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RESEARCH, DEVELOPMENT & ENGINEERING CENTER
U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-452

TOXICOLOGICAL EVALUATION/VERIFICATION OF DECONTAMINATION PROCEDURES/PRODUCTS FROM ALTERNATIVE TECHNOLOGIES FOR CHEMICAL DEMILITARIZATION: DEPARTMENT OF TRANSPORTATION (DOT) TEST RESULTS FOR A MUSTARD (HD) WASTESTREAM

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March 1998

Aberdeen Proving Ground, MD 21010-5423

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1998	Final; 97 Feb - 97 Mar

4. TITLE AND SUBTITLE Toxicological Evaluation/Verification of Decontamination Procedures/Products from Alternative Technologies for Chemical Demilitarization: Department of Transportation (DoT) Test Results for a Mustard (HD) Wastestream

5. FUNDING NUMBERS

None

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

DIR, ERDEC, ATTN: SCBRD-RTL/SCBRD-RTB, APG, MD 21010-5423

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDEC-TR-452

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The Alternative Technology Program was tasked by Congress to research for an acceptable procedure other than incineration to reduce the U.S. Army's mustard (HD) stockpile to non-toxic/hazardous waste. The search for this best destruction method has resulted in the selection of a HD/water reaction in which HD is reacted with 90 °C water to form water, thiodiglycol, and salts. This reaction can then be biodegraded and disposed of. To complete the acceptance for this decontamination method, animal tests were conducted according to DoT (CFR 49) required procedures in rats (orally) and rabbits (dermally). The reacted HD/water by-products produced no deaths in either species, and there were no observable toxic effects and no dermal irritation in rabbits. Therefore, for purposes of the DoT regulations, this material is a Category III material for packaging purposes, but these final results must also be confirmed by the inhalation route in rats.

14. SUBJECT TERMS

Alternative Technology Program
HD/water reaction products
Dermal irritation
Dermal toxicity
Oral toxicity
Rabbit
Mustard (HD)
DoT tests
CFR 49
Rat

15. NUMBER OF PAGES

58

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	**UNCLASSIFIED**	**UNCLASSIFIED**	**UL**

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QUALITY ASSURANCE

This study, conducted as described by Protocol 97-314, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date Reported
Data and Final Report	20 Jun 97	20 Jun 97

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

DENNIS W. JOHNSON
Quality Assurance Coordinator
Research and Technology Directorate

25 JUL 1997

SCBRD-RTL (1p)

MEMORANDUM FOR SCBRD-RTL (J. Manthei)

SUBJECT: Temperature and Humidity Variation in Building E3300, 2 Mar 97

1. During the period in question, the animals in these rooms experienced a slight variance from the recommended parameters for temperature (rats) and humidity (rabbits). These animals were monitored daily and no clinical signs were observed. At the completion of the study, all examinations of sentinel animals and laboratory analyses showed no apparent affects.

2. It is my opinion that these fluctuations in environmental conditions had no impact on this study (protocol number 97-314).

3. The point of contact is the undersigned, x5-3431.

NEWTON H. FOSTER, IV.
MAJ, VC
Team Leader, Vet Spt

Preface

This study was authorized under the Alternative Technology Program, and work began in February 1997 and was completed in March 1997. The experimental data are contained in laboratory notebook 97-0011.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996. These investigations were also performed in accordance with requirements of AR 70-18, Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs. These studies were conducted and records were maintained according to Good Laboratory Practices (GLP) standards. This work was done under approved ERDEC Laboratory Animal Use and Review Committee Protocol 97-314, approved 2 December 1996.

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Acknowledgments

The authors acknowledge the Veterinary Support Team, Research and Technology Directorate, for their assistance in procuring and caring for the animals, conducting the gross necropsies on animals, and preparing the tissues and blood samples for shipment to the pathologists.

Robert S. Lindsay and Fred Baldauf, the Design Evaluation Laboratory, are thanked for preparing and supplying the test sample evaluated. The Analytical Chemistry Team is thanked for analyzing and providing the analytical data for the samples tested.

Estrella M. Cacal is thanked for preparing the draft manuscript for processing.

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1. INTRODUCTION

Public Law 99-145 and its amendments direct the Army to dispose of its stockpile of military chemical weapons.[1] Although incineration was the method of destruction, Congress requested that the Army investigate the potential for alternative disposal procedures because of public concern.

The agent mustard (HD) - Bis(2-chloroethyl) sulfide stockpile is one of the major stockpiles of concern. This material in its pure state is a severe vesicating agent that can be fatal by inhalation and percutaneous routes of entry. It is considered to be a moderately volatile agent with a low solubility in water at 22°C.[2] Although incineration is known to be a very effective and safe method for the destruction of mustard, it was deemed necessary to investigate other alternative methods. These alternative destruction methods were to destroy HD quickly, not create excessive additional bulk residues, be relatively inexpensive, and not create any undue hazard to the workforce and/or the communities surrounding the disposal/storage sites.

Of the alternative disposal methods reviewed/studied/tested for agent HD, one procedure has been selected for final approval. This method is simply referred to as neutralization, followed by biodegradation.[3] In this procedure, a volume of water is heated to 194 °F (90 °C) and 4 wt% HD is fed into it. This reaction produces 90% thiodiglycol and 2 wt% hydrochloric acid. Once the reaction is complete, the pH is adjusted to +12 with the addition of sodium hydroxide. This procedure reduces the HD to < 200 ppb. The hydrolysate is then released from the toxic reaction cubicle and fed to a falling film evaporator. In the evaporator, the volatile organic carbon compounds are stripped out, condensed, and combined with the water recycle stream for subsequent destruction in an ultraviolet oxidation unit.

The remaining hydrolysate is fed to a sequencing batch bioreactor, which consumes more than 90% of the organic carbon, including 99% of the thiodiglycol. The resulting liquid effluent is filtered and recycled through an evaporator. The evaporator bottoms consist of salt brines that are mixed with solidifiers

and disposed of in a landfill. The waste biomass from the sequencing batch bioreactors is fed to an aerobic digester to reduce the volume, dried in a filter press, and then disposed of commercially. Although these two solid wastes have no hazardous constutuents, Maryland law requires that they be treated as hazardous wastes. Approximately 6 lb of solid waste are produced for each pound of HD destroyed.

The Maryland Citizens Advisory Commission, the National Research Council, and Congress have supported this alternative destruction process for HD.

To transport the waste products off post to landfill sites, the destruction products from the HD neutralization process must be tested according to Federal Regulatory requirements known as the Department of Transportation (DOT) and Code of Federal Regulations 49 (CFR 49) Test.[4] This DOT test requires that two groups of albino rats be tested. One group of albino rats is dosed by the oral route, and a second group is dosed by the inhalation route. A group of rabbits is dosed by the dermal route. The criteria states that if one-half or more of the test animals die, the residue is a greater toxic hazard than category III (shipping category) for shipping purposes. Category III is the least restrictive shipping category. These three tests are required to assess the toxic hazard of the HD wastestream for transportation purposes of the wastestream. These tests also support the original requirements for the delisting of chemical surety materials for Maryland as described in several U.S. Army Chemical Research, Development and Engineering Center (CRDEC) [now known as the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC)] technical reports.[5,6]

This report summarizes the procedures and test results following the oral dosage of rats and dermal dosage of rabbits with the liquid HD wastestream.

The inhalation test results will be presented in a separate test report.

2. MATERIALS

2.1 Rats.

A shipment of 18 young adult Sprague-Dawley rats (9 males, 9 females) arrived on 12 Feb 97 from Charles River Laboratories (Raleigh, NC). Upon arrival, these animals were placed on quarantine in Building E3222, Room 107.

Rats were housed individually in large polycarbonate cages (8 in. x 10 in. x 18 in.), with a stainless-steel wire top covered with another filtered plastic top. Beta hardwood chips were used for bedding. The cages were changed on a Mondays, Wednesdays, and Fridays. Certified rodent chow was supplied ad libitum, as was filtered house water. The water was supplied in Nalgene plastic bottles with an attached stainless-steel sipper tube. Water and feed were supplied/checked daily. Animals were observed at least twice daily during quarantine and while on test, except on weekends/holidays when they were observed once per day. Body weights were recorded upon arrival and just prior to testing, as well as at 7 and 14 days after dosage.

