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| Microfiche No. | | |
| DTS0530364-1 | | |
| | | |
| New Dec ID. | Old Dec ID. | |
| 89-910000122 | BEHQ-1591-1150 | |
| | | |
| Date Produced | Date Received | TSCA section |
| 4/22/91 | 5/03/91 | 8E |
| | | |
| Submitting Organization | | |
| ARISTECH CHEMICAL CORP | | |
| | | |
| Contractor | | |
| HAZELTON LABORATORIES | | |
| | | |
| Document Title | | |
| A SUBCHRONIC (4-WEEK) DIETARY ORAL TOXICITY STUDY OF DI (ISONONYL) PHTHALATE IN B6C3F1 MICE (FINAL REPORT) WITH COVER LETTER DATED 042991 | | |
| | | |
| Chemical Category | | |
| DI (ISONONYL) PHTHALATE | | |

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RE: 8EHQ-0191-1150 S

Dear Sir or Madam:

As requested by the Agency in its February response to subject 8(e) submission, Aristech Chemical Corporation is providing a full copy of the final report from "A subchronic (4-Week) Dietary Oral Toxicity Study of Di(isononyl)phthalate in B6C3F1 Mice". We make no confidentiality claims regarding this cover letter or the enclosed study.

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

Sincerely,

John R. Bankston II

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Enclosure

cc: J. J. Pottmeyer III
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FINAL REPORT

Study Title:

A Subchronic (4-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in B6C3F1 Mice

Author:

Barbara A. Myers, Ph.D.

Study Completion Date:

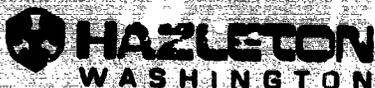
April 22, 1991

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Laboratory Project Identification:

HWA Study No. 2598-100



HWA 2598-100

- 2 -

COMPLIANCE STATEMENT

**A Subchronic (4-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in B6C3F1 Mice**

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. 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:

Barbara A. Myers 4/22/91
BARBARA A. MYERS, Ph.D. /Date
Life Sciences Division

STUDY IDENTIFICATION
A Subchronic (4-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in B6C3F1 Mice

H/A Study Number: 2598-100

Test Material: Di(isononyl)phthalate (INP)

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Study Timetable

| | |
|--------------------------------|--------------------|
| Study Initiation: | March 28, 1990 |
| Initiation of Dosing: | August 15, 1990 |
| Completion of Necropsy: | September 14, 1990 |

STUDY PERSONNEL
A Subchronic (4-Week) Dietary Oral Toxicity Study of
Di(isononyl)phthalate in B6C3F1 Mice

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SUMMARY

This study was designed to evaluate the subchronic toxicity of Di(isononyl)phthalate (DINP) when administered in the feed daily to B6C3F1 mice for at least 4 weeks and to determine dose levels for a subsequent 13-week study using this species. Five groups of 10 mice/sex/group were fed DINP added to feed at dose levels of 0 (Group 1); 3,000 ppm (Group 2); 6,000 ppm (Group 3); 12,500 ppm (Group 4); or 25,000 ppm (Group 5). The control and dosed feed were fed to the animals ad libitum. Criteria evaluated for compound effect included mortality, body weight, food consumption, clinical observations, organ weights, and gross, clinical, and microscopic pathology.

All animals survived to study termination. No compound-related clinical signs were observed. Weekly body weights were significantly lower than respective controls in Group 5 males and females from Weeks 3-5. In Group 5 females, weekly food consumption from Weeks 2-4 and total food consumption were significantly lower than respective controls. Relative to corresponding controls, Group 5 animals had elevated serum alanine aminotransferase activity (both sexes) and blood urea nitrogen levels (males only). Significant dose-related decreases in absolute kidney and testes/epididymides weights in Group 3-5 males were observed. Significantly increased liver/gallbladder weight parameters were observed in all groups of compound-treated animals with the exception of Group 2 females.

There were a number of observed histologic alterations which seemed to be related to DINP exposure. Hepatocellular enlargement was present in livers of Group 3-5 females and Group 2-5 males with a clear dose response relative to severity. Focal coagulative necrosis and/or separate chronic inflammatory foci occurred in livers of Group 5 males and at a lower incidence in Group 4 and 5 females. Tubular nephrosis was observed in kidneys of all Group 5 males and females and one Group 4 male.

Lesions of the reticuloendothelial system were noted in Group 5 males and females and included atrophy of the spleen and lymphoid depletion in the thymus. Lesions of the epididymis were observed in all Group 5 males and were characterized as increased cellular debris. In all Group 5 females, lesions of the reproductive tract included a virtual absence of corpora lutea in the ovaries and atrophic uteri.

In conclusion, dosing of B6C3F1 mice with DINP for 4 weeks at a dose level of 25,000 ppm was associated with decreased body weight, decreased food consumption (females only), hepatic coagulative necrosis and associated elevation in serum alanine aminotransferase activity, renal tubular nephrosis and associated elevated blood urea nitrogen (males only), atrophy of the spleen, lymphoid depletion in the thymus, epididymal lesions, and atrophy of the uterus and ovaries. Dosing at 12,500 ppm was associated with a low incidence of hepatic coagulative necrosis (females only) and a very low incidence of renal tubular nephrosis (one male). Dose-related hepatocytomegaly and associated statistically significant increases in liver/gallbladder weight parameters were observed in all compound-treated groups (with the exception of females receiving 3,000 ppm); these changes were most pronounced in the 25,000 ppm group. Dose-related statistically significant decreases in testes/epididymides weights and kidney weights were observed in males dosed with 6,000, 12,500, and 25,000 ppm. Based upon findings from this study, a high-dose level of 15,000 ppm is suggested for a subsequent 13-week dietary toxicity study of DINP using this species.

INTRODUCTION

This report presents and discusses the methods and results of a study designed to evaluate the subchronic toxicity of a chemical, Di(isononyl)phthalate (DINP), when fed daily to mice for at least 4 weeks. The results of this study were used to determine dose levels for a subsequent 13-Week study using this species. The first day of compound administration was August 15, 1990, and gross necropsies were concluded on September 14, 1990.

This study was designed to meet EPA Toxic Substances Control Act Test Guidelines, 40 CFR Part 798, and conducted in compliance with the EPA Good Laboratory Practice Standards, 40 CFR Part 792, effective December 29, 1983.

TEST AND CONTROL MATERIALS

The test material, Di(isononyl)phthalate (DINP), lot No. QCL 9004-273, described as a clear, colorless liquid, was received from the Sponsor on May 22, 1990, and stored at room temperature. The purity, as supplied by the Sponsor, was reported to be 99.9% active ingredient. 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 and control feed.

