USACHPPM

U.S. Army Center for Health Promotion and Preventive Medicine

(Provisional)

TOXICOLOGICAL STUDY NO. 75-55-YJ81-91
4-AMINO 2 NITROTOLUENE (42ANT) SUMMARY OF MUTAGENICITY STUDIES, AVIAN TOXICITY STUDY AND AQUATIC TOXICITY STUDIES

U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (Provisional)

The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) lineage can be traced back over fifty years to the Army Industrial Hygiene Laboratory. That organization was established at the beginning of World War II and was under the direct jurisdiction of The Army Surgeon General. It was originally located at the Johns Hopkins School of Hygiene and Public Health, with a staff of three and an annual budget not to exceed three thousand dollars. Its mission was to conduct occupational health surveys of Army operated industrial plants, arsenals, and depots. These surveys were aimed at identifying and eliminating occupational health hazards within the Department of Defense's (DOD) industrial production base and proved to be beneficial to the Nation's war effort.

Most recently, it has been nationally and internationally known as the U.S. Army Environmental Hygiene Agency or AEHA. Its mission, by this time, had been expanded to support the worldwide preventive medicine programs of the Army, DOD and other Federal Agencies through consultations/supportive services, investigations and training.

Today, it is redesignated the U.S. Army Center for Health Promotion and Preventive Medicine. Its mission for the future is to provide worldwide technical support for implementing preventive medicine, public health and health promotion/wellness services into all aspects of America's Army and the Army Community anticipating and rapidly responding to operational needs and adaptable to a changing world environment.

The professional disciplines represented at the Center include chemists, physicists, engineers, physicians, optometrists, audiologists, nurses, industrial hygienists, toxicologists, entomologists, and many others as well as sub-specialties within these professions.

The organization's quest has always been one of excellence and continuous quality improvement; and today its vision, to be the nationally recognized Center for Health Promotion and Preventive Medicine, is clearer than ever. To achieve that end, it holds ever fast to its values which are steeped in its rich heritage:

- Integrity is the foundation
- Excellence is the standard
- Customer satisfaction is the focus
- Its people are the most valued resource
- Continuous quality improvement is its pathway

Once again, the organization stands on the threshold of even greater challenges and responsibilities. It is being totally reorganized with a provisional structure and will obtain its first General Officer leadership. As it moves into the next century, new programs are being added related to health promotion/wellness, soldier fitness and disease surveillance. As always, its mission focus is centered upon the Army Imperatives so that we are trained and ready to enhance the Army's readiness for war and operations other than war.

It is an organization fiercely proud of its history, yet equally excited about the future. It is destined to continue it development as a world-class organization with expanded services to the Army, DOD, other Federal Agencies, the Nation and the World Community.

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This report summarizes mutagenicity studies, avian toxicity studies and aquatic toxicity studies associated with the exposure to 4-amino 2 nitrotoluene. These studies were conducted to provide basic information on the potential toxicity of this material. This information will be utilized to predict the probable risks to humans, avian and aquatic species posed by this compound.

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DEPARTMENT OF THE ARMY
U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE (PROVISIONAL)
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REPLY TO ATTENTION OF

MCHB-DL-TE

TOXICOLOGICAL STUDY NO. 75-55-YJ81-91
4-AMINO 2-NITROTOLUENE (4A2NT)
SUMMARY OF MUTAGENICITY STUDIES
AVIAN TOXICITY STUDIES AND AQUATIC TOXICITY STUDIES

1. REFERENCES. See Appendix A.

2. PURPOSE. This report summarizes mutagenicity studies, avian toxicity studies and aquatic toxicity studies associated with the exposure to 4-amino 2-nitrotoluene.

3. GENERAL. 4-amino-2-nitrotoluene (4A2NT) is a photolytic metabolite of 2,4-dinitrotoluene (2,4-DNT). 4A2NT is a frequent groundwater contaminant at army ammunition depots which are historically associated with 2,4-DNT. These studies were conducted to provide basic information on the potential toxicity of this material. This information will be utilized to predict the probable risks to humans, avian and aquatic species posed by this compound.

4. MATERIALS.

a. Test Substance. The test substance, 4A2NT, was purchased from the Aldridge Chemical Co., Inc., 1001 W. Paul Ave., Milwaukee, Wisconsin. It was a rust colored powder with little, if any, odor. It is listed in RTECS as CAS NO. 119-32-4. One lot (07006BX) of 4A2NT 97 percent was received containing 2,475 grams. A sample was analyzed by the Organic Environmental Chemistry Division, U.S. Army Environmental Hygiene Agency (USAEHA), using infrared spectroscopy and gas chromotography. The analysis was a comparison of spectrum with literature and GC chromotography. The IR spectrum is similar to that reported by Aldridge with differences due to sampling techniques.

b. Contract Studies.

(1) Avian Toxicity Studies were performed under commercial contract by Bio-Life Associates, Ltd. Neillsville, Wisconsin.

(2) Mutagenicity Studies were performed under commercial contract by Integrated Laboratory Systems, Durham, North Carolina.

(3) Aquatic Toxicity Studies were performed under commercial contract by Wildlife International Ltd., Easton, Maryland.