As part of the quarantine procedure, two rats were necropsied and also had a complete serology health check 24-hr after arrival. Two additional rats were similarly processed at the end of the 14-day post experimental observation.

All rats that survived the test were euthanatized with inhaled CO_2 and incinerated.

2.2 Rabbits.

Eighteen young adult (9 male and 9 female) New Zealand White (NZW) rabbits were procured from Covance (Denver, PA). Upon arrival at ERDEC, they were immediately placed on quarantine in Building E3222, Room 106. The rats were housed in single unit stainless-steel cages held in a 9-unit stainless-steel self-flushing rack. Filtered house water was supplied via a nipple at the upper-rear of each cage. Certified rabbit chow was supplied on the following schedule per instructions from the rabbit breeder:

Day 1 (First 12-24hr)	- No Food
Day 2	- 25 g
Day 3	- 50 g
Day 4-6	- 75-100 g
Day 7 (End of study)	- 125-150 g

This restricted feeding schedule was maintained to control/prevent a disease known as mucoid enteropathy. This disease is of unknown etiology but is common following shipping stress and a change of diet and occurs mainly in young-adult rabbits.

The quarantine facility was cleaned daily as were the cage racks. The cage racks were automatically flushed to remove fecal and urine waste on a 15-30 min cycle.

Two rabbits were necropsied 48-hr after arrival and both a complete serological check and gross necropsy were done. Two additional rabbits were also handled similarly after the test and 14-day observation were completed. All rabbits that survived the test and observation period were humanely euthanatized with an intravenous administration of Fatal Plus (Pentobarbital - 89 mg/kg) and incinerated.

Rabbits were weighed upon arrival, at test time, and at 7 and 14 days during post test observation. Rabbits were required to weigh 2.3-3.0 kg at the initiation of the test.

2.3 Test Chemical.

A 5-gal black steel, sealed metal bucket was received on 18 Feb 97 from the Design Evaluation Laboratory (DEL), ERDEC. A 1-L sample, provided by DEL, was contained in a glass bottle and was identified as sample #M12-D1-102-HW-0427. This particular aliquot was a portion of a 12-L Mettler HD/H_2O Bench-Scale reaction that they began on 11 Feb 97. The HD used in the reaction was procured on 30 May 96 and identified as TC #D94102, HD-S-5191-CTF-N-8; the water used was tap water from Building E3510, Room 32.

DEL personnel added HD over 30 min to 90 °C water, and the pH reached 0.51 and was adjusted with 18% NaOH to final pH of 12.2. Sample M12-D1-102-HW-0427 was withdrawn at 150 min after the last HD was fed to the reaction. Analysis for residual HD was done by the modified Alternate Technology Program (ATP) method HN-01 and no HD was detected at 0.004 ppm detection limit. The 1-L sample was 90-95% clear liquid, with 5-10% bottoms (salts) of brownish color when supplied to the Toxicology Team for toxicity testing. Additional information about this test sample is found in Appendix A.

3. METHODS AND RESULTS

3.1 Oral Test Procedures in Rats.

Rats were prepared for oral dosage (gavage) by weighing them the afternoon prior to testing to assure that they all weighed between 200 and 300 g. After weighing, the food was removed from each rat (1500 hr); however, water was retained until just prior to dosage. At test time, each rat was reweighed on a calibrated Mettler balance to the nearest gram. Each rat was then identified by ear tag, sex, and a black ink test number was placed on its tail. The oral gavage was done by gently, but firmly holding each rat in a leather gloved hand (left) so that

the head was supported in an upright position. The gavage needle (16-gauge, 3½-4 in. long stainless-steel with a bulbular end) was attached to the dosage syringe and then carefully inserted into the esophagus and down to the level of the stomach. The test substance was then injected directly into the stomach and the rat was returned to its home cage and held off food and water for 6 hr. During this time, each animal was observed for the onset of any observable toxic signs. Because HD or its breakdown products (thiodiglycol, etc.) would be the possible toxic products, we would expect to see toxic signs of gastric distress and possibly diarrhea.

After 6 hr, food and water were returned to the rats. Observation continued for the remainder of the 14-day test, twice per day and once per day on weekends.

3.2 Dermal Test Procedures in Rabbits.

Young adult NZW rabbits of both sexes (6 each sex) were prepared for dermal testing the day prior to the test. Each rabbit was checked to be sure it was in the proper test weight range (2.3-3.0 kg). Its dorsal hair was then gently clipped, using first an A-2 coarse blade attached to small animal electric clippers. A second clipping followed immediately using a #40 blade. This blade was a surgical-preparation blade that, by specification, clips hair to 1/130th of an inch. After the clipping procedures, each rabbit was checked for use suitability (no scratches, bite marks, etc.) and then it was returned to its home cage. The next day (morning) each rabbit was weighed to the nearest 0.01 kg on a calibrated Toledo balance. Each rabbit was then placed into an aluminum stanchion that securely held the rabbit with of an adjustable neck collar. Each stanchion had solid sides to prevent the animal from contacting its test mates. Rabbit ears were held together, folded forward in half and taped with 1½ to 2 in. masking tape. Taping the ears would prevent their contact with the test substance and would also serve as a place to record the animals' test number. The test site was identified and outlined with a back ink magic marker. A two-layer, thick piece of surgical gauze was taped to the skin with hypo-allergenic tape; and a polyethylene film semi-occlusive cover was taped over the gauze. Each animal was dosed with 1,000 mg/kg of the test substance. The substance was deposited on the skin under the gauze, which helped retain the liquid at the test site. The test site was then covered with the polyethylene film and the film was taped to the skin.

This secured test patch/sample was left intact for 24 hr. During the test, observations were continuous until 1530 hr, with additional checks made at 1700, 1800, and 2000 hr

and again the next morning at 0700 until the test was complete (24 hr). After 24 hr, each rabbit was removed from the testing hood and placed into hood #3 (Building E3300, Room 26). The semi-occlusive polyethylene film and gauze patch were removed and placed into a glass jar. The residual test substance (residue) was blotted dry with toweling and the animal was examined for dermal irritation and/or toxic signs. Each animal's back was then rinsed with lukewarm tap water, and blotted dry. The rabbit was then returned to its home cage. Animals were observed at least twice daily for delayed toxic effects. Body weights were taken at 7 and 14 days after usage. After the 14-day weighing, each animal was humanely euthanatized with an intravenous administration of Fatal-Plus (89 mg/kg pentobarbital) and incinerated.

3.3 Test Results.

3.3.1 Results of the Rat Oral Toxicity Test.

The oral gavage of a group of 12 young adult Sprague-Dawley rats was accomplished on 19 Feb 97. Each rat was weighed to the nearest whole gram on a calibrated Mettler balance. The test substance was initially weighed (1.0 mL aliquot) and its density determined as 1.0203 gm/mL.* The dosage per rat was to be 500 mg/kg so the dosage was adjusted for the density to 0.490 mL/kg. Rats were weighed just prior to dosage and the dosage volume per rat was calculated (see Table 1). A 16-gauge stainless-steel bulbular feeding tube was attached to a 1.0 mL glass syringe. The desired dosage volume was loaded, the syringe cleared of air bubbles, the feeding tube was carefully inserted into the rats esophagus to the level of the stomach, and the test substance administered. Rats had been fasted overnight prior to dosage and food and water were withheld for 6 hr after dosage to permit maximum uptake of the HD/water reaction by-products. Because the HD/water reaction product was biphasic, it was agitated just prior to dosage to assure that both the liquid and solids were equally dispersed in the injectant. The aliquot was agitated between each dosage to assure equal distribution of the test substance for each animal.

After dosage, the 12 rats (6 males, 6 females) were observed for the onset of toxic signs until 1500 hr, and again at 1700, 1800, and 2000 hr, and again the next morning. No toxic signs were observed during the initial 24 hr and all rats

* - **The density of the HD/water product was later determined to be 1.0223 g/mL ± 0.01% by the Analytical Chemistry Team. The toxicity data are done using our value of 1.0203. The slight variance at the thousands place would not change the actual dosage volumes given to rats or rabbits in our study.**

appeared to retain normal activity. Observations continued twice daily through 14 days except on weekends when they were done once per day.

Table 1 lists body weights in killograms for all rats tested.

