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Document Title

A SUBCHRONIC (13-WEEK) DIETARY ORAL TOXICITY STUDY OF DI(ISONONYL)PHTHALATE IN FISCHER 344 RATS WITH ATTACHMENTS AND COVER LETTER DATED 082291

Chemical Category

DIISONONYL PHTHALATE (28553-12-0)

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ATTN: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Dear Sir or Madam:

Enclosed is a final report copy of an unpublished Health and Safety Study submitted on behalf of Aristech Chemical Corporation. This study, titled "A Subchronic (13-Week) Dietary Oral Toxicity Study of Di(isononyl)Phthalate in Fischer 344 Rats", is submitted pursuant to the reporting schedule listed at 40 CFR §716.60.

Information required at 40 CFR §716.30(b) is provided below:

Chemical Name: Diisononyl Phthalate

CAS Number: 28553-12-0

Submitting Official: John R. Bankston II

Title: Senior Regulatory Specialist

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If you have any questions regarding this information, please contact me.

Sincerely,

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John R. Bankston II

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HAZLETON
WASHINGTON

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FINAL REPORT

Study Title:

A Subchronic (13-Week) Dietary Oral Toxicity Study of Di(isononyl)phthalate in Fischer 344 Rats

Author:

Barbara A. Myers, Ph.D.

Study Completion Date:

August 12, 1991

Performing Laboratory:

Hazleton Washington, Inc.
1330-B Piccard Drive
Rockville, Maryland 20850

Laboratory Project Identification:

HWA Study No. 2598-102

COMPLIANCE STATEMENT
A Subchronic (13-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in Fischer 344 Rats

This study was conducted in compliance with the Good Laboratory Practice Regulations as set forth in Title 40 of the U.S. Code of Federal Regulations Part 792, issued November 29, 1983 (effective December 29, 1983), and with any applicable amendments. All deviations from the protocol and/or GLPs are listed in Appendix 14. There were no deviations from the aforementioned regulations which affected the quality or integrity of the study or the interpretation of the results in the report.

Study Director:
Michael R. Moore, Ph.D., D.A.B.T.
Assoc. Director, Toxicology
for

8/12/91

Barbara A. Myers, Ph.D.
Life Sciences Division

Date

QUALITY ASSURANCE STATEMENT

Study Title: A Subchronic (13-Week) Dietary Oral Toxicity Study of Di(isononyl)phthalate in Fischer 344 Rats

Project No.: 2598-102

Quality Assurance inspections and reviews of this study were conducted according to the standard operating procedures of the Quality Assurance Unit and according to the Good Laboratory Practice regulations of the Environmental Protection Agency (EPA - TSCA), Title 40 of the U.S. Code of Federal Regulations Part 792, issued November 29, 1983 (effective December 29, 1983) and with any applicable amendments. These inspections and reviews were performed and findings were reported to the Study Director and management as follows:

Dates of Inspection/Review	Dates Findings Reported to Management	Inspector/Reviewer
Protocol Review:		
9/11,14/90	9/14/90	M. Aderiye
Inspection and/or Data Review:		
10/10,17,26 & 11/2,5/90	12/11/90	B. Munch
12/12,13,13,20,21,27/90	1/14/91	B. Munch M. Aderiye
Report and Data Review:		
5/13-17;20,24,31; 6/3/91	6/3/91	S. Geroux

Saundra L. Geroux 8/2/91

Saundra L. Geroux / Date Released
Quality Assurance Unit

STUDY IDENTIFICATION
A Subchronic (13-week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in Fischer 344 Rats

HWA Study Number:	2598-102
Test Material:	Di(isononyl)phthalate
Sponsor:	Aristech Chemical Corporation 600 Grant Street, Room 1140 Pittsburgh, PA 15230-0250
Sponsor's Representative:	Bernard D. Astill, Ph.D. Bernard D. Astill Associates 195 Lyell Street Spencerport, NY 14559
Study Director:	Barbara A. Myers, Ph.D. Hazleton Washington, Inc. 1330-B Piccard Drive Rockville, Maryland 20850 (301) 670-9600
Study Timetable:	
Study Initiation:	March 28, 1990
Initiation of Dosing:	September 26, 1990
Completion of Necropsy:	December 28, 1990

STUDY PERSONNEL
A Subchronic (13-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in Fischer 344 Rats

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SUMMARY

This study was designed to evaluate the toxicity of Di(isononyl)phthalate (DINP) when administered in the diet to Fischer 344 rats for 13 weeks and to provide data necessary for designing longer duration toxicity studies. Ten rats/sex/group received feed to which DINP was added to achieve dose levels of 2,500 (low dose), 5,000, 10,000, or 20,000 (high dose) ppm, corresponding to dosage Groups 2 through 5, respectively. A control group of 10 rats/sex (Group 1) received basal diet only. All animals had ad libitum access to feed and were dosed until the day prior to sacrifice. Criteria evaluated for compound effect included survival, clinical signs, body weight, food consumption, ophthalmology, organ weights, and clinical, gross, and microscopic pathology.

All animals survived to study termination. There were no ophthalmoscopic findings related to compound treatment. An increased incidence of urine stains occurred in female rats receiving 10,000 and 20,000 ppm. Low body weight gain was observed in high-dose animals of both sexes. Relative to the corresponding control value, the depression in body weight gain was more pronounced in high-dose females than in males. Food intake was slightly decreased in high-dose females, but was comparable between control males and high-dose males. Weekly compound consumption in all groups of DINP-dosed females tended to be slightly greater than values in corresponding male dosage groups. Mild anemia occurred in compound-treated animals of both sexes and was more severe in males than females, as select hematology parameters were significantly lower than in corresponding controls in males from the 5,000 through 20,000 ppm groups and females from the 10,000 and 20,000 ppm groups. Increased serum albumin levels and/or decreased serum globulin levels were observed in males and females from the 10,000 and 20,000 ppm groups; serum globulin levels were also decreased in females receiving 5,000 ppm.

Liver, kidney, and nonglandular stomach were observed to be target organs. Mean liver weight parameters were significantly increased in a dose-dependent manner in all groups, with the exception of low-dose females. Hepatocellular enlargement was observed microscopically in animals of both sexes from the 20,000 ppm groups. Mean kidney weight parameters were significantly increased in all DINP-treated groups. Microscopic renal lesions, characterized as an increased severity of regenerative/basophilic tubules and as granular casts in the cortical medullary junction, were observed in males from the 5,000 to 20,000 ppm groups. In addition, mild renal dysfunction in DINP-dosed animals was suggested by effects on blood urea nitrogen levels, which were markedly elevated in males from the 10,000 and 20,000 ppm groups and slightly elevated in females from the 20,000 ppm group. Microscopic lesions of the nonglandular stomach were observed in select animals of both sexes from the 10,000 and 20,000 ppm groups, and were characterized as vesiculation and increased severity of acute inflammation. Uterine weight parameters in high-dose females were markedly lower than the corresponding values in control females; however, no gross or microscopic changes were associated with the altered organ weights for this tissue.

