

OVERVIEW

BENZENE - BP-30-J
CMA-API Tox Studies
Protocol

The appended study designs are the results of a perceived need to coordinate the benzene research programs of the API and the CMA. To this purpose, a small working group of toxicologists (representing the CMA Benzene TRTG and/or the API Toxicology Committee) was established and charged with the responsibility to "rethink" to CMA and API research programs and to develop a technically sound (yet cost-effective) experimental approach. Six specific research objectives were delineated.

1. Can we create a "dose-response curve" for the following parameters (hematologic, cytogenetic, anatomical changes)?
2. Can we demonstrate an "effect level" at low concentrations of benzene vapor?
3. Can we identify a more sensitive indicator of toxic effect?
4. Is there an increased susceptibility to toxicity on repeated (intermittent) exposures?
5. Are there permanent effects from a single, high exposure (pulse)?
6. Are there permanent effects from an occasional high exposure (repeated pulse)?

The study designs presented here address these research objectives. It should be emphasized that these designs are presented as an indication of research direction only, and should not be construed as final protocols.

DO 067933
CONFIDENTIAL

02070

YZ0080914

FIVE-DAY ACUTE BENZENE INHALATION STUDY

Purpose: Dr. J. Dorner (API consultant) has been asked to consider various types of Laboratory Tests that may be useful (and practical) for monitoring benzene toxicity (e.g., δ -ALA, red cell hemolysis, serum enzymes, leukocyte alkaline phosphatase, etc.). He will recommend several tests for API/CMA consideration. The purpose of five-day study described below is 1) to confirm that these parameters are in fact measureable in the rat and mouse, 2) to give a preliminary indication of their responsiveness to toxic benzene exposure, and 3) to validate the contract laboratory's ability to perform these tests. On the basis of these observations, one or more of these parameters will be tested further.

Exposure Concentrations: Control and 2000 ppm

Exposure Duration: 6 hours/day for 5 days

Animals; 5 male and 5 female rats and mice/group.

Clinical Observations: Pre-exposure and after fifth day of exposure.

Sacrifice: At termination, the animals will be sacrificed and necropsied grossly. Tissues will be saved for possible histopathological examination at a later date.

DO 067934
CONFIDENTIAL

YZ0080915

NINETY-DAY SUBCHRONIC STUDY

Purpose: The purpose of the 90-day subchronic study is 1) to identify the most sensitive parameter indicative of a toxic effect due to benzene exposure, 2) to provide a general indication of the level of benzene exposure necessary for this toxic change to occur, and 3) to define the length of time necessary to develop such a toxic change. Such information will be used in selecting the exposure levels and exposure intervals for chronic studies.

Exposure Concentrations: Control and 4 levels

Exposure Duration: 6 hours/day, 5 days/week, for 13 weeks.

Animals: 5 male and 5 female rats and mice per group will be sacrificed at each observation point to provide blood, tissues, etc. for all clinical observations except cytogenetic evaluation. A separate group of 5 male and 5 female rats and mice will be pulsed with colcemid one-hour before sacrifice for cytogenetic analysis at each observation point.

Clinical Observations: The following will be noted at days 0, 1, 15, 30, 60, and 90.

- Hematological Evaluation (eg, RBC count, WBC count, differential, platelets, hematocrit, mean corpuscular volume)
- Clinical Chemistries
- Consultant's battery (selection based on consultant's recommendations and results of the 5-day study)
- Bone marrow chromosome assay (using separate group of animals pretreated with colcemid).

Sacrifice: At each observation point, all animals not pretreated with colcemid will be examined by gross necropsy. Tissues from these animals will be saved for possible future histopathology. At the terminal sacrifice, tissues of the high exposure level group will be prepared for histopathology. If possible signs of benzene-induced toxicity are noted at this level, the CMA may request a complete histopathological survey of all tissues.

