus noting whether the fetus is normal or not and listing any abnormalities present.

#### I. Report

The report should contain the following information for each group:

- a) Dosage (amount + concentration) of the test material
- b) Duration of contact
- c) Number of pregnancies/group
- d) Number of resorptions/litter
- e) Number of corpora lutea of pregnancy/litter
- f) Number of implantations/litter
- g) Number of live fetuses/litter
- h) Number of dead fetuses/litter
- i) Number of fetuses with abnormalities/litter
- j) Number and weight of males and females/litter
- k) Number of fetuses examined for soft tissue abnormalities/litter
- l) Number of fetuses with soft tissue abnormalities/litter
- m) Number of fetuses examined for skeleton defects/litter
- n) Number of fetuses with skeleton defects/litter

- o) Types and detailed description of skeleton or soft tissue defects
- p) For each fetus showing abnormalities, the identification of the male used to inseminate.
- q) Types and detailed description of gross pathological findings in the mothers. The final report will be submitted to the sponsor within 4 weeks after completion of the experimental part of the study.

J. Statistical Evaluation

Any statistical evaluation that may become necessary will be done by the sponsor. It is not planned to use the no-treatment control group (I) for statistical analysis, unless a special reason to do so comes up during the course of the study.

K. On-site Monitoring

A representative of the sponsor will monitor the study at least once. The representative need not inform the contracting laboratory in advance of the intended visit. The names of all persons representing the sponsor and the date of all visits will be documented by the contracting laboratory.

L. Shipping and Handling of Samples

All samples, fetuses and tissues collected during this study will be carefully labeled and stored until the study is completed unless otherwise stated in this protocol. On completion of the study all biological samples and unused test materials will be returned to the sponsor in appropriate containers as specified by the sponsor at that time. The mode and time of shipping must be mutually agreed upon prior to shipping.

- M. All original records will be available to the sponsor at any time during or after this study. They will not be disposed of without written consent of the sponsor.

Notice

The stipulations of this protocol are to be implemented in conformance with current generally accepted standards for this type of research and it is the responsibility of the laboratory management to establish the appropriate controls and review processes for so doing.

If, after the study is underway, it becomes necessary to make changes on the approved protocol, the proposed revisions and reasons for change are to be discussed with the Principal Investigator and are to be confirmed in writing. These documents become part of the permanent file for this study.

Similarly, the Principal Investigator is to be notified as soon as practical whenever an event occurs that is unexpected and may have an effect on the validity of the study, e.g., the unexpected death of an animal; escape of animals from cages; loss of tissues of fetuses. The Principal Investigator will authorize whatever action seems appropriate and will arrange for documentation of the nature and cause of the event and of the actions taken as a result. Among the actions to be considered is a scientific review of the entire study.

H. G. Dummer 7/31/78  
Operations Liaison

G. M. Benke 7-25-78  
G. M. Benke, Principal Investigator

/erw  
4/27/78

## APPENDIX I

### Teratology Methods: The Artificial Insemination of Rabbits

The rabbit is widely used in teratology studies for several reasons: 1, it is known to be susceptible to certain teratogenic agents that are refractory in the rat, i.e. thalidomide; 2, it is a non-rodent (Lagomorph); 3, it is more heterogeneous than the rat and therefore more likely to reveal specific sensitivities to environmental agents and/or unusual anomalies; and 4, produces large numbers of offspring. The rabbit is more expensive to acquire and maintain because of their size and longer maturation time. Rabbits become sexually mature at about 5 months of age. Consequently, studies with them are generally limited to teratology (Segment 11) only.

Unlike the rat, the rabbit is not a spontaneous ovulator and has no apparent estrous cycle. These aspects of its reproductive physiology means that different ways must be devised to document and "time" conceptions. Although natural or "hand mating" systems are employed with success, we have chosen artificial insemination as a way to achieve a good conception rate (about 90%) with a relatively minimum effort (Gibson, J.P., R. E. Staples and J. W. Newberne, Use of the Rabbit in Teratogenicity Studies. *Tox. & Appl. Pharm.* 9, 393-408, 1966). A further advantage of artificial insemination, is that the number of sperm donors can be kept low, thus minimizing the genetic effects of the sires on the outcome of the study.

