



CHEMICAL MANUFACTURERS ASSOCIATION

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March 23, 1995

Dear Vinyl Chloride Health Committee Members:

A March 22, 1995 letter to William Cibulus of ATSDR is enclosed for your review and files. I will contact Mr. Cibulus next week to schedule a meeting. If you have any questions, please call me at (202) 887-1192.

FYI

Sincerely,

Has Shah

Jonathan

Hasmukh C. Shah, Ph.D.
Manager, Vinyl Chloride Panel

Enclosures



CHEMICAL MANUFACTURERS ASSOCIATION

March 22, 1995

William Cibulas, Ph.D.
Chief, Research Implementation Branch
Division of Toxicology
Agency for Toxic Substances & Disease Registry
1600 Clifton Road, N.E.
Mailstop E29
Atlanta, Georgia 30333

Dear Dr. Cibulas:

On January 23, Dr. Christopher DeRosa responded to my letter of November 28, 1994 indicating the intent of the Chemical Manufacturers Association (CMA) Vinyl Chloride Panel to address certain data needs for vinyl chloride identified by the Agency for Toxic Substances and Disease Registry (ATSDR). Dr. DeRosa requested that the Panel take the lead role in developing the protocol for a two-generation reproductive toxicity study of vinyl chloride by the inhalation route.

We have prepared the enclosed protocol of such a study for ATSDR review. Pursuant to your telephone conversation with our counsel, Caffey Norman, we have not attempted to identify dose levels at this time. Dose levels would be determined after a review of existing literature or, if necessary, following a range-finding study, in either event in consultation with ATSDR.

You will note that in a few places information has been redacted from the enclosed protocol. The purpose of these deletions is to remove certain references that would limit the study to a particular laboratory. Such information will be included when a final protocol is submitted for review.

As Mr. Norman discussed with you, selecting dose levels and otherwise providing a completely final protocol for your review would require several additional weeks. We believe that it would be more useful if this time were devoted to considering ways that the enclosed protocol might be enhanced to include measures of

William Cibulas, Ph.D.

March 22, 1995

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developmental toxicity. In this regard, Dr. DeRosa's letter requested clarification of the statement in my letter of November 28 that "[i]n light of the existing two-species inhalation developmental toxicity study, and other available data concerning the developmental toxicity of vinyl chloride, we would like to discuss how [the reproductive toxicity] study **and other available data** might be enhanced to include measures of developmental toxicity as an alternative to the two-species developmental toxicity study referred to EPA." This passage was unfortunately garbled during our internal review and the phrase "and other" available data" was mistakenly repeated. The italicized words should not appear in the sentence. Our intent is to determine in consultation with ATSDR scientists whether it is possible to include measures of developmental toxicity of interest to ATSDR in the protocol for the two-generation reproductive toxicity study that is enclosed for your review.

To facilitate this discussion, we also have enclosed a review of the available data on the developmental toxicity of vinyl chloride. We look forward to meeting with ATSDR scientists to discuss the strengths and weaknesses of the existing developmental toxicity data and options to obtain any needed additional data that do not require a full two-species developmental toxicity study.

We look forward to a meeting between scientists from the Vinyl Chloride Panel member companies and ATSDR in the near future to discuss the enclosed protocol, the utility of the available developmental toxicity data, and the ways the enclosed protocol might be enhanced.

Sincerely,

Has Shah

Hasmukh C. Shah, Ph.D.
Manager, Vinyl Chloride Panel

Enclosures

cc: W. Caffey Norman, Esq.

P&S 154556

INTRODUCTION

Prior Toxicity Data.

(TO BE INSERTED)

Objective.

The objective of the two-generation inhalation reproduction study outlined in this protocol is to evaluate the effects of the test material on the reproductive capability and neonatal growth and survival in rats. This study will be conducted to meet the requirements of the Environmental Protection Agency (EPA): TSCA Test Guidelines (EPA, 1985), the Organisation for Economic Co-Operation and Development (OECD), Guidelines for Testing of Chemicals, Section 4: Health Effects, (OECD, 1981), and the European Economic Community (EEC), Methods for the Determination of Toxicity (EEC, 1988).

Statement of GLP Practice.

This study will be conducted in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations for NonClinical Studies (FDA, 1988), the EPA TSCA Good Laboratory Practice Standards (EPA, 1990), the OECD Good Laboratory Practice Procedures (OECD, 1982), and .

