G.G. 3/7/85

004695

DATA EVALUATION RECORD

STUDY TYPE: Teratology study in rabbits.

CITATION: Korte R, Osterburg I. Embryotoxicity of KWG 0519 in rabbits. (REPROTOX order number 494, Study No. KWG 0519/019). An unpublished study submitted to Bayer AG, April 1980.

ACCESSION NUMBER: 071468.

DP 9/5/85

LABORATORY: REPROTOX GmbH, Vorbergweg 41, 4400 Munster, Bundesrepublik Deutschland. Huntingdon Research Center, Dutschland.

TEST MATERIAL: KWG 0519 (Baytan). The batch number was 1616002/79 Eg. 5/79. The purity and source of the test material were not stated.

PROTOCOL:

1. Seventy two female New Zealand White rabbits 12 to 14 weeks of age and 2.4 to 4.4 kg body weight were acclimated for at least seven days prior to study initiation. The animals were individually housed and the room was maintained at 22-24° C and a relative humidity of 50-60 percent. A 12-hour light/dark cycle was provided. A basal diet of Ssniff K rabbit diet and tapwater were provided ad libitum.

2. KWG 0519 was added to a Cremophor EL distilled water venicle (5 drops Cremophor EL per 10 ml distilled water) to achieve the dose levels of 10, 30, and 100 mg/kg. Cremophor EL/distilled water was administered to the vehicle control animals.

3. The day sperm was observed in a vaginal smear was defined as day 0 of gestation and 50 IU's of human chorionic gonadotropin was administered via intravenous injection. The mated females were then randomly assigned to a treatment group.

   The rabbits were orally dosed (10 ml/kg body weight) with the vehicle, 10, 30, or 100 mg/kg KWG 0519 daily from day 6 to day 18 of gestation. The female body weights were recorded on days 0, 6, 18, and 28 of gestation.

   The rabbits were sacrificed on day 28 of gestation by intravenous injection of T-61. After caesarean section the gravid uterus was removed and opened to determine the number and distribution of total

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implantation sites, early resorptions, late resorptions, dead fetuses, and live fetuses. The corpora lutea were counted and the dam was examined for macroscopic pathologic lesions. The individual fetuses were weighed and subjected to an external examination including a determination of the sex. Each fetus was dissected and examined for visceral abnormalities. The heads were removed and examined for soft tissue abnormalities by Wilson's technique. The remaining portion of each fetus was prepared and stained with Alizarin Red S and examined for skeletal abnormalities.

4. The Student's t-test and Chi-square analysis were used to determine statistical significance. The report does not specify which method was utilized to analyse a particular measurement. All references to statistical significance are based on the statistical analyses performed by REPROTOX.

RESULTS:

Clinical Observations: No clinical observations of the maternal animals were conducted during the study.

Body Weights: The maternal body weights during gestation and the weight gain over days 6-18 of gestation are shown in Table 1. The mean body weights of all groups were similar at each weight interval. The mean weight gain of the 100 mg/kg dose level during days 6 to 18 of gestation was less than the weight gain observed during this period among the control females. REPROTOX has reported this difference between the control and 100 mg/kg females as significant ($p \leq 0.05$) by Student's t-test. It should be noted that the Student's t-test is designed to compare two-treatments and utilizing it to compare four treatments results in a higher alpha value ($p = 0.26$) than assumed by the authors ($p \leq 0.05$). Therefore, the null hypothesis of no treatment difference will be incorrectly rejected in 26 of 100 samples.

TABLE 1. Mean Body Weights (kg) During Gestation

	Dose Level (mg/kg)			
	0	10	30	100
Mean Body Weight-Day 0	3.2	3.1	3.1	3.1
Mean Body Weight-Day 6	3.2	3.2	3.2	3.2
Mean Body Weight-Day 18	3.5	3.5	3.4	3.3
Mean Body Weight-Day 28	3.7	3.7	3.7	3.6
Mean Body Weight Gain-Day 6-18	0.3	0.3	0.2	0.1 S-

S- = Significantly less than the vehicle control at $p \leq 0.05$ (REPROTOX).

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Reproduction Indices: A summary of reproduction indices is presented in Table 2. Statistically significant changes and dose-related effects were not observed in these parameters.

Fetal Examinations: As shown in Table 2, no external or visceral malformations were detected in any of the litters exposed to the test article or in any of the vehicle control litters. The incidence of fetuses with skeletal variations in the treated litters was similar to the vehicle control group. The skeletal abnormalities observed were the presence of developed or reduced 13th rib(s) and delayed ossification of the sternebrae and are not necessarily indicative of chemically-induced terata.

Necropsy: No remarkable or treatment related gross lesions were detected at necropsy.

DISCUSSION:

There were no indications of teratogenicity during the study. The absence of teratogenic effects may be the result of the lack of optimum dose levels. Although a decreased body weight gain was seen at the highest dose level (100 mg/kg) there was no "overt maternal toxicity such as slight weight loss" (EPA, Guidelines, 1982, 83-3-g-3-iii, pg. 127) and there was no maternal death. The highest dose used did not produce any life-shortening or life-threatening effects. Furthermore, clinical observations of the maternal animals were not reported. Consequently, it is apparent from the data that the maternal animals could have tolerated higher doses. If evidence to the contrary are made available, the core classification of the study will be reconsidered.

Several deficiencies in the conduct of the study and in the REPROTOX report were noted during the evaluation of the data. The deficiencies are as follows:

- Although the REPROTOX report states on page 5 that "body weights and dosage volumes were calculated for individual animal (sic) from day 6 to 18 p.c. daily," body weight measurements were recorded only for days 0, 6, 18, and 28 of gestation. Therefore, it appears that the dose volumes were based on day 6 of gestation body weights and not adjusted.

- The REPROTOX report indicated that the fetal body weights of the 100 mg/kg dose group were significantly reduced. The summary and the results section state that this measurement was slightly decreased but that the difference was not significant. A statistical comparison by this reviewer indicated no significant difference ($p \leq 0.05$) between the vehicle control and the 100 mg/kg dose level fetal body weights.

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TABLE 2. Summary of Reproduction and Teratology Data

	Dose (mg/kg)				
	0	10	30	100	Historical Controls
Mean No. of Corpora Lutea/Litter	9.4	8.4[a]	9.1[a]	8.6[a]	9.3
Mean No. of Implantation Sites/Litter	7.7	6.8	6.9	7.4	7.4
Mean No. of Resorption Sites/Litter	0.5	1.4[b]	0.5	0.6	0.7
Mean No. of Live Fetuses/Litter	7.1	5.3	5.8	6.3	6.6
Litters with No Live Fetuses	0/13	2/12	1/11	1/12	-[c]
Live Fetus/Implantation Site Ratio	0.92	0.79	0.84	0.92	0.89
Mean Fetal Body Weight (g)	34.1	35.6	35.1	32.8	35.1
Fetuses with External Malformations	0/92	0/64	0/70	0/82	12/2,238
Fetuses with Visceral Malformations	0/92	0/64	0/70	0/82	1/947
Fetuses with Skeletal Variations	80/92	57/64	63/70	68/82	692/1,111

[a]Based on litters with live fetuses only. In litters with 100 percent resorptions the corpora lutea were reduced and could not be counted.

[b]One litter contained nine resorption sites. The mean excluding this litter was 0.7.

[c]Historical data not supplied on litter basis.

NOTE: One high dose rabbit "littered prior to treatment." It was removed from the study.

- The REPROTOX report indicated in a table that the high dose body weight on day 6 of gestation is significantly less than the vehicle control. This is an error; instead, the report should have indicated that the high dose maternal weight gain from days 6-18 of gestation was significantly reduced. This would then be consistent with the text of the report which state. that the weight gain for this period was significantly reduced.

- No clinical observations of the maternal animals were reported.

- No analysis of the dosing solutions were reported.

- The Student's t-test and Chi-square analysis were utilized to measure significance. Neither test is appropriate to designate significant differences between three or more groups. ANOVA followed by a multirange comparison would have been more appropriate for parametric measurements. The Kruskal-Wallis test would have been more appropriate for non-parametric measurements. The use of the Student's t-test to compare four groups increases the alpha value from 0.05 to 0.26. (See Results - Body Weights for detailed discussion).

CONCLUSIONS:

Under the conditions of this study, KWG 0519 administered by gavage at 100 mg/kg during days 6 to 18 of gestation produced no indications of overt maternal toxicity or of teratogenicity; however, the study is limited because doses permitting optimum test sensitivity were not used.

CORE CLASSIFICATION: Supplementary data.

The study was classified as supplemental because doses permitting optimum test sensitivity were not used.

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G.G. 7/17/85

DATA EVALUATION RECORD

004695

STUDY TYPE: Teratology study in rats.

CITATION: Machemer L. Evaluation for embryotoxic and teratogenic effects on orally dosed rats. Unpublished report number 7038 prepared by Bayer AG-Institut fur Toxikologie for Mobay Chemical Corporation. October 7, 1977.

ACCESSION NUMBER: 071468.

LABORATORY: Bayer AG, Institut fur Toxikologie, Wuppertal-Elberfeld, Deutschland.

TEST MATERIAL: KWG-0519 (Baytan), Batch 16001/76. The purity and source of the test material were not stated.

PROTOCOL:

1. Eighty-two female FB 30 (Long Evans) rats weighing 189-260 g, and 2 1/2 to 3 1/2 months of age were utilized in this study. An unspecified number of males weighing 350-500 g and 3-6 months in age were utilized for breeding purposes only. The rats were individually housed, except during breeding, in Type II Makrolon cages. The room temperature was maintained at 20-23° C with a relative humidity of approximately 60 percent. A 12 hour light/dark cycle was maintained. Altromin R chow and tapwater were available ad libitum.

2. Two females were mated with each male. The presence of sperm in a vaginal smear was considered an indication of insemination and designated as day 0 of gestation. The inseminated females were "continuously allocated in equal numbers" to one of the four groups on day 0 of gestation and were administered the vehicle (0.5 percent aqueous Cremophor EL), or 10, 30, or 100 mg/kg KWG 0519 daily from day 6 to day 15 of gestation. A dose volume of 10 ml/kg body weight was utilized.

3. The females were observed for general appearance, behavior, and mortality at unspecified intervals. Body weights were recorded on days 0, 6, 15, and 20 of gestation.

4. On day 20 of gestation, the females were sacrificed and the gravid uterus removed by caesarean section. The uterus was opened and the number of implantation sites, resorptions, dead fetuses, and live

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fetuses were determined. The placentas and fetuses were both weighed and the fetuses were examined for external abnormalities and sex. Approximately two fifths of the fetuses were examined for visceral abnormalities using a modified Wilson's technique. The remaining fetuses were examined for skeletal abnormalities after preparation and staining with Alizarin Red S.