At each observation point, the bone marrow of colcemid-treated animals will be prepared to a suitable stage and will be saved for possible future cytogenetic analysis. At the terminal sacrifice, the bone marrow of the high exposure level group will be prepared and screened for cytogenetic changes. If positive signs of benzene-induced toxicity are noted at this level, the CMA may request a complete chromosomal analyses of all other bone marrow preparations.

DO 067935
CONFIDENTIAL

YZ0080916

TWO-YEAR CHRONIC STUDY

Purpose: Because of NYU's finding of one case of chronic myelogenous leukemia, as well as a suggestive trend for RBC and lymphocytes in rats exposed to 100 ppm, an evaluation of the toxic effects of low level benzene exposure is proposed.

Exposure Concentrations: Control and 4 exposure levels

Exposure Duration: 6 hours/day, 5 days/week for 24 months

Animals: 10 male and 10 female rats and mice/interim sacrifice group
5 male and 5 female rats and mice/interim sacrifice group
(cytogenetics only)
50 male and 50 female rats and mice/terminal sacrifice group
5 male and 5 female rats and mice/terminal sacrifice group
(cytogenetics only)

Clinical Observations: Cytogenetic, hematologic, and/or chemical observations (selected on basis of 90-day study results) will be made at 0, 6, 12, 18, and 24 months.

Terminal Sacrifice: All animals will be sacrificed at 24 months. A complete necropsy will be performed and a complete set of tissues will be saved from all animals except those used in cytogenetics. High exposure level animal tissues and chromosomes will be evaluated for histopathology. The CMA may request a complete histological survey of all tissues at a later date.

DO 067936
CONFIDENTIAL

YZ0080917

TWO-YEAR INTERMITTENT STUDY

~~Purpose:~~ Purpose: To determine if there is a potential for increased susceptibility to a toxic response after benzene intermittent exposures.

Exposure Concentration: Control and one level (to be determined on basis of 90-day subchronic study).

Exposure Duration: The time interval (X) for exposure and clinical observations will be determined using data from the 90-day subchronic study.

Exposure Cycle 1 will begin with exposures at day 0 and will continue to day X on a 6 hours/day, 5 days/week schedule. Animals will then be held to six months without exposure; this period may be extended if the mean of the treated animals do not show recovery to within 1 S.D. of the control mean.

Exposure Cycle 2 will begin at 6 months (or at the time when animals have recovered to within 1 S.D. of the control mean) and will continue until day 6 months + X. Animals will then be held to one year (or until ~~the recovery~~), *it*. animals will be sacrificed.

Animals: 25 male and 25 female rats and mice/group.
5 male and 5 female rats and mice/group (cytogenetics only).

Clinical Observations: The "most sensitive index" of benzene toxicity (as determined in the 90-day subchronic study) and cytogenetic evaluations will be performed at days 0, (2/3 X), (X), monthly to 6 months (or recovery). 6 months, (6 months + 2/3 X), (6 months + X), monthly to 12 months (or recovery).

*NOTE: The TRTG has approved conducting a 2-Year Intermittent Study but has asked the Protocol Subgroup to reconsider the protocol.

DO 067937
CONFIDENTIAL

YZ0080918

Chronic Two-Year (24-Month) Single High Level Exposure

Purpose: To determine the long-term toxicological effects of a single high exposure to benzene as compared to longer term low-level exposure effects.

Exposure Concentration: LC₅₀ for benzene for prescribed time, preferably 4-6 hours.

Exposure Duration: One exposure of from 4-6 hours and 24 months of no exposure.

Animals: 125 male and 125 female rats and mice/terminal sacrifice treated group
50 male and 50 female rats and mice/terminal sacrifice control group
10 male and 10 female rats and mice/sacrifice group ("most sensitive indicator")
5 male and 5 female rats and mice/sacrifice group (cytogenetics only).

