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REPORT TO

MONSANTO CHEMICAL COMPANY

SUBACUTE DERMAL TOXICITY OF

AROCLOR 4465



REPORT TO

MONSANTO CHEMICAL COMPANY

SUBACUTE DERMAL TOXICITY OF

AROCLOR 4465

I. Introduction and Outline of Procedure

A sample identified as Aroclor 4465 was received from Monsanto Chemical Company for evaluation of 20-day subacute dermal toxicity in albino rabbits.

Skin applications of the test material, in the form of a corn oil solution, were made daily to three test groups consisting of two rabbits each (one male and one female) for a period of 20 days. These test groups corresponded to daily dermal doses of 75, 150, and 300 mg/kg/day respectively.

In addition to the three test groups, a treated control group of two rabbits was also employed. The animals in this group received daily dermal applications of corn oil comparable to the volume administered to the highest test dose group.

The rabbits in all groups were housed individually over the period of investigation and observations were made with respect to body weight effects, incidence of mortality, reactions displayed and status of the

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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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— Monsanto Chemical Company

TABLE I

20-Day Subacute Dermal Toxicity - Albino Rabbits

Dosage Schedule

Group	Number of Animals	Dose * (mg/kg/day)	Number of Scheduled Consecutive Applications
T-C	2	**	20
T-I	2	75	20
T-II	2	150	20
T-III	2	300	20

* Administered as a 4.0 per cent solution (w/v) in corn oil.

** The treated control rabbits received doses of corn oil comparable in volume to that applied to the rabbits of the high dose group.

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II. Investigational Procedure

A. Experimental Animals

The animals employed in the 20-day subacute dermal toxicity study were adult, New Zealand strain albino rabbits. The body weights of the test animals were in the two-to-three kilogram range. A total of 8 adult rabbits were selected from a larger population after examination of each animal for general physical well-being. Each animal selected for the experiment was housed individually in a wire-bottomed stainless steel rabbit cage for the duration of the test. All rabbits were fed a standard laboratory rabbit ration* plus water ad libitum.

B. Method of Application

In preparation for the test, the back of each rabbit was shaved using electric clippers. The exposure site thus prepared on each animal constituted approximately ten per cent of the total body surface area. The animals were then returned to their stock cages, and 24 hours were allowed to elapse before the first percutaneous applications were made. This period permitted the skin to recover from the slight disturbance of the stratum corneum caused by the clipping procedure and also permitted the healing of any microscopic abrasions possibly produced during the shaving procedure.

At the end of the 24-hour recovery period, the calculated doses of the test material in the form of a 4.0 per cent (w/v) corn oil

* Rockland Rabbit Ration, Rockland Farms, New City, New York.

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solution were gently distributed (without rubbing) over the entire application sites on the test rabbits.

The animals in the treated control group were treated in the same manner as those in the test group with the exception that corn oil alone was applied to the skin.

Following the dermal applications, the animals were returned to their individual cages to await testing on the following day.

The above schedule was followed every day, including weekends, for a total of 20 consecutive days.

C. Body Weight Effects

On the morning of the first test day, the body weight of each rabbit in every group was determined and recorded. Thereafter, individual weighings were made weekly and the data recorded as an index to body weight effects. Dose calculations were made weekly to adjust for changes in body weight.

D. Mortality and Reactions

Checks for mortality and abnormal behavioral reactions were made daily during the 20-day test period as well as during the 14-day observation period. Special emphasis was placed on observations for local skin reactions in order to determine the degree of skin irritation produced by the test material.

E. Hematologic Studies and Urine Analyses

Blood studies including determinations of hemoglobin concentration, erythrocyte count, and both total and differential leukocyte

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counts, were made at the beginning of the study and at the end of the 20-day test period. Urine analyses for reducing substances and microscopic elements were also done at these time intervals. The blood studies and urine analyses were done on the individual rabbits in each group.

