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E. I. DU PONT DE NEMOURS AND COMPANY
Haskell Laboratory for Toxicology
and Industrial Medicine
P.O. Box 50, Elkton Road
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DU PONT CENTRAL RESEARCH AND DEVELOPMENT

CONTAINS NO CBI

8EHO-0692-1303 SUPP

June 11, 1992

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Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street SW
Washington, DC 20460



8920000312

:DCN

Dear Coordinator:

8EHO-0791-1303

In our letter of July 24, 1991, we informed you of the preliminary results of a recently completed ninety-day feeding study in rats.

Enclosed please find a copy of the final report.

Sincerely,

Charles F. Reinhardt

Charles F. Reinhardt, M.D.
Director

CFR/AMK:dj
Phone: (302)366-5285

Enclosure (1): Final Report, Du Pont HLR 370-91, "Subchronic Oral Toxicity: 90-Day study with DPX-T3217-107 (Cymecranil) Feeding and Neurotoxicity Study".

No CBI in this submission as per Mr. [unclear] 20
phone call to submitter on 6-15-92 6/15/92

THE DOCUMENT RECEIVED BY
92 JUL 21 AM 11:53

Subchronic Oral Toxicity: 90-Day
Study with DPX-T3217-107 (Cymoxanil)
Feeding and Neurotoxicity Study
in Rats

Volume 1 of 3

0 8 0 4

Study Title

Subchronic Oral Toxicity: 90-Day Study
with DPX-T3217-107 (Cymoxanil)
Feeding and Neurotoxicity Study in Rats

Volume 1 of 3

Data Requirements

U.S. EPA Pesticide Assessment Guidelines
Subdivision F, 82-1

OECD Test Guideline 408
MAFF Japan 59 NohSan No. 4200

Author

Dolores E. Malek, Ph.D.

Study Completed on

June 8, 1992

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Medical Research Project No. 9131-001

Laboratory Project ID

Haskell Laboratory Report No. 370-91

0009

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-1C7

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA FIFRA Good Laboratory Practice Standards (40 CFR 160), OECD Principles of Good Laboratory Practice (C(81)30(Final), Annex 2), and MAFF Japan Good Laboratory Practice Standards (59 NohSan No. 3850). Any areas of noncompliance are documented in the study records. No deviations existed that affected the validity of the study.

Additional analyses were performed to resolve questions pertaining to the stability of the test material in diet maintained at room temperature. Analyses performed by the sponsor, Du Pont Agricultural Products, to address this issue were not part of the original study design. These data, which are considered supplemental, were not subject to quality assurance evaluation. The analyses are included in a report titled "Investigation of Cymoxanil Stability in Rat Diet."

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Du Pont Agricultural Products
E. I. du Pont de Nemours and Company

Date

Study Director: _____

Dolores E. Malek
Dolores E. Malek, Ph.D.
Research Toxicologist
Chronic and Biochemical Toxicology

6/3/92

Company Representative: _____

Registration Specialist

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

FLAGGING OF STUDIES FOR POTENTIAL ADVERSE EFFECTS

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Study Director: *Dolores E. Malek* 6/3/92
Date
Dolores E. Malek, Ph.D.
Research Toxicologist
Chronic and Biochemical Toxicology

Company Representative: _____
Registration Specialist

0008

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

REPORT AND COMPOUND INFORMATION

Material Tested:

Haskell No.

2-Cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)
acetamide

18753

Other Codes and/or synonyms:

DPX-T3217-107
DPX-T3217
IN-T3217-107
cymoxanil
Lot # D91029420 or D291029420

Submitter's Notebook No.: N.B. 0170-126

C.A.S. Registry Number: 57955-95-7

Medical Research Project No.: 9131-001

Purity

Active Ingredient: 96.8% (Initial Analysis)
97.6% (Re-analysis)

Sponsor:

Du Pont Agricultural Products
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19805

Material Submitted by:

John C. Summers
Du Pont Agricultural Products
Experimental Station, Bldg. 402
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19698

Study Initiation/Completion Dates: 11-28-90 / 06-08-92

In-life Study Initiated/Completed: 12-21-90 / 04-12-91

Notebooks: E67387, E67387-AA, -BA, -CA, -DA, -EA, -EB

Distribution:

J. C. Summers (6)
Chronic and Biochemical Toxicology (1)

0004

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
FEEDING AND NEUROTOXICITY STUDY IN RATS

SUMMARY

The objectives of this feeding study were to assess the potential subchronic toxicity and neurotoxicity of DPX-T3217 (cymoxanil) in rats. For approximately 90 days, five groups of 20 male and five groups of 20 female Crl:CDØBR rats each were fed diets that contained 0, 100, 750, 1,500, or 3,000 ppm DPX-T3217. Within each group, the first set of ten rats was designated for the evaluation of subchronic toxicity. The remaining ten rats in each group were assigned to the neurotoxicity substudy. Samples of the prepared diet were collected periodically during the study to verify the concentration of DPX-T3217. The rats were weighed weekly throughout the study. Individual food consumption and clinical signs of toxicity were also assessed. Ophthalmoscopic examinations were conducted on test days -13 and 91. At approximately 45 and 90 days after study initiation, all surviving subchronic rats were evaluated for clinical pathology. On test days 104 and 105, all surviving rats from the subchronic study were sacrificed and necropsied. Selected organs were weighed and tissues examined for the presence of any gross or microscopic lesions. Rats from the neurotoxicity substudy were assessed by a functional observational battery (FOB) and by monitoring motor activity prior to study initiation and during study weeks 5, 9, and 13. All rats in the neurotoxicity substudy were prepared and sacrificed for neuropathology evaluation following the week 13 neurobehavioral assessments.

Body weights and body weight gains in both male and female rats at the 100 and 750 ppm levels were comparable with those of the control group throughout the study. Occasional statistically significant decreases in mean body weight and mean body weight gain were observed in males and females at 1,500 ppm within the first two weeks of the study. Significant decreases, however, were observed in mean body weights throughout the study for male and female rats at the 3,000 ppm level. Both male and female rats at 3,000 ppm also had significant decreases in overall mean body weight gains. These body weight effects at 1,500 and 3,000 ppm were considered compound related.

With the exception of the 3,000 ppm females, the overall mean daily food consumption observed in compound-treated groups was comparable with control. At 3,000 ppm, a statistically significant increase in food consumption was observed in female rats throughout the study. An overall increase in food consumption above control was evident in female rats at 1,500 ppm, however this effect was not significant. These increases in food consumption were coincident with depressed body weight gains and significant decreases in mean food efficiency in the 1,500 and 3,000 ppm female groups. Significant decreases in food efficiency related to compromised body weight gain, in male rats at 1,500 and 3,000 ppm, were also present but to a lesser degree than those observed in female rats. The food consumption effects in females and food efficiency effects in males and females at the 1,500 and 3,000 ppm levels were considered compound related.

The overall mean daily intake of DPX-T3217 for the 100, 750, 1,500, and 3,000 ppm dose groups (subchronic and neurotoxicity rats) was approximately 6.54, 47.6, 102, and 224 mg/kg/day, respectively, for male rats, and 8.00, 59.9, 137, and 333 mg/kg/day, respectively, for female rats. Analyses of diet samples indicated that DPX-T3217 was stable, and the preparations of diets containing the subject test material were judged to have met the targeted concentrations for this study.

There were no adverse clinical signs or ophthalmological findings in male or female subchronic rats attributable to dietary consumption of DPX-T3217. One female rat from the 750 ppm level was found dead on test day 42. This death was not considered compound related. There were no other mortalities throughout the study.

Mean total leukocyte counts in male rats in the 1,500 and 3,000 ppm groups were decreased primarily due to a moderate decrease in lymphocytes and a slight decrease in monocytes. These leukocytic alterations were most likely related to an increased release of endogenous glucocorticoids rather than to a primary,

compound-related effect. Biologically significant alterations were not observed in clinical chemical or urinalysis variables of male rats. No biologically significant clinical pathological alterations were observed in female rats.

In the subchronic study, significant compound-related reductions in mean final body weights resulted in a significant decrease in absolute mean heart weight in the 3,000 ppm males and a significant increase in mean relative testes, kidney, and brain weights in 1,500 and 3,000 ppm males. The 3,000 ppm females had significantly increased mean relative liver and spleen weights. Although the biological significance of the increase in splenic weight was uncertain, the increase in liver weight was judged to be biologically significant and compound related.

Microscopically, increased elongate spermatid degeneration was observed in the seminiferous tubules of the testes. Minimal epididymal changes including intratubular debris, multinucleated spermatids, and bilateral hypospermia were associated with elongate spermatid degeneration. Coincident testicular and most epididymal effects were statistically significant at 3,000 ppm and considered compound related at 1,500 ppm and 3,000 ppm.

In the neurotoxicity substudy, male and female rats given DPX-T3217 in the diet at 3,000 ppm had biologically remarkable and/or statistically significant decreases in mean body weights, relative to controls. In male rats, the 3,000 ppm diet also decreased relative landing foot splay scores and reduced relative forelimb grip strength scores during weeks 9 and/or 13. These non-significant observations were considered to reflect the significantly smaller body weights of the 3,000 ppm male rats and were not considered neurotoxic effects. In female rats, no behavioral parameters were affected by DPX-T3217.

There were no compound-related gross or microscopic lesions within the nervous system or skeletal muscle in any neurotoxicity substudy rats evaluated. These neurobehavioral and neuropathological data indicated that DPX-T3217 was not a neurotoxic agent.

Under the conditions of this study, the no-observable-effect level (NOEL) for subchronic findings was 750 ppm for male and female rats. The NOEL for evidence of neurotoxicity was 3,000 ppm for male and female rats.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
FEEDING AND NEUROTOXICITY STUDY IN RATS

Medical Research Project No. 9131-001

	<u>Date</u>
Authored by: <u><i>Dolores E. Malek</i></u> Dolores E. Malek, Ph.D. Research Toxicologist Chronic and Biochemical Toxicology	<u>6/3/92</u>
Quality Assurance Review by: <u><i>Joseph C. Hamill</i></u> Joseph C. Hamill Coordinator Quality Assurance	<u>6/3/92</u>
Approved by: <u><i>Robert W. Rickard</i></u> Robert W. Rickard, Ph.D., D.A.B.T. Manager Chronic and Biochemical Toxicology	<u>6/5/92</u>
Reviewed and Approved for Issue by Study Director: <u><i>Dolores E. Malek</i></u> Dolores E. Malek, Ph.D. Research Toxicologist Chronic and Biochemical Toxicology	<u>6/8/92</u>

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
FEEDING AND NEUROTOXICITY STUDY IN RATS

Medical Research Project No. 9131-001

QUALITY ASSURANCE DOCUMENTATION

<u>Time Interval</u>	<u>Audit Dates</u>	<u>Audit Report #</u>	<u>Date Findings Reported to Management & Study Director</u>	
Study Audits:				
2-Month	12/20/90; 2/21,22,28/91; 3/1/91	1149	3/1/91	3/1/91
End-of-Testing	3/27/91; 4/5/91; 5/9,13/91	----	5/13/91	5/13/91
Clinical Pathology Report:				
#14-91	7/9,10/91	----	7/10/91	7/22/91
Pathology Report:				
#59-91	9/30/91; 10/1-4/91	----	10/7/91	10/9/91
Neuropathology Report:				
#68-91	10/4,7/91	----	10/7/91	10/7/91
Analytical Reports:				
#HA-91-120	1/29/92; 2/3/92	----	2/20/92	2/25/92
#HA-91-121	" "	----	"	"
#HA-92-003	1/31/92; 2/4,7/92	----	2/11/92	4/13/92
Neurobehavioral Report:				
	2/21,24-26/92; 3/23-26,30/92	----	3/30/92	4/3/92
Final Report:				
HLR #370-91	5/11-15,18,19/92	1262	5/21/92	6/3/92

Joseph C. Hamill 6/3/92
 Joseph C. Hamill
 Coordinator
 Quality Assurance

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DFX-T3217-107

ACKNOWLEDGMENTS

The following individuals participated in the conduct of the study:

Study Directors: Dolores E. Malek, Ph.D. (7/01/91 - present)
Charles A. Mebus, Ph.D. (11/28/90 - 6/30/91)

Manager: Robert W. Rickard, Ph.D.

Operations Supervisor: Stephen D. Nash

Report Writing
Supervisor: Anne Marie Bathon, B.A.

Toxicology Report
Preparation: P. Jeffrey Chapman, B.S.

Primary
Technician: Elizabeth A. Kretzing, B.S.

The following individuals were responsible for the clinical pathology evaluations, gross pathology examinations, slide preparation, and microscopic evaluations:

Managers: James R. Gibson, Ph.D. (1/10/92 - present)
Theodore W. Slone, Jr., D.V.M. (11/28/90 - 1/09/92)

Clinical Pathologists: Glenn S. Elliott, D.V.M., Ph.D. (9/16/91 - present)
Steven R. Frame, D.V.M., Ph.D. (6/22/91 - 9/15/91)
Michael C. Carakostas, D.V.M., Ph.D.
(11/28/90 - 6/21/91)

Pathologist: Mary M. Sommer, V.M.D.

Neuropathologist: Mary M. Sommer, V.M.D.

Supervisor: Joan A. Wolfe (11/28/90 - 1/31/92)

Clinical Pathology
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Donna R. Holt, A.A. (11/28/90 - 5/31/91)

Pathology
Report Preparation: Wanda F. Dinbokowitz

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

ACKNOWLEDGMENTS (Continued)

The following individuals were responsible for neurotoxicity evaluation:

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Senior Research
Biologist: Greg R. Christoph, Ph.D. (5/01/91 - 8/25/91)

Research
Teratologist: Cynthia D. Driscoll, Ph.D. (11/28/90 - 4/30/91)

Neurotoxicity
Report Preparation: Kathleen A. Mikles, B.A.

Primary Technician: Kathleen A. Mikles, B.A.

Secretary: Judith R. Hash

The following individuals were responsible for compound purity and diet analyses performed at Haskell Laboratory:

Managers: Ralph G. Stahl, Ph.D. (2/01/91 - present)
Frank X. Phillips, Ph.D. (11/28/90 - 1/31/91)

Research
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Analytical Chemist: Charlotte H. Lattin, B.S. (7/15/91 - present)

Research Biochemists: Paul H. Lieder, Ph.D. (5/24/91 - 7/14/91)
Daniel R. Vincent, Ph.D. (11/28/90 - 5/23/91)

Technician: Nirmala Raghavan, B.S.

The following individuals were responsible for the conduct and reporting of experimental diet analyses performed at the Experimental Station:

Research Biologist: Peter Horne, Ph.D.