5. The Mann-Whitney (Wilcoxon's Analysis) was used to analyze weight gains, number of implantations, number of fetuses, number of resorptions, and placental weight for statistical significance. The Chi-square test was used to determine the possible statistical significance of the number of fetuses with skeletal alterations, visceral, or external abnormalities, and number of stunted fetuses. The fertility and gestation indices were analyzed using either the Chi-square or Fisher Exact test. A 5 percent probability level was chosen as the level of significance.

RESULTS:

Clinical Observations and Mortality: Although clinical observation data were not presented in the report, it was stated that KWG 0519 did not induce "detrimental effects on the physical appearance and behavioral patterns of the dams." No animals died during the course of the study.

Maternal Body Weights Gain: Maternal weight gain from day 6 to day 15 and day 0 to day 20 of gestation are presented in Table 1.

TABLE 1. Mean Maternal Body Weight Gain (g)

Dose Level (mg/kg)	Gestation Period	
	Days 6-15	Days 0-20
0	53.1	140.5
10	53.5	138.8
30	50.7	141.9
100	40.5*	134.8

* = Significantly less than the control at $p \leq 0.01$. (Bayer).

A significant decrease ($p \leq 0.01$) in maternal body weight gain (days 6-15 of gestation) as compared to the vehicle control was observed at the 100 mg/kg dose level. The maternal body weight gains at 10 and 30 mg/kg during this period were similar to that of the vehicle control group. However, during the entire period of gestation (days 0-20 of gestation), the maternal weight gains of all treated groups were similar to that of the vehicle control.

Reproduction Indices: A summary of reproduction indices is presented in Table 2. A comparison of the gestation index, mean numbers of implantation sites, resorption sites, dead fetuses, and live fetuses, mean fetal weight, mean placental weight, or number of stunted fetuses indicated no KWG 0519 related response. The number of implantation sites and resorption sites were significantly reduced at the 10.0 mg/kg dose level as compared to the vehicle control group. This effect was not seen at the other two higher dose levels. At 100 mg/kg the number of resorptions was significantly decreased. Mean fetal and placental weights of the 100 mg/kg group were significantly higher than that of the control. The number of stunted fetuses (fetuses weighing less than 3 g) was increased at 10 and 30 but not at 100 mg/kg dose levels.

TABLE 2. Summary of Reproduction and Teratology Data

	Dose Level (mg/kg)			
	0	10	30	100
Gestation Index[a]	20/20	20/20	20/20	20/20
Mean no. of implantation sites	12.3	10.5**	11.7	11.4
Mean no. of resorption sites	1.6	0.5*	1.0	0.5*
Mean no. of live fetuses	10.7	10.0	10.7	10.9
Mean fetal body weight (g)	4.05	4.17	4.13	4.27*
Mean placental weight (g)	0.57	0.58	0.58	0.63**
Incidence of stunted fetuses[b]	3/215	6/200	12/215	1/218
Incidence of fetuses with visceral or external abnormalities	3/64	5/59	13/68	0/56
Incidence of abnormal fetuses	4/215	5/200	13/215	0/218
Incidence of litters with abnormal fetuses[c]	3/20	1/20	3/20	0/20

[a] No. of females with live fetuses on day 20 of gestation/no. of pregnant females.

[b] Stunted fetuses were defined as fetuses weighing less than 3 g.

[c] Includes external, visceral, and skeletal abnormalities.

* Significantly different than the control at $p \leq 0.05$.

** Significantly different than the control at $p \leq 0.01$.

Fetal Examinations: A summary of the teratology data is presented in Table 2. An increase in the number of fetuses with visceral or external abnormalities was observed when the 30 mg/kg dose level was compared to the vehicle controls. Despite the increase in abnormal fetuses at 30 mg/kg, the numbers of litters with abnormal fetuses were identical in the vehicle control and 30 mg/kg dose level. The numbers of litters with abnormal fetuses in the 10 and 100 mg/kg dose levels were less than the number observed in the controls. Therefore, no dose-relationship was established for the number of litters with abnormal fetuses.

The visceral/external abnormalities and the number of fetuses (litters) affected are summarized in Table 3.

TABLE 3. Number of Fetuses with Abnormalities*

Malformation	Dose Level (mg/kg)			
	0	10	30	100
Hydrops universalis (edema)	1(1)	0	0	0
Ectopia - left testis	1(1)	0	0	0
Hypoplasia - telencephalon	1(1)	5(1)	10(3)	0
Anopthalmia/Micropthalmia	1(1)	5(1)	6(2)	0
Kinky or shortened tail	0	3(1)	5(1)	0
Hydrocephaly	0	0	3(2)	0
Brachygnathia	0	0	1(1)	0
Ectrodactyly	0	0	5(1)	0
Total Fetuses[a]	3/54	5/59	13/68	0/66
Total Litters	3/20	3/20	3/20	0/20

[a] Some fetuses had more than one malformation.

* The report combined visceral and external abnormalities.

The dams containing the abnormal fetuses had acceptable weight gains and did not appear to have abnormal clinical signs.

DISCUSSION:

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An adequate evaluation of KWG 0519 for teratogenicity was not possible with the data provided. Optimal dose levels to provide maximal test sensitivity were not achieved. Although a decrease in maternal mean body weight gain (days 6-15 of gestation) occurred among the 100 mg/kg dose level females when compared to the controls, the 100 mg/kg dose level did not "induce some overt maternal toxicity such as slight weight loss" (EPA Guidelines, 1982, 83-3-g-3-iii, pg. 127).

Adequate evaluation of the data is further hindered by the incidence of fetal abnormalities among the control fetuses and the severity of the abnormalities observed. The occurrence of subcutaneous edema, ectopia of a testis, hypoplasia of the telencephalon, and anophthalmia in a control population is not normally expected. In the absence of any historical control data and individual dam clinical and body weight observations, it cannot be determined if the abnormalities observed among the control fetuses are expected for the testing laboratory or the product of unusual sensitivity in the dams with abnormal litters. The question of unusual sensitivity in the dams with abnormal litters is particularly important because there were no abnormal litters among the high-dose dams.

In addition, the following deficiencies were noted that, although they did not compromise the study, limited the sensitivity of the test to detect compound-related effects:

- The report did not differentiate between visceral and external abnormalities. Some of the visceral/external abnormalities (kinky tail and ectrodactyly) are usually considered skeletal malformations.
- Fetal crown-to-rump lengths were not determined.
- The number of corpora lutea was not determined.
- Necropsy was not conducted on the maternal animals.
- The Mann-Whitney test was inappropriately utilized. This test is used to determine differences between two independent populations. When used to test three treatment groups against the control group, the level of significance is changed from that assumed by the authors ($p \leq 0.05$) to a $p = 0.26$. The Kruskal-Wallis test is appropriate in testing three or more independent populations.

CONCLUSIONS:

The oral administration of KWG 0519 from days 6-15 of gestation to Long-Evans rats did not produce any overt maternal toxicity or teratogenicity at 100 mg/kg; however, doses permitting optimal test sensitivity were not used.

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CORE CLASSIFICATION: Supplemental data.

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DATA EVALUATION RECORD 004695

STUDY TYPE: Mouse lymphoma forward mutation.

CITATION: Cifone MA, Brusick DJ. (1982). Mutagenicity evaluation of KWG 0519 in the mouse lymphoma forward mutation assay. Final Report. Project No. 20999. Prepared by Litton Bionetics, Inc. Kensington, MD for Bayer AG, West Germany.

ACCESSION NUMBER: 071468.

RD 9/3/85

LABORATORY: Litton Bionetics, Inc. Kensington, MD.

TEST MATERIAL: The test material was identified as KWG-0519 from Batch 816 066 128 with a 97.5 percent purity. It was described as being a white powder.

PROTOCOL:

1. The test material was dissolved in dimethylsulfoxide (DMSO) at 100 mg/ml and prior to each assay, stock solutions were prepared by performing serial dilutions in DMSO. In the mutation assays, the stock solutions were further diluted in the cell media by 100 fold. The test material was miscible in the assay medium from 0.061 µg/ml to 250 µg/ml, but precipitated at 500 and 1000 µg/ml (the maximum applied dose).

2. Mutagenicity testing was based on the procedure of Clive and Spector (1975). Cultures of mouse lymphoma cell line L5178Y $TK^{+/-}$ were treated with KWG 0519 for 4 hours with one of 11 doses in two trials, ranging from 7.8-150 µg/ml; both with and without the addition of rat liver S9 homogenate (from male Sprague-Dawley rats treated with Aroclor 1254 as described by Ames, et al., 1975). The cells were washed and placed in growth medium for two or three days and allowed to recover, grow, and express the induced (mutant) $TK^{-/-}$ phenotype. From each dose level 3×10^6 cells were then seeded on soft agar plates that contained 100 µg/ml of 5-bromo-2'-deoxyuridine, and resistant (mutant) colonies were counted after 10 days in this selective medium. The count of mutant colonies was then compared to the count of colonies derived from cells of the same suspension that were cloned in nonselective medium (total viable cell number). The ratio of resistant colonies to the total viable cell number, for each treatment, was considered to be the mutant frequency, expressed as mutant colonies per viable colonies times 10^{-4}.

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3. Negative and solvent control assays were conducted concurrently with the test material. Similarly, positive control assays were conducted with ethylmethane sulfonate (EMS) at 0.5 µl/ml being used in non-activation assays and dimethylnitrosamine (DMN) at 0.3 µl/ml in activation assays.

4. An assay was considered acceptable only if the following ten criteria were met: (1) The absolute cloning efficiency of the negative control should have been between 70 percent and 130 percent (2) The untreated negative and solvent control cultures should have had the same growth rate and cloning efficiencies within experimental error. (3) The negative control cultures should have reached a growth value of 8.0 (3 population doublings) within 24 hr. (4) The negative control cultures (solvent and untreated control) should have had a background mutation frequency within a range of 5×10^{-6} to 50×10^{-6}. (5) The two positive controls, one for a direct acting mutagen and one for a mutagen that requires metabolic activation, should have yielded mutants within a frequency range specified for each chemical. The mutant frequency expected was 300 to 800×10^{-6} for EMS and 200 to 800×10^{-6} for DMN. (6) Test materials with little or no mutagenic activity should have been assayed at a maximum dose that reduces the suspension growth to 5-10 percent of the solvent control. (7) Treatments that gave fewer than 2.5×10^6 cells after two days of growth were not considered useable for mutant analyses. (8) The cloning efficiency was required to be 10 percent or greater and the number of clones was required to exceed 20 in order to derive an experimental mutant frequency. (9) A minimum of two dishes was required to obtain the colony numbers for viable and mutant counts (ordinarily three was used) and could be used provided the colony numbers did not differ by more than three-fold. (10) A minimum of three concentrations, obtained from five treated cultures, was considered necessary for the acceptance of a single assay used to evaluate the test material.