F. Gross and Microscopic Pathologic Studies

Arrangements were made to subject any animal which might die during the test to a gross autopsy. Also, in those instances where post-mortem changes were not advanced, sections of representative tissues and organs were scheduled to be taken for histopathologic study.

At the conclusion of the investigational period, all surviving rabbits in each group were sacrificed and gross and microscopic studies were performed. The following tissues and organs were included in the microscopic studies: heart, aorta, lung, trachea, liver, gall bladder, pancreas, esophagus, stomach, small intestine, caecum, colon, spleen, cervical and mesenteric lymph nodes, kidney, urinary bladder, testis, ovary, prostate, uterus, pituitary, adrenal gland, submaxillary salivary gland, thyroid, parathyroid, skeletal muscle, bone marrow, peripheral nerve, spinal cord, brain, and skin.

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III. Results

A. Body Weight Data

Weekly individual body weights for each test group and the control group are assembled in Table II.

TABLE II

Body Weight Data

Group	Animal Number and Sex	Individual Body Weights (Kilograms) Test Day Number: 0	7	14	20	Observation Day Number 14
T-C	1-M	2.31	2.35	2.49	2.57	2.74
	2-F	2.50	2.63	2.68	2.80	2.96
T-I	1-M	2.13	2.13	2.30	2.40	2.54
	2-F	2.52	2.55	2.58	2.73	2.91
T-II	1-M	2.04	1.97	1.98	1.98	1.87
	2-F	2.72	2.55	2.55	2.10	(2.04)
T-III	1-M	2.38	2.10	(1.87)	-	-
	2-F	2.78	2.55	1.96	(1.87)	-

Note: Figures in parentheses represent autopsy weights.

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B. Mortality

The mortality data are summarized in Table III. The data indicate the number dead and the total number of doses received prior to death. The female of Group II died on the third day of the 14-day observation period.

The mortality data were statistically analyzed employing the technique of Litchfield and Wilcoxon*, and the mean subacute percutaneous lethal dose (LD_{50}) was calculated together with the $LD_{0.01}$ and the $LD_{99.99}$. For the purpose of these calculations the test period was assumed to be 34 days, i.e. a 20-day application period plus a two-week observation period. All animals that died during the interval, at each dose level, were used in establishing the per cent mortality for the dose level. The data are presented in Table IV and are graphically illustrated in Figure 1.

* J. T. Litchfield, Jr. and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments", J. Pharm. & Exp. Ther., Vol. 96, No. 2; pp. 99-113 (1949).

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TABLE III

Summary of Mortality Data

Group	Number Tested	Number Dead	Sex	Number of Consecutive Doses Received Prior to Death
T-C	2	0	-	-
T-I	2	0	-	-
T-II	2	1	F	20
T-III	2	1	M	11
		1	F	19

