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FINAL

DATA EVALUATION REPORT

CAPTAN

Study Type: Mutagenicity: Heritable Translocation Assay in Mice

Prepared for:

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GUIDELINE SERIES 84: MUTAGENICITY
HERITABLE TRANSLOCATION

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Heritable translocation assay in mice

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 159

MRID Number: [redacted]00131710

TEST MATERIAL: Captan

SYNONYMS: cis-N-trichloromethylthio-4-cyclohexene, 1,2-dicarboximide

SPONSOR: U.S. Environmental Protection Agency, Research Triangle Park, NC/ICI Americas Inc., Wilmington, DE

STUDY NUMBER: LSU-3493; EPA Contract No. 68-01-2458

TESTING FACILITY: SRI International, Menlo Park, CA

TITLE OF REPORT: Mutagenesis Studies of Pesticide Compounds--Mouse Heritable Translocation Test--Captan

AUTHOR: Jorgenson, T.A., Rushbrook, C.A., Jones, D.C.L., and Skinner, W.A.

REPORT ISSUED: May 1980

CONCLUSIONS--EXECUTIVE SUMMARY: No conclusions can be reached regarding the potential of captan to induce reciprocal translocations in the male offspring of F_0 males fed 2500 or 5000 ppm of the test material for 8 consecutive weeks. Owing to an accident, which occurred immediately after the conclusion of mating in the parental generation, approximately half of the high-dose group dams and their offspring were removed from the main group (5000 ppm subgroup A) and carried separately for the remainder of the study (5000 ppm, subgroup B). Adverse compound effects, which included decreased body weight, decreased body weight gain, and decreased food consumption were seen in F_0 males of the high dose group. Similarly, reduced fertility and reduced viable litters were observed in both high-dose subgroups. The evidence of overt compound toxicity at 5000 ppm captan clearly indicated that an appropriate high dose was tested. The low dose did not adversely affect body weight or food consumption; however, possible reproductive effects at 2500 ppm captan could not be assessed because of the lower than expected fertility rate

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(77.5%) in the vehicle control group and the relatively high incidence of F_0 control nonbreeder males (8 of 60).

In the F_1 generation, reproductive data for the vehicle control group were more consistent with the expected results for normal male mice; findings for the captan treatment groups were generally comparable to the vehicle control. Following two additional mating rounds, all identified presumptive translocation heterozygotes in the vehicle control and captan groups were subjected to cytogenetic analysis. The evaluation of meiotic metaphases yielded one positive reciprocal translocation in high-dose subgroup A. However, this finding could not be interpreted because a translocation figure was also observed in the vehicle control group. We assess that the spontaneous heritable translocation frequency noted in this study (i.e., 1 reciprocal translocation in 200 progeny, 5×10^{-3}) was well above the expected background frequency (2.3×10^{-4}-9.1×10^{-4}).[1] This high incidence, in conjunction with the poor reproductive performance of F_0 vehicle control males, seriously compromised the results.

Also of concern was the use of fewer F_1 subgroup B high-dose males (50 from subgroup B versus 200 from subgroup A) in the genetic screening phase of the study. We concede that the parental reproductive data for subgroup B were probably invalid since compound effects on reproductive parameters could not be distinguished from the effects of trauma. However, the rationale for excluding a high percentage of F_1 subgroup B males from critical mating phase of testing was not provided. Although the number of treated F_0 male offspring analyzed for translocation heterozygosity is not specifically addressed by Guidelines, the use of fewer F_1 subgroup B offspring decreased the representation of high-dose parental males and, therefore; reduced the possibility of determining whether the single translocation that was scored was artifactual or related to captan treatment.

Additionally, there was no information on test material purity, stability, homogeneity in dietary preparations, or verification of actual doses used in the study. A quality assurance/compliance with good laboratory practices statement was also not presented.

Based on these considerations, we conclude that the study is unacceptable. The study, therefore, does not satisfy Guideline requirements for genetic effects Category II, Structural Chromosome Aberrations.

STUDY CLASSIFICATION: The study is unacceptable; the results are inconclusive.

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[1]Generoso, W.M., Cain, K.T., Huff, S.W., and Gosslee, D.G. Heritable-Translocation Test in Mice in: Chemical Mutagens--Principles and Methods for Their Detection, A. Hollaender and F.J. de Serres, eds. (1978) Vol. 5:55-77, Plenum, NY.