Technician: Caroline R. Boucher

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

ACKNOWLEDGMENTS (Continued)

The following individuals were responsible for quality assurance:

Coordinator: Joseph C. Hamill (3/1/92 - present)

Manager: Carl W. Erkenbrecher, Jr., Ph.D. (11/28/90 - 2/29/92)

Senior Auditor: Joseph C. Hamill (11/28/90 - 2/29/92)

Ophthalmological examinations were conducted by

James M. Clinton, V.M.D.
Animal Eye Clinic
South Jersey Animal Hospital
Medford, New Jersey

The health status of the animals on study was assessed by the attending
Laboratory Veterinarian, Charles E. Cover, V.M.D.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
FEEDING AND NEUROTOXICITY STUDY IN RATS

INTRODUCTION

The subject compound, DPX-T3217 (cymoxanil), is a fungicide undergoing toxicity evaluation for re-registration. Dose levels selected for this study were based upon a previous 90-day feeding study conducted at 0, 100, 500, and 2,500 ppm (technical material) DPX-T3217 in the diet.(1) Decreases in body weight and body weight gain were observed in both male and female rats from the 2,500 ppm dose groups. Mean body weight decreases for male and female rats were approximately 18% and 14%, respectively. Decreases in mean body weight gain were approximately 22% and 20% for male and female rats, respectively. There was no histologic evidence of toxicity in the tissues of male or female rats fed 2,500 ppm DPX-T3217 in the diet; however, hematological and clinical chemical analyses indicated that this dose may have caused some liver toxicity and mild anemia. The high doses for the present study (1,500 ppm and 3,000 ppm) were selected to establish a maximum tolerated dose (MTD),(2) whereas the intermediate dose (750 ppm) was expected to cause minimal toxicity. The low dose (100 ppm) was expected to be a no-observable-effect level (NOEL).

OBJECTIVE

The objectives of this study were to evaluate the subchronic toxicity and neurotoxicity of DPX-T3217 when incorporated into nutritionally adequate chow and fed to male and female Crl:CD¹BR rats for approximately 90 days. The oral route of administration was selected because it is a potential route of human exposure.

MATERIALS AND METHODS

The protocol and protocol amendments are in Appendix A. This study was conducted in accordance with all applicable Good Laboratory Practice standards.(3,4,5) The study design meets and/or exceeds United States EPA FIFRA(6) and TSCA,(7) OECD,(8) and MAFF Japan(9) test or proposed test guidelines. Additional analyses performed by the sponsor to resolve issues pertaining to the stability of the test material in diet were not outlined in the original study design. These analyses are included in the report titled "Investigation of Cymoxanil Stability in Rat Diet."

A. Test Material

The test material and an analytical standard of known purity were supplied by the sponsor, Du Pont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, Delaware. DPX-T3217 was submitted for analysis of purity at the beginning (12/13/90) and near the end (4/17/91) of the study.

B. Test Species

On 12/4/90, 139 male and 135 female Crl:CD®BR rats, born approximately 11/13/90, were received from Charles River Laboratories, Inc., Raleigh, North Carolina. On 12/5/90, the body weights ranged from 42.6 to 77.7 grams for the male rats, and from 35.3 to 74.9 grams for the female rats. The Crl:CD®BR rat was selected on the bases of extensive experience with this strain and its suitability with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

All rats were housed in stainless steel, wire-mesh cages suspended above Upjohn Deotized Animal Cage Boards (DACB®) or R-2 Reemay®-backed cage boards. Cage racks were relocated within the animal room each week and cages were

repositioned on the racks every two weeks. Animal rooms were maintained on a 12-hour light/dark cycle (fluorescent light) and targeted at a temperature of $23 \pm 2^{\circ}\text{C}$ and a relative humidity of $50 \pm 10\%$. Occasional excursions outside the target ranges were minor and did not affect the study.

Tap water was provided ad libitum. During the pretest period (see Section D), all rats were fed Purina® Certified Rodent Chow® #5002 meal; during the test period, all rats were fed the diet of their respective treatment group (see Section F).

Haskell Laboratory has an animal health monitoring program which consists of periodic food and water analyses for contaminants and sampling freshly washed cages and cage racks for bacteria. This program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records. No deviations existed in variables measured that affected the validity of the study.

D. Pretest Period

Upon arrival at Haskell Laboratory, the rats were removed from shipping cartons and housed three per cage, sexes separate. Each rat was temporarily identified with a colored tail mark and the cages were labeled with the temporary numbers. The rats were weighed three times during the pretest period and observed daily for any gross signs of disease or injury. On 12/7/90, all rats were examined by a veterinary ophthalmologist (see Section J).

On the bases of body weights and clinical observations, all rats were considered healthy and were released from quarantine on 12/12/90 by the laboratory veterinarian.

A neurobehavioral test battery, consisting of motor activity and functional observational battery assessments, was conducted on all neurotoxicity rats prior to study start on days 12/17/90 through 12/20/90.

E. Assignment to Groups and Study Start

After the quarantine period, 100 rats of each sex were selected for study use on the bases of adequate body weight gain and freedom from any ophthalmological abnormalities or clinical signs of disease or injury. The selected rats were divided by computerized, stratified randomization into five groups of 20 males and five groups of 20 females, so that there were no statistically significant differences among group body weight means within a sex. The order of rats within each group was randomized. Each group of rats within a sex was designated as either a control, low-, low-intermediate-, high-intermediate-, or high-concentration group. The first ten rats in each group were designated for subchronic toxicity evaluation. The remaining ten rats in each group were designated for neurotoxicity evaluation (neurobehavioral and neuropathological).

After assignment to groups, each rat was housed individually and permanently identified with a number tattooed on the tail. The study started on 12/21/90. The rats were approximately 38 days of age at study start. Rats that were not assigned to a test group were removed from the animal room approximately two weeks after study start and were sacrificed by carbon dioxide inhalation.

F. Diet Preparation, Administration, and Sampling

During the test period, rats in each group were fed a diet of Purina® Certified Rodent Chow® #5002 meal (PCRC) that contained the following concentrations of DPX-T3217:

<u>Groups</u>		<u>Dietary Concentration of DPX-T3217 (ppm)^a</u>
<u>Male</u>	<u>Female</u>	
I	II	0 (Control)
III	IV	100 (Low)
V	VI	750 (Low-intermediate)
VII	VIII	1,500 (High-intermediate)
IX	X	3,000 (High)

^a Weight/weight concentration of active ingredient DPX-T3217 (adjusted for 96.8% purity).

DPX-T3217 was added to PCRC and thoroughly mixed for three minutes in a high-speed mixer. Control diets were mixed for the same period of time. All diets were prepared weekly.

On test day -1, samples (approximately 50 grams each) were collected from each concentration of diet prepared with DPX-T3217. These samples were analyzed to verify concentration, homogeneity, and stability of DPX-T3217 in the test diets. Fresh-frozen samples of each diet containing DPX-T3217 were collected from the bottom of the diet mixer and frozen until analyzed. Homogeneity samples were collected from the top, middle, and bottom of the diet mixer and frozen until analyzed. Stability samples collected from feed jars were stored at room temperature for 7 and 14 days. Stability samples collected from the bottom of the diet mixer were refrigerated for 14 days.

Samples (approximately 50 grams) were collected from feed jars from diets prepared on test days 48 and 90 (2/7/91 and 3/21/91). These samples were collected from one feeder from each group and frozen on the day of collection. Only one feeder sample per dietary level was analyzed; the other was discarded without analysis.

After all storage conditions were met, samples were frozen and stored frozen until analyzed. Whenever any samples were collected, a sample of control diet was also collected and frozen.

Methods used to analyze the samples are outlined in the Diet Analyses Report.

G. Body Weights

All rats were weighed once per week throughout the study. In addition, the neurotoxicity substudy rats were weighed on the days when FOB and motor activity assessments were made.

H. Food Consumption, Food Efficiency, and Intake of DPX-T3217

The amount of food consumed by each rat over each weighing interval was determined throughout the study. From these determinations and body weight data, mean individual daily food consumption, food efficiency, and intake of DPX-T3217 were calculated.

I. Clinical Observations and Mortality

Cage-site examinations to detect moribund or dead rats and abnormal behavior and appearance among rats were conducted at least once daily throughout the study.

At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. The neurotoxicity substudy rats were also observed during FOB and motor activity monitoring.

J. Ophthalmological Evaluations

Two ophthalmological examinations were conducted by a veterinary ophthalmologist. At least one hour before each examination, one or two drops of 1% atropine sulfate solution were placed in each eye of every rat. Both eyes were examined by focal illumination and indirect ophthalmoscopy. The examinations were conducted under subdued lighting.

The initial examination was performed during the pretest period (test day -13) on all rats received for the study, prior to selection and grouping. Three male rats (pretest identification numbers 27, 63, and 68) were excluded from the study when the examination revealed preexisting ophthalmological abnormalities. All surviving subchronic rats were examined at the end of the study (test day 91), prior to the scheduled sacrifice. A summary of the findings from each ocular examination was submitted by the examining ophthalmologist (Ophthalmology Report).

K. Clinical Laboratory Evaluations

Clinical laboratory evaluations of subchronic rats were conducted approximately 45 and 90 days after initiation of the study. For the 45-day clinical, males were evaluated on test day 52 (2/11/91) and females were evaluated on test day 53 (2/12/91). For the 90-day clinical, males were evaluated on test day 96 (3/27/91) and females were evaluated on test day 97 (3/28/91). For both clinical examinations, only the surviving subchronic rats from each test group were sampled.

Two days prior to collection of blood samples for clinical evaluation, the selected rats were placed in metabolism cages and allowed to acclimate to the new cages. These rats were fasted for at least 16 hours immediately before blood sample collection. Urine was collected from each rat during the fasting period. Blood samples for hematological and clinical chemical measurements were collected from the orbital sinus of each rat while the rat was under light carbon dioxide anesthesia.

Hematology: The hematological parameters examined at each sampling time consisted of erythrocyte, leukocyte, differential leukocyte, and platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration. Blood smears for reticulocyte counts were prepared from each rat at each sampling time, but evaluation was not required.

Clinical Chemistry: In conjunction with each hematological examination, blood serum was evaluated for alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities, and concentrations of blood urea nitrogen, total serum protein, albumin, globulin (calculated), creatinine, total bilirubin, cholesterol, glucose, calcium, sodium, potassium, phosphate, and chloride.

Urine Analyses: Urine collected during each fasting period was measured for volume, osmolality, urobilinogen, and pH. The presence of hemoglobin or occult blood, glucose, protein, bilirubin, and ketone was also determined. Urine color and transparency were recorded. The sediment from each urine

specimen was microscopically examined for number of erythrocytes, leukocytes, epithelial cells, and casts.

L. Neurotoxicity Substudy Procedures

Ten rats from each group were designated for neurotoxicity evaluation. Functional Observational Battery (FOB) and motor activity monitoring were conducted on the neurotoxicity rats prior to study start and during weeks 5, 9, and 13. For each rat, the FOB and motor activity were evaluated on the same day. Rats were counterbalanced by sex and by dose groups over time to minimize the influence of uncontrolled factors. The experimenter conducting the FOB and motor activity monitoring was blind with respect to the treatment group of each rat.

1. Functional Observational Battery (FOB)

A detailed description of the FOB is available in Appendix A (Protocol and Protocol Amendments). FOB testing consisted of a series of quantified behavioral observations conducted in a sequence that proceeded from the least interactive to the most interactive. During the first phase of FOB assessments, each rat was evaluated in three "environments": (1) inside the home cage, (2) outside the home cage, and (3) in a standard "open field" arena (approximately 45 x 31 x 12 cm).

Inside the home cage, the presence of palpebral closure, writhing, circling, biting, and vocalization, as well as any unusual changes in body posture were recorded, if and when observed. Outside the home cage, each rat was assessed for fur appearance, ease of removal, and ease of handling. Presence of piloerection, bite marks, palpebral closure, lacrimation, and salivation were also evaluated. In the open field arena, the rats were evaluated for any unusual responses in level of activity (e.g., arousal), grooming, coordination, locomotion, gait, and righting reflex. The presence of convulsions/tremors, palpebral closure, labored breathing, defecation, and urination was also evaluated. In addition, simple assessments of sensory

function were made including responses to approach/touch, finger snap, and tail pinch.

The remainder of FOB testing involved standardized or calibrated devices. The presence or absence of pupillary constriction was assessed after a beam of light was directed into each eye. Fore- and hindlimb grip strength (kg) were measured by a strain gauge device (Chatillon Dial Push-Pull gauge). Landing foot splay (cm) was evaluated by measuring the distance between fresh ink impressions made on paper by the hindpaws when the rat was dropped from a height of approximately 32 cm.

2. Motor Activity

Motor activity was evaluated using a multi-station automated locomotor activity monitoring device (Omnitech Digiscan). Both horizontal and vertical activity counts were recorded. Motor activity counts were evaluated in four blocks of ten minutes each to assess habituation over a 40-minute test session.

3. Neuropathology Evaluation

After completion of the final (week 13) FOB and motor activity evaluation, rats in the neurotoxicity substudy were anesthetized, perfused with a fixative, and evaluated as described in Section M.2.

M. Pathological Evaluations

1. Subchronic Toxicity Rats

One rat designated for the subchronic substudy was found dead on test day 42 and was necropsied. After approximately 90 days on test (test days 104 and 105 for males and females, respectively), all surviving rats assigned to the subchronic substudy were sacrificed by chloroform anesthesia and exsanguination and necropsied. The order of sacrifice was random among all treatment groups within a sex.

Femur and sternum bone marrow smears were prepared from all rats sacrificed by design, but evaluation was not required.

The brain, heart, liver, spleen, kidneys, adrenals, and testes of rats sacrificed by design were weighed at necropsy and organ weight/final body weight ratios were calculated. The following tissues were collected from rats sacrificed by design: skin, bone marrow, lymph nodes (mesenteric and mandibular), thymus, spleen, aorta, heart, nose, trachea, lungs, salivary glands, esophagus, stomach, liver, pancreas, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), kidneys, bladder, pituitary, thyroid-parathyroid, adrenals, prostate, testes, epididymides, seminal vesicles, mammary gland, ovaries, uterus, vagina, cervix, brain, spinal cord, peripheral nerve (sciatic), bone, muscle (thigh), eyes, exorbital lacrimal glands, Harderian glands, and all gross lesions. Tissues were inadvertently not collected from one female rat found dead.

All tissues were processed to block stage. Tissues collected from rats in the high-concentration groups and control groups were further processed to slides and examined microscopically. Liver, kidneys, lungs, testes, and all organs with gross lesions from rats in the low-, low-intermediate-, and high-intermediate-concentration groups were also examined microscopically.

2. Neurotoxicity Substudy Rats

Following the final FOB assessments, the neurotoxicity substudy rats were sacrificed on test days 108-112. All rats were anesthetized by intraperitoneal injection of pentobarbital and underwent in situ whole body perfusion. The perfusion and necropsy procedures are outlined in the study protocol and amendments (see Appendix A).

The brain, spinal cord, and gastrocnemius muscle were paraffin embedded for preparation of hematoxylin and eosin (H&E) and specially stained slides. Tissues from rats in the control and high-concentration groups were processed and examined. In addition, the dorsal root ganglia, dorsal and ventral root fibers, sciatic and tibial nerves, and the gasserian ganglia were embedded in plastics. Sections (0.5 microns) of the dorsal root ganglia and the dorsal and

ventral root fibers were stained with toluidine blue and examined microscopically. If no lesions were detected in the H&E stained sections, then the nervous tissue stained with H&E was further evaluated using Luxol Fast Blue/PAS for myelin sheaths and neuronal cell bodies and their processes.

N. Statistical Analyses

Body weights, body weight gains, food consumption, organ weights, and clinical laboratory measurements were analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. The Bartlett's test for homogeneity of variances was performed on the organ weight and clinical laboratory data and, if significant ($\alpha = 0.005$), was followed by nonparametric procedures.

Incidence of clinical observations as well as descriptive FOB parameters were evaluated by Fishers's Exact test with a Bonferroni correction.

Continuous data from the FOB (body weight, fore- and hindlimb grip strength, and landing foot splay) and motor activity tests were analyzed by appropriate parametric and/or non-parametric statistics (see Statistical Analysis in the Neurobehavioral Report).