   The assay was considered to be positive for mutagenicity only if the following conditions were met: (1) At a given treatment, a mutant frequency that exceeded 150 percent of the concurrent background by at least 10×10^{-6} must occur. (2) A dose-related or toxicity-related increase must occur in mutant frequency, preferably at three doses (depending on the dose where mutagenic activity occurs). (3) It was not acceptable to have an increase in mutant frequency followed by a decline to a value below the minimum criterion. (4) An increase of two-fold the minimum criterion or greater near the highest testable toxicity. (5) A negative mutagenic correlation with dose was only acceptable if a positive correlation with toxicity exists, but an apparent increase in mutagenicity with decreasing toxicity is not acceptable as evidence of mutagenicity.

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RESULTS:

KWG 0519 was reported to have excessive lethal activity at 250 µg/ml under nonactivating conditions. Results from 2 trials each in the presence and absence of metabolic activation were presented. In the absence of metabolic activation, a concentration range of 7.81 to 150.00 µg/ml was used (Table 1), and in the presence of metabolic activation, a concentration range of 3.91 to 150.00 µg/ml was used (Table 2). The growth of treated cells relative to the solvent control ranged from 8.1-121 percent in the unactivated assay and was 13.9-111.6 percent in the S9-activated assay. Using the the minimum criterion of mutation frequency (150 percent of negative control + 10 x 10^{-6}) to be exceeded, the authors determined that one treatment (37.5 µg/ml) in the second trial of the assay without activation induced mutagenesis, but that higher and more toxic doses were inactive (Table 1). However, the 100 µg/ml treatment also induced essentially the same mutant frequency as the 37.5 µg/ml treatment.

The authors also concluded that the minimum criterion was not exceeded at any dose in the assays with metabolic activation. However, in trial 1 at the 31.3 µg/ml treatment level with metabolic activation the mutation frequency approached the minimum criterion (Table 2). At KWG 0519 concentrations which were toxic, the cloning efficiencies were usually reduced from the ranges of 71.2-79.2 percent obtained in the negative control without activation and 68.4-70.2 percent with activation.

DISCUSSIONS:

In the first trial, none of the treatment levels without metabolic activation induced mutagenic activity in the mouse lymphoma cells. The mutation frequencies were actually lower than those for the negative controls as was cytotoxicity. In the second trial higher cytotoxicities were obtained, and two treatments (37.5 and 100 µg/ml) induced a mutation frequency that reached or exceeded the minimum criterion. However, the mutation frequencies at higher doses were low and comparable to controls, and a dose-related increase was not evident.

The following minor deficiencies were noted in this study. The mutation frequency of the positive control (EMS) in the second trial was much lower when compared to the first. There was also a difference in the relative cell growth between trials at the higher dosages. This difference may reflect significant variations in the cell sensitivity to mutagenesis; a phenomenon that is well documented for cell cultures that are growing at different rates.

CONCLUSIONS:

Baytan (KWG 0519) was tested in the mouse lymphoma (L 5178Y) assay to determine its ability to induce mutation from $TK^{+}/^{-}$ to $TK^{-}/^{-}$ at dosages ranging from 7.81-150.0 µg/ml without metabolic activation and

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3.91-1[illegible]0 µg/ml with metabolic activation. Under the conditions of this study, the results indicated that Baytan was non-mutagenic in both the presence and absence of metabolic activation at the dosage tested.

CORE CLASSIFICATION: Acceptable.

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TABLE 1. Summary of Mouse Lymphoma (L5178Y) Mutagenesis Assay on KWG 0519 Without Metabolic Activation

Assay Material	Viable Colonies (Ave Total x 10^6)	Mutant Colonies (Ave Total)	Cloning Efficiency (Ave)	Relative Growth (percent)	Mutation Frequency ($10E^{-6}$ Units)
Trial 1					
Solvent Control	2.23	53.0	74.3	100.0	23.8
Untreated Control	1.95	61.0	65.0	75.3	31.3
EMS (0.5 µl/ml)	0.71	557.0	23.7	12.3	784.5
KWG 0519 (µg/ml)					
7.81	2.3	34.0	101.3	121.0	15.0
15.60	2.2	38.0	97.7	45.7	17.4
31.30	3.1	57.0	138.0	84.7	18.5
62.50	2.4	38.0	108.9	60.9	15.6
125.00	2.1	40.0	94.1	35.1	19.0
Trial 2					
Solvent Control[a]	2.4	34.0	80.2	100.0	14.2
Untreated Control	2.3	36.0	77.3	59.7	15.5
EMS (0.5 µl/ml)	2.3	640.0	76.3	50.6	279.5
KWG 0519 (µg/ml)					
25.0	2.7	37.0	111.4	47.3	13.8
37.5	1.5	49.0	62.3	20.5	32.7
50.0	1.4	28.0	59.9	29.1	19.4
75.0	1.2	15.0	49.9	25.0	12.5
100.0	1.0	32.0	42.0	8.1	31.7
150.0	1.8	42.0	76.5	15.6	22.8

[a]Average value from two tests.

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TABLE 2. Summary of Mouse Lymphoma (L5178Y) Mutagenesis Assay on KWG 0519 With Metabolic Activation

Assay Material	Viable Colonies (Ave Total x 10^6)	Mutant Colonies (Ave Total)	Cloning Efficiency (Ave)	Relative Growth (percent)	Mutation Frequency ($10E^{-6}$ Units)
Trial 1					
Solvent Control[a]	2.2	57.0	69.9	100.0	27.1
Untreated Control	2.0	64.0	65.7	63.8	32.5
DMN (0.3 µl/ml)	0.64	273.0	21.3	12.5	426.6
KWG 0519 (µg/ml)					
3.91	2.23	52.0	106.3	104.3	23.3
7.81	2.19	52.0	104.4	90.0	23.7
15.60	2.36	51.0	112.5	101.8	21.6
31.30	1.25	60.0	59.6	46.7	48.0
62.50	1.66	50.0	79.2	34.9	30.1
Trial 2					
Solvent Control[a]	1.99	61.5	66.2	100.0	30.9
Untreated Control	2.35	75.0	78.3	126.2	31.9
DMN (0.3 µl/ml)	0.72	192.0	24.0	13.7	266.7
KWG 0519 (µg/ml)					
25.0	2.18	81.0	109.8	111.6	37.2
37.5	2.18	68.0	109.8	101.0	31.2
50.0	3.40	99.0	171.2	110.1	29.1
75.0	3.01	85.0	151.6	99.4	28.2
100.0	2.22	91.0	111.8	27.3	41.0
150.0	1.65	35.0	83.1	13.9	21.2

[a] Average value from two tests.

G.G. 3/7/85

DATA EVALUATION RECORD

004695

STUDY TYPE: Dominant lethal mutagenicity study with male mice.

CITATION: Herbold B. 1978. Dominant lethal study on male mouse to test for mutagenic effects. Report No. 7900. Prepared by Bayer AG, Institute fur Toxikologie, Wuppertal-Elberfeld, Germany. November 6, 1978

ACCESSION NUMBER: 071468.

RDS 9/5/85

LABORATORY: Bayer AG, Institute fur Toxikologie, Wuppertal-Elberfeld, Germany.

TEST MATERIAL: The test material was identified as KWG 0519 (Bay 144924), Batch 16001/76, with a 93.7 percent purity.

PROTOCOL:

1. Mice of the NMRI Strain were supplied by S. Ivanovas GmbH Kisslegg/ Allgau. The animals were 8-12 weeks old and the males weighed 33-37 g, and the females weighed 28-33 grams at the beginning of the study. Animals were housed individually, except during mating where 1 male was caged with one virgin female, in rooms maintained at 20-26° C and average relative humidity of 60 percent with a 12 hour light/dark cycle. Food and tapwater were provided ad libitum.

2. The test material was suspended in a 2 percent Cremophor emulsion (concentration not specified) and administered orally to a group of 50 male mice at a single dose of 500 mg/kg body weight. Fifty males in the control group received 2 percent Cremophor emulsion in a volume of 10 ml/kg body weight, equivalent to that given to the treated mice. Dose selection was based on results from a preliminary study.

3. Following treatment each male was caged with a virgin female for 4-days, then the female was removed and replaced by another female this mating procedure was repeated for 12 times over a period of 48 days. A total of 600 female mice were mated with treated males and 596 females mated with control males.

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4. Twelve days following the mating period, the uterus of each female was examined and pre- and post-implantation losses, total implantations, live and dead implants, and corpora lutea were counted. The following parameters were determined:

$$\text{Fertilization quota} = \frac{\text{No. of fertilized females} \times 100}{\text{No. of females bred}}$$

Pre-implantation loss is estimated by comparing the average number of implantations in each fertilized female from the control and treated groups.

Post-implantation loss is the count of deciduomata or resorptions, dead embryos, and fetuses.

5. The frequency of dead implants was compared between groups using a 2-factor analysis of variance. Statistically significant differences ($p = 0.05$) were further analyzed by the Dunnett's test. Where dose and time factors showed significance in the F-test at $p=0.05$, the least significant difference was calculated using the Tukey test. Frequency distributions for dead implants, viable implants, total implants and preimplantation loss were compared using the Kolmogorov-Smirnov test.

RESULTS:

Males showed no adverse clinical signs following administration of the test compound, except for ruffling of the fur that persisted for 30 minutes. None of the treated males died. However, 6 females and 1 male control mice, and 2 females placed with treated males were eliminated from the study during the mating period as a result of cannibalism, autolysis or technical errors. The final number of females placed with control and treated males was 590 and 598, respectively.

There were no significant differences in the fertilization rates of females mated with treated males when compared to controls (Table 1). Preimplantation losses were also not affected by treatment in a dose- or time- related manner, although a significant increase was noted in females from the first mating.

Statistical analysis of dead implants, the ratio of dead implants: total implantations, and the frequency distribution of dead and viable implants showed that the test compound did not produce any effects on postimplantation loss (Table 1).

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TABLE 1. Summary of Results of the Dominant Lethal Assay in Mice After Treatment with KWG 0519

	Fertilization Quota		Preimplantation Loss/ Fertilized Females		Postimplantation Loss/Fertilized Female			
					Live Implants		Dead Implants	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	78.0	86.0	0.36	1.05	11.7	10.7	0.72	0.65
2	88.0	75.5	0.93	0.46	10.9	11.3	0.52	0.70
3	82.0	72.0	1.12	0.83	12.1	12.1	0.78	0.58
4	71.4	86.0	0.83	0.37	10.6	10.7	1.11	0.35
5	84.0	82.0	0.93	0.78	11.1	11.4	0.83	0.73
6	78.0	86.0	0.64	0.74	11.3	11.6	0.62	0.56
7	81.6	82.0	0.20	0.49	11.7	11.0	0.77	1.17
8	78.7	81.6	0.86	0.45	10.1	11.1	0.81	0.55
9	83.7	74.0	0.51	0.59	11.5	11.8	0.71	0.57
10	81.6	88.0	0.50	1.07	11.8	10.8	0.47	0.52
11	79.2	78.0	0.25	0.41	11.6	11.1	0.66	0.69
12	77.6	76.0	0.46	0.32	11.7	11.6	0.66	0.76
X	80.3	80.6	0.64	0.70	-	-	0.71	0.65

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DISCUSSIONS:

A dominant lethal assay was conducted in NMRI mice treated with a single oral dose of KWG 0519 (Baytan) at a concentration of 500 mg/kg body weight. It was stated in the report that dose selection was based on a range-finding experiment in which groups of 5 male mice received a single oral dose of 500, 700, or 1000 mg/kg, and "in this experiment, the 500 mg/kg dose was tolerated, the only sign being brief slight ruffling of the hair coat." However, dose selection should be based on the highest dose that permits the male to produce fertile mating over an 8-week period[1]. Consequently, it cannot be determined whether an MTD was used in this study.

