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BIOASSAY OF A MIXTURE OF 1, 2, 3, 6, 7, 8-HEXACHLORODIBENZO-p-DIOXIN AND 1, 2, 3, 7, 8, 9-HEXACHLORODIBENZO-p-DIOXIN (Dermal Study) FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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BIOASSAY OF

1,2,3,6,7,8 and 1,2,3,7,8,9-

HEXACHLORODIBENZO-p-DIOXINS

FOR POSSIBLE CARCINOGENICITY

(Dermal Study)

Carcinogenesis Testing Program
National Cancer Institute
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FOREWORD

This report presents the results of the bioassay of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI)/ National Toxicology Program (NTP). This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS

This bioassay was conducted at the Illinois Institute of Technology Research Institute (IITRI), Chicago, Illinois, initially under direct contract to NCI and later under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The project director was Mr. A. Shefner (1); Dr. M. E. King (1) was the principal investigator for this study; and Dr. P. Holmes (1,2) assembled the data. Doses of the test chemical were selected by Dr. O. G. Fitzhugh (3, 4). Mr. T. Kruckeberg (1) and Mr. K. Kaltenborn (1) were in charge of animal care. Histopathologic evaluations were performed by Dr. J. H. Rust (1). The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978).

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. S. Vatsan (4) using methods selected for the bioassay program by Dr. J. J. Gart (6). Chemicals used in this bioassay were synthesized and analyzed under the direction of Dr. A. Gray (1), with the assistance of Mr. S. Cepa (1) and Mr. V. DaPinto (1). Further chemical analyses were conducted at Midwest Research Institute (7). The results of the chemical analytical work were reviewed by Dr. S. S. Olin (4).

This report was prepared at Tracor Jitco (4) under the direction of Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. R. L. Schueler, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI (8) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Jr., Dr. Michael P. Dieter, Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. C. W. Jameson, Dr. Y. Jack Lee, Dr. Harry Mahar, Dr. James McCoy, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins (HCDD) for possible carcinogenicity was conducted by dermal application of a suspension of this substance to Swiss-Webster mice.

HCDD (0.01 μg) suspended in 0.1 ml acetone was applied to the backs of 30 mice of each sex 3 days per week for 104 weeks. During the first 16 weeks, doses were 0.005μg HCDD per application. An additional 30 mice of each sex were pretreated with one application of 50 μg DMBA in 0.1 ml acetone 1 week before the initiation of the HCDD applications. As vehicle controls, 45 mice of each sex received 0.1 ml of acetone three times per week. Thirty animals of each sex served as untreated controls. Mean body weights of all test and vehicle control mice were comparable throughout the bioassay; mean body weights of untreated controls were higher than those of the test and vehicle-control groups.

In male mice, the incidence of alveolar/bronchiolar carcinomas in the group administered only HCDD was significantly higher (P=0.045) than that in the vehicle-control group; however, the incidence was not significantly higher when compared with untreated controls.

In male mice, the incidence of lymphomas or leukemias was significantly lower (P=0.011) in the group administered only HCDD when compared with the untreated controls.

In female mice, the incidences of fibrosarcomas of the skin were significantly higher (P=0.044) in animals administered HCDD (both with and without pretreatment with DMBA) than in the untreated-control group; however, when the incidences were compared with those of the vehicle controls (relative risk=3.037) the results were not significant.

Under the conditions of this bioassay, HCDD was not carcinogenic for male or female Swiss-Webster mice.

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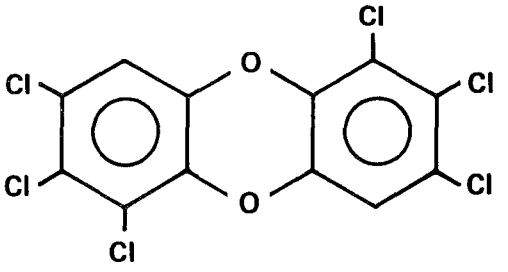
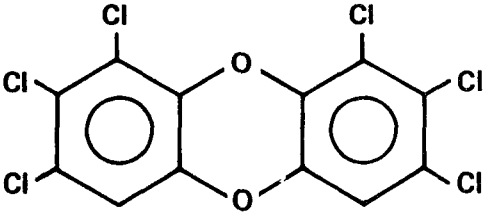


1, 2, 3, 6, 7, 8-HCDD
CAS 57653-85-7



1, 2, 3, 7, 8, 9-HCDD
CAS 19408-74-3

Hexachlorodibenzo-p-dioxin (HCDD) (NCI C03703) is formed as a byproduct during the manufacture of certain chlorophenols and has been found in trichlorophenol, tetrachlorophenol, pentachlorophenol and in the chlorophenol-derived herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Woolson et al., 1972; Firestone et al, 1972). From 1967 to 1970, the concentration of HCDD in commercial pentachlorophenol ranged from 0.03 to 38 ppm (Firestone et al., 1972). Since then, HCDD levels in pentachlorophenol have been reduced to less than 1 ppm (Blaser et al., 1976).

HCDD was first identified in 1967 and was called the "chick edema factor," following 10 years of research into the cause of a buildup of fluid in the pericardial sac and abdominal cavity, or at subcutaneous sites, which killed millions of broilers in the eastern and midwestern United States (Firestone, 1973). Liver damage was also seen in the poisoned birds. The chick embryo later became the animal test system used to detect HCDD or other dioxins in commercial fatty acids (Firestone, 1978). Using x-ray crystallography, Cantrell (1969) identified 1,2,3,7,8,9-hexachloro-p-dibenzodioxin as one of the toxic components in fats used in animal feeds and related this substance specifically to the cause of chick edema. The presence of HCDD in animal feeds was traced to impurities in the pentachlorophenol used in the preservation of animal hides from which fats containing HCDD residues were processed and used in animal feeds (Firestone, 1972).

Schwetz et al. (1973) found that a single oral dose of 100 μg/kg HCDD was lethal to male Sprague-Dawley rats. Severe weight losses and gross evidence of liver damage were observed in pregnant Sprague-Dawley rats administered oral doses of 100 μg/kg day for 10 consecutive days. Doses of 10 or 100 μg/kg/day of HCDD were fetotoxic, and a single dose of 100 μg/kg was teratogenic.

Although much has been published about the structurally related 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD), the literature on HCDD is very limited and not all references specify which isomer was used. Biological effects of HCDD appear to parallel, qualitatively, the biological effects of TCDD. Toxicity appears to be partly correlated with the degree of chlorination at the 2,3,7, or 8 position (McConnell and Moore, 1976). Although less potent than TCDD, HCDD is active in the rabbit ear bioassay for acnegenic activity and in the chick edema bioassay. Studies of aryl hydrocarbon hydroxylase induction showed that TCDD was the most potent, followed by 1,2,3,4,7,8-HCDD, 1,2,3,7,8,9-HCDD, and 1,2,3,6,7,8-HCDD. 1,2,4,5,7,9-HCDD had no effect (Bradlaw, 1975). 1,2,3,7,8,9-HCDD was 20% as effective as TCDD in inducing aryl hydrocarbon hydroxylase (Poland et al., 1976). The isomers of HCDD used in the present study (1,2,3,6,7,8 and 1,2,3,7,8,9) are both chlorinated at the four lateral ring positions considered important for biological activity (McConnell and Moore, 1976).

In the early 1970's, HCDD was one in a series of the chlorodibenzo-p-dioxins selected for testing by the Carcinogenesis Testing Program after TCDD, a contaminant in 2,4,5-T, was found to be a potent teratogen (Courtney et al., 1970; Sparschu et al., 1971). Results of preliminary toxicologic analyses indicated that the dioxins were some of the most toxic substances known. Long-term animal bioassays were initiated for all of the dioxins that were released into the environment by the use of the herbicides and microbicides they contaminated. A companion study of HCDD administered by gavage (NCI, 1980) was conducted concurrently with this HCDD skin paint study.

II. MATERIALS AND METHODS

A. Chemical

HCDD (Lot No. IIT 102) was synthesized at the Chemistry Division of IIT Research Institute (IITRI), Chicago, Illinois (Appendix E). The white crystalline solid was approximately 98.6% hexachlorodibenzo-p-dioxin, consisting of a 1:2 mixture of the 1,2,3,6,7,8-(CAS 57653-85-7) and the 1,2,3,7,8,9-(CAS 19408-74-3) isomers (31% and 67% of the total HCDD, respectively).

After separation and purification, the isomers were identified by comparing x-ray powder patterns with theoretical calculations and with the reported x-ray data for the 1,2,3,7,8,9-isomer (Cantrell et al., 1969), as well as by comparing melting point, gas-liquid chromatography, proton magnetic resonance, and mass spectrometry (Gray et al., 1975). The mixture of the two HCDD isomers used in the present study was similar to the HCDD synthesized by an alternate route (Kende and DeCamp, 1975).

The following impurities were identified by vapor-phase chromatography and mass spectrometry: bromopentachlorodibenzo-p-dioxin, less than 0.004%; dichlorodibenzo-p-dioxin, 0.004%; trichlorodibenzo-p-dioxin, 0.004%; tetrachlorodibenzo-p-dioxin, 0.07-0.09%; and pentachlorodibenzo-p-dioxin (at least two isomers), 0.4%. No octachlorodibenzo-p-dioxin was found in HCDD by either vapor-phase chromatography or mass spectrometry (Appendix F). The chemical mixture will be referred to as HCDD.

The HCDD was stored in brown glass vials at room temperature in a dark glove-box hood and was exposed to light only at 3-month intervals, when samples were removed for preparation of stock suspensions in acetone.

The purity of the 7,12-dimethylbenz(a)anthracene (DMBA) purchased from K & K Laboratories (Cleveland, Ohio) was not determined.

B. Dosage Preparation

Fresh stock suspensions of 2.5 μg/ml HCDD in acetone (Mallinkodt, Inc., St. Louis, Mo.) were prepared every 3 months. At the time of administration of HCDD, the stock suspension was shaken well, and suitable aliquots were

added to additional acetone to give the desired concentrations of the test chemical. DMBA was dissolved in acetone and applied in a volume of 100 μl. The preparations of HCDD or DMBA in acetone were kept in brown glass bottles with Teflon-lined caps. The bottles were sealed with tape, triple-bagged in plastic, and stored at 4°C. The backs of all animals were clipped weekly, and acetone and acetone suspension of HCDD and DMBA were applied to the clipped areas of vehicle control and test groups of mice with automatic pipettes equipped with disposable tips.

To determine the accuracy of the concentration of the HCDD in the stock suspensions in acetone, samples were analyzed at IITRI when the stocks were freshly prepared and at the end of the 3-month periods of use (Appendix G). During the first 16 weeks of the chronic study, the concentration of HCDD in the skin paint stock solution was 1.25 μ g/ml (or half the desired value). Subsequently, the mean concentration of 16 samples containing a theoretical level of 2.5 μ g/ml was 2.83$\pm$0.77 μ g/ml. The coefficient of variation was 27%.

C. Animals

Male and female Swiss-Webster mice, obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, were used in subchronic and chronic studies. The animals used in the chronic studies were approximately 4 weeks old when received and were acclimated in the laboratory for 2 weeks before the start of the bioassay. Those animals with no visible signs of disease were then earmarked for individual identification and assigned to dosed or control groups, using a table of random numbers. Because of animal supply limitations, multiple shipments of mice received within a 2-week period were used. The mice from each shipment were evenly distributed among all test and control groups. All mice were approximately the same age when placed on study.

D. Animal Maintenance

The mice were housed in temperature and humidity-controlled rooms. The temperature was maintained at 20° to 22°C and the relative humidity was

40% to 50%. A system of exhaust ducts maintained a negative air pressure in the animal rooms relative to the hallways and allowed 15 changes of room air per hour. The exhaust system included a series of HEPA filters through which all air from the animal rooms and hoods passed before being released into the exterior atmosphere. Fluorescent lighting was provided 12 hours each day.

The mice were housed 10 per cage in clear 19" x 10-1/2" x 8" polystyrene cages (Maryland Plastics, Federalsburg, Maryland). Each cage was fitted with a special tight-fitting polystyrene lid adapted to hold two metal filter housings and a water bottle. The filter housings contained FG 50 filters; one was left open to the room atmosphere while the other was attached to a hose that led to a pipe running the length of the shelf on the rack. Pipes on each of the four shelves of the rack led to a large vertical pipe at the end of the rack. The large pipe was connected by flexible hose to the HEPA filter exhaust system. This arrangement provided a constant flow of air that was filtered both as it entered and as it left the cages.

Because of the possible toxicity of the test chemical for laboratory personnel, the cages and lids in the rooms housing the groups of animals painted with the HCDD were used only once and were discarded every week. The used cages and lids were triple-sealed in plastic bags and incinerated at 982°C, as was all waste material from the animal rooms and the hoods. The glass water bottles and stainless steel sipper tubes from the used cages were rinsed in the same rooms, using the organic solvent chlorothene N.U.® (Central Solvents, Chicago, Illinois) to dilute out any dioxin present, and were then sanitized at 82°C in an automatic washer. The polystyrene cages in the rooms housing the control groups of animals were recycled three times, and the corresponding water bottles and sipper tubes were not rinsed in chlorothene before washing. After 4 weeks of use, the cages housing the control animals were also incinerated.

Disposable clothing was worn by all personnel and, after use, was incinerated by the procedure used for the cages and other waste material.

The animals were provided with clean cages and fresh Absorb-Dri® hardwood chip bedding (Lab Products, Inc., Garfield, N.J.) once per week. They were fed Wayne® Lab Blox (Allied Mills, Inc., Chicago, Illinois) in pellet form and were provided with fresh food when their cages were changed. Tap water

was made available ad libitum. Clean water bottles were provided once per week, and the bottles were refilled once during the week.

E. Subchronic Studies

Subchronic dermal application studies were conducted to determine the amount of test chemical to be used in the chronic studies. Groups of 10 mice of each sex were administered HCDD by dermal application three times per week for 13 weeks at doses ranging from 0.01 to 50 μg per application. The animals were observed daily for deaths. At the end of the study, necropsies and histologic examinations of tissues were performed on several of the mice in each dosed group. The rates of mortality and the incidences of histopathologic change for the different dosed groups are given in Table 1.

Applications of 50 or 25 μg HCDD caused 100% mortality in both male and female mice. The 10 μg dose caused 80% mortality in female mice. The 25 μg application in male mice and the 10 μg application in female mice caused depletion of cellular elements in lymphoid tissue. Moderate liver damage was present even at the lowest doses (0.01 μg in males and 0.05 μg in females). The degree of liver damage was not strictly dose related in either sex. The dose for the chronic study was chosen so that liver damage would be minimized. The dose selected was 0.01 μg per application. This dose corresponded to approximately 1.5 μg/kg/wk, based on an average mouse weight of 20 g and on the use of three applications of HCDD per week.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic dermal studies are shown in Table 2. Thirty mice of either sex administered HCDD alone, and an additional 30 mice of either sex given one application of 50 μg DMBA (at the same site as subsequent applications of HCDD) 1 week before the initiation of the HCDD application, were housed in one room with untreated control group No. 2. Vehicle controls were administered acetone alone and were housed in a second room with untreated control group No 1. The vehicle-control groups of each sex were shared with a dermal application study of TCDD which was housed in a third room.

Table 1. HCDD Subchronic Dermal Studies in Mice.

