

HUNTINGDON LIFE SCIENCES STUDY NO.: 96-4080

AMENDMENT NO.: 4

STUDY TITLE: Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats

PROTOCOL ISSUE NO.: 3

Changes

1. Page 3, 2 Study Personnel: (Protocol Amendment No. 1)
Change "Primary Technician" from: "Cynthia Lyskowski" to "Linda Reed, B.S."
Effective: 1 March 1997

2. Page 14, 9.8 Animal Identification:
Change the following sentence -

From:
"Following the selection procedure (postweaning) to identify the P₂ parental animals from the F₁ offspring, these selected animals will be eartagged with a unique number."

To:
"Following the selection procedure (postweaning) to identify the P₂ parental animals from the F₁ offspring, these selected animals will be tail tattooed with a unique number."
Effective: 05 May 1997

3. Page 18, 11.5 Chamber Monitoring:
Add the following activity to the chamber monitoring table:
Activity: "In-chamber observation of study animals during exposures"
Frequency/chamber/day: "once - mid-way into the exposures"
Effective: 21 March 1997

4. Page 22, 12.5.4 Pup Body Weights and Sexing Data:
Add the following: "All F₁ generation animals retained as the P₂ parental generation will be weighed on Postnatal Days 32 and 39."
Effective: 12 May 1997

5. Page 24, 13.1.2.4.2 Unselected F₁ Pups:
Delete the following sentence from this section:

"Some control pups not selected into the pool of animals to become the P₂ parental generation will be used in a satellite study with vinyl chloride (see Study No. 96-4092)."

6. Page 25, 13.1.3.3 Parental Animals:
In the list of tissues to be weighed change "right epididymis (total and cauda)" to "left epididymis (total)".

HUNTINGDON LIFE SCIENCES STUDY NO.: 96-4080

AMENDMENT NO.: 4

STUDY TITLE: Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats

PROTOCOL ISSUE NO.: 3

Changes

7. Page 27, 13.1.8 Sperm Count, Motility and Morphology Assessments:
Change the following sentence

From:

"From the first 15 P₁ and P₂ parental males sacrificed at termination in each group, samples of sperm from the vas deferens (left) and distal cauda epididymis (left) will be collected."

To:

"In the P₁ and P₂ parental generation, sperm evaluations (count, motility and morphology) will be performed for a total of 15 males per group. Included in this group will be all males in each group that did not sire a litter. The remaining males identified for sperm assessment will be the first animals in the group until a total of 15 males per group have been evaluated."

Effective: 24 April 1997

Reasons for Changes

1. Cynthia Lyskowski left the company and Linda took over responsibility as "Primary Technician" for the study. Linda had been working with Cynthia on this study.
2. Because the weanling F₁ animals are being handled so much with daily transporting back and forth between the home cage and exposure cages, we recommended identification by tattooing the animal's number on the tail as opposed to eartags. It was our opinion that the animals would be more likely to lose the eartags with such frequent handling and change in caging.
3. In-chamber observation of the animals during exposures was not in the protocol. It was decided in our 21 March phone conversation to start conducting these evaluations. This corresponded to early in the mating period of the P₁ parental animals.
4. In our 12 May 1997 conversation, I recommended that we weigh the F₁ parental animals on Postnatal Days 32 and 39 since animals were being treated. Animals will be weighed again at the formal initiation of the premating treatment period and weekly thereafter.
5. The protocol for the satellite study (96-4092) provides for weanlings to come from a separate population of animals. The original intent was to use the excess F₁ control pups for the satellite study but due to scheduling difficulties and the staggered weaning dates that would be encountered, it was decided not to use these animals.

HUNTINGDON LIFE SCIENCES STUDY NO.: 96-4080

AMENDMENT NO.: 4

STUDY TITLE: Vinyl Chloride Combined Inhalation Two-Generation Reproduction and Developmental Toxicity Study in CD Rats

PROTOCOL ISSUE NO.: 3

Reasons for Changes

6. Both epididymides will be weighed intact. Since the right epididymis was being weighed and processed for sperm assessment (Section 13.1.8), the table in the protocol should have specified weighing of the left epididymis. The left epididymis was weighed and preserved intact. The most recent harmonized OPPTS guidelines (No. 870.3800 - Reproduction and fertility Effects, February 1996) recommends that the epididymis be processed intact as a longitudinal section that would include portions of the caput, corpus and cauda. To weigh the cauda segment of the epididymis would require cutting the organ.

7. The protocol had specified the first 15 P₁ and P₂ males per group for sperm assessment. Following review of the reproductive performance of the P₁ parental males it was decided (conversation with Dean Rodwell 24 April 1997) to include in this number the males that did not sire a litter (i.e., males that did not mate, males that mated but did not successfully impregnate a female). The remaining males identified for sperm assessment in each group were the first animals in the group until a total of 15 had been selected.

Amendment approved by:

 Raymond E. Schroeder, M.S., DABT
 Title: Study Director
 For: Huntingdon Life Sciences

 Date

 Hasmukh C. Shah, Ph.D.
 Title: Sponsor Representative
 For: Chemical Manufacturers Association

 June 17, 1997
 Date

 James B. Knaak, Ph.D.
 Title: Study Monitor
 For: Occidental Chemical Corporation

 6/17/97
 Date

HUNTINGDON LIFE SCIENCES STUDY NO.: 96-4080

AMENDMENT NO.: 4

STUDY TITLE: Vinyl Chloride Combined Inhalation Two-Generation Reproduction and
Developmental Toxicity Study in CD Rats

PROTOCOL ISSUE NO.: 3

The IACUC Protocol Review Subcommittee has reviewed this protocol and found it to be in
compliance with all appropriate regulations:

By: _____ Date: _____
Title: Institutional Animal Care and Use Committee Member

By: _____ Date: _____
Title: Institutional Animal Care and Use Committee Member