In addition, in response to the Final Rules amending the U.S. Animal Welfare Act that were promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activity (ACUA) that is required for the conduct of this study has been reviewed and given full approval by the Institutional Animal Care and Use Committee (IACUC). The IACUC has determined that the proposed Activity is in full accordance with these Final Rules. The IACUC has assigned Activity No. Reproductive Toxicology 01 to this Animal Care and Use Activity.

MATERIALS AND METHODS

The test material used for this study was obtained from X(name), X(city), X(state) and was identified as (Lot number of the test material). The purity of the test material was reported to be xx.x% (reference). A sample of the test material will be taken and stored in a manner consistent with the reference sample retention policy of this laboratory. The test material will be reanalyzed at approximately 6-month intervals to confirm purity and stability.

The test substance has the following properties:

Chemical Name:

Synonyms:

Molecular Formula:

Molecular Weight:

Structures:

Appearance:

Vapor Pressure:

Saturated Atmosphere:

Vapor Density:

Flash Point:

Boiling Point:

Specific Gravity:

Conversion Factors:

Test Species and Husbandry.

Male and female CD rats (Charles River Breeding Laboratory, Kingston, NY) approximately four weeks of age will be purchased for the study. Animals will be ordered to ensure that a sufficient number of animals of acceptable health and

weight are available to conduct the study as designed. This strain of rat has been selected because of its general acceptance and suitability for toxicity testing and the availability of a reliable commercial source. Upon arrival at the laboratory¹, all rats will be examined for health status by a veterinarian and acclimated to the laboratory environment for approximately two weeks

. For the randomization procedure, the rats will be weighed and ranked according to body weight and those from the extremes of the distribution will be identified and removed from the population until only the number of animals required for the study remain. These rats will be randomly assigned by weight to the treatment groups to increase the probability of uniform group mean weights and standard deviations at the initiation of the study. Rats not placed on test will be removed from the test room and the disposition of these animals will be documented in the study file.

Identification of all rats on test will be accomplished by inserting a uniquely coded alphanumeric metal tag in one ear of each rat. In the event that an ear tag becomes dislodged during the course of the study, it will be replaced with one having the same alphanumeric code (i.e., a new alphanumeric code will not be assigned) and noted in the study file.

Rats will be housed singly in wire mesh, stainless steel cages in racks provided with deotized cage board to minimize odor and aid in maintaining a clean environment. Prior to and following daily exposures during late gestation and throughout lactation, females will be housed in plastic nesting boxes provided with ground corn cob nesting material (further details provided in experimental design section). The animal rooms of the facility are designed to maintain humidity at approximately 40-60%, temperature at approximately 22 °C, photoperiod at 12 hrs light:12 hrs dark and air flow at 12-15 changes/hour. A feed crock and a pressure-activated stainless steel water nipple will be components of all cages. A basal diet of Purina Certified Rodent Chow No. 5002 (Purina Mills Inc., St. Louis, MO) will be provided *ad libitum* except during the

¹Fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

six hour per day exposures at which time feed will be withheld. Municipal drinking water will be available *ad libitum* throughout the prestudy and study periods. Analysis of the chow will be performed by Purina Mills Inc. to confirm that the diet provides adequate nutrition, and to quantify the levels of selected contaminants associated with the formulation process. Drinking water obtained from the _____ will be analyzed for chemical parameters and biological contaminants by _____. In addition, specific analyses for chemical contaminants will be conducted at periodic intervals.

Exposure Chamber.

Animals will be housed and exposed to test material vapors in a 14.5 m³ chamber (2.4 m wide x 2.4 m high x 2.4 m deep with a pyramidal top) under dynamic airflow conditions. Chamber airflows will be maintained at approximately 2900 liters/minute, which is sufficient to provide the normal concentration of oxygen to the animals.

Vapor Generating System.

The various concentrations of test material will be generated using a glass J-tube method (Miller et al., 1980). Liquid test material will be metered into a glass J-tube assembly and vaporized by a preheated stream of compressed air (up to 100 l/min) passing through the J-tube. Compressed air will be heated to the minimum extent necessary to facilitate complete vaporization of the test material. The compressed air and test material vapors will be diluted and mixed with room air to achieve a total flow rate of 2900 l/min and the desired concentration of test material vapors.

Chamber Monitoring.