Dose μg/application	% Mortality		Incidence of Histopathologic Change											
			Lymph Node(a)		Spleen(a)		Thymus(a)		Bone Marrow(a)		Lung(a)		Liver(a)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
50	100	100	--	--	1/2	2/2	--	--	1/2	2/2	1/2	--	2/2	2/2
25	100	100	2/3	--	3/3	2/2	2/3	2/2	3/3	2/2	0/3	--	3/3	2/2
10	--	80	--	1/2	--	1/2	--	--	--	2/2	--	--	--	2/2(b)
5	--	0	--	0/2	--	0/2	--	0/2	--	0/2	--	--	--	2/2
1.0	0	10	0/2	0/9	0/2	0/9	0/2	0/9	0/2	0/9	0/2	--	2/2	7/9(c)
0.5	10	0	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	--	2/2	2/2
0.1	0	0	0/8	0/2	0/8	0/2	0/8	0/2	0/8	0/2	0/8	--	8/8(b)	2/2
0.05	0	0	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	--	2/2	2/2
0.01	0	--	0/2	--	0/2	--	0/2	--	0/2	--	0/2	--	2/2(b)	--

(a)Number of animals with effect/number of animals examined
(b)Ascitic-like fluid in abdominal cavity.
(c)One animal examined showed only threshold changes in the liver.
(M = Male, F = Female)

Table 2. HCDD Chronic Dermal Application Studies in Mice

Test Group	Initial No. of Animals(a,b)	Room	Dose (c) μg/ application	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
MALE					
Untreated-Control Group No. 1	15	1C9	0	0	104
Untreated-Control Group No. 2	15	1B0	0	0	104
Vehicle-Control (d)	45	1C9	0	0	104
Dosed	30	1B0	0.01	104	0
Dosed plus DMBA	30	1B0	0.01(e)	101	0
FEMALE					
Untreated-Control Group No. 1	15	1C9	0	0	104
Untreated-Control Group No. 2	15	1B0	0	0	104
Vehicle-Control (d)	45	1C9	0	0	104
Dosed	30	1B0	0.01	104	0
Dosed plus DMBA	30	1B0	0.01(e)	104	0

(a) All animals were approximately 6 weeks of age when placed on study, regardless of shipment.
(b) Mice from multiple shipments covering a 2-week period were evenly distributed among all test and control groups.
(c) The HCDD was administered 3 days per week in acetone at a constant volume of 0.1 ml for each application. During the first 16 weeks, doses were 0.005μg/application.
(d) Vehicle-controls received 0.1 ml acetone for each application.
(e) Each animal was administered 50 μg of dimethylbenzanthracene 1 week before to the initiation of dermal applications of HCDD.

G. Clinical Examinations and Pathology

Animals were observed twice daily for mortality. Body weights were recorded every 2 weeks for the first 12 weeks and every month thereafter. Moribund animals and those that survived to the termination of the study were killed using sodium pentobarbital and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues and organs were taken at necropsy: skin, mandibular lymph node, salivary gland, mammary gland, bone marrow, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, colon, liver, gall bladder, pancreas, spleen, kidney, adrenal, gonads, nasal cavity, brain, pituitary, spinal cord, skeletal muscle, sciatic nerve, and all tissue masses.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Data on this experiment have been recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible compound-related effect on survival were performed using the method

of Cox (1972) to compare each dosed group with the control group. One-tailed P values have been reported for all tests except for the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) before histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The statistical analyses of tumor incidence are used to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals. When results from two dosed groups were compared simultaneously with those for a single group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni test for inequality (Miller, 1966) requires that the P values for any comparison be less than or equal to 0.025. In this case, HCDD and HCDD plus DMBA are compared with the vehicle control group and, therefore, this correction is made where applicable.

Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation.

The approximate 95% confidence interval for the relative risk of each dosed group, compared with its control, was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of this confidence interval have been included in the tables of statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated

from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS

A. Body Weights and Clinical Signs

Throughout the bioassay, mean body weights of treated groups were slightly greater than those of vehicle controls and slightly less than mean body weights of untreated controls. Mean body weights were similar among the groups of male or female mice administered HCDD, or HCDD plus DMBA; and mean body weights of untreated controls were greater than those of the test and vehicle-control groups (Figure 1). No other clinical signs were reported.

B. Survival

Estimates of the probabilities of survival for male and female mice administered HCDD or HCDD plus DMBA by dermal application at the doses of this bioassay, together with those of the controls, are shown by the Kaplan and Meier curves in Figure 2. Five study groups were used for each sex: a group administered HCDD alone, a group administered HCDD plus DMBA, a vehicle-control group, and two untreated control groups. The two untreated-control groups are pooled into one group. In male mice, the Cox test comparing the survival of the pooled untreated-control group and the group administered HCDD plus DMBA is significant ($P=0.036$) as a result of shortened survival in the group with DMBA; however, the survival of each of the test groups and the vehicle-control group are comparable. The results of the Cox test in female mice are not significant, thus indicating comparable survival among all groups.

In male mice, 23/30 (77%) of the pooled untreated-control group, 30/45 (67%) of the vehicle-control group, 25/30 (83%) of the group administered HCDD alone, and 20/30 (67%) of the group administered HCDD plus DMBA lived 60 weeks or more. Nine of the 30 (30%) in the pooled untreated-control group, 7/45 (16%) of the vehicle-control group, 2/30 (7%) in the group administered HCDD alone, and 3/30 (10%) in the group administered HCDD plus DMBA were alive at the end of study. In females, 28/30 (93%) of the combined untreated-control group, 43/45 (96%) of the vehicle-control group, 24/30

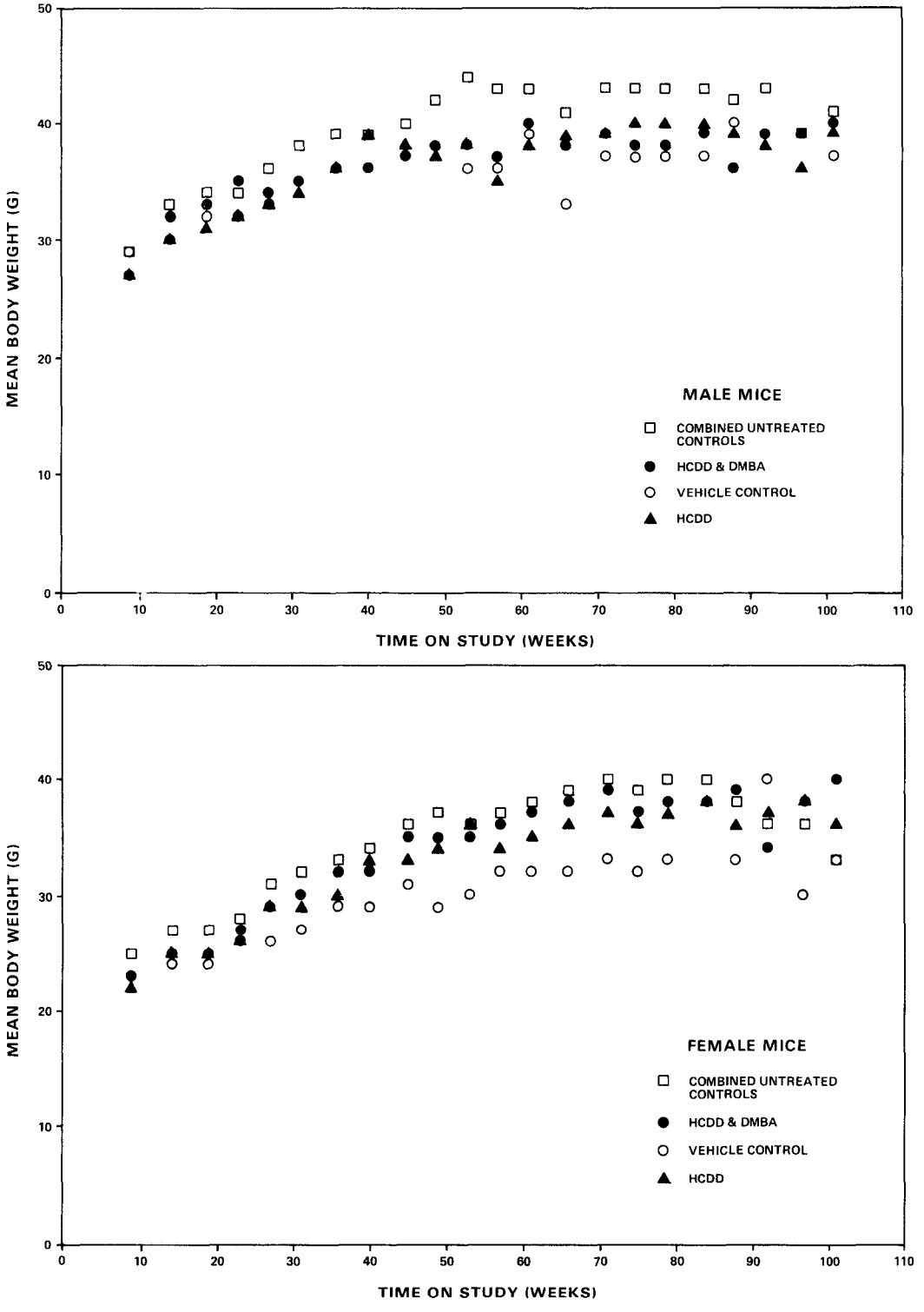


Figure 1. Growth Curves for Mice Administered HCDD or HCDD & DMBA by Dermal Application

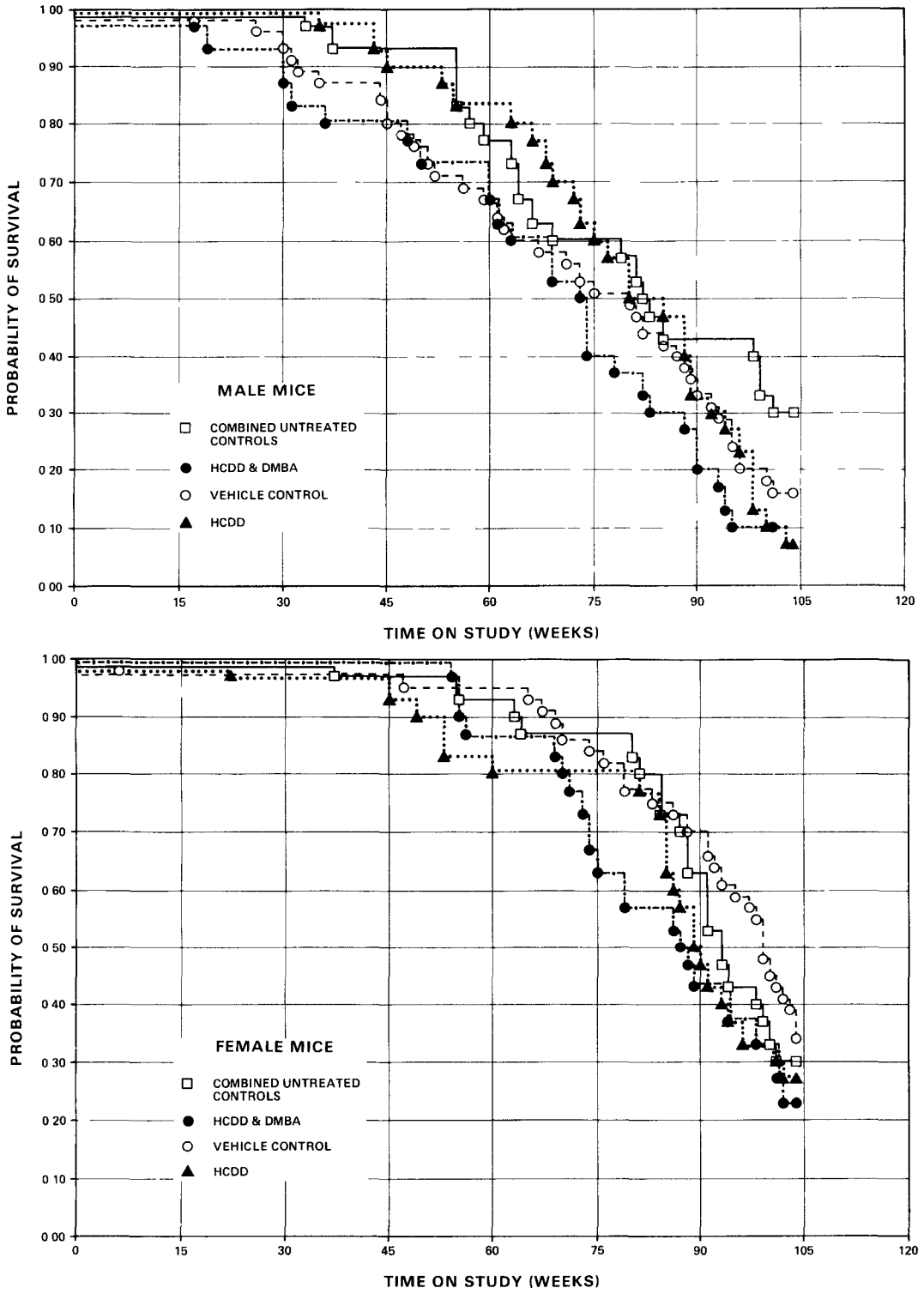


Figure 2. Survival Curves for Mice Administered HCDD or HCDD & DMBA by Dermal Application

(80%) of the group administered HCDD alone, and 26/30 (87%) of the group administered HCDD plus DMBA lived beyond 60 weeks. There were 9/30 (30%) of the pooled untreated-control group, 16/45 (36%) of the vehicle-control group, 8/30 (27%) of the group administered HCDD alone, and 7/30 (23%) of the group administered HCDD plus DMBA alive at the end of the study at 102-106 weeks.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendixes A and C, Tables A1, A2, C1, and C2; findings on nonneoplastic lesions are summarized in Appendixes B and D, Tables B1, B2, D1, and D2. Groups of animals receiving the test chemicals, the vehicle control, and untreated controls are tabulated and summarized separately.

The types of tumors seen were typical for Swiss-Webster mice, except for increased numbers of skin tumors which were not usually located on the back. Most of the skin tumors were fibrosarcomas, with only an occasional fibroma or epithelial tumor. There were two squamous cell carcinomas in male mice given HCDD and DMBA. Although few of these skin tumors were seen, they appeared in slightly higher incidences in dosed mice.

In conclusion, histopathologic examination provided no conclusive evidence for the carcinogenicity of HCDD for the skin or internal organs of female Swiss-Webster mice. Under the condition of this bioassay, however, a slightly increased incidence of skin tumors may be associated with administration of HCDD and HCDD plus DMBA.

D. Statistical Analyses of Results

Tables 3 and 4 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group. The two untreated-control groups are combined into one group in the analysis. Since the doses in the two test groups did not differ in HCDD alone, no trend analysis was made. The differences in tumor incidences between groups receiving HCDD and those receiving HCDD plus DMBA were not statistically significant.

In male mice, the incidence of animals with alveolar/bronchiolar carcinomas in the lung is higher (P=0.045) in the group administered HCDD than in the vehicle-control group. This value of 0.045 is above the level of significance (0.025) used under the Bonferroni criterion to compare two test groups with a common control group. No tests are significant when the incidence of animals with this tumor in the group administered HCDD is compared with that in the untreated-control group. The incidence of this lesion in the untreated-control group is 4/28 (14%) as compared with 1/41 (2%) in the vehicle-control group. When the incidences of animals with either adenomas or carcinomas of the lungs are used in the analysis, none of the results are significant.

Statistical tests conducted upon the time to observation of the lung tumors in the test groups of male rats compared with the matched controls indicate no significant difference.

The two squamous cell carcinomas occurred in male mice administered HCDD plus DMBA at weeks 60 and 94.

In female mice, the incidence of animals with fibrosarcomas of the skin is significantly higher in each of the test groups (P=0.044) than in the untreated control group; however, when the test groups are compared with the vehicle-control group, the results are not significant.

In male mice, the incidence of animals with lymphomas or leukemias is significantly lower (P=0.011) in the group administered HCDD than in the untreated control group.

In each of the 95% confidence intervals for relative risk shown in the tables, the value of one or less than one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals, except for the incidence of lymphomas in male mice, has an upper limit greater than one indicating the theoretical possibility of tumor induction by HCDD which could not be detected under the conditions of this test.