Except for Bartlett's test, all significance was judged at $\alpha = 0.05$.

RECORDS AND SAMPLE RETENTION

All original data and the original of this report will be retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Preserved wet tissues, paraffin blocks, histological slides, and blood and bone marrow smears will be retained at Haskell Laboratory. An archived sample of the test material will be retained at Haskell Laboratory.

RESULTS AND DISCUSSIONA. Compound Purity and Diet Analyses of DPX-T3217 (Diet Analyses Report, Table 1, Appendix B)

A sample of DPX-T3217 was supplied by the sponsor with a stated purity of 96.8% (active ingredient). This purity value was used to prepare diets containing DPX-T3217. Samples of the subject compound were submitted for purity analysis on 12/13/90 and 4/17/91. The samples were subsequently analyzed for percent active ingredient. The measured purities of DPX-T3217 near the beginning and the end of the study were both 95% (Appendix B). This analysis confirms the purity reported by the sponsor (96.8%), within the limits of experimental variation. These data demonstrate that DPX-T3217 was stable over the duration of the study.

Samples of diet from each level were collected on test day -1 to verify concentration, homogeneity, and stability (Table 1). There were no apparent differences among the top, middle, or bottom samples in the measured concentration of DPX-T3217, indicating that the mixing resulted in a homogeneous distribution of test compound in the diet. The mean \pm standard deviation of the homogeneity values (top, middle, bottom) expressed as percent of nominal were 90 ± 2 , 103 ± 3 , 100 ± 1 , and 101 ± 3 for 100, 750, 1,500, and 3,000 ppm of DPX-T3217, respectively.

The stability of DPX-T3217 was assessed at each dietary level by comparing the measured concentration of the compound on the day of diet preparation (fresh frozen) to the following storage conditions: (1) seven and 14-day room temperature, and (2) 14-day refrigeration. With the exception of the 100 ppm room temperature and refrigerated diets, and the 750 ppm room temperature diets, DPX-T3217 concentrations ranged from 84% to 103% of nominal and appeared to be stable under all storage conditions. The 100 and 750 ppm diets were stable when fresh frozen. When stored refrigerated, however, the 100 ppm sample was 78% of nominal (day 14), and at room temperature the measured concentration decreased over time to 52% of nominal by day seven and 35% of nominal by day 14. Similarly, the measured concentration in the 750 ppm room

temperature diet samples decreased over time to 76% of nominal by day seven and to 63% by day 14. Data from replicate analyses of back-up samples confirm these observations. The test compound was not detected in control diet samples.

Cage-site (room temperature) feeder samples of diet formulated on test days 48 (2/7/91) and 90 (3/21/91) were collected to verify the concentration of DPX-T3217 delivered to the rats. With the exception of the 100 ppm samples, the average measured percent of nominal concentration ranged from 80% to 94% (diets prepared test day 48 and sampled after seven days), and 81% to 98% (diets prepared test day 90 and sampled after five days). The measured concentrations of the 100 ppm diets sampled after five days (diet prepared 3/21/91) and seven days (diet prepared 2/7/91) were 63% and 57% of nominal, respectively. The test compound was not detected in control diet samples.

In an analytic experiment (see report titled, "Investigation of Cymoxanil Stability in Rat Diet"), 15 ppm diet samples of radiolabeled DPX-T3217 were prepared and analyzed by a similar method. Analysis of these samples revealed that recovery of DPX-T3217 from the diet decreased after a minimal 24-hour room temperature incubation from that obtained on the day of diet preparation. Approximately 60 to 70% of the test compound could be extracted by a one-hour sonication from samples held at room temperature from one to seven days. In general, recovery for these same samples was increased to greater than 90% with a second extraction and overnight (> 16 hours) sonication. High Performance Liquid Chromatographic analysis of the extracted radiolabeled material indicated that no degradation of DPX-T3217 occurred.

In a subsequent study, rat diets containing DPX-T3217 (50 to 2,000 ppm) were prepared and samples were stored under similar conditions as in the present study. DPX-T3217 was analyzed at each dietary level after one hour and 16 hours sonication. Compared to the one-hour sonication, recoveries were significantly higher (6 to 41%) with vigorous extraction (> 16 hour sonication) in samples held at room temperature for up to 14 days. (10)

Therefore, the low recoveries observed in the 100 and 750 ppm room temperature diet samples (stability and cage-site) in the present study were

likely due to insufficient extraction of the test material from the matrix, rather than chemical instability of DPX-T3217 or improper diet formulation.

Based on the analyses of experimental and actual rat diets from MR 9257 and rat diets in the present study, DPX-T3217 was considered stable and diets containing DPX-T3217 met the targeted concentrations of active ingredient for this study.

3. Mean Body Weights and Body Weight Gains (Figures 1 & 2, Tables 2-5, Appendix C)

Body weights and body weight gains in both male and female rats at the 100 and 750 ppm levels were comparable with those of the control groups throughout the study.

Occasional statistically significant decreases from control in mean body weights and mean body weight gains in male and female rats were present within the first two weeks of the study at the 1,500 ppm level. Significant decreases in mean body weight were observed, however, throughout the study in males and females at the 3,000 ppm level. The overall (test days 0-97) mean body weights were decreased by 15% and 9% for male and female rats, respectively. Occasional significant differences were observed throughout the study in mean body weight gains for these groups of rats. The overall mean body weight gains at the 3,000 ppm level were decreased from control by 22% and 20% for males and females, respectively. The body weight effects for male and female rats at 1,500 and 3,000 ppm were considered compound related. These results were consistent with those observed in rats fed diets containing DPX-T3217 in a previously conducted 90-day study.(1)

C. Food Consumption, Food Efficiency and Intake of DPX-T3217 (Tables 6-11, Appendix D)

With the exception of the 3,000 ppm females, the overall mean daily food consumption observed in compound-treated groups was comparable with control.

At 3,000 ppm, a statistically significant increase in food consumption above control was observed in female rats throughout the study (26% overall increase). Although not significant, an overall increase (9%) in food consumption was also exhibited by the 1,500 ppm female group. These increases in mean food consumption were coincident with depressed aforementioned body weight gains, and significant decreases of 17% and 35% from control in mean food efficiency in the 1,500 and 3,000 ppm female groups, respectively. Significant decreases of 8% and 19% in overall food efficiency, related to compromised weight gains, were also present in male rats at 1,500 and 3,000 ppm, respectively. The food consumption effects in females and food efficiency effects in males and females at the 1,500 and 3,000 ppm levels were considered compound related.

The overall (test days 0-97) mean daily intake of DPX-T3217 for male rats at the 100, 750, 1,500, and 3,000 ppm levels was approximately 6.54, 47.6, 102, and 224 mg/kg/day, respectively (Table 10). The overall mean daily intake of DPX-T3217 for female rats at the 100, 750, 1,500, and 3,000 ppm levels was approximately 8.00, 59.9, 137, and 333 mg/kg/day, respectively. The mean intake differences among male and female groups were attributed to normal variations in body weight gain and food consumption by each sex during the study.

D. Clinical Observations and Mortality (Tables 12-15, Figures 3 & 4, Appendix E)

No compound-related clinical signs were exhibited by any male or female rats throughout the study. No statistically significant differences relative to control were observed.

Except for the 750 ppm female group where one rat (Haskell Animal Number 496173) was found dead on test day 42 (i.e., 95% survival), there was 100% survival in all male and female test groups (Tables 14 and 15). The cause of death of this rat was unknown, however, it was not considered compound related.

E. Ophthalmological Evaluations (Ophthalmology Report, Appendix F)

Two ophthalmological examinations were conducted for the subject study. At the pretest evaluation (test day -13), three male rats had preexisting ocular lesions and were not used in the study. All other rats were considered ophthalmoscopically normal.

Prior to the final sacrifice (test day 91), an ophthalmological examination was conducted on all surviving rats (50 male, 49 female) assigned to the subchronic study. One female rat from the 100 ppm level (Haskell Animal Number 496161) and three male rats from the 750 ppm level (Haskell Animal Numbers 495975, 496028, and 496061) exhibited ocular lesions in the form of left eye phthisis or superficial corneal vascularization. These ocular findings are not considered compound related, but rather are likely the result of stress associated with orbital sinus blood sampling procedures.

F. Clinical Laboratory Evaluations (Clinical Pathology Report, Tables 16-21, Appendix G)

Mean total leukocyte counts were mildly to moderately decreased in the 1,500 and 3,000 ppm male groups at the 45- and 90-day sampling times. Leukocyte counts were decreased primarily due to decreases in lymphocytes and, less importantly, because of decreases in monocytes. These leukocytic alterations were most pronounced at the 90-day sampling time. The causal relationship between treatment and the leukocytic alterations was somewhat equivocal. Decreases in circulating lymphocyte counts could be directly related to compound administration. However, the absence of morphological alterations in spleen, lymph nodes, thymus, and bone marrow did not support the presence of a primary compound-related effect. Furthermore, in rodents the most common cause of decreased lymphocyte counts is an increased release of endogenous glucocorticoids.(11,12) Therefore, the leukopenia observed in the present study was considered to represent a secondary effect associated with "stress" in which endogenous glucocorticoids cause a redistribution of lymphocytes from the circulation into lymphoid tissue.

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Other statistical differences compared with control were observed in hematological variables of males, including decreases in erythrocyte counts and hemoglobin concentration, and in clinical chemical variables of males and females, including decreases in serum aspartate aminotransferase and total protein. These effects, however, were not dose-dependent, were within the range of expected variation, and were considered biologically insignificant.

The no-observable-effect level (NOEL) for clinical pathology in male rats was 750 ppm based on the observed leukocytic alterations in the 1,500 and 3,000 ppm groups. However, since the leukocytic alterations were likely a secondary effect and not toxicologically significant, the no-observable-adverse-effect level (NOAEL) in male rats was considered 3,000 ppm. The NOEL in female rats was 3,000 ppm for the hematological, clinical chemical, and urinalysis variables measured.

G. Neurotoxicity Evaluations (Neurobehavioral Report, Tables 22-35
Figures 5-20, Appendices H-0)

A neurobehavioral test battery, consisting of motor activity (MA) and functional observational battery (FOB) assessments, was conducted on all neurotoxicity substudy rats prior to study start and again during weeks 5, 9, and 13 of the feeding period.

Body weights were significantly decreased ($P \leq 0.05$) in the 3,000 ppm male rats evaluated for neurotoxicity parameters during weeks 5, 9, and 13, as compared with the control group. Body weights in the 3,000 ppm female rats evaluated for neurotoxicity parameters were also reduced during these same intervals, although only the week 9 value was statistically significant ($P \leq 0.05$). No other compound-related effects on body weight occurred.

No compound-related differences occurred in the incidence of clinical observations on the days of neurotoxicity evaluations in either male or female rats. Differences among the four dose groups were neither biologically remarkable nor statistically significant.

Absolute forelimb grip strength scores in the male and female rats were unaffected by the test material. Differences among the four dose groups were not significant and all values observed in the groups fed DPX-T3217 were within $\pm 10\%$ of the control group values.

A small reduction (not significant) in relative forelimb grip strength scores occurred in the 3,000 ppm male rats during week 13; relative scores were only 82% of the control group values during this interval. This observation was considered to reflect the significant decrease ($P \leq 0.05$) in body weight that occurred in the 3,000 ppm male rats and was not considered a neurotoxic effect. Relative forelimb grip strength scores were unaffected in the female rats; differences were neither biologically remarkable nor statistically significant.

Absolute and relative hindlimb grip strength scores in the male and female rats were unaffected by the test material. Differences among the four dose groups were neither statistically significant nor biologically remarkable.

In male rats, relative foot splay scores (percent of baseline) in the 3,000 ppm male rats were decreased by 18% during week 9 and decreased by 23% during week 13, as compared with the respective control group scores. These observations were considered to reflect the significantly smaller ($P \leq 0.05$) body weights of the 3,000 ppm male rats and were not considered neurotoxic effects. Absolute foot splay scores (cm) in the male rats fed DPX-T3217 were neither dosage-dependent nor significantly different, as compared with the control group scores.

In female rats, absolute and relative foot splay scores were increased in the 3,000 ppm dose group (6.2 cm and 148%, respectively) during week 5, as compared with the control group (5.4 cm and 115%, respectively). These observations were considered unrelated to the test material because: (1) the differences were not statistically significant as compared with the concurrent control group values, (2) the scores observed in the 3,000 ppm female rats were either within the range of historical control values (4.2 to 6.5 cm and 106 to 131% for absolute and relative values, respectively) or within 10% of the upper limit of historical control values, and (3) the differences did not recur when

foot splay was evaluated during weeks 9 and 13. Neither absolute nor relative landing foot splay scores were affected in the male or female rats administered dietary levels of 100, 750, or 1,500 ppm.

No compound-related differences in the remaining FOB parameters occurred for either male or female rats. The incidences of FOB findings in the groups given the test material were neither biologically remarkable nor significantly increased, as compared with the control groups.

Horizontal and vertical motor activity in the male and female rats was unaffected by the test compound. Differences among the four dose groups were neither biologically remarkable nor statistically significant. All dose groups demonstrated similar rates of habituation in horizontal and vertical activity levels across each test session as indicated by a significant Block effect ($P \leq 0.01$) and no significant Dose x Block interactions.

On the basis of the parameters evaluated in the neurobehavioral part of the neurotoxicity substudy, the NOEL for DPX-T3217 in both male and female rats was 1,500 ppm. None of the behavioral findings at 3,000 ppm were considered neurotoxic effects.

H. Pathological Evaluations

1. Subchronic Toxicity Rats (Pathology Report, Tables 36-47, Figures 21-51, Appendices P & Q)

The mean final body weights of the 1,500 and 3,000 ppm males and 3,000 ppm females were significantly less than those of respective controls. The mean final body weights of the 1,500 ppm and 3,000 ppm males were decreased by 10% and 16%, respectively. The 3,000 ppm female group had a mean final body weight of 12% less than that of the female control group. These body weight effects were judged to be compound related.

The mean absolute heart weight of the 3,000 ppm males was statistically significantly decreased (Table 36). For heart, this reduction reflects the decreased mean body weight of the group.

The mean relative (percent of body weight) testes, kidney, and brain weights of the 1,500 and 3,000 ppm males were significantly increased. For testes, kidney, and brain, this increase reflects the preservation of organ weight when accompanied by decreased body weight. The mean absolute organ weights of the testes, kidney, and brain for these groups were comparable with those of the control group (Table 36).

The mean relative liver and spleen weights were significantly increased in the 3,000 ppm female group (Table 37). The biological significance of the increased splenic weight was uncertain. The increased relative liver weight was judged to be biologically significant and compound related. No microscopic changes were observed to explain these changes. The weight of the adrenals of one 100 ppm male rat (Haskell Animal Number 496068) was 78 times greater than the average absolute weight of the other adrenals in the group. This organ was judged to have been misweighed, as there were no coincident gross or microscopic pathological observations.

There were no compound-related gross pathological findings in male or female rats (Tables 38-39). Gross observations, such as splenic cysts and mandibular lymph node enlargement or discoloration, were consistent with conditions or changes commonly encountered in the strain and age of rat used in this study. One 750 ppm female rat (Haskell Animal Number 496173) was found dead on test day 42. This rat had a perineal mass. No masses were observed in any other rats.

