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Reproductive Tract Lesions Resulting from Subchronic Administration (63 Days) of Tri-*o*-cresyl Phosphate in Male Rats

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Reproductive Tract Lesions Resulting from Subchronic Administration (63 Days) of Tri-*o*-cresyl Phosphate in Male Rats. SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV, AND ABOU-DONIA, M. B. (1987). *Toxicol. Appl. Pharmacol.* **89**, 49–63. An initial dose–range pilot study where animals were gavaged with between 100 and 1600 mg tri-*o*-cresyl phosphate (TOCP)/kg/day for 14 days resulted in decreased epididymal sperm density and disruption of the seminiferous epithelium in 100% of treated animals. A subchronic 63-day study (reflecting the 49-day length of the rat seminiferous epithelium cycle plus the 14-day transit time of spermatids through the epididymis was initiated. Dose-dependent (10 to 100 mg TOCP/kg/day) decreases in cauda epididymal sperm motility and density, testicular enzyme activities, and alterations in sperm morphology were observed. Concurrent pair-fed controls (matched to the highest dose group, 100 mg TOCP/kg/day) indicated that weight loss resulting from TOCP administration had minimal contributory effects to the testicular toxicity seen. Plasma α-tocopherol acetate (vitamin E) and testosterone concentrations were unaffected. Tri-*p*-cresyl phosphate (TPCP), the nonneurotoxic structural analog of TOCP, produced no toxic effects, demonstrating the necessity of the *ortho*-cresol moiety for induction of damage. A minimum effective (threshold) dose for observable testicular toxicity was determined to be 10–25 mg TOCP/kg in this study. These data suggest that TOCP interferes with spermatogenic processes and sperm motility directly and not via an androgenic mechanism or decreased vitamin E availability.

Tri-*o*-cresyl phosphate (TOCP) is an industrial chemical used as a plasticizer in lacquers and varnishes and as a fire retardant in high-speed brake lubricants (Windholz *et al.*, 1983). Numerous studies have documented the adverse neurological effects that accompany TOCP exposure (Smith *et al.*, 1930; Smith and Lillie, 1931; Barnes and Denz, 1953; Cavanagh, 1954, 1964; Abou-Donia, 1981; Abou-Donia *et al.*, 1986). While investigating the neurotoxic effects of TOCP in roosters, we noted the presence of testicular pathology (Somkuti *et al.*, 1987a). A 14-day pilot study established that the male Fisher 344 rat was also sensitive to reproductive tract toxicity following TOCP oral administration.

Esterases have been shown to be sensitive to inhibition by organophosphorus compounds (Abou-Donia, 1985). Acetylcholinesterase (AChE) and butyrylcholinesterase

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TABLE 1

SUMMARY OF 14-DAY TOCP DOSE-RANGE FINDING PILOT STUDY IN F344 RATS

	Control	Treatment mg TOCP/kg/day 100	200	400	800	1600
Number of surviving animals[a]	10/10	10/10	4/10	3/10	0/10	1/10[b]
Av body weight gain/loss over 14 days[c]	+24.4 ±2.8[d]	+14.2 ±4.2	−23.5 ±9.0	−52.0 ±9.0	N/A[e]	N/A
Clinical signs of cholinergic toxicity[f]	—	—	+	+	+	+
Sperm number/mg cauda (×1000)	63.4 ±5.0	30.5 ±3.1	25.2 ±2.1	12.6 ±2.3	N/A	N/A
Presence of testicular histopathology	—	+	+	+	N/A	+
% Testis of total body weight	0.57 ± 0.01	0.58 ± 0.02	0.62 ± 0.04	0.45 ± 0.05	N/A	N/A

[a] Animals died within the first 5 days of treatment due to acute anti-cholinergic toxicity of TOCP.

[b] A single animal survived 4 days of treatment (100% mortality in the other nine), after which dosing was discontinued and the animal was perfused for histopathological examination.

[c] Grams.

[d] ±SE.

[e] N/A, not applicable.

[f] Eye bulb protrusion, excess salivation, tremors, ocular discharge, diarrhea.

