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THE ACUTE, DELAYED NEUROTOXICITY EVALUATION OF TWO JET ENGINE OIL FORMULATIONS

E. R. Kinkead
S. K. Bunger
R. E. Wolfe
H. G. Wall

NSI TECHNOLOGY SERVICES CORPORATION
101 WOODMAN DRIVE, SUITE 12
DAYTON, OHIO 45431

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HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
HUMAN SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
Chief, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

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E. R. Kinkead, S. K. Bunger, R. E. Wolfe, H. G. Wall

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This study was designed to determine the potential of two jet engine oils to produce acute, delayed neurotoxicity. The hydrocarbon-based ester oil formulation contained 3% tricresyl phosphate isomers including triorthocresyl phosphate (TOCP) in one of the formulations. Hens were orally dosed over a five-day period and then observed for a total period of 30 days. All TOCP-positive control hens demonstrated signs of acute, delayed neurotoxicity. Hens from both jet engine oil groups remained asymptomatic throughout the observation period. No neurotoxic hazard would be expected for military or civilian personnel involved in the manufacture, transportation, or handling of these compounds. Keywords: Experimental; Lubricating oils; Neurotoxicity; (RT)

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PREFACE

This is one of a series of reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, NSI Technology Services. This document serves as a final report on the in-life neurotoxicity of two jet engine oil formulations. The research described in this report began in May 1989 and was completed in December 1989 under U.S. Air Force Contract No. F33615-85-C-0532. Lt Col Michael B. Ballinger served as Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory. This study was sponsored by the U.S. Navy under the direction of CDR David A. Macys, MSC, USN. LT Linda Lininger, MSC, USN, served as coordinator of this study.

This work was supported by the Naval Medical Research and Development Command Task MR04122010006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large.

The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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ABBREVIATIONS

cc	Cubic centimeter
cm	Centimeter
ft	Feet
h	Hour
IR	Infrared
kg	Kilogram
mg	Milligram
mL	Milliliter
NMRI/TD	Navy Medical Research Institute Toxicology Detachment
NTE	Neurotoxic esterase
OPIDN	Organophosphate-induced delayed neuropathy
p	Probability
SEM	Standard error of the mean
TCP	Tricresyl phosphate
TOCP	Triorthocresyl phosphate

SECTION 1

INTRODUCTION

The Navy is interested in evaluating the acute, delayed neurotoxicity of two jet engine oil formulations developed by the Mobil Oil Corporation. These samples could potentially result in axonal degeneration characteristic of organophosphate-induced delayed neuropathy (OPIDN) due to a tricresyl phosphate (TCP) additive. The major component of each oil is a mixture of hydrocarbon-based esters. In the first formulation, NMRI/TD No. 9082-1, the additive is 3% tricresyl phosphate isomers. In the second formulation, NMRI/TD No. 8323-1, the additive is 3% of the ortho derivative of tricresyl phosphate (TOCP), a known neurotoxin. The Navy Medical Research Institute Toxicology Detachment (NMRI/TD) requested that an acute, delayed neurotoxicity study be performed on each of the samples to determine the potential health hazard involved with using these lubricants.

Many organophosphorus compounds have been found to cause delayed neurotoxic effects in man (Norton, 1980). A single exposure to a neurotoxic organophosphorus compound has been reported capable of producing axonal damage after a delay of eight to 10 days. Low level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to TOCP (Beresford and Glees, 1963). Organophosphorus compounds which cause axonal pathology interact with the enzyme neurotoxic esterase (NTE) in the initial step of the delayed neurotoxicity. This interaction occurs within hours of dosing and can be measured in the brain. NTE inhibition in the brain correlates with that in the spinal cord and nerve (Davis and Richardson, 1980).

This study was designed to determine if delayed neurotoxic effects result from exposure of adult chickens to the two jet engine oils of interest, NMRI/TD Nos. 9082-1 and 8323-1. Both engine oil samples, as well as a vehicle control and a TOCP-positive control, were tested concurrently. Final determination of an injury effect was based on a comparison of the test chickens with the TOCP-postive control chickens. An NTE assay was performed on a portion of the test animals for comparison with the non-biochemical assay.

