Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062
AREA CODE 312
TELEPHONE 272-3030

PROTOCOLS FOR

MONSANTO COMPANY

AROCLOR STUDIES

DSW 034238

STLCOPCB4018063

PROTOCOL FOR

MONSANTO COMPANY

A TISSUE RESIDUE STUDY IN ALBINO RATS

WITH AROCLORS

I. Outline of Experiment

A. Type and Length: Tissue Residue Study; duration 30 days

B. Animal Species Tested: Male Albino Rats (approximately 100 g)

C. Organization of Groups:

Group	Number of Animals Male	Female	Dietary Concentration in ppm
Control	15	15	None
I	15	15	Low dose level
II	15	15	High dose level

D. Test Material Administration

Diets will be prepared fresh weekly by mixing the calculated quantity of test material with a standard pulverized rat ration. Food will be allowed ad libitum.

II. Parameters to be Studied

A. Feed Consumption

Feed consumption will be determined weekly for each animal.

B. Reactions

Observations for untoward behavioral reactions will be made.

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C. Gross Pathology

Upon sacrifice, organs and tissues will be examined grossly for pathologic alterations.

III. Sample Collection

At the end of 30 days all animals will be sacrificed and samples of muscle, fat, liver and kidney will be taken from each animal. Samples of each tissue from each animal will be frozen and will either be shipped to Monsanto for analysis or analyses will be done by Bio-Test.

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ABSTRACT

Chronic Toxicity of Three Polychlorinated Biphenyls in Albino Rats. Fancher, Otis E., Keplinger, M. L., Plank, J. B., Wheeler, E. P., and Calandra, J. C. (0000). Toxicol. Appl. Pharmacol. 00, 00 - 00. Two year toxicity studies were conducted in albino rats with 3 polychlorinated biphenyls (Aroclors 1242, 1254 and 1260) at dietary levels of 1, 10 and 100 ppm. Each of the three materials at the 100 ppm level caused liver changes characterized by increased liver weights, increases in liver to body weight and liver to brain weight ratios, and by slight to mild centrilobular hypertrophy with fatty vacuolization. These changes were observed only in animals sacrificed after 24 months of treatment. No other effects were observed at 100 ppm except for a weight depression for female rats with Aroclor 1254 and no effects were observed with any material at 1 or 10 ppm.

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CHICKEN TOXICITY, REPRODUCTION AND RESIDUE STUDY

ON AROCLOR

The object of the study will be to investigate the general toxic effects of the test material on young adult male and female chickens with special emphasis upon hatchability of eggs and viability of offspring. In addition, samples of meat and eggs will be analyzed for test material residues (either by Monsanto or by Bio-Test).

Sixteen male and 80 female 12-week old white Leghorn chickens will be selected for the study. These animals will be equally divided into one control and three test groups. The three test groups will correspond to graded dietary levels of the test material. Dosing will begin immediately and will continue through the egg collection period.

The chickens in the control and test groups will be weighed weekly and observations for mortality, reactions and general food disappearance will be made periodically during the study.

As each group begins laying (at 17 to 18 weeks of age), the eggs will be collected daily and egg weights recorded. When the egg weights are consistently between 50 and 60 grams, and at least 20 per cent of the hens are laying, the daily egg production will be collected and placed in an incubator tray. Egg collection will be continued for 28 days from collection

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Day One or until at least 100 eggs per group are available for hatching.

During the storage period (not to exceed five days for any group of eggs), the eggs will be turned twice daily. The eggs will be placed in an incubator maintained at 99° to 100°F with a wet bulb reading of 85°F.

The egg production for an additional week will be collected for analysis. Total egg production and egg weights will be recorded.

At the end of the egg collection period, one-half of the chickens in each group will be sacrificed, defeathered and quick-frozen. The carcasses and the eggs retained for analysis will be shipped to Monsanto or will be retained for analysis at Bio-Test.

The remaining chickens will be placed on a 30-day recovery regimen during which time the test material will be removed from the test diets. Eggs will be collected once each week. At the end of this period, the remaining chickens will be sacrificed and handled in the same manner as those at the end of the test feeding period. The carcasses and eggs will again be retained for analysis.

The chicks from the first generation will be observed for 30 days post-hatching.