A. MATERIALS:

1. Test Material: Captan

Description: None provided
Identification number: SX-640
Purity: Not reported
Receipt date: Not reported
Stability: Not reported
Contaminants: None listed
Solvent used: Corn oil/Basal diet (Purina "finely ground commercial diet")
Other provided information: Storage conditions of the test material were not furnished. Test material diets were prepared at 2-week intervals and refrigerated at 4°C until use. The report did not indicate whether stability, homogeneity, or actual concentrations of captan in feed were determined at any time throughout the study.

2. Control Materials:

Negative/Route of Administration: None

Vehicle/Final Concentration/Route of Administration: Corn oil at a final concentration of 3% was mixed with basal diet and fed to the vehicle control animals for 8 weeks.

Positive/Final Concentration/Route of Administration: Triethylene-melamine (TEM) was administered in drinking water at 0.32 mg/L for 2 weeks and at 0.124 mg/L for 2 weeks.

Note: Prior to receiving TEM, males in the positive control group were fed the 3% corn oil control diet for 4 weeks.

3. Test compound:

Route of Administration: Dietary preparations

Dose levels used: 2500 and 5000 ppm

4. Test animals:

(a) Species: mouse Strain ICR/SIM Age: 8-10 week (F_0 males); 10-12 weeks (females)
Weight: At dosing: 33.4-33.8 g (males); females not reported.
Source: Simonsen Laboratories, Gilroy, CA.

(b) No. animals used per dose: The methods used to identify and randomize the animals were not reported. Animals were assigned to the following study groups:

Test Group	Dose	F_0 Males/ Group	Females/ F_0 matings
Vehicle Control[a]			
Corn oil	3%	60	120
Positive Control[b]			
Triethylenemelamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	66	132
Test Groups[a]			
Low dose	2500 ppm	60	120
High dose	5000 ppm	61	122

[a]Vehicle and test material doses were administered in the diet for 8 weeks.
[b]TEM was administered in drinking water at 0.32 mg/L for 2 weeks followed by a 2-week administration of 0.124 mg/L. Prior to treatment with TEM, positive control males were fed the 3% corn oil control diet for 4 weeks.

(c) Animals properly maintained? Housing and environmental conditions were not reported.

B. TEST PERFORMANCE:

1. Heritable Translocation Assay:

(a) Dose selection/compound administration: The report stated that the doses used in this study were selected by personnel from the USEPA and the performing laboratory. F_0 males were fed dietary preparations of 3% corn oil or 2500 and 5000 ppm captan for 8 weeks. F_0 males in the positive control group were maintained on the 3% corn oil/basal diet for 4 weeks and administered 0.32 mg/L TEM in drinking water for 2 weeks, followed by a 2-week administration of 0.124 mg/kg TEM also in drinking water.

(b) Animal observations: Body weights and food consumption were recorded weekly throughout the 8-week treatment period. The report did not indicate whether other clinical observations were recorded.

(c) Mating:

(1) F_0 males: After treatment, each male in the two experimental groups and in the vehicle and positive control groups

were mated with two untreated virgin females for 1 week. At the conclusion of mating, F_0 males were discarded by an unspecified procedure. F_0 females were allowed to deliver and raise their litters to weaning age and were discarded. The number of pregnancies, number of nonbreeder F_0 males, average live litter size, and average number of weaned males per litter were determined.

(2) F_1 males (first mating): At maturity (10-12 weeks of age), 200 F_1 males from each group were housed with three untreated virgin females for a maximum of 4 weeks. Males selected for breeding included at least one representative from each liter (maximum of four from one litter) containing one or more males. Within litters, the more vigorous males were selected to improve breeding. Females were examined daily during the first two mating weeks for evidence of a vaginal plug; females with signs of matings were sacrificed 14 days after observation of vaginal plugs. Females with obvious pregnancies but no vaginal plug were sacrificed as soon as the pregnancy was apparent. Females with no signs of mating were sacrificed at week 4 postmating. Uteri from all females were examined for the number of live and dead implants.

(3) Screening of F_1 males for translocation heterozygosity: F_1 males were classified as normal, partially sterile, sterile, or nonbreeders as follows:

Partially sterile: Partially sterile males were identified using one of the three outlined criteria:

- If all three females were pregnant, at least two must have ≤ 9 live implants and one female must have ≤ 6 live implants.
- If two of three females were pregnant, one must have ≤ 9 live implants and the other must have ≤ 6 live implants.
- If one of three females was pregnant, the litter must contain ≤ 6 live implants.