Table 1. Body Weights of Sprague-Dawley Rats Used in DoT Oral Testing

Test ID Number	Sex	Ear Tag	12 Feb 97 Initial wt/kg	18 Feb 97 Pre-Test wt/kg (Prefast)	19 Feb 97 Test wt/kg (Fasted)	26 Feb 97 7-Day	5 Mar 97 14-Day
1	♂	001	.190	.252	.230	.312	.350
2	♂	002	.195	.255	.240	.314	.350
3	♂	003	.185	.255	.235	.320	.380
4	♂	004	.190	.255	.232	.313	.350
5	♂	005	.191	.250	.235	.305	.350
7	♂	007	.183	.250	.230	.305	.352
10	♀	010	.189	.210	.200	.239	.245
11	♀	011	.178	.228	.210	.232	.231
13	♀	013	.175	.210	.200	.215	.222
16	♀	016	.183	.220	.200	.237	.250
18	♀	018	.183	.220	.200	.242	.260
14	♀	014	.180	.210	.197	.227	.235

Rats lose weight rapidly when fasted due to their high metabolic rate. Also, male rats gain weight at a faster rate than do female rats. Therefore, having rats of the same age does not permit the investigator to match body weights.

During the 14-day test, all rats remained healthy with no observable toxic effects from the HD/water by-products. All 12 rats gained weight from their pre-test weight.

3.3.2 Results of the Rabbit Dermal Toxicity Test.

Following their 7-day quarantine, a group of 12 NZW rabbits (6 males, 6 females) were tested to determine the dermal toxicity hazard of the HD/water reaction identified as M12-01-102-HW-0427. Each rabbit was dosed with 1,000 mg/kg of the HD/water product. Actual dosage volume was 0.98 mL/kg as adjusted for the density of the product. Rabbits were held in stanchions and a two-layer thick piece of surgical gauze was taped to the skin. This gauze was 4 x 8 in. and the four outer edges were taped to the rabbits' back with hypo-allergenic tape. The dosage of HD/water product was then applied, and the entire test site was then sealed with a semi-occlusive polyethylene film. This film was also taped to the skin using heavy duty refrigeration duct-tape. This exposure lasted 24 hr and rabbits were held inside a filtered fume hood that had a face velocity of 100 Lfpm ± 10%. The climatic conditions (Room 26, Bldg E3300) were to be 75 ± 2 °F and the relative humidity (RH) was to be 50 ± 20%. Actual recorded climatic conditions were 75.5 °F and 42% RH at the start of the exposure. After 24 hr, each rabbit was removed from the exposure hood and its protective occlusive covering and gauze pad were removed. Each rabbit's back was checked for dermal irritation and toxic signs and then rinsed with lukewarm tap water, blotted dry, and again checked. At 24 hr, none of the 12 rabbits showed any dermal irritation or had any toxic signs. Each rabbit was returned to its home cage and observed for the remainder of the 14-day test. Twice per day, observations were made except on weekends when observations were one/day. At 24 hr, all rabbits, when returned to their home cages, began to eat and ate their entire 150 g food allotment. Body weights of the 12 rabbits tested are listed in Table 2 and include pre-quarantine weights, pre-dose weights, and 7- and 14-day post exposure weights.

4. DISCUSSION

The U.S. Army's stockpile of HD was originally scheduled to be destroyed by incineration. However, public concern for safety resulted in Congress directing the Army to search for an effective alternate destruction method other than incineration. Over the past 3+ years, chemists and engineers have worked together to develop an alternative procedure for the safe destruction of HD agent. This search has culminated in the accepted policy that HD will be destroyed by reacting it with 90 °C water, neutralizing the acidic reaction products with NaOH, and then biodegrading the by-products for final resolve.

Table 2. Body Weights of New Zealand White Rabbits Used in DoT Dermal Testing

Test ID Number	Sex	Ear Tag	11 Feb 97 Initial wt/kg	19 Feb 97 Test wt/kg	26 Feb 97 7-Day wt/kg	5 Mar 97 14-Day wt/kg
1	♂	64230	2.58	2.60	2.82	2.96
2	♂	63215	2.44	2.39	2.60	2.65
4	♂	63210	2.68	2.60	2.65	2.62
5	♂	63221	2.60	2.65	2.84	2.91
6	♂	63217	2.52	2.60	2.78	2.77
8	♂	63213	2.73	2.65	2.83	2.94
10	♀	63208	2.66	2.60	2.76	2.98
11	♀	63209	2.39	2.50	2.70	2.76
13	♀	63223	2.59	2.80	2.90	3.02
14	♀	63207	2.59	2.55	2.72	2.86
17	♀	63211	2.49	2.50	2.66	2.80
18	♀	63205	2.73	2.60	2.76	2.94

Toxicity tests conducted at the U.S. Army Edgewood Research, Development and Engineering (ERDEC) in 1996,[7] had indicated that HD/water reaction by-products did not produce observable dermal irritation in the rabbit or any other observable toxic signs/effects following a 24-hr dermal contact with 1000 mg/kg of the decontaminated HD/water. Further refinement of the decontamination techniques and a better mixing apparatus, adapted to a 12-L Mettler Bench Scale reactor, provided the final HD/water product that was tested in rats (oral) and rabbits (dermal) according to Federal Guidelines as established in Code of Federal Regulations 49 (CFR 49).[4] In recent laboratory tests at ERDEC, a group of 12 young adult Sprague-Dawley rats (6 each sex) were dosed orally with 500 mg/kg of the HD/water product (M12-D1-102-HW-0427) and no toxic signs were observed over the 14-day evaluation. These animals, at no

time, developed any loss of appetite, change in fecal output, or change in observable activity. Body weights were monitored at 7- and 14-days after dosage and they all gained weight at a normal rate.

In addition to the rat oral tests, ERDEC also tested a group of young adult (NZW) rabbits (6 each sex) to determine the dermal effects of the HD/water product. A dose of 1,000.0 mg/kg HD/water product was placed on the skin, semi-occluded for 24 hr. The rabbits were observed for a total of 14-days. There were no toxic signs and no dermal irritation observed in any of the 12 rabbits.

The breakdown products of this HD/water reaction contained about 96% water, 3+% thiodiglycol, and the remainder was various other organics (conversation with Robert Lindsay, Design Evaluation Laboratory, January 1998). Although this by-product had a pH of 12.2, it did not cause dermal irritation or observable gastric distress in rabbits or rats. With test animals undergoing oral and dermal tests, if the test substance in this instance HD/water reaction by-products produced any gastro-intestinal effects, the subjects (animals) could be expected to refuse food intake and thereby lose weight. This effect was not observed in either rats or rabbits as evidenced by their body weights (See Table 3).

Other areas of concern (in addition to testing procedures) during a test as described in this report are the maintenance of climatic conditions during the 14-day procedure, as well as the health status of the test and Quality Assurance (QA)/Quality Control (QC) (Sentinel) animals.

Table 4 lists the day-to-day climatic conditions for the rodent room (Room 28) and the rabbit holding room (Room 32) in Bldg E3300. The day-to-day variables in the temperature and relative humidity were controlled within test parameters with the exception of 2 Mar 97 [temperature 1° low in the rat room (Room 28), and in the rabbit room (Room 32)], where the humidity ran 5% high. This particular day was a very warm day (72 °F) and because the building chillers were not operational, the control system was not able to completely regulate the climate. This minor temporary temperature/humidity variance would not impact on the test results. The QA/QC (Sentinel) animals were maintained in the same rooms as the test animals at all times. The results of their serology workups and the gross and histopathology indicated no health problems. Therefore, the test animals would not have suffered any problems from the one-day variance in climatic conditions. The serology and the gross and histopathology results for rats and rabbits are located in Appendix B and indicate all normal tissues.