Table 3. Analyses of the Incidence of Primary Tumors in Male Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

Topograpy: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Integumentary System: Fibrosarcoma (b)	3/29 (10)	3/42 (7)	6/30 (20)	3/24 (13)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.933	1.208
Lower Limit			0.460	0.176
Upper Limit			10.921	8.191
Relative Risk (Vehicle Control) (e)			2.800	1.750
Lower Limit			0.651	0.250
Upper Limit			15.919	11.932
Weeks to First Observed Tumor	55	90	38	60
Integumentary System: Fibrosarcoma or Fibroma (b)	3/29 (10)	4/42 (10)	6/30 (20)	3/24 (13)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.933	1.208
Lower Limit			0.460	0.176
Upper Limit			10.921	8.191
Relative Risk (Vehicle Control) (e)			2.100	1.313
Lower Limit			0.544	0.206
Upper Limit			9.195	7.005
Weeks to First Observed Tumor	55	46	38	60
Integumentary System: Squamous-cell Carcinoma of the Skin (b)	0/29 (0)	0/42 (0)	0/30 (0)	2/24 (8)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			-	Infinite
Lower Limit			-	0.365
Upper Limit			-	Infinite
Relative Risk (Vehicle Control) (e)			-	Infinite
Lower Limit			-	0.522
Upper Limit			-	Infinite
Weeks to First Observed Tumor	-	-	-	60
Lung:Alveolar/Bronchiolar Adenoma (b)	2/28 (7)	6/41 (15)	5/30 (17)	3/24 (13)
P Value (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			2.333	1.750
Lower Limit			0.421	0.218
Upper Limit			22.894	19.385
Relative Risk (Vehicle Control) (e)			1.139	0.854
Lower Limit			0.301	0.149
Upper Limit			4.023	3.559
Weeks to First Observed Tumor	59	75	76	54

Table 3. Analyses of the Incidence of Primary Tumors in Male Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Lung: Alveolar/Bronchiolar Carcinoma (b)	4/28 (14)	1/41 (2)	5/30 (17)	3/24 (13)
P Values (c,d)	-	-	P=0.045 (Vehicle Controls)	N.S.
Relative Risk (Combined Untreated Control) (e)			1.167	0.875
Lower Limit			0.280	0.141
Upper Limit			5.315	4.630
Relative Risk (Vehicle Control) (e)			6.833	5.125
Lower Limit			0.819	0.437
Upper Limit			310.654	256.604
Weeks to First Observed Tumor	86	84	66	74
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	6/28 (21)	7/41 (17)	9/30 (30)	6/24 (25)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.400	1.167
Lower Limit			0.515	0.358
Upper Limit			4.155	3.752
Relative Risk (Vehicle Control) (e)			1.757	1.464
Lower Limit			0.655	0.454
Upper Limit			4.854	4.397
Weeks to First Observed Tumor	59	75	66	54
Hematopoietic System: All Lymphomas (b)	6/29 (21)	3/42 (7)	0/30 (0)	2/24 (8)
P Values (c,d)	-	-	P=0.011(N) (Untreated Controls)	N.S.
Relative Risk (Combined Untreated Control) (e)			0.000	0.403
Lower Limit			0.000	0.043
Upper Limit			0.590	2.003
Relative Risk (Vehicle Control) (e)			0.000	1.167
Lower Limit			0.000	0.102
Upper Limit			2.286	9.351
Weeks to First Observed Tumor	66	31	--	96
Hematopoietic System: Lymphoma or Leukemia (b)	6/29 (21)	4/42 (10)	0/30 (0)	2/24 (8)
P Values (c,d)	-	-	P=0.011(N) (Untreated Controls)	N.S.
Relative Risk (Combined Untreated Control) (e)			0.000	0.403
Lower Limit			0.000	0.043
Upper Limit			0.590	2.003
Relative Risk (Vehicle Control) (e)			0.000	0.875
Lower Limit			0.000	0.083
Upper Limit			1.482	5.550
Weeks to First Observed Tumor	66	18	--	96

Table 3. Analyses of the Incidence of Primary Tumors in Male Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Liver: Hepatocellular Carcinoma (b)	3/29 (10)	0/42 (0)	1/30 (3)	0/22 (0)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			0.322	0.000
Lower Limit			0.006	0.000
Upper Limit			3.740	2.114
Relative Risk (Vehicle Control) (e)			Infinite	--
Lower Limit			0.075	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	82	--	103	--

(a) Dosed groups received doses of 0.01 μg of HCDD or 0.01 μg of HCDD after pretreatment with 50 μg of DMBA.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the dosed group is the probability level for the Fisher exact Test for the comparison of the dosed group with the combined untreated-control group and with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in the dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between the dosed group and the control group.

Table 4. Analyses of the Incidence of Primary Tumors in Female Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

Topography: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Integumentary System: Fibrosarcoma (b)	0/30 (0)	2/41 (5)	4/27 (15)	4/27 (15)
P Values (c,d)	-	-	P=0.044 (Untreated Controls)	P=0.044 (Untreated Controls)
Relative Risk (Combined Untreated Control) (e)			Infinite	Infinite
Lower Limit			1.054	1.054
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control) (e)			3.037	3.037
Lower Limit			0.467	0.467
Upper Limit			31.347	31.347
Weeks to First Observed Tumor	---	98	57	77
Lung: Alveolar/Bronchiolar Adenoma (b)	2/30 (7)	4/41 (10)	4/27 (15)	1/25 (4)
P Values (c,d)	--	--	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			2.222	0.600
Lower Limit			0.348	0.011
Upper Limit			22.869	10.786
Relative Risk (Vehicle Control) (e)			1.519	0.410
Lower Limit			0.306	0.009
Upper Limit			7.399	3.806
Weeks to First Observed Tumor	102	79	57	104
Lung: Alveolar/Bronchiolar Carcinoma (b)	2/30 (7)	5/41 (12)	1/27 (4)	2/25 (8)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			0.556	1.200
Lower Limit			0.010	0.093
Upper Limit			10.031	15.432
Relative Risk (Vehicle Control) (e)			0.304	0.656
Lower Limit			0.007	0.066
Upper Limit			2.493	3.628
Weeks to First Observed Tumor	91	75	89	100
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	4/30 (13)	8/41 (20)	4/27 (15)	3/25 (12)
P Value (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.111	0.900
Lower Limit			0.228	0.144
Upper Limit			5.378	4.789
Relative Risk (Vehicle Control) (c)			0.759	0.615
Lower Limit			0.183	0.113
Upper Limit			2.511	2.270
Weeks to First Observed Tumor	91	75	57	100

Table 4. Analyses of the Incidence of Primary Tumors in Female Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Hematopoietic System: All Lymphomas (b)	10/30 (33)	14/41 (34)	9/27 (33)	6/27 (22)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.000	0.667
Lower Limit			0.425	0.231
Upper Limit			2.294	1.733
Relative Risk (Vehicle Control) (e)			0.976	0.651
Lower Limit			0.432	0.232
Upper Limit			2.032	1.547
Weeks to First Observed Tumor	67	70	56	58
Hematopoietic System: Lymphoma or Leukemia (b)	10/30 (33)	14/41 (34)	10/27 (37)	6/27 (22)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			1.111	0.667
Lower Limit			0.494	0.231
Upper Limit			2.473	1.733
Relative Risk (Vehicle Control) (e)			1.085	0.651
Lower Limit			0.502	0.232
Upper Limit			2.186	1.547
Weeks to First Observed Tumor	67	70	26	58
Circulatory System: Hemangioma or Hemangiosarcoma (b)	2/30 (7)	3/41 (7)	0/27 (0)	1/27 (4)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			0.000	0.556
Lower Limit			0.000	0.010
Upper Limit			3.673	10.031
Relative Risk (Vehicle Control) (e)			0.000	0.506
Lower Limit			0.000	0.010
Upper Limit			2.468	5.869
Weeks to First Observed Tumor	88	104	--	104
Uterus: Leiomyoma (b)	3/30 (10)	0/36 (0)	1/26 (4)	2/26 (8)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			0.385	0.769
Lower Limit			0.008	0.068
Upper Limit			4.429	6.172
Relative Risk (Vehicle Control) (e)			Infinite	Infinite
Lower Limit			0.075	0.415
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	96	--	104	72

Table 4. Analyses of the Incidence of Primary Tumors in Female Mice Administered HCDD or HCDD plus DMBA by Dermal Application (a)

(continued)

Topography: Morphology	Combined Untreated Control	Vehicle Control	HCDD	HCDD plus DMBA
Ovary: Granulosa-cell Tumor (b)	1/29 (3)	1/33 (3)	0/25 (0)	3/25 (12)
P Values (c,d)	-	-	N.S.	N.S.
Relative Risk (Combined Untreated Control) (e)			0.000	3.480
Lower Limit			0.000	0.301
Upper Limit			21.242	174.657
Relative Risk (Vehicle Control) (e)			0.000	3.960
Lower Limit			0.000	0.340
Upper Limit			24.165	198.698
Weeks to First Observed Tumor	106	70	--	102

(a) Dosed groups received doses of 0.01 μg of HCDD or 0.01 μg of HCDD after pretreatment with 50 μg of DMBA.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the dosed group is the probability level for the Fisher exact Test for the comparison of the dosed group with the combined untreated-control group and with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in the dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between the dosed group and the control group.

IV. DISCUSSION

Throughout the bioassay the mean body weights of the groups of male or female mice administered HCDD or HCDD plus DMBA were similar and were greater than those of corresponding vehicle-control groups but less than those of untreated control groups. The consequences of using only 0.005μg rather than 0.01μg HCDD per application during the first 16 weeks of the chronic study were not considered to be significant.

After 80 weeks, survival was sufficient to detect late appearing tumors.

Alveolar/bronchiolar carcinomas occurred in the male mice administered HCDD at an incidence that was significantly higher (P=0.045) than that in the vehicle-control group, but the incidence was not significant when compared with the untreated control group that had longer survival. Alveolar/bronchiolar carcinomas did not occur in statistically significant incidences in male mice pre-treated with DMBA before being administered HCDD, when compared with either the vehicle or the untreated controls.

Compared with the untreated control group, the incidence of male mice with lymphomas or leukemias was significantly lower (P=0.011) in the group administered HCDD but not in the group administered with DMBA and HCDD.

Two squamous cell carcinomas in male mice administered HCDD plus DMBA occurred in dosed skin near a hind leg or the tail. The possibility that these areas may have been exposed accidentally during application of the test chemical cannot be eliminated.

In female mice, fibrosarcomas in the skin occurred at incidences that were significantly higher (P=0.044) in animals administered HCDD (both those with and without pretreatment with DMBA) than in the untreated control group. Although there were more female mice with fibrosarcomas in the two groups administered HCDD when compared with the vehicle controls, the increased incidences were not statistically significant. Thus, the relationship between incidences of fibrosarcomas in female mice and HCDD administration could not be clearly established.

In the present study, pretreatment with DMBA had no statistically significant effect on skin tumor production in either male or female mice. The significance of the two squamous cell carcinomas of the skin in male

mice administered both HCDD and DMBA cannot be determined in the absence of a group exposed to DMBA alone. Male mice pretreated with DMBA, when compared with male mice not pretreated, had a lower incidence of alveolar/bronchiolar carcinomas and a higher incidence of lymphomas.

A bioassay of HCDD administered by gavage was run concurrently with the present study (NCI, 1980). Under the conditions of that bioassay, HCDD was carcinogenic for female Osborne-Mendel rats and for male and female B6C3F1 mice, inducing increased incidences of hepatocellular carcinomas, adenomas, or neoplastic nodules. In the gavage studies, the B6C3F1 mice received 5, 2.5, or 1.25 μg/kg/week (males) and 10, 5, or 2.5 μg/kg/week (females). These were high doses when compared with 1.5 μg/kg/week for male and female Swiss-Webster mice in the present studies. The mice in the gavage studies lived longer.

A dermal bioassay of TCDD in Swiss-Webster mice was conducted concurrently in the room used for the present study. The protocols were identical except that a lower dose was applied (NCI, 1980a). In the dermal TCDD studies, there were statistically significant increased incidences of fibrosarcomas in the integumentary system in female mice receiving 0.005 μg per application when compared with controls. This significant increase was not found in the male mice receiving 0.001 μg per application. In the present dermal HCDD studies, both male and female mice received 0.01 μg per application. Thus, after dermal application under identical conditions, TCDD was clearly carcinogenic for female mice, whereas HCDD (at a two-fold higher dose) was not.

V. CONCLUSION

Under the conditions of this bioassay, HCDD was not considered carcinogenic for male or female Swiss-Webster mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN MICE ADMINISTERED HCDD BY
DERMAL APPLICATION

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED HCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	15	42	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	42	30
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(42)	(30)
FIBROSARCOMA		1 (7%)	1 (2%)	2 (7%)
*SUBCUT TISSUE	(14)	(15)	(42)	(30)
SEBACEOUS ADENOMA			1 (2%)	
FIBROMA			1 (2%)	1 (3%)
FIBROSARCOMA		2 (13%)	2 (5%)	4 (13%)
LIPOMA		1 (7%)		
RESPIRATORY SYSTEM				
#LUNG	(13)	(15)	(41)	(30)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (8%)	1 (7%)	6 (15%)	5 (17%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (15%)	2 (13%)	1 (2%)	5 (17%)
CORTICAL CARCINOMA, METASTATIC	1 (8%)			
FIBROSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(42)	(30)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)	3 (20%)	2 (5%)	
GRANULOCYTIC LEUKEMIA			1 (2%)	
#BONE MARROW	(14)	(14)	(40)	(28)
ALVEOLAR/BRONCHIOLAR CA, METASTA				1 (4%)
#LYMPH NODE	(8)	(7)	(25)	(21)
FIBROSARCOMA, INVASIVE		1 (14%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#RENAL LYMPH NODE	(8)	(7)	(25)	(21)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (14%)		
#THYMUS	(11)	(10)	(30)	(27)
ALVEOLAR/BRONCHIOLAR CA, METASTA				1 (4%)
THYMOMA				1 (4%)
CIRCULATORY SYSTEM				
#HEART	(13)	(15)	(42)	(30)
HEMANGIOSARCOMA	1 (8%)			
#LIVER	(14)	(15)	(42)	(30)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	1 (3%)
DIGESTIVE SYSTEM				
#LIVER	(14)	(15)	(42)	(30)
HEPATOCELLULAR ADENOMA			1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (7%)	2 (13%)		1 (3%)
#SMALL INTEST./SEROSA	(11)	(12)	(34)	(27)
FIBROMA				1 (4%)
URINARY SYSTEM				
#KIDNEY	(14)	(14)	(42)	(30)
SARCOMA, NOS, UNC PRIM OR META		1 (7%)		
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(15)	(40)	(28)
CORTICAL CARCINOMA	1 (9%)			
PHEOCHROMOCYTOMA				1 (4%)
#THYROID	(13)	(14)	(35)	(28)
ADENOMA, NOS	1 (8%)			
REPRODUCTIVE SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(15)	(42)	(30)
FIBROSARCOMA			1 (2%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATH@	7	13	30	21
MORIBUND SACRIFICE	1		8	7
**SCHEDULED SACRIFICE	5		4	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	2	2	3	2
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

** Animals are in fact early terminal sacrifices, but appear as scheduled sacrifices due to system interpretation.

TABLE A1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	10	15	17
TOTAL PRIMARY TUMORS	9	15	19	22
TOTAL ANIMALS WITH BENIGN TUMORS	2	2	8	8
TOTAL BENIGN TUMORS	2	2	9	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	8	8	12
TOTAL MALIGNANT TUMORS	7	12	10	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2	1
TOTAL SECONDARY TUMORS	1	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC		1		
TOTAL UNCERTAIN TUMORS		1		

