



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

Registration Division

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PROTOCOL FOR HUMAN
HAZARD EVALUATION OF MICROBICIDES
USED IN INDUSTRIAL AIR WASHING
SYSTEMS TO CONTROL BIOLOGICAL
FOULING

A memorandum issued April 7, 1972, by Dr. David Greenman, Pharmacologist, Registration Division, stated "The use of slimicides in air washer systems must be supported by data to indicate that inhalation exposure resulting from this use would be toxicologically insignificant." Due to lack of knowledge of air washer structure and function by Registration Division personnel, Dr. Greenman's memo in effect declared a moratorium on registration of new microbicide products for use in air washer systems.

Subsequent to Dr. Greenman's memo, a comprehensive knowledge of air washer technology has been acquired, permitting Toxicology Branch, Registration Division to endorse the following hazard evaluation protocol. The protocol addresses both quantitation of pesticide to which humans may

be exposed and data required for evaluation of hazard from such exposure.

A. Introduction

Industrial air washing systems are devices employed to control the temperature and relative humidity of air at necessary critical values in the tobacco, textile, synthetic fiber and other industries. Incoming outside air is first heated if necessary, filtered to remove most particulate nutrients and microorganisms, passed through a recirculating, temperature controlled water spray containing microbicide to adjust the relative humidity, and then is subjected to "mist elimination" action to remove water droplets. Treated air is then propelled by fans through distribution ducts serving enclosed manufacturing or processing areas. The air is then recycled back through the air washing system, along with some added outside air. Unpublished results of a study conducted by an industrial firm indicate that air washing system mist eliminators effectively remove water droplets, thus protecting humans working in plant areas from water-borne microbicides used in spray water to control biological fouling. However, the Agency is concerned with any volatile parent compound or breakdown products of the microbicide that may be stripped by air from spray water and carried to humans.

In addition to presenting a low order of hazard potential, a second important concern of the Agency is definition of use condition under which the hazard was assessed.

Thus microbicide use is considered only in air washing systems that incorporate efficient outside air filtration and mist eliminating components, and that air washing systems be maintained in a clean condition.

B. Hazard Evaluation Protocol

1. Basic acute toxicity tests (product formulation):

- a. Acute oral LD₅₀.
- b. Acute dermal LD₅₀.
- c. Acute primary dermal irritation.
- d. Acute primary eye irritation.

Exposure of technicians to microbicide chemical dusts while treating air washer spray water is estimated to be minimal.

2. Identity, quantification and hazard evaluation of volatile materials from microbicides or microbicide breakdown products generated in industrial air washing systems.

Laboratory test systems utilizing air bubbled through microbicide treated water at air/water volume ratios approximating intended use values may be employed for microbicide volatile fraction studies. A complete description of the exposure chamber and test equipment should be provided.

A laboratory test of this type should include:

- a. Determination of the presence and amounts of volatile microbicide product active ingredient(s) and/or breakdown products in the test water reservoir, as well as in the exit air.

Air should be bubbled separately through relatively low and high aqueous concentrations of candidate microbicide at a rate of approximately 5 volumes of air to 1 volume of water per minute, for a total of 5 hours at room temperatures.

The microbicide treated test solution and air exiting the test system should be sampled and tested for original active microbicide ingredient(s) and/or predicted and other detected breakdown products at the beginning of the experiment, and at intervals frequent enough to permit characterization of volatile materials. Appropriate analytical procedures must be employed to identify and measure volatile materials.

The lower microbicide test concentration should approximate intended air washer label use dosage rates.

The higher microbicide test concentration should contain as much product solute as possible. Relatively nontoxic volatile materials demonstrated at use and much higher dosage rates will aid in establishing an adequate margin of safety for this use.

- b. Depending upon the presence, amounts, and if possible the identity of volatile materials detected in part a. above, subacute inhalation toxicity tests may be required.

- (i) If volatile materials generated from microbicides in part a. above can be identified and are determined by the Agency to be relatively nontoxic, further toxicity tests may not be required.
- (ii) If volatile materials generated from microbicides in part a. above can not be identified, or are known to be toxic, inhalation toxicity tests will be required as follows:

A minimum of two groups of at least 15 male and 15 female rats each should be exposed separately for one hour per day, 5 days per week, for each of 12 consecutive weeks to aerosols generated from at least 2 different microbicide concentrations as outlined in part a. above.

The highest dose-level tested should elicit some toxic or pharmacological effects; a lower dose-level should not show any such effects.