SECTION 2

MATERIALS AND METHODS

TEST MATERIALS

The two engine oil samples supplied by NMRI/TD are listed below.

Jet engine oils:

No. 9082-1
Additive: 3% TCP
Density: 1.003 g/mL at 60°F
Initial Boiling Point: >600°F
Vapor Pressure: <0.1 mm Hg at 37.8°C

No. 8323-1
Additive: 3% TOCP
Density: 1.003 g/mL at 60°F
Initial Boiling Point: >600°F
Vapor Pressure: <0.1 mm Hg at 37.8°C

Positive and Negative Control Materials:

Triorthocresyl Phosphate (TOCP), practical grade,
Lot #C9B obtained from Eastman Kodak, Co.,
CAS #78-30-8.

Corn oil, commercial grade, purchased locally. The corn oil was tested for the presence of peroxides prior to use.

An infrared (IR) spectrum of each sample was obtained prior to the start of the study. The THRU/NSI Chemistry Section retained an archive sample of each test material.

ANIMALS

Delayed neurotoxicity potential was evaluated using leghorn hens (*Gallus domestica*, Carey Nick 320 hybrid, Carey Farms, Inc., Marion, Ohio), eight to 14 months of age, weighing between 1.1 and 2.1 kg. The debeaked hens were identified by leg bands and group housed in 3 ft x 6 ft pens to allow free movement. Food (MannaPro, Eggmaker 15 Crumbles) and water were provided *ad libitum*. Hens were maintained on a 15-h light/dark cycle starting at 0300 h.

Verbal communication with the supplier (Carey Farms, Inc.) provided additional flock history and husbandry practices. **Table 1** lists the vaccinations administered to the flock. No pesticides were applied to hens used in this study, nor were disinfectants used while birds were in the poultry houses. When poultry houses became vacant, they were cleaned and disinfected with formaldehyde. The supplier indicated that the flock had not experienced any disease problems.

TABLE 1. FLOCK VACCINATION HISTORY[a]

Vaccination	Age of hen
Marek's disease	1 day
Infectious bronchitis	2 weeks
Infectious bursal disease	2 weeks
Newcastle disease	2 weeks
Infectious bronchitis (booster)	10-12 weeks
Newcastle disease (booster)	10-12 weeks
Fowlpox	20-24 weeks

[a]Provided verbally by Carey Farms, Inc., Marion, OH

ACUTE, DELAYED NEUROTOXICITY

The design of this study followed the requirements of military specification MIL-H-19457B. All hens were weighed prior to the start of the study and weekly thereafter. Test substances were administered to unfasted adult hens by oral intubation employing a 3-cc syringe fitted with a 15-cm infant feeding catheter. Doses were administered on five consecutive days beginning on Monday. The engine oils and TOCP were diluted in corn oil to obtain the appropriate dose. Each hen was weighed prior to the initial dose and 1.0 mL/kg body weight was administered by gavage. The dosing regimen was as follows.

Engine oils	Groups of four hens each were treated with 420, 360, 300, or 240 mg/kg/day for five days.
TOCP Positive Controls	Groups of four hens each were treated with 90, 75, or 60 mg/kg/day for five days.
Corn oil	Twelve hens were given the maximum total volume of fluid equal to that given test animals (i.e., 1.0 mL/kg).

Observations and scoring began seven days after the first dose and continued three times a week (Monday, Wednesday, and Friday) until 30 days after the initial dose. The following scoring system was used.