The concentration of the test material in each chamber will be measured at least once per hour using a MIRAN 1A infrared spectrophotometer (Foxboro Analytical, Norwalk, CT). Analytical equipment will be calibrated using standards having a known test material vapor concentration contained in 90 liter

SARAN* film gas bags prior to the first exposure and at least monthly thereafter. Daily checks of the analytical equipment will be performed prior to each exposure period using a single the test material standard concentration. In addition, the amount of the test material used each day will be recorded and the nominal concentrations (amount of the test material used/total chamber airflow) of the test material will be calculated. Prior to the start of the study, each of the chambers to be used will be checked to ensure that a uniform distribution of vapors occurs within the breathing zone.

Airflow through each chamber will be determined at hourly intervals using a differential pressure transducer (Model C264, Setra Systems, Inc., Acton, MA) or a Universal Venturi tube (Series 180, BIF, 345 Harris Ave., Providence, RI). The manometer and differential pressure transducer will be calibrated with a gas meter (Singer Aluminum Diaphragm Meter, Model AL-1400, American Meter Division, Philadelphia, PA) prior to the start of the study. The Universal Venturi tubes have been calibrated at the factory.

Chamber temperatures will be measured with a thermometer or resistance temperature device (RTD) and relative humidities will be measured with relative humidity gauges or humidity sensors (HMP 112A, Vaisala, Helsinki, Finland) at least once each hour. Calibration of the hygrometers, RTD's and humidity sensors will be documented in the study file. The temperature and relative humidity in each chamber will be controlled by a system designed to maintain temperature at approximately $22\pm 2^{\circ}\text{C}$ and relative humidity at approximately 40-60%.

Output from the differential pressure transducer, RTD, humidity sensor and infrared spectrophotometer will be collected by the CAMILE* Data Acquisition and Control System.

Exposure Concentrations.

* Trademark of The Dow Chemical Company

* Trademark of SAGIAN Indianapolis, Indiana.

Rats will be exposed to target concentrations of 0, X, Y or Z ppm of the test material. These dose levels correspond to an oral equivalent dose of approximately 0, X, Y and Z mg/kg/day assuming ventilation rates of 1 l/min/kg and 100 percent absorption. The chosen concentrations were selected by the sponsor and based upon the results of the inhalation toxicity studies previously discussed.

Experimental Design.

Groups of 30 male and 30 female rats will be exposed to 0, X, Y or Z ppm of the test material via inhalation, for 6 hours/day, 5 days/week prior to mating and 6 hours/day, 7 days/week during mating, gestation and lactation. The overall chronology of events for this study is depicted in Table 1. The treatment of the first generation parental (P1) rats will begin at approximately 6 weeks of age. After approximately 10 weeks of exposure (5 days/week, excluding holidays), P1 rats will be mated (one male to one female of the respective treatment group) to produce the F1 litters. Following weaning (3 weeks of age) of the F1 litters, 30 males and 30 females from each treatment group will be randomly selected and assigned to the respective treatment group to become the second generation parents (P2). After approximately 10 weeks of treatment following weaning of the last F1 litter, the P2 adults will be bred to produce the F2 litters. Exposures of P1 and P2 adult rats to the test material will continue until the adults are sent to necropsy. All rats will be housed continuously in exposure chambers following the initial exposure to the test material except during late gestation and throughout lactation periods, when female rats will be housed outside of the exposure chambers. Maternal rats will not be exposed to the test material after day 20 of gestation (as calculated from day 0 of gestation via sperm-positive vaginal lavage) through the fourth day postpartum, in order to allow for parturition and initiation of lactation. During the lactation period, pups will not be placed in the exposure chambers, but will remain in the nesting boxes separated from the dam for approximately 7 hours/day on lactation days 5 through 21.

Breeding Procedure

Breeding of the P1 and P2 adults will commence after approximately 10 weeks of treatment. Each female will be placed with a single male from the same dose level (1:1 mating) until pregnancy occurs or either three estrous cycles or two weeks has elapsed. During each breeding period, daily vaginal lavage samples will be evaluated for the presence of sperm as an indication of mating. The day on which sperm are detected or a vaginal plug is observed *in situ* will be considered day 0 of gestation. Sperm- and plug-positive females will then be separated and placed back into wire mesh, stainless steel cages. If mating has not occurred after two weeks, the animals will be separated without further opportunity for mating. For the P2 mating, cohabitation of male and female litter mates will be avoided.