Table 3. Mean Body Weights in Kilogram (Kg) of Rats and Rabbits Following Dosage with HD/Water Reaction By-Products - M12-D1-102-HW-0427

Species	Sex	Mean Body Weight of Test Animals Arrival wt/kg	Pre-Test wt/kg	Post-Test wt/kg 7-Day	Post-Test wt/kg 14-Day
	Test Animals				
Rats	Males	$\bar{x}$-0.187[a]	0.253	0.312	0.355
		S.D.-0.006[b]	0.005	0.006	0.012
	Females	$\bar{x}$-0.180	0.215	0.232	0.241
		± S.D.-0.005	0.007	0.010	0.014
	Controls QA/QC				
	Males	$\bar{x}$- ---[c]	---	0.313	0.360
		± S.D.- ---	---	0.018	0.025
	Females	$\bar{x}$- ---	---	0.225	0.228
		S.D.- ---	---	0.006	0.011
	Test Animals				
Rabbits	Males	$\bar{x}$-2.63	2.53	2.75	2.80
		± S.D.-0.11	0.10	0.10	0.15
	Females	$\bar{x}$-2.49	2.53	2.75	2.89
		± S.D.-0.16	0.09	0.08	0.10
	Controls QA/QC				
	Males	$\bar{x}$- ---	---	2.92	2.90
		± S.D.- ---	---	0.04	0.07
	Females	$\bar{x}$- ---	---	2.69	2.78
		± S.D.- ---	---	0.13	0.11

[a] - $\bar{x}$ Mean body weight in kg
[b] - Standard deviation
[c] - No data at this time frame

Table 4. Day-to-Day Climatic Records for Testing Facility - Bldg E3300

Date		Room 28 (Rats) Temp °F	R.H.%	Room 32 (Rabbits) Temp °F	R.H.%
Feb	19	70.0[a]	49.0[b]	75.5	42.0(Room 26)*
	20	70.0	45.0	65.0[c]	52.0[c]
	21	70.0	46.0	65.0	55.0
	22	70.0	45.0	65.0	55.0
	23	70.0	45.0	65.0	50.0
	24	69.7	38.0	64.0	50.0
	25	70.6	34.0	65.0	40.0
	26	71.8	40.0	64.0	52.0
	27	70.0	60.0	65.0	70.0
	28	70.0	47.0	65.0	47.0
Mar	1	70.0	46.0	70.0	45.0
	2	65.0**	62.0	66.0	75.0**
	3	69.0	37.0	65.0	65.0
	4	70.0	45.0	65.0	55.0
	5	69.7	41.0	64.0	60.0

* - Rabbit test facility (Room 26, Bldg E3300) was to be 75 ± 2 °F and 30-70% RH - these rabbits were in Room 26 for 24-hr, the length of the dermal "DoT" test exposure.

[a] - Room 28, Bldg E3300 (rat room) temperature was to be 70 ± 4°F, and

[b] - 30-70% RH

[c] - Room 32, Bldg E3300 (rabbit holding room) temperature was to be 65 ± 5 °F, and

[d] - 30-70% RH

** - On 2 Mar 97, Sunday, the outdoor temperature rose to about 72 °F with high humidity. This slight overage in humidity range of 5% was a temporary effect and would have no adverse effects on the rabbits. During the cold months, the building chillers are not operational so unusually mild/humid days make it difficult to control humidity with 100% fresh makeup air intake.

5. CONCLUSIONS

It is concluded from the toxicological evaluation of this mustard/water reaction by-product (M12-D1-102-HW-0427) that:

- 3.8% HD, when reacted with 90 °C water as described in this report and the supporting analytical

documentation, is reduced to a Category III toxic substance, according to guidelines established in the Federal Register and Code of Federal Regulations (CFR) 49 (otherwise known as Department of Transportation (DoT) 49).

- The HD/water product as configured and evaluated herein is less toxic than a Category III material as described in CFR 49. Therefore, the HD/water product should pose no serious health risk to workers provided they exercise normal safety procedures for working with materials of a high pH such as 12 plus.

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APPENDIX A

ANALYSIS DATA FOR HD/WATER REACTION BY-PRODUCT

NOTE: These analytical data are supplied as information only and are not to be construed as final data. Only the final analytical report may be cited as final documented chemistry data. This data will be reported by Analytical Chemistry Team.

ANALYTICAL REQUEST AND RESULTS

TO: (REQUESTOR, PLEASE FILL IN NAME AND ADDRESS)	DATE
R. S. Lindsay, Design Evaluation Lab SCBRD-ENM-S E3510	2/11/97
	PHONE NO. X2801

FROM: Analytical Chemistry Team

ANALYSIS OF (structure or further description IF UNCLASSIFIED on reverse side)

HD/Water Reaction Products

SAMPLE NO.

M12-D1-101-HW-0427

TOXIC NON-TOXIC MOL. WT. EMP. FORM

DETERMINE

Low level HD - 0.2ug/ml in solution.

RESULTS AS FOLLOWS BY: Sumpter, K. B.
James, L. G.

Extraction.

Followed procedures outlined in the modified ATP method HN-01.

Analysis.

Sample M12-D1-101-0427 was analyzed by the modified ATP method HN-01 and found to contain no HD.

Instrument Detection Level - 0.099ppm

Method Detection Limit - 0.004ppm

TEAM LEADER	DATE
Michael W. Ellzy	13 Feb 1997

SCBRD Form 49, 15 Jun 94 replaces SMCCR Form 49, 1 May 85 which is obsolete.

ANALYTICAL REQUEST AND RESULTS

TO: (REQUESTOR, PLEASE FILL IN NAME AND ADDRESS)	DATE
SCBRD-ENM-S, R.S. Lindsay Design Evaluation Lab, E3510	19 Feb 97
	PHONE NO. X2801

FROM: Analytical Chemistry Team (ACT)

ANALYSIS OF (structure or further description IF UNCLASSIFIED on reverse side)

HD/H_2O Reaction Product

SAMPLE NO.

M-12-D1-103-HW-0427 OTH01897-2

TOXIC NON-TOXIC MOL. WT. EMP. FORM.

DETERMINE:

Thiodiglycol (TDG) concentration by ACT Method 030

RESULTS AS FOLLOWS BY: B. S. Ince

TDG concentration = 23,600. mg/L

TEAM LEADER Richard W. Kelley DATE 20Feb97

SCBRD Form 49, 15 Jun 94 replaces SMCCR Form 49, 1 May 85 which is obsolete.

ANALYTICAL REQUEST AND RESULTS

TO: (REQUESTOR, PLEASE FILL IN NAME AND ADDRESS)
R. S. Lindsay, Design Evaluation Lab
SCBRD-ENM-S
E3510

DATE: 2/11/97

PHONE NO.: X2801

FROM: Analytical Chemistry Team

ANALYSIS OF (structure or further description IF UNCLASSIFIED on reverse side)

HD/Water Reaction Products

SAMPLE NO.

M12-D1-103-HW-0427

TOXIC NON-TOXIC MOL. WT. EMP. FORM.

Toxicity Undetermined

DETERMINE:

Organics

RESULTS AS FOLLOWS BY: L. Janes/21Feb97
D. Rohrbaugh/13Feb97

Extraction.

Twenty-five milliliters of the aqueous sample was filtered, pH adjusted and extracted with two milliliters of chloroform. The extracted sample was submitted for analyses.

Analysis.

See attached sheets for organic characterization.

Organic composition using GC/FID consisted of the following compounds:

Compound	Retention Time	Conc.(in extract)	Conc. (in aqueous)
1,4-Thioxane	4.218min	0.236mg/mL	37.76ppm
1,4-Dithiane	7.780min	1.769mg/mL	283.04ppm
TDG	9.746min	0.036mg/mL	5.76ppm
HO-CH2CH2-S-CH2CH2-S-CH2CH2-OH	16.895min	0.038mg/mL	6.08ppm
HO-CH2CH2-S-CH2CH2-O-CH2CH2-S-CH2CH2-OH	20.092min	0.023mg/mL	3.68ppm
HO-CH2CH2-S-CH2CH2-S-CH2CH2-S-CH2CH2-OH	22.593min	0.074mg/mL	11.84ppm

TEAM LEADER
Michael W. Ellzy

DATE
3 Mar 1997

SCBRD Form 49, 15 Jun 94 replaces SMCCR Form 49, 1 May 85 which is obsolete.

