One-generation extension study:
Overview of materials distributed to EDMVS
November 2001

I. Reason for this Study

The "one-generation extension study" explores whether sensitivity to endocrine effects in the standard two-generation study can be increased simply by allowing the F1 generation to develop beyond puberty rather than being sacrificed at weaning. Effects that are present but not observable at weaning – due, for example, to normally incomplete development of organs – might become observable if organ development were allowed to continue.

In the standard two-generation study, one male and one female from the F1 generation *are* raised beyond weaning, but because this number is so small, false negatives could easily occur for effects, which have low penetration. Similarly, false positives might occur due to random variation. By increasing the number of animals that are raised to adulthood, the reliability of the assay may be increased.

A concise explanation of the concern that this study addresses is given in the **attached excerpt from the National Toxicology Program's Low-Dose Peer Review Report**, especially the last two paragraphs.

Since attention in this proof-of-concept study is focused on the F1 generation only, it was considered unnecessary to breed and examine the F2 generation that would normally be a part of a two-generation study. For this reason, EPA is referring to this study as the *"one-generation* extension study". It should be remembered, however, that if extension of the F1 generation is seen to be worthwhile, this modification would be applied to the two-generation study design; the one-generation extension study is not being developed as a separate assay.

II. Study protocol

The study protocol is attached.

The study focuses on males because it is the male reproductive organs that are undeveloped (unobservable) at the time of weaning. Vinclozolin (0, 50, or 100 mg/kg/day) or di-n-butyl phthalate (0, 100, or 500 mg/kg/day) will be administered to pregnant rats by gavage on gestational day (GD) 6 through postnatal day (PND) 21. Litters will be culled to 10 animals per litter on PND 4, retaining as many males as possible. Three males per litter will be sacrificed at PND 21 and the remainder sacrificed on day PND 95±5. Careful observation of the reproductive organs will be made, and the weights of the following organs will be taken:

- Each testis individually
- Each corpus plus caput epididymides

- Each cauda epididymides
- Entire seminal vesicle, plus coagulating glands with fluid as a unit, if possible
- Entire prostate, then ventral and dorsal lateral lobes separately
- Paired adrenals
- Liver
- Levator ani plus bulbocavernosus
- Cowper's (bulbourethral) glands as a pair
- Glans penis

III. Expert review

The one-generation extension study was developed by EPA researchers at the National Health and Environmental Effects Research Laboratory.

Comments from Dr. Paul Foster, CIIT Centers for Health Research, on a previous draft of this protocol are attached. Several changes to the protocol were made on the basis of Dr. Foster's comments; his comments do not necessarily apply to the final protocol. Most significantly, the study was extended so that the observation period goes to PND 95 rather than PND 60.

Comments from Research Triangle Institute, the laboratory which will be conducting the study for EPA through EPA's main support contract with Battelle Memorial Institute, were also solicited. They are not attached here because they deal primarily with logistical issues pertaining to endpoints (sperm measurements, hormone measurements) that were dropped from the final protocol. RTI also strongly recommended extending the study from PND 60 to around PND 90. RTI's comments can be distributed to EDMVS upon request.

IV. Timeline

This protocol was approved by EPA on August 23, 2001 and has been waiting to go into the lab since that time. The fire in the animal care facility on August 25 delayed the study. The subsequent start date of Nov. 14 was postponed so that EDMVS could review the protocol. A final report is expected approximately 9 months after initiation of the study in the lab.

V. Questions for EDMVS

1. Should a similar study be undertaken for females?
2. Are there other endpoints that should be considered?