Reserve samples of the test (20 g) and control articles (200 g) were taken at initiation and stored at room temperature and frozen, respectively. These samples, as well as any 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 65 male and 63 female B6C3F1 mice was received from Charles River Laboratories, Inc., Raleigh, North Carolina, on July 31, 1990. The mice were approximately 4 weeks of age at receipt and were acclimated to laboratory conditions for approximately 2 weeks before compound administration was initiated.

Upon receipt, the animals were assigned temporary numbers and housed a maximum of two per cage (same sex) in stainless-steel, hanging, wire-mesh cages with food (Purina[®] Certified Rodent Chow[®] #5002) and tap water (via an automatic watering system) available ad libitum. Appropriate dietary mixtures were available ad libitum during the study period, except when noted otherwise. The basal feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water is routinely analyzed for contaminants and specific microorganisms. No contaminants were known to be present in the diet or water at levels which might interfere with achieving the objectives of this study.

The mice were maintained in a 12-hour light and 12-hour dark cycle. During the study, the room temperature and humidity were recorded twice daily and ranged from 70 to 74°F and 60 to 63%, respectively. Air changes ranged from 9.4 to 11.8 changes/hour.

On the last day of the quarantine/acclimation period, all animals were examined by a veterinarian for general physical condition and any clinical signs of disease. A total of 50 mice/sex was used on the study.

METHODS**Groups and Dietary Levels**

Prior to compound administration, all clinically acceptable animals were randomized using a body-weight-dependent computerized randomization procedure which eliminated the animals with extreme body weights and produced homogeneity of the variance and mean body weights (± 2 standard deviations) among groups/sex. Following randomization, each animal was individually housed, uniquely identified by individual eartag, and assigned to the following groups and dietary levels:

| Group Number | Dietary Levels ppm | Number of Animals | | Identification Number | |
|--------------|-----------------------|-------------------|---------|-----------------------|---------------|
| | | Males | Females | Male | Female |
| 1 (Control) | 0 | 10 | 10 | A34441-A34450 | A34451-A34460 |
| 2 (Low) | 3,000 | 10 | 10 | A34461-A34470 | A34471-A34480 |
| 3 (Mid-low) | 6,000 | 10 | 10 | A34481-A34490 | A34491-A34500 |
| 4 (Mid) | 12,500 | 10 | 10 | A34501-A34510 | A34511-A34520 |
| 5 (High) | 25,000 | 10 | 10 | A34521-A34530 | A34531-A34540 |

At initiation of treatment, the animals were approximately 6 weeks old and the body weights ranged from 21 to 26 g for the males and 16 to 24 g for the females.

All animals not selected for the study were removed from the study room and used for training purposes.

The mouse was selected for use on this study because mice have been used historically in safety evaluation studies and are recommended by the appropriate regulatory agencies.

Compound Formulation and Administration

The necessary amount of test material and feed per level were weighed on an appropriate balance (mg/kg) in series with an Epson computer with printout tape. Each level was prepared by adding the proper amount of test material (per level) into a glass beaker filled with feed. Each level was then premixed in a Waring blender with approximately 200 g of feed for approximately 2 to 3 minutes to ensure an apparent homogeneous mixture. Based on the amount of test material required to be added to each diet level, it was necessary to do multiple premixes at those levels.

Waring blender premixes were added to feed to total 5 kg (per level) and mixed for 10 minutes in a Hobart mixer. The remaining preweighed bulk feed was placed in a Patterson-Kelley twin-shell mixer fitted with an intensifier bar. The 5-kg premixes (per level) were added to the bulk feed and mixed for 1 minute/kg.

Fresh diets were prepared once for the 4-week study and stored refrigerated (2-6°C) in glass jars with teflon-coated lids. A sufficient 7-day diet was presented at the start of each week. The purity of the test material was assumed to be 100% for dose preparation purposes.

The oral route was chosen because potential human exposure is by the oral route.

Analysis of Diet Samples

Homogeneity and initial stability of the test diet (at levels of 1,000 and 27,000 ppm) were determined prior to compound administration and compared to the results of a 4-week refrigeration plus 1-week room temperature stability analysis that was determined after compound administration. Homogeneity samples were taken in duplicate from the top, middle, and bottom of the 1,000 and 27,000 ppm mixtures. Concentration analyses were performed on each dietary level of the feed prior to treatment.

Analyses were performed by reverse phase, high-performance liquid chromatography, as described in Appendix 1.

Observations and Records

All mice were observed twice daily for mortality and moribundity. In addition, careful cageside observations were performed for obvious indications of a toxic effect once daily. Individual body weights were recorded at initiation and weekly thereafter. Food consumption was recorded weekly and compound consumption calculated. Thorough physical examinations were performed at each weighing interval.

Throughout the course of the study, feed spillage/waste was observed in all treated groups and the control groups. In order to accurately reflect food consumption values, the spilled feed was collected from beneath each cage and weighed. The spilled/wasted feed weight was recorded and added to the feeder weight at the conclusion of the appropriate week.

Clinical Pathology

During Week 5, the following parameters were determined on blood samples collected via orbital sinus puncture without anesthesia on all animals after an overnight fast from food (but not water):

Hematology

| | |
|-------------------------------------|---------------------------|
| leukocyte count (WBC) | erythrocyte count (RBC) |
| hemoglobin (HGB) | platelet count (PLATELET) |
| hematocrit (HCT) | leukocyte differential |
| corrected leukocyte count (COR WBC) | cell morphology |

Clinical Chemistry

| | |
|------------------------|---------------------------|
| sodium (SODIUM) | total bilirubin (T BILI) |
| potassium (POTAS) | blood urea nitrogen (BUN) |
| chloride (CHLORIDE) | creatinine (CREAT) |
| total protein (T PROT) | glucose (GLUCOSE) |

albumin (ALBUMIN)
calcium (CALCIUM)
inorganic phosphorus (IN PHOS)

aspartate aminotransferase (AST)
alanine aminotransferase (ALT)
globulin (GLOBULIN)

Bone marrow smears were prepared at the time of necropsy for the evaluation of myeloid/erythroid ratio (M/E).

Due to the small size of the animals, it was probable that enough blood could not be collected for both hematology and clinical chemistry evaluations. Therefore, on the first day of clinical sampling, blood was collected for hematology first, then chemistry. On the second day of sampling, blood was collected for chemistry first, then hematology.

Sacrifice and Gross Pathology

Necropsies were conducted at termination on all animals by trained personnel using procedures approved by board-certified pathologists. Immediately following blood collection, all animals were weighed, anesthetized with an injection of sodium pentobarbital, and exsanguinated. A board-certified pathologist was present at the scheduled sacrifices. The necropsy included an examination of the following:

- external surface of the body
- 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 at the time of tissue trimming (only if trimming was performed)
- nasal cavity and paranasal sinuses
- thoracic, abdominal, and pelvic cavities and their viscera
- cervical tissues and organs

All findings were recorded.

