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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005345

AUG 13 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA File Symbol 476-1872, OF2334
Vernam-Petition for Tolerances on Alfalfa and Beans
Accession Nos. 255948, 255947, 247151, 249703, 249704, 071713, 245293

Caswell No. 711

FROM: William Woodrow, Ph.D. 8-12-86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D. ABK 8/13/86
Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Stauffer Chemical Company
1200 South 47th Street
Richmond, CA 94804

Action Requested:

Stauffer Chemical Company requests an amended registration for Vernam 7E Selective Herbicide to establish a tolerance of 0.1 ppm on alfalfa and beans (dry and green) and bean forage.

Recommendations:

1. A tolerance of 0.1 ppm for Vernam 7E Selective Herbicide on alfalfa and beans (dry and green) and bean forage is not toxicologically supported.

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An August 18, 1981 Woodrow memorandum to Robert Taylor listed the following (then current) Vernam data gaps to be satisfied prior to further Vernam tolerance consideration:

a. A 1-year dog feeding study.

Present status - Stauffer has not made mention to the Agency regarding this data request.

b. A second chronic feeding/oncogenic study (using the rat) - Stauffer acknowledges an Agency-approved timeframe to submit a final rat chronic feeding/ oncogenic study by August 1987.

c. Toxicity studies reviewed in the present report (reviewed on a contract basis).

(1) Teratogenicity study in rabbits.

The NOEL for maternal and fetal toxicity of Vernam in rabbits was 200 mg/kg/day (the HDT). LOEL's for maternal and fetal toxicity could not be determined; an absence of maternal toxicity at the HDT precluded study acceptance to determine teratogenic potential.

Classification: Supplementary Data.

(2) Teratogenicity study in mice.

Deficiencies such as insufficient number of litters for teratological evaluation, absence of individual data on fetal abnormalities, and lack of adequate information on the animals used precluded determination of NOEL's or LOEL's for maternal or fetal toxicity, or assessment of teratogenic potential.

Classification: Core Invalid study.

(3) A two-generation reproduction study in rats.

The parental NOEL of 20 ppm and LOEL of 100 ppm for rats administered Vernam is based on a statistically significant depression in the mean body weight for both parental males and females. The reproductive performance was comparable among control and Vernam-dosed groups, thus no effects on mating, fertility,

gestational or lactational indices were observed. The NOEL for reproductive effects is, therefore, considered to be 506 ppm.

Separately, there appeared to be an increase in the number of incidences of urinary tract variants in pups from all Vernam-dosed groups of the P_0 and P_1 second matings when compared to controls, including the lowest level tested at 20 ppm Vernam, and a possible indication of developmental toxicity or teratogenesis was noted. The LEL for possible developmental toxicity, therefore, appears to be 20 ppm (LDT). However, in order to clarify this matter we are asking the registrant to provide historical control data for this strain of rat from the lab that conducted this study. We are also requesting that the required teratology study be conducted in the same strains of rat and preferably from the same supplier as was used in the reproduction study (see p. 5, item 3c).

Classification: Guideline for reproductive effects. This classification supersedes Dynamac's.

(4) A metabolism study.

^{14}C-Vernam and its sulfoxide are readily absorbed, metabolized, and excreted when administered to rats. Urinary metabolites identified included s-cystine, mercapturate, 3-mercapatolactate, and mercaptoacetate conjugates of S-(N,N-dipropyl)thiocarbamate, dipropylamine, and propylamine.

Classification: Core Minimum Data.

(5) Micronucleus mutagenicity assay in mice.

The study authors did not report a clastogenic effect; however, male or female mice at the highest doses tested (1200 mg/kg for males, and 1400 mg/kg for females) did not show any toxic effects. Therefore an MTD was not established, and the mice may not have received an adequate high-dose challenge.

Classification: Unacceptable study.

(6) Ames mutagenicity assay.

Litton Bionetics study No. T-6311, October 1977. The data provided by the study authors were insufficient to establish whether a cytotoxic dose level had been approached, and thus the dose range employed may have been inadequate. Single plates per test dilution only were used; plates should have been prepared at least in duplicate to show test reproducibility.

Classification: Unacceptable study.

(7) Ames mutagenicity assay.

Andersen, K.J., et al., Evaluation of Herbicides for Possible Mutagenic Properties. J. Agr. Food Chem. Vol. 20, No. 3, January 10, 1972.

Individual plate counts and results for each test strain were not presented in the study. (The number of revertents for the positive controls and for the test material were not given.) In addition, metabolic activation with S9 was not routinely used in assays.

Classification: Unacceptable study.

(8) DNA-repair and reverse mutation study.

Shirasu, Y., et al. Mutagenicity Screening of Pesticides in the Microbial System. Mut. Research 40:19-30, 1976.

This study was intended to serve as a large preliminary screening of 166 pesticides for mutagenicity potential; thus, individual data were not reported for Vernam, including no mutagenesis testing with the addition of the metabolic activation complex, S9.

Classification: Unacceptable study.

2. The label signal word and precautionary statements are satisfactory.

3. The following Vernam toxicity data requirements remain outstanding:

   a. A 1-year dog feeding study;

   b. A second chronic feeding/oncogenic study (using the rat);

   c. Teratology studies using two mammalian species;

      NOTE: Since the two-generation reproduction study evaluated in the present report indicated the possibility of a teratogenic effect manifested as increased incidences of urinary tract variants in rat pups, one of the two requested teratology studies should be conducted with the same strain of rat that displayed the urinary tract variants; the Crl/CD (SD) BR rat.

   d. A battery of mutagenicity studies.

      (Gene mutation study, structural chromosomal study, other genotoxic effects, e.g., chromosomal aberrations, direct DNA damage, and repair.)

Previously reviewed data - See attached one-liner lists.

New Vernam toxicity data reviewed in the present report.

   a. Bryan, J., et al. Teratogenic potential (Segment 11) oral study in rabbits with Vernam. (Unpublished Study No. WIL-80207 by Wil Research Laboratories, Inc., Cincinnati, OH for Stauffer Chemical Co., April 6, 1981. Accession No. 245293.

      Reviewed by Dynamac Corp., December 12, 1985, Dynamac No. 1-36D. EPA: 68-02-4225.

      The present reviewer agrees with Dynamac's conclusions: The study should be classified Supplementary Data. (LOEL's for maternal and fetal toxicity could not be determined at the HDT tested [200 mg/kg/day]. This dose was the NOEL; however, a lack of maternal toxicity indicated that dose levels were not high enough; therefore, the teratogenic potential for Vernam in rabbits could not be determined.)

   b. Benson, B., et al. Vernam, safety evaluation by teratological study in the mouse. (Unpublished Study No. T-2132, prepared by Woodard Research Corp., Herndon, VA, for Stauffer Chemical Co., Richmond, CA, April 28, 1967.) Accession No. 247151.

Reviewed by Dynamac Corp., November 6, 1985, Dynamac No. 038C. EPA: 68-02-4225.

The present reviewer agrees with Dynamac's conclusions: The study should be classified Core Invalid. (Major study deficiencies included insufficient number of litters for teratological evaluation in absence of individual data on fetal abnormalities, and inadequate information on the animals used.)

c. Minor, J.L., et al. A two-generation rat reproduction study with Vernam technical (unpublished study No. T-10124, prepared by Stauffer Chemical Co., Environmental Health Center, Farmington, CT, January 27, 1983, Accession Nos. 249703, 249704.

Reviewed by Dynamac Corp., November 21, 1985, Dynamac No. 038-31,2. EPA: 68-02-4225. (Rereviewed July 7, 1986. Dynamac No. 1-038-31,2. EPA 68-02-4225).

The present reviewer disagrees with Dynamac's conclusions: This study should be classified Guideline Data. Parental NOEL and LOEL in rats were 20 and 100 ppm of Vernam, respectively, for a statistically significant depression in the mean body weights. The reproductive performance was comparable between control and Vernam-treated groups, including indices for mating, fertility, gestational, or lactational parameters. Therefore, the reproductive NOEL based on reproductive indices was 500 ppm. However, the developmental toxicological LOEL in rats was 20 ppm Vernam, the lowest dose level tested, at which increased incidence of urinary tract variants occurred. Therefore, the NOEL for developmental toxicity was apparently not established. Historical control data on these findings have been requested. A teratology study in the same strain of rat has also been requested.

d. Micuillis, J.B., et al. Balance, tissue residue and metabolism study with N-[1-^{14}C] propyl Vernam in the rat. Unpublished study (No. MRC-82-03) prepared by Stauffer Chemical Co., Mountain View, CA, April 1982.

Reviewed by Dynamac Corp., September 12, 1984, Dynamac No. 75. EPA: 68-01-6561.

The present reviewer agrees with Dynamac's conclusions: The study should be classified Core Minimum Data. (^{14}C-Vernam and its sulfoxide are readily absorbed, metabolized, and excreted when administered orally to rats. Urinary metabolites identified included

s-cystine, mercapturate, 3-mercaptolactate, and mercaptoacetate conjugates of S-(N,N-dipropyl)thiocarbamate, dipropylamine, and propylamine.)

e. Shirasu, Y., et al. (1976) Mutagenicity screening of pesticides in the microbial system. A published report in Mutation Research 40:19-30. Prepared by the Toxicology Division of the Institute of Environmental Toxicology, Tokyo, and the Department of Induced Mutation, National Institute of Genetics, Japan. Accepted for publication on September 16, 1975 (DNA-repair and reverse mutation).

Review by Dynamac Corp., September 7, 1984; Dynamac No. 75. EPA: 68-01-6561.

The present reviewer agrees with Dynamac's conclusions: The study should be classified Unacceptable. (This study was intended to screen 166 pesticide for mutagenic potential via the Ames assay. Individual test data were not presented in the report for any chemical, including Vernam. No results with S9 metabolic activation were included in this study.)

f. Andersen, K.J., et al. Evaluation of Herbicides for Possible Mutagenic Properties. (Published: J. Agri. Food Chem. Vol. 20, No. 3, 1972.) Prepared by Columbus Laboratories, Batelle Memorial Institute, Columbus, OH, January 10, 1972 (Ames study).

Reviewed by Dynamac Corp., October 4, 1984, Dynamac No. 75. EPA: 68-01-6561.

The present reviewer agrees with Dynamac's conclusions: The study should be classified Unacceptable. (Individual plate counts and results for each tester strain were not presented. S9 metabolic activation assay tests were not routinely used in the assays.)

g. Jagannath, D.R., et al. Mutagenicity evaluation of Vernam Tech CBG-2601. Unpublished Study No. T-6311, prepared by Litton Bionetics, Inc., Kensington, MD, for Stauffer Chemical Corp., Western Research Centers, Richmond, CA, October 1977 (Ames study).

Reviewed by Dynamac Corp., September 7, 1984 Dynamac No. 75. EPA: 68-01-6561.

The present reviewer agrees with Dynamac's conclusions. The study should be classified Unacceptable. (Data were not presented to show that the low dose was

nontoxic, and that the highest dose tested was close to limit of cell toxicity. If cytotoxicity could not be determined, the compound should have been assayed at the solubility limit. No attempt was made to check the test result reproducibility; only one plate per test concentration was used.)

h. Majeska, J.B., et al. Vernam technical mutagenicity evaluation in bone marrow micronucleus. (Unpublished Study No. T-11821, prepared and submitted by Stauffer Chemical Co., Farmington, CT, May 30, 1984. Accession No. 25594-7.

Reviewed by Dynamac Corp., November 6, 1985 Dynamac No. 038-A. EPA: 68-02-4225.

The present reviewer agrees with Dynamac's conclusions: The study should be classified Unacceptable. (No clastogenic effects were noted when male mice were exposed to three doses ranging from 800 to 1200 mg/kg, or when female mice were exposed to three doses of Vernam ranging from 1000 to 1400 mg/kg, at 24, 48, and 72 hours exposure. However, no toxic effects at the highest dose range of test Vernam should have included higher doses.)