Sterile: Males were categorized as sterile if evidence of mating (vaginal plug) was found but no females were pregnant.

Nonbreeder: Males were considered nonbreeders if there was no evidence of mating and no females were pregnant.

Normal: Males that did not fit one of the above categories were considered normal and were discarded.

(4) Remating of F_1 males: Males presumptively classified as sterile, partially sterile, or nonbreeders by either set of classification criteria were remated to three untreated females, evaluated, and screened as described. Questionable cases (i.e., if no distinction could be made between live or dead implants) were also remated.

Males classified as normal from the second mating were discarded; males classified as presumptive positives by the screening criteria were either carried through a third mating round or subjected to a cytogenetic evaluation. The same procedure was followed for the third mating round; however, all presumptive sterile, partially sterile, questionable, or nonbreeders were examined cytogenetically.

(d) Cytogenetic evaluation: After three rounds of mating, all vehicle control group males and all treated males suspected of translocation heterozygosity were subjected to a cytogenetic evaluation. Meiotic metaphases from representatives of the positive control group were also examined.

(1) Chromosome harvest/slide preparation: Testes were removed, weighed and placed in 2.2% sodium citrate; testes' weights were not reported. The tunica of each testis was punctured and tubules were rolled on a glass plate to release cells. Cell suspensions were centrifuged, resuspended in 1% sodium citrate fixed in methanol:acetic acid (3:1), dropped onto slides, and air dried. Slides were stained with 2% Giemsa, coverslipped, and coded.

(2) Slide analysis: Fifty meiotic metaphase plates (25/testis) were examined from each animal. If a translocation figure was seen before 50 cells were examined, the slide analysis was discontinued.

(e) Statistical analysis: There was no indication that the data were analyzed statistically.

C. REPORTED RESULTS

1. Animal observations: No animal deaths were reported. Throughout the 8-week premating period, the body weight for high-dose males was consistently lower than control; average body weight gain over the 8-week period was ~40% lower than control (Table 1), and was largely due to the transient effects on body weight that were seen during weeks 0 to 4. Our reviewers noted that body weight gain in the vehicle control appeared low; however, food consumption for this group was normal. The study authors stated that the body weight depression in the 5000 ppm group appeared to be due to the inability of the male mice to acclimate to the high level of captan in their diet. The decreased food consumption

TABLE 1: Representative F_0 Male Body Weight and Food Consumption During the Premating Period for Male Mice Fed Captan for 8 Consecutive Weeks in the Heritable Translocation Assay

Substance	Dose	Average Body Weight (g) at Week 0	Average Body Weight (g) at Week 4	Average Body Weight (g) at Week 8	Average Body Weight Gain (g)[a]	Average Food Consumption (gm/mouse/day at Week) 1	Average Food Consumption (gm/mouse/day at Week) 4	Average Food Consumption (gm/mouse/day at Week) 8
Vehicle Control								
Basal diet/3% Corn oil	--	33.8	34.6	39.2	5.4	4.01	5.28	5.55
Positive Control[b]								
Triethylenemelamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	33.4	35.8	39.8	6.4	4.05	5.33	5.74
Test Material								
Captan	2500 ppm	33.8	34.4	38.9	5.1	3.74	4.93	5.13
	5000 ppm	33.6	31.4	36.9	3.3	3.25	4.45	4.83

[a]Calculated by our reviewers.
[b]Males in the positive control group were maintained on basal diet containing 3% corn oil for 4 weeks prior to TEM administration.

observed in high-dose males, particularly during the first 4 weeks, supports this statement. In general, average body weight for low-dose males was comparable to the vehicle control throughout premating. At the majority of dosing weeks, however, food consumption for mice feed the 2500-ppm diet was slightly lower than control.

The positive control (TEM), administered to F_0 males in drinking water had no adverse effects on body weight, body weight gain, or food consumption.

2. Fertility in F_0 mice: Owing to an accident immediately following the end of mating, approximately 50% of the females bred to the high-dose males and their offspring could not be identified with a specific male; these animals were removed from the main group, carried separately throughout the study and identified as sub group B. The remaining high-dose group animals were refered to as subgroup A.