Organ Weights

For each terminally killed animal, the following organs (when present) were weighed after careful dissection and trimming to remove fat and other contiguous tissue in a uniform manner:

liver with gallbladder
kidneys
testes with epididymides

Organ-to-terminal-body-weight ratios were calculated.

Tissue Preservation

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

List A

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

The following tissues were preserved for possible future examination:

List B

| | |
|--------------------------|---------------|
| sternum with bone marrow | mammary gland |
| skeletal muscle | eyes |

femur (including articular surface)
thoracic spinal cord
exorbital lacrimal glands

cervical spinal cord
lumbar spinal cord

Histopathology

The tissues specified in List A were embedded in paraffin, sectioned, stained, and examined microscopically from all control and high-dose animals. In addition, liver, testes with epididymides (males), spleen, and kidneys from the low-, mid-low-, and mid-dose groups and gross lesions from all animals were examined in a like manner.

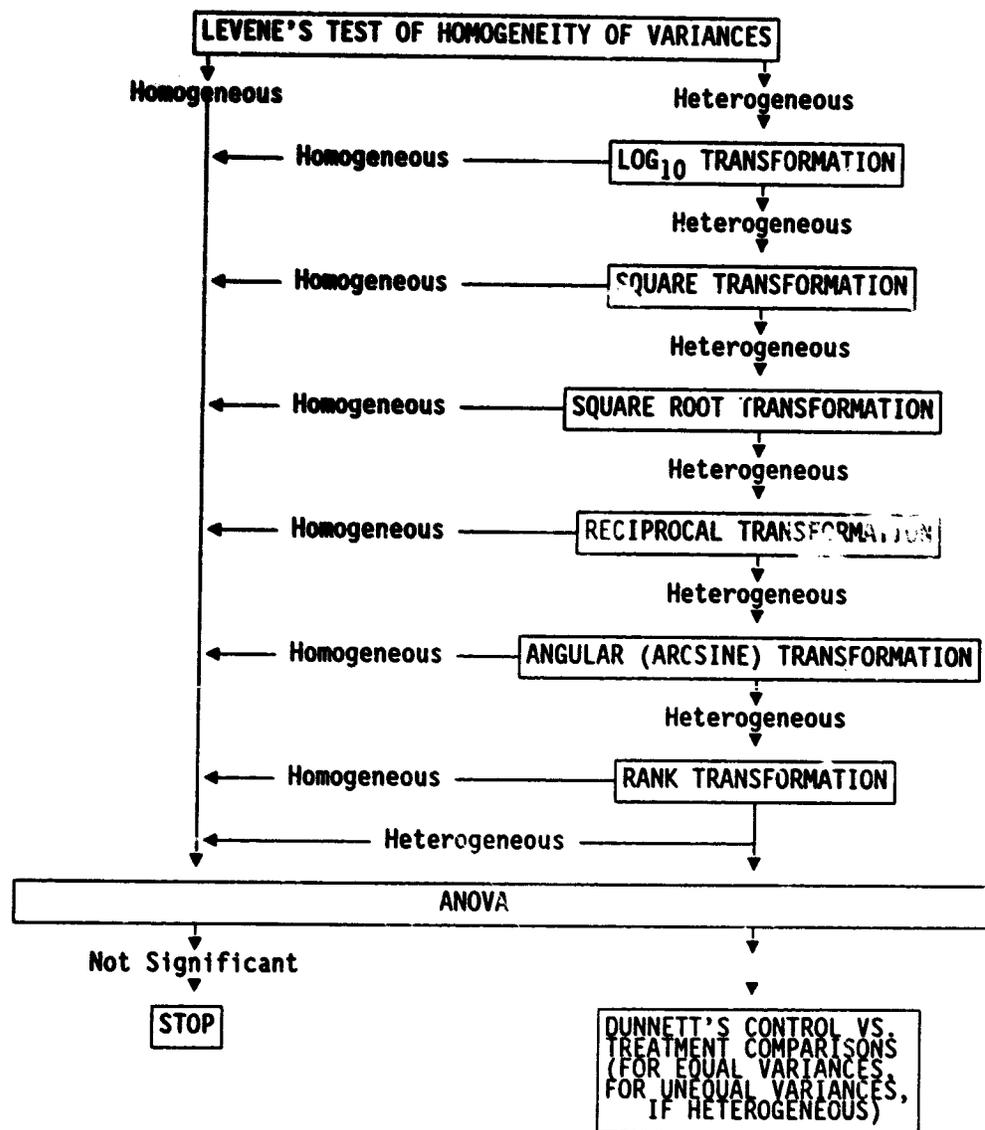
Target organs noted microscopically at the high dose, e.g., the thymus, ovaries, and uterus, were also examined (as previously described) from all animals.

Statistical Analysis

Weekly absolute body weights, body weight change (Weeks 1-4), weekly food consumption, total food consumption (Weeks 1-4), clinical pathology data (except cell morphology gradings), fasted terminal body weights, and organ weight data were evaluated by analysis of variance (ANOVA) using Dunnett's procedure for comparing treated means to the appropriate control means, as diagrammed in Figure 1.

If variances of untransformed data were heterogeneous, the data were transformed to achieve variance homogeneity. When the series of transformations was unsuccessful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed at the 5.0% two-tailed probability level. Data transformations are presented in Appendix 9. Statistically significant differences are designated throughout the report by the term significant.

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.

0 0 2 0



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Specimen, Raw Data, and Final Report Storage

All tissue specimens, blocks and slides, all raw data, and the final report will be retained by Hazleton Washington, Inc., according to GLP requirements.

RESULTS**Analytical Chemistry**

Results of analyses for homogeneity, stability, and concentration of DINP in the diet are presented in Table 1.

Analysis for homogeneity indicated that the test material was homogeneously mixed (mean concentration within 5% and coefficient of variation within 10%) in the diet at 1,000 and 27,000 ppm. Initial stability and 4-week refrigeration plus 1-week room temperature stability was established for 1,000 and 27,000 ppm. Concentration analysis for each level of the test diet was within 2% of target.