A similar group of at least 30 rats should serve as untreated control animals.

Treated and control animals should be observed throughout the 12 week test period.

OBSERVATION OF TREATED ANIMALS

General

Animals should be checked daily for evidence of toxic effects, detection and removal of dead and moribund animals. Moribund animals should be sacrificed. Statistical analyses should be performed when appropriate, to assist in the reporting and evaluation of data.

When average tests results are required, they are to be accompanied by the standard error or standard deviation. All statistical methods should be identified by reference and/or fully described.

Growth and food consumption at various intervals should be reported, with averages for each sex and dosage-level listed in tabular form.

Behavioral abnormalities and other toxicological or pharmacological manifestations should be reported for each sex and dosage-level group or if appropriate, for individual animals. A description of the time range over which such abnormalities or manifestations occurred together with an indication of the approximate number of animals involved.

The occurrence of infectious diseases and administration of drug treatment, if any, should be reported for each individual animal.

The time of mortality with individual values for each animal in tabular form should be reported.

The results of residue analyses should be reported if performed; including individual values for each animal and averages for each sex and dosage-level group in tabular form.

Clinical

Clinical determinations should be made on at least 10 animals of each sex in each group. Whenever possible the determinations should be made on the same animals. Results of all hematological, biochemical and other tests performed should be reported; including individual values

for each animal and averages for each sex and dosage-level group in tabular form.

Hematology

The following hematology determinations should be made initially and at the termination of the testing period: hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, and, if there is evidence of anemia, reticulocyte count.

Blood Chemistry

The following blood chemistry determinations should be performed initially and at the termination of the study: calcium, phosphorus, fasting glucose, urea nitrogen, total protein, hepatic enzymes and such other determinations as may be necessary for adequate toxicological evaluation. If the active ingredient is an organophosphate or a carbamate, or otherwise inhibits cholinesterase activity, cholinesterase activity should be determined.

Urinalysis

Standard urinalyses should be made during testing and at the termination of the testing period. Samples may be pooled according to sex and dosage-level group.

Gross necropsy

Organs which should be weighed include the liver, kidneys, spleen, heart, testes, adrenals, lungs, and brain.

Individual organ weights and organ to body weight ratios with averages accompanied by the standard error or standard deviation for each sex and dosage-level group should be reported.

All gross abnormalities must be adequately described. Such descriptions, wherever appropriate, should include measurements. Descriptions of commonly occurring lesions may be summarized, but the deviations from the average and some attempt at grading should be indicated for each animal.

Individual data for each animal and summary data for each sex and dosage-level group in tabular form, together with an indication of the number of animals showing each gross abnormality should be reported.

Gross dissection and examination should be performed by or under the supervision of a qualified pathologist, preferably the one who will also be responsible for the microscopic examination.

Histopathology

Adequate descriptions and diagnoses of all lesions including grading wherever appropriate should be reported. Individual data for each animal and summary data with average grades for each sex and dosage-level group in tabular form, together with an indication of the number of animals showing each type of lesion should be reported.

Microslides made from tissues fixed in 10% neutral buffered formalin or another generally recognized fixative and stained with hematoxylin and eosin are suitable for routine microscopic examination. Special stains, such as fat stains to determine the nature of cytoplasmic vacuoles in hepatic cells, may be necessary at times.

The following list of organs and tissues should be examined microscopically:

All animals in control and high dosage groups. All gross lesions, brain (at least 3 levels), spinal cord (at least 2 levels), eye, pituitary, salivary gland, heart, thymus, thyroid, lungs with mainstem bronchi, trachea, esophagus, stomach, small and large intestine, adrenals, pancreas, liver, kidneys, testes, prostate, ovaries, corpus and cervix uteri, spleen, lymph node, bone with marrow, skeletal muscle, skin, sciatic nerve, and mammary gland. Sections of bone should be taken from sternabrae, vertebrae, or the tibia-femoral joint (attached muscle).

All animals in intermediate and low dosage groups: Liver, kidneys, heart, any gross lesion, and any target organ (one showing any evidence of an effect of treatment, either at the high dose or from laboratory tests or clinical observations at any treatment level).

c.

Label Statements

The following statement or similar phraseology will be required on labels for microbicides used in industrial air washing systems, in addition to the required label statements for recirculating cooling water systems:

"For use only in industrial air washing systems that maintain effective mist eliminating components."

Part or all of the present document may be superseded upon publication of the Guidelines for Registration of Pesticides in the United States.