Male animals that received 500 µg/kg of KWG 0519 were bred with 12 untreated females each for a 4-day interval over a 48-day period. The increase in preimplantation loss in females from the first mating suggest some treatment-related effect on epididymal sperm. However, the report stated that the value obtained was within normal range and further statistical analyses by the Kolmogorov-Smirnov test for distribution frequency did not corroborate this finding. Although, this may be true the statistics could not be evaluated by this reviewer because they were presented in German.

CONCLUSIONS:

The test compound produced no clinical signs in treated males other than a ruffling of the fur, and there were no mortalities. Analysis of fertility quotas, and pre- and post-implantation loss data in comparison with controls, indicated that reproduction and fetal survival were not affected by test compound treatment. However, more information is needed with respect to dosage selection and whether on 'MTD' was used before the mutagenic potential of the test compound can be assessed.

CLASSIFICATION: Unacceptable in the present form. However, if additional information is made available, the report can be reevaluated and reconsidered for an acceptable classification.

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[1] Bateman AJ. "The Dominant Lethal Assay in the Male Mouse," in Handbook of Mutagenicity Test Procedures, ed. Kilbey BJ, 1977, p. 324-334.

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity, salmonella/microsome test.

CITATION: Herbold B. 1979. Salmonella/microsome test for detection of point-mutagenic effects. Report No. 8189, prepared by Institut Fur Toxikologie, Bayer AG, Wuppertal-Elberfeld, Germany.

ACCESSION NUMBER: 071468.

LABORATORY: Bayer AG, Institut fur Toxikologie, Wuppertal-Elberfeld, Germany.

TEST MATERIAL: The test material was identified as KWG 0519 from batch 16001/76, with a 93.7 percent purity.

PROTOCOL:

1. The assay was conducted according to the procedure of Ames et al. (1973, 1975) with Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100. The test material was dissolved in DMSO and concentrations of 4, 20, 100, 500, and 2500 ug per plate were used in the assay. Four replicate plates were prepared per strain per dose, and the total number of bacteria in each group determined from two plates. The assay was conducted in the pesence and absence of metabolic activation using S9 mix prepared from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The positive controls used were, endoxane used at a concentration of 145 ug/plate with strains TA1535 and TA100, andtrypaflavin at a concentration of 200 ug/plate with strains TA1537 and TA98.

2. A positive result was defined as the observation of a dose-dependent increase in the number of revertants to a level double (or greater) that of the solvent control in at least one strain.

RESULTS:

The results indicated that the assays without metabolic activation were conducted at only the highest dose of 2500 ug/plate (Table 1). Assays with metabolic activation were conducted as indicated in the protocol.

Cytotoxicity resulted at the highest dose tested (2500 ug/plate) in all strains, and also at the 500 ug/plate concentrations in strain TA98

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TABLE 1. Summary of Mean Revertants Produced in the Salmonella/ Microsome Assay as a Result of KWG 0519 Treatment

Compound	Concentration (µg/plate)	Metabolic Activation	TA1535	TA1537	TA98	TA100
KWG 0519	2500	-	10.8	10.5	8.3	96.8
	500		-	-	-	-
	100		-	-	-	-
	20	-	-	-	-	-
	4	-	-	-	-	-
DMSO	-	-	11.8	10.0	33.5	133.8
Endoxane	135	-	11.7	-	-	-
Trypaflavin	200		-	9.3	28.5	-
KWG 0519	2500	+	10.5 (0.91)[a]	9.0 (0.60)	30.3 (0.08)	107.0 (0.16)
	500	+	11.3 (3.77)	10.5 (2.89)	49.0 (0.80)	157.5 (2.48)
	100	+	12.5 (3.92)	9.3 (3.09)	39.3 (0.88)	127.3 (2.57)
	20	+	9.3 (3.91)	11.8 (3.18)	49.5 (0.84)	130.8 (2.31)
	4	+	9.5 (3.83)	9.0 (3.03)	30.0 (0.94)	144.3 (2.61)
DMSO	-	+	12.5 (4.17)	10.0 (3.18)	43.3 (1.13)	143.5 (2.45)
Endoxane	135	+	301.3 (3.79)	-	-	381.8 (2.19)
Trypaflavin	200	+	-	25.8 (2.21)	305.5 (0.91)	-

[a] Total number of bacteria per ml x 10^9 are represented in parenthesis.

(Table 1). The survival of plasmid-bearing S. typhimurium strains TA98 and TA100 was approximately 6 or 7 percent at 2,500 µg/plate compared to about 20 percent in TA1535 and TA1537 at the same concentration and was about 7 percent in TA98 at 500 µg/plate. The number of revertants produced after KWG 0519 treatment was not increased to levels twice that of the solvent control in any of the tester strains in the presence of S9 metabolic activation and was not increased at 2,500 µg/plate when S9 was absent. However, the positive controls endoxane and trypaflavin showed significantly increased numbers of revertants in all tester strains for the activated assay only.

DISCUSSIONS:

Although no rationale was presented for the dosage selection, a wide concentration range encompassing a dosage high enough to produce cytotoxicity was used. Adequate data were produced indicating that the test compound was not mutagenic in any of the tester strains in the presence of metabolic activation. Positive controls tested in the presence of S9 produced an increase in the number of revertants, demonstrating that the assay was capable of producing a postive response in the presence of rat liver S9.

In the absence of metabolic activation the full range of concentrations were not tested. Since cytotoxicity occurred at the single dose tested, other concentrations where growth innibition was not interfering with the results should have been tested. In addition, no positive control suitable for determining a mutagenic response in the absence of metabolic activation was included.

CONCLUSIONS:

The assay compound, KWG 0519, was not mutagenic in the presence of S9 activation, however cytotoxicity was excessive in all tester strains at 2,500 µg/plate, and also at 500 µg/plate in strain TA98. The assays in the absence of metabolic activation included only one dosage and at that dose, 2,500 µg/plate, cytotoxicity was excessive. Hence, no conclusion may be drawn from the data in the absence of S9.

CLASSIFICATION: Unacceptable. The data base is inadequate for proper assessment of mutagenicity.

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DATA EVALUATION RECORD

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STUDY TYPE: Mutagenicity, DNA Damage Using The E. coli Pol A_1^- Test.

CITATION: Herbold B. 1981. Study of DNA damage using the E. coli POL A_1^- test. Report No. 10255. prepared by Bayer AG, Institut fur Toxikologie, Wuppertal, Germany. October 19, 1982.

ACCESSION NUMBER: 071468.

LABORATORY: Institut Fur Toxikologie, Bayer AG, Wuppertal, Germany.

TEST MATERIAL: The test material was identified as KWG 0519, beta-(4-chloropnenoxy)-alpna-(1,1 dimethylethyl)-1H-1,2,4-triazole-1-ethanol, from batch 816066128, with a 97.5 percent purity.

PROTOCOL:

1. The assay was conducted according to the method of Rosenkranz and Leifer (1980)[1] using E. coli, repair deficient strain (K12)p3478 (PolA_1^-) and repair efficient strain W 3110(PolA^+). The test compound (KWG 0519) the antibiotic control (chloramphenical) and the positive control (methylmetnane sulfonate), were used to treat the E. coli strains, both in the presence and absence of metabolic activation (S9 mix). The S9 mix was prepared from the livers of male Sprague-Dawley rats pretreated with Aroclor 1254 and was added to cofactors described by Ames, et al.[2] Each dose of the test compound or control substance was placed on a paper disc (unspecified diameter)

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[1]Rosenkranz HS and Z. Leifer. 1980. Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient Escherinia coli. In E.J. de Serres and A. Hollaender (eds) Chemical Mutagens, Principles and Methods for their Detection, Vol 6, S. 109-147, Plenum Press, New York and London.

[2]Ames BN, McCann J, and Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31:347.

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which was then placed on a nutrient agar plate containing the test bacteria in a soft agar overlay. Zones of inhibition were measured after 24 hours of incubation at 37° C. The test material was dissolved in DMSO and tested at 62.5, 125, 250, 500, and 1,000 ?g/ plate. Four replicates of each dose were used. A positive result was defined as a reproducible increase of more than 2 mm in the difference between the diameter of the zones of inhibition between the $PolA^+$ and $PolA_1^-$ strains.

RESULTS:

No increase in the diameter of the inhibition zone between strains resulted from treatment with the test compound in the presence or absence of metabolic activation (Table 1). The negative control compound,

TABLE 1 Mean Diameter of Inhibition Zone (mm) Resulting From the DNA Damage Assay in E. coli Tester Strains

Compound Strains	Concentration (µg/plate)	Metabolic Activation (S9)	Mean Diameter (mm) in Tester Strain		Difference Between
			$PolA_1^-$	$PolA^+$	
Solvent Control	0.0	-	0	0	0
KWG 0519	62.5	-	0	0	0
	125.0	-	0	0	0
	250.0	-	0	0	0
	500.0	-	0	0	0
	1,000.0	-	0	0	0
Chloramphenicol	30.0	-	29	29	0
MMS	10.0 µl	-	67	44	23
Solvent Control	0.0	+	0	0	0
KWG 0519	62.5	+	0	0	0
	125.0	+	0	0	0
	250.0	+	0	0	0
	500.0	+	0	0	0
	1000.0	+	0	0	0
Chloramphenicol	30.0	+	27	28	≤0
MMS	10.0 µl	+	67	44	23

chloramphenical, produced equal inhibition in both strains. The positive control, MMS, caused inhibition in both strains, but the $PolA_1^-$ strain developed an inhibition zone which was 23 mm greater in diameter than that of the $PolA^+$ strain. This positive response is considered a consequence of DNA-damaging activity.

DISCUSSIONS:

The assay was adequately conducted and appropriate controls were used. The positive compound, MMS, produced a clear positive effect indicating that the assay was capable of detecting DNA damage.

CONCLUSIONS:

Under the conditions of this E. coli $PolA_1^-$ test, technical Baytan did not produce detectable DNA damage.

CLASSIFICATION: Acceptable.

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DATA EVALUATION RECORD 004695

STUDY TYPE: Mutagenicity, rat hepatocytes unscheduled DNA-synthesis.