Symptom-free	0 points
Doubtful or minor symptoms	2 points
Positive paralytic symptoms	8 points
Advanced paralytic symptoms	12 points
Death	16 points

During observation and scoring, the chickens were removed from their enclosures and placed on a rubber mat to provide sure footing. Symptoms observed in test hens during the observation period were compared with those seen in the TOCP-treated hens. Reported scores represented an average of the scores of three observers. The mean symptom scores noted on Day 21 after the initial dose were used to calculate a TOCP equivalent as required by the military specification. This calculation is as follows.

$$\text{TOCP Equivalent (\%)} = \frac{\text{mg/kg TOCP}}{\text{mg/kg Test Material}} \times \frac{\text{Total Score for Test Material} \times 100}{\text{Total Score for TOCP}}$$

Hens that died during the 30-day study were examined for gross pathology at death. All surviving chickens were sacrificed upon completion of the observation period. The entire brain, spinal cord, and both sciatic nerves (with attached gastrocnemius muscles), were collected for histopathologic examination. Histologic sections were prepared of the medulla, cerebellum, optic lobes, and frontal cortex of the brain; cervical, thoracic, and lumbosacral segments of the spinal cord; the proximal, middle, and distal thirds of one sciatic nerve; the entire gastrocnemius, and any observed gross legions. Duplicate sciatic nerve and spinal cord sections from at least three hens per treatment group were stained with Bodian's stain to demonstrate cytoplasm in neuron cell bodies and processes, and Luxol Fast Blue to demonstrate myelin.

NTE Assay

Additional hens (four per group) were added, as well as an additional treatment level (1000 mg/kg) to be used in the NTE assays. Doses were administered in the same manner as that described for the acute, delayed neurotoxicity hens. Twenty-four hours following the fifth treatment all hens were euthanatized and the brain of each removed for NTE assay. The dosing was as follows.

Jet Engine Oil Samples	Groups of four hens each were treated with 1000, 420, 360, 300, or 240 mg/kg/day for five days.
TOCP-Positive Controls	Groups of four hens each were treated with 90, 75, or 60 mg/kg/day for five days.
Corn Oil	Four hens were given the maximum total volume of fluid equal to that given test animals (i.e., 1.0 mL/kg).

Statistical Analysis

Body weight plus or minus standard error of the mean (SEM) was calculated using a two-factorial analysis of variance. Body weights were compared using the Ryan-Einot-Gabriel-Welsch Multiple F-test (SAS Institute, Inc., 1985). Fischer's Exact test and the Yates' Corrected Chi-Square test were used to compare histopathologic lesions (Zar, 1974). Severity of lesions were compared using ANOVA and the Scheffe Multiple Comparison test (Zar, 1974).

SECTION 3

EXPERIMENTAL RESULTS

The NTE assay was performed by the Mobil Oil Corporation laboratories under an agreement with NMRI/TD. Results of that assay are unavailable and will not be included in this report.

Mean body weights of the hens during the course of the study are listed in **Table 2**. All test groups, including the corn oil controls, maintained body weight (± 5%) throughout the study. Only the TOCP-treated hen groups showed a decrease in mean body weights throughout the study. Mean body weights of the two higher TOCP-treatment groups were different statistically at three weeks ($p<0.05$) while all were different ($p<0.05$) at four weeks.

Neurotoxic signs were observed in all of the hens that received TOCP. Neither of the two jet engine oil test groups nor the corn oil control groups showed signs of acute, delayed neurotoxicity. One test hen (8323-1), and one TOCP-dosed hen died during the observation period; however, neither animal showed neurotoxic symptoms prior to death.

Gross observations at necropsy showed six of the twelve hens exposed to TOCP at doses from 60 to 90 mg/kg had grossly reduced skeletal muscle mass. Among hens dosed with 9082-1 oil, two had cystic ovarian lesions, one had 0.5 x 0.2 x 0.2-cm white nodules in the oviduct mesentery, and one had a 0.6-cm diameter ulcer in the midline breast skin. One hen that was dosed with 360 mg/kg/day of 8323-1 oil had a pale liver. Pale kidneys were observed in two TOCP-dosed hens. A hen that was dosed with TOCP at 90 mg/kg/day and died spontaneously on Day 9 had atrophic myocardial and mesenteric fat and numerous ova in the abdominal cavity. A hen that was dosed with 8323-1 oil at 300 mg/kg/day had multiple yellow-to-tan, firm nodules, 0.2 to 1.0-cm diameter in the liver; dark red-to-purple lung parenchyma; a firm tan mass with adhesions to the liver, ovary and proventriculus; multiple firm, white-to-tan 0.1 to 0.3-cm diameter ovarian nodules; and pale kidneys.