89818:Woodrow:HED-15:KENCO:7/18/86:7/30/86:dej:VO
R:89823:Woodrow:HED-15:KENCO:8/1/86:10/1/86:sj:VO
R:89824:Woodrow:HED-15:KENCO:8/7/86:10/30/86:TAR:VO

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DRAFT

EPA: 68-02-4225
TASK: 038-A
November 6, 1985

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DATA EVALUATION RECORD

VERNAM

Mutagenicity--Micronucleus Assay in Mice

STUDY IDENTIFICATION: Majeska, J. B., and Matheson, D. W. Vernam technical mutagenicity evaluation in bone marrow micronucleus. (Unpublished study No. T-11821 prepared and submitted by Stauffer Chemical Company, Farmington, CT; dated May 30, 1984.) Accession No. 25594-7.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

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1. CHEMICAL: Vernam.

2. TEST MATERIAL: Vernam technical from lot No. WRC 4921-24-6 was a yellow liquid stored in the dark at room temperature; its purity was not specified.

3. STUDY/ACTION TYPE: Mutagenicity--micronucleus assay in mice.

4. STUDY IDENTIFICATION: Majeska, J. B., and Matheson, D. W. Vernam technical mutagenicity evaluation in bone marrow micronucleus. (Unpublished study No. T-11821 prepared and submitted by Stauffer Chemical Company, Farmington, CT; dated May 30, 1984.) Accession No. 25594-7.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
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Date: _____

Nancy McCarroll, B.S.
Independent Reviewer
Dynamac Corporation

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Date: _____

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

William Woodrow, Ph.D.
EPA Reviewer

Signature: _____
Date: _____

Albin Kocialski, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of the assay, exposure of mice to three doses of Vernam, ranging from 800 to 1200 mg/kg in male mice and 1000 to 1400 mg/kg in females, for 24, 48, and 72 hours did not elicit a clastogenic effect. However, the authors did not report a toxic effect at the highest dose tested. Therefore, we are unable to assess whether the selected dose range was appropriate and if the maximum tolerated dose (MTD) was achieved.

B. The study is unacceptable.

8. RECOMMENDATIONS:

A. The study should be repeated, and the results must provide evidence for a toxic response at the highest dose assayed in the male and female mice.

B. Careful attention should be given to the staining quality of the slides. We suggest that the authors use the staining procedure of Schmid[1] or an equivalent technique.

Items 9 and 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Vernam technical, lot No. WRC 4921-24-6, was described as a yellow liquid that was stored in the dark at room temperature. The purity was not specified. Vernam was diluted in corn oil, the solvent control.

2. Test Animals: Six- to 7-week-old male and female CD-1 mice were obtained from Charles River Breeding Laboratories. The animals weighed between 24-30 g at study initiation.

a. Animal Maintenance: Animals were housed five per cage. Food and water were available ad libitum.

b. Group Assignment: Animals were randomly assigned to groups. Prior to study initiation, all animals were

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[1] Schmid, W., Chemical mutagen testing on in vivo somatic mammalian cells, Agents and Actions: 3 (1973) 77-85.

[2] Only items appropriate to this DER have been included.

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weighed and acceptable weight limits were established. Animals within the appropriate weight range were uniquely identified with metal ear tags.

3. Test Material Administration: The test material was administered by oral gavage in 0.5 mL of corn oil.

4. Range-finding Study: Animals (number and sex unspecified) were administered by gavage with either two doses of the test material at 600, 800, 1000, 1200, or 1400 mg/kg at 24-hour intervals or one dose at 1500, 2000, 2500, 3000, 3500, or 4000 mg/kg. Animals were observed for toxic effects. In addition, 72 hours after dosing, selected animals were sacrificed and bone marrow slides were evaluated for cytotoxicity.

5. Micronucleus Assay:

   a. Dose Selection: Based on the results of the range-finding study, the MTD for each sex and the two lower doses were selected. Doses administered to males were 1200, 1000, and 800 mg/kg and to females were 1400, 1200, and 1000 mg/kg.

   b. Animal Sacrifice/Bone Marrow Harvest: Ten mice (five males and five females) were administered the selected doses of the test material or solvent control as a single oral administration. At 24, 48, and 72 hours, animals were sacrificed by cervical dislocation. The positive control groups (50 mg/kg cyclophosphamide for males and 100 mg/kg for females) were sacrificed 48 hours after dosing. Bone marrow cells were harvested from the tibia of both legs by aspiration into fetal calf serum. The marrow pellet was collected by centrifugation and resuspended in calf serum. A drop of the suspension was placed onto slides; slides were fixed and stained (2% Giemsa).

   c. Slide Analysis: One thousand polychromatic erythrocytes (PCE) per mice were scored for the number of micronuclei. The frequency of PCE in 1000 normochromatic erythrocytes (NCE) was determined as a measure of cytotoxicity.

6. Evaluation Criteria: A test material was considered positive if it induced a statistically significant increase in micronucleated PCE (MPE) over the solvent control at $p < 0.01$.

7. The statistical method used was the Kastenbaum-Bowman test.

B. Protocol: No protocol was submitted.

12. REPORTED RESULTS:

Range-finding Study: It was reported that toxic signs and deaths occurred in male mice at ≥ 1200 mg/kg and in female mice at ≥ 1500 mg/kg. The MTD (1200 mg/kg for males and 1400 mg/kg for females) were therefore based on the presence of "clinical signs with little or no animal deaths"; the specific types of clinical signs were not reported. Bone marrow slides were prepared at 72 hours after dosing; however, the authors stated, "the data were insufficient to conclude that there was toxicity in the bone marrow as indicated by PCE frequency."

No clinical signs were reported in either sex at any time period following exposure to the selected doses of the test material.

Micronucleus Assay: No statistically significant increases in MPE over the solvent control occurred at any sampling interval at the dose levels tested in males (800, 1000, or 1200 mg/kg) or in females (1000, 1200, or 1400 mg/kg). Representative data are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Vernam Technical is not clastogenic in vivo as indicated by the absence of an increase frequency of micronuclei in polychromatic erythrocytes."

B. A quality assurance statement was signed and dated November 30, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that at the doses tested, Vernam did not cause a clastogenic response in the bone marrow of CD-1 mice. In the range-finding study, the authors stated that the MTDs for males (1200 mg/kg) and females (1400 mg/kg) were "determined by the presence of clinical signs with little or no animal deaths." However, in the micronucleus assay at these same MTDs, no clinical signs or mortalities were reported. To properly evaluate the clastogenic/mutagenic effects of a substance, the highest dose selected should elicit toxic or cytotoxic responses. A criterion frequently used to set the highest dose level is 80 percent of the $LD_{50/7}$; by this criterion the dose used in this study was insufficient. Without some evidence of toxicity, we are unable to assess whether the dose range selected to evaluate the clastogenic potential of Vernam technical in the bone marrow of mice was adequate.

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with Vernam Technical

Substance	Dose (mg/kg)	Time of Sacrifice (h)	No. of Animals per Group	No. of PCE[a] per Group	Total No. of MPE per Group	% MPEs[b] per Group	Average No. PCE/1000 NCE	Average Group[b] PCE:NCE
Males								
Solvent Control Corn Oil	0	24	5	5000	7	0.14	265	0.3:1
		48	5	5000	13	0.26	354	0.4:1
		72	5	5000	8	0.16	420	0.4:1
Positive Control Cyclophosphamide	50	48	5	5000	42	0.84*	301	0.3:1
Test Material Vernam	1200[c]	24	5	5000	12	0.24	295	0.3:1
		48	5	5000	7	0.14	296	0.3:1
		72	5	5000	8	0.16	389	0.4:1
Females								
Solvent Control Corn Oil	0	24	5	5000	12	0.24	262	0.3:1
		48	5	5000	5	0.10	323	0.3:1
		72	5	5000	7	0.14	404	0.4:1
Positive Control Cyclophosphamide	100	48	5	5000	164	3.3*	218.4	0.2:1
Test Material Vernam	1400[c]	24	5	5000	4	0.08	374	0.4:1
		48	5	5000	5	0.10	439[d]	0.4:1
		72	5	5000	6	0.12	316	0.3:1

[a] PCE = Polychromatic erythrocytes.
MPE = Micronucleated polychromatic erythrocytes.
NCE = Normochromatic erythrocytes.

[b] Calculated by reviewers.

[c] Highest dose tested; lower doses (800 and 1000 mg/kg for males and 1000 and 1200 mg/kg for females) had similar results.

[d] Data calculated from four mice.

*Significantly greater than control ($p < 0.01$) by the Kastenbaum-Bowman test.

It has been reported by Schmid[3] and others that the ratio of PCE:NCE is quite constant, about 1:1. A shift in this ratio is indicative of cytotoxicity; therefore, it is a critical measurement. The ratio of PCE:NCE for the controls and dosed groups ranged from 0.3:1 to 0.4:1. It was apparent from the data that the number of PCE/1000 NCE reported by the authors was much lower than expected.[4] It is our assessment that the decrease in PCE was not a cytotoxic response because the ratio was similar for all groups.

From the examination of PCE:NCE ratios that were reported, we conclude that the slides may have been improperly stained or improperly scored for PCE. If the staining is inadequate, then the bluish-staining PCE cannot be distinguished from reddish-staining NCE, causing erroneous interpretations in the micronucleus assay.

Cyclophosphamide (50 mg/kg in males and 100 mg/kg in females), the positive control, induced a clastogenic effect as indicated by the significant ($p < 0.01$) increase in the frequency of MPE, over the solvent control at 48 hours, which demonstrates the sensitivity of the assay for detecting a clastogenic response.

Item 15--see footnote 2.

16. CBI Appendix: Appendix A, Materials and Methods, CBI pp. 6-9.

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[3] Schmid, W., pp. 77-85.

[4] Ibid.

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APPENDIX A

Materials and Methods

Vernam Scientific Reviews

Page _____ is not included in this copy.

Pages 18 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 impurities

___ Description of the product manufacturing process

___ Description of product 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

_x_ FIFRA registration data

___ The document is a duplicate of page(s) _____

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA: 68-01-6561
TASK: 75
September 7, 1984

DATA EVALUATION RECORD

VERNAM TECH CBG-2601

Mutagenicity

CITATION: Jagannath, D.R., Brusick, D.J. 1977. Mutagenicity evaluation of Vernam Tech CBG-2601. Unpublished study No. T-6311 prepared by Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, MD 20795 for Stauffer Chemical Corporation Western Research Centers, Richmond, California 94804. Dated October 1977.

REVIEWED BY:

Brenda Worthy, M.T.
Project Scientist
Dynamac Corporation

Signature: Brenda Worthy
Date: 9-7-84

I. Cecil Felkner, Ph.D.
Mgr. Genetic Toxicology Dept.
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-7-84

Cipriano Cueto, Ph.D.
Department Director
Dynamac Corporation

Signature: Cipriano Cueto
Date: 9-7-84

APPROVED BY:

W. Woodrow, Ph.D.
EPA Scientist

Signature: _____
Date: _____

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Ames assay.

CITATION: Jagannath, D.R., Brusick, D.J. 1977. Mutagenicity evaluation of Vernam Tech CBG-2601. Unpublished Study No. T-6311 prepared by Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, MD 20795 for Stauffer Chemical Corporation Western Research Centers, Richmond, California 94804. Dated October 1977.

ACCESSION NUMBER: 071713.

LABORATORY: Litton Bionectics, Inc., 5516 Nicholson Lane, Kensington, MD 20795.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: Vernam Tech CBG-2601 was described as a light brown liquid received on September 29, 1977. No purity was given.