GC/MS/CI Characterization of M12-D1-103-HW-0427 $CHCl_3$ Extract

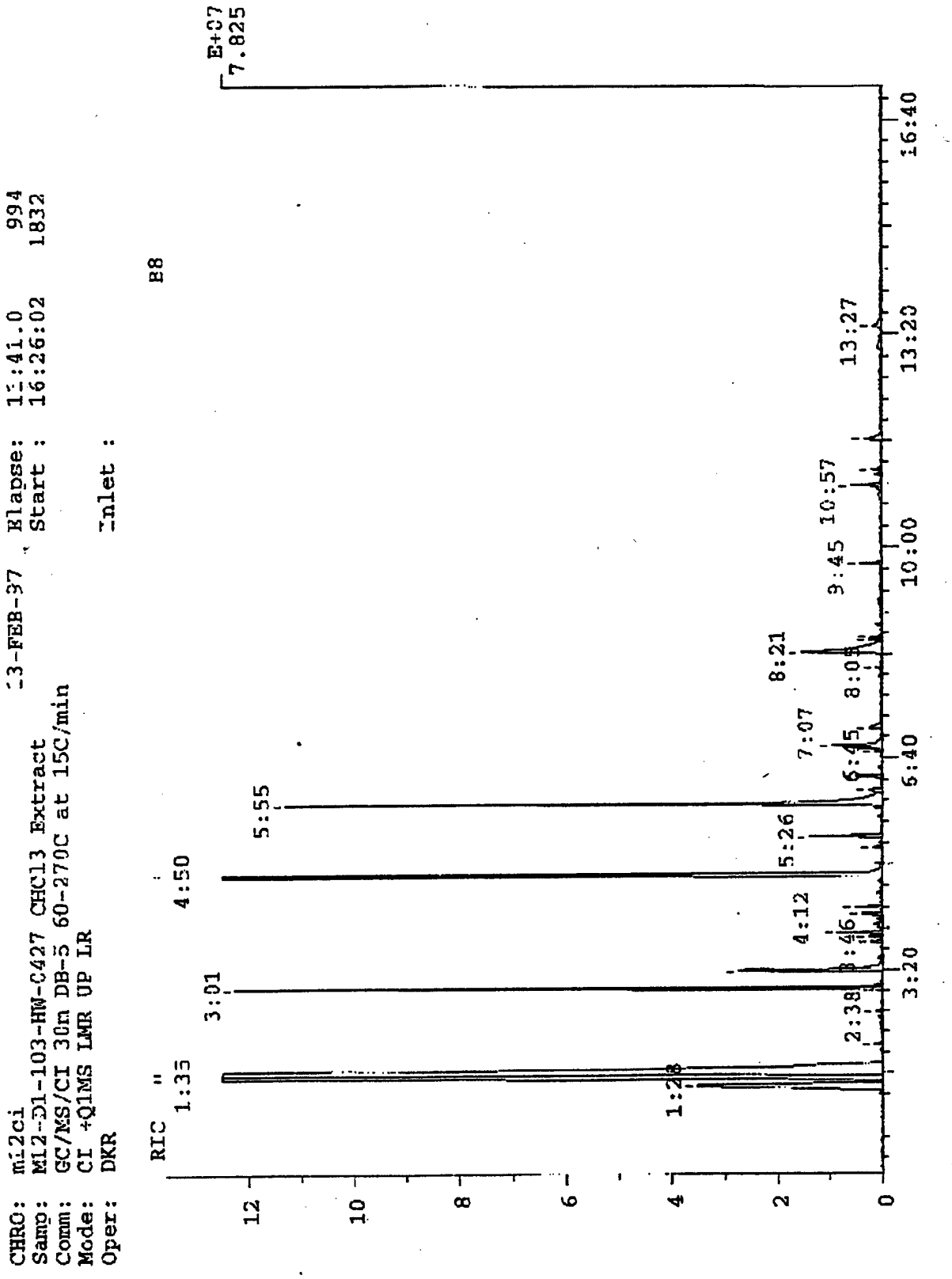
Scan No	RT (min)	MW	Compound	Area %
256	3:01	104	1,4-Thioxane	6.87
280	3:18	104,106	CH_2=CHSCH_2CH_2OH, EtSCH_2CH_2OH	2.36
320	3:46	182	$Cl_2CHOCHCl_2$	0.17
327	3:51	142	$ClCH_2CH_2OCH_2CH_2Cl$	0.16
332	3:55	132	Unknown	0.48
357	4:12	132	Unknown	0.22
367	4:19	132	Unknown	0.26
411	4:50	120	1,4-Dithiane	74.3
424	5:00	118	Unknown	0.07
446	5:15	134	Unknown	0.18
461	5:26	156	ClCH=CHSCH_2CH_2Cl or Isomer	0.72
503	5:55	122	Thiodiglycol	8.04
523	6:09	148	Unknown	0.26
541	6:22	148	Unknown	0.28
579	6:49	187	RSCH=CH_2	0.29
582	6:51	189	RSEt	0.51
605	7:07	132	Unknown	0.34
710	8:21	164	CH_2=CHS$CH_2CH_2SCH_2CH_2OH$	2.42
727	8:33		Unknown	0.16
731	8:36	162	Unknown	0.17

829	9:45	222	Diethylphthalate	0.34
931	10:57	206	DCCDI	0.37
946	11:07	247	$RSCH_2CH_2SCH=CH_2$	0.09
953	11:12	208	$CH_2=CHSCH_2CH_2OCH_2CH_2SCH_2CH_2OH$	0.17
994	11:41	288	RSR	0.40
1145	13:27	224	DCCDI Urea	0.38

R = 2-diisopropylaminoethyl
DCCDI = Dicyclyclohexylcarbodiimide

Run obtained 13 Feb 97 on TSQ-7000 (File m12ci)

Note: Presence of VX degradation products and DCCDI suggests reactor may have been used for VX prior to this run without complete cleaning.


CHRO: mi2ci
Samp: M12-D1-103-HW-0427 CHCl3 Extract
Comm: GC/MS/CI 30m DB-5 60-270C at 15C/min
Mode: CI +Q1MS LMR UP LR
Oper: DKR
13-FEB-37
Elapse: 15:41.0 994
Start : 16:26:02 1832
Inlet :
E8
E+07
7.825
RIC "
1:35
3:01
4:50
5:55
1:28
2:38
3:46
4:12
5:26
6:45
7:07
8:21
8:08
9:45
10:57
13:27
3:20
6:40
10:00
13:20
16:40
12
10
8
6
4
2
0

APPENDIX A

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APPENDIX B

SEROLOGICAL AND BOTH GROSS AND HISTOPATHOLOGICAL DATA FOR QUALITY ASSURANCE/QUALITY CONTROL RATS AND RABBITS

SECTION A: HEALTH EVALUATION OF RATS AND RABBITS PRE-EXPERIMENTAL (QUARANTINE)

REQUEST FOR GROSS NECROPSY/LAB PROCEDURES

[X]Pretest Screening [ ] Post Test Screening

Date of Procedure: 2/13/97 Location: Necropsy/3222

Number of animals needed: 2 rats Time: 9 AM

Investigators Name: Jim Manthei Phone Number: 5-3727

Protocol Number: 97-314 Delivery Date: 2/12/97

Species\Strain: Rat/Sprauge Dawley Source: Charles River/Canada Rm 15

If there is a problem with above scheduled times please contact MAJ Newt Foster (5-3431) or a Vet Support Member (5-2273). Please have these animals clearly marked as the animals for these procedures by the morning of the scheduled procedures.

COPIES FURNISHED TO: M.Foster, J Rickerl, J Scotto, J Manthei

ANIMAL NECROPSY REC[redacted]PMENT NO. 9711-3 / 9713-3

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
Charles River Rm 15	J. MANTHEY	97-314		2/12/97
SPECIES	BREED/STRAIN	SEX	AGE	ANIMAL I.D. NO.
rat	Sprague Dawley	♂		006/6
DATE/TIME OF DEATH	DATE/TIME OF NECROPSY	PROSECTOR		
2/13/97	2/13/97	N FOSTER		

HISTORY

DISPOSITION
A. SCHEDULED
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
(A.) FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS) BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
1.74					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing remarkable found on necropsy

anal tape check showed nothing remarkable

fecal test done in house negative.