(BuChE) activities have purportedly been identified in mouse and rat Sertoli cells and spermatozoa (Chakraborty and Nelson, 1974, 1976). Neurotoxic esterase (NTE) activity, reported by Lotti *et al.* (1985) as present in rooster testis, was found in the Fischer 344 rat testis (Somkuti, unpublished). NTE has been suggested to be a target esterase for delayed neuropathic compounds (Johnson, 1982). A cholinergic component of sperm motility has also been suggested (Harbison *et al.*, 1976; McGrady and Nelson, 1976) and changes in sperm morphology have been positively associated with mutagenic and antifertility effects (Soares *et al.*, 1979; Wyrobek and Bruce, 1975).

Testosterone, responsible for maintenance of accessory sex glands and normal progression of spermatogenesis, was measured in testicular interstitial fluid. Because testosterone is secreted by Leydig cells into this extratubular fluid, interstitial fluid testosterone is more sensitive than plasma testosterone for determining available testosterone concentrations (Sharpe and Cooper, 1983). Testicular atrophy has been shown to be produced by vitamin E deficiency (Mason, 1933). TOCP was reported to decrease dietary vitamin E ab-

TABLE 2

AVERAGE FOOD CONSUMPTION/PER WEEK AND TOTAL BODY WEIGHT GAIN OVER 63 DAYS[a]

Treatment Group	Av food consumed/ week/animal (g ± SD)	Total weight gain (g ± SD)
Control	132 ± 17	112 ± 35
Corn oil control	126 ± 10	122 ± 32
10 mg TOCP/kg/day	126 ± 6	122 ± 14
25 mg TOCP/kg/day	128 ± 7	113 ± 20
50 mg TOCP/kg/day	122 ± 8	96 ± 21
75 mg TOCP/kg/day	118 ± 12	78 ± 18
100 mg TOCP/kg/day	114 ± 16	62 ± 27
Pair-fed control	114 ± 16	67 ± 9
100 mg TPCP/kg/day	132 ± 4	145 ± 17

[a] Animals were treated daily with varying concentrations of TOCP (n = 10 animals/group). Food consumption in pair-fed and control animals was monitored daily; all others were monitored weekly.

TABLE 3

BODY AND TESTIS WEIGHTS (WITH TESTIS WEIGHT AS PERCENTAGE OF TOTAL BODY WEIGHT) IN RATS GIVEN DAILY ORAL DOSES OF TOCP[a]

Treatment group[b]	Av body wt[c] (g)	Av testis wt[c] (g)	Testis/body wt (%)	Percentage of control
Control	327 ± 4[d]	1.43 ± 0.02	0.438 ± 0.005	100.0
Corn oil control	304 ± 6	1.35 ± 0.04	0.444 ± 0.001	101.4
10 mg TOCP/kg/day	317 ± 13	1.33 ± 0.05	0.419 ± 0.001	95.7
25 mg TOCP/kg/day	318 ± 10	1.40 ± 0.11	0.439 ± 0.003	100.2
50 mg TOCP/kg/day	293 ± 6	1.13 ± 0.08	0.380 ± 0.003	86.8
75 mg TOCP/kg/day	281 ± 9	0.94 ± 0.17	0.333 ± 0.006*	76.0
100 mg TOCP/kg/day	272 ± 12	0.73 ± 0.11	0.264 ± 0.003*	60.3
Pair-fed control	256 ± 5	0.86 ± 0.09	0.334 ± 0.004*	76.3
100 mg TPCP/kg/day	334 ± 4	1.48 ± 0.02	0.442 ± 0.001	100.9

[a] Animals were killed 63 days after treatment.
[b] $n = 5$ animals/group; animals were dosed for 63 consecutive days.
[c] Weight recorded at end of experiment.
[d] ±SE.
* Significantly different from control ($p < 0.05$).

sorption from the gastrointestinal tract (Meyers *et al.*, 1953; Meyers and Mulder, 1953).

An investigation was initiated to characterize the biochemical and morphologic effects of oral TOCP administration on the male rat reproductive system. We examined the effect of TOCP on testicular esterase activities (AChE, BuChE, NTE) and assessed spermatid count, motility, and morphology in cauda epididymides. Testis weight was used as a general indicator of germ cell loss. Both plasma and interstitial fluid testosterone concentrations were measured, along with plasma levels of α-tocopherol.