MATERIAL AND METHODS:

Microbial Strains: Five strains of the bacterium Salmonella typhimurium were used: TA1535, TA1537, TA1538, TA98, and TA100 and the yeast strain Saccharomyces cerevisiae D4 were used in this study.

Metabolic Activation: The S9 homogenate was prepared from the liver of an adult male Sprague-Dawley rat induced by injection with Aroclor 1254. Components of the S9 mix are listed in Table 1.

TABLE 1. Components of S9 Mix

TPN	4 µmoles/ml
Glucose-6-phosphate	5 µmoles/ml
Sodium phosphate (dibasic)	100 µmoles/ml
$MgCl_2$	8 µmoles/ml
KCl	33 µmoles/ml
S9 fraction	0.1-0.15 ml/ml

Preparation of Test Material: Vernam was diluted with dimethylsulfoxide (DMSO) to prepare a stock solutions which were added to the plates at five final dose levels: 0.001, 0.01, 0.1, 1.0 and 5.0 µl/plate.

Controls: The negative (solvent) control was DMSO at 50 µl/plate. The positive controls were those given in Table 2.

TABLE 2. Positive Controls

Strain	Activation	Chemical	Concentration (µg plate)
TA1535	-	N-methyl N'-Nitro-nitrosoguanidine (MNNG)	10
	+	2-Anthramine (ANTH)	100
TA1537	-	Quinacrine mustard (QM)	10
	+	8-Aminoquinoline (AMQ)	100
TA1538	-	2-Nitrofluorene (NF)	100
	+	2-Acetylaminofluorene (AAF)	100
TA98	-	NF	100
	+	AAF	100
TA100	-	MNNG	10
	+	ANTH	100
D4	-	MNNG	10
	+	DMNA*	100 µmoles

* Not identified by author, but presumed to be dimethylnitrosamine because these are common initials.

Nonactivated Assay: Five dose levels of the test material (0.001, 0.01, 0.1, 1.0 and 5.0 µl/plate) and the control substances were mixed with 10^8 cells from each of the 6 tester strains, in 2.0 ml of molten agar supplemented with biotin and a trace of histidine; this mixture was overlaid onto a selective agar plate, one plate per treatment (from Table 1, page 4 in the report). The plates were incubated for 48 hrs at 37°C and the revertant colonies were scored.

S9 Activated Assay: Similar procedures were used, except that 0.5 ml of the S9 mixture was added to the 2.0 ml of molten agar.

Evaluation Criteria: If a chemical produced a positive dose-response over three concentrations with the lowest increase equal to twice the solvent control in S. typhimurium strains TA1535, TA1537 or TA1538, the chemical was considered to be mutagenic. If a chemical produced a positive dose-response over three concentrations with the highest increase equal to twice the solvent control in strains TA98, TA100, or S. cerevisiae D4, it was considered to be mutagenic.

RESULTS:

The number of revertants reported for the assays conducted on Vernam technical, with and without metabolic activation were essentially equal to those reported for the negative control. Results obtained for the negative and positive controls are presented in Table 3.

DISCUSSION:

The authors reported that all tester strains gave a positive response with and without S9-activation for the positive control and the negative (solvent) control produced the expected numbers of revertant colonies. They stated that none of the plates treated with the test material produced positive responses. The investigators concluded that Vernam Tech CBG-2601 was not mutagenic.

Our assessment is that the data are insufficient to conclude that Vernam is not mutagenic. Although the authors stated that a toxicity test had been conducted and that the low dose was nontoxic, no data were presented. Thus, it could not be confirmed that the highest dose tested was close to the limit of cytotoxicity and that the lowest dose was nontoxic. If there was no cytotoxicity, then the compound should have been assayed at the solubility limit. In addition, no attempt was made to assure the reproducibility of the results, since only single plates were assayed. According to the standard Ames assay[1], a minimum of duplicate plates per dose per strain should be performed in order to assess reproducibility of the data.

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[1] Ames et al., Mutat. Res., 31:347-364 (1975).

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TABLE 3. Results for Negative and Positive Control Substances

Strain	Chemical	Activation	Revertants Per Plate
TA1535	DMSO	–	18
		+	27
	MNNG	–	>1000
	ANTH	+	234
TA1537	DMSO	–	22
		+	19
	QM	–	577
	AMQ	+	424
TA1538	DMSO	–	19
		+	34
	NF	–	>1000
	AAF	+	673
TA98	DMSO	–	31
		+	47
	NF	–	>1000
	AAF	+	>1000
TA100	DMSO	–	196
		+	198
	MNNG	–	>1000
	ANTH	+	>1000
D4	DMSO	–	32
		+	23
	MNNG	–	573
	DMNA	+	48

CONCLUSIONS:

No conclusions can be drawn from this study because it could not be confirmed that the dose range used was high enough, or whether the results were reproducible.

CLASSIFICATION: Unacceptable.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION

DRAFT

005345
EPA: 68-01-6561
TASK: 75
October 4, 1984
005345

DATA EVALUATION RECORD

VERNAM

Mutagenicity

CITATION: Andersen, K.J., Leighty, E.G., Takahashi, M.T. Evaluation of Herbicides for Possible Mutagenic Properties. (Published: J. Agr. Food Chem. Vol. 20, No. 3, 1972). Prepared by Columbus Laboratories, Battelle Memorial Institute, Columbus, Ohio. Dated January 10, 1972.

REVIEWED BY:

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Date: _____

DATA EVALUATION RECORD 005345

STUDY TYPE: Mutagenicity.

CITATION: Andersen, K.J., Leighty, E.G., Takahashi, M.T. Evaluation of Herbicides for Possible Mutagenic Properties. (Published: J. Agr. Food Chem. Vol. 20, No. 3, 1972). Prepared by Columbus Laboratories, Battelle Memorial Institute, Columbus, Ohio. Dated January 10, 1972.

ACCESSION NUMBER: 071713.

LABORATORY: Columbus Laboratories, Battelle Memorial Institute, Columbus Ohio 43201.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: The test material was identified as Vernolate (Vernam) technical and it was 90-99% pure. Vernolate was obtained from Stauffer Chemical Co. San Francisco, CA 94108.

MATERIALS AND METHODS:

Bacterial Strains: Eight mutant strains of Salmonella typhimurium, described by Whitfield, et al. 1966[1], were used in this study. The strains were obtained from Dr. B.N. Ames (University of California, Berkeley).

Preparation of Test Material: The authors reported that approximately 1 to 5 µl of the test material was used neat.

Controls: Untreated cultures of eight Salmonella typhimurium mutant strains served as the negative controls. The positive controls were diethyl sulfate, N-Methyl-N'-nitro-N-nitrosoguanidine (NG) and ICR-191 (an acridine-half mustard compound). Concentrations of the positive control chemicals were not specified.

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[1] Whitfield Jr. H.J., Martin R.G. and Ames B.M. J. Mol. Biol. 21, 355 1966.

Assay: From freshly grown bacterial cultures of each Salmonella mutant strain (2×10^6 bacteria/ml), 0.2 ml was added to 2 ml of soft agar supplemented with 0.20 µmole of histidine. The mixture was then poured onto a histidine-free minimal agar plates and allowed to solidify. The test material, at 1 to 5 µl, was then applied onto each assay plate.

Mutation Scoring: The number of revertant colonies were counted for each negative control and for each test material.

Evaluation Criteria: The mutagenicity of the test material was evaluated by measuring the frequency of reversion to histidine independence, according to the procedure of Ames and Whitfield (1966).[1] The reversion frequency was compared to the spontaneous reversion frequency for each of the untreated bacterial strains.

RESULTS:

The results of the study, as concluded by the authors, was that the known mutagens, diethylsulfate, NG, and ICR-191, gave positive responses, while the test material, Vernolate technical, gave a negative response. Thus the results indicated that Vernolate technical was not mutagenic in the 8 strains of Salmonella typhimurium. Table 1.

TABLE 1. Response of Histidine-Requiring Mutants of Salmonella typhimurium to Known Mutagens and Herbicides

Compound	Test Result
Diethylsulfate (mutagen)	+
NG (mutagen)	+
ICR-191 (mutagen)	+
Vernolate	-

DISCUSSION:

The authors reported that Vernolate technical was not mutagenic to the tester bacteria; reversion to histidine independence was not induced by the test material under the conditions of the assay.

Our assessment is that the results presented, cannot be interpreted because individual plate counts and results for each strain were not presented. The number of spontaneous revertants for the untreated mutant strains (negative control) were reported to be 0 to 20 colonies/plate, however, the number of revertants for the positive controls and for the test material were not given. In order to make a proper assessment of the positive

controls and the test material, colony counts for each tester strain are needed and they should correspond to concentrations of the materials being assayed. Furthermore, at the time these assays were performed metabolic activation with S9 was not routinely used in assays. Hence, quantitative data with and without metabolic activation for mutants that are now routinely used in the Ames assay, were not produced by this study.

CONCLUSIONS:

Based on the results as presented in this report, it cannot be concluded if Vernam (Vernolate) technical was or was not mutagenic.

CLASSIFICATION: Unacceptable.

005345

EPA: 68-01-6561
TASK: 75
September 7, 1984

DATA EVALUATION RECORD

VERNAM

Mutagenicity

CITATION: Shirasu, Y., et al. 1976. Mutagenicity screening of pesticides in the microbial system. A published report in Mutation Research 40:19-30. Prepared by the Toxicology Division of the Institute of Environmental Toxicology, Tokyo and the Department of Induced Mutation, National Institute of Genetics, Japan. Accepted for publication on September 16, 1975.

REVIEWED BY:

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity, DNA-repair and reverse mutation.

CITATION: Shirasu, Y., et al. 1976. Mutagenicity screening of pesticides in the microbial system. A published report in Mutation Research 40:19-30. Prepared by the Toxicology Division of the Institute of Environmental Toxicology, Tokyo and the Department of Induced Mutation, National Institute of Genetics, Japan. Accepted for publication on September 16, 1975.

ACCESSION NUMBER: 071713.

LABORATORY: Toxicology Division, Institute of Environmental Toxicology, 2-772 Susuki-cho, Kodaira-shi, Tokyo 187 and Department of Induced Mutation, National Institute of Genetics, Mishima, Shizuoka-kin 411, Japan.

QUALITY ASSURANCE STATEMENT: Not applicable to this report.

TEST MATERIAL: The test material, Vernam, was reported to in this study as Vernolate and has the chemical name, S-propyldipropylthiocarbamate. The sample was obtained through Dr. T. Suzuki of the Agricultural Chemicals Inspection Station of the Ministry of Agriculture and Foresty (Japan). No purity was given.

METHODS:

Bacterial Strains: *Bacillus subtilis* strains H17 Rec^+ and M45 Rec^- were used for the rec-assay (DNA repair). Reversion assays were performed using *Escherichia coli* strains WP2 *trp* B/r and WP2 *trp* hcr and *Salmonella typhimurium* strains TA1535, TA1536, TA1537, and TA1538.

Preparation of the Test Material: The test material was dissolved in either dimethylsulfoxide (DMSO) or ethylacetate (ETAc) to achieve a final concentration of 1 mg/ml. The vehicle for the test article was not identified.

Rec-Assay: The *B. subtilis* strains were grown overnight in B-2 broth, and each of the two cultures were streaked onto a plate of B-2 agar. The starting points of these streaks were covered with a paper disc, 10 mm in diameter, that contained 0.02 ml of the test solution. After incubation at 37° C for 24 hours, the length of the inhibition zone was measured.

Reversion Assay: A 0.1 ml sample of each overnight bacterial culture was spread onto the surface of MB[1] or 9BB[2] agar with a glass spreader. From the test solution a 0.02 ml aliquot was applied to a 10 mm paper disc which was placed on the center of the bacterial lawn. After incubation at 37° C for 2 days, revertants colonies around each disc were counted.