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED HCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	15	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	41	27
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(15)	(41)	(27)
FIBROSARCOMA				2 (7%)
*SUBCUT TISSUE	(15)	(15)	(41)	(27)
FIBROSARCOMA			2 (5%)	2 (7%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(15)	(41)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (7%)	4 (10%)	4 (15%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (13%)		5 (12%)	1 (4%)
FIBROSARCOMA, METASTATIC			1 (2%)	1 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (27%)	2 (13%)	9 (22%)	7 (26%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (13%)	1 (7%)	4 (10%)	1 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE				1 (4%)
GRANULOCYTIC LEUKEMIA				1 (4%)
#BONE MARROW	(13)	(13)	(37)	(24)
FIBROMA			1 (3%)	
#SPLEEN	(15)	(15)	(40)	(26)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
FIBROMA			1 (3%)	
#PANCREATIC L.NODE	(12)	(12)	(30)	(22)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MALIGNANT LYMPHOMA, MIXED TYPE		1 (8%)		
#THYMUS	(15)	(15)	(39)	(21)
THYMOMA, MALIGNANT			1 (3%)	
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(12)	(12)	(30)	(22)
HEMANGIOMA		1 (8%)		
#LIVER	(15)	(15)	(41)	(26)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
#UTERUS	(15)	(15)	(36)	(26)
HEMANGIOMA			1 (3%)	
#OVARY	(14)	(15)	(33)	(25)
HEMANGIOMA			1 (3%)	
DIGESTIVE SYSTEM				
#PAROTID GLAND	(15)	(12)	(36)	(26)
MIXED TUMOR, BENIGN			1 (3%)	
#LIVER	(15)	(15)	(41)	(26)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
#PANCREAS	(15)	(15)	(40)	(26)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
LIPOMA			1 (3%)	
#PANCREATIC DUCT	(15)	(15)	(40)	(26)
SARCOMA, NOS			1 (3%)	
#GASTRIC SEROSA	(15)	(15)	(41)	(27)
SARCOMA, NOS			1 (2%)	
#DUODENUM	(13)	(15)	(40)	(26)
LIPOMA				1 (4%)
*RECTUM	(15)	(15)	(41)	(27)
SQUAMOUS CELL CARCINOMA		1 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
URINARY SYSTEM				
*GENITOURINARY TRACT	(15)	(15)	(41)	(27)
FIBROSARCOMA			1 (2%)	
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(14)	(41)	(26)
CORTICAL ADENOMA	1 (7%)			
PHEOCHROMOCYTOMA				1 (4%)
REPRODUCTIVE SYSTEM				
*VAGINA	(15)	(15)	(41)	(27)
LEIOMYOMA				1 (4%)
#UTERUS	(15)	(15)	(36)	(26)
ADENOCARCINOMA, NOS			1 (3%)	
LEIOMYOMA	2 (13%)	1 (7%)		1 (4%)
#CERVIX UTERI	(15)	(15)	(36)	(26)
LEIOMYOMA	1 (7%)			
#OVARY	(14)	(15)	(33)	(25)
LUTEOMA	1 (7%)			
GRANULOSA-CELL TUMOR		1 (7%)	1 (3%)	
LIPOMA			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
THYMOMA, METASTATIC			1 (2%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATHa	8	11	28	20
MORIBUND SACRIFICE	1	1	1	2
**SCHEDULED SACRIFICE	3	1	15	3
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	3	2	1	5
ANIMAL MISSING				
a INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	25	18
TOTAL PRIMARY TUMORS	15	9	39	23
TOTAL ANIMALS WITH BENIGN TUMORS	6	3	10	7
TOTAL BENIGN TUMORS	6	3	11	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	5	23	14
TOTAL MALIGNANT TUMORS	9	5	27	15
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	1
TOTAL SECONDARY TUMORS			6	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1	
TOTAL UNCERTAIN TUMORS		1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN
** Animals are in fact early terminal sacrifices, but appear as scheduled sacrifices due to system interpretation.

APPENDIX B

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED HCDD BY DERMAL APPLICATION