5. METHODS.

a. In Vitro Mutagenicity Assays.

(1) Micronucleus Assay (In Vivo). The potential for 4A2NT to cause mutagenicity was assessed in the Rodent Bone Marrow Micronucleus Assay in male B6C3F1 mice. Mice were treated by intraperitoneal injection of 4A2NT in corn oil on 3 consecutive days. Treatment doses were 500, 1,000, and 2,000 mg/kg. The positive control was dimethylbenz[a]anthracene. All animals were euthanized 24 hours after the final treatment and the bone marrow removed. The frequency of polychromatic erythrocytes (immature red cells) was scored (1).

(2) CHO Cell Assay. 4A2NT was tested for its mutagenic potential in the Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells. In the nonactivated study, duplicate cultures were exposed to 4A2NT at 25, 50, 100, 200, and 400 micrograms/mL for 18 hours. In the S9 activated study, cells were exposed 100, 250, 500, 700, and 900 micrograms/mL for 4 hours, washed and incubated for another 16 hours. Metaphase cells were then harvested, stained, and examined. The percent of damaged cells in the total population was examined and the frequency of aberrations per cell was calculated. The positive control was Mitomycin C in the nonactivated portion and cyclosphamide in the S9 activated portion (2).

(3) Ames Test. The 4A2NT was evaluated for mutagenic activity using the Ames Salmonella/Microsome Reverse Mutation Plate Assay. The Ames test used Salmonella typhimurium indicator strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100. The sample was tested directly and in the presence of liver homogenates (S9 fraction) from male rats. Concurrent positive and solvent controls were run along with five dose points of the test sample. All tests were run in triplicate at doses of 5, 1, .5, .1, and .05 mg/plate 9(3).

(4) Mouse Lymphoma Test. The 4A2NT was assessed for mutagenic activity in the L5178Y+/- Mouse Lymphoma Mutagenesis Assay in the presence and absence of Aroclor induced rat liver S9. The nonactivated cultures selected for cloning were treated with 4A2NT at a maximum dose of 625 micrograms/mL. The S9 activated cultures selected for cloning were treated with doses up to 450 micrograms/mL. The diluent was sterile DMSO. The cultures were evaluated for elevated mutation frequency and clatogenic response (4).

b. Avian Toxicity.

(1) LC50 Bobwhite Quail. To determine the effects of 4A2NT toxicity on wildlife, a 9-day dietary exposure in bobwhite quail was conducted. The test material was in the diet at 312, 625, 1,250, 2,500, and 5,000 ppm to five groups of ten 12-day-old bobwhite quail of indeterminate sex. There were also five control groups which received stock diet only. Treatment was planned for 5 days followed by a 4-day recovery period. All the test birds were dead by the beginning of test day 4. Gross pathological examinations were performed on the 50 test birds that died during the study. Selected surviving control birds were subjected to gross pathological examinations at the end of the study (5).

(2) LC50 Mallard Ducklings. To determine the effects of 4A2NT on wildlife, an 8-day dietary exposure in mallard ducklings was conducted. The test material was in the diet at 312, 625, 1,250, 2,500, and 5,000 ppm to five groups of ten 5-day-old mallard ducklings of indeterminate sex. There were also five control groups which received stock diet only. Treatment was for 5 days followed by a 3-day recovery period. Birds that died during the investigation were subjected to gross pathological examinations as were selected survivors (one test bird and the control birds survived to study termination) (6).

(3) LD50 Bobwhite Quail. To determine the effects of graded oral doses of 4A2NT in bobwhite quail. Six groups of ten birds (five males and five females) approximately 57-weeks old were given a single oral dose of 4A2NT via gelatin capsules. Dose levels were 0, 3.16, 6.81, 10.0, 14.7, and 21.5 mg a.i./kg of body weights. There was one control group (five males and five females), with each bird receiving one empty gelatin capsule. Birds that died during the study were subjected to gross pathological examinations as were selected survivors from the remaining test groups and control groups (7).

c. Aquatic Toxicity.

(1) 96-Hour Static Acute Toxicity Test With Rainbow Trout. To evaluate the acute toxicity of 4-amino, 2-nitrotoluene to rainbow trout during a 96-hour exposure period under static test conditions. Fish were exposed to five test concentrations, a solvent control, and a negative control (well water). Two replicate test chambers were maintained for each treatment and control group, with 10 fish in each test chamber. Nominal test concentrations were 1.9, 3.2, 5.4, 9.0, and 15 mg a. I./L. Observations of clinical signs including mortality were made approximately 4.5, 24, 48, 72, and 96 hours. LC50 values were calculated at 24, 48, 72, and 96 hours. The no mortality concentration was determined by visual examination of data (8).

(2) 96-Hour Static Acute Toxicity Test With The Bluegill. To evaluate the acute toxicity of 4-amino, 2-nitrotoluene to bluegill trout during a 96-hour exposure period under static test conditions. Fish were exposed to five test concentrations, a solvent control, and a negative control (well water). Two replicate test chambers were maintained for each treatment and control group, with 10 fish in each test chamber. Nominal test concentrations were 13, 22, 36, 60, and 100 mg a. I./L. Observations of clinical signs including mortality were made approximately 2, 24, 48, 72, and 96 hours. LC50 values were calculated at 24, 48, 72, and 96 hours. The no mortality concentration was determined by visual examination of data (9).