For confirmation of positive responses in the spot (reversion) assay, the plate incorporation procedure of Ames et al.[3] was then performed. Overnight bacterial culture (0.1 ml) and 0.1 ml of the test material solution were added to 2 ml soft agar at 45° C and overlayed onto 9BB agar. After incubation at 37° C for 2 days the revertant colonies were scored. None of the assays were conducted with S9-activation.

RESULTS:

The authors reported overall results of the mutagenic screen of 166 pesticides one of which was Vernam. Negative results in the rec- and reversion assays were not reported on an individual compound basis. Because Vernam did not induce positive response in the rec- or reversion assays, the data were not reported.

DISCUSSION:

This study was intended as a preliminary screen for mutagenicity of a large number of compounds, and individual test data were not included in the report for the pesticide Vernam; therefore no assessment of the reported findings can be made. It should also be noted that the data reported did not include mutagenesis testing with the addition of rat liver S9 for metabolic activation.

CONCLUSIONS:

Due to the limited test data presented in this report, no conclusion can be made concerning the mutagenicity of Vernam.

CLASSIFICATION: Unacceptable.

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[1] Ames, B. N. 1971 in A Hollaender (ed.) Chemical Mutagen, Plenum Press, N.Y., pp. 267-282.

[2] Witkin, E. M. 1956. Cold Spring Harbor Quant. Biol. 21:123-140.

[3] Ames, B. N.; Lee, F. D.; Durston, W. E. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. (U.S.) 70:782-786.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DRAFT

005345

EPA: 68-01-6561
TASK: 75
September 12, 1984

DATA EVALUATION RECORD

VERNAM

METABOLISM

CITATION: Miaullis, J.B., Vispetto, A.R. Balance, tissue residue and metabolism study with N-[1-^{14}C]propyl vernam in the rat. Unpublished study (No. MRC-82-03) prepared by Stauffer Chemical Co., Mountain View, CA. Dated April 1982.

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005345

DATA EVALUATION RECORD

STUDY TYPE: Metabolism.

CITATION: Miaullis, J.B., Vispetto, A.R. Balance, tissue residue and metabolism study with N-[1-^{14}C]propyl vernam in the rat. Unpublished study (No. MRC-82-03) prepared by Stauffer Chemical Co., Mountain View, CA. Dated April 1982.

ACCESSION NUMBER: 071713.

LABORATORY: Mountain View Research Center, Mountain View, CA.

QUALITY ASSURANCE STATEMENT: Not present for this report.

TEST MATERIAL: The test material was identified as N-[1-^{14}C] propyl vernolate, ^{14}C-Vernam (S-propyl N,N-dipropylthiocarbamate) with a specific activity of 10 mCi/mM, and radiochemical purity of 98.0% as determined by TLC. A [^{14}C] Vernam-sulfoxide stock solution was prepared from the ^{14}C-Vernam, and had a radiochemical purity of 99.0%, as determined by TLC.

PROCEDURES:

Fourteen male and 14 female Sprague-Dawley albino rats weighing between 156-212 g were treated by oral intubation with either [^{14}C] Vernam (at nominal concentrations of 8, 100, or 550 mg/kg) or [^{14}C] Vernam-sulfoxide (at a nominal concentration of 100 mg/kg). The animals were housed in individual glass metabolism cages to allow for separate collection of urine, feces and $^{14}CO_2$. These samples were collected daily for 7 days.

Groups of animals were sacrificed at 1, 3 and 7 days and the following tissues/organs were weighed and prepared for combustion and LSC analysis: urinary bladder, blood, brain, carcass, cecum, cecum contents, intestine-distal/proximal, esophagus, fat, femur, heart, hide, kidney, liver, lung, spleen, stomach, gonad and thigh muscle.

Urine and feces were counted directly in Ready-Solv (Beckman). Tissue samples were combusted and $^{14}CO_2$ trapped in Carbosorb (Packard). Liquid scintillation counting was performed with a Packard 3380 instrument, determining efficiency by internal standardization and channels ratio.

^{14}C-Vernam sulfoxide was synthesized from ^{14}C-Vernam by the method of Casida et al. (1974)[1] and purified by thin layer chromatography (TLC); the ^{14}C-sulfone was also synthesized with the same method but by using an excess of m-chloroperbenzoic acid. Unlabeled standards for chromatography were either synthesized by Stauffer or obtained from commercial sources.

Thin layer chromatography was performed on 250 μ silica gel plates (E. Merck) to separate metabolites or on 500 or 1000 μ silica gel GF plates for preparative purposes. Labeled compounds were located by autoradiography and unlabeled compounds by UV quench, I_2 vapor, or ninhydrin. A gas chromatography-radioactive monitor (GC-RAM) system was used with a high temperature splitter and CuO_2 combustion furnace, monitoring effluents by flame photometry and radioactivity. Gas chromatography-mass spectrometry was performed with a Finnigan 4000 series computerized system.

RESULTS:

Clinical Observations and Mortality: No overt signs of toxicity were noted except for possible lethargy in the animals at the 550 mg/kg dose. One male and two females from the 550 mg/kg dose group accidentally suffocated due to mechanical pump failure. Excretion data for these animals was available, but tissue analyses were not performed.

Absorption and Excretion: Table 1 summarizes a balance study with male and female rats given a single oral dose of 105 mg/kg Vernam. These results indicated that the compound was rapidly absorbed since there was only a small percentage of the dose recovered in feces and indicate that the major route of excretion was urinary. There was lower recovery in this experiment than expected and in a separate experiment it was found that 13 percent of urinary radioactivity was lost upon air drying of the sample. The initial rate of urinary excretion was rapid; 37.5, 63.7, 68.0 and 71.7 percent of the dose in males and 30.7, 59.7, 65.2 and 73.9 percent of the dose in females was found in the urine at 12, 24, 72 and 168 hours, respectively (average of 2 animal/sex). There was no difference in excretion patterns between males and females.

Table 2 shows radioactivity levels expressed as percent of dose excreted at 72 hours in expired air, urine, and feces in rats dosed at 8, 100, and 550 mg/kg. There were no dose-related effects on the rates of excretion. The percent of the high dose excreted in urine at 12 hours was lower at the high dose (12.3%) than at the lowest dose (46.5%).

Vernam-sulfoxide was excreted similarly to Vernam. However, the initial rate of urinary excretion (12 hours) was slightly greater (48.7%) than with Vernam (35.7%) and less $^{14}CO_2$ was recovered with the sulfoxide (Table 2). Males and females had similar excretion profiles (Table 2).

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[1] Casida, J.E., Gray, R.A., Tilles, H. Science 184:573 (1974).

TABLE 1. Recovery of Radioactivity as Percent of Dose in Animals Given a Single Oral Dose of Approximately 105 mg/kg ^{14}C-Vernam[a]

	Males/hours 24	Males/hours 168	Females/hours 24	Females/hours 168
CO_2	8.2	11.2	13.2	16.1
Urine	65.9	71.7	58.7	73.9
Feces	3.0	5.3	3.1	1.8
Recovery[b]	83.9	88.6	82.8	93.9

[a]Data at each time point are average of 2 animals/sex.
[b]Includes cage wash, but does not include recovery from tissues.

TABLE 2. Percent of Radioactivity Recovered at 72 Hours as a Function of Dose and Chemical[a]

Dose mg/kg	Urine	Feces	CO_2	Recovery
Vernam				
8	66.2	4.0	14.2	87.6
100	57.0	5.2	18.2	92.5
550	71.5	4.9	9.9	87.5
Vernam-sulfoxide				
100	80.2	1.9	7.3	91.9

[a] Data were combined by this reviewer for 2 males and 2 females at each dose, since there were no essential differences between sexes in excretion.

Tissue Distribution: Measurable levels of ^{14}C-residues were found in all tissues. Table 3 summarizes residues in some tissues at 24 hours. The highest levels were found in liver and blood, with lower levels in digestive tract and related organs. With the exception of blood, the tissue levels declined rapidly between 1 and 7 days. The residues in blood at 1-, 3-, and 7-day periods were 20.9, 18.1, 15.9 ppm in males and 23.7, 18.1, and 18.4 ppm in females at a dose of 105 mg/kg. Total radioactivity in tissues from rats receiving 105 mg/kg was 4.6, 2.1 and 1.4 percent of the dose at 1, 3, and 7 days (males and females combined). At doses of 8, 105 and 550 mg/kg the percent of dose in the tissues at 3 days was 4.0, 2.7 and 3.7, respectively.

When ^{14}C-Vernam-sulfoxide (112 mg/kg) was administered, tissue distribution was similar to that found with Vernam. At 72 hours the following levels were found: blood 36.5 ppm, liver 7.6 ppm, kidney 5.3 ppm, lung 5.6 ppm, and brain 1.7 ppm (2 males and 2 females combined).

TABLE 3. Tissue Levels of Radioactivity (ppm Equivalent) 24 Hours After a Dose of Approximately 105 mg/kg ^{14}C-Vernam[a]

	Males	Females
Blood	20.9	23.7
Liver	25.5	26.1
Intestine/distal	9.2	8.0
Intestine/proximal	7.2	7.2
Kidney	7.3	8.8
Lung	6.5	6.8

[a]Only tissues with the highest levels are included in this summary. Data for 2 males and 2 females were combined by this reviewer since there were no apparent sex related differences and averages are presented.

Characterization of Urinary Metabolites: Thin layer chromatography in several solvent systems indicated that no unchanged Vernam was found in the urine and that the pattern of metabolites was similar in males and females.

Extraction of urine at pH 7.0 with ethyl acetate showed that less than 2% of the radioactivity was organo-soluble. Thin layer chromatography of the extract showed that there was no unchanged Vernam or its sulfone or sulfoxide.

Ethyl acetate extraction of acidified urine yielded 41% of the urinary radioactivity from Vernam treated animals and 63% of that from Vernam-sulfoxide dosed animals. The metabolites of this extract were separated by TLC, recovered, methylated, separated by GLC, and their identity confirmed by comparison of mass spectra with standard compounds. The major metabolites identified were the mercaptoacetic, mercaptolactic and N-acetylcysteine derivatives of S-(N,N-dipropyl)thiocarbamate.

After extraction of the acidified urine, the pH of the aqueous phase was adjusted to pH 12 and it was then extracted with ethyl acetate. These extracts contained 25% of the radioactivity of the Vernam urine and 11% of that in the Vernam-sulfoxide urine. Thin layer cochromatography with standards identified the metabolites as dipropylamine, propylamine, and dipropylacetamide. Derivitization with heptafluorobutyric acid followed by GC-MS confirmed the identify of propylamine.

The residual aqueous phase contained small amounts of previously identified metabolites in addition to a newly identified metabolite, S-(N,N-dipropylcarbamoyl) cysteine.

Table 4 gives a quantitative summary of the urinary metabolites.

It was concluded from the identification and quantification of metabolites that the major pathway of metabolism of Vernam was sulfoxidation. This may be followed by oxidation of the sulfoxide to the sulfone. The sulfone can react spontaneously with nucleophiles (which might explain the binding of radioactivity to blood) or it may react spontaneously with the sulfhydryl group of glutathione.

The sulfone can also be enzymatically conjugated with glutathione (GSH). Following conjugation, the glutamate and glycine residues of the GSH are expected to be enzymatically cleaved to form the cysteine conjugate. The cysteine conjugate may be directly excreted in urine or acetylated to form the mercapturate. The cysteine conjugate may also form the mercaptolactic derivative by transamination and this derivative may be oxidized to the mercaptoacetic derivative.