In conclusion, dosing of male and female Fischer 344 rats with Di(isononyl)phthalate in feed for 13 weeks at a level of 20,000 ppm was associated with low body weight gain, mild anemia, elevated kidney weights and BUN levels, and microscopic lesions of the liver and nonglandular stomach in animals of both sexes; microscopic renal lesions in males; and a slight decrease in food intake, an increased incidence of urine stains, and decreased uterine weight in females. Effects observed at the dietary level of 10,000 ppm included mild anemia, microscopic lesions of the nonglandular stomach, and elevated liver and kidney weights in animals of both sexes; microscopic renal lesions and elevated BUN levels in males; and an increased incidence of urine stains in females. The dietary level of 5000 ppm was associated with elevated liver and kidney weights in animals of both sexes, and with mild anemia and microscopic renal lesions

in males. At 2500 ppm, effects included significantly elevated kidney (both sexes) and liver (males) weight parameters. Body weight changes were more pronounced in DINP-dosed females than in males. With respect to hematology changes and renal pathology, males were more sensitive to the effects of DINP than females, particularly in light of the observation that compound consumption was lower in males than in females during the study. A no-observable-effect-level was not established for DINP at the levels used in this study.

INTRODUCTION

This study was designed to characterize the potential subchronic toxicity of Di(isononyl)phthalate when administered daily in the diet to Fischer 344 rats for 13 weeks and to provide data necessary for designing longer duration toxicity studies. Dosing began on September 26, 1990, and terminal sacrifices were completed on December 28, 1990.

The protocol was designed in accordance with the EPA Toxic Substances Control Act test guidelines, 40 CFR Part 798. The study was conducted in compliance with the Good Laboratory Practice Standards, 40 CFR Part 792. Any deviations from protocol and/or GLP are listed in Appendix 14.

TEST AND CONTROL MATERIALS

The test material, Di(isononyl)phthalate (DINP), Lot No. QCL 9004-273, was received from Aristech Chemical Corporation on May 22, 1990, and was stored at room temperature. The test article was described as a clear colorless liquid, with a purity of 99.9%. Information on the methods of synthesis and stability, as well as data on composition or other characteristics which define the test material, are on file with the Sponsor.

Purina® Certified Rodent Chow® #5002 was used as the basal diet and served as the control material.

Reserve samples of the test and control materials were taken and stored at room temperature. The reserve samples and all remaining test material will be forwarded to the Sponsor upon completion of the test program encompassed by the study agreement.

TEST ANIMALS AND HUSBANDRY

A total of 126 (64 male and 62 female) 29 day-old CDF®(F-344)/CrlBR rats was received on September 11, 1990, from Charles River Laboratories, Inc., Raleigh, North Carolina. Rats were the species of choice for this study because they have historically been used in safety evaluation studies and are recommended by appropriate regulatory agencies.

Caging Conditions - Upon receipt, the animals were housed two per cage in stainless-steel, hanging, wire-mesh cages. The animals were randomized following an acclimation period of nine days, were assigned permanent identification numbers, and were individually housed for the remainder of acclimation and the duration of the study.

Feed and Water - Purina® Certified Rodent Chow® #5002 was available ad libitum during both the acclimation and study periods, unless otherwise noted. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. Tap water was available ad libitum, via an automatic watering system, during both the acclimation and study periods. The water was retrospectively analyzed for contaminants and specific microbes. Results of feed and water analyses are on file at Hazleton Washington, Inc.

The Study Director and/or Sponsor have considered possible interfering substances potentially present in animal feed and water, including the test material itself or possible structurally related materials. None of these contaminants are reasonably expected to be present in animal feed or water at levels sufficient to interfere with this study.

Environmental Conditions - Controls were set to maintain the temperature and relative humidity at 72 ± 6°F and 50 ± 20%, respectively. Temperature and humidity were monitored throughout the acclimation and dosing periods. A 12-hour light/12-hour dark cycle was maintained in the animal room. There were ten or more air changes/hour in the animal room.

METHODS

Group Assignment and Dosage Levels

Animals were initially accepted for potential study use based upon physical and ophthalmologic examinations. Animals which exhibited either clinical signs or ocular abnormalities were eliminated from the randomization pool. A total of 100 rats (50/sex) were assigned to the study by first eliminating the animals with extreme body weights and then selecting the random assignment which produced homogeneity of both the variance and the means by Bartlett's test (1937) and one-way analysis of variance (ANOVA). At the time of randomization, the weight variation of the animals selected did not exceed ± 2 standard deviations of the mean body weight for each sex, and the mean body weight for each group of each sex was not statistically different. Animals were assigned to groups as follows:

Group	Dosage Level (ppm)	Number of Animals		Animal Numbers	
		Male	Female	Male	Female
1 (Control)	0	10	10	11600-11609	11610-11619
2 (Low)	2500	10	10	11620-11629	11630-11639
3 (Mid-low)	5000	10	10	11640-11649	11650-11659
4 (Mid)	10,000	10	10	11660-11669	11670-11679
5 (High)	20,000	10	10	11680-11689	11690-11699

Animals were uniquely identified by permanent identification numbers tattooed on the tail. At initiation of dosing, the animals were approximately six weeks of age with body weights ranging from 110 to 131 g for the males and 90 to 108 g for the females.

Excess animals were removed from the study room and subsequently euthanized.

Compound Formulation and Administration

The test diets were available to the test animals ad libitum seven days per week, for at least 13 weeks, until the day before necropsy. Fresh test diets were prepared at least every four weeks. The purity of the test material was assumed to be 100% for formulation of the test diets.

The correct amount of test material and feed for each dose level was weighed on an appropriate balance in series with an Epson computer and printer. Each level was prepared by placing feed into an appropriately sized glass beaker. A depression was made in the center of the feed and the weighed test material was added to the depression. This test material-feed combination was mixed with approximately 200 g of feed for 2-3 minutes in a Waring blender to form a homogeneous premix. Additional premixes were needed as the amount of test material increased at the higher dose levels. The premix(es) were then added to 5 kg of feed in a Hobart blender and mixed for 10 minutes. The premix from the Hobart blender was added to the required amount of feed for the dose level and mixed in a Patterson-Kelly Twin Shell mixer (2 cu. ft.) fitted with an intensifier bar for 1 min/kg. At no time during the mixing procedure was plastic allowed to come in contact with the test material. The mixed test diets were held in refrigerated storage until release to the technical group. Fresh test diets were presented weekly to the test animals.

Reserve samples from each mixed batch were retained for possible analysis and stored under refrigeration. These samples will be discarded after the final report has been issued.

The oral route was chosen because it is a potential route of human exposure.

Analysis of Prepared Formulations

Stability - Stability determinations were conducted on the first

and third test diet mixes for the low- and high-dose formulations. Each mix was assayed for Day 0 stability[a], after four weeks in refrigerated storage, and after four weeks in refrigerated storage plus one week of storage in the animal room.