CITATION: Myhr BC, Brusick DJ. 1982. Evaluation of KWG 0519 in the primary rat hepatocyte unscheduled DNA-synthesis assay. Project No. 21001. Report prepared by Litton Bionetics, Kensington, MD, for Bayer AG, Institute fur Toxikologie, West Germany.

ACCESSION NUMBER: 071468.

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LABORATORY: Litton Bionetics, Inc., Kensington, MD.

TEST MATERIAL: The test material was identified as "KWG 0519 (Batch 816 066 128, Content 97.5 percent)," and described as a pale yellow powder.

PROTOCOL:

1. The test material was dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mg/ml and stock solutions were prepared by serial dilutions in DMSO prior to use in the UDS assay. The stock solutions were further diluted (1:100) in Williams' Medium E (WME) containing 5 percent fetal bovine serum and treatments initiated by replacing the media on the cultures with media containing the different concentrations of test material. Concentrations of 250-1000 ug/ml were completely lethal and a second trial was conducted with 10 treatments ranging from 0.25-250 ug/ml.

2. Hepatocytes from adult male Fischer 344 rats were obtained by *in situ* perfusion of the liver with a collagenase solution. Monolayer cultures of the hepatocytes were established on plastic coverslips in the culture dishes, and the UDS assay was initiated on the same day.

3. The UDS assay was based on a procedure that was developed by Williams.[1,2] The hepatocytes established in monolayer cultures on coverslips (attachment period of 1.5 to 2 hr at 37 °C in a humidified atmosphere containing CO_2), were removed and refed WME. After

[1]Williams GM. 1977. Cancer Res. 37, 1845-1851.

[2]Williams GM. 1980. In Chemical Mutagens, Vol. 6, De Serres F and Hollaender A, eds., Plenum Press, NY. pp. 61-79.

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approximately 3 hr of incubation, the WME was replaced with 2.5 ml of WME containing 1 percent fetal bovine serum, 1 μCi/ml [^{3}H]-thymidine, and the test material at the appropriate concentrations. Each treatment, including negative and positive controls, was performed on 5 replicate cultures; two were used for determinations of cytotoxicity and three for the UDS assay. After 17 hr treatment [the design called for 18 - 19 hr], the assay was terminated by washing the cell monolayers twice with WME that contained 1mM unlabeled thymidine. The 2 cultures for cytotoxicity assessment were washed with WME, returned to the incubator for 20 - 24 hr, and their viable cell counts estimated by the trypan blue exclusion test.

To the [^{3}H]-thymidine labeled cells, 1 percent sodium citrate was added to cause the nuclei to swell, and the cells were then fixed in acetic acid : ethanol (1:3) and dried for a minimum of 24 hr. The coverslips were then mounted on glass slides so that the cell preparation was up, after which the slides were dipped in Kodak N782 emulsion and dried. After 7 to 10 days exposure at 4°C in light-tight boxes the emulsions were developed in D19 and stained by the modified hematoxylin-eosin procedure of Williams.

Microscopic examination of the cells under oil immersion at 1500x, displayed on a video screen of an automatic counter, was employed to score nuclear grains for 50 randomly selected cells on each coverslip. To measure UDS, the nuclear grains were counted and background counts were subtracted. Three areas adjacent to the nucleus and of similar size were chosen, and their average counts provided the background count.

4. A positive UDS assay should have met one or all of the following criteria:

   a. A mean nuclear grain count of at least 6 grains per nucleus in excess of the concurrent negative control.

   b. More than ten percent of the population of treated cells counted should have 6 or more grains in excess of the concurrent negative control.

   c. More than 2 percent of the population of treated cells with 20 or more grains.

   Generally, if condition a is satisfied, conditions b and c will also be met. If only conditions b and c were met, an acceptable decision can be made that there was UDS activity. If none of the conditions above were met within the limits of the concentrations the test material was considered inactive. If any of the conditions tested were met with a dose-related increase in UDS activity, the test material was considered to be positive for UDS. If, however, the negative control cells gave an average of 6 grains per nucleus or if 1 percent or more cells had 20 grains per nucleus, the assay would be rejected. It was also recognized that not all types of DNA-damage

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could be detected by this assay and that toxicity and/or solubility problems could lead to inconclusive or noninterpretable results.

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5. Statistical treatment of the data was not described.

RESULTS:

Two trials were performed because in the first trial concentration of 250-100 µg/ml were lethal and cells that were viable (e.g., 81.6 percent at 50 µg/ml) did not adhere to the slide long enough to complete the autoradiographic procedure. In the second trial, there was somewhat better retention and an analysis could be completed (Table 1). Toxicity was severe at the higher dosages and no cells survived at 250 µg/ml, and although 38.1 percent survived at 100 µg/ml they failed to adhere to the coverslip at a high enough frequency to permit autoradiography. At 50 µg/ml where survival was 53 percent, the cells had become rounded, but the grain analysis could be made with some difficulty.

The minimum criteria for a positive assay in this trial was set at a mean net nuclear grain count of 6.87 on at least 14 percent of the nuclei containing 6 or more grains, or at least 2 percent of the nuclei containing 20 or more grains. Treatment related changes failed to exceed these criteria at 50 and 10 µg/ml where the highest response was obtained. The positive control chemical (2-acetylaminofluorene) produced detectable UDS and survival was at 94.9 percent. Heavy labeling, indicative of DNA replication and not repair, occurred in only 1.1 percent (168/15,500) of the cells screened.

DISCUSSIONS:

The following deficiencies in the study were noted It is not clear why the minimum criterion in the protocol for a positive result was 6 grains in 10 percent of the cells while the criterion applied to the assay was 6 grains in 14 percent of the cells. However, by either criterion, Baytan did not induce unscheduled DNA-synthesis in primary hepatocytes at concentration of 0.25 - 50 µg/ml. The treatment period was reduced from 18 to 17 hours (5 percent); its effect on the test is not known. In addition, results from individual replicates were not presented in order to determine the extent of variability among replicates.

CONCLUSIONS:

KWG 0519 (Baytan) did not induce unscheduled DNA-synthesis (UDS) in primary rat hepatocytes at a concentration range between 50 and 0.25 µg/ml suggesting that the test material did not cause DNA-damage. Higher concentrations of the test material were too cytotoxic to permit interpretation.

CLASSIFICATION: Acceptable.

TABLE 1. Summary of Data on UDS in Rat Hepatocytes

Test Material	Concentration	UDS Avg. grains/ nucleus[a]	Percent nuclei[b] with ≥ 6 grains	Percent nuclei[b] with ≥ 20 grains	Percent Survival
DMSO (Solvent Control)	1.0 percent	0.87	4.0	0.0	100.0
2-Acetylamino-fluorene (Pos. Control)	0.05 µg/ml	11.19	83.3	12.0	94.9
KWG 0519 (Baytan)	250.00	No hepatocytes survived		---	--
in µg/ml	190.00	Insufficient cells for analysis		--	38.1
	50.00	1.85	8.7	0	53.0
	25.00	1.43	5.3	0	82.2
	10.00	1.38	7.4	0	81.7
	5.00	1.15	3.3	0	90.7
	2.50	1.12	2.7	0	93.1
	1.00	1.23	1.3	0	95.6
	0.50	0.87	2.3	0	89.4
	0.25	0.63	0.7	0	94.0

[a]Average net from triplicate coverslips (150 cells).

[b]Average values of triplicate coverslips (50 cells/coverslip).

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DATA EVALUATION RECORD

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STUDY TYPE: Mutagenicity, micronucleus test with mice.

CITATION: Herbold B. 1979. Micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects. Report No. 8584, prepared by Bayer AG, Institute fur Toxikologie, Wuppertal-Elberfeld, Germany.

ACCESSION NUMBER: 071468.

LABORATORY: Bayer AG, Institute fur Toxikologie, Wuppertal - Elberfeld, Germany.

TEST MATERIAL: The test material was identified as KWG 0519 from batch 16010-77, with a purity of 96.5 percent.

PROTOCOL:

1. Mice of the NMRI strain were supplied by S. Ivanovas GmbH, Kisslegg/ Allgau. The animals were 8 -12 weeks old and weighed between 21 and 32 g. Animals were housed 3 to a cage and kept in rooms maintained at 23 - 25° C, and about 60 percent relative humidity with a 12-hour light/dark cycle. Food and water were provided ad libitum.

2. The test material was mixed in 0.5 percent Cremophor emulsion and administred by stomach tube to groups of 5 animals/sex/dose at 0, 350, and 500 mg/kg. Endoxane, the positive control compound, was dissolved in distilled water and administered orally at 100 mg/kg, based on the active ingredient cyclophosphamide.

3. The animals were treated again after 24 hours with the same dosing regimen and sacrificed 5 hours later. The femurs from each mouse were excised and bone marrow smears were prepared according to the method of Schmid.[1] From each mouse, 1,000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei and the number of normochromatic erythrocytes per 1000 PCEs determined.

[1] Schmid W. 1979. The micronucleus test. In Kilbey BJ, et al. Handbook of Mutagenicity Test Procedures, Elsevier Sci. Pub. Co. Amsterdam, NY., Oxford 1979, pp. 235-242.

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4. The results were statistically evaluated using Wilcoxcon's non-parametric ranking test and a difference was considered significant at $p < 0.05$.

RESULTS:

It was stated that all KWG 0519 treated mice lost weight, about 1 - 6 g per animal, but individual data were not presented for all mice. In addition, a male from the 350 mg/kg group was reported to be very drowsy and a second male from the 500 mg/kg group died from acute toxicity following the second dosing. However, the physical appearance, motor activity and appetite of the other animals were similar to those of the negative control animals. The results indicated that the number of normochromatic erythrocytes per 1,000 PCEs in KWG 0519 treated animals were similar to the negative controls (Table 1). This ratio in positive control was significantly different from the negative control ($p < 0.05$, Wilcoxon ranking test). There were no biologically or statistically significant differences between KWG 0519 and negative control treatment groups for the number of micronucleated normochromatic or polychromatic erythrocytes (Table 1). Endoxan, the positive control, produced a significant increase in micronucleated cells at 100 mg/kg, indicating increased chromosome breakage.

DISCUSSIONS:

This study was conducted because a previous study (Report No. 7588 dated June 9, 1978) showed some evidence of bone marrow depression at KWG 0519 dosage of 2 x 350 mg/kg. Bone marrow depression was not obtained in the present study at treatment levels of 2 x 350 and 500 mg/kg. Although one animal in the 2 x 500 mg/kg treatment group had 2945 normochromic erythrocytes per 1,000 PCE's. This elevated ratio was considered to be a result of other pathological processes not related to compound administration. As indicated also in the next study the sampling periods in this experiment should have been extended to 8-12 hours, as recommended in the assay of Heddle and Salamone[2]. Consequently, the sampling intervals used in this study may have not been adequate for micronucleus production.