A direct oxidative pathway yielding S-3-hydroxypropyl metabolites does not represent a significant route of metabolism. A second major pathway is oxidation of the S-propyl-α carbon group to finally yield propylamine and dipropylamine.

TABLE 4. Percent of Urinary ^{14}C in Identified Metabolites from 24 Hour Urine Samples of Rats Administered Vernam or Vernam-Sulfoxide

Metabolite	Vernam	Vernam-Sulfoxide
Mercaptoacetic-DPTC[a]	4.2	5.1
3 mercaptolactic-DPTC	3.2	3.5
N-acetylcysteine-DPTC	26.8	36.5
Cysteine-DPTC	6.8	14.6
Other neutrals and acids	27.1	26.3
Dipropylamine	25.4	11.5
Propylamine	8.1	4.7
Total	101.6	101.2

[a] DPTC: S-(N,N-dipropyl)thiocarbamate derivative.

DISCUSSION:

There was some variability in recovery when individual animal data on excretion were examined. The authors commented on the lower than expected percent recovery of radiolabel and in subsequent experiments showed that losses occurred during air concentration of urine samples. However, these losses did not compromise the interpretation of the results. Since there were no apparent differences between sexes in the excretion or tissue distribution, this reviewer combined data for males and females, where appropriate, in summarizing the data; consequently, the mean values at different time points were on four determinations rather than two.

The characterization of urinary metabolites was well designed and complete. Since there was no ^{14}C-label in the S-propyl group, the fate of this residue cannot be determined. The elimination of $^{14}CO_2$ in expired air suggests the dipropylamine and propylamine produced metabolically are further degraded in addition to being excreted in the urine.

The discussion of metabolism by the authors was clear and the proposed metabolic scheme was supported by the data.

CONCLUSIONS:

^{14}C-Vernam and its sulfoxide are readily absorbed, metabolized, and excreted when administered orally to rats. Recovery in urine, feces, and respired CO_2 in males were about 66, 3, and 8% of the administered dose, respectively after 24 hours. Tissue residues were in proportion to the dose at 8, 105 and 550 mg/kg levels and accounted for approximately 4.0, 2.7 and 3.7 percent of the dose at 72 hours. Highest levels were found in the blood and liver (e.g., 21.8 and 25.8 ppm 24 hours after a 105 mg/kg dose) and levels disappeared rapidly from the liver but slowly from the blood. Urinary metabolites were identified and quantified. The metabolites identified included the s-cystine, mercapturate, 3-mercaptolactate, and mercaptoacetate conjugates of S-(N,N-dipropyl)thiocarbamate, dipropylamine and propylamine.

CLASSIFICATION: ~~Acceptable~~. ~~Confidential Data~~ Acceptable.

CONFIDENTIAL BUSINESS INFORMATION

NATIONAL SECURITY INFORMATION (EO 12065)

005345

EPA: 68-02-4225
DYNAMAC No. 1-038-81,2
July 7, 1986

DATA EVALUATION RECORD

VERNAM

A Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Minor, J. L., Downs, J. R., Zwicker, G. M., and Freudenthal, R. I. A two-generation rat reproduction study with Vernam Technical. (Unpublished study No. T-10124, prepared by Stauffer Chemical Company, Environmental Health Center, Farmington, CT, dated January 27, 1983.) Accession Nos. 249703-249704.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-7-86

1. CHEMICAL: Vernam, S-propyldipropylthiocarbamate.

2. TEST MATERIAL: Vernam technical (lot No. WRC 4921-24-6, code No. EHC-0139-25) was 98.3 percent pure by weight and described as an amber liquid.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Minor, J. L., Downs, J. R., Zwicker, G. M., and Freudenthal, R. I. A two-generation rat reproduction study with Vernam Technical. (Unpublished study No. T-10124, prepared by Stauffer Chemical Company, Environmental Health Center, Farmington, CT, dated January 27, 1983.) Accession Nos. 249703-249704.

5. REVIEWED BY:

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Date: July 7, 1986

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William Woodrow, Ph.D.
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Date: _____

Albin Kocialski, Ph.D.
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Date: _____

7. CONCLUSIONS:

A. The parental NOEL and LOEL are rats is 20 and 100 ppm of Vernam, respectively, based on a statistically significant depression in the mean body weight for both parental males and females given 100 ppm Vernam. Reproductive performance was comparable among control and Vernam-dosed groups; no effects on mating, fertility, gestational, or lactational indices were noted. The developmental toxicological LOEL in rats is 20 ppm Vernam, the lowest level tested, based on increased incidences of urinary tract variants in pups from all Vernam-dosed groups of the P_0 and P_1 second matings when compared to controls.

B. Since NOEL for developmental toxicity was not established, this study is classified Core Supplementary.

8. RECOMMENDATIONS: The presence of increased incidences of urinary tract variants in rat pups fed Vernam serve as a preliminary indication of possible developmental toxicity or teratogenesis; thefore we suggest that additional tests be conducted to evaluate the teratogenic potential of Vernam.

Items 9-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Diet Preparation: Vernam technical lot No. WRC 4921-24-6, code No. EHC-0139-25) was reported to be 98.3 percent pure by weight. The test material was mixed with corn oil and blended into rodent chow to yield the appropriate concentrations. Each test diet contained 1 percent added corn oil, and test diets were prepared at approximately 4-week intervals. During the gestational and lactational phases, diets were prepared in double batches, and all diets were analyzed for the test material concentrations approximately once a month. The stability of the test material was determined; however, methods for determining of concentration and stability were not specified.

B. Animals and Test System: After a 14-day quarantine period, 42-day-old CrlCD(SD) BR rats (from Charles River) were randomly assigned to one of four treatment groups on the basis of body weight. Each group consisted of 15 males and 30 females. Vernam was administered at concentrations of 0, 20, 100 or 500 ppm in the diet. After 62 days of treatment, the animals were mated (one male to two females) to yield the F_{1a} generation. The F_{1a} pups were weaned and necropsied. The parental rats, still on treatment, were remated the first week following the F_{1a}

[1] Only items appropriate to this DER have been included.

45

weaning period to produce the F_{1b} litters. Following weaning, animals from litters of females from each group who delivered viable offspring from both matings were randomly selected and continued on their respective treatments as the P_1 animals. An additional five rats/sex/dose were selected to replace any weanlings lost before day 40. The P_0 animals were sacrificed and necropsied following the F_{1b} weaning. When the P_1 rats were 105-115 days of age, they were mated to produce the F_{2a} and F_{2b} litters as was described for the F_1 animals. The F_{2b} litters were handled as described for the F_{1b} litters. P_1 animals were sacrificed and necropsied 21 days after F_{2b} weaning, and tissues were collected.

At termination, all parental animals and weanlings scheduled for necropsy and histopathology were anesthetized with pentobarbitol and exsanguinated. Remaining weanlings and preweaning pups were sacrificed by carbon dioxide inhalation. Scheduled necropsies were performed under the supervision of a veterinary pathologist. Specified organs were weighed and tissues were collected for possible histopathological examination. The organs of animals found dead or sacrificed in poor condition were not weighed; otherwise, necropsy procedures were similar to those described for animals sacrificed on schedule.

Complete necropsies were performed on all P_0 animals, but tissues were collected for the control and high-dose animals only. Tissues were collected for all P_1 animals following complete necropsies.

Ten F_{1a} weanlings/sex from the control and high-dose groups and the same number of F_{1b} and F_{2b} weanlings/sex from all dose groups were subjected to complete necropsies. Selected organs from these weanlings were weighed. The remaining weanlings were given a partial necropsy. Necropsies were performed on abnormal F_{2a} weanlings only.

Preweaning pups and stillborn pups were necropsied under the supervision of the study director either before or after fixation in Bouin's solution and examined by the Wilson method. The remaining viscera were examined by the Staples' technique for fresh specimens and by the Wilson method for Bouin's-fixed specimens.

C. Parameters Measured: All parental rats were observed daily for overt signs of toxicity or ill health. During the growth phases, the body weight and food consumption of each rat were determined weekly.

All animals were examined externally prior to cohabitation, and male copulatory behavior was noted as present or absent when a female was added to the male's cage. Each morning of cohabitation, a vaginal smear was performed on each female to determine the stage of estrus or to confirm a positive mating.

The day sperm or a copulatory plug was observed was designated as gestational day (GD) 0. Weekly body weight measurements were continued on males and unmated females.

Body weights were measured on GD 0, 6, 13, and 20. Food consumption was determined for the GD intervals 0-6, 6-13, and 13-20. Beginning on GD 21, the females were monitored for normal behavior during the day. The day of delivery was designated postpartum or lactational day 0. The dam and litter were examined as soon as possible after delivery (lactational day 0) and on lactational days 4, 7, 14, and 21. For each examination period, the following data were recorded: dam body weight, total litter size, number of live pups, number of dead pups, gross anomalies, weight, and sex of each pup. After F_{1a}, litters were weighed collectively after delivery. The following maturational landmarks were determined: day 4, unfolding of the external pinna of the ear; day 7, incisor eruption; and day 14, opening of the eye. On days 0, 4, and 7, the absence of milk in the pup's stomach was noted, and on day 7, the retrieval behavior of the female was determined. Food consumption was determined for the female during lactation.

D. Statistical Analysis: Enumerated data were analyzed by Fisher's Exact test, and data collected during gestation and lactation were analyzed using the Mann-Whitney nonparametric rank test. Continuous data were analyzed by Dunnett's procedure. All tests were two-tailed, and 0.05 was selected as the level of significance.

The authors also stated that "animals which were not characteristic of the major portion of the animals, and values which were judged to be outliers were removed, and the statistics reported based on this edited subset." The authors' criteria for selection of animals included pregnancy at the last mating for inclusion of organ weights; a litter size greater than five pups for all parameters during lactation (except litter size) and "true" second litters from the second mating. Minimum and maximum values were judged to be outliers based on the standard deviation within the group, which included the outlier values.

E. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Test Diet Analyses: The concentrations and homogeneity of the test material in 12 of the 14 batches of diet used in this study were analytically confirmed. Measured mean concentrations of Vernam in the diet were 19.4, 102, and 505 ppm for theoretical concentrations of 20, 100, and 500 ppm, repectively. Preliminary studies demonstrated that the test material was stable after 2

weeks when stored at 4° or 60°C. After 4 weeks, a 10 percent loss in chemical activity was noted at 60°C and a 5 percent loss was reported at ambient temperature.

B. General Effects in Parental Males:

1. Survival, Appearance, or Behavior: All P_0 and P_1 males survived until scheduled time of sacrifice. No compound-related effects on appearance or behavior were noted in these animals.

2. Body Weights: Mean body weights for P_0 and P_1 males fed 500 and 100 ppm Vernam were significantly decreased ($p < 0.05$) when compared to controls (Table 1). These decreases were observed consistently after weeks 8 and 16 for P_0 males at the 500- and 100-ppm levels, respectively. No differences were noted for animals fed 20 ppm Vernam when compared to controls.

3. Feed Intake: Feed intake was significantly reduced ($p < 0.05$) for male P_0 and P_1 rats fed 500 ppm Vernam when compared to controls (Table 2). Sporadically decreased food consumption occurred in P_0 and P_1 males fed 100 ppm, and no effects were noted in males at the 20-ppm level. Average intake values for Vernam in males for the P_0 and P_1 rats are given in Table 3.

   The authors stated that dividing the ppm levels by an accepted factor of 20 to obtain intakes in mg/kg/day gave expected Vernam dosages of 1, 5, and 25 mg/kg/day.

4. Findings at Termination: The authors stated that all necropsy findings were considered to be incidental, including significant decreases ($p < 0.05$) in liver weights and kidney-to-body weight ratios at the 500-ppm level in P_0 males. No effects were noted for males given 20 ppm Vernam. No organ weights were reported for P_1 males.