The histopathologic diagnoses for each tissue alteration and their corresponding incidences have been listed in **Table 3**. The average severity scores of neural lesions are listed in **Table 4**. Of the two hens that died spontaneously, the one that received 300 mg/kg/day of oil 8323-1 had a cholangiocarcinoma that was a primary tumor of the gall bladder and metastatic to the liver, an ovarian papillary adenocarcinoma, and chronic active peritonitis. These findings correlated to the gross findings. The sciatic nerve of this hen had isolated segmental demyelination that involved a single node of the nerve fiber. Histologic findings in the 90 mg/kg/day TOCP hen that died spontaneously were limited to isolated segmental demyelination of the sciatic nerve that was minimally severe.

The incidence data indicate the frequent occurrence of demyelination of the sciatic nerve in all experimental animal groups, even the controls. The average severity score for demyelination never exceeded 2.0 (slight) for any dose group. Although the incidence data indicate more frequent occurrence of axonal degeneration in the sciatic nerve of TOCP-dosed hens than in other dose groups, the difference in incidence was not statistically significant. The statistical analyses did not disclose intergroup differences in the occurrence of any histopathologic lesions on the basis of compound or dose level comparisons.

SECTION 4

DISCUSSION

The lesions observed in this study that may occur as a morphologic manifestation of OPIDN include demyelination, axonal degeneration, myofiber degeneration, and myositis. These lesions also may occur as minimally to mildly severe background lesions. Lesions seen in controls and most hens dosed with the two jet engine oils were minimally severe, and were only slightly more severe in TOCP-dosed hens. Consequently, most of the lesions observed, including neoplasms and inflammatory changes, have been considered background lesions. Clearly, the statistical analyses of histopathologic lesion incidence data or neural lesion average severity data did not reveal significant intergroup differences. The occurrence of background neural lesions in mature hens has been documented (Bickford and Sprague, 1982). Weakly pathogenic persistent viral infections may account for the background neural lesions. The frequent findings of lymphocytic inflammatory changes in tissues of hens used in this study suggested a persistent viral infection. Despite the lack of more marked neural lesions, or statistically significant dose group differences, the clinical signs merit consideration in the final judgment of the the OPIDN potential of the chemicals tested. Presently, no definitive quantitative correlation of the severity of neural lesions with the clinical signs of OPIDN is applied in regulatory-type testing of the OPIDN potential of chemicals.

The exact cause of death of the two hens that died spontaneously is unknown. Both the cholangiocarcinoma and peritonitis which occurred in one animal may have contributed to the demise of that animal. Surviving hens in this study, receiving five consecutive oral doses of up to 420 mg test material/kg body weight, remained neurologically asymptomatic throughout the 30-day period.

Under the conditions of this study, neither of the two fluids tested can be considered neurotoxic. If human response to these engine oils parallels that of the hen, no neurotoxic hazard would be expected for military or civilian personnel involved in the manufacture, transportation, or handling of these compounds.

