

BENZENE SUBCHRONIC TOXICITY STUDY WITH EVALUATIONS OF REPRODUCTIVE FUNCTION AND DOMINANT LETHALITY

P U R P O S E

DETERMINE THE TOXICITY ASSOCIATED WITH EXPOSURE OF
MICE AND RATS TO GRADED LEVELS OF BENZENE BY INHALATION
FOR 90 DAYS. MORE SPECIFICALLY, DEFINE THE TARGET ORGANS
FOR TOXICOLOGIC RESPONSE AND THE CONCENTRATION AT WHICH
THERE IS NO APPARENT ADVERSE EFFECT. IN ADDITION,
MEASURE THE REPRODUCTIVE CAPACITY OF FEMALE RATS DURING
EXPOSURE TO BENZENE, AND, AS AN IN VIVO MEASURE OF
MUTAGENICITY, TEST FOR A POSSIBLE DOMINANT LETHAL EFFECT
IN MALE RATS EXPOSED TO BENZENE.

EXPERIMENTAL DESIGN

CONCENTRATIONS: 0 PLUS 3 LEVELS OF BENZENE (MAYBE 4)

ANIMALS: CD-1 MICE AND SPRAGUE-DAWLEY RATS

EXPOSURE: 6 HR/DAY, 5 DAYS/WEEK

GROUP SIZE:

STUDY A - 15/SEX/GROUP - MICE & RATS

STUDY B - 20 FEMALES/GROUP (RATS ONLY)
PLUS UNEXPOSED MALES FOR MATING

STUDY C - 20 MALES/GROUP (RATS ONLY)
PLUS UNEXPOSED FEMALES FOR
MATING (1:1)

STUDY A:

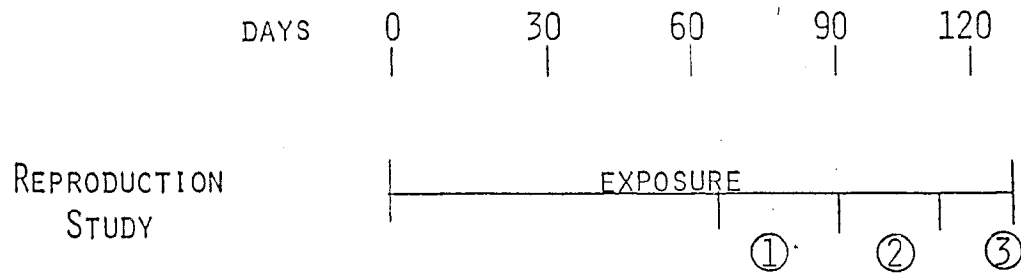
DAYS 0 30 60 90 120
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SUBCHRONIC
STUDY



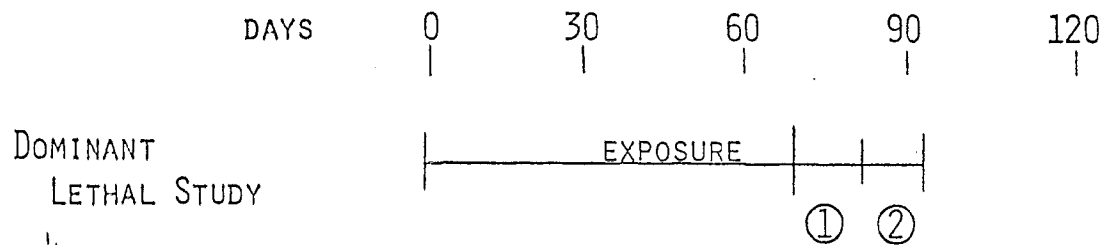
SACRIFICE, COMPLETE
HISTOPATHOLOGY AT 0 AND HIGH
CONC. PLUS TARGET ORGANS AT
LOWER LEVELS TO DEFINE A NO-
ADVERSE EFFECT CONC. -
COLLECT TISSUES FOR BENZENE
AND METABOLITE LEVELS.

STUDY B:



- ① MATE WITH UNEXPOSED MALES AT DAY 70
- ② GESTATION
- ③ LACTATION

STUDY C:



- ① ② MATE EXPOSED MALES WITH
UNEXPOSED FEMALES (1:2);
SACRIFICE FEMALES 10 DAYS
AFTER SEPARATION FROM MALES
AND COUNT NUMBER CORPORA LUTEA,
IMPLANTATIONS AND RESORPTIONS.

OBSERVATIONS

A. SUBCHRONIC STUDY

DEMEANOR DAILY

BODY WEIGHTS 2X WEEKLY FIRST 2 WEEKS, 1X WEEKLY THEREAFTER

what? HEMATOLOGY - 10 MICE AND RATS/GROUP DURING 13TH WEEK
OF EXPOSURE

what? URINALYSIS - 10 MICE AND RATS/GROUP DURING 13TH WEEK
urinalysis reported? OF EXPOSURE

what? 3 CLINICAL CHEMISTRY - ALL MICE AND RATS AT SACRIFICE
ditto!

PATHOLOGY - GROSS NECROPSY EXAM ALL ANIMALS

- HISTOPATHOLOGY COMPLETE ON ALL CONTROLS AND
HIGH EXPOSURE LEVEL PLUS TARGET ORGANS ON
LOWER CONCENTRATIONS TO DEFINE A NO-ADVERSE
EFFECT LEVEL

ORGAN WEIGHTS - ALL ANIMALS AT NECROPSY - LIVER, KIDNEY,
BRAIN, HEART, TESTES, THYMUS, *spleen*

B. REPRODUCTION STUDY

DEMEANOR DAILY

BODY WEIGHTS 2X WEEKLY FIRST 2 WEEKS, 1X WEEKLY THEREAFTER

MATE WITH PROVEN UNEXPOSED MALES AFTER DAY 70

DETERMINE - PERCENT PREGNANT

- MEAN LITTER SIZE ON DAYS 1, 7, 14, 21 OF LACTATION
- MEAN PUP WEIGHTS ON DAYS 1, 7, 14, 21 OF LACTATION
- GROSS NECROPSY FINDINGS IN ALL WEANLINGS AT DAY 21 OF AGE
- ORGAN WEIGHTS OF WEANLINGS AT NECROPSY - LIVER, KIDNEY, TESTES
- GROSS NECROPSY FINDINGS IN ADULT FEMALES

when does exposure clear? Consider an F₁B litter

C. DOMINANT LETHAL STUDY

DEMEANOR DAILY

BODY WEIGHTS 2X WEEKLY FIRST 2 WEEKS, 1X WEEKLY THEREAFTER

STOP EXPOSURE AT 70 DAYS AND MATE EXPOSED MALES WITH
UNEXPOSED FEMALES (1 MALE WITH 2 FEMALES) FOR 2 WEEKS -
ONE SET OF FEMALES PER WEEK

SACRIFICE FEMALES 10 DAYS AFTER SEPARATION FROM MALES
AND COUNT NUMBER OF CORPORA LUTEA PLUS IMPLANTATIONS
AND RESORPTIONS PER FEMALE. CALCULATE FERTILITY,
PRE-IMPLANTATION LOSS AND RESORPTION RATE

*usually mate for 6-8
weeks so that adverse
effects from any phase of
spermatogenic cycle
can be detected.*