Homogeneity - Evaluation for homogeneity was performed on the low- and high-dose formulations prior to initiation of dosing. Duplicate samples from the top, middle, and bottom of the formulations were analyzed for concentration of the test material. A remix of the low-dose formulation was analyzed for homogeneity due to a greater than 10% variation between duplicate samples from the middle of the first formulation of that level.

Concentration Analyses - Samples of the formulations from all test diet mixes for all groups were analyzed for concentration of the test material.

Analytical Method - The analytical chemistry methods used for sample analyses are presented in Appendix 1.

Observations and Records

Mortality and Clinical Observations - The rats were observed for mortality and moribundity twice daily. A careful cageside observation for obvious indications of toxic effects was performed once daily. A thorough physical examination was conducted once each week.

Body Weight and Food Consumption - Body weights were measured and recorded at randomization, prior to treatment, and weekly thereafter. Food consumption was measured and recorded weekly.

Ophthalmoscopic Examinations - An indirect ophthalmoscopic examination was performed on all animals prior to treatment and during Week 13, using 1% Mydriacyl® as the mydriatic agent.

[a] Stability analysis of the first test diet mix was performed on the day following diet preparation (Day 1), however the analysis was completed within 24 hours of sample preparation.

Clinical Pathology

Following at least 13 weeks of treatment, all animals were placed in urine collection racks and fasted overnight, with water available, prior to clinical sampling. Samples for hematology and serum chemistry were obtained from the orbital sinus of the animals under CO_2/O_2 anesthesia. Urine specimens were collected during the overnight fast (approximately 16 hours) in individual urine collection cages. A rotating pattern through the groups and sexes was used as the order for blood collection. The following parameters were evaluated:

Hematology

Absolute Reticulocyte Count (A RETIC)
Cell Morphology
Corrected Leukocyte Count (COR WBC)
Erythrocyte Count (RBC)
Hematocrit (HCT)
Hemoglobin (HGB)
Leukocyte Count (WBC)
Leukocyte Differential
Myeloid/Erythroid Ratio[a] (M/E)
Platelet (PLATELET)
Reticulocyte Count (RETIC)

Clinical Chemistry

Alanine Aminotransferase (ALT)
Albumin (ALBUMIN)
Albumin/Globulin Ratio (A/G RATIO)
Aspartate Aminotransferase (AST)
Blood Urea Nitrogen (BUN)
Calcium (CALCIUM)
Chloride (CHLORIDE)
Creatinine (CREAT)
Gamma Glutamyltransferase (GGT)
Globulin (GLOBULIN)
Glucose (GLUCOSE)
Inorganic Phosphorus (IN PHOS)
Potassium (POTAS)
Sodium (SODIUM)
Total Bilirubin (T BILI)
Total Protein (T PROT)

[a] Bone marrow obtained from the femur.

Urinalysis

Appearance (UTRANS, UCOLOR)
Bilirubin (BILI)
Glucose (GLUCOSE)
Ketones (KETONES)
Microscopic Examination of Sediment
Occult Blood (OC BLD-U)
pH (PH)
Protein (PROTEIN)
Specific Gravity (SP GR)
Urobilinogen (UROBIL)

Urine Calcium (UCALCIUM, U CA EX)
Urine Chloride (U CHLOR, U CL EX)
Urine Creatinine (U CREAT, U CRE EX)
Creatinine Clearance (CRECLEAR)
Urine Osmolality (U OSMOL)
Urine Phosphorus (U PHOS, UPHOS EX)
Urine Potassium (U POTAS, U K EX)
Urine Sodium (U SODIUM, U NA EX)
Urine Volume (U VOL)

Terminal Studies

Sacrifice and Gross Pathology - All animals were weighed on the day of scheduled necropsy and were sacrificed by exsanguination under sodium pentobarbital anesthesia. Necropsies were performed on all animals by appropriately trained personnel using procedures approved by board-certified pathologists; a board-certified pathologist was present at necropsy. The order of necropsy followed the order of blood collection whenever possible and necropsies included examination of the following:

The external surface
All orifices
Cranial cavity
Carcass
External surface of the brain (at necropsy); the external surface of the spinal cord and cut surfaces of the brain and spinal cord will be examined at the time of tissue trimming (in animals examined histologically)

The nasal cavity and paranasal sinuses
The thoracic, abdominal and pelvic cavities and their viscera
The cervical tissues and organs

Organ Weights - The following organs (when present) were weighed following careful dissection and trimming of fat and other contiguous tissue:

brain with stem
kidneys
liver
lung
spleen
testes with epididymides
uterus

Organ-to-terminal body weight and organ-to-brain weight ratios were calculated using the terminal body weight and brain weight, respectively, of the fasted animals, which were recorded at the time of necropsy.

Tissue Preservation - The following tissues (when present) from each animal were preserved in 10% neutral-buffered formalin:

adrenals
aorta
bone marrow (femur)
brain with brainstem (medulla/pons, cerebellar cortex, and cerebral cortex)
duodenum, jejunum, ileum, colon, cecum, rectum
esophagus
heart
kidneys
lesions
liver[a]
lungs
mesenteric lymph node
ovaries
pancreas
pituitary
salivary glands (mandibular)
sciatic nerve
spleen
stomach
testes with epididymides
thymus
thyroid (parathyroids)
trachea
urinary bladder
uterus

[a] Livers from the first five males from Groups 1, 2, 4, and 5 were weighed immediately after exsanguination. A section collected from each lobe, weighing approximately 1 g, was frozen in liquid nitrogen, and stored at approximately -70°C for use in validation of enzyme assays for HWA Study 2598-101.

The following tissues from each animal were preserved in 10% neutral-buffered formalin for possible future examination if indicated by signs of toxicity or target organ involvement:

cervical spinal cord
eyes
exorbital lacrimal glands
femur including articular surface
lumbar spinal cord
mammary gland
mid-thoracic spinal cord
thigh musculature
sternum with bone marrow

Histopathology - The tissues presented in the first listing above from all animals in Groups 1 and 5, and the liver, testes with epididymides (males), spleen, kidneys, nonglandular stomach, and gross lesions from all study animals, were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analyses

Mean body weight (Weeks 1, 5, 9, and 14), mean body weight change (Weeks 1-13), mean food consumption (Weeks 1, 4, 8, and 13), mean total food consumption (Weeks 1-13), hematology (except cell morphology gradings), serum chemistry, urine chemistry, and organ weight data of the control group were compared statistically to the data from the same sex of the treated groups. Statistical analyses were performed as diagrammed in Figure 1.