Increased elongate spermatid degeneration was observed in one 0 ppm, three 750 ppm, five 1,500 ppm, and seven 3,000 ppm male rats (Tables 40, 42, and 44). No spermatid degeneration was observed at 100 ppm. The increased incidence was statistically significant by Fisher's Exact test for the 3,000 ppm group. The lesion consisted of rounded cells with condensed nuclei and granular eosinophilic cytoplasm at the luminal border of the germinal epithelium in

tubules of approximate stages XII through XIV and I through IV (Figures 42, 44, 46, and 48). Morphologically normal elongate spermatids and degenerate spermatids were both found in affected tubules. Not all tubules of stages XII through XIV and I through IV were equally affected. The architecture of the testes was not disturbed. The lesion was graded minimal or mild in all cases. All testicular sections were from Bouin's fixed tissue and were stained with hematoxylin and eosin. No special fixatives or stains were used to aid in staging.

One male rat each from the 1,500 and 3,000 ppm groups (Haskell Animal Numbers 496027 and 495970, respectively) had multinucleated spermatids in the seminiferous tubules (Figure 49). Although this lesion is a common pathological finding⁽¹³⁾ and alone it has questionable significance, in conjunction with elongate spermatid degeneration in these groups (significant increase at 3,000 ppm) and epididymal changes, it supports a compound-related male reproductive organ effect.

Various epididymal pathological findings were minimal and included intratubular debris, multinucleated spermatids, and bilateral hypospermia. These changes reflect the seminiferous tubule changes. Hypospermia was seen in four 3,000 ppm males. Cell debris was observed in one 1,500 ppm and six 3,000 ppm males. The incidence of cell debris in the epididymides was significantly increased for the 3,000 ppm group when compared with the control group. Multinucleated spermatids were present in the epididymides of one 1,500 and one 3,000 ppm male. None of these epididymal changes were observed in the 0, 100, or 750 ppm males. One 750 ppm male had mild subacute inflammation of the epididymal interstitium, a non-specific change frequently encountered as a spontaneous lesion in male rats.

The coincident testicular and epididymal effects in the 1,500 and 3,000 ppm males were judged to be compound related. No epididymal effects were seen in the 750 ppm males and the incidence of degeneration of the elongate spermatids in this group compared with control was not statistically significant.

Other microscopic findings, such as cardiomyopathy in the control and 3,000 ppm males (Figures 29 and 30, respectively) and odontal inflammation in 3,000 ppm females, are commonly encountered lesions in this strain of rat. No toxicological significance was attributed to these findings

Based on final body and organ weights and histopathology, the NOEL for pathology was 750 ppm for male rats and 1,500 ppm for female rats.

2. Neurotoxicity Substudy Rats (Neuropathology Report, Tables 48-51, Appendix R)

All neurotoxicity substudy rats survived to the terminal sacrifice. No gross lesions were observed in female rats. The gross abnormalities observed in male rats were liver discoloration and small testes (Table 48). Microscopically, this latter testicular finding (3,000 ppm male) corresponded to severe atrophy and degeneration of the seminiferous tubules. The lesion did not resemble the elongate spermatid degeneration seen in the subchronic part of the study. This lesion was judged to be spurious and not related to compound administration. No other males were affected.

There were no compound-related lesions detected within the nervous system or skeletal muscle of male or female rats.

Similar types and incidences of histological lesions were observed in all of the rats examined microscopically (0 and 3,000 ppm groups). There was no significant difference in incidence between the 0 ppm groups and the 3,000 ppm groups when evaluated by Fisher's Exact test. The lesions were all minimal, consisted of myelin degeneration, myelin phagocytosis, skeletal muscle degeneration, and macrophages in the interstitium of the skeletal muscle, and were interpreted to represent spontaneous incidental lesions of no biological significance in this strain and age of rat. One 3,000 ppm male had minimally dilated ventricles. No significance was attributed to this finding.

The NOEL for neuropathology was 3,000 ppm for male and female rats.

CONCLUSION

For the subchronic study, the no-observable-effect level (NOEL) was 750 ppm for male and female rats fed diets containing DPX-T3217 for 97 days. All toxicological effects in rats administered compound were relative to respective control group findings. The subchronic NOEL was based primarily on compound-related body weight and food efficiency decreases at 1,500 and 3,000 ppm in females, and body weight decreases and testicular and epididymal histological effects at 1,500 and 3,000 ppm in males.

There were no adverse clinical pathological findings, clinical signs of toxicity, ophthalmological effects, or mortality that were compound related or toxicologically significant.

Statistically significant compound-related body weight decreases in male and female neurotoxicity rats at 3,000 ppm set the NOEL for this substudy at 1,500 ppm. Analyses of neurobehavioral and neuropathological data indicated that DPX-T3217 was not a neurotoxic agent. There were no clinical signs of toxicity or mortality that were compound related or toxicologically significant.

Under the conditions of this study, the NOEL for subchronic findings was 750 ppm for male and female rats. The NOEL for evidence of neurotoxicity was 3,000 ppm for male and female rats.

0 0 3 6

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Du Pont HLR 370-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

DIET ANALYSES REPORT

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANIL)
FEEDING AND NEUROTOXICITY STUDY IN RATS

ANALYSIS FOR THE ACTIVE INGREDIENT IN DPX-T3217-107 IN DIET SAMPLES

Medical Research Project Number:	9131-001
Haskell Sample Number:	18,753
Analytical Test Code:	430
Analytical Report Number:	HA-92-003

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p2

SUMMARY

Concentrations of DPX-T3217 in diets prepared December 20, 1990; February 7 and March 21, 1991, were determined by high-performance liquid chromatography (HPLC). Nominal concentrations of prepared diets were 0, 100, 750, 1500 and 3000 ppm. A one hour sonication was used to extract DPX-T3217 into acetonitrile from the diet samples. Based on analysis, DPX-T3217 was distributed homogeneously in the diet at all concentration levels. At the 1500 and 3000 ppm levels the test compound was stable when fresh frozen, or upon refrigeration or room temperature storage. Stability data at the 100 and 750 ppm levels were deemed inconclusive due to low recoveries. At all dietary levels the measured concentrations of DPX-T3217 decreased over time when stored at room temperature; however, the percent recoveries at the two high dose levels were within an acceptable range of $\pm 20\%$ of nominal. This effect was particularly exaggerated at 100 ppm, averaging 52% (day 7) to 35% (day 14) of nominal and 750 ppm, averaging 76% (day 7) to 63% (day 14) of nominal.

A similar trend in measured concentrations was observed in samples obtained from cage-site, where feed was maintained at room temperature for 5 to 7 days. Although the cage-site recoveries for 750 ppm (80 and 81% of nominal), 1500 ppm (83 and 89% of nominal), and 3000 ppm (94 and 98% of nominal) samples were within an acceptable range of $\pm 20\%$ of nominal, the effect was exaggerated at the 100 ppm (57 and 63% of nominal) level.

A separate study (MR 9257) has shown that total percent recovery of DPX-T3217 was generally greater than 90% with vigorous extraction (> 16 hours sonication) from experimental diet samples stored at room temperature for up to 7 days. Analysis of the extracted material indicated that no degradation of DPX-T3217 occurred. Analytical results (MR 9257) have also shown that percent recoveries of DPX-T3217 from diet samples (50 to 2000 ppm), prepared similar to those in the present study, and stored at room temperature for up to 14 days, were significantly higher through vigorous extraction, compared to those resulting from a one hour sonication extraction. Therefore, the low recoveries observed primarily in the 100 and 750 ppm room temperature diets in the present study were likely due to insufficient extraction of the test material rather than chemical instability of DPX-T3217 or improper diet formulation.

Based on the analysis of purity samples submitted December 13, 1990 and April 17, 1991, DPX-T3217 was judged to be stable during the course of this study.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p3

METHODS

DIET SAMPLE ANALYSIS

Analysis for DPX-T3217 in diet samples was according to the following methods.

CHROMATOGRAPHIC CONDITIONS

Instrument.....	Hewlett-Packard 1090 liquid chromatograph
Column.....	Hyperasil ODS, 60 x 4.6 mm
Mobile Phase.....	35% acetonitrile, 65% water adjusted to pH 2.6 with H ₃ PO ₄
Flow Rate.....	1 mL/min
Injection Volume.....	5 µL
Oven Temperature.....	40°C
Detection.....	UV absorbance at 243 nm

CALIBRATION AND QUANTITATION

H-18,753 (DPX-T3217) was used to make calibration solutions of approximately 5.0 to 100 µg DPX-T3217/mL acetonitrile. DPX-T3217 peak heights from HPLC analysis of these solutions were used to construct separate calibration curves by least squares regression to cover different segments of the concentration range. Measured concentrations for recovery and diet samples were determined by applying the replicate peak heights for each sample to the appropriate calibration curve.

RECOVERY SAMPLE ANALYSIS

Recovery of DPX-T3217 from diet was tested at 100 and 3000 ppm to confirm analytical method performance. DPX-T3217 solution (approximately 250 µg DPX-T3217/mL in acetonitrile) or DPX-T3217 solid was added to samples of control diet (2.5 g). These samples were evaporated to dryness and then processed and analyzed in the same manner as diet samples submitted at the respective concentrations.

DIET SAMPLE PREPARATION

Acetonitrile (25 or 50 mL) was added to a single aliquot (2.5 or 5.0 g) of each diet sample to extract DPX-T3217. These mixtures were sonicated for one hour. Each extract was filtered (0.45 µm) and aliquots of the filtrates either analyzed directly (100 ppm and 750 ppm) or diluted to a nominal concentration of 75 µg/mL with acetonitrile prior to analysis (1500 ppm and 3000 ppm diets).

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p4

PURITY SAMPLE ANALYSIS

Methods and results for the analyses of DPX-T3217 purity are documented in reports HA-91-120 and HA-91-121.

RESULTS

DPX-T3217 eluted from the HPLC column as a symmetric peak with a retention time of approximately 1.4 minutes. Measured concentrations of recovery samples were 87% and 98% of nominal at the 100 ppm level and 98% and 101% of nominal at the 3000 ppm level (Table I), indicating that the method performed satisfactorily over this concentration range.

HOMOGENEITY

Analytical results from homogeneity samples are summarized in Table II. Measured concentrations of DPX-T3217 in these samples ranged from 88% to 92% of nominal at the 100 ppm level, 100% to 105% of nominal at the 750 ppm level, 99% to 101% of nominal at the 1500 ppm level, and 98% to 104% of nominal at the 3000 ppm level indicating that the test compound was distributed homogeneously in the diet.

STABILITY

Analytical results from stability samples are summarized in Table III. With the exception of the 100 ppm room temperature and refrigerated diets, and the 750 ppm room temperature diets, DPX-T3217 concentrations ranged from 84% to 103% of nominal and appeared to be stable in diets that were stored frozen, refrigerated, or at room temperature. The 100 and 750 ppm diets appeared to be stable when fresh frozen. However, when stored refrigerated the measured concentration of DPX-T3217 in the 100 ppm sample was 78% (day 14). When stored at room temperature, the average percent nominal decreased over time to 52% (day 7) and 35% (day 14). The average measured concentration in the 750 ppm diets also decreased over time to 76% (day 7) and 63% (day 14) of nominal when stored at room temperature. Reported data from replicate analysis of backup samples confirm these observations. The 100 ppm refrigerated sample was not reanalyzed. DPX-T3217 was not observed in control diet.

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p5

STABILITY (Continued)

In a subsequent study (MR 9257) experimental radiolabeled 15 ppm diet samples were prepared and analyzed by a similar method. Analysis of these samples revealed that recovery of DPX-T3217 from the diet decreased after a minimal 24-hour room temperature incubation from that obtained on the day of diet preparation. Approximately 60 to 70% of the test compound could be extracted by a one hour sonication from samples held at room temperature for up to 7 days. In general, recovery for these same samples was increased to greater than 90% with a second extraction and overnight, (> 16 hours) sonication. Analysis of the extracted radiolabeled material indicated that no degradation of DPX-T3217 occurred.

As part of MR 9257, animal diets (50 to 2000 ppm) were prepared and samples were stored under similar conditions as in the present study. DPX-T3217 was analyzed at each dietary level after one hour and 16 hours sonication. Compared to the one hour sonication, recoveries were significantly higher with vigorous extraction (> 16 hour sonication) in samples held at room temperature for up to 14 days. Therefore, the low recoveries observed primarily in the 100 and 750 ppm recoveries in the room temperature diet samples in the present study were likely due to insufficient extraction of the test material from the matrix, rather than chemical instability of DPX-T3217 or improper diet formulation.

CAGE-SITE

Results from the analysis of feeder samples are summarized in Table IV. With the exception of the 100 ppm diets, concentrations were 80% (average of 750 ppm backup results) to 94% of nominal for diets prepared February 7, 1991, and 81% (average of 750 ppm backup results) to 98% of nominal for diets prepared March 21, 1991. These data indicate that DPX-T3217 was at acceptable concentrations for feeder samples for levels above 100 ppm. Measured concentrations of the 100 ppm cage-site sample prepared February 7, 1991, and sampled after 7 days at room temperature, was 57% of nominal; and the 100 ppm sample prepared March 21, 1991, and sampled after 5 days at room temperature, was 63% of nominal. Reported data from replicate analysis of backup samples confirm this observation. Test compound was not observed in control diet.

Based on the technical data cited above from MR 9257, the low recoveries observed in the cage-site diet samples for 100 ppm in the present study were likely due to insufficient extraction of the test material, rather than chemical instability of DPX-T3217 or improper diet formulation.

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p6

PURITY SAMPLES

The average purity of the DPX-T3217 sample submitted December 13, 1990 was 95% (HA-91-120), and that of a sample obtained April 17, 1991 was 95% (HA-91-121). DPX-T3217 was reported by the sponsor to be 96.8% pure. Analyses conducted during this study confirmed the results of the sponsor's analysis, within the limits of experimental variation, and indicated that the compound was stable.

ACKNOWLEDGMENTS

Nirmala Raghavan analyzed the samples. P. Horne and C. R. Boucher (Du Pont Agricultural Products) prepared and analyzed the experimental radiolabeled DPX-T3217 diets.

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Analytical Report Number: HA-91-003

Notebook references: E-67399: 12, 13, 27 - 40, 52 - 62
E-67367: 40, 70, 107

Number of pages in this report: 13

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p7

TABLE I

RECOVERY OF DPX-T3217 ADDED TO CONTROL DIET

SAMPLE TYPE	NOMINAL	ppm DPX-T3217		PERCENT NOMINAL
		MEASURED	AVERAGE	
RECOVERY(A)	100	87 86	87	87
RECOVERY(B)	100	98 97	98	98
RECOVERY(A)	3000	2928 2933	2931	98
RECOVERY(B)	3000	2988 3073	3031	101

(A) Processed with samples prepared December 20, 1990.