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED HCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	15	42	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	42	30
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(42)	(30)
CYST, NOS				1 (3%)
EDEMA, NOS			1 (2%)	
INFLAMMATION, NOS			2 (5%)	
ULCER, NOS		1 (7%)	3 (7%)	1 (3%)
ULCER, ACUTE			1 (2%)	1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
ABSCESS, NOS				1 (3%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
INFLAMMATION, CHRONIC	1 (7%)			1 (3%)
ULCER, CHRONIC		1 (7%)		
INFLAMMATION PROLIFERATIVE			1 (2%)	
NECROSIS, NOS				1 (3%)
MELANIN				1 (3%)
HYPERPLASIA, NOS			1 (2%)	1 (3%)
HYPERPLASIA, PAPILLARY			1 (2%)	
HYPERKERATOSIS	1 (7%)		3 (7%)	4 (13%)
ACANTHOSIS		1 (7%)	1 (2%)	
KERATIN-PEARL FORMATION				1 (3%)
*SUBCUT TISSUE	(14)	(15)	(42)	(30)
EPIDERMAL INCLUSION CYST			1 (2%)	
ULCER, NOS			1 (2%)	2 (7%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (3%)
ABSCESS, NOS				3 (10%)
ULCER, CHRONIC				1 (3%)
GRANULATION, TISSUE			1 (2%)	1 (3%)
NECROSIS, NOS			2 (5%)	2 (7%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(13)	(15)	(41)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (8%)		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LUNG/BRONCHIOLE	(13)	(15)	(41)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR	6 (46%)	4 (27%)	13 (32%)	11 (37%)
HYPERPLASIA, EPITHELIAL			1 (2%)	
#LUNG	(13)	(15)	(41)	(30)
ATELECTASIS			2 (5%)	1 (3%)
CONGESTION, NOS		3 (20%)	3 (7%)	3 (10%)
INFLAMMATION, NOS				1 (3%)
INFLAMMATION, FOCAL			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (7%)		
INFLAMMATION, INTERSTITIAL	1 (8%)	2 (13%)	3 (7%)	6 (20%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (7%)	1 (2%)	
EMPYEMA			1 (2%)	
INFLAMMATION, ACUTE FOCAL				1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
NECROSIS, NOS			1 (2%)	
ALVEOLAR MACROPHAGES			1 (2%)	
HEMATOPOIETIC SYSTEM				
*SUBCUT TISSUE	(14)	(15)	(42)	(30)
MASTOCYTOSIS				1 (3%)
#SPLEEN	(13)	(13)	(40)	(30)
AMYLOIDOSIS		3 (23%)		1 (3%)
HYPOPLASIA, NOS	1 (8%)		5 (13%)	5 (17%)
ATROPHY, NOS		1 (8%)		
HYPERPLASIA, NOS	2 (15%)		4 (10%)	1 (3%)
HEMATOPOIESIS			5 (13%)	3 (10%)
#SPLENIC FOLLICLES	(13)	(13)	(40)	(30)
HYPOPLASIA, NOS				1 (3%)
ATROPHY, NOS				1 (3%)
HYPERPLASIA, NOS		2 (15%)	2 (5%)	3 (10%)
#SPLENIC RED PULP	(13)	(13)	(40)	(30)
HYPOPLASIA, NOS			2 (5%)	5 (17%)
HYPERPLASIA, NOS				1 (3%)
MEGAKARYOCYTOSIS			1 (3%)	
HEMATOPOIESIS		2 (15%)		
ERYTHROPOIESIS				1 (3%)
GRANULOPOIESIS			1 (3%)	
#LYMPH NODE	(8)	(7)	(25)	(21)
HYPERPLASIA, NOS			2 (8%)	3 (14%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#CERVICAL LYMPH NODE	(8)	(7)	(25)	(21)
ABSCESS, NOS				1 (5%)
HYPERPLASIA, NOS	4 (50%)	3 (43%)	10 (40%)	7 (33%)
#TRACHEAL LYMPH NODE	(8)	(7)	(25)	(21)
HYPERPLASIA, NOS	1 (13%)			
#PYLORIC LYMPH NODE	(8)	(7)	(25)	(21)
HEMORRHAGE		1 (14%)		
#PANCREATIC L.NODE	(8)	(7)	(25)	(21)
HYPERPLASIA, NOS				1 (5%)
#LUMBAR LYMPH NODE	(8)	(7)	(25)	(21)
HYPERPLASIA, LYMPHOID			1 (4%)	
#MESENTERIC L. NODE	(8)	(7)	(25)	(21)
ABSCESS, NOS			1 (4%)	
INFLAMMATION PROLIFERATIVE			1 (4%)	
NECROSIS, CASEOUS			1 (4%)	
#RENAL LYMPH NODE	(8)	(7)	(25)	(21)
HYPERPLASIA, NOS	1 (13%)			1 (5%)
#LUNG/BRONCHIOLE	(13)	(15)	(41)	(30)
HYPERPLASIA, LYMPHOID			1 (2%)	1 (3%)
#PAROTID GLAND	(12)	(15)	(36)	(28)
FIBROSING ADENOSIS				1 (4%)
#LIVER	(14)	(15)	(42)	(30)
HYPERPLASIA, NEUTROPHILIC				1 (3%)
HEMATOPOIESIS			1 (2%)	
#KIDNEY	(14)	(14)	(42)	(30)
HYPERPLASIA, LYMPHOID			1 (2%)	
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(8)	(7)	(25)	(21)
LYMPHANGIECTASIS			1 (4%)	
#MESENTERIC L. NODE	(8)	(7)	(25)	(21)
LYMPHANGIECTASIS	1 (13%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#HEART	(13)	(15)	(42)	(30)
MINERALIZATION	1 (8%)			
ABSCESS, NOS				1 (3%)
#HEART/ATRIUM	(13)	(15)	(42)	(30)
THROMBOSIS, NOS		2 (13%)		
#MYOCARDIUM	(13)	(15)	(42)	(30)
MINERALIZATION		1 (7%)		
INFLAMMATION, CHRONIC				1 (3%)
INFLAMMATION, CHRONIC FOCAL		2 (13%)	3 (7%)	1 (3%)
INFLAMMATION PROLIFERATIVE		1 (7%)		
NECROSIS, FOCAL		1 (7%)		
#ENDOCARDIUM	(13)	(15)	(42)	(30)
INFLAMMATION PROLIFERATIVE	1 (8%)			
HYPERPLASIA, NOS	1 (8%)			
*RENAL ARTERY	(14)	(15)	(42)	(30)
ARTERIOSCLEROSIS, NOS			1 (2%)	
#URINARY BLADDER	(13)	(14)	(39)	(27)
THROMBOSIS, NOS				1 (4%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(12)	(15)	(36)	(28)
INFLAMMATION, FOCAL		1 (7%)		
#PAROTID GLAND	(12)	(15)	(36)	(28)
INFLAMMATION, NOS		1 (7%)	3 (8%)	1 (4%)
INFLAMMATION, FOCAL	1 (8%)			
NECROSIS, FOCAL			1 (3%)	
#LIVER	(14)	(15)	(42)	(30)
CONGESTION, NOS	1 (7%)			
INFLAMMATION, FOCAL				1 (3%)
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	
INFLAMMATION, ACUTE FOCAL		2 (13%)		
INFLAMMATION, ACUTE DIFFUSE			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		1 (7%)		
CIRRHOSIS, NOS	1 (7%)			
DEGENERATION, HYDROPIC			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NECROSIS, NOS	1 (7%)		1 (2%)	
NECROSIS, FOCAL		2 (13%)	1 (2%)	
NECROSIS, COAGULATIVE		2 (13%)		2 (7%)
AMYLOIDOSIS		3 (20%)		1 (3%)
LIPOIDOSIS	1 (7%)			1 (3%)
FOCAL CELLULAR CHANGE				1 (3%)
HEPATOCYTOMEGALY	1 (7%)		2 (5%)	1 (3%)
#PORTAL TRACT	(14)	(15)	(42)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
#LIVER/CENTRILOBULAR	(14)	(15)	(42)	(30)
METAMORPHOSIS FATTY	1 (7%)			
HEPATOCYTOMEGALY	5 (36%)		1 (2%)	1 (3%)
#LIVER/PERIPORTAL	(14)	(15)	(42)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (7%)	2 (5%)	
#LIVER/HEPATOCYTES	(14)	(15)	(42)	(30)
CLOUDY SWELLING	1 (7%)			
ATROPHY, NOS		1 (7%)		
#STOMACH	(14)	(13)	(42)	(29)
HYPERPLASIA, EPITHELIAL		1 (8%)		
HYPERKERATOSIS		1 (8%)	3 (7%)	3 (10%)
#DUODENUM	(11)	(12)	(34)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (9%)			
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)
#COLON	(11)	(15)	(36)	(29)
IMPACTION, NOS		2 (13%)		
URINARY SYSTEM				
*GENITOURINARY TRACT	(14)	(15)	(42)	(30)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
#KIDNEY	(14)	(14)	(42)	(30)
HYDRONEPHROSIS	1 (7%)		1 (2%)	2 (7%)
GLOMERULONEPHRITIS, NOS		1 (7%)		
PYELONEPHRITIS, NOS		1 (7%)	5 (12%)	5 (17%)
LYMPHOCYTIC INFLAMMATORY INFILTR	8 (57%)	10 (71%)	20 (48%)	17 (57%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
GLOMERULONEPHRITIS, ACUTE				3 (10%)
INFLAMMATION, CHRONIC		1 (7%)	1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	9 (64%)	3 (21%)	6 (14%)	5 (17%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	1 (3%)
GLOMERULOSCLEROSIS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	
#KIDNEY/CORTEX	(14)	(14)	(42)	(30)
MINERALIZATION				1 (3%)
ABSCESS, NOS				2 (7%)
#KIDNEY/GLOMERULUS	(14)	(14)	(42)	(30)
AMYLOIDOSIS			1 (2%)	
#KIDNEY/TUBULE	(14)	(14)	(42)	(30)
CALCULUS, NOS			1 (2%)	
CALCIFICATION, NOS			1 (2%)	
#KIDNEY/PELVIS	(14)	(14)	(42)	(30)
ABSCESS, NOS			1 (2%)	
METAPLASIA, SQUAMOUS			1 (2%)	
#URINARY BLADDER	(13)	(14)	(39)	(27)
CAST, NOS			1 (3%)	2 (7%)
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, ACUTE FOCAL		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE		1 (7%)		1 (4%)
INFLAMMATION, ACUTE/CHRONIC				1 (4%)
INFLAMMATION, CHRONIC	4 (31%)		3 (8%)	10 (37%)
INFLAMMATION, CHRONIC FOCAL				1 (4%)
INFLAMMATION, CHRONIC DIFFUSE		1 (7%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
INFLAMMATION PROLIFERATIVE		1 (7%)	3 (8%)	1 (4%)
FIBROSIS		1 (7%)		
HYPERPLASIA, EPITHELIAL		2 (14%)	1 (3%)	
POLYPOID HYPERPLASIA			1 (3%)	1 (4%)
ENDOCRINE SYSTEM				
#PITUITARY	(8)	(12)	(26)	(19)
MINERALIZATION				1 (5%)
#ADRENAL	(11)	(15)	(40)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (3%)	1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
DEGENERATION PIGMENTARY	5 (45%)	2 (13%)	4 (10%)	2 (7%)
AMYLOIDOSIS			1 (3%)	
LIPOIDOSIS	1 (9%)			
#ADRENAL CORTEX	(11)	(15)	(40)	(28)
HAMARTOMA		2 (13%)		2 (7%)
#ZONA GLOMERULOSA	(11)	(15)	(40)	(28)
METAPLASIA, NOS	5 (45%)	6 (40%)	6 (15%)	9 (32%)
#ZONA FASCICULATA	(11)	(15)	(40)	(28)
HYPERTROPHY, FOCAL	1 (9%)			
#ADRENAL MEDULLA	(11)	(15)	(40)	(28)
CONGESTION, NOS			1 (3%)	
HEMORRHAGE		1 (7%)		
REPRODUCTIVE SYSTEM				
*GENITAL SYSTEM	(14)	(15)	(42)	(30)
RETENTION OF CONTENT	1 (7%)	1 (7%)	2 (5%)	4 (13%)
INFLAMMATION, SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
PLASMA-CELL INFILTRATE			1 (2%)	
*BULBOURETHRAL GLAND	(14)	(15)	(42)	(30)
CAST, NOS				1 (3%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	2 (7%)
INFLAMMATION PROLIFERATIVE				1 (3%)
POLYPOID HYPERPLASIA			1 (2%)	
*PREPUTIAL GLAND	(14)	(15)	(42)	(30)
EPIDERMAL INCLUSION CYST			1 (2%)	
ABSCESS, NOS				1 (3%)
GRANULATION, TISSUE			1 (2%)	
#PROSTATE	(13)	(14)	(39)	(28)
RETENTION OF CONTENT	9 (69%)	7 (50%)	18 (46%)	16 (57%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	1 (4%)
INFLAMMATION, CHRONIC				1 (4%)
INFLAMMATION, CHRONIC FOCAL		2 (14%)		
INFLAMMATION, CHRONIC SUPPURATIV			2 (5%)	1 (4%)
INFLAMMATION PROLIFERATIVE				1 (4%)
*SEMINAL VESICLE	(14)	(15)	(42)	(30)
DILATATION, NOS	2 (14%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
RETENTION OF CONTENT	9 (64%)	7 (47%)	18 (43%)	15 (50%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (3%)
INFLAMMATION, CHRONIC	1 (7%)			
*COAGULATING GLAND	(14)	(15)	(42)	(30)
INFLAMMATION PROLIFERATIVE				2 (7%)
#TESTIS	(14)	(14)	(41)	(28)
MINERALIZATION	3 (21%)	1 (7%)		3 (11%)
ABSCESS, NOS				1 (4%)
DEGENERATION, NOS			1 (2%)	1 (4%)
NECROSIS, NOS				3 (11%)
NECROSIS, FOCAL			1 (2%)	
NECROSIS, CASEOUS			1 (2%)	
ATROPHY, NOS	4 (29%)	5 (36%)	5 (12%)	10 (36%)
ATROPHY, FOCAL	1 (7%)			
ASPERMATOGENESIS				1 (4%)
SPERMATOGENIC ARREST	4 (29%)	3 (21%)	7 (17%)	6 (21%)
HYPOSPERMATOGENESIS		1 (7%)		5 (18%)
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)	
#TESTIS/TUBULE	(14)	(14)	(41)	(28)
DEGENERATION, NOS			1 (2%)	
*EPIDIDYMIS	(14)	(15)	(42)	(30)
RETENTION OF CONTENT				1 (3%)
SPERMATOCELE				1 (3%)
COLLAPSE				1 (3%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
PLASMA-CELL INFILTRATE				1 (3%)
INFLAMMATION PROLIFERATIVE				1 (3%)
ASPERMATOGENESIS	3 (21%)	4 (27%)	3 (7%)	10 (35%)
HYPOSPERMATOGENESIS	2 (14%)	3 (20%)	4 (10%)	5 (17%)
*VAS DEFERENS	(14)	(15)	(42)	(30)
SPERMATOCELE				1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(15)	(42)	(30)
INFLAMMATION PROLIFERATIVE			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(15)	(42)	(30)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)			1 (3%)
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				2
HYPERPLASIA, NOS				1
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/HISTO PERF			1	
AUTOLYSIS/NO NECROPSY	1		3	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED HCDD BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	15	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	41	27
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(15)	(41)	(27)
INFLAMMATION, CHRONIC				1 (4%)
HYPERPLASIA, FOCAL	1 (7%)			
HYPERKERATOSIS	1 (7%)		1 (2%)	2 (7%)
ACANTHOSIS				1 (4%)
VERRUCA				1 (4%)
*SUBCUT TISSUE	(15)	(15)	(41)	(27)
EDEMA, NOS		1 (7%)		
ULCER, NOS			1 (2%)	
ABSCESS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		2 (5%)	
#LUNG/BRONCHIOLE	(15)	(15)	(41)	(27)
INFLAMMATION, NOS				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (27%)	10 (67%)	22 (54%)	11 (41%)
#LUNG	(15)	(15)	(41)	(27)
ATELECTASIS			1 (2%)	1 (4%)
CONGESTION, NOS	2 (13%)			1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
INFLAMMATION, INTERSTITIAL	3 (20%)	3 (20%)	5 (12%)	2 (7%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE				1 (4%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
HYPERPLASIA, ADENOMATOUS	1 (7%)	3 (20%)	2 (5%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HEMATOPOIETIC SYSTEM				
*MAMMARY GLAND	(15)	(15)	(41)	(27)
ADENOSIS	2 (13%)		2 (5%)	4 (15%)
#BONE MARROW	(13)	(13)	(37)	(24)
FIBROUS OSTEODYSTROPHY	3 (23%)	1 (8%)	7 (19%)	5 (21%)
HYPERPLASIA, NOS			1 (3%)	
RETICULOCYTOSIS	1 (8%)			
#SPLEEN	(15)	(15)	(40)	(26)
CYST, NOS			1 (3%)	
NECROSIS, NOS				1 (4%)
HYPOPLASIA, NOS	1 (7%)	1 (7%)	3 (8%)	
ATROPHY, NOS		1 (7%)		
HYPERPLASIA, NODULAR			1 (3%)	
HYPERPLASIA, NOS	3 (20%)	2 (13%)		3 (12%)
MEGAKARYOCYTOSIS			1 (3%)	
HYPERPLASIA, LYMPHOID	1 (7%)			1 (4%)
HEMATOPOIESIS			2 (5%)	1 (4%)
#SPLENIC FOLLICLES	(15)	(15)	(40)	(26)
HYPERPLASIA, NOS	2 (13%)		4 (10%)	3 (12%)
#SPLENIC RED PULP	(15)	(15)	(40)	(26)
HYPOPLASIA, NOS	1 (7%)	1 (7%)	1 (3%)	3 (12%)
HEMATOPOIESIS	2 (13%)	1 (7%)	1 (3%)	
ERYTHROPOIESIS				1 (4%)
#LYMPH NODE	(12)	(12)	(30)	(22)
NECROSIS, FOCAL				1 (5%)
HYPERPLASIA, NOS	2 (17%)	3 (25%)	6 (20%)	1 (5%)
#CERVICAL LYMPH NODE	(12)	(12)	(30)	(22)
HYPERPLASIA, NOS	1 (8%)	4 (33%)	17 (57%)	6 (27%)
#PANCREATIC L.NODE	(12)	(12)	(30)	(22)
PIGMENTATION, NOS			1 (3%)	
HYPERPLASIA, NOS		1 (8%)	1 (3%)	1 (5%)
#LUNG/BRONCHIOLE	(15)	(15)	(41)	(27)
HYPERPLASIA, LYMPHOID		1 (7%)	1 (2%)	
#PAROTID GLAND	(15)	(12)	(36)	(26)
FIBROSING ADENOSIS			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LIVER	(15)	(15)	(41)	(26)
HEMATOPOIESIS	1 (7%)			1 (4%)
#ADRENAL	(15)	(14)	(41)	(26)
MYELOPOIESIS			1 (2%)	
#THYMUS	(15)	(15)	(39)	(21)
HYPERPLASIA, NOS		2 (13%)	2 (5%)	1 (5%)
#THYMIC MEDULLA	(15)	(15)	(39)	(21)
HYPERPLASIA, NOS	2 (13%)			
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(12)	(12)	(30)	(22)
LYMPHANGIECTASIS			1 (3%)	
#MESENTERIC L. NODE	(12)	(12)	(30)	(22)
LYMPHANGIECTASIS			2 (7%)	
#HEART	(15)	(14)	(40)	(26)
ENDOCARDIOSIS	2 (13%)	1 (7%)		
#HEART/ATRIUM	(15)	(14)	(40)	(26)
THROMBOSIS, NOS	1 (7%)		2 (5%)	
#MYOCARDIUM	(15)	(14)	(40)	(26)
INFLAMMATION, CHRONIC FOCAL	1 (7%)			
*CENTRAL VEINS/LIVER	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(12)	(36)	(26)
INFLAMMATION, NOS		2 (17%)	1 (3%)	1 (4%)
#PAROTID GLAND	(15)	(12)	(36)	(26)
INFLAMMATION, NOS			1 (3%)	2 (8%)
INFLAMMATION, CHRONIC FOCAL				1 (4%)
HYPERPLASIA, NOS				1 (4%)
#LIVER	(15)	(15)	(41)	(26)
HEMORRHAGIC CYST				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
INFLAMMATION, NOS	1 (7%)			
INFLAMMATION, FOCAL			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		3 (20%)	3 (7%)	
INFLAMMATION, ACUTE		1 (7%)		
NECROSIS, COAGULATIVE	1 (7%)	1 (7%)	1 (2%)	
AMYLOIDOSIS			1 (2%)	1 (4%)
LIPOIDOSIS		1 (7%)		
FOCAL CELLULAR CHANGE	1 (7%)			
CYTOLOGIC DEGENERATION				1 (4%)
#LIVER/CENTRILOBULAR	(15)	(15)	(41)	(26)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
NECROSIS, NOS	1 (7%)			
HEPATOCYTOMEGALY		1 (7%)		1 (4%)
#LIVER/PERIPORTAL	(15)	(15)	(41)	(26)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)			
HYPERPLASIA, NOS	1 (7%)			
#LIVER/KUPFFER CELL	(15)	(15)	(41)	(26)
HYPERPLASIA, NOS			1 (2%)	
*GALLBLADDER	(15)	(15)	(41)	(27)
CALCULUS, NOS				1 (4%)
INFLAMMATION, CHRONIC	1 (7%)			1 (4%)
#BILE DUCT	(15)	(15)	(41)	(26)
HYPERPLASIA, NOS	2 (13%)			1 (4%)
#PANCREAS	(15)	(15)	(40)	(26)
DILATATION/DUCTS				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (3%)	
HYPERPLASIA, NOS	1 (7%)			
#PANCREATIC DUCT	(15)	(15)	(40)	(26)
INFLAMMATION PROLIFERATIVE				1 (4%)
#STOMACH	(15)	(15)	(41)	(27)
INFLAMMATION, ACUTE	1 (7%)			
HYPERKERATOSIS			2 (5%)	4 (15%)
#GASTRIC MUCOSA	(15)	(15)	(41)	(27)
HYPOPLASIA, NOS		1 (7%)		
#FORESTOMACH	(15)	(15)	(41)	(27)
HYPERKERATOSIS		1 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#DUODENUM	(13)	(15)	(40)	(26)
NECROSIS, NOS		1 (7%)		
*RECTUM	(15)	(15)	(41)	(27)
ULCER, ACUTE		1 (7%)		
DESMOPLASIA		1 (7%)		
NECROSIS, NOS		1 (7%)		
URINARY SYSTEM				
#KIDNEY	(15)	(15)	(41)	(26)
HYDRONEPHROSIS	1 (7%)		1 (2%)	
GLOMERULONEPHRITIS, NOS	1 (7%)			1 (4%)
PYELONEPHRITIS, NOS		2 (13%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	5 (33%)	6 (40%)	26 (63%)	14 (54%)
PYELONEPHRITIS SUPPURATIVE		1 (7%)		
GLOMERULONEPHRITIS, ACUTE			1 (2%)	1 (4%)
INFLAMMATION, CHRONIC	2 (13%)	1 (7%)	1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	10 (67%)	12 (80%)	16 (39%)	7 (27%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS	2 (13%)	2 (13%)	2 (5%)	3 (12%)
INFARCT, HEALED			1 (2%)	
#KIDNEY/GLOMERULUS	(15)	(15)	(41)	(26)
AMYLOIDOSIS			1 (2%)	
#URINARY BLADDER	(13)	(13)	(30)	(19)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (8%)		
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, ACUTE NECROTIZING		1 (8%)		
INFLAMMATION, ACUTE/CHRONIC		2 (15%)		
INFLAMMATION, CHRONIC	4 (31%)	5 (38%)	10 (33%)	10 (53%)
INFLAMMATION, CHRONIC FOCAL			2 (7%)	
INFLAMMATION PROLIFERATIVE		1 (8%)		
ATROPHY, NOS			1 (3%)	
METAPLASIA, SQUAMOUS				1 (5%)
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(14)	(41)	(26)
CONGESTION, NOS			2 (5%)	1 (4%)
DEGENERATION PIGMENTARY	9 (60%)	8 (57%)	22 (54%)	16 (62%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
AMYLOID, NOS				1 (4%)
HYPERPLASIA, NOS				1 (4%)
#ADRENAL CORTEX	(15)	(14)	(41)	(26)
HAMARTOMA	1 (7%)	1 (7%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (2%)	
#ZONA GLOMERULOSA	(15)	(14)	(41)	(26)
METAPLASIA, NOS	6 (40%)	9 (64%)	20 (49%)	12 (46%)
#THYROID	(15)	(15)	(39)	(20)
INFLAMMATION, NOS		1 (7%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)	
#THYROID FOLLICLE	(15)	(15)	(39)	(20)
GOITER COLLOID			1 (3%)	
#PANCREATIC ISLETS	(15)	(15)	(40)	(26)
HYPERPLASIA, NOS		1 (7%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(41)	(27)
DILATATION/DUCTS	3 (20%)	1 (7%)	3 (7%)	5 (19%)
*VAGINA	(15)	(15)	(41)	(27)
INFLAMMATION, ACUTE/CHRONIC		1 (7%)		
INFLAMMATION PROLIFERATIVE				2 (7%)
HYPERPLASIA, EPITHELIAL		1 (7%)		
#UTERUS	(15)	(15)	(36)	(26)
MINERALIZATION			1 (3%)	
HEMORRHAGE			1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)
ABSCESS, NOS			1 (3%)	
NECROSIS, NOS			1 (3%)	
#CERVIX UTERI	(15)	(15)	(36)	(26)
INFLAMMATION, CHRONIC		1 (7%)		
#UTERUS/ENDOMETRIUM	(15)	(15)	(36)	(26)
HYPERPLASIA, NOS	1 (7%)	5 (33%)	8 (22%)	2 (8%)
HYPERPLASIA, CYSTIC	12 (80%)	9 (60%)	22 (61%)	23 (88%)
#OVARY	(14)	(15)	(33)	(25)
CYST, NOS	1 (7%)		4 (12%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ATRESIA	10 (71%)	12 (80%)	20 (61%)	18 (72%)
HEMORRHAGIC CYST	1 (7%)	3 (20%)	2 (6%)	3 (12%)
AMYLOIDOSIS				1 (4%)
CHOLESTEROL DEPOSIT				1 (4%)
ATROPHY, NOS	8 (57%)	11 (73%)	25 (76%)	20 (80%)
ATROPHY, CYSTIC	4 (29%)		1 (3%)	2 (8%)
LUTEINIZATION	2 (14%)	3 (20%)	2 (6%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(15)	(15)	(41)	(27)
ABSCESS, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
BODY CAVITIES				
*ABDOMINAL WALL	(15)	(15)	(41)	(27)
HERNIA, NOS			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
HEMORRHAGE				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (20%)		1 (2%)	1 (4%)
AMYLOIDOSIS	1 (7%)		3 (7%)	2 (7%)
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				1
SPECIAL MORPHOLOGY SUMMARY				
AUTOLYSIS/NO NECROPSY			4	3

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX C

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

TABLE C1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	15	42	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	42	24
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(42)	(24)
SQUAMOUS CELL CARCINOMA				2 (8%)
TRICHOEPITHELIOMA				1 (4%)
FIBROSARCOMA		1 (7%)	1 (2%)	
*SUBCUT TISSUE	(14)	(15)	(42)	(24)
SEBACEOUS ADENOMA			1 (2%)	
FIBROMA			1 (2%)	
FIBROSARCOMA		2 (13%)	2 (5%)	3 (13%)
LIPOMA		1 (7%)		
RESPIRATORY SYSTEM				
#LUNG	(13)	(15)	(41)	(24)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (8%)	1 (7%)	6 (15%)	3 (13%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (15%)	2 (13%)	1 (2%)	3 (13%)
CORTICAL CARCINOMA, METASTATIC	1 (8%)			
FIBROSARCOMA, METASTATIC			1 (2%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(42)	(24)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (7%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)	3 (20%)	2 (5%)	2 (8%)
GRANULOCYTIC LEUKEMIA			1 (2%)	
#SPLEEN	(13)	(13)	(40)	(23)
FIBROMA				1 (4%)
#LYMPH NODE	(8)	(7)	(25)	(15)
FIBROSARCOMA, INVASIVE		1 (14%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#RENAL LYMPH NODE	(8)	(7)	(25)	(15)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (14%)		
CIRCULATORY SYSTEM				
#HEART	(13)	(15)	(42)	(22)
HEMANGIOSARCOMA	1 (8%)			
#LIVER	(14)	(15)	(42)	(22)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(14)	(15)	(42)	(22)
HEPATOCELLULAR ADENOMA			1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (7%)	2 (13%)		
URINARY SYSTEM				
#KIDNEY	(14)	(14)	(42)	(23)
SARCOMA, NOS, UNC PRIM OR META		1 (7%)		
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(15)	(40)	(22)
CORTICAL CARCINOMA	1 (9%)			
#THYROID	(13)	(14)	(35)	(22)
ADENOMA, NOS	1 (8%)			
#PARATHYROID	(5)	(2)	(6)	(6)
ACINAR-CELL ADENOMA				1 (17%)
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(15)	(42)	(24)
FIBROSARCOMA			1 (2%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATH@	7	13	30	23
MORIBUND SACRIFICE	1		8	4
**SCHEDULED SACRIFICE	5		4	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	2	2	3	3
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** Animals are in fact early terminal sacrifices, but appear as scheduled sacrifices due to system interpretation.