Mortality and Clinical Observations

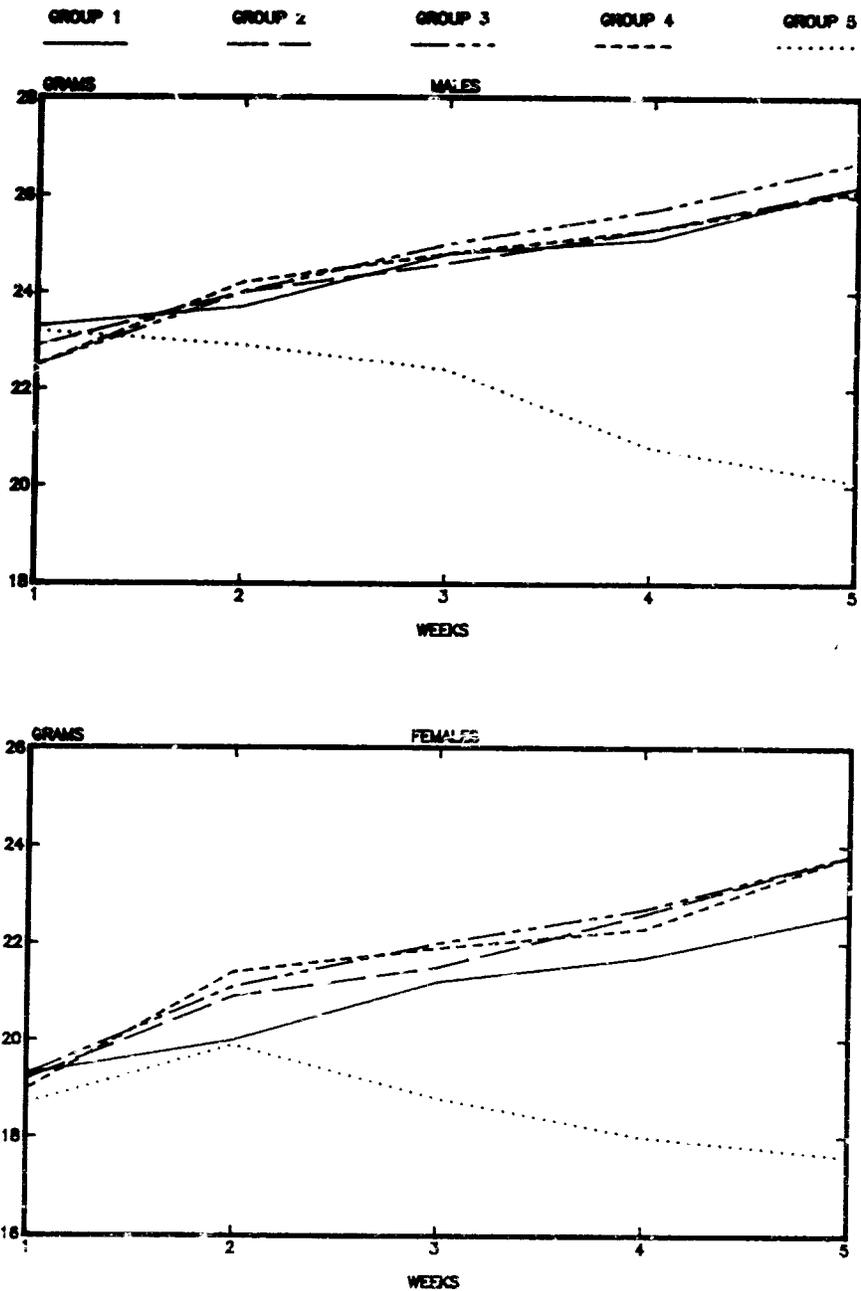
Observations noted during the weekly physical examinations are summarized in Table 2 and presented individually in Appendix 2.

No premature deaths occurred during the course of the study. There were no observations noted at the daily cageside observations and those noted at the weekly physicals were considered incidental and not related to compound administration.

Body Weights, Food Consumption, Compound Consumption, and Efficiency of Food Utilization

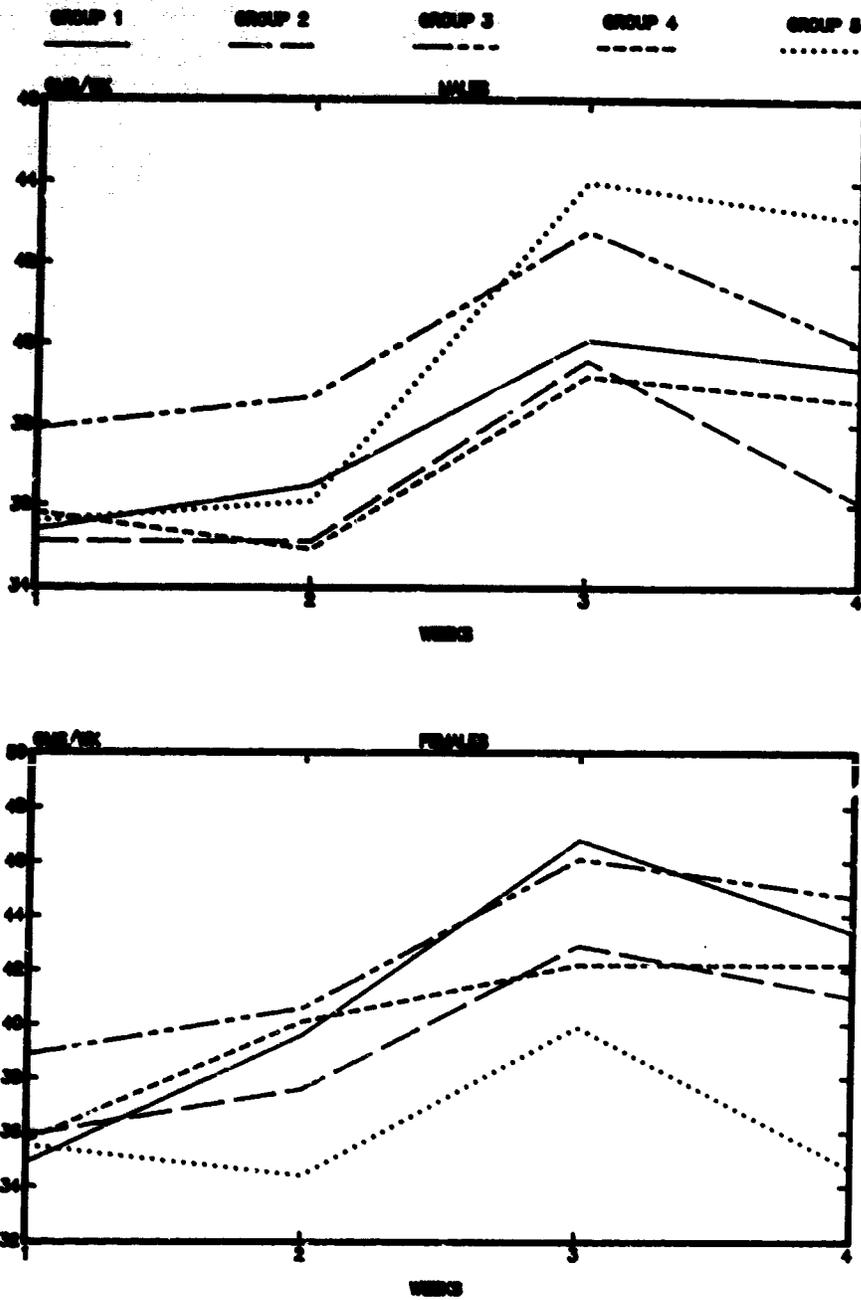
Mean body weights and food consumption values are presented graphically in Figures 2 and 3, respectively. Mean weekly body weights (Weeks 1-5) and weekly and total body weight changes (Weeks 1-4) are presented in Tables 3A and 3B, respectively. Mean weekly and total food consumption values (Weeks 1-4) and weekly and total food conversion efficiency (Weeks 1-4) are presented in Tables 4A and 4B, respectively. Mean compound consumption values are presented in Table 5. Individual body weight, body weight change, food consumption, food efficiency, and compound consumption values are presented in Appendices 3A, 3B, 4A, 4B, and 5, respectively.