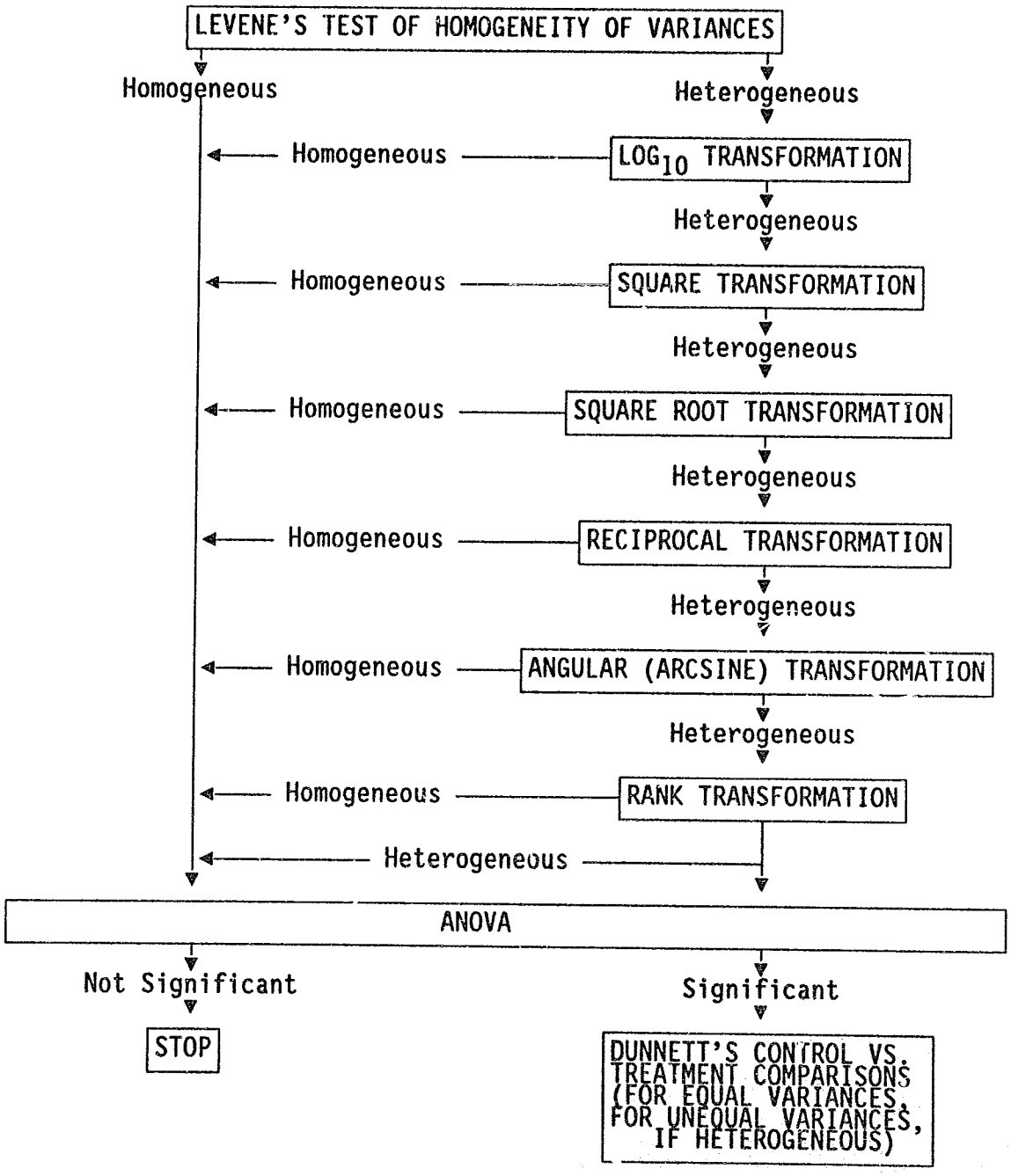
If variances of untransformed data were heterogeneous, a series of transformations were performed in an effort to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were routinely performed at the 5% two-tailed probability level.

Statistical significance is designated throughout the text of this report by the term, significant. Data transformations are presented in Appendix 12.

Specimen, Raw Data, and Final Report Storage

All tissue specimens, blocks and slides, raw data, and the final report (or copies thereof) will be retained by Hazleton Washington, Inc., in accordance with GLP requirements.

Figure 1
Flowchart of ANOVA and Related Methods



All parametric comparisons take variance homogeneity/heterogeneity into consideration. All transformations indicated in the flowchart are done on untransformed data.

RESULTS

Analytical Chemistry

Analytical chemistry results are presented in Table 1.

The percent of target concentration was low (less than 10% of target) and varied by more than 10% from its duplicate sample for one prestudy homogeneity sample taken from the middle of the mix for Group 2. A remix of the Group 2 test diet was performed and analysis of homogeneity samples produced results within 10% of the target concentration and with acceptable variance between duplicate samples. The percent of target concentration was high (greater than 10% of target) at the Day 35 stability analysis of the Group 5 test diet mix for Weeks 1-3 and low at the Day 35 stability analysis of the Group 2 test diet mix for Weeks 8-11. All other analyses were within ± 10% of the target concentration, with acceptable variance between duplicate samples.

In-Life Observations

Mortality and Clinical Observations - A summary of clinical and cageside observations is presented in Table 2 and individual clinical and cageside observations are presented in Appendix 2.

All animals survived to study termination.

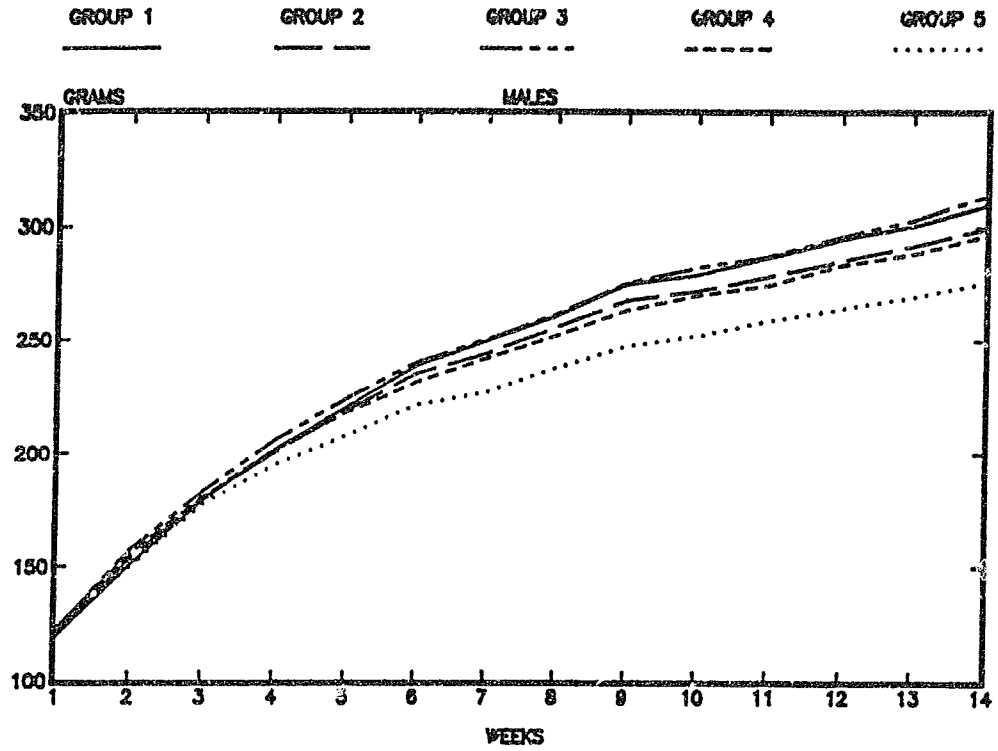
During the study, urine stains were observed in one Group 5 male and in one Group 2, one Group 3, six Group 4, and nine Group 5 females. Two Group 5 females appeared thin at the Week 13 physical examination. The remaining clinical signs were sporadic, of low frequency, and appeared to be incidental in nature.