MATERIALS AND METHODS

Chemicals

Tri-*o*-cresyl phosphate (TOCP, 99%) and tri-*p*-cresyl phosphate (TPCP, 97%) were purchased from Eastman

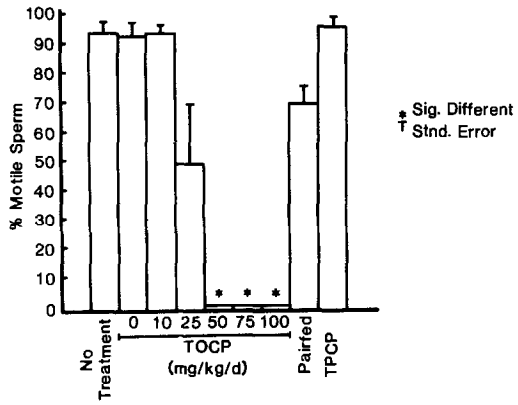

FIG. 1. Subchronic study (63 day). Cauda epididymal sperm motility from various control, TOCP- and TPCP-treated animals.

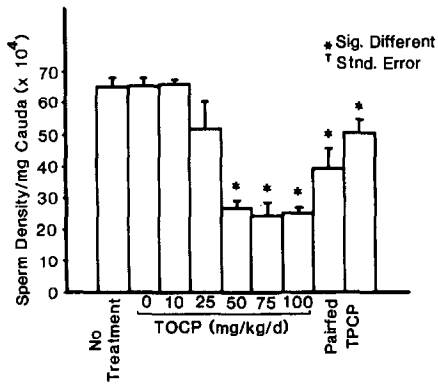

FIG. 2. Subchronic study (63 day). Sperm density/mg cauda ($\times 10^4$) epididymis.

TABLE 4

SPERM MORPHOLOGY ANALYSIS

Treatment group[a]	% Abnormal sperm (±SE)	% Abnormal sperm by type	
		No hook	Amorphous
Control	0.19 ± 0.09[b]	100.0	0
Corn oil control	0.17 ± 0.08	100.0	0
10 mg TOCP/kg/day	0.25 ± 0.13	100.0	0
25 mg TOCP/kg/day	1.20 ± 0.31	84.2	15.8
50 mg TOCP/kg/day	33.80 ± 2.38[c]	60.7	39.3
75 mg TOCP/kg/day	38.71 ± 5.82[c]	68.4	31.6
100 mg TOCP/kg/day	45.64 ± 4.12[c]	67.0	33.0
Pair-fed control	0.88 ± 0.23	100.0	0
100 mg TPCP/kg/day	0.21 ± 0.08	100.0	0

[a] n = 5 animals/group; animals were dosed daily for 63 days.
[b] Values are the mean number abnormal observed in 200 sperm counted by two independent observers.
[c] Mostly headless sperm observed.

Kodak Co. (Rochester, NY). *O,O*-Diethyl-*O*-4-nitrophenyl phosphate (paraoxon) was obtained from Sigma Chemical Co. (St. Louis, MO), and *N,N'*-diisopropylphosphorodiamidic fluoride (mipafox) was synthesized by the Midwestern Research Institute (Kansas City, MO). The following enzyme substrates were purchased from Sigma Chemical Co.: acetylthiocholine iodide, butyrylthiocholine iodide, and 1-naphthyl acetate. 4-Nitrophenyl valerate was synthesized by Dr. A. Nomeir.

Animals

Fischer 344 male rats (190–210 g, 10–11 weeks old) were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). All animals were housed two per cage in a 21–23° C controlled environment with a 12-hr light cycle (on 0600 until 1800) and provided with NIH-07 open formula diet feed pellets (Purina, St. Louis) and tap water *ad libitum*. Animals were allowed to acclimatize for 1 week prior to experimentation.

Experimental Design

14-Day study. This pilot experiment was designed to establish the dose range necessary for induction of testicular toxicity and for setting the upper dose for the 63-day subchronic exposure study. Animals (n = 8/group) received either 100, 200, 400, 800, or 1600 mg TOCP/kg body wt orally by gavage suspended in corn oil as vehicle for a period of 14 days. A corn oil-treated group served as vehicle control. Two animals from each group were selected and prepared for histopathological examination. Sperm density per milligram cauda was assessed in the remaining animals.