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[2]Heddle JA, Salamone MF. 1981. The micronucleus assay. I. *In vivo*. In Stich, HF and San RHC, eds. Short-term tests for chemical carcinogen, Springer-Verlag, NY pp. 247-254.

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TABLE 1. Group Mean Results of the Micronucleus Test in Mice with KWG 0519 Treatment

Test Group	Dosage (mg/kg)	Number of Normochromatic Erythrocytes 1000 PCEs	Micronucleated Cells/1000	
			Normochromatic-Erythrocytes	PCEs
Negative Control	0	845.8	1.08	2.40
KWG-0519	350	759.9	1.85	2.50
KWG-0519	500	1047.2	1.55	1.67
Endoxane	100	1116.4**	1.03	40.20*

* $p \leq 0.05$ (Wilcoxon ranking test).
** $p \leq 0.01$ (Wilcoxon ranking test).

CONCLUSIONS:

Under the condition of this mouse micronucleus test, Baytan did not have a mutagenic effect at 2 x 350 or 500 mg/kg. However, the sampling intervals of 6 hours, used in this study, may have not been adequate for micronucleus production. Moreover, methodology providing a positive response with a potent clastogen such as endoxane, does not ensure a similar response by a test material with a lesser clastogenic potential.

CORE CLASSIFICATION: Unacceptable.

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity, Rec-assay with Bacillus subtilis and Reversion assay with Salmonella typhimurium.

CITATION: Nagane M, Hatanaka J, Iyatomi A. 1982. KWG 0519 mutagenicity test on bacterial system. Report No. 220 from Nihon Tokushu Noyaku Seizo KK. Agricultural Chemicals Institute, Toxicological Laboratory, Japan.

ACCESSION NUMBER: 071468.

LABORATORY: Nihon Tokushu Noyaku Seizo K.K. Agricultural Chemicals Institute, Toxicological Laboratory, Japan.

TEST MATERIAL: The test material was identified as KWG 0519, beta-4(4-chlorphenoxy)-α-(1,1-dimethylethyl)-1H,1,2,4-triazole-1-ethanol, technical grade from batch Pt. 816066128, with a 97.5 percent purity.

PROTOCOL:

1. Rec-assay. Overnight cultures (unspecified media and temperature) of B. subtilis strains NIG17 (Rec+) and NIG45 (Rec-) were streaked onto a solid agar plate. The test compound was absorbed on to a paper disc [presumably, the disc was placed in the center of the streaks from strain NIG17 and NIG45] at a concentration of 200 µg/disc, and the plates were incubated at 37° C overnight. After incubation, the distance of growth inhibition was measured for NIG17 and NIG45, and compared with results of the furylfuramide (AF-2) positive control test.

2. Reverse Mutation Assay. Using the battery of S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and E. coli B/r Try-Hcr-, the authors performed the plate incorporation assay essentially as described by Ames et al. Concentrations of 0, 5, 10, 100, 500, 1,000, or 5,000 µg/plate were used in the assay and all strains scored for mutation reversion without metabolic activation. Strains TA98 and TA100 were also scored with metabolic activation. Activation was achieved using the S9 fraction from liver homogenates of rats treated with a polychlorinated bipnenyl (unspecified concentration and type). The following chemicals were used as positive controls for the indicated strains: strain TA1535, beta-propiolactone (beta-PL); strain TA1537, 9-aminoacridine (9-AA); strain TA1538, 2-nitrofluorene (2-NF); strains TA98 and TA100, and E. coli, AF-2. Mutagenicity Assay (with metabolic activation): strains TA98 and TA100, 2-acetylaminofluorene (2-AAF).

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RESULTS:

In the Rec-assay, KWG 0519 failed to inhibit either B. subtilis strain NIG17 (Rec^+) or NIG45 (Rec^-) at a concentration of 200 µg/disc (Table 1). AF-2 innibited the growth of both strains at 0.2 µg/disc with innibition in NIG45 (Rec^-) being five-fold that of NIG17(Rec^+). The assay was performed only in the absence of metabolic activation.

In the reverse mutation assay (Table 2), there was no evidence that KWG 0519 induced mutagenic activity in the various mutant strains of

TABLE 1. Rec-assay with KWG 0519 in Bacillus subtilis

Compound	Concentration in Disc (µg)	Inhibition Zone (mm)		Difference (mm)
		NIG17	NIG45	
KWG 0519	200.0	0	0	0
AF-2 (Positive Control)	0.2	4	22	18

Salmonella and E. coli without S9 activation. The mutagen/carcinogen 2-AAF was mutagenic for strains TA98 and TA100 only after S9 activation. These results provided evidence that the tester bacteria were capable of responding to a mutagenic insult. At a concentration of 5,000 µg/plate, KWG 0519 was extremely cytotoxic, but at lower concentrations it was not, thereby, indicating that the treatment emcompassed an adequate testing range.

DISCUSSIONS:

The Ames assays adequately demonstrated that KWG 0519 lacked mutagenic activity in this test system at the dose concentrations used. Within the limitations of the Rec-assay, DNA damage did not occur at the single concentration test of 200 µg/disc. However, a range of concentrations should have been used in the Rec-assay and the study should have included a test with metabolic activation.

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TABLE 2. Reverse Mutation Induced in Salmonella Strains by KWG 0519

Concentration (µg/plate)	S9	Average Revertants Induction by KWG 0519					
		TA100	TA1535	TA98	TA1537	TA1538	B/r
KWG 0519							
0.0	-	115.5	13.5	17.0	8.5	16.0	13.5
0.0	+	224.0	24.0	35.0	9.0	25.0	30.0
5.0	-	133.5	12.5	20.5	2.5	10.0	16.5
5.0	+	217.5	26.5	26.0	7.0	14.5	20.5
10.0	-	110.0	10.0	16.5	4.5	11.0	14.0
10.0	+	198.5	20.0	33.5	6.5	18.0	32.5
100.0	-	123.5	12.0	18.0	5.5	8.0	18.0
100.0	+	209.5	22.5	28.5	11.0	18.0	38.5
500.0	-	121.0	12.0	18.5	4.5	6.5	15.5
500.0	-	186.5	18.0	31.5	7.0	21.5	43.0
1,000	-	126.5	9.0	20.5	3.5	5.0	16.0
1,000	+	152.0[b]	[a]	17.0	1.5	13.0	28.5
5,000	-	[a]	[a]	[a]	[a]	[a]	[a]
5,000	+	[a]	[a]	[a]	[a]	[a]	[a]
Positive Controls[c]							
AF-2 0.10	-	1020	-	-	-	-	
B-PL 200	-	-	>2,000	-	-	-	-
AF-2 0.05	-	-	-	147	-	-	
9-AA 50	-	-	-	-	146.7	-	-
2-NF 50	-	-	-	-	-	>2,000	-
AF-2 0.20	-	-	-	-	-	-	523
2AAF 50	+	541.5	0	766	-	-	-
2AAF 50	-	97.5	0	14	-	-	-

[a] Excessive lethality, therefore unscorable.

[b] Only 1 plate could be scored.

[c] See protocol for abbreviations.

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More inclusive DNA-damaging assays such as those described by Felkner[1] could have provided more conclusive evidence of the absence or presence of DNA-damaging activity.

CONCLUSIONS:

In the Rec-assay, the test material KWG 0519 did not produce a DNA-damaging effect in the absence of metabolic activation with B. subtilis strains NIG17 [M-17] and NIG45 [M-45] at a single concentration of 200 µg/disc. However, only one dosage was used and the assay did not include a test with metabolic activation. In an Ames assay, the test material was not mutagenic in the presence or absence of metabolic activation to five S. typhimurium strains or E. coli strain B/r Try⁻ Hcr⁻ at five concentrations ranging from 5 to 1,000 µg/plate. Cytotoxicity occurred at 5,000 µg/plate.

CLASSIFICATION: Reverse Mutation Assay: Acceptable. Rec-assay: Unacceptable.

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[1] Felkner, IC et al., in I.C. Felkner, ed. Microbial Testers: Probing Carcinogenesis. Dekker, N.Y./Basel 1981, pp. 93-119.

C.G. 3/7/85

004695

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity, micronucleus test with mice.

CITATION: Herbold B. 1978. Micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects. Report No. 7588 prepared by Bayer AG, Institute fur Toxikologie, Wuppertal-Elberfeld, Germany.

ACCESSION NUMBER: 071468.

LABORATORY: Bayer AG, Institute fur Toxikologie, Wuppertal - Elberfeld, Germany.

TEST MATERIAL: The test material was identified as KWG 0519 from batch 16001/76, with a purity of 93.7 percent.

PROTOCOL:

1. Mice of the NMRI strain were supplied by S. Ivanovas GmbH, kisslegg/Allgau. The animals were 8 - 12 weeks old and weighed between 24 and 36 grams. Animals were housed 5 to a cage and kept in rooms maintained at 22 ± 2° C and an average relative humidity of 60 percent with a 12 hour light/dark cycle. Food and water were provided ad libitum.

2. The test material was mixed in 0.5 percent Cremophor emulsion and administered by stomach tube to groups of 5 animals/sex/dose at 0, 175 and 350 mg/kg. Endoxane, the positive control compound, was dissolved in distilled water and administered orally at 80 mg/kg to 5 males and 5 females.

3. The animals were treated again after 24 hours with the same dosing regimen. Six hours following the second oral administration of the test compounds, all mice were decapitated and their femurs excised. Bone marrow smears were prepared according to the method of Schmid[1]. From each mouse, 1000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei and the number of normochromatic erythrocytes per 1,000 PCEs determined.

4. The results were statistically evaluated using Wilcoxon's non-parametric ranking test where a difference was considered significant at $p < 0.05$.

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[1] Schmid W. The micronucleus test in Kilbey BJ, et al. - Handbook of Mutagenicity Test Procedures, Elsevier Sci. Pub. Co. Amsterdam, NY, Oxford 1979, pp. 235-242.

RESULTS:

The results indicated an increased number of normochromatic erythrocytes per 1,000 polychromatic erythrocytes at the 350 mg/kg test group (Table 1). However, this increase was not statistically significant when compared to the control. There was a statistically erythrocytes at the 175 mg/kg test group when compared to controls.

There were no biologically or statistically significant differences in the number of micronucleated polychromatic erythrocytes between KWG 0519 and control treatment groups, indicating no mutagenic effect (Table 1). Endoxane, the positive control, produced a significant increase in micronucleated polychromatic cells at 80 mg/kg, indicating increased chromosome breakage.

DISCUSSION:

The number of micronucleated cells per normochromatic erythrocytes (normalized to 1,000 cells) was increased to a significant level at a dosage of 2 x 175 mg/kg ($p \leq 0.05$) compared to negative control. The authors stated that this parameter was not relevant to an assessment of mutagenic effect due to the duration of the polychromatic phase. However, nuclei are not expelled from erythrocytes in these animals until 8-12 hours following the last mitosis forming erythrocytes[1]. Therefore, by sacrificing only 6 hours after the last treatment the full effect of the last dose administered could not have been assessed. In our opinion, the single sacrifice time of 6 hours following the last dosage does not allow for adequate determination of micronucleus production in either polychromatic or normochromatic cells.