(3) 48-Hour Static Acute Toxicity Test With The Cladoceran (Daphnia). To evaluate the acute toxicity of 4-amino, 2-nitrotoluene to the cladoceran during a 48-hour exposure period under static test conditions. Daphnids were exposed to five test concentrations and a negative control (well water). Two replicate test chambers were maintained for each treatment and control group, with 10 daphnids in each chamber. Nominal test concentrations were 0.65, 1.1, 1.8, 3.0, and 5.0 mg a. I./L. Observations of clinical signs including mortality were made approximately 16.5, 24, and 48 hours. EC50 values were calculated at 24 and 48 hours. The no mortality/immobility concentration was determined by visual examination of data (10).

6. RESULTS.

a. In Vitro Mutagenicity Assays.

(1) Ames Test. The test article, 4-amino-2-nitrotoluene, exhibited a positive mutagenic response in strains TA1537, TA1538, and TA98 without metabolic activation and in all strains except TA100 with exogenous metabolic activation (3).

(2) Mouse Lymphoma. The test article, 4A2NT, was clastogenic as well as mutagenic in the absence and presence of metabolic activation. There were clear concentration-related depressions in RTG values and increases in induced mutation frequencies were observed in the absence and presence of metabolic activation (4).

(3) CHO Assay. The test article 4-amino-2-nitrotoluene, was highly clastogenic in CHO cells. In the absence of metabolic activation, the lowest effective dose was 400 μg/mL, in the presence of metabolic activation, the lowest effective dose was 700 μg/mL (2).

(4) Micronucleus Test. Multiple treatments with 4-amino-2-nitrotoluene did not result in a significantly increased frequency of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of male B6C3F1 mice and did not significantly depress the percentage of PCE. The test was negative (1).

b. Avian Toxicity.

(1) LD50 Bobwhite Quail. A no-observed-effect level (NOEL) was not achieved in this study. The 14-day acute oral LD50 of 4A2NT was determined to be 9.13 mg a.i./kg of body weight, with 95 percent confidence limits of 7.33 and 11.4 mg a.i./kg of body weight. Gross pathology of 28 birds dying during the investigation revealed internal hemorrhaging in all birds. In 27 birds, a white, fibrous substance circumscribed one or more of the following organs: heart, liver, lungs, gizzard, and intestines. Pink-colored intestines were noted in 15 birds. The livers of eight birds were friable. Pale pectoral muscles were found in six birds, and very pale kidneys were present in one bird. Gross pathological examinations of 16 arbitrarily selected surviving birds revealed abnormal findings in five birds. The heart of one bird was enlarged 3X. There was a blood clot on the right lobe of the liver of one bird. The liver of one bird was friable and enlarged 2X. A 3X enlarged gallbladder and enlarged intestines were noted in one bird. The liver of a control bird was mottled (9).

(2) LC50 Mallard Ducklings. A NOEL was not achieved in this study. The 8-day acute dietary LC50 of 4A2NT was determined to be less than 312 ppm a.i. Only one 312 ppm a.i. bird and the control birds survived at study termination. Gross pathological examinations noted internal hemorrhaging, gizzards and/or intestines void of contents, and pale livers as some common findings in the birds that died during the study. Gross pathological examinations of arbitrarily selected survivors at termination revealed no abnormal findings (6).

(3) LC50 Bobwhite Quail. A NOEL was not achieved in this study. The 9-day acute dietary LC50 of 4A2NT was determined to be less than 312 ppm a.i. No mortalities were recorded in the control groups throughout the investigation. At the beginning of test day 4, all test birds were dead. Gross pathological examinations of all the 17 birds that were found dead on day 4, had a white, fibrous substance circumscribing the heart, the intestines were dark colored, and the crop and gizzard were void of feed. Gross pathological examinations of arbitrarily selected survivors at termination revealed no abnormal findings (5).

c. Aquatic Toxicity.

(1) LC50 Rainbow Trout. The 96-hour LC50 of 4A2NT was 12 mg 4A2NT/L. The 95 percent confidence limits were 9.0 and 15 mg 4A2NT/L. The 96-hour no mortality concentration was determined to be 9.0 mg 4A2NT/L (8).

(2) EC50 Daphnia. The 48-hour EC50 value for daphnids exposed to 4A2NT was 2.7 mg/L. The 95 percent confidence limits were 2.0 and 4.2 mg/L. The slope of the concentration-response curve was 2.2. The 48-hour no mortality concentration was determined to be 0.65 mg/L (10).

(3) LC50 Bluegill. The 96-hour LC50 of 4A2NT was 44 mg 4A2NT/L. The 95 percent confidence limits were 39 and 49 mg 4A2NT/L. The slope of the concentration-response curve was 12.1. The 96-hour no mortality concentration was determined to be 22 mg 4A2NT/L (9).

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APPENDIX A

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