TABLE C1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	10	15	11
TOTAL PRIMARY TUMORS	9	15	19	16
TOTAL ANIMALS WITH BENIGN TUMORS	2	2	8	5
TOTAL BENIGN TUMORS	2	2	9	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	8	8	7
TOTAL MALIGNANT TUMORS	7	12	10	10
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2	
TOTAL SECONDARY TUMORS	1	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC		1		
TOTAL UNCERTAIN TUMORS		1		

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE C2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	15	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	41	27
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(15)	(41)	(27)
SQUAMOUS CELL CARCINOMA				1 (4%)
*SUBCUT TISSUE	(15)	(15)	(41)	(27)
BASAL-CELL CARCINOMA				1 (4%)
FIBROSARCOMA			2 (5%)	3 (11%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(15)	(41)	(25)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (7%)	4 (10%)	1 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (13%)		5 (12%)	2 (8%)
SARCOMA, NOS, METASTATIC				1 (4%)
FIBROSARCOMA, METASTATIC			1 (2%)	1 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (27%)	2 (13%)	9 (22%)	2 (7%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (13%)	1 (7%)	4 (10%)	3 (11%)
#BONE MARROW	(13)	(13)	(37)	(23)
FIBROMA			1 (3%)	
#SPLEEN	(15)	(15)	(40)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
FIBROMA			1 (3%)	
#PANCREATIC L.NODE	(12)	(12)	(30)	(16)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	1 (6%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#SMALL INTESTINE	(13)	(15)	(40)	(26)
ADENOMA, NOS				1 (4%)
*RECTUM	(15)	(15)	(41)	(27)
SQUAMOUS CELL CARCINOMA		1 (7%)		
URINARY SYSTEM				
*GENITOURINARY TRACT	(15)	(15)	(41)	(27)
FIBROSARCOMA			1 (2%)	
#URINARY BLADDER	(13)	(13)	(30)	(19)
ADENOMATOUS POLYP, NOS				1 (5%)
ENDOCRINE SYSTEM				
#ADRENAL	(15)	(14)	(41)	(26)
CORTICAL ADENOMA	1 (7%)			
REPRODUCTIVE SYSTEM				
#UTERUS	(15)	(15)	(36)	(26)
ADENOCARCINOMA, NOS			1 (3%)	
LEIOMYOMA	2 (13%)	1 (7%)		2 (8%)
LEIOMYOSARCOMA				1 (4%)
#CERVIX UTERI	(15)	(15)	(36)	(26)
LEIOMYOMA	1 (7%)			
#OVARY	(14)	(15)	(33)	(25)
LUTEOMA	1 (7%)			
GRANULOSA-CELL TUMOR		1 (7%)	1 (3%)	3 (12%
LIPOMA			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MALIGNANT LYMPHOMA, MIXED TYPE		1 (8%)		
#LUMBAR LYMPH NODE	(12)	(12)	(30)	(16)
CARCINOMA, NOS, METASTATIC				1 (6%)
#THYMUS	(15)	(15)	(39)	(27)
THYMOMA, MALIGNANT			1 (3%)	
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(12)	(12)	(30)	(16)
HEMANGIOMA		1 (8%)		
#LIVER	(15)	(15)	(41)	(27)
HEMANGIOSARCOMA	1 (7%)		1 (2%)	
#UTERUS	(15)	(15)	(36)	(26)
HEMANGIOMA			1 (3%)	
#OVARY	(14)	(15)	(33)	(25)
HEMANGIOMA			1 (3%)	1 (4%)
DIGESTIVE SYSTEM				
#PAROTID GLAND	(15)	(12)	(36)	(23)
MIXED TUMOR, BENIGN			1 (3%)	
#LIVER	(15)	(15)	(41)	(27)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
SARCOMA, NOS, INVASIVE				1 (4%)
FIBROSARCOMA, INVASIVE				1 (4%)
#PANCREAS	(15)	(15)	(40)	(26)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
LIPOMA			1 (3%)	
#PANCREATIC DUCT	(15)	(15)	(40)	(26)
SARCOMA, NOS			1 (3%)	
#STOMACH	(15)	(15)	(41)	(26)
SARCOMA, NOS				1 (4%)
#GASTRIC SEROSA	(15)	(15)	(41)	(26)
SARCOMA, NOS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(15)	(15)	(41)	(27)
LIPOMA				1 (4%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
THYMOMA, METASTATIC			1 (2%)	
FIBROSARCOMA				1 (4%)
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	45	30
NATURAL DEATHə	8	11	28	21
MORIBUND SACRIFICE	1	1	1	2
**SCHEDULED SACRIFICE	3	1	15	5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	3	2	1	2
ANIMAL MISSING				

ə INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** Animals are in fact early terminal sacrifices, but appear as scheduled sacrifices due to system interpretation.

TABLE C2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	25	20
TOTAL PRIMARY TUMORS	15	9	39	26
TOTAL ANIMALS WITH BENIGN TUMORS	6	3	10	6
TOTAL BENIGN TUMORS	6	3	11	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	5	23	16
TOTAL MALIGNANT TUMORS	9	5	27	16
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	4
TOTAL SECONDARY TUMORS			6	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1	3
TOTAL UNCERTAIN TUMORS		1	1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	14	15	42	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	42	24
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(42)	(24)
EDEMA, NOS			1 (2%)	
INFLAMMATION, NOS			2 (5%)	
ULCER, NOS		1 (7%)	3 (7%)	8 (33%)
INFLAMMATION, NECROTIZING				1 (4%)
ULCER, ACUTE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	1 (4%)
INFLAMMATION, CHRONIC	1 (7%)			2 (8%)
ULCER, CHRONIC		1 (7%)		
INFLAMMATION, CHRONIC NECROTIZIN				1 (4%)
INFLAMMATION PROLIFERATIVE			1 (2%)	
NECROSIS, NOS				1 (4%)
EPIDERMODYSPLASIA VERRUCIFORMIS				1 (4%)
HYPERPLASIA, NOS			1 (2%)	1 (4%)
HYPERPLASIA, PAPILLARY			1 (2%)	
HYPERKERATOSIS	1 (7%)		3 (7%)	2 (8%)
ACANTHOSIS		1 (7%)	1 (2%)	5 (21%)
VERRUCA				1 (4%)
*SUBCUT TISSUE	(14)	(15)	(42)	(24)
EPIDERMAL INCLUSION CYST			1 (2%)	
INFLAMMATION, NOS				1 (4%)
ULCER, NOS			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
ABSCESS, NOS				1 (4%)
GRANULATION, TISSUE			1 (2%)	
SCAR				1 (4%)
NECROSIS, NOS			2 (5%)	2 (8%)
RESPIRATORY SYSTEM				
*NASAL CAVITY	(14)	(15)	(42)	(24)
INFLAMMATION, ACUTE SUPPURATIVE				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#LUNG/BRONCHUS	(13)	(15)	(41)	(24)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (8%)		1 (2%)	1 (4%)
#LUNG/BRONCHIOLE	(13)	(15)	(41)	(24)
LYMPHOCYTIC INFLAMMATORY INFILTR	6 (46%)	4 (27%)	13 (32%)	5 (21%)
HYPERPLASIA, EPITHELIAL			1 (2%)	
#LUNG	(13)	(15)	(41)	(24)
ATELECTASIS			2 (5%)	
CONGESTION, NOS		3 (20%)	3 (7%)	6 (25%)
INFLAMMATION, FOCAL			1 (2%)	1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (7%)		2 (8%)
INFLAMMATION, INTERSTITIAL	1 (8%)	2 (13%)	3 (7%)	4 (17%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (7%)	1 (2%)	
EMPYEMA			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
NECROSIS, NOS			1 (2%)	
ALVEOLAR MACROPHAGES			1 (2%)	
HEMATOPOIETIC SYSTEM				
*SKIN	(14)	(15)	(42)	(24)
PARAKERATOSIS				1 (4%)
#BONE MARROW	(14)	(14)	(40)	(24)
MYELOFIBROSIS				1 (4%)
#SPLEEN	(13)	(13)	(40)	(23)
AMYLOIDOSIS		3 (23%)		4 (17%)
HYPOPLASIA, NOS	1 (8%)		5 (13%)	2 (9%)
ATROPHY, NOS		1 (8%)		
HYPERPLASIA, NOS	2 (15%)		4 (10%)	
MEGAKARYOCYTOSIS				1 (4%)
HYPERPLASIA, GRANULOCYTIC				1 (4%)
HYPERPLASIA, RETICULUM CELL				1 (4%)
HYPERPLASIA, LYMPHOID				1 (4%)
HEMATOPOIESIS			5 (13%)	6 (26%)
#SPLENIC FOLLICLES	(13)	(13)	(40)	(23)
HYPOPLASIA, NOS				3 (13%)
HYPERPLASIA, NOS		2 (15%)	2 (5%)	1 (4%)
#SPLENIC RED PULP	(13)	(13)	(40)	(23)
HYPOPLASIA, NOS			2 (5%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MEGAKARYOCYTOSIS			1 (3%)	
HEMATOPOIESIS		2 (15%)		
ERYTHROPOIESIS				1 (4%)
GRANULOPOIESIS			1 (3%)	
#LYMPH NODE	(8)	(7)	(25)	(15)
NECROSIS, CASEOUS				1 (7%)
HYPERPLASIA, NOS			2 (8%)	1 (7%)
HYPERPLASIA, LYMPHOID				1 (7%)
#CERVICAL LYMPH NODE	(8)	(7)	(25)	(15)
HYPERPLASIA, NOS	4 (50%)	3 (43%)	10 (40%)	6 (40%)
#TRACHEAL LYMPH NODE	(8)	(7)	(25)	(15)
HYPERPLASIA, NOS	1 (13%)			
#PYLORIC LYMPH NODE	(8)	(7)	(25)	(15)
HEMORRHAGE		1 (14%)		
#LUMBAR LYMPH NODE	(8)	(7)	(25)	(15)
HYPERPLASIA, LYMPHOID			1 (4%)	1 (7%)
#MESENTERIC L. NODE	(8)	(7)	(25)	(15)
ABSCESS, NOS			1 (4%)	
INFLAMMATION PROLIFERATIVE			1 (4%)	
NECROSIS, CASEOUS			1 (4%)	
#RENAL LYMPH NODE	(8)	(7)	(25)	(15)
HYPERPLASIA, NOS	1 (13%)			
#BRACHIAL LYMPH NODE	(8)	(7)	(25)	(15)
ABSCESS, NOS				1 (7%)
#INGUINAL LYMPH NODE	(8)	(7)	(25)	(15)
INFLAMMATION, CHRONIC				1 (7%)
#LUNG/BRONCHIOLE	(13)	(15)	(41)	(24)
HYPERPLASIA, LYMPHOID			1 (2%)	
#LIVER	(14)	(15)	(42)	(22)
HEMATOPOIESIS			1 (2%)	1 (5%)
#KIDNEY	(14)	(14)	(42)	(23)
HYPERPLASIA, LYMPHOID			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
CIRCULATORY SYSTEM				
#PANCREATIC L.NODE	(8)	(7)	(25)	(15)
LYMPHANGIECTASIS			1 (4%)	
#MESENTERIC L. NODE	(8)	(7)	(25)	(15)
LYMPHANGIECTASIS	1 (13%)			1 (7%)
#HEART	(13)	(15)	(42)	(22)
MINERALIZATION	1 (8%)			
#HEART/ATRIUM	(13)	(15)	(42)	(22)
THROMBOSIS, NOS		2 (13%)		1 (5%)
#MYOCARDIUM	(13)	(15)	(42)	(22)
MINERALIZATION		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE				2 (9%)
INFLAMMATION, CHRONIC FOCAL		2 (13%)	3 (7%)	1 (5%)
INFLAMMATION PROLIFERATIVE		1 (7%)		
NECROSIS, FOCAL		1 (7%)		
#ENDOCARDIUM	(13)	(15)	(42)	(22)
INFLAMMATION PROLIFERATIVE	1 (8%)			
HYPERPLASIA, NOS	1 (8%)			
*RENAL ARTERY	(14)	(15)	(42)	(24)
ARTERIOSCLEROSIS, NOS			1 (2%)	
#HEPATIC SINUSOID	(14)	(15)	(42)	(22)
RETENTION OF CONTENT				1 (5%)
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(12)	(15)	(36)	(21)
INFLAMMATION, FOCAL		1 (7%)		
#PAROTID GLAND	(12)	(15)	(36)	(21)
INFLAMMATION, NOS		1 (7%)	3 (8%)	
INFLAMMATION, FOCAL	1 (8%)			1 (5%)
NECROSIS, FOCAL			1 (3%)	
#LIVER	(14)	(15)	(42)	(22)
CONGESTION, NOS	1 (7%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
LYMPHOCYTIC INFLAMMATORY INFILTR			3 (7%)	
INFLAMMATION, ACUTE FOCAL		2 (13%)		
INFLAMMATION, ACUTE DIFFUSE			1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		1 (7%)		
CIRRHOSIS, NOS	1 (7%)			
DEGENERATION, HYDROPIC			1 (2%)	
NECROSIS, NOS	1 (7%)		1 (2%)	
NECROSIS, FOCAL		2 (13%)	1 (2%)	
NECROSIS, COAGULATIVE		2 (13%)		
AMYLOIDOSIS		3 (20%)		1 (5%)
AMYLOIDOSIS, FOCAL				1 (5%)
LIPOIDOSIS	1 (7%)			
HEPATOCYTOMEGALY	1 (7%)		2 (5%)	
#PORTAL TRACT	(14)	(15)	(42)	(22)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
#LIVER/CENTRILOBULAR	(14)	(15)	(42)	(22)
METAMORPHOSIS FATTY	1 (7%)			
HEPATOCYTOMEGALY	5 (36%)		1 (2%)	3 (14%)
#LIVER/PERIPORTAL	(14)	(15)	(42)	(22)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (7%)	2 (5%)	
INFLAMMATION, ACUTE SUPPURATIVE				1 (5%)
#LIVER/HEPATOCYTES	(14)	(15)	(42)	(22)
CLOUDY SWELLING	1 (7%)			
ATROPHY, NOS		1 (7%)		
ATROPHY, DIFFUSE				1 (5%)
#BILE DUCT	(14)	(15)	(42)	(22)
INFLAMMATION, CHRONIC				1 (5%)
#STOMACH	(14)	(13)	(42)	(23)
HYPERPLASIA, EPITHELIAL		1 (8%)		
HYPERKERATOSIS		1 (8%)	3 (7%)	1 (4%)
#GASTRIC MUCOSA	(14)	(13)	(42)	(23)
HYPERPLASIA, NOS				1 (4%)
#DUODENUM	(11)	(12)	(34)	(19)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (9%)			
#COLON	(11)	(15)	(36)	(19)
IMPACTION, NOS		2 (13%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
NEMATODIASIS				1 (5%)
URINARY SYSTEM				
*GENITOURINARY TRACT	(14)	(15)	(42)	(24)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
ABSCESS, NOS				1 (4%)
INFLAMMATION ACUTE AND CHRONIC				1 (4%)
#KIDNEY	(14)	(14)	(42)	(23)
HYDRONEPHROSIS	1 (7%)		1 (2%)	
GLOMERULONEPHRITIS, NOS		1 (7%)		
PYELONEPHRITIS, NOS		1 (7%)	5 (12%)	2 (9%)
LYMPHOCYTIC INFLAMMATORY INFILTR	8 (57%)	10 (71%)	20 (48%)	13 (57%)
INFLAMMATION, INTERSTITIAL				1 (4%)
PUS				2 (9%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)	
INFLAMMATION, CHRONIC		1 (7%)	1 (2%)	2 (9%)
GLOMERULONEPHRITIS, CHRONIC	9 (64%)	3 (21%)	6 (14%)	5 (22%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	1 (4%)
GROWTH, ALTERATION				1 (4%)
#KIDNEY/GLOMERULUS	(14)	(14)	(42)	(23)
AMYLOIDOSIS			1 (2%)	1 (4%)
#KIDNEY/TUBULE	(14)	(14)	(42)	(23)
CALCULUS, NOS			1 (2%)	
CALCIFICATION, NOS			1 (2%)	
#KIDNEY/PELVIS	(14)	(14)	(42)	(23)
ABSCESS, NOS			1 (2%)	
METAPLASIA, SQUAMOUS			1 (2%)	
#URINARY BLADDER	(13)	(14)	(39)	(20)
CAST, NOS			1 (3%)	
INFLAMMATION, ACUTE			1 (3%)	1 (5%)
INFLAMMATION, ACUTE FOCAL		1 (7%)		
INFLAMMATION, ACUTE SUPPURATIVE		1 (7%)		
INFLAMMATION, CHRONIC	4 (31%)		3 (8%)	5 (25%)
INFLAMMATION, CHRONIC DIFFUSE		1 (7%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
INFLAMMATION PROLIFERATIVE		1 (7%)	3 (8%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
FIBROSIS		1 (7%)		
HYPERPLASIA, EPITHELIAL		2 (14%)	1 (3%)	1 (5%)
POLYPOID HYPERPLASIA			1 (3%)	
ENDOCRINE SYSTEM				
#ADRENAL	(11)	(15)	(40)	(22)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (3%)	
DEGENERATION PIGMENTARY	5 (45%)	2 (13%)	4 (10%)	
AMYLOIDOSIS			1 (3%)	1 (5%)
LIPOIDOSIS	1 (9%)			
#ADRENAL CORTEX	(11)	(15)	(40)	(22)
HAMARTOMA		2 (13%)		
#ZONA GLOMERULOSA	(11)	(15)	(40)	(22)
METAPLASIA, NOS	5 (45%)	6 (40%)	6 (15%)	3 (14%)
#ZONA FASCICULATA	(11)	(15)	(40)	(22)
HYPERTROPHY, FOCAL	1 (9%)			
#ADRENAL MEDULLA	(11)	(15)	(40)	(22)
CONGESTION, NOS			1 (3%)	
HEMORRHAGE		1 (7%)		
REPRODUCTIVE SYSTEM				
*GENITAL SYSTEM	(14)	(15)	(42)	(24)
RETENTION OF CONTENT	1 (7%)	1 (7%)	2 (5%)	
INFLAMMATION, SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
PLASMA-CELL INFILTRATE			1 (2%)	
*BULBOURETHRAL GLAND	(14)	(15)	(42)	(24)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	1 (4%)
POLYPOID HYPERPLASIA			1 (2%)	
*PREPUTIAL GLAND	(14)	(15)	(42)	(24)
EPIDERMAL INCLUSION CYST			1 (2%)	
GRANULATION, TISSUE			1 (2%)	
#PROSTATE	(13)	(14)	(39)	(20)
RETENTION OF CONTENT	9 (69%)	7 (50%)	18 (46%)	8 (40%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	2 (10%)
INFLAMMATION, ACUTE NECROTIZING				1 (5%)
INFLAMMATION, CHRONIC FOCAL		2 (14%)		
INFLAMMATION, CHRONIC SUPPURATIV			2 (5%)	
*SEMINAL VESICLE	(14)	(15)	(42)	(24)
DILATATION, NOS	2 (14%)			
RETENTION OF CONTENT	9 (64%)	7 (47%)	18 (43%)	8 (33%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	2 (8%)
INFLAMMATION, CHRONIC	1 (7%)			
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)
#TESTIS	(14)	(14)	(41)	(24)
MINERALIZATION	3 (21%)	1 (7%)		2 (8%)
DEGENERATION, NOS			1 (2%)	
NECROSIS, FOCAL			1 (2%)	
NECROSIS, CASEOUS			1 (2%)	
ATROPHY, NOS	4 (29%)	5 (36%)	5 (12%)	4 (17%)
ATROPHY, FOCAL	1 (7%)			
ATROPHY, DIFFUSE				1 (4%)
SPERMATOGENIC ARREST	4 (29%)	3 (21%)	7 (17%)	4 (17%)
HYPOSPERMATOGENESIS		1 (7%)		
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)	1 (4%)
#TESTIS/TUBULE	(14)	(14)	(41)	(24)
DEGENERATION, NOS			1 (2%)	
*EPIDIDYMIS	(14)	(15)	(42)	(24)
DILATATION, NOS				1 (4%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)	
ASPERMATOGENESIS	3 (21%)	4 (27%)	3 (7%)	4 (17%)
HYPOSPERMATOGENESIS	2 (14%)	3 (20%)	4 (10%)	1 (4%)
*VAS DEFERENS	(14)	(15)	(42)	(24)
SPERMATOCELE				1 (4%)
INFLAMMATION, CHRONIC				1 (4%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(14)	(15)	(42)	(24)
INFLAMMATION PROLIFERATIVE			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(14)	(15)	(42)	(24)
TRAUMATIC ABNORMALITY				1 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)			2 (8%)
BACTERIAL SEPTICEMIA				1 (4%)
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				3
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/HISTO PERF			1	
AUTOLYSIS/NO NECROPSY	1		3	6