C. General Effects in Parental Females:

1. Survival, Appearance, and Behavior: No Vernam-related effects on the survival, appearance, or behavior were observed for the parental females. Four parental females (two P_0 females at 500 ppm and two P_1 females at 100 ppm) died during the study, but these deaths were not attributed to Vernam ingestion.

2. Body Weights: There were significant depressions ($p < 0.05$) in body weight for the P_1 females at all dose levels and for the P_0 females fed 100 and 500 ppm Vernam when compared to controls (Table 4).

TABLE 1. Effect of Vernam Ingestion on Mean Body Weights (g) of Male Rats

Dietary Level (ppm)	Study Day 0	21	56	91	112	68
P_0 Males						
0	200[a] ±13	350 ±24	472 ±37	520 ±44	560 ±48	622 ±59
20	201 ±9	351 ±19	477 ±27	526 ±34	570 ±45	634 ±60
100	198 ±11	341 ±18	448 ±28	484* ±34	523* ±41	577* ±44
500	200 ±10	339 ±17	442* ±27	476* ±29	509* ±33	547** ±31
	Study Day 176	197	232	281	302	371
P_1 Males						
0	126 ±12	296 ±21	453 ±31	546 ±35	585 ±39	642 ±45
20	120 ±17	287 ±29	451 ±32	555 ±45	594 ±48	662 ±66
100	116 ±15	275 ±23	413** ±33	498** ±42	525** ±46	581** ±53
500	115 ±16	267** ±26	399** ±34	483** ±37	505 ±45	542** ±55

[a]Mean ± SD.

*Significantly different from controls, $p < 0.05$.

**Significantly different from controls, $p < 0.01$.

TABLE 2. Effect of Vernam Ingestion on Mean Daily Feed Intake (g) in Male Rats

Dietary Level (ppm)	Study Day 21	56	91	112	146	168
P_0 Males						
0	24[a] ±2	24 ±3	23 ±6	24 ±3	25 ±3	24 ±3
20	25 ±3	24 ±3	24 ±3	23 ±3	25 ±3	24 ±2
100	24 ±2	23 ±2	22 ±3	22 ±2	22 ±2	23 ±2
500	23 ±2	22 ±2	22 ±2	21 ±2	22** ±2	21** ±2

Dietary Level (ppm)	Study Day 197	232	281	302	344	371
P_1 Males						
0	22 ±2	25 ±2	24 ±2	24 ±2	25 ±2	25 ±2
20	23 ±2	25 ±2	24 ±3	23 ±3	25 ±3	25 ±3
100	22 ±2	22** ±2	22 ±2	23 ±2	24 ±2	22* ±2
500	20* ±2	21** ±2	21** ±2	21** ±2	22** ±2	21** ±3

[a]Mean ± SD.

*Significantly different from controls, $p < 0.05$.

**Significantly different from controls, $p < 0.01$.

TABLE 3. Mean Vernam Intake (mg/kg/day) of Two Generations of Male Rats

	Dietary Levels (ppm)					
	20		100		500	
	P_0	P_1	P_0	P_1	P_0	P_1
Maximum	2.0	2.2	9.7	11.3	25	24
At First Mating	1.0	1.1	5.1	5.3	25	24
Minimum	0.8	0.8	3.9	3.9	19.7	19.0

TABLE 4. Effect of Vernam Ingestion on Mean Body Weights (g) of Female Rats

Dietary Level (ppm)	Study Day 0	21	56	118	175
P_0 Females					
0	140[a] ±9	208 ±19	263 ±30	291 ±29	319 ±36
20	140 ±9	205 ±20	259 ±32	288 ±29	317 ±40
100	141 ±9	204 ±15	253 ±21	277 ±20	300 ±24
500	139 ±7	197* ±13	239** ±16	263** ±16	289** ±17

	Study Day 176	197	282	309	372
P_1 Females					
0	108 ±8	194 ±17	262 ±24	314 ±31	335 ±38
20	39** ±12	108** ±15	249 ±24	299 ±25	322 ±34
100	103 ±13	183* ±19	245* ±27	286* ±30	300** ±28
500	95** ±10	173** ±17	229** ±24	269** ±23	286** ±25

[a]Mean ± SD.

*Significantly different from controls, $p < 0.05$.

**Significantly different from controls, $p < 0.01$.

3. Feed Intake: Mean feed intake values were significantly reduced ($p < 0.05$) at the 500- and 100-ppm levels in both the P_0 and P_1 generations when compared to controls (Table 5). Consistent decreases were noted during weeks 2 and 4 for P_0 females receiving 500 and 100 ppm Vernam, respectively.

   Average Vernam intake values for the P_0 and P_1 female rats are presented in Table 6.

4. Findings at Termination: The authors stated that all findings at termination were considered incidental occurrences and not related to Vernam administration. Statistically significant decreases ($p < 0.05$) in liver weights and liver-to-body weight ratios noted in females given 20 ppm of Vernam and statistically significant increases ($p < 0.05$) in the organ-to-body weight ratios for liver, kidney, heart, and brain noted in females given 500 ppm were not considered biologically significant.

D. Reproductive Effects:

1. Fertility and Behavior: There were no adverse effects on fertility or reproductive performance related to Vernam ingestion (Table 7). Only two males (P_1 control and 100-ppm groups) were judged to be sterile in this study. The numbers of P_0 females failing to produce offspring after two matings were two, two, one, and zero and for P_1 females, four, four, two, and one for groups given 0, 20, 100, or 500 ppm of Vernam, respectively.

2. Dam Body Weights and Feed Intake During Gestation: A statistically significant reduction ($p < 0.05$) in dam body weights was noted consistently during gestation in rats given 500 ppm when compared to controls (Table 8). Decreased body weights (significant at $p < 0.05$) were also noted in P_0 females given 100 ppm Vernam during the second gestational period and in P_1 females during both gestational periods when compared to controls. Feed intake was significantly decreased ($p < 0.05$) in pregnant rats given 100 or 500 ppm Vernam when compared to controls. No differences were noted in the feed intake or body weights of females fed 20 ppm Vernam.

3. Dam Body Weights and Feed Intake During Lactation: During lactation, body weight values were significantly reduced ($p < 0.05$) in females at the 500- and 100-ppm dose levels when compared to controls (Table 8). Occasional significant reductions in food consumption were noted in females given 100 or 500 ppm Vernam when compared to controls, but a consistent pattern was not apparent. There were no effects on either body weight or feed intake during lactation in rats given 20 ppm Vernam.

TABLE 5. Effect of Vernam Ingestion on Mean Daily Feed Intake of Female Rats in a Two-Generation Reproduction Study

Dietary Level (ppm)	Study Day 21	35	56	118	175
P_0 Females					
0	17[a] ±2	17 ±2	17 ±2	19 ±4	20 ±4
20	16 ±2	17 ±2	16 ±2	19 ±2	19 ±3
100	16 ±2	16** ±2	16* ±2	18 ±3	18 ±3
500	16* ±1	16** ±1	14** ±2	16** ±2	16** ±2

	Study Day 197	218	232	309	372
P_1 Females					
0	16 ±2	17 ±3	17 ±2	19 ±2	19 ±3
20	15 ±2	16 ±2	16* ±2	20 ±2	19 ±3
100	15** ±1	16 ±2	16* ±2	18 ±3	18* ±3
500	14** ±1	15** ±2	15** ±2	17* ±2	17** ±2

[a]Mean ± SD.

*Significantly different from controls, $p < 0.05$.

**Significantly different from controls, $p < 0.01$.

TABLE 6. Mean Vernam Intake (mg/kg/day) of Two Generations of Female Rats

	Dietary Levels (ppm)					
	20		100		500	
	P_0	P_1	P_0	P_1	P_0	P_1
Maximum	2.1	2.2	10.2	10.6	51.0	53.7
At First Mating	1.3	1.3	6.0	6.3	30.2	32.0
Minimum	1.3	1.1	6.0	5.1	28.7	28.3

TABLE 7. Effects of Vernam Ingestion on Reproductive Parameters for Two Generations of Rats

Litter Generation	Dietary Level (ppm)	No. Females Mated	No. Females Pregnant	Fertility Index (%)	No. Litters Born	Gestation Index (%)	Mean No. Pups/Litter	Born Viable
F_{1a}	0	30	26	83	25	96	12.9	97
	20	30	24	77	23	96	13.8	99
	100	30	28	87	26	93	12.1	99
	500	29	28	90	26	93	12.0	98
F_{1b}	0	30	24	77	23	96	12.9	97
	20	30	27	83	25	93	14.0	96
	100	30	28	93	28	100	12.3	93
	500	29	26	76	22	85	12.9	98
F_{2a}	0	30	23	70	21	91	13.8	98
	20	30	27	87	26	96	12.8	98
	100	30	27	87	26	96	13.0	96
	500	30	28	93	28	100	12.1*	99
F_{2b}	0	30	28	83	25	89	13.9	99
	20	30	27	70	21	78	14.1	97
	100	30	27	73	22	81	13.2	94
	500	30	29	90	27	93	13.0	98

*Significantly different from controls, $p < 0.05$.

TABLE 8. Effect of Vernam Ingestion on Mean Body Weights (g) of Female Rats during Gestation and Lactation

Litter Generation	Dietary Level (ppm)	Gestation Day				Lactation Day				
		0	6	13	20	0	4	7	14	21
F_{1a}	0	264	290	314	376	293	304	310	325	305
	20	269	285	310	376	290	304	316	324	305
	100	252	276	299	360	281	286*	302	311*	299
	500	238*	262*	284*	345*	271*	282*	294*	310*	301
F_{1b}	0	290	316	341	410	333	335	352	353	325
	20	287	314	342	412	335	338	350	341	330
	100	277	301	325*	388*	316	317*	330*	337*	318
	500	266*	289*	314*	379*	311*	314*	328*	336*	321
F_{2a}	0	274	299	323	386	310	317	328	334	314
	20	271	298	317	384	305	309	320	335	314
	100	258*	284*	307*	375	291*	299*	309*	321	304
	500	239*	266*	287*	354*	278*	280*	290*	304*	292*
F_{2b}	0	310	335	358	434	347	357	364	372	341
	20	301	323	347	424	336	338	350	361	331
	100	286*	309*	332*	409*	326	335*	348	348*	326*
	500	267*	289*	312*	386*	304*	313*	323*	336*	320*

*Significantly different from controls, $p < 0.05$.

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4. Litter Size: The authors stated that neither litter size nor sex distribution were affected consistently or significantly by Vernam ingestion. Further, the reduction of litter size at the 500-ppm level for the first litter of the P_1 generation was a "secondary" effect to the decreased body weights for the P_1 females; it was therefore not a biologically significant reproductive effect. Tables 7 and 9 present the effects of Vernam on several reproductive parameters.

5. Mean Litter Weights: The authors stated that consistent decreases in litter weights in the 500-ppm dose group were noted but the only significant reduction ($p < 0.05$) was observed for the F_{1b} offspring when compared to controls (Table 9). Significant decreases were also noted for F_{1b} offspring in the 20-ppm dose group, but these were not considered biologically significant.

6. Pup Survival and Development: As seen in Table 9, no differences in survival of pups from any generation in the control and treatment groups were apparent. The authors noted a significant increase ($p < 0.05$) in the viability index and in the precentage of pups with eyes open on lactational day 14 in the 500-ppm dose group of the F_{2a} generation when compared to controls. All other developmental indices were comparable among control and treatment groups for both generations.

7. Macroscopic Findings: The authors stated that no effects directly attributable to Vernam administration were found in offspring obtained from either generation. Increases in the numbers of weanlings with macroscopic findings from the 500-ppm dose group were noted in the second matings of both generations. These findings included dilation of the renal pelves and/or convoluted ureters. These changes were statistically significant ($p < 0.05$) in the F_{2b} generation at the 500-ppm dietary level when compared to controls.