The rabbit normally is induced to ovulate by the mounting activities of the male, which stimulates the release of pituitary luteinizing hormone. However, many other activities such as handling, shipping or inadvertant exposure to the sight and smell of males may trigger pseudopregnancy. In addition, females are very territorial animals and will resist, even kill, males brought to their housing. Thus, the two sexes must be housed in separate quarters or rooms and the females must be oriented for at least 18 days in the case that some may have become pseudopregnant during shipping and receiving. In any kind of mating procedure, the females must be taken to the males' territory.

Male rabbits may be purchased at the time a study is initiated or may be maintained for longer periods. In practice, several of the better sires, may be kept and used for many months. Despite their reputations, some males may have a low libido or have low sperm counts (oligospermia). In general, 10-15 males are obtained for a study involving 100-120 females.

To obtain the semen, the males (bucks) are trained to accept an artificial vagina designed by Bredderman, et al. (Bredderman, P.J., R. H. Foote and Yassen, A.M. An improved artificial vagina for collecting rabbit semen. *J. Reprod. Fert.* 7, 401-403, 1964). The artificial vagina is washed and the space between the inner and outer linings is refilled with ethylene glycol and then the intact device is warmed to 40°C before using. The tip of a calibrated centrifuge tube is fitted into the smaller end of the AV to collect the semen. The buck and a "teaser" doe are placed together on a laboratory table in the room housing the bucks. They are allowed a few minutes to get acquainted; when the buck attempts to mount, the artificial vagina is held between the does rear legs and if necessary, the buck is helped to enter it. Usually a few days of training are all that is necessary.

During this training period, the volume of the ejaculate, after removing the sperm plug, is recorded and the sperm count made by drawing .05 ml of semen into a red cell pipette, diluting with warm normal saline and counting the number of sperm in a hemacytometer. From this count, the total number of motile sperm is determined. Any undesirable bucks can be eliminated in this period, but there should be 4-8 donors for the teratology study. Well performing bucks can ejaculate 2-3 times per day for 5-7 days with only a very small reduction in semen volume and sperm count.

For the teratology study, two or three does from each group will be inseminated daily until all are inseminated. This allows the work involved in the insemination and in the final sacrifices to be distributed among several days. Also, it allows the use of fewer bucks, since they can be alternately used and rested.

The insemination tube is a pipette made from a 16 cm length of 5 mm glass tubing. At a distance 4 cm from one end there is a 150° angle. The tip on this end is fire-polished. The other end is fitted with a female glass locking joint, into which is inserted a 3 cc syringe. This allows precise control over the volume of semen delivered and prevents contamination.

Many investigators use diluted or extended semen. However, studies in our laboratory indicate that extended semen may be killed or lose its motility. Therefore, we use undiluted semen. In addition, it is known that the number of sperm per volume of semen can be critical and that at least 12 million sperm/ml of semen are needed for successful inseminations. The higher concentration of sperm in undiluted semen insures a higher conception rate.

For the insemination of the does on the study, the semen is kept in a 37°C water bath after collecting. The inseminator sits on a chair and holds the doe, head down, between his legs, with the animal's hind feet pointed away from him. The animal's forequarters are restrained between the inseminator's calves and the animal's hindquarters between the thighs. The tail is pulled toward the inseminator and the vulva is cleaned with cotton or gauze. A clean pipette is filled with 1/4 ml of undiluted semen and then inserted in the vagina, with the tip directed at the dorsal wall to avoid the urethral orifice. After the tip has been inserted as far as the angle, it is rotated 180° so that the tip points to the ventral surface and the insertion is continued until a slight resistance is felt. The semen is then expelled and the tube withdrawn. If the animal urinates, she should be re-inseminated 15-30 minutes later.