TABLE 2. EFFECTS OF ORAL INTUBATION OF JET OILS ON CHICKEN BODY WEIGHTS (kg)[a]

Treatment Group	Day 0	Day 14	Day 21	Day 28
Corn oil[b]	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
TOCP (mg/kg)				
90	1.6 ± 0.1	1.5 ± 0.2[c]	1.3 ± 0.1[c,d]	1.2 ± 0.1[c,d]
75	1.6 ± 0.1	1.6 ± 0.2	1.4 ± 0.1[d]	1.2 ± 0.2[d]
60	1.8 ± 0.2	1.7 ± <0.1	1.4 ± 0.1	1.3 ± 0.2[d]
9082 (mg/kg)				
420	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
360	1.6 ± 0.1	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
300	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
240	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.2
8323 (mg/kg)				
420	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.2
360	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
300	1.6 ± 0.1	1.8 ± <0.1	1.7 ± 0.1[c]	1.7 ± 0.1[c]
240	1.6 ± 0.1	1.6 ± 0.2	1.5 ± 0.2	1.4 ± 0.2

[a]Mean ± SEM, N = 4.
[b]N = 12.
[c]N = 3.
[d]Significantly different than control at $p < 0.05$, using a two-factorial analysis of variance.

TABLE 3. HISTOPATHOLOGY INCIDENCE SUMMARY

Material:	9082-1				8323-1				TOCP			Corn Oil
Dose (mg/kg)	420	360	300	240	420	360	300	240	90	75	60	1 mL/kg
Animals on Study:	4	4	4	4	4	4	4	4	4	4	4	12
Animals Necropsied:	4	4	4	4	4	4	4	4	4	4	4	12
Brain[a]	4	4	4	4	4	4	4	4	4	4	4	11
Lymphocytic perivasculitis	2[b]	1	2	2	2	2	1	2	2	1	0	3
Lymphocytic leptomeningitis	0	0	0	0	0	0	0	0	0	0	0	1
Gliosis	1	0	1	1	1	1	0	2	1	0	0	4
Mineralization, leptomeningeal vein	0	0	0	0	0	0	0	0	0	1	0	0
Cervical spinal cord	4	4	4	3	4	4	2	4	3	4	4	12
Lymphocytic perivasculitis	1	0	0	0	0	0	0	0	0	0	0	1
Demyelination	0	0	0	0	0	0	0	0	1	1	1	0
Axonal degeneration	0	0	0	0	0	0	1	0	1	1	1	0
Thoracic spinal cord	4	4	4	3	4	4	3	4	3	4	4	12
Axonal degeneration	0	0	0	0	1	0	0	1	0	0	0	0
Lymphocytic perivasculitis	0	0	0	0	0	0	0	0	0	0	0	1
Gliosis	0	0	0	0	1	0	0	0	0	0	0	0
Perikaryal eosinophilic cytoplasmic granules	0	0	0	0	0	0	1	0	0	0	0	0
Lumbosacral spinal cord	4	4	4	4	4	4	3	4	3	4	4	12
Lymphocytic perivasculitis	0	0	0	0	1	0	0	0	0	0	0	1
Gliosis	1	0	0	0	0	0	0	0	0	0	1	0
Lymphocytic leptomeningitis	0	0	0	0	0	0	0	1	0	0	0	0
Axonal degeneration	0	0	0	0	0	0	0	0	0	0	0	0
Perikaryal eosinophilic cytoplasmic granules	0	0	0	2	0	1	1	2	0	1	0	2
Sciatic nerve	4	4	4	4	4	4	3	4	3	4	4	12
Lymphocytic perineuritis	1	1	0	1	1	1	0	1	1	3	1	2
Demyelination	4	3	4	4	3	4	3	4	3	4	3	11
Inflammation, interstitial	1	0	0	2	1	1	0	2	1	2	1	2
Lymphocytic perivasculitis	0	0	1	1	2	0	0	1	0	0	1	0
Axonal degeneration	0	0	0	0	1	1	0	0	2	2	0	0
Schwann cell hyperplasia	0	0	0	1	1	0	1	1	0	1	0	0
Lymphocytic medial arteriolitis	0	0	0	1	0	0	0	0	0	0	0	0

[a]The number of animals in which the organ was examined appears in the row of data for each organ.
[b]The number of animals with each lesion is in the corresponding space for each lesion and treatment group.