9. Absolute and Relative Organ Weights in Pups: Significant decreases ($p < 0.05$) in organ weights were noted at 500 ppm for kidneys in F_{1a} females, lungs in F_{1a} males and females, and the brain and lung in F_{1b} females when compared to controls. In addition, significant decreases in organ-to-body weight ratios were noted for lungs in the F_{1a} males and females and F_{2b} females at this dose level when compared to controls. However, the authors stated that these changes were not attributable to Vernam administration.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The assessment of the toxicological effects of Vernam on rats was based on clinical observations, mortality, changes in body weight and feed intake, gross abnormalities at necropsy, and changes in organ weights. The potential effect of Vernam on reproductive

TABLE 9. Effect of Vernam Ingestion on Pup Survival and Body Weights During Lactation in Two Generations of Rats

Generation by Litter	Dietary Level (ppm)	Pup Survival (%) Days 0-4	Pup Survival (%) Days 0-21	Mean Pup Body Weight (g) Day 4	Mean Pup Body Weight (g) Day 21 Male	Mean Pup Body Weight (g) Day 21 Female
F_{1a}	0	98.6	96.3	9.4	45.2	43.0
	20	97.7	97.7	9.3	42.5	41.0
	100	99.0	98.1	9.2	43.3	41.2
	500	99.7	98.6	9.5	42.9	40.8
F_{1b}	0	98.5	98.5	9.9	47.0	44.0
	20	98.5	97.4	9.3	41.9*	40.3*
	100	98.1	97.1	9.6	44.4	42.7
	500	99.0	99.0	9.6	42.9*	40.9*
F_{2a}	0	97.2	96.3	9.5	45.1	42.9
	20	98.7	98.5	9.9	45.8	44.1
	100	98.0	97.0	9.4	45.0	43.8
	500	100.0	99.3	9.9	43.8	42.1
F_{2b}	0	96.4	94.0	9.0	43.2	41.7
	20	99.4	97.3	9.3	43.5	41.4
	100	99.7	98.7	9.6	44.7	42.3
	500	99.2	98.1	9.2	42.3	40.2

*Significantly different from controls, $p < 0.05$.

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TABLE 10. Effect of Vernam Ingestion on the Incidence of Macroscopic Findings in Two Generations of Rat Pups

Litter Generation	Dietary Concentration (ppm)	No. of Litters Examined	No. Litters w/ Macroscopic Alterations	No. Litters w/ Urinary Tract Variants
F_{1a}	0	25	15 (60)[a]	8 (32)[a]
	20	23	8 (35)	5 (22)
	100	26	7 (27)	6 (23)
	500	26	12 (46)	8 (31)
F_{1b}	0	23	6 (26)	4 (17)
	20	25	13 (52)	10 (40)
	100	28	12 (43)	10 (36)
	500	22	12 (55)	9 (41)
F_{2b}	0	25	12 (48)	6 (24)
	20	21	13 (62)	11 (52)
	100	22	17 (77)	8 (36)
	500	27	18 (67)	18 (67)*

Note: Only abnormal-appearing pups were examined for the F_{2a} generation. Therefore, data from the F_{2a} generation were not presented.

[a]Values in parentheses represent the percent incidence.

*Significantly different from controls, $p < 0.05$.

parameters was assessed on the basis of changes in mating and fertility indices, length of gestation, changes in body weights and feed intake of pregnant and lactating animals, litter size and viability, pup body weight, developmental abnormalities, and internal examination and organ weights of pups. From data obtained, the authors concluded that the only effects attributable to Vernam administration were decreases in body weight and feed intake for male and female rats fed 100 or 500 ppm. In parental animals, significant decreases in body weights were noted in females after 2 weeks and males after 11 weeks of test material administration at dietary levels of 500 and 100 ppm, respectively. Intrauterine development was not affected. Significant decreases in body weight noted during the first 5 weeks after weaning of P_1 females were, according to the authors, due to this group's (F_{1b}) large mean litter size and subsequent reduced nutrient intake during lactation and were therefore not attributed to Vernam administration. The authors concluded that increases in relative organ weights in P_0 females were attributed to body weight losses. Increases in absolute and relative liver weights in P_0 females were due to lactational stress because of large mean litter sizes. Changes in the body weights of pups were noted only after direct ingestion of the test material. Intrauterine development was not affected; litter sizes, live born indices, and abnormalities were comparable among control and treatment groups. The authors concluded that although significant increases in the incidence of renal and ureter variants were noted in F_{2b} weanlings fed 500 ppm Vernam, these changes were not attributable to Vernam administration because of previous high incidences of similar urinary tract variants observed in control animals received from the same source.

The authors reported the NOEL for parental rats to be 20 ppm Vernam in the diet based on decreased body weights and feed intake in animals given 100 or 500 ppm Vernam. They assessed that a dietary level of 500 ppm Vernam did not cause any negative reproductive effects.

B. A signed quality assurance statement was presented but not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We agree with the study authors' assessment of the NOEL for general toxicological effects after Vernam administration. However, we disagree with the study authors' assessment that the increased incidence of urinary tract findings was not biologically significant. Comparisons of control with the Vernam-dosed groups revealed an approximately twofold increase in the incidence of urinary tract findings among the F_{1b} and F_{2b} litters. We consider the increased incidences of urinary tract variations at all doses to be compound-related developmental effects in the

offspring. Therefore, the NOEL for developmental toxicity could not be assessed.

B. We agree with the authors' assessment of the maternal NOEL being 20 ppm. However, it should be noted that at 20 ppm, the P_1 females had significantly decreased body weights ($p < 0.05$) from study days 176 to 211 (approximately 7 to 11 weeks of age). Since the mean body weight gain from weeks 12 to 21 was the same as controls (118 g versus 121 g) and these same females were also smaller at weaning, we do not consider this transient weight change to be toxicologically significant.

C. The authors concluded that significant decreases in size of the first litter of the 500-ppm dosed P_1 females was a secondary effect of decreased body weights for these females. However, we assess that the difference was the result of analyzing a small sample size.

D. Deficiences in the study report were as follows:

1. Organ weights for P_1 parental animals were not reported. According to protocol amendments, these organs were inadvertently not weighed at necropsy. Since significant differences were obtained in organ weights and organ-to-body weight ratios for P_0 animals in the 500-ppm dose group, the addition of P_1 organ weights would have enabled us to assess whether these decreases were biologically insignificant as the authors stated.

2. The majority of F_{2a} pups were not examined internally after sacrifice. We question this omission especially since increases in urinary tract variants occurred in pups from the F_{1b} and F_{2b} generations. Additional data would have better enabled us to evaluate the biological significance of the incidences of urinary tract variants found in F_{1b} and F_{2b} animals.

3. Although results of analyses of concentration and stability of Vernam in test diets were presented, methodology used for analyses were not reported. We were therefore unable to determine if methods used were acceptable for determination of these parameters.

4. Individual data of necropsy finding for weanlings were not completely presented; sex of pups with abnormal findings was not reported and unilateral urinary tract variants were not defined as being present on the right or left side. Therefore, summary tables could not be verified.

Item 15-see footnote 1

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. 93-121.

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APPENDIX A

Study Protocol

Vernam Scientific Reviews

Page _____ is not included in this copy.

Pages 64 through 85 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product 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

_x_ FIFRA registration data

___ The document is a duplicate of page(s) _____

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005345

EPA: 68-02-4225
TASK: 038C
November 6, 1985

005345

DATA EVALUATION RECORD

VERNAM

Teratogenicity Study in Mice

STUDY IDENTIFICATION: Benson, B., Scott, W. J., and Beliles, R. P. Vernam, safety evaluation by teratological study in the mouse. (Unpublished study No. T-2132 prepared by Woodard Research Corporation, Herndon, VA, for Stauffer Chemical Company, Richmond, CA; dated April 28, 1967.) Accession No. 247151.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

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005345

1. CHEMICAL: Vernam; S-propylidpropylthiocarbamate; vernolate.

2. TEST MATERIAL: Vernam Tech 96%, Lot No. 6902805, was described as a clear liquid.

3. STUDY/ACTION TYPE: Teratogenicity study in mice.

4. STUDY IDENTIFICATION: Benson, B., Scott, W. J., and Beliles, R. P. Vernam, safety evaluation by teratological study in the mouse. (Unpublished study No. T-2132 prepared by Woodard Research Corporation, Herndon, VA, for Stauffer Chemical Company, Richmond, CA; dated April 28, 1967.) Accession No. 247151.

5. REVIEWED BY:

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EPA Reviewer

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Date: _____

Albin Kocialski, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. We could not assess NOELs or LOELs for maternal or fetal toxicity and teratogenicity after dietary administration of Vernam to pregnant mice because there were major deficiencies in study conduct and the final report. Deficiencies included insufficient number of litters for teratological evaluation, absence of individual data on fetal abnormalities, and lack of adequate information about the animals used.

B. This study did not meet the minimum criteria for a teratogenicity study as suggested in the EPA guidelines (see item 14). Therefore, the teratogenic potential of Vernam in mice cannot be assessed and this study is classified Core invalid.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Article: Vernam was dissolved in acetone and mixed in a basal ration (acetone was allowed to evaporate) at concentrations of 0, 53, and 160 ppm; these levels corresponded to target dosages of 0, 8, and 24 mg/kg/day. The animals were fed the test diets from gestation day (GD) 6 through 18 (for animals Caesarean sectioned) or until natural delivery (for the remaining animals).

2. Animal and Test System: Sixty female mice received from Charles River Mouse Farms, Inc., were mated with male mice from the same source and assigned to three groups of 20 animals each. The authors did not specify the method of selection. The presence of a vaginal plug was considered evidence of mating, and the day that plugs were noted was designated GD 0. Mice were weighed and observed for changes in behavior and general appearance on GD 6, 11, 15, and 18. On GD 18, one-half of the females were anesthetized with chloroform, and their litters were delivered by Caesarean section. The remaining animals were allowed to deliver naturally and pregnancy duration to the nearest half day was recorded.

[1] Only items appropriate to this DER have been included.

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Internal organs of each dam were examined for abnormalities. The uteri were examined for number of live and dead fetuses, fetal body weights, and number of resorptions. Animals were considered nonpregnant if no resorptions or fetuses were present. External abnormalities in the fetuses were noted.

One-half of the fetuses from each litter was preserved in Bouin's solution. Limbs were removed, and the heads were sectioned and examined for abnormalities. Viscera were examined and unusual findings were recorded. The remaining fetuses were macerated in KOH, stained with Alizarin Red S, and examined under 1.5 x magnification for skeletal abnormalities.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Test Material: The authors did not report any analyses of Vernam concentration, homogeneity, or stability in the basal diet.

B. Maternal Effects: The authors reported that all females survived, and that no maternal dose-related pharmacological signs were observed. No differences were noted in body weight gains between control and treated groups (Table 1). The authors reported no differences in pregnancy rates (Table 2) or length of gestation between control and treated groups. Necropsies revealed no visceral abnormalities.

C. Embryonic/Fetal Effects: There were no group differences reported for sex ratios, numbers of viable and dead fetuses, or number of resorptions. The authors reported that two fetuses from one dam fed 8 mg/kg/day Vernam "exhibited swelling in the parieto-occipital area of the skull with subcutaneous hemorrhage." After clearing the specimens, irregular stain deposits were noted in parietal bones. No other significant dose-related findings were noted from the visceral or skeletal examinations when compared to controls.

13. AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that based on study results, the dietary administration of Vernam at dosages of 8 or 24 mg/kg/day had no maternal or fetal effects. The authors did not assess the NOELs or LOELs for this study.