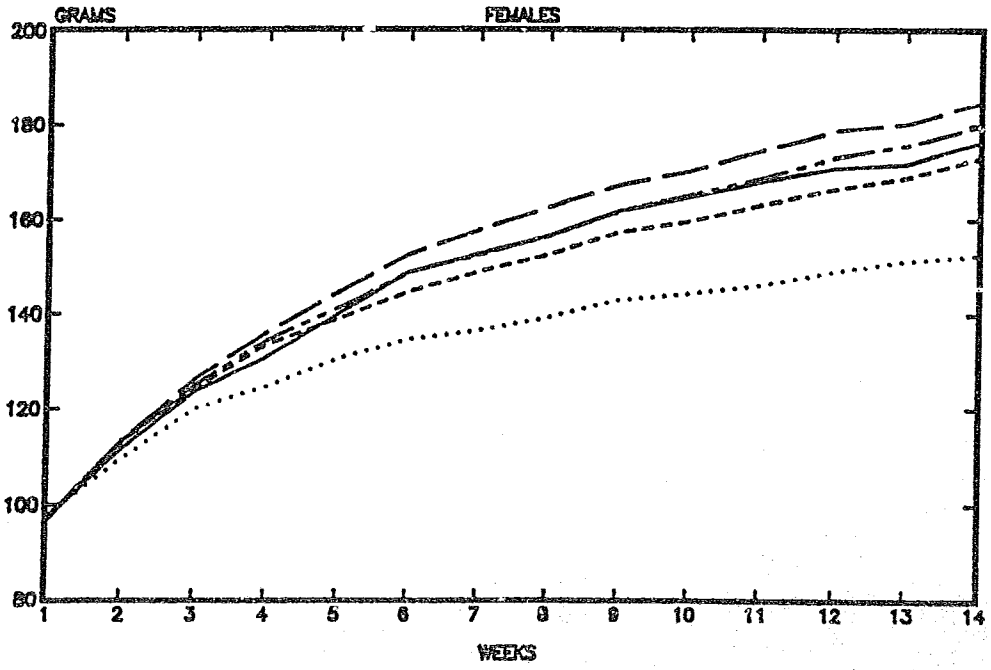
Body Weight - Mean body weight data are presented in Table 3A and are depicted graphically in Figure 2. Body weight change data are presented in Table 3B. Individual data are presented in Appendix 4.

The Group 5 male body weights were significantly lower than the respective control values at Weeks 9 and 14, and for Weeks 1-13. The

HWA 2598-102

FIGURE 2 – MEAN BODY WEIGHTS





Group 5 female body weights were significantly lower than the respective control values at Weeks 5, 9, and 14, and for Weeks 1-13. Body weight data for the remaining groups and sexes were comparable at the intervals analyzed.

Food Consumption - Mean food consumption and mean total food consumption (Weeks 1-13) are presented in Table 4. Mean food consumption data are depicted graphically in Figure 3. Individual food consumption data are presented in Appendix 5.

The Group 5 male mean food consumption was significantly higher than the respective control value at Week 1 and the Group 5 female food consumption was significantly lower than the respective control values at Weeks 4 and 8. Food consumption data for the remaining groups and sexes were comparable at the intervals analyzed.

Efficiency of Food Utilization and Compound Consumption - Mean and individual efficiency of food utilization are presented in Table 5 and Appendix 6, respectively. Mean and individual compound consumption are presented in Table 6 and Appendix 7, respectively.

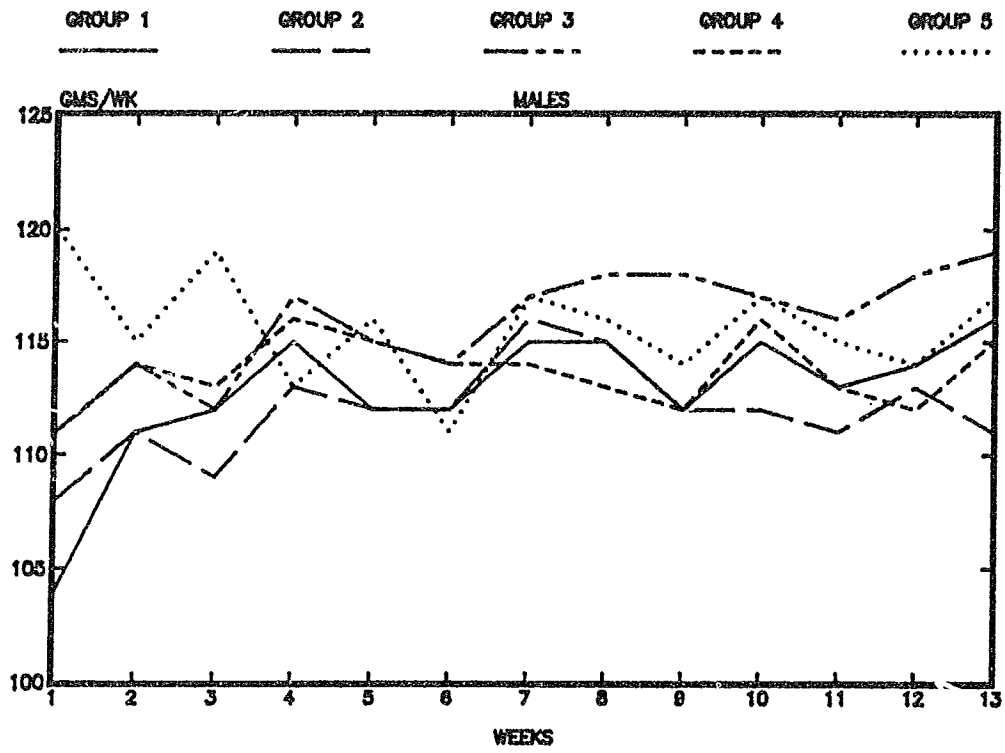
Mean efficiency of food utilization (%) ranged from 3.6 (Week 9) to 30.4 (Week 1) in the Group 1 males, from 3.7 (Week 9) to 30.7 (Week 1) in the Group 2 males, from 4.4 (Week 10) to 31.6 (Week 1) in the Group 3 males, from 4.0 (Week 10) to 29.8 (Week 1) in the Group 4 males, and from 3.9 (Week 9) to 24.1 (Week 1) in the Group 5 males. In the females, mean efficiency of food utilization ranged from 1.1 (Week 12) to 17.4 (Week 1) in Group 1, from 1.4 (Week 12) to 18.7 (Week 1) in Group 2, from 3.0 (Week 12) to 18.0 (Week 1) in Group 3, from 2.9 (Weeks 9 and 12) to 18.5 (Week 1) in Group 4, and from 1.7 (Week 13) to 12.8 (Week 2) in Group 5.