Newton Foster DVM

APPENDIX B

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9711-4 | 9713-4

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
Charles River Lab	J MANTHEI	97-314		2/12/97
SPECIES	BREED/STRAIN	SEX	AGE	ANIMAL I.D. NO.
RAT	Sprague Dawley	♀		012/12
DATE/TIME OF DEATH	DATE/TIME OF NECROPSY	PROSECTOR		
2/13/97	2/13/97	N FOSTER		

HISTORY

DISPOSITION
(A) SCHEDULED
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
(A) FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS) BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
247					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing remarkable found in necropsy

Anal tape check showed nothing remarkable

fecal test done in house negative

Newton Foster DVM

APPENDIX B

REQUEST FOR GROSS NECROPSY/LAB PROCEDURES

[X]Pretest Screening [_] Post Test Screening

Date of Procedure: 2/13/97 Location: Necropsy/3222

Number of animals needed: 2 rabbits Time: 9 AM

Investigators Name: Jim Manthei Phone Number: 5-3727

Protocol Number: 97-314 Delivery Date: 2/11/97

Species\Strain: Rabbit (NZW)/c eartags Source: Covance/Denver PA

If there is a problem with above scheduled times please contact MAJ Newt Foster (5-3431) or a Vet Support Member (5-2273). Please have these animals clearly marked as the animals for these procedures by the morning of the scheduled procedures.

COPIES FURNISHED TO: M.Foster, J Rickerl, J Scotto, J Manthei

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9711-1 | 9712-1 | 9713-1

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
COVANCE / DENVER PA	J. MANTHEI	97-314		2/11/97
SPECIES: RABBIT	BREED/STRAIN: NZW	SEX: ♀	AGE:	ANIMAL I.D. NO.: #16 / 63214
DATE/TIME OF DEATH: 2/13/97	DATE/TIME OF NECROPSY: 2/13/97	PROSECTOR: N FOSTER		

HISTORY

DISPOSITION: (A) SCHEDULED; B. SPONTANEOUS DEATH; C. OTHER:

CONDITION: (A) FRESH; B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒

BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS) BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
2.22					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing remarkable found in necropsy.

fecal Test done in house results negative

Newton Foster DVM

APPENDIX B

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9711-2 | 9712-2 | 9713-2

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
COVANCE / DENVER PA	J. MANTHEI	97-314		2/11/97
SPECIES	**BREED/STRAIN**	**SEX**	**AGE**	**ANIMAL I.D. NO.**
RABBIT	NZW	♂		#7/63217
DATE/TIME OF DEATH	**DATE/TIME OF NECROPSY**	**PROSECTOR**		
2/13/97	2/13/97	N FOSTER		

HISTORY

DISPOSITION
(A) SCHEDULED
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
(A) FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS) BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
2.77					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing remarkable found in necropsy

fecal test done in house results negative.

Newton Foster DVM

APPENDIX B

Our Code MV M718
Page 1 DAAD0596P-4060

TO: Major N. H. Foster
USA APG SA
USA, ERDEC, SCBRD-RTL
Bldg. E3222
APG-EA, MD 21010-5423

FROM: Robert L. Peters, Ph.D.
Microbiological Associates
9900 Blackwell Road
Rockville, MD 20850
(301) 738-1000

DATE: February 21, 1997
SPECIMEN: 2 rabbit and 2 rat sera
RECEIVED: February 14, 1997

RESULTS: None of the tests were positive.

	IFA			MHA
Sample ID	CARB	E.cun	Tyz-Rb	T.cun
9711-1	-	-	-	-
9711-2	-	-	-	-

	ELISA					
Sample ID	CARB	MPul	PVM	RCV/SDA	Reo	Sendai
9711-3	0.01	0.00	0.00	0.01	0.00	0.02
9711-4	0.01	0.01	0.00	0.01	0.00	0.00

	IFA		HAI	
Sample ID	LCM	Parvo	H-1	KRV
9711-3	-	-	-	-
9711-4	-	-	-	-

ELISA: Positive value is >= 0.17 OD units
IFA : + = positive; - = negative
HAI : Numerical value = positive; - = negative
MHA : Numerical value = positive; - = negative

MICROBIOLOGICAL ASSOCIATES, INC.

Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Fax (301) 738-1036

COMPREHENSIVE ANIMAL HEALTH SERVICE
Microbiology Laboratory

DATE RECEIVED: February 14, 1997

DATE REPORTED: February 24, 1997

REPORT TO:

Major N.H. Foster
USA ERDEC
SCBRD-RTL/ N.H. Foster
BLDG E3222
APG-EA, MD 21010-5423

REPORT FROM:

Anton M. Allen, DVM, Ph.D.
Director of
Veterinary Services

CAHS LOG#	SPECIMEN DESIGNATION	SOURCE	ORGANISM ISOLATED
B1006-1	Rabbit ID# 9712-1	Oropharyngeal Swab	Negative*
B1006-2	Rabbit ID# 9712-2	Oropharyngeal Swab	Negative*

*Primary bacterial pathogens were not detected by aerobic culture techniques.

NOTE:
Coagulase negative Staphylococcus sp., Group D alpha streptococcus-enterococcus, Escherichia coli, and Oligella ureolytica were isolated. These bacteria are common components of the rabbit microflora.

MICROBIOLOGICAL ASSOCIATES, INC.

Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Fax (301) 738-1036

PATHOLOGY REPORT

ACCESSION NO: CAHS-3959

DATE RECEIVED: February 14, 1997
DATE REPORTED: March 10, 1997

SOURCE: Major N.H. Foster
USAA ERDEC
SCBRD-RTL/N.H. Foster
BLDG E3222
APG-EA, MD

P.O. DAAD05-96-P-4060

DESCRIPTION:

Rabbit Tissue, ID # 9713-1, CAHS-3959 (1)
Rabbit Tissue, ID # 9713-2, CAHS-3959 (2)
Rat Tissue, ID # 9713-3, CAHS-3959 (3)
Rat Tissue, ID # 9713-4, CAHS-3959 (4)

RESULTS:

CAHS-3959 (1)

Tissues examined microscopically: Heart, lung, liver, kidney, adrenal, spleen, pancreas, small intestine (x3), stomach, cecum, colon, eye and lacrimal gland, skin, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Liver- necrosis, focal, minimal (single lesion)

CAHS-3959 (2)

Tissues examined microscopically: Heart, lung, liver, kidney, spleen, pancreas, small intestine (x2), cecum, colon, stomach, brain, eye, lacrimal gland, middle ear, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Kidney- mineralization, tubular, minimal

PATHOLOGY REPORT
ACCESSION NO: CAHS-3959

CAHS-3959 (3)

Tissues examined microscopically: Heart, lung, trachea, thymus, liver, kidney, spleen, pancreas, small intestine, cecum, colon, brain, eyes, lacrimal glands, brain, middle ears, nasal passages, testicle, epididymus, seminal vesicle, bladder

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings: No lesions found

CAHS-3959 (4)

Tissues examined microscopically: Heart, lung, trachea, liver, kidney, spleen, pancreas, duodenum, ileum, cecum, colon, ovary, fallopian tube, uterus, bladder, brain, middle ear, eye, lacrimal gland, salivary gland, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings: No lesions found

Comment: The above minimal lesions are considered to be incidental and not indicative of infection by adventitial agents.

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services

SECTION B: HEALTH EVALUATION OF RATS AND RABBITS POST EXPERIMENTAL (14-DAYS)

REQUEST FOR GROSS NECROPSY/LAB PROCEDURES

[_]Pretest Screening [x] Post Test Screening

Date of Procedure: 3/6/97 Location: NECROPSY /3222

Number of animals needed: 2 Time: AM

Investigators Name: J MANTHIE Phone Number: 5-3724

Protocol Number: 97-314 Delivery Date: 2/12/97

Species\Strain: RAT/ SPRAUGE DAWLEY Source: CHARLES RIVER (Canada room 15)

If there is a problem with above scheduled times please contact MAJ Newt Foster (5-3431) or a Vet Support Member (5-2273). Please have these animals clearly marked as the animals for these procedures by the morning of the scheduled procedures.

COPIES FURNISHED TO: _____

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9714-3 / 9716-3

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
CHARLES RIVER	J. MANTHEI	97-314		2/12/97
SPECIES	**BREED/STRAIN**	**SEX**	**AGE**	**ANIMAL I.D. NO.**
RAT	SPRAGUE DAWLEY	♂		008/8
DATE/TIME OF DEATH	**DATE/TIME OF NECROPSY**	**PROSECTOR**		
3/6/97	3/6/97	N FOSTER		

HISTORY

DISPOSITION
(A) SCHEDULED
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
(A) FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS)	BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
38						

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing noteworthy found during necropsy
no lesions found
fecal - neg.
Skin scrapings - neg
tape test - neg.