63-Day study. This dose–response study was designed to determine the minimal effective dose of TOCP. Animals were dosed daily for 63 days, which represented the cycle of the seminiferous epithelium in the rat (49 days) plus epididymal transit time (14 days). Groups of 10 animals each received 10, 25, 50, 75, or 100 mg TOCP/kg/day. An additional group of 10 rats was treated with 100 mg TPCP/kg/day. Control groups (n = 10/group) consisted of either untreated or vehicle (corn-oil)-treated animals. From each group, 5 animals were perfused for histopathologic examination, while tissues from the remaining five were used in the various assays described below. Treatment-related effects due to weight loss were established by inclusion of a pair-fed group (n = 10 rats) whose dietary intake was matched on a daily basis to the previous day's consumption of the 100 mg TOCP/kg experimental animals. All animals were housed 2 per cage with the exception of the singly housed pair-fed and 100 mg TOCP/kg groups. Animals were observed daily for neurological dysfunction and signs of acute cholinergic toxicity (excess salivation, tremors, diarrhea, ocular discharge). Body weights of all animals were monitored weekly. Dose schedules were adjusted to reflect changes in body weight. Food consumption was determined weekly in the non-pair-fed groups.

Enzyme Assays

After 63 days of treatment, the rats were sacrificed by decapitation. Trunk blood was collected, and the testes were quickly removed and held at 4°C until assayed. Tes-

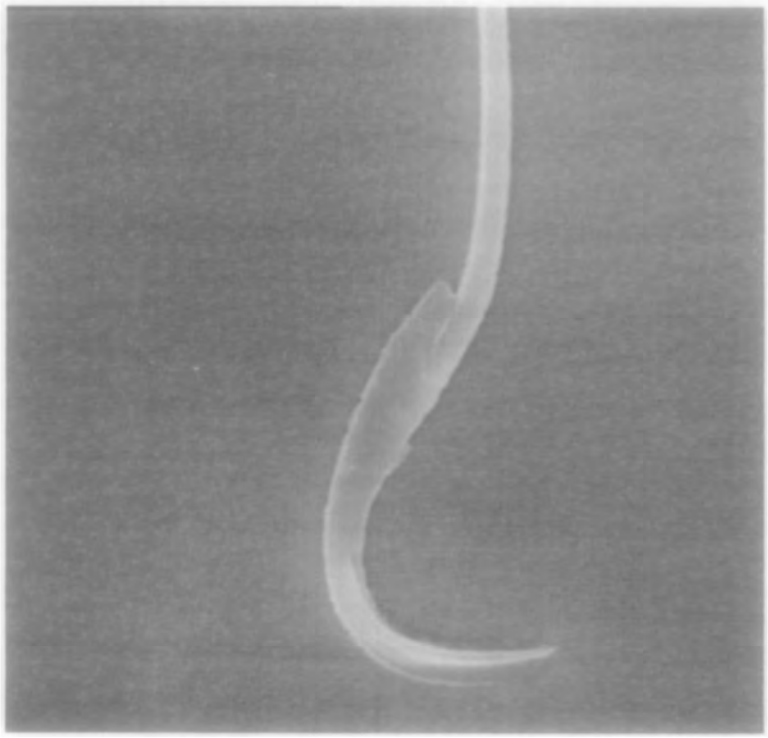

FIG. 3. Control SEM of spermatozoan displaying normal head morphology (2900×).

tis-nonspecific esterase (NSE) and presence of AChE were assayed as previously described (Lapadula *et al.*, 1984). NTE was determined by the method of Johnson (1977). Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Evaluation of Sperm Motility, Morphology, and Density

Cauda epididymal sperm samples were used to assess motility. A small sample was expressed into egg yolk buffer at 37°C (Turner and Giles, 1981). A sample of this suspension was placed on a 37°C slide and motility was assessed on a warmed microscope stage by two independent observers who categorized all sperm in 10 fields (40× magnification) as either motile or nonmotile. Motility was determined within 5 min of death.

A weighed section of right cauda epididymis (ca. 25 mg) was minced in 2 ml of pH 7.4 Na-phosphate-buffered saline (PBS) and the cells were dispersed by repeated pipeting. One milliliter of this suspension was stained with 1% eosin Y for 45 min, dried, and evaluated for morphology. Intact sperm were categorized as either normal, no hook, excessive hook, amorphous, pin-head, two heads/two tails, or short sperm head (Wyrobek and Bruce, 1975). A 0.5-ml vol of the remaining suspension was diluted to 2 ml with PBS and heated to kill the sperm, and the cells were counted using a calibrated cell counter (Elzone Model 80 XY, Particle Data, Elmhurst, IL). Sperm size was analyzed by the Elzone 80 XY and calibrated with latex beads supplied by Particle Data laboratories. The data are expressed as size distribution histograms.