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DUCK TOXICITY AND RESIDUE STUDY

ON AROCLOR

The object of the study will be to investigate the general toxic effects of the test material on young adult male and female mallard ducks and to determine residue levels in specific organs and whole carcass.

Twenty male and twenty female young adult mallard ducks will be selected for the study. These animals will be equally divided into one control and three test groups. The three test groups will correspond to graded dietary levels of the test material.

The ducks in the control and test groups will be weighed weekly and observations for mortality, reactions and general food disappearance will be made periodically during the study.

At the end of a 30-day feeding period, one-half of the animals in each group will be sacrificed, defeathered and subjected to a gross pathological examination. The liver, kidneys and samples of skeletal muscle and fat will be removed from each bird and packaged separately. These tissues will be quick-frozen and retained for analysis for the test material

The remaining ducks will be placed on a 30-day recovery regimen during which time the test material will be removed from the test diets.

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At the end of this period, the remaining ducks will be sacrificed and handled in the same manner as those at the end of the test feeding period.

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PROTOCOL FOR

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THREE-GENERATION REPRODUCTION STUDY
OF
AROCLOR

IN ALBINO RATS

I. General

A three-generation reproduction study will be conducted with Sprague-Dawley derived albino rats. A total of 96 weanlings (32 males and 64 females) will be equally subdivided into three test groups and one control group. The three test groups will correspond to the feeding of three graded dietary levels of the test material.

II. First Generation

A. Parental Animals

1. Diets and Feeding

All diets will be prepared in the central diet room of the laboratories. The basic ration with which all diets are prepared is a standard, pulverized rat stock diet*. The diets for any given test group will be prepared by adding a calculated weight of the test material to a pre-weighed portion of the stock diet.

The amount of food allotted per week to each rat is to be sufficient for ad libitum feeding. However, checks will be made daily to ensure that the food jars are not empty.

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* Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri.

2. Body Weights and Weight Gains

The body weight of each rat in every group will be determined and recorded initially, and weekly thereafter until the animals are 100 days old.

3. Mortality, Reactions and General Observations

Mortality and abnormal behavioral reactions will be recorded daily. The animals also will be observed for fertility, length of gestation and lactation performance.

4. Mating Procedure

When the animals reach 100 days of age, each male will be randomly mated with two females from the same group. After successful copulation, determined by the presence of a copulation plug or blood in the vagina, the male will be removed from the females and returned to his original cage. Any male failing to copulate within two estrus cycles of the female will be replaced by another male of the same group. However, no more than two males will be used per female during a given breeding cycle. Records will be kept of the number of successful copulations observed, the number of estrus cycles required to obtain a mating, and the number of resulting pregnancies. These data will be utilized to calculate mating and fertility indices.

The F1a litters obtained will be weaned at 21 days postpartum. The females will then be given a 10-day rest period and again mated, the above procedure being repeated to obtain F1b weanlings.

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5. Pathologic Studies

After the second litter has been weaned (following approximately 33 weeks on test), 8 male and 8 female animals from each group will be sacrificed and gross pathologic observations will be made. Organ weights will be taken on the liver, kidneys, spleen, gonads, heart, brain and any organs which appear abnormal. A complete set of tissues will be removed and fixed in a ten per cent formalin solution.

In addition, microscopic examinations will be conducted upon five males and five females from both the control and the high test groups. If abnormalities are noted, the affected organs of the lower test group animals will also be examined.

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The following tissues and organs will be examined:

Tissues and Organs Examined Grossly	Tissues and Organs Examined Microscopically
Heart	Heart (right and left ventricles)
Trachea	Trachea
Lungs	Lungs
Liver	Liver
Pancreas	Pancreas
Esophagus	Esophagus
Stomach	Stomach (cardiac, fundic and pyloric regions)
Intestinal tract	Small intestine (duodenum, jejunum and ileum)
Spleen	Caecum
Lymph nodes	Colon
Kidneys	Spleen
Urinary bladder	Lymph node (cervical and mesenteric)
Testes	Kidney
Ovaries	Urinary bladder
Prostate	Testis
Seminal vesicles	Ovary
Uterus	Prostate
Pituitary gland	Seminal vesicles
Adrenal glands	Uterus
Salivary glands	Pituitary gland
Thyroid glands	Adrenal gland
Skeletal muscle	Salivary gland (submaxillary)
Bone	Thyroid gland
Peripheral nerve	Parathyroid gland
Eyes	Bone marrow (sternum and femur)
Brain	Peripheral nerve (sciatic)
Thymus	Brain (cerebrum, cerebellum and pons)
Optic nerves	Spinal cord (three levels)
Aorta	
Spinal cord	

Post-mortem animals will be examined in the same manner but organ weights will not be recorded.