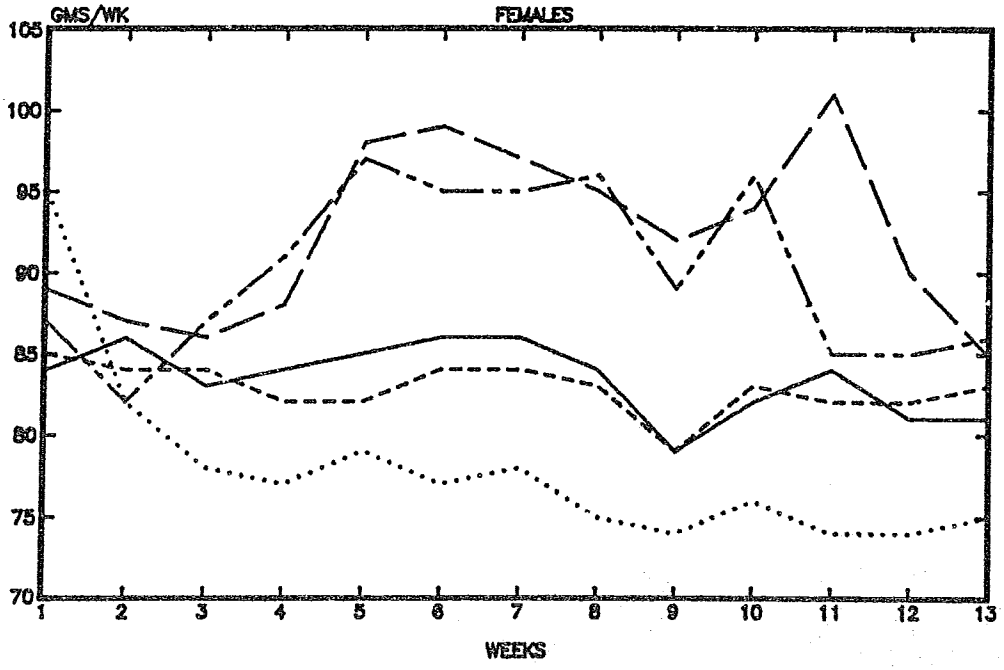
Mean overall compound consumption (mg/kg) for Weeks 1-13 in the males was 175.8 for Group 2, 354.6 for Group 3, 719.6 for Group 4, and 1544.7 for Group 5. In the females, mean overall compound consumption for Weeks 1-13 was 218.9 for Group 2, 438.0 for Group 3, 823.8 for Group 4, and 1687.1 for Group 5.

Ophthalmology - Individual ophthalmology findings are presented

HWA 2598-102

FIGURE 3 – MEAN FOOD CONSUMPTION





in Appendix 3. Findings are further discussed in the Ophthalmology Report.

Any rats with prestudy ophthalmic lesions were excluded from the study. A bilateral corneal opacity, which is a common finding in Fischer 344 rats, was present in all animals at Week 13. The ophthalmoscopic observations noted were considered to be incidental findings.

Clinical Pathology

Mean hematology values are presented in Table 7 and individual data are presented in Appendix 8. Mean serum chemistry and urine chemistry values are presented in Table 8 and individual serum chemistry data are presented in Appendix 9. Individual urinalysis values are presented in Appendix 10. Findings are further discussed in the Clinical Pathology Report.

Hematology - Mean erythrocyte count, mean hematocrit, and mean hemoglobin were significantly lower in the Group 4 and 5 males and females and in the Group 3 males when compared to the respective control values. There were no other significant differences in the hematology data.

Clinical Chemistry - Evaluation of the clinical chemistry data revealed statistically significant findings, as detailed in Text Table 1.

Text Table 1
Statistically Significant Findings
Serum Chemistry Data

Parameter	Group:	Males 2	Males 3	Males 4	Males 5	Females 2	Females 3	Females 4	Females 5
BUN				↑	↑				↑
CREAT									↓
T PROT									↓
ALBUMIN				↑	↑			↑	
GLOBULIN				↓	↓		↓	↓	↓
A/G RATIO				↑	↑		↑	↑	↑
T BILI						↓	↓		↓

KEY: ↓ = Significantly decreased, $p \leq 0.05$.
↑ = Significantly increased, $p \leq 0.05$.

Urinalysis - Evaluation of urine chemistry data revealed significantly increased mean urine sodium and potassium excretion values in the Group 4 females when compared to respective control values. There were no other significant difference in urine chemistry data.

Evaluation of urinalysis data revealed a slight increase in urine specific gravity in the Group 5 males, accompanied by increases in urine protein, leukocytes, and amorphous material. Increases in incidence and grade for leukocytes and amorphous material were also noted in the Group 4 males. Urinalyses in the females were generally comparable between control and treated groups.

Terminal Studies

Gross Pathology - Gross pathology findings are summarized in Table 9 and individual gross pathology findings are presented in Appendix 11.

Enlarged and dark livers were observed in two Group 5 males. Dark areas of the glandular stomach were observed in three Group 5 males, one Group 1 female, and three Group 5 females. Remaining gross pathology findings were sporadic, of low frequency, and appeared to be incidental in nature.

Organ Weights - Mean absolute organ weight, organ-to-terminal body weight, and organ-to-brain weight data are presented in Tables 10, 11, and 12, respectively. Individual organ weight data are presented in Appendix 11.

Evaluation of the organ weight data revealed statistically significant findings, as detailed in Text Table 2.

Text Table 2
Statistically Significant Findings
Organ Weight Data

Parameter	Group:	Males 2	Males 3	Males 4	Males 5	Females 2	Females 3	Females 4	Females 5
Absolute									
Terminal body weight					↓				↓
Lung					↓				↓
Kidney			↑	↑	↑	↑	↑	↑	↑
Liver			↑	↑	↑		↑	↑	↑
Uterus									↓
Organ-to-Body									
Lung							↓		
Brain					↑				↑
Kidney		↑	↑	↑	↑		↑	↑	↑
Liver		↑	↑	↑	↑		↑	↑	↑
Uterus									↓
Testis/Epidid				↑	↑				
Organ-to-Brain									
Lung					↓				↓
Kidney			↑	↑	↑	↑	↑	↑	↑
Liver			↑	↑	↑		↑	↑	↑
Uterus									↓

KEY: ↓ = Significantly decreased, $p \leq 0.05$.
↑ = Significantly increased, $p \leq 0.05$.