FIGURE 2 - MEAN BODY WEIGHTS



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FIGURE 3 - MEAN FOOD CONSUMPTION



The mean body weight values of the Group 5 males and females at Weeks 3, 4, and 5 were significantly lower than controls. Mean body weight values of Group 2, 3, and 4 females were significantly elevated for at least one interval compared to the mean female control value at sporadic intervals, but the differences were slight. The mean weekly body weight changes in both sexes of Group 5 were negative or equal to zero at all weeks with the exception of the Week 1 value in the females. The mean total body weight changes (Weeks 1-4) were also negative in the Group 5 males and females, and significantly lower than corresponding controls for both sexes. Although the Group 4 female total body weight change value was significantly elevated, it was not considered to be treatment related.

The mean weekly food consumption (Weeks 2, 3, and 4) and total food consumption (Weeks 1-4) values were significantly decreased for the Group 5 females, but not for the Group 5 males. Weekly and overall (Weeks 1-4) mean food efficiency values were negative in both sexes of Group 5, with the exception of a positive value for the females at Week 1.

Compound consumption within each group of the same sex was comparable at Weeks 1-4 for Groups 2, 3 and 4, and at Weeks 1 and 2 for Group 5. In Group 5 animals at Weeks 3 and 4, compound consumption was 1.1 to 1.4 times greater than at Weeks 1 and 2.

Clinical Pathology

Hematology (including myeloid/erythroid ratios) and clinical chemistry data are presented in Tables 6 and 7 (mean data) and Appendices 6 and 7 (individual data), respectively. Statistically significant findings are presented in Text Table 1. A detailed discussion of clinical pathology findings is presented in the Clinical Pathology Report.

Due to the small size of the young mice on this study, the required volume of blood needed to evaluate all hematology and chemistry parameters could not be obtained. In many cases, less than half the animals in each group were used in statistical analyses, thus, making it difficult to interpret the data.

Text Table 1
 Statistically Significant Clinical Pathology Findings

| Group: | Male | | | | Female | | | |
|-------------------|------|---|---|---|--------|---|---|---|
| | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 |
| Hematology | | | | | | | | |
| RBC | | | | | | | | ↓ |
| HGB | | | | | | | | ↓ |
| HCT | | | | | | | | ↓ |
| WBC | | | | ↓ | | | | |
| CDR WBC | | | | ↓ | | | | |
| LYMPH | | | | | | | ↑ | ↑ |
| Chemistry | | | | | | | | |
| BUN | | | | ↑ | | | | |
| ALT | | | | ↑ | | | | ↑ |

Key: ↑/↓ = Significantly increased/decreased mean values as compared to corresponding control values, $p \leq 0.05$.

The significant differences in clinical hematology parameters noted in Text Table 1 are not considered to be treatment related. The elevated levels of blood urea nitrogen noted in Group 5 males and alanine aminotransferase activity noted in Group 5 males and females suggest renal and hepatic involvement, although the statistics are based on values from four or fewer animals in Group 5.

Gross Pathology

Gross pathology incidence is summarized in Table 8 and presented individually in Appendix 8.

Gross observations were noted in the livers of Group 3 and 5 males and Group 4 and 5 females, and were characterized as enlarged, dark, and/or having pale areas.

Other gross findings were sporadic among the groups or of singular occurrence, and therefore, not considered to be treatment related.

Terminal Body Weights and Absolute and Relative Organ Weights

Mean terminal body weight and absolute organ weight data, and organ-to-terminal-body-weight ratios are presented in Tables 9 and 10, respectively, and individually in Appendix 8. Statistically significant findings are presented in Text Table 2.

Text Table 2
Statistically Significant Terminal Body Weight and Organ Weight Data

| Group: | Male | | | | Female | | | |
|--------------------------|------|---|---|---|--------|---|---|---|
| | 2 | 3 | 4 | 5 | 2 | 3 | 4 | 5 |
| Terminal Body Weight | | | | ↓ | ↑ | ↑ | | ↓ |
| Absolute Organ Weights | | | | | | | | |
| Kidney | | ↓ | ↓ | ↓ | | | | |
| Liver with gallbladder | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | |
| Testes with epididymides | | ↓ | ↓ | ↓ | | | | |
| Relative Organ Weights | | | | | | | | |
| Kidney | | ↓ | ↓ | ↑ | | | | ↑ |
| Liver with gallbladder | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Testes with epididymides | | ↓ | ↓ | ↓ | | | | |

Key: ↑/↓ = Significantly increased/decreased mean values as compared to control values, $p \leq 0.05$.

Mean terminal body weights were significantly reduced in the Group 5 males and females when compared to the respective controls. Significant and dose-related decreases were seen in the mean absolute and relative kidney and testis weights of the Group 3, 4, and 5 males, with the exception of the increased relative kidney weight in Group 5. This and the significantly increased mean relative kidney weight of the Group 5 females were most likely secondary to the body weight loss of these animals. Mean absolute and relative liver weights were significantly increased in all treated male groups and all but Group 2 of the females as a result from treatment with DINP.

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Histopathology

Histopathology findings are summarized in Table 11 and presented individually in Appendix 8. A detailed discussion of the histopathology findings is presented in the Pathology Report.

Suggested treatment-related microscopic findings were observed in the liver, spleen, ovary, uterus, kidney, testes with epididymides, and thymus.

Hepatocytomegaly of the liver was noted in all treated male groups and all but Group 2 of the female groups. Other liver findings, focal necrosis and focal chronic inflammation, were noted in only the Group 5 males and Group 4 and/or 5 females. The finding termed atrophy was noted in the spleen of several Group 5 males and females, and also in the ovary and uterus of all the Group 5 females. Tubular nephrosis of the kidney was seen in all animals of both sexes in Group 5. Although there were no microscopic findings in the testes, an increased amount of cellular debris was present in the tail of the epididymides of all the Group 5 males. The thymus revealed possible compound-related findings of necrosis and/or depletion of the lymphocytes in primarily the Group 4 and 5 males, but sporadic incidences were noted in all of the female groups, including the control group.