Throughout gross and microscopic examination, particular attention will be paid to the reproductive organs.

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B. Progeny

1. Body Weight Data

Pups will be weighed at weaning.

2. Mortality, Reactions and General Observations

All pups will be examined for physical abnormalities at birth and the number of viable and stillborn members of each litter recorded. Records of survival at periodic intervals during the lactation period will be maintained and a final examination for physical abnormalities will be made at the weaning of each litter.

3. Survival Data

Survival indices will be calculated for various points during the lactation period. On the fifth day of lactation, litters of greater than 10 pups will be reduced to that number.

4. Pathologic Studies

A gross internal pathologic examination will be made upon any pup appearing abnormal. No such examinations will be made upon progeny which appear normal.

III. Second and Third Generations

The procedures followed for the second and third generations will be identical to those described for the first generation except that the parental animals for the F2 generation in each group will be selected from F1b weanlings of that group. Parents for the F3 generation will be selected from F2b litters.

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In addition, ten male and ten female pups from the F3b litters of each test group and the control group will be sacrificed at weaning (21 days post-partum) and subjected to complete gross and microscopic examination.

IV. Reports

A complete formal report will be submitted upon completion of each generation.

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hematopoietic and urinary systems. Special emphasis was placed on observations for skin irritation produced by the test material.

Following the 20-day application period, the rabbits were kept under observation until an additional 14 days had elapsed. At the end of this period, final mortality tabulations were made and all surviving rabbits from each test and control group were sacrificed for pathologic studies. Gross autopsies were performed and a complete set of representative tissues and organs was taken from each rabbit and preserved in formalin. Microscopic examinations of selected tissues and organs were made to ascertain the presence or absence of histopathologic change as a result of dermal applications of the test material at the dose levels employed.

The dosage schedule and other pertinent data are presented in Table I.

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PROTOCOL FOR

MONSANTO COMPANY

TWO-YEAR CHRONIC ORAL TOXICITY
OF
AROCLOR

IN ALBINO RATS

I. Outline of Investigation

A. Type and Length: Two-Year Chronic Oral Toxicity

B. Animal Species Tested: Charles River Strain Albino Rats

C. Organization:

Group	Number of Animals Male	Female	Dietary Level of Test Material (ppm)
Control	30	30	None Administered
Test I	30	30	Low Level
Test II	30	30	Middle Level
Test III	30	30	High Level

D. Means of Administration: Voluntary oral ingestion; ad libitum feeding of diets containing various levels of test material.

II. Parameters To Be Investigated DSW 034251

A. Body Weights

All animals will be weighed weekly during the first three months of the study and monthly for the remainder of the two-year test period.

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B. Food Consumption

Weekly food consumption will be recorded for the first three months of the investigation. Thereafter, monthly spot checks will be made.

C. Mortality and Reactions

Checks for mortality and untoward behavioral reactions will be conducted daily.

D. Hematologic Studies, Clinical Blood Chemistry Studies and Urine Analyses

The studies listed below will be conducted on five rats of each sex from the Control Group and Test Group III at the beginning of the study and after 3, 6, 9, 12, 18 and 24 months of feeding:

1. Hematology: Hematocrit value, hemoglobin concentration, erythrocyte count and total and differential leukocyte counts.
2. Clinical Blood Chemistry: Blood urea nitrogen concentration (BUN), serum alkaline phosphatase activity (SAP), blood glucose concentration and serum glutamic-pyruvic transaminase activity (SGPT).
3. Urine Analyses: Albumin concentration, glucose concentration, microscopic elements examination, pH determination and specific gravity.

The above listed studies will be conducted on animals in the lower dose groups if significant findings appear among high dose animals.

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E. Pathologic Studies

1. Gross

Complete gross autopsies will be conducted upon all post-mortem animals unless precluded by post-mortem autolysis, all animals sacrificed in extremis, five males and five females from each group sacrificed after three months of testing and upon all animals surviving 24 months of testing. At the time of each autopsy, representative tissues and organs will be taken and fixed in 10 per cent formalin solution.