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED HCDD PLUS DMBA BY DERMAL APPLICATION

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
ANIMALS INITIALLY IN STUDY	15	15	45	30
ANIMALS NECROPSIED	15	15	41	27
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	41	27
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(15)	(41)	(27)
EDEMA, NOS				1 (4%)
NECROSIS, CASEOUS				1 (4%)
HYPERPLASIA, FOCAL	1 (7%)			
HYPERKERATOSIS	1 (7%)		1 (2%)	
*SUBCUT TISSUE	(15)	(15)	(41)	(27)
EDEMA, NOS		1 (7%)		
ULCER, NOS			1 (2%)	
ABSCESS, NOS			1 (2%)	
NECROSIS, NOS			1 (2%)	2 (7%)
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(15)	(15)	(41)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		2 (5%)	1 (4%)
#LUNG/BRONCHIOLE	(15)	(15)	(41)	(25)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (27%)	10 (67%)	22 (54%)	11 (44%)
#LUNG	(15)	(15)	(41)	(25)
ATELECTASIS			1 (2%)	2 (8%)
CONGESTION, NOS	2 (13%)			3 (12%)
INFLAMMATION, INTERSTITIAL	3 (20%)	3 (20%)	5 (12%)	6 (24%)
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)	
HYPERPLASIA, ADENOMATOUS	1 (7%)	3 (20%)	2 (5%)	
HEMATOPOIETIC SYSTEM				
*MAMMARY GLAND	(15)	(15)	(41)	(27)
ADENOSIS	2 (13%)		2 (5%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#BONE MARROW	(13)	(13)	(37)	(23)
FIBROUS OSTEODYSTROPHY	3 (23%)	1 (8%)	7 (19%)	7 (30%)
HYPERPLASIA, NOS			1 (3%)	
RETICULOCYTOSIS	1 (8%)			
#SPLEEN	(15)	(15)	(40)	(27)
CYST, NOS			1 (3%)	
PIGMENTATION, NOS				1 (4%)
HEMOSIDEROSIS				1 (4%)
HYPOPLASIA, NOS	1 (7%)	1 (7%)	3 (8%)	3 (11%)
ATROPHY, NOS		1 (7%)		
HYPERPLASIA, NODULAR			1 (3%)	
HYPERPLASIA, NOS	3 (20%)	2 (13%)		3 (11%)
MEGAKARYOCYTOSIS			1 (3%)	
HYPERPLASIA, LYMPHOID	1 (7%)			2 (7%)
HEMATOPOIESIS			2 (5%)	1 (4%)
#SPLENIC FOLLICLES	(15)	(15)	(40)	(27)
HYPOPLASIA, NOS				1 (4%)
HYPERPLASIA, NOS	2 (13%)		4 (10%)	
#SPLENIC RED PULP	(15)	(15)	(40)	(27)
HYPOPLASIA, NOS	1 (7%)	1 (7%)	1 (3%)	
HEMATOPOIESIS	2 (13%)	1 (7%)	1 (3%)	1 (4%)
#LYMPH NODE	(12)	(12)	(30)	(16)
HYPERPLASIA, NOS	2 (17%)	3 (25%)	6 (20%)	
#CERVICAL LYMPH NODE	(12)	(12)	(30)	(16)
HYPERPLASIA, NOS	1 (8%)	4 (33%)	17 (57%)	6 (38%)
#PANCREATIC L.NODE	(12)	(12)	(30)	(16)
PIGMENTATION, NOS			1 (3%)	
HYPERPLASIA, NOS		1 (8%)	1 (3%)	1 (6%)
#RENAL LYMPH NODE	(12)	(12)	(30)	(16)
HYPERPLASIA, NOS				1 (6%)
#LUNG/BRONCHIOLE	(15)	(15)	(41)	(25)
HYPERPLASIA, LYMPHOID		1 (7%)	1 (2%)	
#PAROTID GLAND	(15)	(12)	(36)	(23)
FIBROSING ADENOSIS			1 (3%)	
#LIVER	(15)	(15)	(41)	(27)
HEMATOPOIESIS	1 (7%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#ADRENAL	(15)	(14)	(41)	(26)
MYELOPOIESIS			1 (2%)	
#THYMUS	(15)	(15)	(39)	(27)
ECTOPIA				1 (4%)
HYPERPLASIA, NOS		2 (13%)	2 (5%)	1 (4%)
#THYMIC MEDULLA	(15)	(15)	(39)	(27)
HYPERPLASIA, NOS	2 (13%)			
CIRCULATORY SYSTEM				
#BONE MARROW	(13)	(13)	(37)	(23)
PERIARTERITIS				1 (4%)
#PANCREATIC L.NODE	(12)	(12)	(30)	(16)
LYMPHANGIECTASIS			1 (3%)	
#MESENTERIC L. NODE	(12)	(12)	(30)	(16)
LYMPHANGIECTASIS			2 (7%)	
#HEART	(15)	(14)	(40)	(27)
ENDOCARDIOSIS	2 (13%)	1 (7%)		
#HEART/ATRIUM	(15)	(14)	(40)	(27)
THROMBOSIS, NOS	1 (7%)		2 (5%)	
#MYOCARDIUM	(15)	(14)	(40)	(27)
INFLAMMATION, CHRONIC FOCAL	1 (7%)			1 (4%)
*CENTRAL VEINS/LIVER	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(15)	(12)	(36)	(23)
INFLAMMATION, NOS		2 (17%)	1 (3%)	
#PAROTID GLAND	(15)	(12)	(36)	(23)
INFLAMMATION, NOS			1 (3%)	2 (9%)
#LIVER	(15)	(15)	(41)	(27)
INFLAMMATION, NOS	1 (7%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
INFLAMMATION, FOCAL			1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		3 (20%)	3 (7%)	
INFLAMMATION, ACUTE		1 (7%)		
INFLAMMATION, ACUTE DIFFUSE				1 (4%)
INFLAMMATION, CHRONIC				1 (4%)
NECROSIS, COAGULATIVE	1 (7%)	1 (7%)	1 (2%)	1 (4%)
AMYLOIDOSIS			1 (2%)	
LIPOIDOSIS		1 (7%)		
FOCAL CELLULAR CHANGE	1 (7%)			
#LIVER/CENTRILOBULAR	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
NECROSIS, NOS	1 (7%)			
METAMORPHOSIS FATTY				1 (4%)
HEPATOCYTOMEGALY		1 (7%)		
#LIVER/PERIPORTAL	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)			
HYPERPLASIA, NOS	1 (7%)			
#LIVER/KUPFFER CELL	(15)	(15)	(41)	(27)
HYPERPLASIA, NOS			1 (2%)	
*GALLBLADDER	(15)	(15)	(41)	(27)
INFLAMMATION, CHRONIC	1 (7%)			
#BILE DUCT	(15)	(15)	(41)	(27)
HYPERPLASIA, NOS	2 (13%)			
#PANCREAS	(15)	(15)	(40)	(26)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (3%)	
HYPERPLASIA, NOS	1 (7%)			
#STOMACH	(15)	(15)	(41)	(26)
INFLAMMATION, ACUTE	1 (7%)			
HYPERKERATOSIS			2 (5%)	1 (4%)
#GASTRIC MUCOSA	(15)	(15)	(41)	(26)
HYPOPLASIA, NOS		1 (7%)		
#FORESTOMACH	(15)	(15)	(41)	(26)
HYPERKERATOSIS		1 (7%)		
#SMALL INTESTINE	(13)	(15)	(40)	(26)
PARASITISM				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#DUODENUM	(13)	(15)	(40)	(26)
NECROSIS, NOS		1 (7%)		
*RECTUM	(15)	(15)	(41)	(27)
ULCER, ACUTE		1 (7%)		
DESMOPLASIA		1 (7%)		
NECROSIS, NOS		1 (7%)		
URINARY SYSTEM				
#KIDNEY	(15)	(15)	(41)	(27)
HYDRONEPHROSIS	1 (7%)		1 (2%)	1 (4%)
GLOMERULONEPHRITIS, NOS	1 (7%)			
PYELONEPHRITIS, NOS		2 (13%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	5 (33%)	6 (40%)	26 (63%)	16 (59%)
PYELONEPHRITIS SUPPURATIVE		1 (7%)		
GLOMERULONEPHRITIS, ACUTE			1 (2%)	
INFLAMMATION, CHRONIC	2 (13%)	1 (7%)	1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	10 (67%)	12 (80%)	16 (39%)	11 (41%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)	
GLOMERULOSCLEROSIS, NOS	2 (13%)	2 (13%)	2 (5%)	
INFARCT, HEALED			1 (2%)	
#KIDNEY/GLOMERULUS	(15)	(15)	(41)	(27)
AMYLOID, NOS				1 (4%)
AMYLOIDOSIS			1 (2%)	
#KIDNEY/PELVIS	(15)	(15)	(41)	(27)
DILATATION, NOS				1 (4%)
#URINARY BLADDER	(13)	(13)	(30)	(19)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (8%)		
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, ACUTE NECROTIZING		1 (8%)		
INFLAMMATION, ACUTE/CHRONIC		2 (15%)		
INFLAMMATION, CHRONIC	4 (31%)	5 (38%)	10 (33%)	5 (26%)
INFLAMMATION, CHRONIC FOCAL			2 (7%)	
INFLAMMATION PROLIFERATIVE		1 (8%)		
ATROPHY, NOS			1 (3%)	
METAPLASIA, SQUAMOUS				1 (5%)
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(11)	(35)	(13)
HYPERPLASIA, CHROMOPHOBE-CELL				1 (8%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
#ADRENAL	(15)	(14)	(41)	(26)
CONGESTION, NOS			2 (5%)	
DEGENERATION PIGMENTARY	9 (60%)	8 (57%)	22 (54%)	16 (62%)
#ADRENAL CORTEX	(15)	(14)	(41)	(26)
HAMARTOMA	1 (7%)	1 (7%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)		1 (2%)	
#ZONA GLOMERULOSA	(15)	(14)	(41)	(26)
METAPLASIA, NOS	6 (40%)	9 (64%)	20 (49%)	12 (46%)
#THYROID	(15)	(15)	(39)	(22)
INFLAMMATION, NOS		1 (7%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)	
#THYROID FOLLICLE	(15)	(15)	(39)	(22)
GOITER COLLOID			1 (3%)	
#PANCREATIC ISLETS	(15)	(15)	(40)	(26)
HYPERPLASIA, NOS		1 (7%)		1 (4%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(41)	(27)
DILATATION/DUCTS	3 (20%)	1 (7%)	3 (7%)	2 (7%)
*VAGINA	(15)	(15)	(41)	(27)
INFLAMMATION, ACUTE/CHRONIC		1 (7%)		
HYPERPLASIA, EPITHELIAL		1 (7%)		
#UTERUS	(15)	(15)	(36)	(26)
MINERALIZATION			1 (3%)	
HEMORRHAGE			1 (3%)	
INFLAMMATION, ACUTE FOCAL				1 (4%)
ABSCESS, NOS			1 (3%)	
INFLAMMATION, CHRONIC				1 (4%)
NECROSIS, NOS			1 (3%)	
METAPLASIA, SQUAMOUS				1 (4%)
#CERVIX UTERI	(15)	(15)	(36)	(26)
INFLAMMATION, CHRONIC		1 (7%)		
#UTERUS/ENDOMETRIUM	(15)	(15)	(36)	(26)
INFLAMMATION, CHRONIC SUPPURATIV				1 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
HYPERPLASIA, NOS	1 (7%)	5 (33%)	8 (22%)	7 (27%)
HYPERPLASIA, CYSTIC	12 (80%)	9 (60%)	22 (61%)	17 (65%)
#OVARY	(14)	(15)	(33)	(25)
CYST, NOS	1 (7%)		4 (12%)	
ATRESIA	10 (71%)	12 (80%)	20 (61%)	13 (52%)
HEMORRHAGIC CYST	1 (7%)	3 (20%)	2 (6%)	2 (8%)
ATROPHY, NOS	8 (57%)	11 (73%)	25 (76%)	15 (60%)
ATROPHY, CYSTIC	4 (29%)		1 (3%)	6 (24%)
LUTEINIZATION	2 (14%)	3 (20%)	2 (6%)	1 (4%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(15)	(15)	(41)	(27)
ABSCESS, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
*SKELETAL MUSCLE	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)	
BODY CAVITIES				
*ABDOMINAL CAVITY	(15)	(15)	(41)	(27)
NECROSIS, FOCAL				1 (4%)
*ABDOMINAL WALL	(15)	(15)	(41)	(27)
HERNIA, NOS			1 (2%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(15)	(41)	(27)
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (20%)		1 (2%)	1 (4%)
AMYLOID, NOS				1 (4%)
AMYLOIDOSIS	1 (7%)		3 (7%)	
CONNECTIVE TISSUE				
INFLAMMATION PROLIFERATIVE				1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL NO. 1	UNTREATED CONTROL NO. 2	VEHICLE CONTROL	TEST GROUP
SPECIAL MORPHOLOGY SUMMARY				
AUTOLYSIS/NO NECROPSY			4	3