Representative results from the F_0 fertility phase of the heritable translocation assay are presented in Table 2. No explanation was given for the use in the captan groups of fewer than the specified number of females (i.e., 2 females/treated males). As shown in Table 2, the pregnancy rate for high-dose females of both subgroups was ≥20% less than control. No apparent effects on pregnancy rates were seen in the low-dose group. It was noted, however, that the pregnancy rate for the vehicle control group (77.5%) was lower than the expected rate of at least 80-85%. Similarly, the number of nonbreeder males in the vehicle control group was high; therefore, the biological significance of the 8 nonbreeder males in the 2500-ppm group and the 5 nonbreeder males in subgroup A of the high-dose cannot be determined. Mean live litter sizes for both 5000-ppm groups were reduced compared to the vehicle control; 2500 ppm captan had no effect on the mean litter size. The study authors stated that there was a definite pattern of decreasing litter size and increasing variance between the experimental groups. However, the percentage of females with small litter (i.e., ≤9 live offspring/litter) was higher in the negative control than in the low-dose females and subgroup A of the high-dose. The 18.1% incidence of small litters in subgroup B cannot be clearly interpreted as an effect of treatment with 5000 ppm captan because of the accident and probable trauma experienced by the mothers. Neither the number of pups born dead nor the number of F_0 dams with resorbed litters were reported. Therefore, the overall reproductive toxicity induced by 5000 ppm captan in the parental generation could not be evaluated. The findings do suggest, however, that 5000 ppm captan had an adverse effect on fertility and litter size. No conclusions can be reached for the males fed the 2500-ppm diet. It is conceivable that the low pregnancy rate and high incidence of nonbreeder males in the vehicle control group masked compound effects at 2500 ppm.

TABLE 2: Representative Results of the F_0 Fertility Phase of the Heritable Translocation Assay Conducted with Male Mice Fed Captan for 8 Consecutive Weeks

Substance	Dose	Number of Treated Males	Number of Mated Males	Number of Non-breeder Males	Percent Non-breeder Males	Number of Mated Females	No. of Pregnant Females	% Pregnancies	Mean Live Litter Size ± S.D.	Percent Females with Litter Sizes ≤9 Live Pups[a]	Average Number of Males Weaned/ Litter
Vehicle Control											
Basal diet/3% Corn oil	--	60	60	8	13.3	120	93	77.5	12.30±1.97	12.9	5.81
Positive Control[b]											
Triethylenemelamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	66	66	19	28.8	132	77	58.3	6.55±2.42	87.0	3.26
Test Material											
Captan	2500 ppm	60	60	8	13.3	119	91	76.5	12.16±2.23	9.9	5.68
	5000 ppm										
	Subgroup A	31	31	5	16.1	61	38	62.3	11.68±2.39	7.9	6.24
	Subgroup B[c]	30	30	0	0.0	59	33	55.9	11.33±2.47	18.2	5.22

[a]Calculated by our reviewers.
[b]Males in the positive control group were maintained on basal diet containing 3% corn oil for 4 weeks prior to TEM administration.
[c]Results for the high dose group were separated. Owing to an accident, females and their offspring in this group could not be identified with a specific male.

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3. F_1 matings: Summarized reproduction data from the first round of breeding in the F_1 generation are presented in Table 3. Pregnancy rates for the F_1 animals in the vehicle and captan treatment groups were increased compared to the parental generation. Although the percentage of nonbreeder males in the vehicle control, 2500-ppm group, and 5000-ppm subgroup A was lower than the percentage seen in the F_0 breeding phase, there was an ~50% increase in nonbreeder males in 5000-ppm subgroup B compared to the vehicle control. Combining the results for both 5000-ppm subgroups yielded a nonbreeder rate of 6.4%. The results further indicated that the two dietary preparations of captan did not adversely affect pregnancy rates, average litter sizes, average dead implants, or the percentage of dead implants. Similarly, the percentage of F_1 dams delivery litters with ≤ 9 live offspring was lower in the treatment groups than in the control group.