B. No quality assurance statement was presented.

TABLE 1. The Effect of Dietary Vernam on Mean Body Weights (g) in Pregnant Mice[a]

Vernam Dietary Concentration (mg/kg/day)	Mean Body Weights (g) at GD 6	11	15	18
0	35.2 ±2.2	35.4 ±3.5	42.5 ±4.5	49.3 ±7.7
8	35.0 ±1.6	34.9 ±2.8	42.4 ±4.3	51.2 ±7.5
24	34.9 ±1.7	35.1 ±2.6	42.6 ±5.1	48.4 ±7.4
	Body Weight Gains at GD Interval 6-11	11-15	15-18	6-18
0	0.2	7.1	6.8	14.1
8	-0.1	7.5	8.8	16.2
24	0.2	7.5	5.8	13.5

[a]In this table, data from Caesareanand naturally delivered dams are combined.

[b]Mean ± SD.

TABLE 2. Mean Reproductive and Fetal Parameters of Mice Fed Vernam During Gestation

Parameter	Vernam Dietary Concentration (mg/kg/day)		
	0	8	24
Pregnancy Rate (%)[a]	85	100	100
Implantations[a]	11.9	12.4	12.8
Resorptions[a]	1.6	0.9	1.6
Postimplantation Loss (%)[a]	13.4	7.3	12.5
Live Fetuses[a]	9.9	10.4	11.4
Fetal body wt.(g)			
Caesarean Delivered	1.39	1.05	1.10
Naturally Delivered	1.56	1.41	1.39

[a]For these parameters, data from Caesarean- and naturally delivered females are combined.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Because of the following deficiencies in study design and in presentation of data in the study report, the teratogenic potential of Vernam could not be assessed.

1. No maternal toxicity was seen at any dosage level tested, indicating that the dosages were improperly selected. No background data were reported to indicate the reason for dose selections. Without evidence of maternal toxicity, the teratogenic potential of Vernam cannot be assessed.

2. We could not verify the stability, homogeneity, or concentration of the test material in the test diets because no results of analyses were reported. Furthermore, the amount of test material the animals received could not be determined because no food consumption data were reported.

3. The number of litters available for teratological evaluation was insufficient because half of the mated females were allowed to deliver naturally. The naturally born pups and Caesarian-delivered fetuses could not be grouped together for evaluation because of differing stages of development resulting from differing lengths of gestation. Therefore, only 8-10 litters/group (Caesarean delivered) could actually be compared for teratological evaluation.

4. We question the accuracy of the reproductive parameters such as numbers of viable and dead fetuses, resorptions due to discrepancies between these numbers, and the reported number of total implantations. These discrepancies occurred most frequently for the naturally born litters.

   Accurate counts of dead fetuses may not have been reported for naturally born litters since mice often cannibalize malformed or dead fetuses upon delivery. The animals were not observed continuously, and therefore accurate counts of the number of fetuses may not have been made. Furthermore, since malformed fetuses are often cannibalized, data on the number of malformed fetuses and types of malformations observed may be inaccurate.

5. No individual data for external, visceral, or skeletal abnormalities in fetuses were presented. Therefore, data in the summary tables could not be verified by the reviewers.

6. The report did not specify whether daily mortality checks and observations were performed. The authors stated that on GD 6, 11, 15, and 18 the animals were observed for changes in behavior and general appearance; no daily observations were reported. Without daily observation, overt signs of maternal toxicity can go undetected.

7. The strain, age, and reproductive history of mice used in the study were not reported. Thus, we could not assess the acceptability of the animals used. Knowledge of the strain of mouse used in the study would also be helpful since some fetal visceral and skeletal variants occur more frequently in certain mouse strains. With this information, the results of visceral and skeletal examinations could be better evaluated.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 3 and 4.

APPENDIX A

Materials and Methods

Vernam Scientific Reviews

Page _____ is not included in this copy.

Pages 95 through 96 are not included in this copy.

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___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product 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

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DRAFT

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EPA: 68-02-4225
DYNAMAC No. 1-380
December 12, 1985

005345

DATA EVALUATION RECORD

VERNAM

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Bryan, J., Werchowski, K. M., Rodwell, D. E., and Mayhew, D. Teratogenic potential (segment II) oral study in rabbits with Vernam. (Unpublished study No. WIL-80207 by Wil Research Laboratories, Inc., Cincinnati, OH, for Stauffer Chemical Company; dated April 6, 1981.) Accession No. 245293.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: _____

1. CHEMICAL: Vernam; S-propylidpropylthiocarbamate.

005345

2 TEST MATERIAL: Vernam technical, an amber liquid from lot No. CGB-2601 WRC 4921-24-3, consisted of an unspecified percentage of active herbicidal ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

4. STUDY IDENTIFICATION: Bryan, J., Werchowski, K. M., Rodwell, D. E., and Mayhew, D. Teratogenic potential (segment II) oral study in rabbits with Vernam. (Unpublished study No. WIL-80207 by Wil Research Laboratories, Inc., Cincinnati, OH, for Stauffer Chemical Company; dated April 6, 1981.) Accession No. 245293.

5. REVIEWED BY:

Guillermo Millicovsky, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____

Date: _____

Robin B. Phipps, B.S.
Independent Reviewer
Dynamac Corporation

Signature: _____

Date: _____

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: _____

Date: _____

William Woodrow, Ph.D.
EPA Reviewer

Signature: _____

Date: _____

Albin Kocialski, Ph.D.
EPA Section Head

Signature: _____

Date: _____

7. CONCLUSIONS:

A. Based on the data presented, we assess that the NOEL for maternal and fetal toxicity of Vernam in rabbits is 200 mg/kg/day, the highest dosage used. The LOELs for maternal and fetal toxicity could not be determined. A definitive assessment of the teratogenic potential of Vernam in rabbits was precluded by the absence of maternal toxicity at the highest dose level used in this study.

B. This study is classified as Core Supplementary due to the unacceptable dosages used. In addition, information on the purity of the test material and on the concentration and stability of the dosage solutions was not reported as required by standards set by Good Laboratory Practice.

8. RECOMMENDATIONS:

We recommend that future tests to assess the teratogenic potential of Vernam in rabbits include a dosage (above 200 mg/kg/day) that is capable of producing maternal toxicity. We also recommend that uteri from females appearing to be nonpregnant at necropsy be stained with ammonium sulfide to determine their pregnancy status.

The identity and percentage of active ingredient in the test material and analytical data on the concentration and stability of dosing solutions should be reported.

9. BACKGROUND: The study authors reported that 400 mg/kg of Vernam produced severe maternal toxicity in a range-finding study; however, the animal model used, the number of dosages given, and the treatment period were not specified.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material was described as technical grade Vernam, an amber liquid from lot No. CGB-2601 WRC 4921-24-3. The active ingredient, the percentage of active ingredient, and the stability of the test material were not indicated. Solutions for gavage administration were prepared daily, using corn oil

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[1] Only items appropriate to this PER have been included.

as the vehicle. The dosages used in this study were 0, 2, 20, and 200 mg/kg/day. The dosage volume was 1 mL/kg, based on day 6 body weights.

2. Twenty-two females were randomly assigned by weight to each study group. Artificially inseminated New Zealand white rabbits were dosed from gestation days (GD) 6 through 21. The day of insemination was designated GD 0. Laparotomies were conducted on GD 30.

3. Inseminated females were observed twice daily from GD 0-30 for mortality, toxicity, general health, and behavior. Body weights were recorded on GD 0, 6, 10, 14, 18, 21, 24, 29, and 30. Females were euthanized by injection of T-61 on GD 30 and then subjected to gross necropsies. Ovaries were examined to determine the number of corpora lutea, and uterine contents were examined to assess the presence, type, location, and number of implantations. Fetal viability, body weight, transumbilical distance, crown-to-rump length, and the quantity and color of amniotic fluid were noted.

   Each fetus was examined grossly for external abnormalities. One half of the fetuses were fixed, sectioned, and examined for visceral abnormalities by the method described by Wilson; the remaining fetuses were stained with Alizarin Red S and examined for skeletal abnormalities.

12. REPORTED RESULTS:

A. No results were presented on the concentration or stability of the test material in dosing solutions; in addition, the percentage of active ingredient in the test material was not reported. The study authors indicated that the stability of the test material was determined by the sponsor; however, the study authors did not provide these data.

B. No compound-related clinical signs or mortalities were noted in this study. One animal in the 20-mg/kg/day group died of fibrous pleuritis, and another in the 200-mg/kg/day group died as a result of a gavage accident. One rabbit in each of the dosage groups aborted late in gestation; however, these events were not considered compound related. Maternal body weight gains during gestation were comparable among all groups. Slight reductions in gestational body weights for the 20- and 200-mg/kg/day groups were attributed to lower mean body weights for these animals at the initiation of the study (Table 1). Data for reproductive parameters were reportedly comparable for all groups. Reductions in the number of viable fetuses in the dosage groups were attributed to reductions in the number of corpora lutea in these groups and not to compound effects (Table 2). The incidences of malformations and variations among all groups were biologically and statistically comparable.

TABLE 1. Effects of Vernam on Group Mean Gestational Body Weight and Body Weight Gain in Rabbits

	Dosage (mg/kg/day)			
	0	2	20	200
	Body Weight (g)			
Gestational Day (GD)				
0	3932	3800	3728	3695
6	4047	3903	3817	3789*
21	4174	4047	3892*	3892*
30	4218	4062	3976	3985
	Body Weight Gain (g)			
Study Period				
GD 0- 6 (predosing)	115	103	89	95
GD 6-21 (dosing)	126	144	75	106
GD 21-30 (postdosing)	44	50	62	59
GD 0-30 (gestation)	285	303	247	282

*Reported as significantly different from control value ($p < 0.05$).

TABLE 2. Effects of Vernam on Group Mean Values for Reproductive Parameters in Rabbits

	Dosage (mg/kg/day)			
	0	2	20	200
No. females initially assigned	22	22	22	22
No. females at GD 0	21	21	22	22
No. pregnant (%)	17 (81)	17 (81)	18 (82)	17 (86)
No. pregnant that died (%)	0 (0)	0 (0)	0 (0)	1 (5)
No. aborted (%)	0 (0)	1 (5)	1 (5)	1 (5)
No. females w/ viable fetuses (%)	17 (81)	16 (76)	17 (77)	17 (77)
No. corpora lutea per female	12	9*	10	10
No. implantations per female	10	8	8	9
No. viable fetuses per female	10	8*	8*	8*
Fetal body weight (g)	37	42	42	40

*Significantly different from control value ($p < 0.05$) when analyzed by reviewers using ANOVA followed by Duncan's test for group comparison.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that dosages of 2, 20, and 200 mg/kg/day of Vernam did not produce any maternal or fetal effects in rabbits.

B. A quality assurance statement was signed and dated April 6, 1981.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We assess that the data presented in the study report indicate that no maternal or fetal effects were produced at any dosage level. Statistically significant reductions in the number of viable fetuses in the dosage groups appear to be related to decreases in the mean number of corpora lutea in these groups compared with controls. Therefore, we conclude that 200 mg/kg/day, the highest dosage used, is the NOEL for maternal and fetal toxicity. We further conclude that a definitive assessment of the teratogenic potential of Vernam in rabbits cannot be made until the test material is tested at a dosage high enough to produce some systemic maternal toxicity. Based on the available data, the LOEL for maternal and fetal toxicity could not be determined.

B. Following are the deficiencies that have negatively impacted on the scientific validity of this study:

1. The highest dosage level used was unacceptably low.

2. The percentage of active ingredient in the test material was not reported; this is required by standards set by Good Laboratory Practice.

3. The concentration and stability of dosing solutions were not reported; this is also required by standards set by Good Laboratory Practice.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-4.

APPENDIX A

Materials and Methods

Vernam Scientific Reviews

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