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

PREPARATION OF HCDD

APPENDIX E

Preparation of HCDD

3,4,5-Trichlorophenol (Aldrich Chemical Co., Milwaukee, Wis.) was brominated at 80°-90°C in glacial acetic acid and the product, 2-bromo-3,4,5-trichlorophenol, was recrystallized from methanol-water or benzene-hexane. The potassium salt of 2-bromo-3,4,5-trichlorophenol was prepared by treating the phenol with potassium hydride in benzene. The dry potassium salt of 2-bromo-3,4,5-trichlorophenol was covered with a layer of dry potassium carbonate and heated under sublimation conditions at 240°-270°C at 1 mm pressure for 20 hours. The crude product contained hexachlorodibenzo-p-dioxins, bromohexachlorodibenzo-p-dioxins (from the condensation of the 2,6-dibromo-3,4,5-trichlorophenol impurity in 2-bromo-3,4,5-trichlorophenol), and other minor impurities. The crude product was debrominated by hydrogenolysis with lithium aluminum hydride at 0°C to yield HCDD (Gray et al., 1975).

APPENDIX F

ANALYSES OF HCDD

APPENDIX F

Analysis of HCDD

Midwest Research Institute

A. Vapor-Phase Chromatography

Lot No. IIT 102

a. System 1:

Instrument:	Bendix 2500
Column:	3% OV-1, 1.8 m x 4 mm I.D.
Detector:	Electron capture, ^{63}Ni
Oven Temperature I:	160°C, isothermal
Compound Concentration:	0.032 mg/ml in benzene
Results:	One peak with a retention time identical to that of an authentic sample of tetrachlorodibenzo-p-dioxin. By comparison of the area of this peak with that of a weighed solution of tetrachlorodibenzo-p-dioxin, it was calculated that the tetrachloro compound was present at a concentration of 0.07%.

b. System 2:

Instrument:	Varian 1400
Column:	3% OV-1, 1.8 m x 4 mm I.D.
Detector:	Electron capture, Sc^3H_3
Oven Temperature I:	160°C, isothermal
Compound Concentration:	Saturated (2 mg/ml in benzene)
Results:	Major peak was not eluted in 45 minutes. Eight minor impurities detected.

Peak	Retention Time (min)	Retention Time (Relative to Tetrachloro-dibenzo-p-dioxin)	Possible Identity	Percent by Weight
1	4.2	0.11	Unknown	-
2	7.9	0.20	Dichlorodibenzo-p-dioxin	0.004
3	9.5	0.24	Unknown	-
4	11.7	0.29	Unknown	-
5	17.3	0.43	Trichlorodibenzo-p-dioxin	0.004
6	25.6	0.64	Unknown	-
7	34.5	0.87	Unknown	-
8	39.8	1.00	Tetrachlorodibenzo-p-dioxin	0.01

Possible identities were assigned to peaks which had retention times identical to those for authentic samples of other chlorinated dibenzo-p-dioxins. Percentage compositions by weight were calculated by comparison of the areas of the impurity peaks in the weighed sample with the area of the tentatively identified chlorinated dibenzo-p-dioxin in a weighed solution of similar concentration. No authentic sample of monochlorodibenzo-p-dioxin was available. It is possible that the first peak is the monochloro compound. No percentage compositions were calculated for the unknown peaks because of the great variation in response of electron capture detectors to different compounds.

c. System 3:

Instrument:	Varian 1400
Column:	3% OV-1, 1.8 m x 4 mm I.D.
Detector:	Electron capture, Sc^3H_3
Oven Temperature II:	225°C, isothermal
Compound Concentration:	0.032 mg/ml in benzene
Results:	Major peak and three impurities.

Peak	Retention Time (min)	Retention Time (Relative to Hexachloro-dibenzo-p-dioxin)	Identity	Area Relative to that of Hexachlorodi-benzo-p-dioxin
1	4.0	0.25	Tetrachlorodibenzo-p-dioxin	0.08
2	7.8	0.50	Unknown	-
3	8.6	0.54	Unknown	-
4	15.7	1.00	Hexachlorodibenzo-p-dioxin	100.00

Nothing else eluted in 50 minutes.

The first peak was again quantitated against an authentic sample of tetrachlorodibenzo-p-dioxin. No authentic sample of pentachlorodibenzo-p-dioxin was available. The two peaks with retention times intermediate between tetra- and hexachlorodibenzo-p-dioxin could be pentachloro compounds, but there was no way to verify this. Octachlorodibenzo-p-dioxin was not detected; the detection limit in this sample is less than 0.004%.

d. System 4:

Instrument:	Bendix 2500
Column:	3% Dexsil 400, 1.8 m x 2 mm I.D.
Detector:	Electron capture, ^{63}Ni
Oven Temperature III:	275°C, isothermal
Compound Concentration:	0.032 mg/ml in benzene
Results:	Major peak and two impurities with longer retention times.

Peak	Retention Time (min)	Retention Time (Relative to Hexachloro-dibenzo-p-dioxin)	Identity
1	2.1	1.00	Hexachlorodibenzo-p-dioxin
2	3.5	1.70	Unknown
3	4.8	2.30	Unknown

Nothing else eluted in 20 minutes.

Under these same conditions, octachlorodibenzo-p-dioxin had a retention time of 5.5 minutes and thus was not detected in the sample. This column separated two impurities with retention times intermediate between those of hexa- and octachlorodibenzo-p-dioxin, but no authentic sample was available.

2. Flame Ionization Detection (Lot No. IIT 102)

a. System 1:

Instrument:	Tracor MT 220
Column:	Stainless steel capillary coated with OV-101, 50 ft. x 0.62 in. I.D.
Oven Temperature:	190°C, isothermal
Results:	Major peak and three impurities.

Peak	Retention Time (min)	Retention Time (Relative to that of Hexachloro-dibenzo-p-dioxin)	Area (Relative to that of Hexachloro-dibenzo-p-dioxin)
1	6.0	0.50	0.2
2	6.8	0.57	0.6
3	11.3	0.94	1.40
4	12.0	1.00	100

Peak No. 3 was a shoulder on the major peak and probably did not separate from the major peak on the packed columns used with the electron capture detectors. Peaks 1 and 2 were too large to be due to the chlorinated dibenzo-p-dioxins (di-, tri-, and tetra-) observed and quantitated by electron capture, but it is possible that these are the unknown peaks detected by electron capture.

b. System 2:

Instrument:	Tracor MT 220
Column:	3% Dexsil 400, 1.8 m x 2 mm I.D.
Oven Temperature:	150° to 285°C at 10°C/minute
Results:	Major peak and one impurity.

Peak	Retention Time (min)	Retention Time (Relative to that of Hexachloro-dibenzo-p-dioxin)	Area (Relative to that of Hexachloro-dibenzo-p-dioxin)
1	9.6	0.85	0.2
2	11.3	1.00	100

c. System 3:

Instrument:	Tracor MT 220
Column:	5% N,N'-bis(p-methoxybenzylidine)- α, α'-bi-p-toluidine (liquid crystal), 1.2 m x 2 mm I.D.
Oven Temperature:	235°C, isothermal
Results:	Two peaks (indicating the presence of two isomers).

Peak	Retention Time (min)	Retention Time (Relative to that of Larger Peak)	Area (Relative to that of Larger Peak)
1	10.9	0.81	46
2	13.4	1.00	100

B. Mass Spectrometry

1. Vapor-Phase Chromatography/Mass Spectrometry (Lot No. IIT 102)

Instrument: Varian MAT CH4B mass spectrometer interfaced via a Watson-Biemann helium separator to a Tracor MT 2000 MF gas chromatograph. Data were processed by a Varian 620/i computer.

Column:	3% OV-1, 1.8 m x 2 mm I.D.
Oven Temperature:	210°C, isothermal
Results:	Only one peak, that for the major component, was detected on the ion current monitor. Specific ion searches for other possible impurities indicated the presence of pentachlorodibenzo-p-dioxin and bromopentachlorodibenzo-p-dioxin; the searches gave no evidence for the presence of other chlorinated dibenzo-p-dioxins or tetrabromomonochloro- or bromohexachlorodibenzo-p-dioxin.

Peak	Mass	Relative Intensities	Calculated Relative Intensities
Pentachlorodibenzo-p-dioxin	354	74	61
	356	100	100
	358	85	66
Bromopentachloro-dibenzo-p-dioxin	434	138	98
	436	100	100
	438	51	53

Peak	Mass	Intensity Relative to Base Peak	Relative Intensities of Parent Ion Cluster	Calculated Relative Intensities
Hexachlorodi-benzo-p-dioxin	28(N_2)	100	-	-
	262	5	-	-
	264	9	-	-
	325	12	-	-
	327	20	-	-
	329	12	-	-
	356	7	-	-
	388	47	57	51
	390	82	100	100
	391	10	12	13
	392	72	88	82
	394	30	37	36
	396	11	13	9

2. Direct Inlet Mass Spectrometry (Lot No. IIT 102)

Instrument:	Varian MAT CH4B mass spectrometer. Data processed by a Varian 620/i computer.
Results:	Mass spectrum consistent with the structure of the major component. Specific ion searches for the two most intense masses in the parent ion cluster of pentachloro-dibenzo-p-dioxin were positive, but these masses also occur in the fragmentation of hexachlorodibenzo-p-dioxin, so the presence of pentachlorodibenzo-p-dioxin could be neither confirmed nor denied. Specific ion searches did not detect any of the other chlorinated dibenzo-p-dioxins or bromo-, pentachloro-, tetrabromomonochloro-, or bromohexachlorodibenzo-p-dioxins or the anion of 2-bromo-3,4,5- trichlorophenol, the starting material in the synthesis of hexachlorodibenzo-p-dioxin. Peaks were detected with masses at 436, 485, 487, 492, 513, 515, 545, and 547-554, which could not be due to the major component. The origin of these peaks was not determined.

C. Special Analyses

Subsequent to the analyses described in (A) and (B) above, the following special analyses were performed.

1. Vapor-Phase Chromatography/Mass Spectrometry with Solid Injection

Instrument:	Varian MAT CH4B mass spectrometer interfaced via a Watson-Biemann helium separator to a Tracor MT 2000 MF gas chromatograph. Data processed by a Varian 620/i computer.
Column:	3% Dexsel 400, 1.8 m x 4 mm I.D. on Chromosorb W (AW)

Oven Temperature:	300°C, isothermal
Inlet Temperature:	320°C
Helium Separator Temperature:	340°C
Sample Injection:	0.5 mg hexachlorodibenzo-p-dioxin was loaded into a solid sampler (Analabs) and injected directly onto the column.
Results:	Two minor peaks were detected on the ion current monitor before the major peak was eluted. Specific ion searches for the masses in the parent ion cluster indicated that the first minor peak was tetrachlorodibenzo-p-dioxin.

TETRACHLORODIBENZO-P-DIOXIN

Mass	Relative Intensities	Calculated Relative Intensities
320	74	76
322	100	100
324	55	50

2. Vapor-Phase Chromatography with Electron Capture Detection

Instrument:	Varian Aerograph 1400
Detector:	Electron capture, Sc^3H_3
Column:	3% Dexsil 400 on Chromosorb W(AW) 1.8 m x 2 mm I.D., glass
Inlet Temperature:	230°C
Detector Temperature:	270°C
Oven Temperature:	220°C
Concentration of Hexachlorodibenzo-p-dioxin:	1.1 mg/ml in benzene
Percent Tetrachlorodibenzo-p-dioxin:	$0.09 \pm 0.03(\delta)$%

APPENDIX G

QUARTERLY ANALYSES OF HCDD STOCK SOLUTIONS

APPENDIX G

Quarterly Analyses of HCDD Stock Solutions

Stock solutions of HCDD in acetone were analyzed at the beginning and at the end of each quarter by the IITRI Chemistry Division. The method of analysis consisted of adding an internal standard (pentachlorodibenzo-p-dioxin, PCDD) to samples so that the internal standard concentration was approximately the same as that of the sample being analyzed. The solution containing sample and standard was then injected onto an electron capture/gas chromatography system. The column was a 2 m x 1/8 in. Dexsil 300 with a N_2/CH_4 carrier - gas flow rate of 50 ml/min and an oven temperature of 275°C. Quantitation was achieved by manually measuring the area under the resultant peaks with a planimeter and comparing with standard curves for the internal standard and test compound. The standard curve was represented by a third order polynomial equation fitting response to amounts.

The theoretical concentration for the stock solution was 2.5 μg/ml. The actual concentration as measured by the above method varied from 1.96 to 2.97 μg/ml. The mean was 2.83 μg/ml and the coefficient of variation was 27%.

Review of the Bioassay of 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HCDD)* (dermal) for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

February 15, 1980

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin (HCDD) for carcinogenicity.

The primary reviewer for the report on the bioassay of HCDD said that HCDD was not carcinogenic, under the conditions of test. An experimental limitation of the study may have been the relatively short lifespan of the animals. The reviewer pointed out the reduced incidence of lymphomas and leukemias among treated male mice.

The secondary reviewer noted that the dosages administered were almost a thousand-fold lower than those given in the gavage study of HCDD. He commented on the likelihood that a certain amount of the HCDD was taken in orally as a result of the animals licking one another. The toxic hepatitis observed may have been caused by the ingestion of the test material. In response to a question, a Program staff member said that no information was available on the amount of HCDD absorbed through the skin.

The primary reveiwer moved that the report on the bioassay of HCDD by dermal application be accepted as written. The motion was seconded and approved unanimously.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, Eppley Institute for Research in Cancer
Joseph Highland, Environmental Defense Fund
William Lijinsky, Federick Cancer Research Center
Henry C. Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Louise Strong, University of Texas Health Sciences Center

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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