Newton Foster DVM

APPENDIX B

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9714-4 / 9716-4

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT	DATE RECEIVED
CHARLES RIVER	J. MANTHEI	97-34	2/12/97
SPECIES	**BREED/STRAIN**	**SEX / AGE**	**ANIMAL I.D. NO.**
RAT	SPRAGUE DAWLEY	♀	015 / 15
DATE/TIME OF DEATH	**DATE/TIME OF NECROPSY**	**PROSECTOR**	
3/6/97	3/6/97	N FOSTER	

HISTORY

DISPOSITION: (A) SCHEDULED; B. SPONTANEOUS DEATH; C. OTHER:

CONDITION: (A) FRESH; B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☐

BACTERIOLOGY ☐ PARASITOLOGY ☐

WEIGHT (GRAMS) BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
2.3					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing noteworthy found in necropsy
no lesions found.
fecal - neg.
skin scrapings - neg
tape test - neg

Newton Smith DVM

APPENDIX B

REQUEST FOR GROSS NECROPSY/LAB PROCEDURES

[_]Pretest Screening [x] Post Test Screening

Date of Procedure: 3/6/97 Location: NECROPSY /3222

Number of animals needed: 2 Time: AM

Investigators Name: J MANTHIE Phone Number: 5-3724

Protocol Number: 97-314 Delivery Date: 2/12/97

Species\Strain: RABBIT(NZW) Source: Covance/Denver PA

If there is a problem with above scheduled times please contact MAJ Newt Foster (5-3431) or a Vet Support Member (5-2273). Please have these animals clearly marked as the animals for these procedures by the morning of the scheduled procedures.

COPIES FURNISHED TO: _____

MICROBIOLOGICAL ASSOCIATES, INC.

Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Fax (301) 738-1036

COMPREHENSIVE ANIMAL HEALTH SERVICE
Microbiology Laboratory

DATE RECEIVED: March 7, 1997

DATE REPORTED: March 12, 1997

REPORT TO:

Major N.H. Foster
USA ERDEC
SCBRD-RTL/ N.H. Foster
BLDG E3222
APG-EA, MD 21010-5423

REPORT FROM:

Anton M. Allen, DVM, Ph.D.
Director of
Veterinary Services

CAHS LOG#	SPECIMEN DESIGNATION	SOURCE	ORGANISM ISOLATED
B1024-1	Rabbit ID# 9715-1	Oropharyngeal Swab	Negative*
B1024-2	Rabbit ID# 9715-2	Oropharyngeal Swab	Bordetella bronchiseptica

*Primary bacterial pathogens were not detected by aerobic culture techniques.

NOTE:
Staphylococcus aureus, Group D alpha streptococcus-enterococcus, and Bacillus sp. were also isolated. These bacteria are common components of the rabbit microflora.

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9714-2 / 9715-2 / 9716-2

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
COVANCE / DENVER PA	J. MANSTHEY	97-314		2/12/97
SPECIES	**BREED/STRAIN**	**SEX**	**AGE**	**ANIMAL I.D. NO.**
RABBIT	NZW	♀		12/63219
DATE/TIME OF DEATH	**DATE/TIME OF NECROPSY**	**PROSECTOR**		
3/6/97	3/6/97	N. FOSTER		

HISTORY

DISPOSITION
~~A. SCHEDULED~~
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
A. FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS)	BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
2130	.					

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

nothing noteworthy discovered during necropsy
no lesions found

fecal - neg
Skin scrapings - neg
Tape Test - not done.

Newton Foster DVM

ANIMAL NECROPSY RECORD

SHIPMENT NO. 9714-1 / 9715-1 / 9716-1

SOURCE	INVESTIGATOR	PROTOCOL NO./PROJECT		DATE RECEIVED
COVANCE/DENVER PA	J. MANTHEI	97-314		2/12/97
SPECIES	BREED/STRAIN	SEX	AGE	ANIMAL I.D. NO.
RABBIT	NZW	♂		9/63218
DATE/TIME OF DEATH	DATE/TIME OF NECROPSY	PROSECTOR		
3/6/97	3/6/97	N FOSTER		

HISTORY

DISPOSITION
(A) SCHEDULED
B. SPONTANEOUS DEATH
C. OTHER:

CONDITION
(A) FRESH
B. AUTOLYZED

PHOTOGRAPHS ☐ SEROLOGY ☒
BACTERIOLOGY ☒ PARASITOLOGY ☒

WEIGHT (GRAMS)	BODY	HEART	LUNGS	LIVER	KIDNEY	GONADS
307						

ORGANS/TISSUES	
BRAIN	
SPINAL CORD	
PITUITARY GLAND	
PERIPHERAL NERVE	
EYE	
ADRENAL GLAND	
TRACHEA	
ESOPHAGUS	
THYROID W/PARATHYROID	
SALIVARY GLAND	
MANDIBULAR LN	
LUNGS AND BRONCHI	
HEART	
AORTA	
THYMUS	
SPLEEN	
KIDNEYS	
LIVER	
GALL BLADDER	
STOMACH	
SMALL INTESTINE	
PANCREAS	
LARGE INTESTINE	
URINARY BLADDER	
TESTES	
PROSTATE	
OVARIES	
UTERUS	
CERVIX	
MAMMARY GLAND	
THIGH MUSCLE	
STERNUM (bone marrow)	
HARDARIAN GLAND	
TONGUE	
TURBINATES	
SKIN	

GROSS EXAMINATION:

findings on necropsy were normal and not noteworthy. no lesions.

fecal - neg.
skin scrapings - neg
tape test - not done

Newton Manthei DVM

RODENT AND RABBIT LABORATORY ORDER FORM

Questions? Call 1-800-804-3586

CUSTOMER NAME Major N.H. Foster

USA ERDEC
SCBRD-RTL/ N.H. Foster
BLDG E3222
APG-EA, MD 21010-5423
PHONE (410) 671-3431
FAX # (410) 612-7235

2 SAMPLES

P.O. NUMBER DAADO5-96-P-4060

DFAS ROCK ISLAND OPERATING LOCATION
ATTN: DFAS-RI-FPV/ B. Border
BLDG 68
ROCK ISLAND, ILL 61299-8000
(309) 782-9204/FAX (309) 782-9994

DATE SHIPPED 3-18-97

If the animal(s) from which your test samples were derived is known or suspected to be infected with a human pathogen, please identify the pathogen : _____

SAMPLE ID (Per vial Label)	SPECIES	TEST CATALOG #	CATALOG DESCRIPTION
9715-1	RABBIT	80-610	OROPHARNGEAL
9715-2	RABBIT	80-610	SWABS

SPECIAL INSTRUCTIONS: _____

PLEASE PROVIDE THE FOLLOWING INFORMATION FOR RODENT/RABBIT SERUM SAMPLES:

DILUTED 1:5 - YES ____ NO ____
(IF NO, EXTRA CHARGE APPLIES)

HEAT INACTIVATED:
YES ____ (SHIP AT ROOM TEMPERATURE)
NO ____ (SHIP TO ARRIVE WITHIN 48 HOURS)
(Extra charge applies)

See important Shipping Instructions and Terms and Conditions of Sale on the reverse.

MICROBIOLOGICAL ASSOCIATES INC

RODENT AND RABBIT LABORATORY ORDER FORM

Questions? Call 1-800-804-3586

CUSTOMER NAME Major N.H. Foster
USA ERDEC
SCBRD-RTL/ N.H. Foster
BLDG E3222
APG-EA, MD 21010-5423
PHONE (410) 671-3431
FAX # (410) 612-7235

P.O. NUMBER DAAD05-96-P-4060
DFAS ROCK ISLAND OPERATING LOCATION
ATTN: DFAS-RI-FPV/ B. Border
BLDG 68
ROCK ISLAND, ILL 61299-8000
(309) 782-9204/FAX (309) 782-9994

DATE SHIPPED 3/6/97

6 SAMPLES

If the animal(s) from which your test samples were derived is known or suspected to be infected with a human pathogen, please identify the pathogen : _____

SAMPLE ID (Per vial Label)	SPECIES	TEST CATALOG #	CATALOG DESCRIPTION
9714-1	RABBIT	80-211	RABBIT ANTI BODY PROFILE
9714-2	RABBIT	80-211	RABBIT ANTI BODY PROFILE
9714-3	RAT	80-219	RAT LEVEL II COMPLETE
9714-4	RAT	80-219	RAT LEVEL II COMPLETE
9714-5	MOUSE	80-218	MOUSE LEVEL II COMPLETE
9714-6	MOUSE	80-218	MOUSE LEVEL II COMPLETE

SPECIAL INSTRUCTIONS: _____

PLEASE PROVIDE THE FOLLOWING INFORMATION FOR RODENT/RABBIT SERUM SAMPLES:

DILUTED 1:5 - YES X NO ___
(IF NO, EXTRA CHARGE APPLIES)

HEAT INACTIVATED:
YES X (SHIP AT ROOM TEMPERATURE)
NO ___ (SHIP TO ARRIVE WITHIN 48 HOURS)
(Extra charge applies)

See important Shipping Instructions and Terms and Conditions of Sale on the reverse.