(B) Processed with samples prepared February 7, and March 21, 1991.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p8

TABLE II

HOMOGENEITY OF DPX-T3217 IN DIETS PREPARED DECEMBER 20, 1990

SAMPLE TYPE	ppm DPX-T3217			PERCENT NOMINAL
	NOMINAL	MEASURED	AVERAGE	
CONTROL	0	0 0	---	---
TOP	100	91 92	92	92
MIDDLE	100	88 89	89	89
BOTTOM	100	88 88	88	88
TOP	750	787 787	787	105
MIDDLE	750	750 751	751	100
BOTTOM	750	778 763	781	104
TOP	1500	1499 1488	1494	100
MIDDLE	1500	1513 1520	1517	101
BOTTOM	1500	1471 1483	1477	99
TOP	3000	3018 3003	3011	100
MIDDLE	3000	3116 3093	3105	104
BOTTOM	3000	2966 2924	2945	98

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p9

TABLE III

STABILITY OF DPX-T3217 IN DIETS PREPARED DECEMBER 20, 1991

SAMPLE TYPE	ppm DPX-T3217			PERCENT NOMINAL
	NOMINAL	MEASURED	AVERAGE	
CONTROL	0	0 0	---	---
FRESH FROZEN	100	93 93	93	93
7 DAYS ROOM ^(A) TEMPERATURE	100	50 47	49	49
7 DAYS ROOM ^(A) TEMPERATURE	100	52 53	53	53
7 DAYS ROOM ^(A) TEMPERATURE	100	54 53	54	54
14 DAYS ROOM ^(B) TEMPERATURE	100	35 36	36	36
14 DAYS ROOM ^(B) TEMPERATURE	100	33 35	34	34
14 DAYS ROOM ^(B) TEMPERATURE	100	34 33	34	34
14 DAYS REFRIGERATED	100	78 78	78	78

(A) Backup results reported and confirm the recovery in the original analysis (48 ppm = 48% of nominal).

(B) Backup results reported and confirm the recovery in the original analysis (33 ppm = 33% of nominal).

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
HA-92-003p10

TABLE III (Continued)
STABILITY OF DPX-T3217 IN DIETS PREPARED DECEMBER 20, 1991

SAMPLE TYPE	NOMINAL	ppm DPX-T3217		PERCENT NOMINAL
		MEASURED	AVERAGE	
FRESH FROZEN	750	731 754	743	99
7 DAYS ROOM ^(c) TEMPERATURE	750	565 575	570	76
7 DAYS ROOM ^(c) TEMPERATURE	750	557 572	565	75
7 DAYS ROOM ^(c) TEMPERATURE	750	577 562	570	76
14 DAYS ROOM ^(d) TEMPERATURE	750	475 474	475	63
14 DAYS ROOM ^(d) TEMPERATURE	750	471 466	469	63
14 DAYS ROOM ^(d) TEMPERATURE	750	485 475	480	64
14 DAYS REFRIGERATED	750	733 718	726	97

^(c) Backup results reported and confirm the recovery in the original analysis (593 ppm = 79% of nominal).

^(d) Backup results reported and confirm the recovery in the original analysis (474 ppm = 63% of nominal).

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p11

TABLE III (Continued)

STABILITY OF DPX-T3217 IN DIETS PREPARED DECEMBER 20, 1991

SAMPLE TYPE	ppm DPX-T3217			PERCENT NOMINAL
	NOMINAL	MEASURED	AVERAGE	
FRESH FROZEN	1500	1506 1475	1491	99
7 DAYS ROOM TEMPERATURE	1500	1324 1306	1315	88
14 DAYS ROOM TEMPERATURE	1500	1261 1258	1260	84
14 DAYS REFRIGERATED	1500	1554 1531	1543	103
FRESH FROZEN	3000	2999 3027	3013	100
7 DAYS ROOM TEMPERATURE	3000	2797 2805	2801	93
14 DAYS ROOM TEMPERATURE	3000	2746 2739	2743	91
14 DAYS REFRIGERATED	3000	2888 2933	2911	97

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p12

TABLE IV
CONCENTRATION OF DPX-T3217 IN FEEDER SAMPLES

DATE PREPARED	ppm DPX-T3217			PERCENT NOMINAL
	NOMINAL	MEASURED	AVERAGE	
2/7/91	0	0 0	---	---
	100(A)	55 57 56	56	56
	100(A)	58 55 58	57	57
	750(B)	591 613 606	603	80
	750(B)	589 601 615	602	80
	1500	1214 1262	1238	83
	3000	2783 2866	2825	94

(A) Backup results reported and confirm the recovery in the original analysis (49 ppm = 49% of nominal).

(B) Backup results reported due to the recovery in the original analysis (541 ppm = 72% of nominal).

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

HA-92-003p13

TABLE IV (Continued)

CONCENTRATION OF DPX-T3217 IN FEEDER SAMPLES

<u>DATE PREPARED</u>	<u>ppm DPX-T3217</u>			<u>PERCENT NOMINAL</u>	
	<u>NOMINAL</u>	<u>MEASURED</u>	<u>AVERAGE</u>		
3/21/91	0	0 0	---	---	
	100(c)		63	64	64
			65		
			64		
	100(c)		60	62	62
			62		
			64		
	750(d)		602	585	78
			589		
			565		
750(d)		618	626	83	
		632			
		627			
1500		1334	1328	89	
		1321			
3000		2923	2945	98	
		2966			

(c) Backup results reported and confirm the recovery in the original analysis (53 ppm = 53% of nominal).

(d) Backup results reported due to the recovery in the original analysis (529 ppm = 71% of nominal).

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SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

INVESTIGATION OF CYMOXANIL STABILITY IN RAT DIET

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Du Pont HLR 370-91

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November 5, 1991

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INVESTIGATION OF CYMOXANIL STABILITY IN RAT DIET

INTRODUCTION

In the course of analyzing diet samples from the 2-year chronic feeding study in rats, it was discovered that poor recoveries (50-60%) of the test substance (cymoxanil) were obtained 2-3 days after the diet had been exposed to room temperature. The question was raised as to whether the cymoxanil was degrading, presumably by hydrolysis due to moisture in the diet, or whether the test substance was becoming tightly bound to the diet matrix, making it difficult to be efficiently extracted.

Attempts were made to address these questions by preparing cymoxanil/diet mixtures and extracting samples of these each day over the course of 7 days. By using ¹⁴C-cymoxanil, the efficiency of extraction of the applied radioactive material could be easily measured and the extracts subsequently analyzed to determine whether degradation had occurred.

MATERIALS AND METHODS

A stock solution of [¹⁴C]DPX-T3217 (cymoxanil) was prepared by dissolving 1 mg (14 μCi/mg) of the compound in 1 mL of acetonitrile. Samples of Purina® Rodent Chow (1 g) were weighed out into sixteen vials which would represent 8 duplicate samples. Aliquots of the stock solution (15 μL) representing 15 μg of test substance were pipetted onto the rat chow to give a concentration of approximately 15 ppm in the diet. The vials were then capped, shaken, and stored

in the freezer over the weekend. The vials were removed and allowed to stand at room temperature for up to 7 days. Vials (2) were extracted immediately (Day 0) and duplicates were sampled each day thereafter. Approximately 10 mL of acetonitrile were added to each vial and the mixture sonicated for 10 minutes. The acetonitrile was then pipetted off and placed in a centrifuge tube. Following centrifugation the supernatant was sampled (3 x 100 μ L) and counted by Liquid Scintillation Counting (LSC) to determine material balance. For the Day 0 samples no further extraction was necessary. For Day 1 onwards, a second extraction step occurred by adding 5 mL of acetonitrile to the diet pellet, resuspending the pellet, then centrifuging. The supernatants from these first two steps were combined and called "extract 1". An additional 5 mL of acetonitrile was added to the pellet and the mixture was sonicated overnight. The resulting supernatant following centrifugation of this mixture was called "extract 2".

Samples of the rat chow were also mixed with water and the pH of the mixture was found to be approximately pH 5.9.

RESULTS

As indicated in Table 1, essentially 90% plus material balance of applied radioactivity was achieved in this study. With the exception of the Day-4 samples, an overnight sonication in acetonitrile was required after the feed was incubated for more than one day at room temperature. In general, after 1 day, approximately 60-70% could be extracted without overnight sonication and the remainder recovered following sonication overnight.

Analysis of the extracts (1 and 2) showed that there was no discernible degradation of the test substance, [14 C]DPX-T3217. This was somewhat surprising since, we expected the aggressive, overnight sonication to result in some breakdown. However, this was not observed. Moreover, the pH of the rat chow/water mixture was approximately 6. From previous studies we know that the breakdown of cymoxanil is rapid in aqueous media of pH >7, however, the compound is relatively stable at pH 5-6.

CONCLUSION

The results indicate that extraction of DPX-T3217 became more difficult following incubation for 1 day at room temperature. However, material balance could be achieved through extensive sonication in acetonitrile. Moreover, HPLC analysis of the extract indicated that no significant degradation of the DPX-T3217 occurred in the rat diet matrix. We, therefore, conclude that the low recoveries observed in the analysis of samples from the 2 year feeding study are more likely to be due to inefficient extraction than to chemical degradation of the test substance.

TABLE I
PERCENT RECOVERY OF APPLIED RADIOACTIVITY

<u>DAY</u>		<u>EXTRACT 1</u>	<u>EXTRACT 2</u>	<u>TOTAL</u>
0	A	86.8*	-	86.8
	B	90.6*	-	90.6
1	A	62.5	27.4	89.9
	B	61.8	28.2	90.0
2	A	65.5	37.1	102.6
	B	65.1	43.6	108.7
3	A	71.8	32.7	104.5
	B	71.0	29.1	100.1
4	A	95.8*	-	95.8
	B	96.1*	-	96.1
5	A	62.0	44.5	106.5
	B	64.1	44.4	108.5
6	A	71.3	13.4	84.7
	B	69.9	13.9	83.8
7	A	63.5	35.7	92.2
	B	59.0	33.0	92.0

* No overnight sonication was required to achieve an acceptable material balance.

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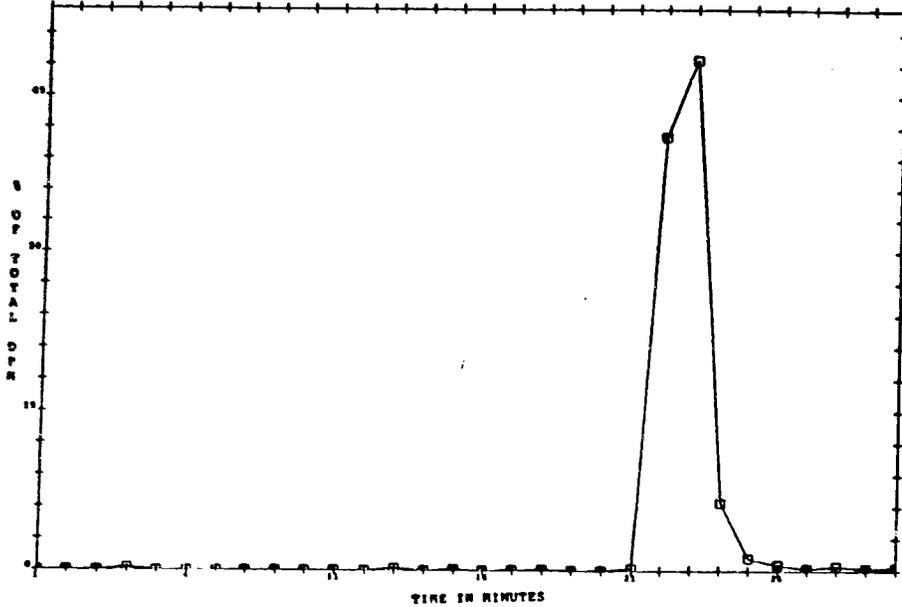
FIGURE 1

Du Pont HLR 370-91

RADIOCHROMATOGRAMS OF EXTRACTS 1 AND 2 ON DAY 1

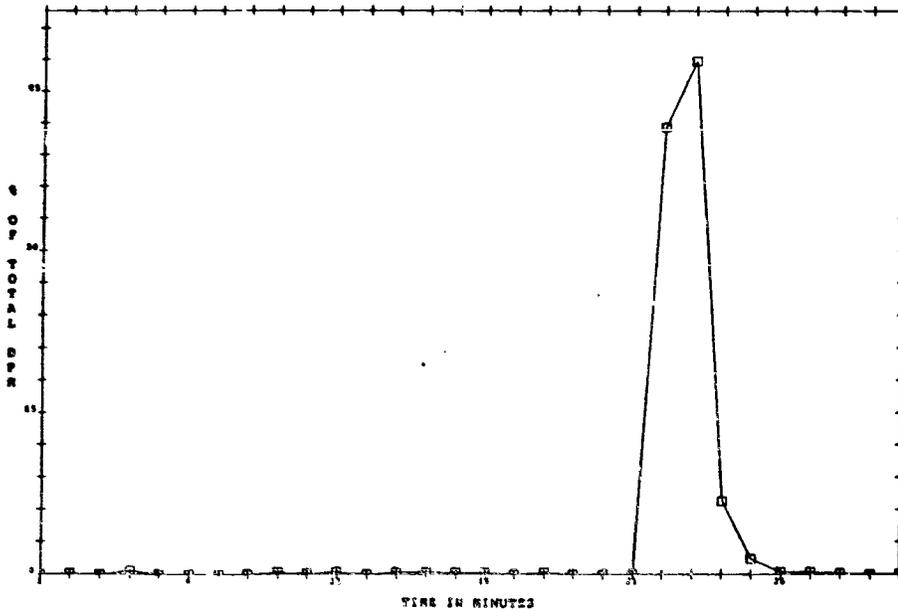
DPR-73217 RAY CROW STABILITY
DAY 1-8 EXTRACTS 1+2
100UL INJ.

Extract 1 (Day 1)



DPR-73217 RAY CROW STABILITY
DAY 1-8 OVERNIGHT SONICATION
100UL

Extract 2 (Day 1)

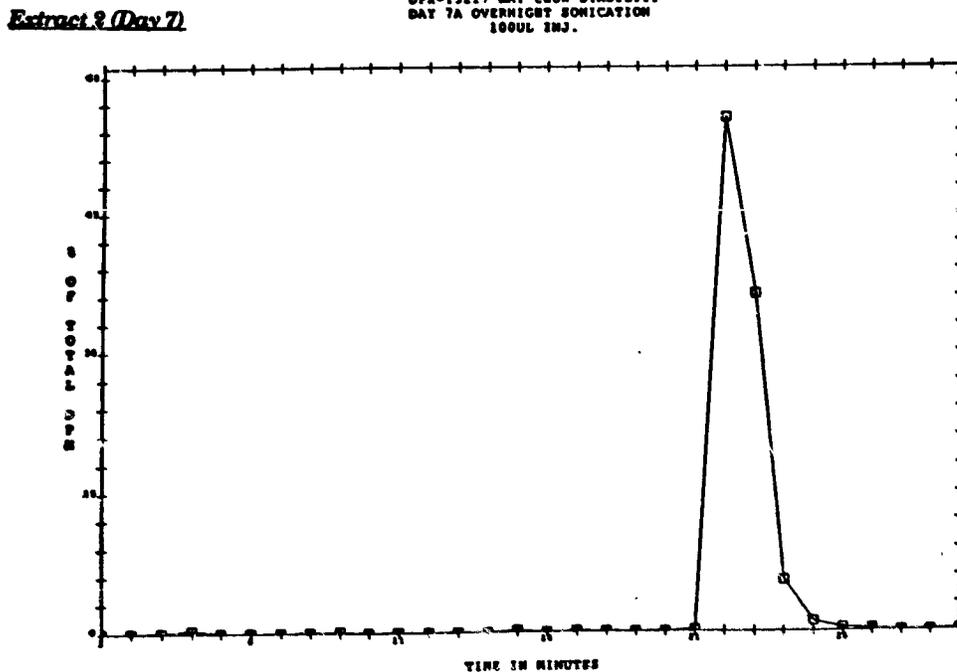
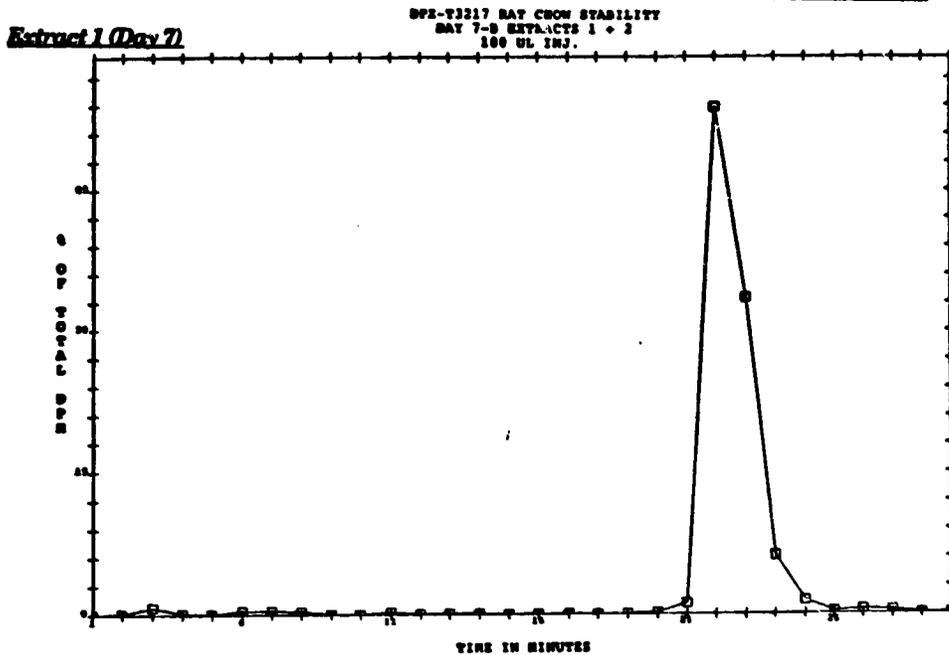