Culling and Weaning

To reduce the variation in the growth of the pups, the F1 and F2 litters with a total number of pups exceeding eight will be culled on day 4 postpartum. Culled litters will be reduced to a total of eight pups, four males and four females, if possible. Pups to be culled will be selected using a computer generated randomization procedure. Litters with eight or fewer pups will not be culled. Preferential culling of runts will not be performed. Culled pups will be examined grossly for abnormalities and euthanized by the deposition of Beuthanasia-D Special (Schering Corporation, U.S.A., Kenilworth, NJ) into the oral cavity. Weaning of all litters will be done 21 days after delivery. Weanlings not held for prospective generations or selected for necropsy will be examined grossly for abnormalities and euthanized by CO₂ inhalation.

Physical Observations

Each rat on study will be observed twice daily (a.m. and p.m.) for mortality, morbidity and moribundity as well as availability of feed and water. In addition, changes in behavior or demeanor and indications of overt toxicity will be evaluated during the a.m. or p.m. observation. In addition, a thorough clinical examination will be conducted on all animals prior to the start of the study and weekly thereafter. This examination will include thorough evaluations of the skin and fur, mucous membranes, respiration, nervous system and behavior pattern. All adult rats found dead or in moribund condition will be submitted

for a gross pathologic examination. Adult rats found dead after normal working hours will be refrigerated until a necropsy can be performed. All pups found dead or pups that are euthanized in moribund condition will be examined to the extent possible for defects and/or cause of death and preserved in neutral, phosphate-buffered 10% formalin. Cannibalized pups will be examined to the extent possible and discarded.

Body Weights and Feed Consumption

All P1 animals will have body weights and feed consumption recorded weekly during the 10-week pre-breeding treatment period, beginning on or before the first week of the study. Body weights for males will be recorded weekly throughout the course of the study. Sperm and plug positive females will be weighed on Days 0, 7, 14 and 21 of gestation. Females that deliver litters will be weighed on Days 1, 4, 7, 14, and 21 of lactation. During breeding, feed consumption will not be measured in males or females due to cohousing. Following completion of the breeding periods, weekly feed consumption again will be measured in males. During gestation, feed consumption will be measured at weekly intervals in sperm and plug positive females. After parturition, feed consumption will be measured twice during the first and second week of lactation and at 2 - 3 day intervals during the last week of lactation. A similar schedule will be followed for the P2 generation.

Litter Data

All litters will be examined as soon as possible after delivery. The following parameters will be recorded for each litter: total litter size on the day of parturition (day 0), the number of live and dead pups on days 0, 1, 4, 7, 14, and 21 postpartum, and the sex and the weight of each pup on days 1, 4 (before and after culling), 7, 14, and 21 of lactation. Any visible physical abnormalities or demeanor changes in the neonates will be recorded during the lactation period.

Physical Maturation Landmarks

All F1 weanlings selected for mating will be observed daily for vaginal opening beginning on postnatal day 30 (Adams *et al.*, 1985) or preputial separation beginning on day 35 (Korenbrodt *et al.*, 1977). If there is a treatment-related effect

observed on the F1 sex ratio, age of vaginal opening or age of preputial separation, then anogenital distance will be measured on post natal day 4 for all F2 pups.

Estrous Cycling

Estrous cycle length and normality will be evaluated daily by vaginal lavage (Cooper et al., 1993) for all P1 and P2 females starting three weeks prior to mating and continuing throughout cohabitation.

Pathology - Adult Rats

A complete necropsy will be conducted by a team of trained individuals under the direct supervision of a veterinary pathologist on all P1 and P2 adults. The scheduled necropsy will be performed after the last litter of the respective generation has been weaned. Adult males will be fasted overnight, anesthetized with methoxyflurane and euthanatized. Adult females will be necropsied on day 2 of diestrus whenever possible. This will be accomplished by monitoring (by vaginal lavage) for the occurrence of at least one estrous cycle after which time females found to be in day one of diestrus will be fasted overnight and necropsied on the following morning. The expected, subsequent stage of the estrous cycle (day 2 of diestrus) will be confirmed by vaginal lavage on the day of necropsy, prior to euthanasia. Based upon the results of these smears, exclusion of appropriate data parameters used for statistics will be performed for females not found to be in the appropriate stage of the estrous cycle (day 2 of diestrus) on the day of necropsy. The fasted females will be euthanized as described for the males. The eyes of both males and females will be examined *in situ* by gently pressing a moistened glass slide against the cornea and observing the eyes under fluorescent light. The uteri of all cohabitated females will be examined for the presence and number of implantation sites. Tissues routinely collected (Table 2) will be saved from these rats and preserved in neutral, phosphate-buffered 10% formalin, with the following exceptions. The testes and epididymides will be preserved in Bouin's fixative. The lungs will be infused with formalin to their approximate normal inspiratory volume. The nasal cavity will be flushed with formalin via the pharyngeal duct to ensure rapid fixation of

the tissue. Moribund rats and those dying spontaneously will be necropsied in a similar manner. However, body and organ weights will not be recorded.