MICROBIOLOGICAL ASSOCIATES INC

RODENT AND RABBIT LABORATORY ORDER FORM

Questions? Call 1-800-804-3586

CUSTOMER NAME Major N.H. Foster
USA ERDEC
SCBRD-RTL/ N.H. Foster
BLDG E3222
APG-EA, MD 21010-5423
PHONE (410) 671-3431
FAX # (410) 612-7235

P.O. NUMBER DAAD05-96-P-4060
DFAS ROCK ISLAND OPERATING LOCATION
ATTN: DFAS-RI-FPV/ B. Border
BLDG 68
ROCK ISLAND, ILL 61299-8000
(309) 782-9204/FAX (309) 782-9994
DATE SHIPPED 3/6/96

6 samples

If the animal(s) from which your test samples were derived is known or suspected to be infected with a human pathogen, please identify the pathogen : _____

SAMPLE ID (Per vial Label)	SPECIES	TEST CATALOG #	CATALOG DESCRIPTION
9716-1	RABBIT	80-502	HISTOPATHOLOGY
9716-2	RABBIT	80-502	HISTOPATHOLOGY
9716-3	RAT	80-502	HISTOPATHOLOGY
9716-4	RAT	80-502	HISTOPATHOLOGY
9716-5	MOUSE	80-502	HISTOPATHOLOGY
9716-6	MOUSE	80-502	HISTOPATHOLOGY

SPECIAL INSTRUCTIONS: _____

PLEASE PROVIDE THE FOLLOWING INFORMATION FOR RODENT/RABBIT SERUM SAMPLES:

DILUTED 1:5 - YES ____ NO ____
(IF NO, EXTRA CHARGE APPLIES)

HEAT INACTIVATED:
YES ____ (SHIP AT ROOM TEMPERATURE)
NO ____ (SHIP TO ARRIVE WITHIN 48 HOURS)
(Extra charge applies)

See important Shipping Instructions and Terms and Conditions of Sale on the reverse.

MICROBIOLOGICAL ASSOCIATES INC

Our Code MV M918
Page 1 DAAD0596P-4060

TO: Major N. H. Foster
USA APG SA
USA, ERDEC, SCBRD-RTL
Bldg. E3222
APG-EA, MD 21010-5423

FROM: Robert L. Peters, Ph.D.
Microbiological Associates
9900 Blackwell Road
Rockville, MD 20850
(301) 738-1000

DATE: March 12, 1997
SPECIMEN: 2 rabbit, 2 rat and 2 mouse sera
RECEIVED: March 7, 1997

RESULTS: None of the tests were positive.

	IFA			MHA
Sample ID	CARB	E.cun	Tyz-Rb	T.cun
9714-1	-	-	-	-
9714-2	-	-	-	-

	ELISA					
Sample ID	CARB	MPul	PVM	RCV/SDA	Reo	Sendai
9714-3	0.00	0.03	0.00	0.00	0.00	0.01
9714-4	0.01	0.03	0.00	0.01	0.00	0.01

	IFA		HAI	
Sample ID	LCM	Parvo	H-1	KRV
9714-3	-	-	-	-
9714-4	-	-	-	-

Our Code MV M918
Page 2 DAAD0596P-4060

Sample ID	ELISA					
	MAd-FL	CARB	Ectro	EDIM	GDVII	LCM
9714-5	0.00	0.01	0.00	0.01	0.04	0.00
9714-6	0.00	0.02	0.01	0.00	0.01	0.00

Sample ID	ELISA					IFA
	MHV	MPul	PVM	Reo	Sendai	MCMV
9714-5	0.00	0.01	0.01	0.03	0.00	-
9714-6	0.00	0.04	0.00	0.00	0.05	-

Sample ID	IFA	HAI		
	Parvo	K	MVM	Polyoma
9714-5	-	-	-	-
9714-6	-	-	-	-

ELISA: Positive value is >= 0.17 OD units
IFA : + = positive; - = negative
HAI : Numerical value = positive; - = negative

MICROBIOLOGICAL ASSOCIATES, INC.

Life Sciences Center
9900 Blackwell Road • Rockville • Maryland 20850
(301) 738-1000 • Fax (301) 738-1038

FINAL PATHOLOGY REPORT

ACCESSION NO: CAHS-3988

DATE RECEIVED: March 7, 1997
DATE REPORTED: April 9, 1997

SOURCE: Major N.H. Foster
USAA ERDEC
SCBRD-RTL/N.H. Foster
BLDG E3222
APG-EA, MD

P.O. DAAD05-96-P-4060

DESCRIPTION:

Rabbit Tissue, ID # 9716-1, CAHS-3988 (1)
Rabbit Tissue, ID # 9716-2, CAHS-3988 (2)
Rat Tissue, ID # 9716-3, CAHS-3988 (3)
Rat Tissue, ID # 9716-4, CAHS-3988 (4)
Mouse Tissue, ID # 9716-5, CAHS-3988 (5)
Mouse Tissue, ID # 9716-6, CAHS-3988 (6)

RESULTS:

CAHS-3988 (1)

Tissues examined microscopically: Heart, thymus, lung, trachea, kidney, liver, spleen, pancreas, small intestine (x2), stomach, large intestine, brain, middle ear, eye, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings: No significant lesions observed

Comment: The cerebral leptomeninges is heavily stained with hematoxylin, indicating that it may be calcified. The bronchial cilia appear slightly "bluish". If desired, the Warthin Starry silver stain can be used to determine whether CAR bacillus is present. CAR bacillus usually produces little or no histologic alteration in rabbits.

PATHOLOGY REPORT
ACCESSION NO: CAHS-3988

CAHS-3988 (2)

Tissues examined microscopically: Heart, lung, trachea, liver, kidney, spleen, pancreas, small intestine (x2), cecum, brain, middle ears, eye, nasal passages, salivary glands

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Heart- aortic calcification, focal, minimal

CAHS-3988 (3)

Tissues examined microscopically: Heart, thymus, lung, trachea, liver, spleen, pancreas, small intestine, cecum, colon, stomach, brain, middle ears, lacrimal glands, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Lung- mineralization of the pulmonary artery, intimal, focal, (single small lesion)
Liver- lymphocytic infiltrates, focal, minimal
Kidney- mineralization, tubular, moderate

CAHS-3988 (4)

Tissues examined microscopically: Heart, lung, trachea, thymus, liver, spleen, pancreas, small intestine, cecum, colon, stomach, brain, middle ears, eyes, lacrimal glands, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Liver- lymphocytic infiltrates, perivascular, minimal
Kidney- mineralization, tubular, moderate
- lymphocytic infiltrates, peripelvic, moderate

Comment: The above lesions are considered to be incidental and not indicative of infection by adventitial agents.

PATHOLOGY REPORT
ACCESSION NO: CAHS-3988

CAHS-3988 (5)

Tissues examined microscopically: Heart, lung, liver, kidney, spleen, pancreas, small intestine, large intestine, brain, eyes, lacrimal glands, middle ears, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings:

Liver- lymphocytic infiltration, perivascular, minimal (single focus)

CAHS-3988 (6)

Tissues examined microscopically: Heart, lung, kidney, liver, small intestine, brain, middle ear, eyes and lacrimal glands, nasal passages

Gross findings: N.A. Formalin-fixed tissues received

Microscopic findings: No lesions observed

Anton M. Allen

Anton M. Allen, DVM, Ph.D.
Director of Veterinary Services