TABLE 3. (CONT.) HISTOPATHOLOGY INCIDENCE SUMMARY

Material:	9082-1				8323-1				TOCP			Corn Oil
Dose (mg/kg)	420	360	300	240	420	360	300	240	90	75	60	1 mL/kg
Animals on Study:	4	4	4	4	4	4	4	4	4	4	4	12
Animals Necropsied:	4	4	4	4	4	4	4	4	4	4	4	12
Gastrocnemius muscle[a]	4	4	4	4	4	4	2	4	3	4	4	12
Subacute myositis	0[b]	0	0	0	0	0	0	0	0	0	2	1
Inflammation, interstitial	2	1	0	3	4	0	1	0	0	1	0	0
Myofiber degeneration	0	0	0	1	0	0	0	0	0	0	0	0
Liver	1	2	1	2	2	1	1	1	2	2	1	5
Lymphocytic aggregate, periportal	1	0	0	0	0	1	0	0	1	0	0	0
Kidney	0	2	1	0	2	1	1	1	1	2	1	1
Lymphocytic interstitial nephritis	0	0	0	0	0	0	0	0	0	1	1	0
Lymphocytic ureteritis	0	0	0	0	0	0	0	0	0	0	1	0
Ovary	2	4	3	3	4	4	2	4	3	4	4	8
Ovarian cyst	0	1	0	0	0	0	0	0	0	0	0	0
Oviduct	2	3	3	3	4	4	2	4	3	4	4	8
Chronic active salpingitis	0	0	0	1	0	0	0	0	0	0	0	0
Lymphocytic salpingitis	0	1	0	0	0	0	0	0	0	0	0	0
Skin	2	3	3	2	4	3	1	2	2	3	1	5
Fibrosarcoma	0	0	1	0	0	0	0	0	0	0	0	0

[a]The number of animals in which the organ was examined appears in the row of data for each organ.
[b]The number of animals with each lesion is in the corresponding space for each lesion and treatment group.

TABLE 4. NEURAL HISTOPATHOLOGIC LESIONS AVERAGE SEVERITY SCORES

Material:	9082-1				6323-1				TOCP			Corn Oil
Dose (mg/kg)	420	360	300	240	420	360	300	240	90	75	60	1 mL/kg
Animals on Study:	4	4	4	4	4	4	4	4	4	4	4	12
Animals Necropsied:	4	4	4	4	4	4	4	4	4	4	4	12
Brain[a]	4	4	4	4	4	4	4	4	4	4	4	11
Lymphocytic perivasculitis	.5[a]	.3	.5	1	.5	.5	.5	.5	.7	.3	0	.3
Lymphocytic leptomeningitis	0	0	0	0	0	0	0	0	0	0	0	.1
Gliosis	.5	0	.3	.3	.3	.3	0	.8	.3	0	0	.4
Mineralization, leptomeningeal vein	0	0	0	0	0	0	0	0	0	.3	0	0
Cervical spinal cord												
Lymphocytic perivasculitis	.3	0	0	0	0	0	0	0	0	0	0	.1
Demyelination	0	0	0	0	0	0	0	0	.7	.3	.3	0
Axonal degeneration	0	0	0	0	0	0	.5	0	.7	.3	.3	0
Thoracic spinal cord												
Axonal degeneration	0	0	0	0	.3	0	0	.3	0	0	0	0
Lymphocytic perivasculitis	0	0	0	0	0	0	0	0	0	0	0	0
Gliosis	0	0	0	0	.3	0	0	0	0	0	0	0
Perikaryal eosinophilic cytoplasmic granules	0	0	0	0	0	0	.3	0	0	0	0	0
Lumbosacral spinal cord												
Lymphocytic perivasculitis	0	0	0	0	.3	0	0	0	0	0	0	.1
Gliosis	.3	0	0	0	0	0	0	0	0	0	.3	0
Lymphocytic leptomeningitis	0	0	0	0	0	0	0	.3	0	0	0	0
Axonal degeneration	0	0	0	0	0	0	0	0	0	0	0	0
Perikaryal eosinophilic cytoplasmic granules	0	0	0	.5	0	.3	.3	5	0	.3	0	.2
Sciatic nerve												
Lymphocytic perineuritis	3	5	0	.8	3	3	0	.3	.3	.8	.5	.2
Demyelination	1 3	8	1	1	.8	1.3	1.3	1 7	1 8	.8	.5	.2
Inflammation, interstitial	.3	0	0	.8	.3	.3	0	.5	.3	.5	.3	.2
Lymphocytic perivasculitis	0	0	.3	.8	.5	0	0	.3	0	0	.5	0
Axonal degeneration	0	0	0	0	.5	.3	0	0	1	.8	0	0
Schwann cell hyperplasia	0	0	0	.5	3	0	1	.3	0	.3	0	0
Lymphocytic medial arteriolitis	0	0	0	5	0	0	0	0	C	0	0	0