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TABLE IV

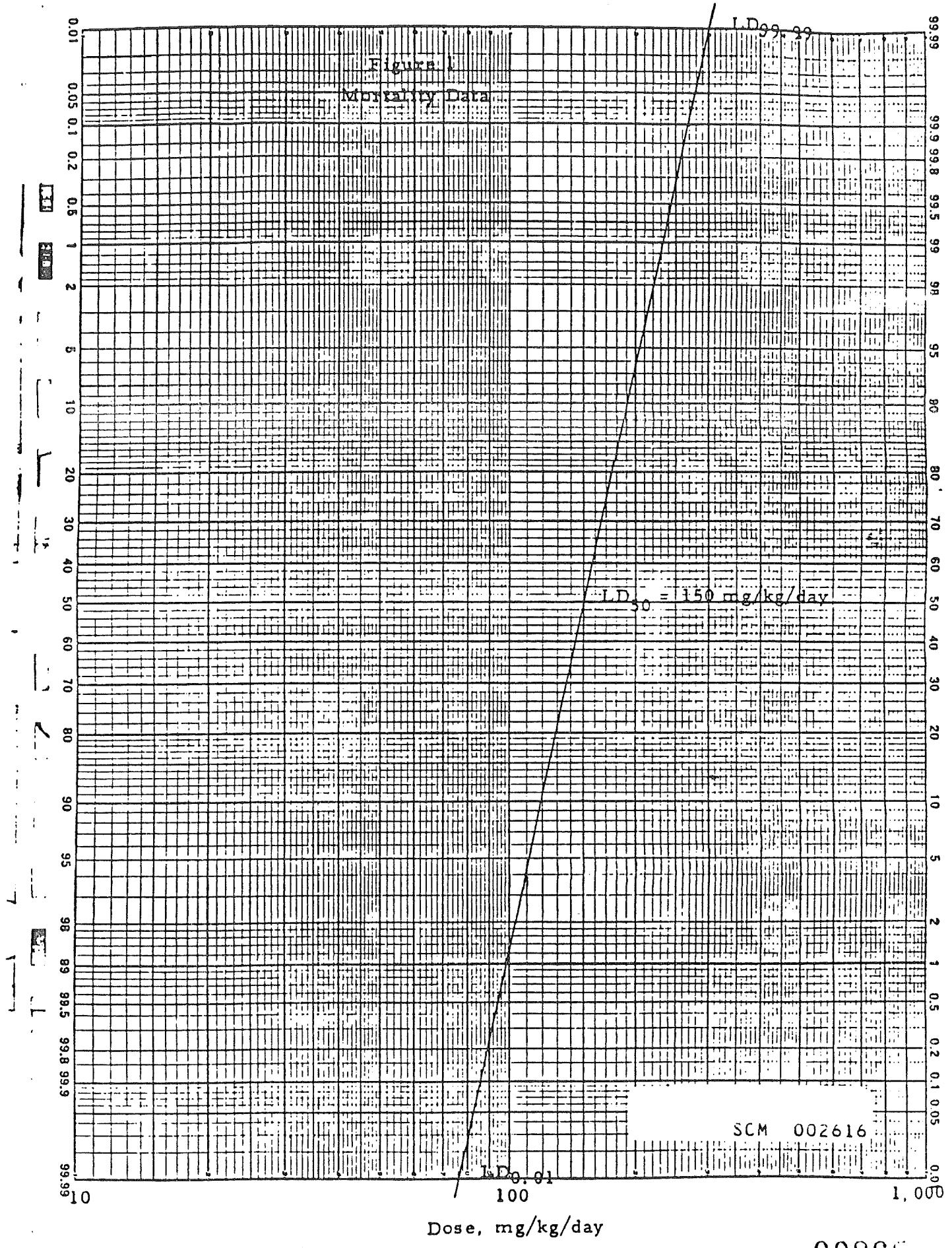
Statistical Analysis of Mortality Data

Dose mg/kg/day	Number Dead	Number Tested	Observed Per Cent Dead	Expected Per Cent Dead
75	0	2	0	0
150	1	2	50	50
300	2	2	100	100

Mean Subacute Dermal Lethal Dose (LD_{50}) = 150 mg/kg/day*
95 Per Cent Confidence Limits of LD_{50} = 105 to 214 mg/kg/day
Subacute Dermal $LD_{0.01}$ = 76 mg/kg/day
Subacute Dermal $LD_{99.99}$ = 300 mg/kg/day

* The LD_{50} specifically refers to the dose which, administered each day over a 20-day period, would be expected to kill 50 per cent of the animals so dosed within the 20-day dosing period plus an additional 14 days during which the test material is not applied.

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C. Reactions

1. Behavioral Reactions

No untoward behavioral reactions were noted among the animals receiving doses of 75 mg/kg/day.