Absolute organ weights will be recorded and organ - body weight ratios and organ - brain weight ratios will be calculated and subjected to statistical analysis, viz. Analysis of Variance and/or "t"-tests. The following organs will be included: liver, kidneys, spleen, gonads, heart, brain and any other organ appearing abnormal upon gross examination.

2. Microscopic

Microscopic examinations will be conducted upon tissues and organs taken from selected animals sacrificed in extremis, and five males and five females from the control and highest dietary dose groups sacrificed after three months and 24 months of testing. The tissues and organs to be examined will include: heart, liver, lung, pancreas, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), caecum, colon, spleen, lymph node, kidney, urinary bladder, testes, ovary, prostate, uterus, pituitary gland, adrenal gland, salivary

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gland (submaxillary), thyroid gland, parathyroid gland, skeletal muscle, bone marrow, peripheral nerve, trachea, spinal cord, eye, optic nerve and brain (cerebrum, cerebellum and pons).

Any significant tissue or organ changes at the high dose level would also be examined at the lower levels.

F. Reports

Interim status reports will be submitted after 3, 12 and 18 months of testing. These reports will contain pertinent data collected to that date.

At the conclusion of the study, three copies of a final formal report will be submitted.

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PROTOCOL FOR

MONSANTO COMPANY

TWO-YEAR CHRONIC ORAL TOXICITY
OF
AROCLOR

IN BEAGLE DOGS

I. Outline of Experiment

A. Type and Length: Two-Year Chronic Oral Toxicity

B. Animal Species Tested: Pure-Bred Beagle Dogs

C. Test Material: Aroclor

D. Organization: Three Test Groups and One Control Group

1. Number of Dogs/Group: Eight (four males and four females)

2. Dose Levels: Control Group - None
Test Group I - Low level
Test Group II - Middle level
Test Group III - High level

3. Means of Administration: Voluntary oral ingestion; ad libitum feeding of test material in diet.

II. Parameters To Be Investigated

A. Body Weights

All animals will be weighed weekly.

B. Food Consumption

Weekly food consumption will be recorded.

C. Mortality and Reactions

Mortality and untoward behavioral reactions will be recorded daily.

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D. Hematologic Studies, Blood Chemistry Studies and Urine Analyses

The studies listed below will be conducted upon all dogs prior to the inception of the test, after 1, 3, 6, 12 and 18 months of testing and just prior to its conclusion.

1. Hematology: Hematocrit, hemoglobin, erythrocyte count, and both total and differential leukocyte counts.
2. Blood Chemistry: Blood urea nitrogen, blood glucose, serum alkaline phosphatase, serum glutamic-pyruvic transaminase and serum glutamic-oxalacetic transaminase.
3. Urine Analyses: Albumin, glucose, pH, microscopic elements and specific gravity.

E. Pathologic Studies

1. Gross Pathologic Studies

Complete gross autopsies will be conducted upon animals sacrificed in extremis and upon all post-mortem animals during the investigation. At the conclusion of the 24-month study, all surviving dogs will be sacrificed and examined. At the time of each sacrifice, a complete set of tissues will be removed from each dog and preserved in ten per cent formalin solution for histopathologic examination. Also at the time of each sacrifice, the weight of the liver, kidneys, spleen, gonads, thyroid, adrenals, pituitary and heart will be recorded.

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2. Histopathologic Studies

Histopathologic examinations will be conducted on tissues taken from animals sacrificed in extremis, from post-mortem animals

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and from all animals sacrificed after 24 months of feeding. The tissues to be included in the examinations are as follows: lung, heart, liver, pancreas, mesenteric lymph node, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), colon, caecum, kidney, adrenal gland, urinary bladder, testes, ovaries, prostate, thyroid gland, submaxillary salivary gland, parathyroid gland, uterus, brain (cerebrum, cerebellum and pons), bone and bone marrow (femur), and skeletal muscle (gastrocnemius).

F. Reports

Interim status reports will be submitted after 3, 12 and 18 months of testing. These reports will contain pertinent data collected to that date.

At the conclusion of the study, three copies of a final formal report will be submitted.

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