Histopathology - Histopathology findings are summarized in Table 13 and individual findings are presented in Appendix 11. The findings are further discussed in the Pathology Report.

Microscopic evaluation revealed hepatocellular enlargement in the Group 5 males (periportal) and females (centrilobular). Treatment-related renal findings were present in the Group 3-5 males and consisted of granular casts and a higher severity of regenerative/basophilic tubules. Vesiculation and an increased severity of acute inflammation were present in the stratified squamous epithelium of the nonglandular stomach in select animals from the Group 4 and 5 male and female rats.

DISCUSSION AND CONCLUSION

Weekly body weights and total body weight change values at selected intervals in animals of both sexes receiving 20,000 ppm of DINP in the diet (high dose, Group 5) were lower than values in corresponding control animals (Group 1). The low body weight gain in Group 5 animals was more pronounced in females than in males; the total body weight change was about 81% and 69% of corresponding control values for males and females, respectively. Slightly lower food intake in the high-dose females may have contributed to the greater relative decrease in body weight gain. Total food consumption in Group 5 females was about 92% of corresponding controls, and significantly lower weekly food consumption was observed for this group at selected intervals during the study. In Group 5 males total food consumption and weekly food consumption were generally comparable to corresponding control values.

In general, the efficiency of food utilization, defined as the ratio of body weight gain to food consumption, was lower in Group 5 males and females than in respective controls. Efficiency of food utilization at Weeks 4, 8, and 13 in Group 5 animals ranged from about 67 to 75% of corresponding controls in males and from about 30 to 80% of corresponding controls in females. In general, during the study, for each DINP-dosed group, compound consumption was greater in females than in males. For example, at Week 13, compound consumption means for Group 2, 3, and 4 females (2500, 5000, and 10,000 ppm, respectively) were greater than the values in the corresponding male DINP-dosed groups by about 24%, 26%, 23%, and 14%, respectively.

The only compound-related clinical sign observed in animals during the study was urine stains, noted at an increased incidence primarily in Group 4 and 5 females.

Mild anemia occurred in animals of both sexes dosed with DINP and was more pronounced in males than in females. In general, in both sexes, red cell counts, hematocrit, and hemoglobin levels decreased with

increasing dose level of DINP; mean values for these three parameters were significantly lower than corresponding control values in Group 3 through 5 males and in Group 4 and 5 females.

Effects on certain serum protein levels were also observed in animals dosed with DINP. Dose-related changes were observed in serum globulin levels (decreases) and the albumin-to-globulin (increases) ratios in DINP-dosed animals; these differences were statistically significant for Groups 4 and 5 in males and for Groups 3 through 5 in females. Increases in serum albumin levels in DINP-treated animals showed a dose-response in males but not in females; levels were significantly greater than corresponding control values in Group 4 and 5 males and in Group 4 females. As there was no correlation between the above changes in serum proteins and body weight changes, it is not clear as to whether these changes reflect the nutritional status of the animals or represent compound-related effects.

Results from this study indicate that liver, kidney, and nonglandular stomach are target organs to DINP toxicity. Marked dose-related increases were observed in absolute liver weights, liver-to-body weight ratios, and liver-to-brain weight ratios in DINP-treated animals. Compared with corresponding control values, these differences were statistically significant for Groups 3 through 5 in animals of both sexes; the liver-to-body weight ratio was also significantly greater in Group 2 males. Microscopically, hepatocellular enlargement varying in severity from slight to minimal was observed in Group 5 males and females. Slight hepatocellular enlargement was also observed in one Group 4 female. The distribution of hepatocellular enlargement varied between sexes; specifically, this change was periportal in males and centrilobular in females.

Effects of DINP on the kidney were reflected by elevated organ weight parameters, microscopic lesions, and elevated blood urea nitrogen (BUN) levels. Absolute kidney weights, kidney-to-body weight ratios, and kidney-to-brain weight ratios were significantly greater than

corresponding control values in Groups 3 through 5 (both sexes). Selected kidney weight parameters were significantly greater than corresponding control values in Group 2 animals.

Microscopic renal lesions, observed only in males, were characterized as increased severity of regenerative/basophilic tubules, primarily in Groups 3 through 5. Granular casts, thought to represent a collection of degenerate/necrotic cortical renal tubular epithelial cells, were also observed at the cortical medullary junction in kidney sections from Group 3 through 5 males. Effects on BUN levels were more pronounced in males than in females. BUN levels in males showed a marked dose-response; relative to corresponding controls, Group 4 and 5 levels were about 33% and 60% greater, and these differences were statistically significant. No clear dose-response for BUN levels was observed in females, and, only in Group 5, where the mean BUN level was about 19% greater than control, was the difference statistically significant.

Microscopic lesions of the nonglandular stomach were observed in select animals from Groups 4 and 5 (both sexes). These lesions were characterized as vesiculation and increased severity of acute inflammation and were graded as minimal to slight in nearly all cases.

Uterine weight parameters were low in high-dose females; mean values for absolute, organ-to-body weight, and organ-to-brain weight ratios were about 61%, 70%, and 62%, respectively, of corresponding control values and the differences from control were statistically significant. No gross or microscopic observations were associated with these organ weight changes. The significantly decreased absolute lung weights and lung-to-brain weight ratios, observed in high-dose males and females, were most likely secondary to the low body weights in these groups, as evidenced by comparable lung-to-body weight ratios between all compound-treated groups and control groups within the same sex.

In conclusion, dosing of male and female Fischer 344 rats with Di(isononyl)phthalate in feed for 13 weeks at a level of 20,000 ppm was associated with low body weight gain, mild anemia, elevated kidney weights

and BUN levels, and microscopic lesions of the liver and nonglandular stomach in animals of both sexes; microscopic renal lesions in males; and a slight decrease in food intake, an increased incidence of urine stains, and decreased uterine weight in females. Effects observed at the dose level of 10,000 ppm included mild anemia, microscopic lesions of the nonglandular stomach, and elevated liver and kidney weights in animals of both sexes; microscopic renal lesions and elevated BUN levels in males; and an increased incidence of urine stains in females. The dose level of 5000 ppm was associated with elevated liver and kidney weights in animals of both sexes, and with mild anemia and microscopic renal lesions in males. At 2500 ppm, effects included significantly elevated kidney (both sexes) and liver (males) weight parameters. Body weight changes were more pronounced in DINP-dosed females than in males. With respect to hematology changes and renal pathology, males were more sensitive to the effects of DINP than females, particularly in light of the observation that compound consumption was lower in males than in females during the study. A no-observable-effect-level was not established for DINP at the dose levels used in this study.