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FIGURE 2

Du Pont HLR 370-91

RADIOCHROMATOGRAMS OF EXTRACTS 1 AND 2 ON DAY 7



00151
Du Pont HLR 370-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

OPHTHALMOLOGY REPORT

JAMES M. CLINTON, V. M. D.
ANIMAL EYE CLINIC AT SOUTH JERSEY ANIMAL HOSPITAL
204 ROUTE 641
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TELEPHONE (609) 664-0304

DuPont Stine Haskell Laboratory
Study: MR 9131
C. Mebus, Ph.D.

Examination Date:
7 December 1990
Test Day (-13)

Pre-test Ophthalmoscopic Examination Summary

Both eyes of 139 male and 135 female rats were examined by focal illumination and indirect ophthalmoscopy. Mydriasis was produced with 1% atropine and the eyes examined in subdued light. Lesions were identified in the following rats:

<u>Temp. ID No.</u>	<u>Sex</u>	<u>Observations</u>
27	Male	Right Eye: Mid-vitreous hemorrhage.
63	Male	Right Eye: Mid-vitreous hemorrhage.
68	Male	Right Eye: Anterior synechia.

Comments

Ideally, the three rats listed above should not be used in the forthcoming study. The remaining rats are ophthalmoscopically normal.

James M. Clinton
James M. Clinton, V.M.D.

0072

JAMES M. CLINTON, V. M. D.
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DuPont Stine - Haskell Laboratory
Study: MR 9131
C. Mebus, Ph.D.

Examination Date:
22 March 1991

Ophthalmoscopic Examination Summary

Both eyes of all of the rats remaining in the study were examined by focal illumination and indirect ophthalmoscopy. Mydriasis was produced with 1% atropine and the eyes examined in subdued light. The dose levels and group identifications were not disclosed to me until after my examinations.

Lesions were identified in the following rats:

<u>Group</u>	<u>Sex</u>	<u>Rat No.</u>	<u>Observations</u>
IV	Female	6161	Left Eye: Phthisis.
V	Male	5975	Left Eye: Phthisis.
V	Male	6028	Left Eye: Superficial corneal vascularization.
V	Male	6061	Left Eye: Superficial corneal vascularization.

Comments

In my opinion, there is no evidence to indicate that there are any ocular lesions that are secondary to exposure to an ocular toxicant. The lesions noted in the four rats listed above are probably due to trauma.

James M. Clinton

James M. Clinton, V.M.D.

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Du Pont HLR 370-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

CLINICAL PATHOLOGY REPORT

0 0 5 1
Du Pont HLR 370-91

CLINICAL PATHOLOGY REPORT NO. 14-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANIL)
FEEDING AND NEUROTOXICITY STUDY IN RATS

MEDICAL RESEARCH PROJECT NO. 9131

HASKELL LABORATORY NO. 18753

Sponsor: Du Pont Agricultural Products

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
FEEDING AND NEUROTOXICITY STUDY IN RATS

Summary

Male and female Cr1:CD®BR rats were fed diets containing DPX-T3217 at concentrations of 0 (control), 100, 750, 1,500, or 3,000 ppm for approximately 90 days.

Mean leukocyte counts in male rats in the 1,500 and 3,000 ppm groups were decreased due primarily to a moderate decrease in lymphocytes and a slight decrease in monocytes. These leukocytic alterations were most likely related to increased release of endogenous glucocorticoids rather than a primary, treatment-related effect. Biologically significant alterations were not observed in clinical chemical or urinalysis parameters from males. No biologically significant clinical pathologic alterations were observed from female rats.

Under the conditions of this study, the no-observable-effect-level was considered to be 750 ppm in male rats based on the observed leukocytic alterations. However, because these alterations were believed to be secondary effects, the no-observable-adverse-effect-level was considered to be 3,000 ppm in male rats. The no-observable-effect-level was considered to be 3,000 ppm in female rats for the hematologic, clinical chemical and urinalysis parameters measured.

Prepared by: C. William Detwiler
C. William Detwiler
Technician

Report by: Glenn S. Elliott
Glenn S. Elliott, D.V.M., Ph.D.
Coordinator, Clinical Pathology

Approved by: J. Robert Gibson
J. Robert Gibson, Ph.D., D.A.B.T.
Assistant Director

Date issued: June 1, 1992

Procedure

Five groups of ten male and five groups of ten female Crl:CD®BR rats were fed diets that contained DPX-T3217 at concentrations of 0 (control), 100 (low), 750 (low-intermediate), 1,500 (high-intermediate), or 3,000 (high) ppm for approximately 90 days. Clinical pathology evaluations were performed at 52 and 53 days on test (45-DAY) and 96 and 97 days on test (90-DAY) for males and females, respectively.

At each sampling time, blood was taken from the orbital sinus of each fasted rat for enumeration of erythrocytes (RBC), leukocytes (WBC), and platelets (PLAT); analysis of hemoglobin (Hb) concentration and hematocrit (Ht); and determination of relative numbers of neutrophils (Neut), band neutrophils (Band), lymphocytes (Lymph), atypical lymphocytes (Alym), monocytes (Mono), eosinophils (Eosin), and basophils (Baso). Absolute values for various types of leukocytes were calculated from the leukocytic data. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the erythrocytic data. Blood cell counts, hemoglobin concentration, hematocrit, and Wintrobe erythrocyte indices were determined on an Ortho® model ELT-8/ds hematology analyzer. Differential cell counts were determined on a Hematrak® Automated Differential System cell counter. Reticulocyte (Retic) smears were prepared but evaluation was not required.

Bone marrow smears were prepared at sacrifice but evaluation was not required.

Serum activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and serum concentrations of glucose (GLUCO), urea nitrogen (BUN), calcium (CALC), phosphate (PHOS), bilirubin (BILRN), cholesterol (CHOL), creatinine (CREAT), total protein (TPROT), albumin (ALBMN), sodium (Na), potassium (K), and chloride (Cl) were measured on a Coulter DACOS® clinical chemistry analyzer using Coulter DART® reagents. Serum globulin (GLOBN) concentration was calculated from the total protein and albumin concentrations.

One day prior to each bleeding time, an overnight (approximately 16-hour) urine specimen was collected from each rat to measure volume (VOL), osmolality (OSMOL), urobilinogen (UROBL), and pH; and to determine the presence of hemoglobin or occult blood (BLOOD), glucose, protein, bilirubin, and ketone (acetoacetic acid). Osmolality was determined on a model A0-10 osmometer. Urine biochemical constituents were measured on a Clinitek® 200 urine chemistry analyzer using Ames Multistix® urine chemistry dipsticks. Urine appearance (color and transparency) was recorded and the sediment from each specimen was microscopically examined.

Statistical Analyses

A one-way analysis of variance (ANOVA) and Bartlett's test were calculated for each sampling time. When the value of the F-test statistic from ANOVA was significant, the Dunnett test was used to compare means from the control groups and each of the groups exposed to DPX-T3217. When the results of the Bartlett test were significant ($p \leq 0.005$), the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control groups and each of the groups exposed to DPX-T3217. Significance was judged at the 5% probability level.

Results

Statistically significant results for male and female rats are summarized in Clinical Pathology Text Tables I and II, respectively. Group means and standard deviations for hematologic (Tables 16 and 17), clinical chemical (Tables 18 and 19), and urinalysis (Tables 20 and 21) data (males and females, respectively) are presented in HLR 370-91 following the supplemental reports. Data for individual animals as well as terms and criteria used for urinalyses and missing data are contained in Appendix G.

H-18753 SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
 MR-9131
 HC-31

CLINICAL PATHOLOGY TEXT TABLE I

SUMMARY OF STATISTICALLY SIGNIFICANT HEMATOLOGIC
 AND CLINICAL CHEMICAL FINDINGS FOR MALE RATS

Sampling Time	45-DAY	90-DAY
<u>Measurement</u>		
<u>Hematology</u>		
RBC	↓ V, VII, IX	-
Hb	↓ V, IX	-
Ht	↓ V, VII	-
MCHC	↑ III	-
WBC	↓ VII, IX	↓ VII, IX
Lymph	-	↓ VII, IX
Mono	↓ III, V, VII, IX	↓ IX
<u>Clinical Chemistry (Serum)</u>		
ALT	-	↓ IX+
AST	↓ V, VII, IX	↓ IX+
CALC	↓ V, VII, IX	↓ IX
PHOS	↓ IX	-
CHOL	-	↓ V, IX
CREAT	↓ IX	-
TPROT	↓ III, V, VII, IX	↓ IX
GLOB	↓ V, VII, IX	↓ IX
Na	↓ VII, IX	-
<u>Clinical Chemistry (Urine)</u>		
VOL	-	↑ V

↑ = Significantly higher than control by Dunnett or Mann-Whitney U (+) criteria

↓ = Significantly lower than control by Dunnett or Mann-Whitney U (+) criteria

- = Not statistically significant

Group Designation and Concentration (ppm)

- III - Low (100)
- V - Low-Intermediate (750)
- VII - High-Intermediate (1,500)
- IX - High (3,000)

Due to analytical and inter-animal variability, the number of animals per group, and other experimental design factors, statistical significance should not be interpreted as denoting biological or toxicological significance. See the discussion section for an interpretation of biologically significant results.

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H-18753 SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107
MR-9131
YC-31

CLINICAL PATHOLOGY TEXT TABLE II

SUMMARY OF STATISTICALLY SIGNIFICANT HEMATOLOGIC
AND CLINICAL CHEMISTRY FINDINGS FOR FEMALE RATS

Sampling Time	45-DAY	90-DAY
<u>Measurement</u>		
<u>Hematology</u>		
RBC	-	↑ VI+
Hb	-	↑ VI+
Ht	-	↑ VI+
MCHC	-	↓ X
Eosin	-	↑ VI
<u>Clinical Chemistry (Serum)</u>		
AST	↓ X+	-
CALC	-	↓ IV,VI,VIII
PHOS	-	↑ X+
CREAT	-	↓ X
TPROT	↓ IV,X	↓ VI,VIII,X
ALBMN	-	↓ VI+,X+
GLOB	↓ VIII,X	-
Na	↓ X	↓ VI
K	-	↓ VIII

↑ = Significantly higher than control by Dunnett or Mann-Whitney U (+) criteria

↓ = Significantly lower than control by Dunnett or Mann-Whitney U (+) criteria

- = Not statistically significant

Group Designation and Concentration (ppm)

- IV - Low (100)
- VI - Low-Intermediate (750)
- VIII - High-Intermediate (1,500)
- X - High (3,000)

Due to analytical and inter-animal variability, the number of animals per group, and other experimental design factors, statistical significance should not be interpreted as denoting biological or toxicological significance. See the discussion section for an interpretation of biologically significant results.

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Discussion

Mean total leukocyte counts were mildly to moderately decreased in the 1,500 and 3,000 ppm male groups at the 45- and 90-day sampling times. Leukocyte counts were decreased primarily because of decreases in lymphocytes and, less importantly, because of decreases in monocytes. These leukocytic alterations were most pronounced at the 90-day sampling time. The causal relationship between treatment and the leukocytic alterations is somewhat equivocal. Decreases in circulating lymphocyte counts could be a primary, treatment-related effect. However, absence of morphological alterations in spleen, lymph nodes, thymus and bone marrow does not indicate that such a primary, treatment-related effect is present.¹ Furthermore, in rodents, the most common cause of decreased lymphocyte counts is increased release of endogenous glucocorticoids.^{2,3} This latter mechanism represents a secondary effect associated with "stress" in which endogenous glucocorticoids cause redistribution of lymphocytes from the circulation into lymphoid tissue.

Other statistically significant hematologic findings in male rats (RBC, Hb, Ht, MCHC, Mono in groups III and V at 45 days) are within the expected range of normal biological variation. All statistically significant hematologic findings in female rats (RBC, Hb, Ht, MCHC, Eosin) were within the expected range of normal biological variation.

Numerous statistically significant differences in mean ALT and AST activity, mean concentrations of calcium, phosphate, cholesterol, creatinine, total protein, globulin, and sodium and urine volume were observed in groups of male rats at the 45-day and the 90-day sampling period. These differences from the control results were small, usually were not dose-related and were often present at only the 45-day sampling period. Therefore, these findings were considered biologically insignificant. Similar statistically significant differences in female rats for mean AST activity, mean concentrations of calcium, phosphate, creatinine, total protein, albumin, globulin, sodium and potassium were also considered within the range of expected variation and biologically insignificant.

Conclusions

Under the conditions of this study, the no-observable-effect-level in male rats was considered to be 750 ppm based on the observed leukocytic alterations in the 1,500 and 3,000 ppm groups. However, the leukocytic alterations were considered likely to be secondary to increased release of endogenous glucocorticoids. Therefore, the no-observable-adverse-effect-level was considered to be 3,000 ppm. The no-observable-effect-level in female rats was considered to be 3,000 ppm for the hematologic, clinical chemical and urinalysis parameters measured.

References

1. MR 9131, H#18753, Pathology Report No. 59-91, Du Pont Co., Haskell Laboratory.
 2. Jensen, M.M., Changes in Leukocyte Counts Associated with Various Stressors, J. Reticuloendothel. Soc., 6:457-465, 1969.
 3. Brondeau, M.T., Bonnet, P., Guenier, J.P., Simon, P., deCeaurriz, J., Adrenal-dependent Leucopenia after Short-term Exposure to Various Airborne Irritants in Rats, J. Appl. Toxicol., 10:83-86, 1990.
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Du Pont HLR 370-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

NEUROBEHAVIORAL REPORT

0083

Du Pont HLR 370-91

NEUROBEHAVIORAL REPORT

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANI!)
FEEDING AND NEUROTOXICITY STUDY IN RATS

MEDICAL RESEARCH PROJECT NO. 9131-001

Haskell Number: 18753

Sponsor: Du Pont Agricultural Products
E. I. du Pont de Nemours and Company
Wilmington, Delaware

NEUROBEHAVIORAL REPORT

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANIL)
FEEDING AND NEUROTOXICITY STUDY IN RATS

SUMMARY

Groups of 10 male and 10 female Crl:CD®BR rats administered DPX-T3217 (cymoxanil) in the feed at dietary concentrations of 0, 100, 750, 1,500 or 3,000 ppm for a minimum of 90 days were assigned to a neurotoxicity substudy. These rats were evaluated for behavioral effects and neuropathology.