The ratio of polychromatic to normochromatic erythrocytes was also investigated to determine the general activity of the test compound on bone marrow erythropoiesis. A depression in erythropoiesis resulted from test compound treatment at the highest dose level (350 mg/kg) and also from endoxane (positive control) treatment, as demonstrated by the elevated number of normochromic erythrocytes per 1,000 PCEs. This effect, although not statistically significant, reflected a general toxic effect but not mutagenic activity. Since toxic effects were produced at the highest concentration administered, the dosage was at an acceptable level.

[1] Heddle JA, Salamone MF. 1981. The micronucleus assay. I. *In vivo*. In Stich HF and San RHC, Eds. Carcinogen, Springes-Verlag, NY, pp. 247-254.

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TABLE 1. Group Mean Results of the Micronucleus Test in Mice with KWG 0519 Treatment

Test Group	Dosage (mg/kg)	Number of Normochromatic Erythrocytes/1000 PCEs	Micronucleated Cells Per 1000	
			Normochromatic-Erythrocytes	PCEs
Negative Control	0	804	0.66	1.9
KWG-0519	175	592.4	2.64*	2.6
KWG-0519	350	1548.2	1.69	1.9
Endoxane	80	1130.4	1.47	42.5**

* $p \leq 0.05$ (Wilcoxon ranking test).
** $p \leq 0.01$ (Wilcoxon ranking test).

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CONCLUSIONS:

Under the conditions of this mouse micronucleus test, Baytan did not have a mutagenic effect at 2 x 175 or 350 mg/kg. However, the number of normochromatic cells per 1000 polychromatic cells increased at 350 mg/kg, suggesting a depression of erythropoiesis. The positive control, endoxane, produced a significant increase ($p \leq 0.01$) in micronucleated cells at 80 mg/kg, as compared to negative control, thereby, indicating that the assay was capable of producing a positive response. However, the sampling intervals of 6 hours, used in this study, may have not been adequate for micronucleus production. Moreover, methodology providing a positive response with a potent clastogen, such as endoxane, does not ensure a similar response by a test material with a lesser clastogenic potential.

CORE CLASSIFICATION: Unacceptable.

G.C. 3/1/85

DATA EVALUATION RECORD

00469

STUDY TYPE: Three-generation reproduction study.

CITATION: Loser E. 1979. MEB 6447 (Bayleton, the parent compound of Baytan): Multigeneration reproduction study on rats. An unpublished report prepared by Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, FRG, for Mobay Chemical Corporation, Stilwell, Kansas.

ACCESSION NUMBER: 071468.

SEE 9/3/85

LABORATORY: Bayer AG, Institut fur Toxikologie, Wuppertal-Elberfeld, Federal Republic of Germany; Consultox Laboratories Ltd., London, England (histopathology).

TEST MATERIAL: The test material was identified as Bayleton (MEB 6447) which has the chemical name 1-(1,2,4-triazolyl-1)-1-(4-chlorophenoxy)-3, 3-dimethyl-butanone-2. This compound has as one of its metabolites, Baytan, the chemical of interest. Bayleton was provided by Bayer AG as a technical grade (batch 16002/75) in April, 1975.

PROTOCOL:

1. The test diets were prepared by blending a 90 percent premix of the test material in [redacted] with powdered rodent food (Altromin R). A control diet and 3 test diets (0, 50, 300, and 1800 ppm Bayleton) were prepared weekly and supplied to the animals ad libitum. Gas chromatographic analysis performed by the investigators at 4 points during the study indicated that the administered diets were within 20 percent of their desired concentrations.

2. At the initiation of the study, 120 SPF Wistar W. 74 rats (40 males and 80 females) were provided by Winkelmann. The rats were 32-39 days old, 45-55 g in weight, and were evenly divided into 4 groups (10 males, 20 females in each group). During the non-mating periods, the rats were housed individually in plastic cages; during mating, 2 females and one male were housed in a single cage.

3. Each group maintained on one of the 4 test diets (0, 50, 300, or 1800 ppm). ting was initiated when the rats were 100 days old; two females and one male were housed together for 19-20 days. The males in one group were rotated so that each female was paired with 3 different males for a period longer than one estrus cycle.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

4. After delivery of the first litter, the pups were counted and weighed. On the fifth day after delivery, litters of more than 10 pups were reduced to 10; weights were measured again. Lactation was allowed for 4 weeks after which time the pups of the first litter were sacrificed. A second mating was conducted, and the offspring weighed and counted as before during a 4-week lactation period. After weaning, the sexes were separated, and 4 weeks later 10 males and 20 females were selected from each group to be parents of the next generation. After the weaning of the second litters, dams were sacrificed. This procedure was repeated for a total of 3 generations.

5. The following parameters were monitored at the specified points during the study: 1) body weights of parents, weekly; 2) body weights of offspring, at birth, 5 and 7 days after birth, weekly thereafter; 3) examination of offspring for malformations, at birth and during lactation period; 4) fertility (no. of pregnancies/no. of mated rats), at each mating; 5) litter size, at delivery and at 5, 7, 14, 21, and 28 days after delivery; 6) gross pathology, on any animal dying during the study and on one male and female pup of each third generation litter at weaning; 7) histopathology, on one male and female pup of each third generation litter at weaning.

6. Differences between values obtained for control and treated groups were tested for statistical significance using the U test of Wilcoxon, Mann, and Whitney.

RESULTS:

First Generation (F_0)

Clinical Observations/Mortality: No differences in the behavior or appearance of the parental animals were noted between the control and treated groups. One female of the 300-ppm group died after the second mating; no cause of death was given.

Body Weights: No differences between control and treated groups were apparent in the mean body weights during the premating period or during the first mating. Mean body weights were lower than control ($p<0.01$) in the females of the 1800-ppm group during the second premating, mating, gestation, and lactation periods. The weights of these animals were approximately 15 percent lower than control at sacrifice.

Fertility: No differences occurred in the fertility index (no. of pregnant/no. of mated female rats) among the groups during the first mating. In the second mating, the fertility index of the 1800-ppm group was lower than control (12/20 vs 19/20), but the difference was not statistically significant.

Litter Size, Weight and Appearance: A significant decrease in the number of offspring per animal was noted for the 1800-ppm group for the first litter at birth and 5 days later, and for the second litter at 5 days

post-partum (Table 1). No differences in average body weight at birth were observed among the groups in either the first on second litters. No mention was made of the results of the examination of the offspring for malformations.

TABLE 1. Litter Size - F_1 Litters

Group/Litter	Average Number of Pups Per Litter		
	At Birth	At 5 days[a]	
		Before Reduction	After Reduction
Control/1st	10.3	10.1	9.2
/2nd	9.2	8.9	8.3
50-ppm/1st	9.7	9.4	9.1
/2nd	9.2	9.6	8.3
300-ppm/1st	9.6	9.3	8.5
/2nd	8.9	8.8	7.7
1800-ppm/1st	8.7**	7.3**	7.3**
/2nd	8.6	7.1*	6.9-

*p<0.05.
**p<0.001.

[a]Litters of more than 10 pups were reduced to 10, 5 days after birth.

Lactation: A decrease in survival of the pups of the 1800-ppm group was noted during the lactation periods of both litters. For the first litter, the control group survival (pups alive at 4 weeks/pups alive after reduction on day 5) was 97.8 percent; the survival for the 1800-ppm group was 90.7 percent. For the second litter, the values for control and 1800-ppm group were 84.2 and 56.4 percent, respectively. These effects on the survival of the 1,800-ppm litters were significant at p<0.01. Weight gain during lactation was also depressed for the 1800-ppm litters of the first mating (but not the second); weight gain was approximately 10 percent less than control (p<0.05).

Second Generation (F_1)

Clinical Observations/Mortality: No clinical observations were reported. Four mortalities occurred (3 males, one each in the control, 50, and 1800-ppm groups; and 1 female in the 50-ppm group) and were attributed to bronchopneumonia.

Body weights: Depression of body weight gain was seen throughout the premating, gestation, and lactation periods for both litters in the male and female animals in the 1800-ppm group (p<0.01). Weight gain in the other groups was similar to control.

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Fertility: Fertility values were significantly lower in both matings for the animals of the 1800-ppm group. Only one 1800-ppm group female became pregnant in the first mating; none of the 20 females became pregnant in the second. Although significant differences did not occur in the other dosed groups, the fertility rate of the 50-ppm animals was lower than control in the second mating (63.2 percent vs 85 percent).

Litter Size, Weight and Appearance: The single litter of the 1800-ppm group had a similar number of pups and a similar average weight to the control litters. Litters of the other dosed groups produced by the first and second matings also were of similar size and average weight to the control litters. No signs of malformations were noted in any of the litters.

Lactation: Survival during lactation of the offspring of the treated animals (including the single 1800-ppm litter) was similar to the control survival for both the first and second litters. Weight gain during lactation of the offspring of the first litters was also similar among the control and treated groups. During the lactation period of the second litters, weight gain of the pups of the 300-ppm group was significantly lower ($p<0.05$) at 5 days, 1 week, 2 weeks, and 3 weeks post-partum. The difference was approximately 15 percent.

Third Generation (F_2)

Clinical Observations/Mortality: No clinical observations were reported. One female of the 300-ppm group died prior to the first mating; no cause of death was established by the investigators.

Body Weights: No significant differences were noted in weight gain between the control and 50-ppm and 300-ppm groups. Because none of the 1800-ppm animals reproduced in the F_1 generation matings, there was no 1800-ppm group in this generation.

Fertility: No differences were evident in the fertility values calculated for the remaining groups for either the first or second mating.

Litter Size, Weight and Appearance: No differences were seen in the numbers of pups per litter or the average weight per pup between the control and treated groups in either mating. No signs of malformations were noted in any of the litters.

Lactation: Survival during the lactation periods of both matings was similar between the control and treated groups. Weight gain of the pups during lactation of the first litters was similar among the groups; however, weight gain of the pups of the 300-ppm group was significantly lower ($p<0.05$) during the second lactation period. The average weight of the 300-ppm pups was approximately 10 percent lower than control 4 weeks post-partum.

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Pathological Examinations: One male and one female pup from each of 10 litters produced by the F_2 generation animals (i.e., the F_{3b} litters) were sacrificed 4 weeks after birth and examined. No lesions were found at necropsy that could be attributed to the test material. Histologic examination of selected tissues (thyroid, heart, thymus, lung, liver, spleen, kidneys, adrenals, and gonads) did not reveal any compound-related lesions.

DISCUSSION:

Discussion of the results of this study should be prefaced with the comment that the study was conducted on Bayleton of which Baytan is a metabolite. The applicability of the results to assessing the reproductive toxicity of Baytan is questionable.