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Life Sciences Division

8/12/91
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Study Coordinator:

Denise Bays

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8/12/91
Date

OPHTHALMOLOGY REPORT

The animals observed prior to test material administration that displayed ophthalmoscopic lesions were rejected from study. At the termination of this study, there did not appear to be any ophthalmoscopic abnormalities related to the compound or dose level. The ophthalmoscopic observations noted are considered to be incidental findings. The bilateral corneal dystrophy seen in all animals is a common finding in Fischer 344 rats.

Veterinarian:

Steven M Kuhlman VMD

STEVEN M. KUHLMAN, V.M.D., M.S.
Life Sciences Division

Date 8/2/91

CLINICAL PATHOLOGY REPORT

Evaluation of the hematology data revealed slight, but significant decreases in red cell mass (erythrocyte count, hemoglobin, and hematocrit) in the Group 3 males and Group 4 and 5 males and females. These changes may be secondary to the nutritional status of the animals. The remaining hematology data were generally comparable between control and treated groups.

Evaluation of the clinical chemistry data revealed slight, but significant increases in blood urea nitrogen, albumin, and albumin to globulin ratio, and significant decreases in globulin in the Group 4 and 5 males.

In the females, significant increases were noted for blood urea nitrogen in Group 5, albumin in Group 4, and albumin to globulin ratio in Groups 3 through 5. Serum creatinine was minimally, but significantly decreased in Group 5, due to the body weight loss. Globulin was significantly decreased in Groups 3 through 5. The increase in blood urea nitrogen may be partially related to the nutritional status of the animals; however, prerenal or renal factors may have also contributed to the increases. The significant decreases in total bilirubin in the Group 2, 3, and 5 females were thought to be incidental based on the low magnitude of the differences. The significant increases in urine sodium and potassium excretion in Group 4 were considered to be spurious. The remaining serum and urine chemistry were generally comparable between control and treated groups.

Evaluation of the urinalyses data revealed a slight increase in urine specific gravity (i.e., increase in urine concentration) in the Group 5 males, accompanied by increases in urine protein, leukocytes, and amorphous material. Increases in incidence and grade for leukocytes and amorphous material were also noted in the Group 4 males. Urinalyses in the females were generally comparable between control and treated groups.

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PATHOLOGY REPORT

One hundred young adult Fischer 344 rats (50 males and 50 females) were randomly placed into the following treatment groups:

Group	Number of Rats Males	Number of Rats Females	Dietary Level ppm
1 (Control)	10	10	0
2 (Low)	10	10	2,500
3 (Mid-low)	10	10	5,000
4 (Mid)	10	10	10,000
5 (High)	10	10	20,000

Following 13 weeks of dietary compound administration, all rats were sacrificed and all tissues from high-dose and control animals were examined microscopically. In addition, gross lesions from all animals and sections of liver, spleen, kidney, nonglandular stomach, and testes with epididymides (males) from Groups 2-4 were examined microscopically.

Treatment-related histopathologic changes were present in the liver, kidney, and nonglandular stomach. Compound-induced hepatic findings included hepatocellular enlargement in the Group 5 male and female rats. This change was graded minimal to slight and likely correlates to the increased liver weights for these animals. Although liver weights were also increased in the mid-dose groups, there was no unequivocal microscopic evidence of hepatocellular enlargement in these animals. The distribution of hepatocellular enlargement varied between sexes; specifically, this change was periportal in the Group 5 males and centrilobular in the Group 5 females. The etiology for this difference in distribution between sexes is unclear. There was no microscopic evidence of hepatocellular degeneration, necrosis, or inflammation associated with this compound.

Treatment-related histopathologic changes consisting of granular casts and an increased severity of regenerative/basophilic tubules were present in the kidneys from the Group 3-5 male rats. Minimal regenerative/basophilic tubules is not an uncommon finding in control Fischer 344 male rats; however, the increased severity (slight to moderate) in the Group 3-5 male rat kidneys suggests it is related to Di(isononyl)phthalate administration. Granular casts, which were present at the cortical medullary junction, represent a collection of degenerate/necrotic cortical renal tubular epithelial cells. This change is not generally seen as a spontaneous finding, but more likely represents compound-induced renal toxicity.

Treatment-related changes consisting of vesiculation and an increased severity of acute inflammation were present in the nonglandular stomachs from select animals in the Group 4 and 5 male and female rats. These changes were graded minimal to slight in nearly all animals and were characterized by vesicle formation in the nonglandular squamous epithelium and a more severe submucosal neutrophil infiltrate (acute inflammation) in several high-dose rats. These findings were consistently present in the limiting ridge of nonglandular stomach.

In conclusion, the dietary administration of Di(isononyl)-phthalate in rats for 13 weeks resulted in compound-related changes in the liver, kidney, and nonglandular stomach. Hepatocellular enlargement was present in the Group 5 male (periportal) and female (centrilobular) rats. Treatment-related renal findings were present in the Group 3-5 males and consisted of granular casts and higher severity of regenerative/basophilic tubules. Vesiculation and an increased severity of acute inflammation were present in the stratified squamous epithelium of the nonglandular stomach in select animals from the Group 4 and 5 male and female rats.

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REFERENCES

Hematology

Absolute Reticulocyte Count (A RETIC)

% Reticulocyte Count/100 x Erythrocyte Count = Absolute Reticulocyte Count (Calculated).

Cell Morphology and Leukocyte Differential

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(WBC x 100) ÷ (NRBC + 100) = COR WBC (Calculated).

Coulter Counter® Model S+IV System

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Erythrocyte Count (RBC)
Hematocrit (HCT)
Hemoglobin (HGB)
Leukocyte Count (WBC)
Platelet (PLATELET)

Myeloid/Erythroid Ratio (M/E)

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Clinical Chemistry

Albumin/Globulin Ratio (A/G)

Albumin ÷ Globulin = A/G RATIO (Calculated).

BMD/Hitachi® 737 Chemistry Analyzer

Boehringer Mannheim Diagnostics, Indianapolis, IN.

	Insert # (date)
Alanine Aminotransferase (ALT)	054501100-0188 (1988)
Albumin (ALBUMIN)	052205300-0686 (1985)
Aspartate Aminotransferase (AST)	054500400-0188 (1988)
Blood Urea Nitrogen (BUN)	052205403-0187 (1987)
Calcium (CALCIUM)	052215002-0985 (1985)
Chloride (CHLORIDE)	052777803-0787 & 052776603-0787 (1987)
Creatinine (CREAT)	051187200-0586 (1986)
Gamma Glutamyltransferase (GGT)	051188600-0686 (1986)
Glucose (GLUCOSE)	052213301-0985 (1985)
Inorganic Phosphorus (IN PHOS)	052212802-0985 (1985)
Potassium (POTAS)	052777803-0787 & 052776603-0787 (1987)
Sodium (SODIUM)	052777803-0787 & 052776603-0787 (1987)
Total Bilirubin (T BILI)	052226401-1188 (1988)
Total Protein (T PROT)	055212202-0985 (1985)

Globulin (GLOBULIN)

Total Protein - Albumin = Globulin (Calculated).

Urinalysis

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