Clinical Observations: At 0, 7 and 14 days, and 12 and 24 months, interim clinical observations will consist of the "most sensitive indicator" for benzene exposure and cytogenetics (preparation only). Five male and five female animals will be run for each sacrifice period to provide animals for cytogenetics. Ten male and 10 female animals will be run to provide for determination of the "most sensitive" benzene exposure parameter plus necropsy and tissue storage at the interim sacrifices.

Sacrifice: All surviving animals will be terminated at 24 months. A gross necropsy will be performed, and tissues will be saved and histopathologically evaluated. The sponsor may request all other tissues to be evaluated at a later date. Cytogenetic analysis will be done on animals from the high exposure group. Others will be done if positive results are found.

DO 067938
CONFIDENTIAL

YZ0080919

Chronic Two-Year (24-Month) Pulse Dose

~~Purpose:~~ To determine the toxicological effects of high level, short duration exposures to benzene.

Exposure Concentration: 1000 ppm benzene and a negative control

Exposure Duration: 2 hours/day, two days/week for 24 months

Animals: 50 male and 50 female rats and mice/terminal sacrifice group
10 male and 10 female rats and mice/interim sacrifice group
5 male and 5 female rats and mice/interim sacrifice group (cytogenetics only).

Clinical Observations: In all animals, at 0, 6, 12, 18 and 24 months the "most sensitive index" of benzene toxicity as determined in the 90-day study and cytogenetics.

Bone marrow preparations suitable for cytogenetic assays will be made and stored for 5 male and 5 female animals which will be separate from the main animal population in the study. These animals will not be necropsied, and tissues will not be saved.

Sacrifices: At all interim observation periods, 10 male and 10 female animals (which will not be part of the main population of the study) will be sacrificed and necropsied; tissues will be saved. At the 24 month sacrifice, complete necropsies will be done and tissues will be saved. Histopathology will be done on all terminal tissues with the possibility that the sponsor may request that the interim sacrifice tissues also be evaluated. Cytogenetic analysis will be done on animals from the terminal sacrifice. Further analysis will be done if positive results are found.

*The TRTG approved the pulse-dose studies in principal, but has referred to Protocol Subgroup

DO 067939
CONFIDENTIAL

YZ0080920

DOW CHEMICAL U.S.A.

TEXAS DIVISION
FREEPORT, TEXAS 77541

June 14, 1978

Mr. Steven Williams
Office of The General Counsel
American Petroleum Institute
2101 L Street N. W.
Washington, D. C. 20037

Dear Mr. Williams:

BENZENE CYTOGENETIC STUDY - PICCIANO

Enclosed are four versions of the Dow benzene cytogenetic study: "A", "B", and "C" by D. J. Picciano, et. al. and "D" by D. J. Kilian and R. L. Daniel. The cytogenetic data are the same, i.e., they are from a 52 employee population at our Freeport, Texas Benzene and Ethyl benzene-A plants. The "D" version was submitted by Dow to EPA and to NIOSH and contains our best assessment of the employee exposure history. We do not know how many of these were turned over to Rachel Scott or to OSHA. Since Picciano deleted the last sentence from paragraph 2, page 3 which states "at least one positive UEP test indicating a possible exposure to benzene greater than 25 ppm" from versions "B" and "C", it is unlikely that Scott is working from version "A" or that OSHA would submit version "A" in evidence.

You should be aware of the fact that Drs. Jacobson, Legator, and Wharton had no reason to question the completeness of the environmental exposure section of Picciano's study. Therefore, their comments were mostly laudatory.

We will be glad to attempt to answer any questions that your review of these documents may raise.

Yours very truly,

R. L. Daniel

R. L. Daniel
Environmental Health Services

bbs

Attachments (4)

cc: Andrew T.A. Macdonald
Wilmer, Cutler & Pickering
1666 K St., N.W.
Washington, D.C. 20006

AN OPERATING UNIT OF THE DOW CHEMICAL COMPANY

00771

DO 067940
CONFIDENTIAL

YZ0003057