Listed in Table 4 are the number of nonbreeder males after three mating rounds and the number of males from each group that were classified as questionable, partially sterile, or sterile after each mating. As shown, the number of nonbreeders in the low-dose group was higher than the control. Analysis of meiotic metaphases for the presumptive positives in this group (five nonbreeders, one questionable, one partially sterile, and one sterile) showed no abnormal configurations (Table 5). Similarly, no translocation figures were seen in the two high-dose subgroup B males (1 questionable and 1 partially sterile) examined cytogenetically. However, analysis of the eight suspect translocation heterozygotes in high-dose subgroup A (2 nonbreeders, 5 partially steriles, and 1 sterile) yield one positive reciprocal translocation. The significance of this finding cannot be assess because a translocation figure was also observed in the vehicle control group. The study authors stated that there would have been no statistical significance between the vehicle control and high-dose translocation rate "even if the control incidence were zero." Similarly, the study authors indicated that the incidence rate in the 5000-ppm group was not significantly increased compared to the reporting laboratory's historical spontaneous rate (6.25×10^{-4}). Nevertheless, the investigators concluded:

> "Since it is impossible to establish whether the single positive F_1 male in the high-level Captan group represents a spontaneous translocation or a marginal effect of the Captan treatment, we interpret the present results as indicating that Captan may have some low-level mutagenic potential, but that this potential could not be demonstrated on a statistical basis with the numbers of animals used in this heritable translocation test."

TABLE 3: Representative Reproductive Data from the First F_1 Mating Phase of the Heritable Translocation Assay Conducted with Male Mice Fed Captan for 8 Consecutive Weeks

Substance	Dose in F_0 Exposure[a]	Number of F_1 Males	Number of Females	Number of Non-breeder Males	Percent Non-breeder Males	Number of Pregnant Females	Percent Pregnancy	Percent Females with Litter Sizes ≤9 Live Pups[a]	Average Live Implants[b]	Average Dead Implants/ Female[a]	Average Total Implants/ Female[a]	Percent Dead Implants[a]
Vehicle Control												
Basal diet/3% Corn oil	--	200	599	14	7.0	492	82	17.0	11.7	>0.5[c]	>12.2	4.1
Positive Control[d]												
Triethylenemelamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	200	599	39	19.5	384	64	32.6	10.1	>1.4	>11.5	12.2
Test Material												
Captan	2500 ppm 5000 ppm	200	600	16	8.0	472	79	16.5	11.7	>0.6	>12.3	4.9
	Subgroup A	200	599	9	4.5	477	80	15.3	11.6	>0.6	>12.2	4.9
	Subgroup B[e]	50	150	7	14.0	112	75	13.4	11.7	>0.4	>12.1	3.3

[a]Percent females with ≤9 live pups/litter, average dead implants, total implants, and percent dead implants were calculated by our reviewers.
[b]Minor calculation errors were uncovered; the presented results were recalculated by our reviewers.
[c]All groups contained litters with >5 dead implants.
[d]Males in the positive control group were maintained on basal diet containing 3% corn oil for 4 weeks prior to TEM administration.
[e]Results for the high-dose group were separated. Owing to an accident, females and their offspring in this group could not be identified with a specific male.

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TABLE 4. Representative Results of Mating and Fertility Classification of Suspect Translocation F_1 Males After Three Rounds of Mating in the Heritable Translocation Assay Conducted with Male Mice Fed Captan for 8 Consecutive Weeks

Substance	Dose in F_0 Exposure	Number F_1 Males Mated	Number of Non-breeder Males[b]	Fertility Classification								
				Questionable[a]			Partially Sterile			Sterile		
				(After 1st Breeding)	(After 2nd Breeding)	(After 3rd Breeding)	(After 1st Breeding)	(After 2nd Breeding)	(After 3rd Breeding)	(After 1st Breeding)	(After 2nd Breeding)	(After 3rd Breeding)
Vehicle Control												
Basal Diet/ 3% Corn oil	--	200	1	4	3	3	5	3	3	1	1	1
Positive Control[c]												
Triethylene-melamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	200	4	11	8	8	28	23	23	13	14	14
Test Material												
Captan	2500 ppm	200	5	3	1	1	3	1	1	1	1	1
	5000 ppm											
	Subgroup A	200	2	5	0	0	7	5	5	0	1	1
	Subgroup B[d]	50	0	2	1	1	2	1	1	0	0	0

[a]Questionable refers to males that had at least one female with implants that were in an early stage of development; no distinction could be made between live and dead implants.
[b]After three rounds of mating
[c]Males in the positive control group were maintained on basal diet containing 3% corn oil for 4 weeks prior to TEM administration.
[d]Results for the high-dose group were separated. Owing to an accident, females and their offspring in this group could not be identified with a specific male.