The study adequately identified the following effects of the dietary administration of Bayleton:

1. A toxic effect on parental animals at 1800 ppm. During the premating, gestation, and lactation periods, the female animals of the F_0 generation, and animals of both sexes in the F_1 generation, showed significant depressions in body weight gain at the 1800-ppm level.

2. An effect on reproduction at 1800-ppm. The average number of pups per litter at birth was significantly lower for the first litters of the F_0 generation, and the survival and weight gain of these pups during lactation were significantly lower than that of the control animals. In the second litters, the number of offspring per litter was significantly lower than control at birth and at 5 days postpartum. Although weight gain was not affected in the second litters, survival among the offspring of the 1800-ppm group was 56.1 percent compared to a control rate of 84.2 percent (significant at $p < 0.01$). In the matings of the animals of the 1800-ppm group, only one female because pregnant in the first mating and none in the second. Hence, by the second generation, reproduction in the 1800-ppm group had ceased.

3. An effect on reproduction at 300 ppm. Offspring of the second litters of the F_1 and F_2 generations (the F_{2b} and F_{3b} litters) showed significant depressions in weight gain during their respective lactation periods.

Although the experimental design of this study was adequate in that effects were observed at 300 and 1800 ppm, the design was limited in that several important parameters that are usually included in reproduction studies were not included in this study. These were as follows:

1. The number of attempts at mating during the mating periods was not examined. Thus, the fertility index had as its denominator the number of paired animals rather than the number of matings and was not a sensitive measure of fertility.

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2. The number of stillborn and/or cannibalized pups at birth was not reported.

3. The lengths of the gestation periods were not reported.

4. Pups were weighed as a group at birth and during lactation, rather than individually; hence, the number of undersize offspring was not reported.

In addition, selection bias may have been introduced into the study at three points, further lessening its sensitivity to detect an effect. The method of selecting the animals as parents for subsequent generations was not specified, the method of selecting the pups to be sacrificed during the reduction of litter size was not given, nor was the method of selecting the pups from the F_{3b} litters for histopathologic examination stated. Furthermore, the number of tissues examined histopathologically is in question. The report procedures specified 15 tissues and gonads were examined; the results section stated that 8 tissues and gonads were examined; the histopathology appendix listed 16 tissues plus gonads. The results of the histopathology examination could not be used to determine the number of tissues examined because only positive findings were reported.

The biological mechanisms of the reproductive effects that were detected in this study cannot be fully evaluated because of limitations in the experimental design. As an example, the decreased fertility noted at 1800-ppm may have resulted from a reduction in viable sperm in the males, or from an effect on the ability of the females to ovulate or sustain pregnancy. In addition, the observed decrease in weight gain in the offspring of the F_1 and F_2 generations may have been a result of decreased lactation or a direct toxic effect on the offspring.

Overall, although this study clearly indicated adverse effects on certain reproduction parameters at 300 and 1800 ppm, other parameters essential to a more complete evaluation of potential reproductive toxicity were not assessed. These included the ratio of unsuccessful and successful mating, the number of stillborn and/or cannibalized pups, the lengths of gestation, individual pup weights, as well as any information permitting the assessment of the reduction in fertility and poor survival of the offspring.

<u>CONCLUSIONS</u>:

The applicability of the results of this reproduction study on Bayleton to the assessment of Baytan is questionable. The results of this study indicated that dietary administration of Bayleton to rats had an adverse effect on reproduction at 300 and 1800-ppm, but due to experimental design limitations of the study a no-effect level could not be identified.

<u>CORE CLASSIFICATION</u>: Supplementary data.

005337

DATA EVALUATION RECORD

Reverse Mutation Test

Tanahashi, N., and Moriya, M. (1982) Triadimenol microbial mutagenicity studies. An unpublished report submitted to the Agency by Mobay Chemical Corporation, prepared by Institute of Environmental Toxicology. Report No. 2578, dated December 3, 1982. EPA Accession No. 073381.

Test Chemical:

Triadimenol [1-(4-chlorophenyl)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanol]. Technical 97.5% ai.

Experimental Protocol:

The test chemical was dissolved in dimethyl sulfoxide (DMSO), and was tested up to 5000 ug/plate at which the bacteria showed toxicity in the preliminary toxicity test. The test was performed with and without metabolic activation. According to the author "five strains of the Ames' TA series of S. typhimurium were used to investigate the mutagenic potential of triadimenol in reverse mutation tests. These strains were TA 1535, TA1537, TA1538, TA98, and TA100. In addition, E. coli WP2 hcr (uvrA) requiring tryptophan was also employed.

"These six strains stored at -80°C were inoculated on nutrient broth liquid medium and cultured by shaking at 37°C over night. For the S. typhimurium strains a sterile solution of 0.5 mM L-histidine-0.5 mM D-biotin was added to molten soft agar (0.6% agar and 0.5% NaCl) at the rate of 1/10 (v/v), and for the E. coli strain a 0.5 mM L-tryptophan solution was added at the same rate. The prepared soft agar was referred to 'top agar.'

"For the preparation of a liver metabolic activation system, Sprague-Dawley male rats (7 weeks old, average body weight 244 g) were given a single intraperitoneal injection of a polychlorinated biphenyl (PCB) mixture (Aroclor 1254) at a dosage of 500 mg/kg. In the evening of the fourth day after the injection, the food was removed. On the fifth day the animals were killed by cervical dislocation and immediately livers were removed. The livers were perfused with an ice-cold 0.15 M KCl solution and were homogenized in three volumes of the same solution (3 ml/g wet liver). The homogenate was centrifuged for 10 minutes at 9000 x g. The 9000 x g supernatant (S-9 fraction) was used in the experiment. All the steps were performed below 5°C with cold and sterile solutions and glassware. The components of 1 ml of the metabolic activation system (S-9 mix) were as follows: 0.1 ml S-9 fraction, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADPH, 4 mM NADH, and 100 mM sodium phosphate (pH 7.4).

"To 2 ml of the molten top agar were added 0.1 ml of bacteria suspension, 0.1 ml of a solution of the compound, and 0.5 ml of 100 mM sodium phosphate (pH 7.4) or the S-9 mix. The contents were mixed uniformly and poured onto the surface of a minimal agar plate. All plates were incubated at 37°C for 2 days, after which the number of revertant colonies was counted. The following compounds were used as positive controls: AF-2(2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide); ENNG(N-ethyl-N'-nitro-N-nitrosoguanidine); 9-AA(9-aminoacridine); 2-NF(2-nitrofluorene); 2-AA(2-aminoanthracene). The results were induced based on reproducibility and relation between doses and reactions."

Results and Discussion:

The results are shown in table 1. In general, at concentrations ranging between 5 and 1000 ug/plate, the test chemical did not show definitive increase in the number of revertant colonies under the test conditions, in the presence and absence of the metabolic activation system. The next higher concentration was toxic to the test organisms.

Sporadic increase in the number of revertant colonies was observed for S. typhimurium TA 1535. In this strain the number of revertant colonies appeared to be doubled at 500 ug/plate without metabolic activation. Slight increase (less than 2X) in the number of revertant colonies for the same strain was observed at the concentration of 1000 ug/plate. However, because of the lack of a dose-response relationship, the limited number of replicates, and the dose spacing, it could not be ascertained as to whether this increase is a result of mutagenic potential of the test chemical.

At 1000 ug/plate, the number of revertant colonies the E. coli WP2 hcr was doubled without metabolic acti[illegible]ion. This increase may be considered as a possible mutagenic response. If an adequate number of replicates had been used, and concentration ranging between 500 and 2000 ug/plate were tested with E. coli, a more definitive conclusion could have been made with respect to the mutagenic potential of this chemical.

Table 1. Reverse mutation tests with or without a liver metabolic activation system (S-9 Mix)**

Compound	ug/plate	S9-Mix	No. of revertant colonies/plate					
			base-change type			frameshift type		
			TA100	TA1535	WP2 hcr	TA98	TA1537	TA1538
Control (DMSO)		-	108 110	4 3	10 8	31 31	5 8	16 9
triadimenol	5	-	113 107	5 8	10 9	35 22	2 6	17 12
	10	-	111 101	7 3	7 8	35 32	6 8	12 13
	50	-	120 126	7 3	8 18	29 22	3 2	16 17
	100	-	108 98	3 3	11 11	24 27	2 5	19 16
	500	-	92 107	5 9	11 12	16 18	7 4	14 8
	1000	-	98 120	5 3	21 18	20 26	7 6	13 11
	5000	-	* *	* *	7* 9*	1* 5*	* *	* *
		-						
Control (DMSO)		+	94 108	7 5	11 10	39 29	5 7	34 29
triadimenol	5	+	117 104	5 6	12 14	43 37	6 5	16 38
	10	+	115 94	7 7	10 11	50 39	9 9	36 29
	50	+	100 108	1 7	8 8	17 42	6 8	29 26
	100	-	106 106	8 6	17 14	35 34	5 4	38 27
	500	-	106 109	6 5	16 9	44 32	9 9	18 26

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Table 1. Reverse mutation tests with or without a liver metabolic activation system (S-9 Mix)** (cont'd)

Compound	ug/plate	S9-Mix	No. of revertant colonies/plate					
			base-change type			frameshift type		
			TA100	TA1535	WP2 hcr	TA98	TA1537	TA1538
triadimenol	1000	-	103	10	11	34	3	22
			83	8	15	42	9	39
	5000	-	*	*	5*	8	*	27
			*	*	8*	11	*	16
		+						
Compound			AF-2	ENNG	AF-2	AF-2	9-AA	2-NF
g/plate			0.01	10	0.04	0.1	80	2
No. of col/ plate		-	407	1178	175	299	2436	230
			402	788	200	279	1956	302
Compound			2-AA	2-AA	2-AA	2-AA	2-AA	2-AA
g/plate			0.5	2	40	0.5	2	0.5
No. of col/ plate		+	348	197	>1000	137	67	160
			351	198	>1000	176	66	162
No. of col/ plate		-	125	4	11	16	4	21
			123	9	22	35	9	25

*Cytotoxic concentrations.

**This table was taken from the original report with slight modifications.

Slight increase (less than 2X) in the number of revertant colonies was observed for E. coli with metabolic activation at concentration ranging between 100 and 1000 ug/plate but was not dose-dependent.

Conclusion:

The test as performed is inconclusive. The test may be repeated for S. typhimurium TA 1535 and for E. coli WP2 hcr at concentrations well spaced between 500 and 2000 ug/plate, with an adequate number of replicates, in the presence and absence of metabolic activation. Variable concentrations of the metabolic activation systems are also recommended.

Core Classification:

Unacceptable.

References:

1. Ames, B.N.; McCann, J., and E. Yamasaki: Mutation Res., 31, 347-364, 1975.

2. Guidebook for Microbial Mutagenicity Study (Chem. Res. Sec., Industrial Safety and Health Dept., Ministry of Labor, eds.), Central Workers' Accident Prevention Association, 1980.

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