A neurobehavioral test battery, consisting of motor activity (MA) and functional observational battery (FOB) assessments, was conducted on the neurotoxicity substudy rats prior to test diet exposure in order to obtain baseline measurements. This neurobehavioral test battery was conducted again during Weeks 5, 9 and 13 of compound administration. Following 90 days of exposure to DPX-T3217 in the diet, all rats in the neurotoxicity subset were sacrificed and underwent in situ whole body perfusions and neuropathology evaluations.

Male and female rats given DPX-T3217 in the diet at 3,000 ppm had biologically remarkable and/or statistically significant decreases in mean body weights, as compared with the respective control groups. In male rats, the 3,000 ppm diet also decreased (not significant) relative landing foot splay scores during Weeks 9 and 13 and reduced (not significant) relative forelimb grip strength scores during Week 13. These observations were considered to reflect the significantly smaller body weights of the 3,000 ppm group male rats and were not considered neurotoxic effects. In female rats, no behavioral parameters were affected by DPX-T3217.

On the basis of the parameters evaluated in the neurotoxicity substudy, the no-observable effect level (NOEL) for DPX-T3217 in both the male and female rats is 1,500 ppm. None of the behavioral findings in this study at 3,000 ppm were considered neurotoxic effects.

Prepared by: Kathleen A. Mikles 5/28/92
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Technician

Approved by: Elizabeth A. Lochry 5/28/92
Elizabeth A. Lochry, Ph.D. Date
Senior Research Toxicologist

MATERIALS AND METHODS

A. General Experimental Design

Groups of 10 male and 10 female Crl:CD®BR rats administered DPX-T3217 (cymoxanil) in the feed at dietary concentrations of 0, 100, 750, 1,500 or 3,000 ppm for a minimum of 90 days were assigned to a neurotoxicity substudy. These rats were evaluated for behavioral effects and neuropathology.

B. Neurotoxicity Evaluations

1. Testing Intervals

A neurobehavioral test battery, consisting of motor activity (MA) and functional observational battery (FOB) assessments, was conducted on the neurotoxicity substudy rats prior to test diet exposure in order to obtain baseline measurements. This neurobehavioral test battery was conducted again during Weeks 5, 9 and 13 of compound administration. Following 90 days of exposure to DPX-T3217 in the diet, all rats in the neurotoxicity subset were sacrificed and underwent in situ whole body perfusions and neuropathology evaluations (see Neuropathology Report for a description of neuropathology procedures and evaluations).

2. Control of Bias

To facilitate behavioral testing, the rats were subdivided into three replicates that were tested on one of four consecutive days during the weeks that the FOB and MA assessments were conducted (the week prior to the onset of treatment and Weeks 5, 9 and 13 of treatment). An equal number of male and female rats from each dose group were assigned to each replicate. Within each replicate, the testing sequence was counterbalanced by dose group and sex to minimize the influence of uncontrolled factors. The observers conducting the FOB evaluations were blind with respect to the dose group of each animal.

On the morning of neurobehavioral assessment, the rats were weighed and examined for clinical signs by an observer not associated with any further neurotoxicity testing on that day. The animals were then transferred to individual holding cages that had coded cage tags to ensure that further testing on that day was conducted without knowledge of dietary level.

Motor activity assessments were conducted in a multi-station automated device (Omnitech Digiscan). Individual rats were assigned to the various test chambers such that an equal number of male and female rats from each dose group were tested in each chamber.

3. Functional Observational Battery (FOB)

A detailed description of the functional observational battery (FOB) is available in APPENDIX A (Protocol and Protocol Amendments). FOB testing consisted of a series of quantified behavioral observations conducted in a sequence that proceeded from the least interactive to the most interactive. During the first phase of FOB assessments, each rat was evaluated in three "environments": 1) inside the home cage; 2) outside the home cage; and 3) in a standard "open field" arena (approximately 45 x 31 x 12 cm).

Inside the home cage, the presence of palpebral closure, writhing, circling, biting and vocalization, as well as any unusual changes in body posture were recorded, if and when observed. Outside the home cage, each rat was assessed for fur appearance, ease of removal and ease of handling. Presence of piloerection, bite marks, palpebral closure, lacrimation and salivation were also evaluated. In the open field arena, the rats were evaluated for any unusual responses in level of activity (e.g., arousal), grooming, coordination, locomotion, gait and righting reflex. The presence of convulsions/tremors, palpebral closure, labored breathing, defecation and urination was also evaluated. In addition, simple assessments of sensory function were made including responses to approach/touch, finger snap and tail pinch.

The remainder of FOB testing involved standardized or calibrated devices. The presence or absence of pupillary constriction was assessed after a beam of light was directed into each eye. Fore- and hindlimb grip strength (kg) were measured by a strain gauge device (Chatillon Dial Push-Pull gauge). Landing foot splay (cm) was evaluated by measuring distance between fresh ink impressions made on paper by the hindpaws when the rat was dropped from a height of approximately 32 cm.

4. Motor Activity

Motor activity was evaluated by a multi-station automated locomotor activity monitoring device (Omnitech Digiscan). Both horizontal and vertical activity counts were recorded. Motor activity counts were evaluated in four blocks of 10 minutes each to assess habituation over a 40-minute test session.

C. Statistical Analysis

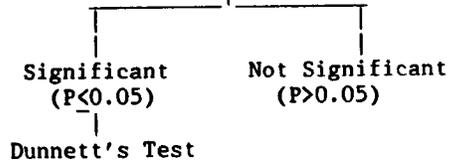
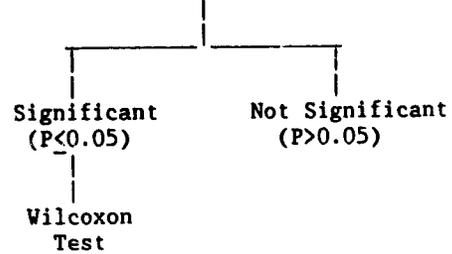
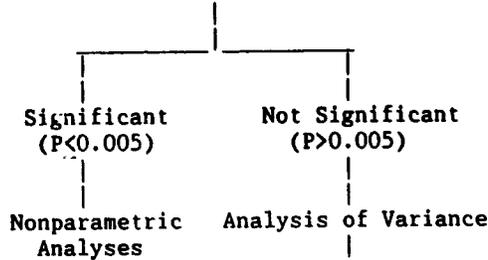
Statistical analysis of the data was performed as follows:

I. PARAMETRIC ANALYSES

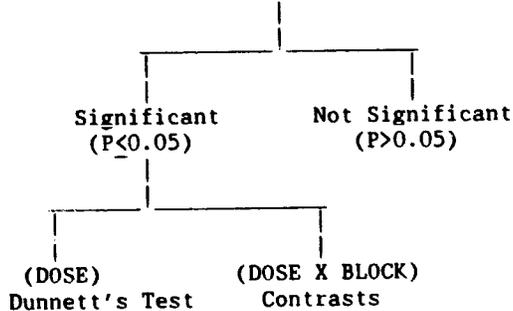
II. NONPARAMETRIC ANALYSES

A. Bartlett's Test

A. Kruskal-Wallis Test

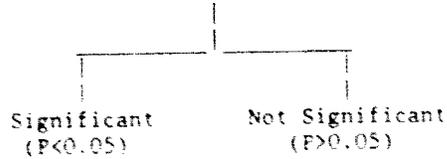


B. Analysis of Variance With Repeated Measures



III. PROPORTION ANALYSES

Fisher's Exact Test
(Bonferroni Correction)



Clinical sign incidence data, as well as descriptive FOB parameters were evaluated by Fisher's Exact test (with a Bonferroni correction) to determine significant experimental group differences with respect to the control group.

Body weights and body weight gains, as well as continuous data from the FOB (fore- and hindlimb grip strength, landing foot splay), were analyzed as parametric data. Bartlett's Test of Homogeneity of Variances was used to estimate the probability that the dose groups had different variances. If Bartlett's Test was not significant, the data were then analyzed via univariate Analysis of Variance (ANOVA), with Dunnett's Test used to identify which dose groups, if any, were significantly different from the control group. If Bartlett's Test was significant, data were analyzed via a Kruskal-Wallis Test, with the Wilcoxon Test used to identify which dose groups, if any, were significantly different from the control group.

Motor activity data were analyzed via univariate Analysis of Variance with DOSE as a between subjects factor and BLOCK as a repeated measure. In the event of a significant effect of DOSE, totals for the control group and groups given the test substance were compared using Dunnett's test. In the event of a significant interaction of DOSE and BLOCK, contrasts were used to identify which dose groups within each block, if any, were significantly different from the control group.

Analysis of grip strength data was conducted on the mean of the absolute scores collected on each of three trials conducted at each test interval (baseline and Weeks 5, 9 and 13 of exposure), as well as the mean of the normalized scores collected on the same three trials at each interval (calculated as a percent of the baseline grip strength). Similarly, analysis of landing foot splay data was also conducted on both absolute and normalized scores as described previously for the grip strength data.

All significance levels were judged at alpha of Bartlett's test which was judged at alpha "significant" or "highly significant" indicates a difference between the control and the experimental group. The individual rat as the control analyses were performed on data for male and

RESULTS AND DISCUSSION

B. Body Weights (Tables 22 and 23)

Body weights were significantly reduced in group male rats evaluated for neurobehavioral test 11, as compared with the control group. These group female rats evaluated for neurobehavioral test 11 during these same intervals, although significantly reduced (P<0.05). No other test body weight occurred.

C. Clinical Observations (Tables 14 and 15)

No test substance-related differences occurred in clinical observations on the days of neurobehavioral test 11. Differences among the biologically reasonable and statistically significant

C. Functional Observational Battery

1. Forelimb Grip Strength (Tables 16 and 17, Figures 2 through 4)

Absolute forelimb grip strength scores were not affected by the test substance. Differences were not significant and all values observed were within 100% of the control group.

All significance levels were judged at $\alpha = 0.05$, with the exception of Bartlett's test which was judged at $\alpha = 0.005$. The use of the word "significant" or "significantly" indicates a statistically significant difference between the control and the experimental groups. Data were evaluated with the individual rat as the unit of analysis. Separate analyses were performed on data for male and female rats.

RESULTS AND DISCUSSION

A. Body Weights (Tables 22 and 23; Appendix H)

Body weights were significantly reduced ($P < 0.05$) in the 3,000 ppm dose group male rats evaluated for neurobehavioral parameters during Weeks 5, 9 and 13, as compared with the control group. Body weights in the 3,000 ppm dose group female rats evaluated for neurobehavioral parameters were also reduced during these same intervals, although only the Week 9 value was significantly reduced ($P < 0.05$). No other test substance-related effects on body weight occurred.

B. Clinical Observations (Tables 24 and 25; Appendix I)

No test substance-related differences occurred in the incidence of clinical observations on the days of neurobehavioral evaluations in either male or female rats. Differences among the four dose groups were neither biologically remarkable nor statistically significant.

C. Functional Observational Battery

1. Forelimb Grip Strength (Tables 26 and 27; Appendix J; Figures 5 through 8)

Absolute forelimb grip strength scores in the male and female rats were unaffected by the test substance. Differences among the four dose groups were not significant and all values observed in the groups given the test substance were within $\pm 10\%$ of the control group values.

A small reduction (not significant) in relative forelimb grip strength scores occurred in the 3,000 ppm dose group male rats during Week 13; relative scores were only 82% of the control group values during this interval. This observation was considered to reflect the significant decrease ($P < 0.05$) in body weight that occurred in the 3,000 ppm dose group male rats and was not considered a neurotoxic effect. Relative forelimb grip strength scores were unaffected in the female rats; differences were neither biologically remarkable nor statistically significant.

2. Hindlimb Grip Strength (Tables 28 and 29; Appendix K; Figures 9 through 12)

Absolute and relative hindlimb grip strength scores in the male and female rats were unaffected by the test substance. Differences among the four dose group were neither statistically significant nor biologically remarkable.

3. Landing Foot Splay (Tables 30 and 31; Appendix L; Figures 13 through 16)

In male rats, relative foot splay scores (percent of baseline) in the 3,000 ppm dose group male rats were decreased by 18% during Week 9 and decreased by 23% during Week 13, as compared with the respective control group scores. These observations were considered to reflect the significantly smaller ($P < 0.05$) body weights of the 3,000 ppm dose group male rats and were not considered neurotoxic effects. Absolute foot splay scores (cm) in the male rats given the test substance were neither dosage-dependent nor significantly different, as compared with the control group scores.

In female rats, absolute and relative foot splay scores were increased in the 3,000 ppm dose group (6.2 cm and 148%, respectively) during Week 5, as compared with the control group (5.4 cm and 115%, respectively). These

observations were considered unrelated to the test substance because: (1) the differences were not statistically significant, as compared with the concurrent control group values; (2) the scores observed in the 3,000 ppm dose group female rats were either within range of historical control values or within 10% of the upper limit of historical control values*; and (3) the differences did not recur when foot splay was tested during Weeks 9 and 13.

Neither absolute nor relative landing foot splay scores were affected in the male or female rats given the 100, 750 or 1,500 ppm diets.

4. Other FOB Endpoints (Tables 32 and 33; Appendix M)

No test substance-related differences in the remaining FOB parameters occurred for either male or female rats. The incidences of FOB findings in the groups given the test substance were neither biologically remarkable nor significantly increased, as compared with the control group.

D. Motor Activity (Tables 34 and 35; Appendices N and O; Figures 17 through 20)

Horizontal and vertical motor activity in the male and female rats was unaffected by the test substance. Differences among the four dose groups were neither biologically remarkable nor statistically significant.

All dose groups demonstrated similar rates of habituation in horizontal and vertical activity levels across each test session as indicated by a significant Block effect ($P \leq 0.01$) and no significant Dose x Block interactions.

* In four subchronic studies (28-day or 90-day) conducted at this test facility during 1991 (HLR Numbers 550-91, 663-91, 852-91 and 853-91), absolute and relative foot splay scores in 40 control group Crl:CD®BR female rats tested during Week 4 of exposure were 5.5 cm and 114%, respectively (ranges of 4.2 to 6.5 cm and 106% to 131%, respectively).

CONCLUSION

Male and female rats given DPX-T3217 in the diet at 3,000 ppm had biologically remarkable and/or statistically significant decreases in mean body weights, as compared with the respective control groups. In male rats, the 3,000 ppm diet also decreased (not significant) relative landing foot splay scores during Weeks 9 and 13 and reduced (not significant) relative forelimb grip strength scores during Week 13. These observations were considered to reflect the significantly smaller body weights of the 3,000 ppm dose group male rats and were not considered neurotoxic effects. In female rats, no behavioral parameters were affected by DPX-T3217.