[a]The average serverity score of each lesion is in the corresponding space for each lesion and treatment group. The scoring codes for assessing lesion severity were progressively:

1 - minimal, 2 - slight (mild), 3 - moderate, 4 - significant, and 5 - severe.

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APPENDIX A

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF CORN OIL

Animal Number	Dose (mL/kg)	21-Day Mean[a] Observation Score	Total Score Per Group	TOCP Equivalent
07030002	1	0		
07030006	1	0		
07030013	1	0		
07030028	1	0		
07030037	1	0	0	<1
07030044	1	0		
07030056	1	0		
07030069	1	0		
07030072	1	0		
07030100	1	0		
07030114	1	0		
07030124	1	0		

[a]Mean score of three observers.

APPENDIX B

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF CORN OIL

Animal Number	Dose (mL/kg)	21-Day Mean[a] Observation Score	Total Score Per Group
07030057	90	8	
07030060	90	[b]	24
07030026	90	8	
07030095	90	8	
07030120	75	8	
07030020	75	8	32
07030101	75	8	
07030045	75	8	
07030008	60	8	
07030024	60	8	32
07030086	60	8	
07030018	60	8	

[a]Mean score of three observers.
[b]Animal died of extraneous causes; no neurotoxic signs observed prior to death.

APPENDIX C

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF 9082-1

Animal Number	Dose (mL/kg)	21-Day Mean[a] Observation Score	Total Score Per Group	TOCP Equivalent
07030007	420	0		
07030017	420	0	0	<1
07030053	420	0		
07030088	420	0		
07030030	360	0		
07030068	360	0	0	<1
07030115	360	0		
07030121	360	0		
07030029	300	0		
07030041	300	0	0	<1
07030052	300	0		
07030119	300	0		
07030016	240	0		
07030055	240	0	0	<1
07030090	240	0		
07030105	240	0		

[a]Mean score of three observers.

APPENDIX D

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF 8323-1

Animal Number	Dose (mL/kg)	21-Day Mean[a] Observation Score	Total Score Per Group	TOCP Equivalent
07030042	420	0		
07030063	420	0	0	<1
07030064	420	0		
07030078	420	0		
07030014	360	0		
07030049	360	0	0	<1
07030061	360	0		
07030081	360	0		
07030035	300	0		
07030065	300	0	0	<1
07030103	300	0		
07030047	300	[b]		
07030011	240	0		
07030022	240	0	0	<1
07030038	240	0		
07030094	240	0		

[a]Mean score of three observers.
[b]Animal died of extraneous causes; no neurotoxic signs observed prior to death.

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QUALITY ASSURANCE

The study, "The Acute Delayed Neurotoxicity Evaluation of Two Jet Engine Oil Formulations," was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of these guidelines. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
June 19, 1989	Animal weighing and initial dosing.
June 28, 1989	Second observation and scoring session.
July 10, 1989	Animal weighing and seventh observation and scoring session.
July 21, 1989	Animal sacrifice.
January 10-12, 1990	Final report and data audit.

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report.

M G Schneider Jr

M. G. Schneider, Jr.
QA Coordinator
Toxic Hazards Research Unit

Date 12 Jan 90