DISCUSSION AND CONCLUSION

Weekly body weights were markedly decreased in both sexes of Group 5 (25,000 ppm) beginning at Week 3 of the study; at Week 5, the body weight means in these groups were about 77 and 78% of the corresponding control means for males and females, respectively. Food consumption in Group 5 females was markedly lower than in the corresponding controls; total food consumption (Weeks 1-4) was 88% of control. Thus, in the Group 5 females, the low body weights are associated with low food consumption. In the Group 5 males, however, there were no changes in food consumption which could be correlated with the low body weights.

Weekly and overall (Weeks 1-4) food efficiency values were negative in Group 5 animals (both sexes), with the exception of a positive value at Week 1 in the females. As food efficiency is calculated by dividing body weight change by food consumption and multiplying by 100 to obtain a percent value, the negative values for weekly body weight change at Weeks 1-4 in males and Weeks 2-4 in females and for total body weight change from Weeks 1-4 resulted in negative food efficiency values at these intervals.

At Weeks 3 and 4 of the study, Group 5 males and females received a higher dose level of DINP per kg of body weight than predicted based on the dietary concentrations (ppm) of DINP. The steady weight loss of Group 5 animals over the course of the study probably contributed in part to the higher compound consumption per kg during Weeks 3 and 4. In general, females received a slightly greater dose of DINP per kg body weight than did the males.

In this species and under the conditions of this study, the target tissues to DINP were liver, kidney, and testes. Results of this study also suggest that ovaries, uterus, and the reticuloendothelial system may be affected by exposure to DINP, but these associations are not as clear as for the aforementioned target tissues.

Liver weights were significantly increased in relation to dose level in all groups of compound-treated mice (both sexes, Group 2 [3,000 ppm] females excepted). The changes in liver weights correlated with the microscopically observed hepatocellular enlargement, which also showed a clear dose response (in terms of severity and incidence), and was present in all groups of compound-treated animals, with the exception of Group 2 females. Focal areas of coagulative necrosis and separate areas of chronic inflammatory foci, observed in Group 5 males and at a lower incidence in Group 4 (12,500 ppm) and 5 females, may be correlated with the observation that serum alanine aminotransferase activity was elevated in Group 5 males and females.

Significant dose-related decreases in absolute kidney weights were observed in Group 3 (6,000 ppm), 4, and 5 males. Relative kidney weights were also decreased in Group 3 and 4 males but increased in Group 5 males, most likely due to the relatively low mean terminal body weight in this group. Histologically, kidneys of all Group 5 males and females and one Group 4 male were characterized by tubular nephrosis. The significantly elevated blood urea nitrogen levels observed in the Group 5 males may have been a reflection of these renal changes.

Testes/epididymides weights were decreased in a dose-dependant manner in all groups of compound-treated males. This effect was most pronounced in the Group 5 males. In this group, the mean absolute testes/epididymides weight was about 69% of the corresponding control mean value. Histologically, there were no testicular lesions which could be correlated with the low organ weight, although an increase in debris was observed in the epididymides of all Group 5 males.

Ovaries of all Group 5 females were distinguished by a lack of corpora lutea, although normal follicles were present. Atrophy of the uterus was observed in all Group 5 females and was characterized as moderate to moderately severe. Similar findings have been reported in female mice which were food-restricted to a degree comparable to that

occurring in the Group 5 females in this study (see Pathology Report for reference). Thus, it is not clear as to whether these are direct compound-related effects or consequences of the depressed food intake in this group.

Atrophic spleens were noted in several Group 5 males and females and a moderate incidence of lymphoid depletion of the thymus was also observed in Group 5 animals of both sexes.

In conclusion, dosing of B6C3F1 mice with DINP for 4 weeks at a dose level of 25,000 ppm was associated with decreased body weight, decreased food consumption (females only), hepatic coagulative necrosis and associated elevation in serum alanine aminotransferase activity, renal tubular nephrosis and associated elevated blood urea nitrogen, atrophy of the spleen, lymphoid depletion in the thymus, epididymal lesions, and atrophy of the uterus and ovaries. Dosing at 12,500 ppm was associated with a low incidence of hepatic coagulative necrosis (females only) and a very low incidence of renal tubular nephrosis (one male). Dose-related hepatocytomegaly and associated statistically significant increases in liver/gallbladder weight parameters were observed in all compound-treated groups (with the exception of females receiving 3,000 ppm); these changes were most pronounced in the 25,000 ppm group. Statistically significant dose-related decreases in testes/epididymides weights and kidney weights were observed in males dosed with 6,000, 12,500, and 25,000 ppm. Based upon findings from this study, a high-dose level of 15,000 ppm is suggested for a subsequent 13-week dietary toxicity study of DINP using this species.

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CLINICAL PATHOLOGY REPORT

Evaluation of the hematology data revealed significantly decreased leukocyte count and corrected leukocyte count in the Group 5 males, due to nonsignificant decreases in the absolute segmented neutrophil and lymphocyte counts. In the females, the absolute lymphocyte counts were slightly, but significantly increased in Groups 4 and 5. Minimal, but significant decreases in erythrocyte count, hemoglobin, and hematocrit were noted in the Group 4 females, however, the corresponding values from the Group 5 females were comparable to control. The aforementioned differences in the hematology data were felt to be incidental to the administration of the test material, based on the low magnitude of the change and/or the lack of a dose response.

Evaluation of the clinical chemistry data revealed significantly increased blood urea nitrogen in the Group 5 males and alanine aminotransferase in the Group 5 males and females. Due to the small specimen volume available from mice insufficient numbers of values were obtained for some parameters, rendering interpretation difficult or impossible. Although the above findings suggest renal and hepatic involvement, the statistics were based on values from four or fewer animals in Group 5.

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PATHOLOGY REPORT

Male (10) and female (10) B6C3F1 mice were exposed to Di(isononyl)phthalate (DINP) in the diet for approximately 4 weeks at each of the following dose groups: Group 1 (untreated diet), 0 ppm; Group 2 (low), 3000 ppm; Group 3 (mid-low), 6000 ppm; Group 4 (mid) 12,500 ppm; Group 5 (high) 25,000 ppm.

Methods

All animals were examined grossly, and each animal was weighed prior to necropsy. Sacrifice was by exsanguination under sodium pentobarbital anesthesia. The most recent clinical observations were reviewed at necropsy, and all grossly observed abnormalities were entered, as encountered, directly into the computerized data capture system. Liver, kidneys, and testes with epididymides were weighed at the time of necropsy.

After gross examination, samples of the following organs were preserved in 10% neutral-buffered formalin:

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

Each lobe of the liver was incised several times to enhance penetration of the fixative, and each kidney was bisected-left longitudinally, right transversely. The lungs were inflated with formalin via the trachea, and contracted bladders were inflated with formalin. After fixation, bony tissues were decalcified prior to processing. Tissues to be examined histologically were embedded in paraffin, sectioned at approximately 5 microns, and stained with hematoxylin and eosin. Histopathological evaluations were performed on those tissues listed above from all animals of Groups 1 and 5. The liver, kidneys, spleen, testes with epididymides, and gross lesions were also examined from all animals of the remaining groups. During the initial histologic examination of tissues, additional target organs were defined which included thymus, ovaries, and uterus. As required by the protocol, these tissues were then examined histologically from all remaining animals in which they were present.