On the basis of the parameters evaluated in the neurotoxicity substudy, the no-observable effect level (NOEL) for DPX-T3217 in both the male and female rats is 1,500 ppm. None of the behavioral findings in this study at 3,000 ppm were considered neurotoxic effects.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

PATHOLOGY REPORT

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PATHOLOGY REPORT NO. 59-91

MEDICAL RESEARCH PROJECT NO. 9131

HASKELL LABORATORY NO. 18753

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANIL)

FEEDING AND NEURCTOXICITY STUDY IN RATS

DU PONT AGRICULTURAL PRODUCTS

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

SUMMARY

Groups of 10 male and 10 female Crl:CD ϕ BR rats were fed diets containing DPX-T3217 at concentrations of 0, 100, 750, 1500, or 3000 ppm. All surviving rats were sacrificed by design after approximately 3 months on test. Animals were examined grossly and selected tissues were weighed and/or examined microscopically.

Statistically significant, compound-related reductions in mean final body weights were found in the 1500 and 3000 ppm males and the 3000 ppm females. These reductions were 10%, 16%, and 12% for the 1500 ppm males, the 3000 ppm males, and the 3000 ppm females, respectively. Lower mean final body weights resulted in decreased absolute mean heart weight in the 3000 ppm males and increased mean relative testes, kidney, and brain weights in the 1500 and 3000 ppm males.

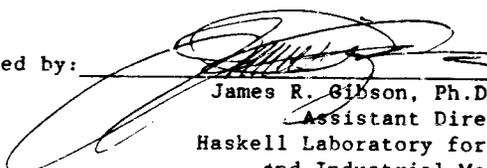
The 3000 ppm females had statistically significantly increased mean relative liver and spleen weights. The biological significance of the increased mean spleen weight was uncertain. The increase in mean relative liver weight was judged to be biologically significant and compound related. No microscopic lesions to explain these changes were seen.

There were no compound-related deaths or gross observations in this study. One 750 ppm female was found dead on day 42. No gross lesions explained the death; tissues were inadvertently not saved for histopathologic evaluation.

Microscopically, increased elongate spermatid degeneration was observed in the seminiferous tubules of the testes. The incidence and severity of the lesion increased with increasing dietary concentration of DPX-T3217. An increase in the amount of degeneration was seen in one control and several treated males. Associated with the degeneration of the elongate spermatids were seminiferous tubule multinucleated spermatids, epididymal cell debris, epididymal hypospermatia, and epididymal multinucleated spermatids. These changes were judged to be compound-related effects.

Under the conditions of this study, the no-observable-effect level for DPX-T3217 is 750 ppm for male rats and 1500 ppm for female rats based on mean final body and organ weights and histopathology.

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INTRODUCTION AND METHODS

This report summarizes final body and organ weight data, and gross and microscopic findings from a subchronic oral toxicity study with DPX-T3217 in Crl:CD®BR rats. Treatment groups were as follows:

<u>Dietary Concentrations</u> DPX-T3217 (ppm)	<u>Group Designation</u>		<u>Number per</u> <u>Dietary Concentration</u> <u>per Sex</u>
	<u>Male</u>	<u>Female</u>	
0 (Control)	I	II	10
100 (Low)	III	IV	10
750 (Low-intermediate)	V	VI	10
1500 (High-intermediate)	VII	VIII	10
3000 (High)	IX	X	10

The rats were sacrificed by design after approximately 90 days. Animals were euthanatized by chloroform anesthesia and exsanguination. Tissues were saved and processed for microscopic examination as specified by the protocol.* Brain, heart, liver, spleen, kidneys, adrenals, and testes of rats sacrificed by design were weighed at necropsy. Testes, epididymides, eyes, and skin with mammary gland (female) were fixed in Bouin's solution. All other tissues were fixed in 10% neutral buffered formalin. Processed tissues were embedded in paraffin, cut at a nominal thickness of 5 micrometers, stained with hematoxylin and eosin and examined with a light microscope. All tissues from animals in the 0 and 3000 ppm groups received a full microscopic examination. Only liver, kidneys, lungs, testes, and gross lesions were examined from rats in the 100, 750, 1500, and 3000 ppm groups that were sacrificed by design at the end of the study.

Mean final body and organ weights were analyzed with a one-way analysis of variance (ANOVA). When the F-test from ANOVA was significant, the Dunnett's test was used to compare means from the control group with the treated group. The p-value for significance was 0.05.

Fisher Exact Test was performed on group incidences of gross and histopathologic findings. The p-value for significance was 0.05.

* adrenals, aorta (thoracic), bone and marrow (femur, sternum), brain, cecum, colon, duodenum, epididymides, esophagus, exorbital lacrimal glands, eyes, harderian glands, heart, ileum, jejunum, kidneys, liver, lymph nodes (mesenteric, mandibular), mammary gland (female), nose, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, spleen, stomach, testes, thymus, thyroids, trachea, urinary bladder, uterus, cervix, vagina, and gross lesions.

RESULTS AND DISCUSSION

Tables, Figures, and Appendices

In this report the tables and appendices provide summary and individual animal data, respectively. The figures include photomicrographs of representative sections of liver, heart, spleen, lung, and kidney of 0 and 3000 ppm males and females and representative sections of testes and epididymides of 0 and 3000 ppm males.

The mean final body and organ weights are summarized in Tables 36 and 37 for males and females, respectively. Summaries of incidences of gross observations in males and females are in Tables 38 and 39, respectively. Summaries of incidences of microscopic observations with lesion grades are in Tables 40 and 41 for males and females, respectively. Summaries of the incidences of non-neoplastic microscopic observations without lesion grades are in Tables 42 and 43 for males and females, respectively. Summaries of incidences of microscopic observations with individual animal identification are in Tables 44 and 45 for males and females, respectively. Summaries of animal disposition and causes of death are in Tables 46 and 47 for males and females, respectively.

Figures 21-40 are photomicrographs of representative sections of liver, kidney, lungs, heart, and spleen for 0 and 3000 ppm males and females. Figures 41-51 are photomicrographs of representative sections of testes and epididymides.

The individual animal final body and organ weights and the individual gross and microscopic findings are listed in Appendices P and Q, respectively.

Mortality

All animals survived until scheduled termination of the study except for one 750 ppm female that was found dead on day 42. The cause of death was not determined. Inadvertently, tissues from this animal were not saved.

Final Body and Organ Weight Data

The mean final body weights of the 1500 and 3000 ppm males and 3000 ppm females were significantly less than their respective control mean final body weights. The mean final body weights of the 1500 ppm and 3000 ppm males were decreased 10% and 16%, respectively. The mean final body weight of the 3000 ppm females was 12% less than the female 0 ppm mean final body weight. These effects were judged to be compound related.

The mean absolute heart weight of the 3000 ppm males was statistically significantly decreased. For heart, this reduction reflects the decreased body weight of the group.

The mean relative testes, kidney, and brain weights of the 1500 and 3000 ppm males were significantly increased. For testes, kidney, and brain, this increase reflects the preservation of organ weight when accompanied by decreased body weight. The mean absolute organ weights of the testes, kidney, and brain for these groups were comparable to the control group weights.

The mean relative liver and spleen weights were significantly increased in the 3000 ppm females. The biological significance of the increased splenic weight was uncertain. The increased relative liver weight was judged to be biologically significant and compound related. No microscopic changes were observed to explain these changes.

The weight of the adrenals of one 100 ppm male, 496068, was 78 times greater than the average absolute weight of the other adrenals in the group. No gross observation was made. This organ was judged to have been misweighed.

Gross Observations

Compound-related gross changes were not observed. The gross observations made were consistent with conditions or changes commonly encountered in this strain and age of rat. One 750 ppm female, 496173, was found dead at 42 days. In error, no tissues were saved from this rat. This 750 ppm female had a perineal mass. No other masses were seen in any other group.

Microscopic Observations

Increased elongate spermatid degeneration was observed in one 0 ppm, three 750 ppm, five 1500 ppm, and seven 3000 ppm males. The incidence and severity of the lesion increased with increasing dietary concentration of DPX-T3217. The increased incidence was statistically significant by the Fisher Exact Test for the 3000 ppm group. The lesion consisted of rounded cells with condensed nuclei and granular eosinophilic cytoplasm at the luminal border of the germinal epithelium in tubules of approximate stages XII through XIV and I through IV (see Figures 42, 44, 46 & 48). Morphologically normal elongate spermatids and degenerate spermatids were both found in affected tubules. Not all tubules of stages XII through XIV and I through IV were equally affected. The architecture of the testes was not disturbed. The lesion was graded minimal or mild in all cases. All testicular sections were from Bouin's fixed tissue and were stained with hematoxylin and eosin. No special fixatives or stains to aid in staging were performed.

One 3000 ppm and one 1500 ppm male had multinucleated spermatids in the seminiferous tubules (see Figure 49). This lesion is a common pathologic finding that can be caused by a variety of agents. Alone this change was so infrequent as to be of questionable significance. The presence of the multinucleated spermatids in conjunction with the elongate spermatid degeneration in the 1500 and 3000 ppm groups indicated probable biological significance as part of the compound-related effect.

The observation of the lesion in the 0 ppm male 496025 indicated that this lesion to a minimal extent occurs spontaneously (see Figures 45 & 47).

The observed epididymal changes were all minimal and consisted of intratubular debris, multinucleated spermatids, and bilateral hypospermia (see Figure 51). These changes reflect the seminiferous tubule changes. Hypospermia, meaning decreased numbers of mature spermatozoa in the epididymal tubules, was seen in four 3000 ppm males. Cell debris was observed in one 1500 ppm and six 3000 ppm males. The incidence of cell debris in the epididymides was statistically significantly increased for the 300 ppm group when compared to the control group. Multinucleated spermatids were in the epididymides of one 1500 and one 3000 ppm male. None of these epididymal changes affected the 0, 100, or 750 ppm males. One 750 ppm male had mild subacute inflammation of the epididymal interstitium, a non-specific change frequently encountered as a spontaneous lesion in male rats.

The coincident testicular and epididymal effects of the 1500 and 3000 ppm males were judged to be compound related. No epididymal effects were seen in the 750 ppm males and the incidence of degeneration of the elongate spermatids in that group was not significantly increased over the 0 ppm males.

The remaining microscopic findings were spurious or common lesions found in this strain and age rat.

The incidence of minimal cardiomyopathy in the 3000 ppm males was statistically less by the Fisher Exact Test than the 0 ppm group. This decrease was of no biological or toxicological significance; cardiomyopathy is a common spontaneous change seen in this strain of rats.

The incidence of odontal inflammation was statistically significantly increased for the 3000 ppm females. There were zero 0 ppm females affected and four 3000 ppm females affected. This lesion is a commonly encountered lesion in this strain of rat; no biological or toxicological significance was attributed to this finding.

CONCLUSION

For pathology, the no-observable-effect level for rats fed DPX-T3217 in the diet under the conditions of this study was 750 ppm for males and 1500 ppm for females based on body and organ weights and histopathology.

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

NEUROPATHOLOGY REPORT

Du Pont HLR 370-91

PATHOLOGY REPORT NO. 68-91

MEDICAL RESEARCH PROJECT NO. 9131

HASKELL LABORATORY NO. 18753

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107 (CYMOXANIL)

FEEDING AND NEUROTOXICITY STUDY IN RATS

DU PONT AGRICULTURAL PRODUCTS

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

NEUROPATHOLOGY

SUMMARY

This report summarizes the gross and microscopic findings for a neurotoxicity 90-day study in male and female Crl:CD®BR rats fed 0, 100, 750, 1500, and 3000 ppm of DPX-T3217 in the diet.

There were no compound-related lesions detected within the nervous system or skeletal muscle of the 0 or 3000 ppm rats.

For neuropathology, the no-observable-effect level for this feeding study with DPX-T3217 in male and female rats was 3000 ppm.

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Date issued: _____

June 1, 1992

MMS/JRG/wfd
WPSP-SOMMER

INTRODUCTION AND METHODS

This report will summarize the gross and microscopic findings for a neurotoxicity 90-day feeding study with DPX-T3217 in male and female Crl:CD¹BR rats. Treatment groups and design, compound concentrations were as follows:

Dietary Concentrations DPX-T3217 (ppm)	Group Designation		Number per Dietary Concentration per Sex
	Male	Female	
0 (Control)	I	II	10
100 (Low)	III	IV	10
750 (Low-intermediate)	V	VI	10
1500 (High-intermediate)	VII	VIII	10
3000 (High)	IX	X	10

After approximately 90 days on test, all surviving rats were sacrificed and perfusion fixed according to the protocol. Tissue samples from the nervous system and muscle were saved, processed for histopathology, and examined as specified by the protocol.* Tissues were examined grossly at the time of perfusion and subsequent dissection.

Sections of the brain, spinal cord, muscle, and gross lesions were embedded in paraffin, sectioned at a nominal thickness of 5 microns, and stained with hematoxylin and eosin. Additional sections of brain and spinal cord were sectioned and stained with Luxol Fast Blue and Periodic Acid Schiff (LFB/PAS) according to standard methods. Sections of sciatic and tibial nerves, and Gasserian ganglia were embedded in glycol methacrylate, sectioned at a nominal thickness of 3 microns, and stained with hematoxylin and eosin. Sections of dorsal root fibers and ganglia, and ventral root fibers were post fixed in osmium tetroxide, embedded in epoxy, sectioned at a nominal thickness of one half micron, and stained with toluidine blue.

All tissues from the males and females in the 0 ppm and 3000 ppm groups were read. Subsequent groups were to be read as required to determine a no-observable-effect level.

RESULTS AND DISCUSSION

Table 48 summarizes the incidences of gross observations for males. The

* Brain (cerebrum, cerebellum, medulla), spinal cord (cervical and lumbar), sciatic nerves, tibial nerves, Gasserian ganglia, cervical and lumbar dorsal root fibers, ganglia (DRF&G), and ventral root fibers (VRF), and gastrocnemius muscle.

females had no gross observations (Table 49). Tables 50 and 51 summarize the incidences and severity of microscopic observations for males and females, respectively. Appendix R contains individual gross and microscopic observations.

Mortality

All animals survived to the terminal sacrifice.

Gross Lesions

The gross abnormalities encountered in the males were common spontaneous lesions in this strain and age of rat. No gross abnormalities were observed in the female rats.

Microscopic Lesions

There were no compound-related lesions detected within the nervous system or skeletal muscle.

Similar types and incidences of microscopic lesions were observed in the 0 and 3000 ppm groups. There was no significant difference in incidence between the 0 ppm groups and the 3000 ppm groups with the Fisher Exact Test. The lesions were all minimal, consisted of myelin degeneration, myelin phagocytosis, skeletal muscle degeneration, and macrophages in the interstitium of the skeletal muscle, and were interpreted to represent spontaneous incidental lesions of no biological significance in this strain and age of rat. One 3000 ppm male had minimally dilated ventricles; as with the other lesions, no significance was attributed to this finding.

Of the 0 and 3000 ppm rats, only one male had a gross lesion. That male had small, soft testes. Microscopically, this corresponded to severe atrophy and degeneration of the seminiferous tubules. This male was in the 3000 ppm group, but no other males were affected. The lesion did not resemble the elongate spermatid degeneration seen in the subchronic part of the study. This lesion was judged to be spurious and not related to compound administration.

CONCLUSION

For neuropathology, the no-observable-effect level for this feeding study with DPX-T3217 in male and female rats was 3000 ppm.

0 0 7 5
Du Pont HLR 370-91

SUBCHRONIC ORAL TOXICITY: 90-DAY STUDY WITH DPX-T3217-107

TABLES