Organ Weights - Adult Rats

The following organs of all P1 and P2 parental animals will be weighed: uterus, ovaries, testes, single epididymis (total and cauda), seminal vesicles (with coagulating glands and their fluids), prostate, brain, liver, kidneys, lungs, adrenal glands, spleen, and thymus, and the organ-to-body weight ratios calculated.

Histology - Adult Rats

Histologic examination of potential target organs and reproductive tissues (Table 2) will be performed on the control and high dose groups. Examination of tissues from the low and middle groups will be limited to those tissues which demonstrate treatment-related histologic changes in the high dose group. Only the right ovary will be routinely processed for standard microscopic examination. The left ovary will be saved for possible oocyte quantification. If deemed necessary by the study sponsor, oocyte quantification will include evaluation of a minimum of ten sections, randomly selected from one completely sectioned ovary per female of the high-dose and control groups. Ovarian follicles will be placed into one of three categories as described by Plowchalk et al., (1993). The total number of follicles and the number of follicles in each of the three categories will be evaluated. Ovaries from the low and middle dose groups may be evaluated if treatment-related changes are observed in the high dose group. A complete set of tissues (excluding the left ovary for females), encompassing all organs listed in Table 2, will be prepared from all rats dying spontaneously or euthanized in a moribund condition and examined in an attempt to determine cause of death.

Sperm Count, Motility and Morphology

For all P1 and P2 males at termination, samples of sperm from the distal cauda epididymis or the proximal vas deferens will be collected for evaluation of percent progressively motile sperm and possible evaluation of sperm morphology. The entire right cauda epididymis will be weighed and then

minced in saline to enumerate the total number of sperm (cauda reserves). Sperm motility and count will be determined with the use of the Hamilton-Thorn (HTM) Integrated Visual Optical System (IVOS) motility analyzer (Hamilton-Thorn Research, Beverly, Massachusetts). All samples for motility analyses will be videorecorded and the recording kept as raw data. Sperm samples will be prepared for morphological evaluation and saved, but will not be evaluated unless deemed necessary by the study sponsor.

Pathology - Weanling Rats

At the time of weaning, 1 pup/sex/litter/dose from the F1 and F2 litters will be randomly selected for a complete necropsy by a team of trained individuals under the direct supervision of a veterinary pathologist. In order to control for variation in body and organ weight, all F1 and F2 pups selected for a complete necropsy will be euthanized at the same age. Pups will be anesthetized with methoxyflurane and euthanatized. Terminal body weights will be recorded. Gross pathologic examination and preservation of tissue samples (Table 2) will be performed as described above for adults.

Organ Weights - Weanling Rats

For all F1 and F2 pups that are examined macroscopically (one/sex/litter), the following organs will be weighed: ovaries, testes, brain, liver, kidneys, adrenal glands, spleen and thymus.

Histology - Weanling Rats

Organs that demonstrate treatment-related effects in weanlings will be examined microscopically in the control and high-dose groups. Examination of tissues from the low and middle groups will be limited to those tissues which demonstrate treatment-related histologic changes in the high dose group. Microscopic examination will also be made of all tissues showing gross pathologic changes.

Statistical Evaluation.

Descriptive statistics (means and standard deviations) will be reported for feed consumption. Body weights, gestation/lactation body weight gains, organ

weights, and sperm count per gram cauda epididymis and percent motile sperm will first be evaluated by Bartlett's test for equality of variances. Based upon the outcome of Bartlett's test, either a parametric or nonparametric analysis of variance (ANOVA) will be performed. If the ANOVA is significant, a Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction will be performed.

Gestation length, average time to mating, litter size, age at vaginal opening and age at preputial separation will be analyzed using a nonparametric ANOVA. If the ANOVA is significant, the Wilcoxon Rank-Sum test with Bonferroni's correction will be performed. Statistical outliers will be identified by the method of Grubbs (1969) and will be routinely excluded from analysis for feed consumption only. Outliers for other endpoints will only be excluded from analysis for documented, scientifically sound reasons. Fertility indices will be analyzed by the Fisher exact probability test and Bonferroni's correction will be used for multiple testing of groups in comparison to a single control. Evaluation of the neonatal sex ratio will be performed by the binomial distribution test. Survival indices and other incidence data among neonates will be analyzed using the litter as the experimental unit by the Wilcoxon test as modified by Haseman and Hoel (1974).