To insure ovulation, the doe is given 0.1 ml of pituitary luteinizing hormone (PLH) per kg of body weight in the marginal ear vein just prior to insemination. Ovulation will occur about 10 hours later. Because the actual conception will occur later, some investigators call the day of insemination day 1 of pregnancy. However, this lead to some confusion, since the day of conception in other animals was labelled day 0. Therefore we as well as others, now designate the day insemination occurs, day "0". With this designation, the does are sacrificed on day 28, instead of day 29. Organogenesis, and therefore the treatment period, are days 7-18.

sab

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6/15/77

## APPENDIX II

### Teratology Methods: Preparation of Fetuses for Skeletal Staining and Examination

In order to examine the fetal skeleton for any possible defects, the fetus is cleared with KOH and stained with Alizarin Red Stain, a calcium-positive-stain. The method employed is a slightly modified version of the one by Staples and Schnell (Stain Technol. 39, 62, 1964). The method works equally well for either rat or rabbit fetuses, although there are some preliminary preparations needed for the rabbit, that are not required for the rat. These differences are noted below.

#### Rat Fetuses

Immediately after the fetuses have been weighed and examined (see previous methods on laparotomies), those selected for clearing and staining will be sacrificed by excessive ether. These fetuses will be carefully eviscerated and the carcasses placed in acetone (50 ml per fetus). They will then proceed through the clearing and staining steps.

#### Rabbit Fetuses

The procedure for preparing rabbit fetuses is somewhat similar to the rats, but includes several additional steps in the preliminary stages. After being sacrificed with 0.1 ml of a 10% solution of Nembutal per fetus, they too are carefully eviscerated, but in this process, the sex of the fetus should be determined internally. Then, the rabbits are skinned and all subcutaneous fat pads are removed, being careful not to separate any bones, such as the toes. The eyes and the ears are removed. The fetuses will then be air-dried for 24 hours. These rabbit fetuses will not go into the acetone, but will go directly into a solution of KOH and Alizarin.

#### Rat Fetuses

1. The fetuses are received in acetone, minimum time - 24 hours, or may be stored for a short period of time, up to about a week.
2. 70% 3A (95% Ethanol, 5% Methanol) - 24 hours
3. 1X KOH + Alizarin Red S - 48 hours
4. Rinse
5. Solution A - 24 hours
6. Solution B - 24 hours
7. Glycerine #2 - 24 hours
8. Pure Glycerine - 24 hours
9. Mount in pure glycerine

#### 1X KOH + Alizarin Red S

3 ml of .6% Alizarin Red S per liter of 1X KOH. The Alizarin Red S should not be added to the 1X KOH until just prior to use. It is advisable to agitate fetuses during this stage. Time element critical.

To rinse discard KOH + Alizarin Red S, run tap water over fetuses for approximately 5 minutes. Then lay stomach down on paper towel to dry off excess water approximately 3-5 minutes.

Solution A

2 parts 70% 3A (95% Ethanol, 5% Methanol)  
2 parts Glycerine  
1 part Benzyl Alcohol

Solution B

1 part 70% 3A  
1 part Glycerine

Solutions A & B may be mixed in advance and kept for long periods of time. Each solution can be used twice.

Glycerine #2

Pure glycerine in Step 8 may be re-used as glycerine #2.

Ex. 1. Puppies

Steps 1 & 2 are omitted, fetuses are air-dried for just 24 hours. Procedure is then same as rate beginning with Step #3 going directly into the KOH and Alizarin Red S.

After the fetuses have been stained and mounted in glass vials, which have been labelled with the study number, treatment, sample number and animal number, they will be examined using a strong light behind them. Each fetal skeleton will be examined carefully, while slowly rotating the vial through one or more 360° turns. The numbers of sternbrae, ribs and vertebrae will be counted. The number of sternbrae and ribs will be recorded as well as the number of any defective or missing part, i.e. 5th sternbrae absent. All abnormalities and observations will be recorded in the laboratory notebooks.

Since there is always considerable variation in the size of the skeleton and the degree of calcification, the comparisons will always be with the control animals in the same study. Thus, the incidences of "dumbbell" vertebrae or unossified sternbrae may vary with different batches of parent animals.