Gross and Related Findings

Gross findings are summarized in detail in the accompanying tables.

All animals survived until the scheduled terminal sacrifice during which there were few observed abnormalities. Of interest were descriptions of altered hepatic size (enlargement) and color which were applied mostly to males or females of Groups 3, 4, and 5. These findings appear to have been manifestations of histological alterations that will be described later in this report.

"Dark areas" were described a number of times in the mucosa of the glandular stomach, but without relation to exposure level.

All other gross abnormalities were of singular occurrence and unrelated to exposure level.

Mean absolute body weight values were significantly depressed in both males and females of Group 5 compared to Group 1 at the weighings

that occurred during Weeks 3, 4, and 5 of the study. Group 5 animals of both sexes also lost body weight as a function of time on study. All other groups gained weight as the study progressed.

The possible role of food consumption in weight depressions is not clear, and, although there are definite groupwise differences, with a couple of exceptions they are unrelated to dose. In females, food consumption was clearly depressed in Group 5 for most of the study, which may help to explain why that group lost weight. Group 5 males, on the other hand, consumed more food than any but Group 3 starting shortly after 2 weeks and more than any other group from 3 weeks until the end of the study; and yet Group 5 males still lost weight as described, so that the role of caloric intake is not clear.

Overnight fasting prior to necropsy caused large decreases in mean body weights of all the groups which are reflected in both the terminal body weight figures and the relative organ weight values calculated on the basis of terminal (post fasting) absolute body weights.

Significant, dose-related decreases in absolute mean organ weight were seen in kidneys and testes of males of Groups 3, 4, and 5. Expressed as organ to terminal body weight ratios, significantly decreased values are still present for kidneys of Groups 3 and 4 males; however, the kidneys of Group 5 show a significant increase in value. This is likely the result of the disproportionately large loss of body weight that occurred in this group and is not the result of any direct effect of the high dose on the kidneys.

The fact that kidney weights were decreased in a dose-related pattern in three groups, two of which showed no evidence of body weight loss, suggest that those decreases occurred as a direct result of exposure to the test substance. Histologic findings tend to corroborate this observation.

Testicular weights are usually quite stable and do not respond quickly to losses of body condition. In this case the decreases in

testicular weight were large enough to strongly suggest a direct effect of compound exposure in Groups 3, 4, and 5.

Mean liver weights, both absolute and relative, were significantly increased in response to DINP exposure in males of Groups 2, 3, 4, and 5 and females of Groups 3, 4, and 5. Histologic correlates will be described.

Histologic Findings

Histologic findings are summarized in detail in the accompanying tables.

There were a number of observed histologic alterations which seem to be related to DINP exposure.

Hepatocellular enlargement was present in the livers of males of Groups 2, 3, 4, and 5, and females of Groups 3, 4, and 5, with a clear dose response relative to incidence and severity. The change, which correlates nicely with organ weight data, was generally diffuse; but in some less developed cases it was more centrilobular in distribution. Affected hepatocytes were swollen with dense, hypereosinophilic cytoplasm. The normal, trabecular architecture was somewhat obscured by the swollen hepatocytes. In many cases, particularly in Group 5 males and also, though less commonly Groups 4 and 5 females, focal areas of coagulative necrosis, mostly devoid of inflammatory reaction, accompanied these cytoplasmic changes; and, in addition, separate chronic inflammatory foci were also seen. These latter changes, particularly the presence of hepatocellular necrosis, may explain an increase in serum levels of alanine aminotransferase that was also reported in the group 5 males and females.

The kidneys of all Group 5 males and females showed compound-related changes characterized by areas of atrophic tubules, sometimes dilated, and often containing granular proteinaceous material which sometimes contained cellular debris. Degenerative cytoplasmic changes,

and often regenerative foci were present; but tubular necrosis was not characteristic. Although these lesions did not as a whole appear morphologically to be very severe, morphologic appearance is not necessarily an accurate indicator of functional impairment. It is, therefore, possible that significantly elevated BUN values reported for Group 5 males did occur as a result of renal damage. BUN values were not significantly elevated for females, and that may or may not be a reflection of histologic lesions that were of a less severe nature than in males.

A single Group 4 male had a small renal lesion compatible with those described, but one which could just as easily be attributed to early chronic progressive nephropathy, a spontaneous kidney disease of mice. The Group 4 level would appear to be a no observable effect level (NOEL) relative to described renal effects, but that is not conclusive without further study.

Some compound related-effect on the lymphoreticular system is suggested by the finding, in several males and females of Group 5, of distinct hypoceullularity in the interfollicular areas of the spleen with little or no evidence of extramedullary hematopoiesis. This finding was termed atrophy. Seen also, in most females of all groups but primarily in Groups 4 and 5 of the males was an increase in the necrosis of lymphocytes in the thymus. In the most mild cases this was seen as an increase in the number of debris-laden macrophages, but in the more severely affected animals there was a great deal of karyorrhectic debris besides that in phagocytes. There was no noticeable change in the bone marrow. Any conclusions drawn between these observations and their possible relationship to DINP exposure would be tenuous; however, there were some rather vague but suggestive changes to hematologic parameters which support the suggestion that the hematopoietic system should be carefully watched in any further work with this compound. Below the Group 4 level

there is no suggestion of effect; at the Group 4 level the suggestion of effect is very slight.

The testes are interesting in that while there were no histologic alterations to the testes themselves suggestive of a correlation with the dose related decrease in organ weight, and while spermatogenesis seemed not to have been affected as evidenced only by subjective histologic observations of the numbers of spermatozoa in testes and epididymides, there was a clear increase in the amount of cellular debris present in the tail of the epididymides of all Group 5 males. The testes are the presumed origin of this detritus which serves as indirect evidence that spermatogenesis was, somehow affected by the test substance, and that the viability of some spermatogenic cells was somehow compromised. The limitation of this finding to Group 5 correlates nicely with organ weight data which also showed a much larger effect on Group 5 males. Taken together, however, the data suggest that organ weights are, in this case, a more sensitive indicator of testicular effect than histopathology by itself.

Reproductive system effects were not limited to the male, however. The ovaries of all Group 5 females appeared smaller in section (they were not weighed); and, interestingly, the main distinguishing histologic feature was the virtually complete absence of corpora lutea. Normal follicles were plentiful, but the lack of luteinization would suggest an arrest of ovulation. This finding was termed "atrophy" for purposes of this report. The uteri of all Group 5 females were also affected by DINP-exposure. They were smaller than normal in cross-sectional area; and the endometrium was virtually devoid of glands.