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TABLE 5. Results of the Cytogenetic Analysis of Suspect Translocation Heterozygotes in F_1 Males After Three Rounds of Mating in the Heritable Translocation Assay Conducted with Male Mice Fed Captan for 8 Consecutive Weeks

Substance	Dose in F_0 Exposure	Number F_1 Males Mated	Total Number of Suspect Translocation Heterozygotes	Cytogenetic Analysis[a]	
				Number of F_1 Analyzed	Number of F_1 with Reciprocal Translocation Figures
Vehicle Control					
Basal diet/ 3% Corn oil	--	200	8(1NB; 3Q;[b] 3PS; 1S)	8	1
Positive Control[c]					
Triethylene-melamine (TEM)	0.32 mg/L (2 weeks) 0.124 mg/L (2 weeks)	200	49(4NB; 8Q; 23P; 14S)	6	6
Test Material					
Captan	2500 ppm	200	8(5NB; 1Q; 1PS; 1S)	8	0
	5000 ppm				
	Subgroup A	200	8(2NB; 5PS; 1S)	8	1
	Subgroup B[d]	50	2(1Q; 1PS)	2	0

[a]Fifty meiotic metaphase preparations or until a translocation figure was observed were analyzed for all F_1 males in the vehicle and treatment groups that were classified as suspect translocation heterozygotes. Meiotic metaphases were analyzed from the following representative F_1 positive control males: 5PS; 1Q.

[b]Questionable refers to males that had at least one female with implants that were in an early stage of development; no distinction could be made between live and dead implants.

[c]Males in the positive control group were maintained on basal diet containing 3% corn oil for 4 weeks prior to TEM administration.

[d]Results for the high-dose group were separated. Owing to an accident, females and their offspring in this group could not be identified with a specific male.

Abbreviations used: NB = nonbreeder; Q = questionable; PS = partially sterile; S = sterile.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that there was sufficient evidence (e.g., reduced body weight, reduced body weight gain, and reduced food consumption in F_0 males and decreased fertility and viable litter sizes in both high-dose subgroups) to conclude that captan was assayed to an appropriate high dose (5000 ppm). However, the biological significance, if any, of the single translocation figure in the high-dose group was obscured by the high spontaneous rate recorded for this study (i.e., 1 reciprocal translocation in 200 control progeny, 5×10^{-3}). It was also noted that the spontaneous frequency in this study was well above the expected background incidence of heritable translocations (2.3×10^{-4} to 9.1×10^{-4})[2]. The following technical deficiencies were of additional concern:

1. No information was provided on test material purity, stability, or homogeneity of the prepared diets; verification of the actual concentrations used in the study was not performed.

2. The reproductive performance of the parental vehicle control group was poor. Although 5000 ppm captan clearly exerted an adverse effect on fertility in the F_0 generation, possible effects at 2500 ppm captan could not be evaluated because of the reduced pregnancy rate and the high incidence of nonbreeders in the vehicle control group. With the exception of the reported accident, environmental conditions were not provided. Therefore, we are unable to ascertain whether preexisting laboratory conditions contributed to the poor mating performance of the vehicle control animals.

3. We concede that the accident, which resulted in an inability to identify approximately half of the dams and their offspring with a specific male, rendered the reproductive data from these animals invalid. However, the rationale for using fewer F_1 males from subgroup B in the genetic screening phase of the study was not clear. The disproportionately lower number of F_1 males selected from subgroup B, in effect, reduced the actual sample size of progeny from individual F_0 males, thereby decreasing the possibility of determining whether the single translocation figure scored in the high-dose group was artifactual or related to captan exposure.

Based on the above considerations, we conclude that the study is unacceptable.

E. QUALITY ASSURANCE MEASURES: Was the study performed under GLP? No.

F. --- APPENDIX: Appendix A, Materials and Methods 221-225.

[2]Generoso, W.M., et al. (1978) Chemical Mutagens--Principles and Methods for Their Detection, A. Hollaender and F.J. de Serres, eds. Vol. 5:55-77, Plenum, NY.

APPENDIX A

MATERIALS AND METHODS
221-225

_____Captan review_____

Page _____ is not included in this copy.

Pages __17_ through __21_ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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