The skeletal data, expressed as the numbers of and percentage of abnormal pups per group will be analyzed by the Chi-square method. All abnormalities seen will be listed and their number and percentage by group will be reported. No statistical treatment of the latter will be done unless deemed appropriate by the investigator.

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6/15/77

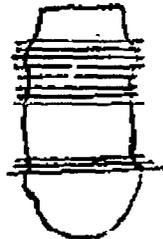
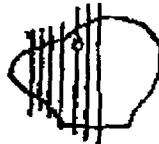
### APPENDIX III

#### Teratology Methods: Preparation and Examination of Fetuses for Soft-Tissue Defects.

Immediately following the laparatomies, the fetuses selected from each litter for soft-tissue examination by the Wilson method (Wilson, J. G., *Teratology: Principles and Techniques*, p. 251, 1965) are immersed in Bouins fixative. The method is equally applicable for rat and rabbit fetuses, with only minor changes necessary for the latter. The fetuses are killed as they enter the Bouins, and this is a necessary step, that draws the fixative into the lungs and stomach for proper fixation. Death is instantaneous. Before immersing the rabbits, a small incision is made in the abdomen to allow the Bouins to enter the abdominal cavity. Otherwise, the pancreatic lysozymes would be released by death and start decomposing adjacent tissues before the fixative could reach them.

Each litter of fetuses is put into a 1/4 to 1/2 gallon plastic container, with about 100 ml of Bouins per fetus and allowed to "fix" for at least two weeks in the case of the rat and three weeks for the rabbit although may remain in the fixative for longer periods without damage. The fixative may be changed, if deemed necessary, but is not usually required. The bottles containing the fetuses will be labelled with the study number, sample number, treatment and color code and the dam number.

After the proper fixation, the fetuses either rat or rabbit, will be removed from the Bouins and rinsed with several washings of tap water. Then, the legs, tail and ears will be removed and the head and torso sectioned with a razor-blade in the following manner:



Each slice will be approximately 1 mm thick and the average number taken from each fetus will be about 15. These slices are transferred to "spot" plates and the slices covered by 70% alcohol to prevent drying. Each side of each slice will be examined under low power (7.5-15X) and all abnormalities and variations will be recorded in the fetal examination notebook, and later transcribed into the proper study notebook.

The sections of the head must be taken back to the ventricles of the brain and should include sections across the nares and palate, the eyes and the fore brain. This will reveal internal defects such as retinocoloboma and internal hydrocephalus. The neck region is not sectioned, as there is little to see with

this type of examination and defects very seldom occur in this area (Wilson, J. G., personal communication).

The sections taken at the top of the torso should begin at the apex of the lungs and will show the thymus, esophagus, trachea and the carotid arteries. As the sections are taken downward, the branching sub-clavian artery, the bronchial trees and the top of the aortic arch will be seen. Then the ductus arteriosus, the pulmonary veins and the atria appear. Next, the ventricles with their valves and septa can be seen. These sections reveal abnormalities such as patent ductus arteriosus, co-actation of the aorta and other valve and septal defects.

The stomach section is not sectioned, but the diaphragm is examined for hernias and the intestines, liver and spleen teased out with forceps and examined. The next two slices are taken across the kidneys, which are examined for normal development of the renal papilla and enlargement of the renal pelvis and ectopia.

Finally, the lower part of the trunk, containing the bladder, ureters and gonads is examined intact for abnormalities such as pseudohermaphroditism, undescended testes, ectopias and ureter anomalies. At this time, the sex, determined earlier, can be confirmed or corrected. After the sections have been examined, they will be discarded, unless some unusual or questionable fetus is seen.

The data will be expressed as the number of and percentage of abnormal pups by groups and will be examined statistically by the Chi-square method. In addition, each abnormality will be listed and expressed as the numbers of and percentage of the pups examined, again by groups. These individual anomalies will not be statistically treated as a routine procedure, but may be in special cases, when the investigator deems it is appropriate.

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6/15/77