Generalized inactivity, weakness, lassitude, and loss of appetite were exhibited by rabbits receiving doses of 150 and 300 mg/kg/day respectively. These symptoms progressed in intensity and eventually ended in profound prostration and death in all but one rabbit in these dose groups. The surviving male in the 150 mg/kg/day dose group continued to show the above reactions during the 14-day observation period, but to a lesser degree than during the dosing period.

2. Local Skin Reactions

The skin at the application site of animals in all test groups became dry after the tenth application of the test material and remained in this condition throughout the test period. During the 14-day observation period, desquamation occurred and by the end of this period the skin of surviving animals appeared normal.

D. Hematologic Studies and Urine Analyses

Values for hematologic data and the results of urine analyses obtained on surviving test animals at the completion of the dosing period did not differ significantly either from those recorded initially or from the corresponding data secured from the treated control animals.

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E. Pathology

1. Gross Pathologic Findings

Complete necropsies were performed upon all animals that died during the test and upon all animals sacrificed at the end of the observation period. No gross pathologic findings were noted among rabbits receiving doses of 75 and 150 mg/kg/day respectively. Rabbits in the 300 mg/kg/day dose group showed liver discoloration (lighter in color) but otherwise no hepatic alterations were seen upon gross examination. All other tissues and organs examined from these animals were comparable to those of treated control animals.

2. Microscopic Pathologic Findings

Microscopic examinations were conducted upon the tissues and organs taken from all sacrificed animals. Animals that died during the test were examined grossly before advanced post-mortem changes had taken place and, consequently, tissue and organ specimens could be and were examined microscopically from these rabbits as well.

No significant histopathologic alterations were noted in any of the tissues and organs of rabbits receiving doses of 75 mg/kg/day.

Animals in the 150 mg/kg/day dose group exhibited mild to moderate hepatic necrosis characterized by multiple areas of individual or groups of hepatic cell degeneration with associated mononuclear infiltration. Vacuolization of liver cells, indicative of incipient liver

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necrosis, was noted among rabbits receiving doses of 300 mg/kg/day. These findings were considered to be directly related to the percutaneous absorption of the test material. All other tissues and organs examined from these animals were comparable to those of treated control animals.

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IV. Summary

A. Body Weight Data

Examination of the body weight data revealed a moderate to severe adverse effect among animals receiving doses of 150 and 300 mg/kg /day respectively. The body weight data of animals in the 75 mg/kg/day dose group compared well with those of the treated control group.

B. Mortality and Reactions

The 20-day subacute dermal mean lethal dose (LD_{50}) of Aroclor 4465 for the albino rabbit was found to be 150 mg/kg/day.

Generalized inactivity, weakness, lassitude, and loss of appetite were exhibited by rabbits receiving doses of 150 and 300 mg/kg/day respectively. No untoward reactions were noted among animals in the 75 mg /kg/day dose group.

The skin at the application site of animals in all test groups became dry during the application period. Desquamation occurred during the 14-day observation period and by the end of this period the skin of surviving animals appeared normal.

C. Hematologic Studies and Urine Analyses

Hematologic data and results of the urine analyses for surviving test group animals compared well with those of the treated control group animals.

D. Pathology

1. Gross Pathologic Findings

No significant gross pathologic findings were noted among

animals in the 75 and 150 mg/kg/day dose groups. Rabbits receiving doses of 300 mg/kg/day revealed liver and discoloration (lighter in color). All other tissues and organs examined from these animals were comparable to those of treated control animals.

2. Microscopic Pathologic Findings

No significant histopathologic tissue alterations were noted among animals receiving doses of 75 mg/kg/day.

Mild to moderate hepatic necrosis, characterized by multiple areas of individual or groups of hepatic cell degeneration with associated mononuclear infiltration, was noted among rabbits in the 150 mg/kg /day dose group. Animals in the 300 mg/kg/day dose group exhibited vacuolization of liver cells indicative of incipient liver necrosis. These findings were considered to be directly related to the percutaneous absorption of the test material. All other tissues and organs examined from these animals were comparable to those seen in the treated control animals.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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