Discussion

The finding of hepatocytomegaly is not surprising, as phthalate esters have, for some time, been recognized to induce proliferation of

specific cytoplasmic organelles called peroxisomes in liver cells resulting in the enlargement of those cells and the liver itself.¹

Similarly, testicular damage has been reported in rodents in response to certain phthalate esters.^{2,3,4} This effect is not seen with all phthalate esters, however, and is dependent on certain structural characteristics shared by those exerting the effect.^{3,4} In this study, testicular damage per se was not seen histologically; but an effect was clearly demonstrated by decreases in testicular weight and by the presence of increased amounts of cellular debris in the tail of the epididymides. While it is important to note that the epididymidal finding was present only in Group 5 animals that had been exposed to an extremely high level of DINP, it should also be mentioned that mice have been shown to be less sensitive to the testicular effects of some phthalate toxicities than rats;⁴ consequently, rats exposed to equivalent levels of this compound might be expected to exhibit a more severe response.

The apparent anovulatory effect and uterine atrophy/hypoplasia observed in the female reproductive tract of animals exposed to the highest level of the test substance have not, to my knowledge, been reported in previous toxicity studies of this class of compounds. These changes are, potentially, very important; however similar morphophysiological effects have been described in female mice as a result of feed restriction of a degree comparable to that which occurred within the Group 5 females of this study,⁵ and, using only the available data, it would be very difficult to rule out nutritional deficiency as the proximate cause.

Renal tubular nephrosis was seen with certainty only at the highest dose level; however it will be important to study the kidneys under conditions of longer term exposure to lesser dose levels of this compound.



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Effects on the lymphoreticular system were suggested as a result of exposure to DINP; however the changes were not consistent enough to draw clear conclusions.


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PATHOLOGY REFERENCES

1. Warren JR, Labwani ND, Reddy JK: Phthalate esters as peroxisome proliferator carcinogens. *Environmental Health Perspectives* 45:35-40, 1982.
2. Gangoli SD: Testicular effects of phthalate esters. *Environmental Health Perspectives* 45:77-84, 1982.
3. Foster PMD, Thomas LV, Cook MW, Gangoli SD: Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. *Toxicology and Applied Pharmacology*: 54:392-398, 1980.
4. Curto KA, Thomas JA: Comparative effects of diethylhexyl phthalate or monoethylhexyl phthalate on male mouse and rat reproductive organs. *Toxicology and Applied Pharmacology*: 62:121-125, 1982.
5. Dierckman TA, Stitzel KA, McConnell RF, Frank RF: The effects of 12-week dietary restriction on ovarian function determined by vaginal smear, organ weights and histopathology with 4 and 8 interim and 12 week terminal sacrifice in B6C3F1 mice. Abstract presented at Biological Effects of Dietary Restriction, an international conference. Washington, D.C., March, 1990.

REFERENCES

Hematology

Cell Morphology and Leukocyte Differential

BROWN, B. (1988). HEMATOLOGY: Principles and Procedures, 5th ed. Lea and Febiger, Philadelphia, PA.

DAVIDSOHN, I., AND HENRY, J. (1974). Todd-Sanford Clinical Diagnosis by Laboratory Methods, 15th ed. W. B. Saunders Co., Philadelphia, PA.

Hemomatic[®] Slide Stainer, Model 70-9. Instruction, Operation, and Service Manual #H-008 (1980). Gam Rad West, Inc., San Juan Capistrano, CA.

KAPFF, C., AND JANDL, J. (1981). BLOOD, Atlas and Sourcebook of Hematology, 1st ed. Little, Brown and Company, Boston, MA.

MIALE, J. (1977). Laboratory Medicine, Hematology, 5th ed. C. V. Mosby Co., St. Louis, MO.

O'CONNOR, B. (1984). A Color Atlas and Instruction Manual of Peripheral Blood Cell Morphology. Williams and Wilkins Co., Baltimore, MD.

PATRICK, C. (1978). Red Blood Cells: Current Aspects. ASCP Regional Continuing Education Programs.

SCHALM, O. W., JAIN, N. C., AND CARROLL, E. J. (1975). Veterinary Hematology, 3d ed. Lea and Febiger, Philadelphia, PA.

SCHERMER, S. (1967). The Blood Morphology of Laboratory Animals, 3d ed. F. A. Davis Co., Philadelphia, PA.

WILLIAMS, W., BEUTLER, E., ERSLEV, A., AND RUNDLES, R. (1977). Hematology, 2d ed. McGraw-Hill Book Co., New York, NY.

WINTROBE, M. (1981). Clinical Hematology, 8th ed. Lea and Febiger, Philadelphia, PA.

ZUCKER-FRANKLIN, D., GREAVES, M., GROSSI, C., AND MARMONT, A. (1981). Atlas of Blood Cells, Vols. I and II. Lea and Febiger, Philadelphia, PA.

Coulter Counter[®] Model S+IV System

Product Reference Manual 4235328B (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Data Terminal with Data Handling PN4235456D (1986, January). Coulter Electronics, Inc., Hialeah, FL.

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

Corrected Leukocyte Count (COR WBC)

$(WBC \times 100) \div (NRBC + 100) = COR\ WBC\ (Calculated).$

Mveloid/Erythroid Ratio (M/E)

Hemomatic Slide Stainer, Model 70-9. Instruction, Operation, and Service Manual #H-008 (1980). Gam Rad West, Inc., San Juan Capistrano, CA.

SCHALM, O. W., JAIN, N. C., AND CARROLL, E. J. (1975). Veterinary Hematology, 3d ed. Lea and Febiger, Philadelphia, PA, pp. 68-69, 321.

Clinical Chemistry

BMD/Hitachi[®] 737 Chemistry Analyzer

Boehringer Mannheim Diagnostics, Indianapolis, IN.

| | <u>Insert # (date)</u> |
|----------------------------------|--|
| 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) |
| 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).

Statistical Methods/Laboratory Procedures

Bartlett's Test

BARTLETT, M. S. (1937). Some examples of statistical methods of research in agriculture and applied biology. J. Royal Statist. Soc. Suppl. IV, pp. 137-170.

Dunnett's t-Test for Control vs. Treatment Comparisons

DUNNETT, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, pp. 1096-1121.

DUNNETT, C. W. (1964). New tables for multiple comparisons with a control. Biometrics 20, pp. 482-491.

Levene's Test

DRAPER, N. R., AND HUNTER, W. G. (1969). Transformations: some examples revisited. Technometrics 11, pp. 23-40.

LEVENE, H. (1960). Robust tests for equality of variances. In Contributions to Probability and Statistics (I. Olkin, ed.). Stanford University Press, Palo Alto, pp. 278-292.