The nominal alpha levels to be used are as follows:

Bartlett's Test (Winer, 1971)	$\alpha=0.01$
Parametric ANOVA (Steel and Torrie, 1960)	$\alpha=0.10$
Nonparametric ANOVA (Hollander and Wolfe, 1973)	$\alpha=0.10$
Dunnett's Test (Winer, 1971)	$\alpha=0.05$, two-sided
Wilcoxon Rank-Sum Test (Hollander and Wolfe, 1973)	$\alpha=0.05$, two-sided with Bonferroni correction (Miller, 1966)
Fisher's Test (Siegel, 1956)	$\alpha=0.05$, two-sided
Censored Wilcoxon Test (Haseman and Hoel, 1974)	$\alpha=0.05$, two-sided
Outlier Test	$\alpha=0.02$, two-sided

(Grubbs, 1969)
Binomial Distribution Test $\alpha=0.05$, two-sided
(Steel and Torrie, 1960)

Because numerous measurements are statistically compared in the same group of animals, the overall false positive rate (Type I errors) will be much greater than the cited alpha levels would suggest. Thus, the final interpretation of numerical data will consider statistical analyses along with other factors such as dose-response relationships and whether the results are significant in the light of other biologic and pathologic findings.

Safety Precautions.

Standard safety precautions will be followed during the conduct of this study.

Quality Assurance.

Permanent records of all data generated during the course of this study, the protocol, any addenda to the protocol, and the final report will be available for inspection by .. All data generated including the protocol, addenda, and final report will be archived at

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TABLE 1
A TWO-GENERATION INHALATION REPRODUCTION STUDY
IN CD RATS

CHRONOLOGY OF EVENTS

WEEKS ON STUDY	P1	F1/P2	F2
1-10	Exposure of P1 males and females prior to first mating.		
11-12	P1 mating period for F1 litters.		
14-15		F1 born and litters culled on day 4 post-partum to 8 pups each.	
17-18		F1 litters weaned on day 21 post-partum; offspring selected for P2 adults; 1 F1 pup/sex/dose/litter selected for necropsy; remaining pups euthanized.	
19-28	Necropsy P1 adults.	Exposure of P2 males and females prior to first mating.	
29-30		Mating period of P2 for F2 litters.	
32-33		F2 born and litters culled on day 4 post-partum to 8 pups each.	
35-36		F2 litters weaned on day 21 post-partum; 1 F2 selected for necropsy; remaining pups euthanized.	
pup/sex/dose/litter			
37		Necropsy P2 adults.	

TABLE 2

Two-Generation Inhalation Reproduction Study in CD Rats

TISSUES COLLECTED AND PRESERVED AT NECROPSY

ADRENALS	KIDNEYS	PROSTATE
AORTA	LACRIMAL/HARDERIAN GLANDS	RECTUM
AUDITORY SEBACEOUS GLANDS	LARYNX	SALIVARY GLANDS
BONE (INCLUDING JOINT)	LIVER	SEMINAL VESICLES
BONE MARROW	LUNGS	SKELETAL MUSCLE
BRAIN (CEREBRUM, BRAINSTEM, CEREBELLUM)	MAMMARY GLAND	SKIN
CECUM	MEDIASTINAL LYMPH NODE	SPINAL CORD (CERVICAL, THORACIC,
LUMBAR)		
CERVIX*	MEDIASTINAL TISSUES	SPLEEN
COAGULATING GLANDS*	MESENTERIC LYMPH NODE	STOMACH
COLON	MESENTERIC TISSUES	TESTES
DUODENUM	NASAL TISSUES*	THYMUS
EPIDIDYMIDES*	ORAL TISSUES	THYROID GLAND
ESOPHAGUS	OVARIES	TONGUE
EYES	OVIDUCTS	TRACHEA
GROSS LESIONS*	PANCREAS	URINARY BLADDER
HEART	PARATHYROID GLANDS	UTERUS
ILEUM	PERIPHERAL NERVE	VAGINA
JEJUNUM	PITUITARY*	

*TISSUE SELECTED FOR HISTOPATHOLOGIC EVALUATION.