Interstitial Fluid Testosterone Determinations

Interstitial testicular fluid was collected as follows: Immediately after removal of the testis, the caudal end of the testicular capsule was incised carefully and the testis was placed upright in an 83 × 13-mm polystyrene tube such that the testis was suspended 1–2 cm above the tube bottom. Fluid was then allowed to percolate out of the testis into the tube bottom for the next 16–20 hr at 4°C (Sharpe and Cooper, 1983). The testis was then removed and the tubes were centrifuged for 5 min at 1000*g* to pellet any contaminating erythrocytes. The remaining fluid was diluted with 10 vol of 0.01 M phosphate-buffered saline (pH 7.4) containing 0.2% bovine serum albumin and stored at −20°C until assayed by radioimmunoassay (Leeco Diagnostics Corp.). Inter- and intraassay variations were 5.4 and 1.5%, respectively.

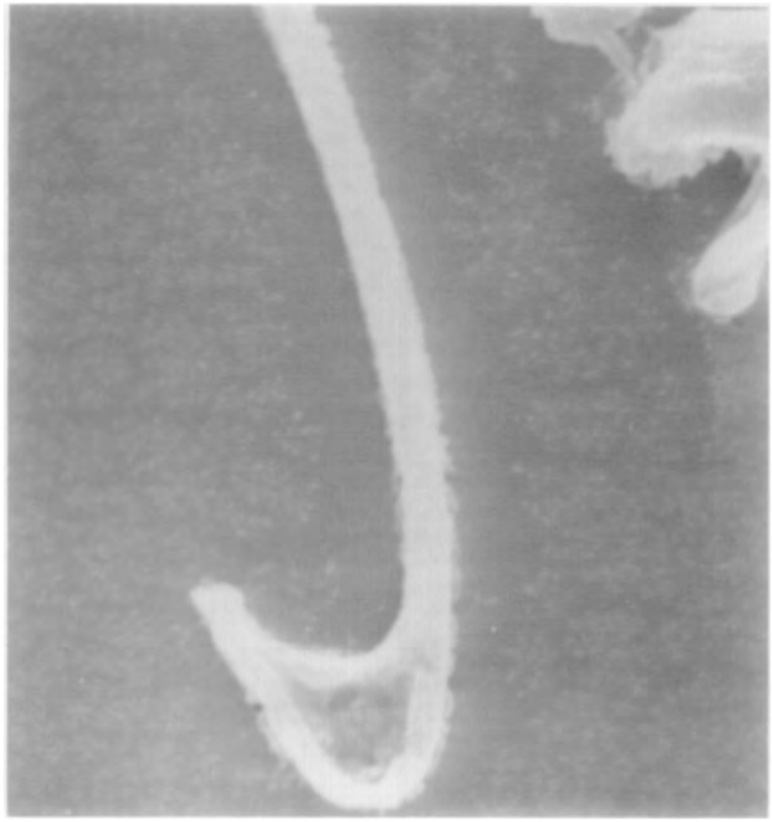

FIG. 4. High power SEM showing detail of typical amorphous head (4300×).

Plasma Vitamin E Determination

Plasma was extracted directly by the method of Howell and Wang (1982). *n*-Hexane (500 μl) was added to a mixture of 200 μl of ethanol (95%) and 200 μl of plasma and mixed for 30 sec. Then 400 μl of the hexane extract was removed and evaporated under nitrogen to dryness. Methanol (150 μl) was added and vortexed for 20 sec. A 100-μl aliquot was injected into a high-performance liquid chromatograph (Glenco System I, Houston, TX) equipped with a Whatman 10-μm 250 × 4.6-mm reversed-phase column and absorbance was recorded at 292 nm.

Histopathology

Upon conclusion of treatments, five animals per group were anesthetized with 50 mg of sodium pentobarbital and perfused through the ascending aorta with warmed (38°C) Ringer's balanced salts containing 0.1% procaine HCl and 0.1% sodium nitroprusside (to facilitate vasodilation) for approximately 1 min. Tissues were fixed by *in situ* perfusion (ca. 3–4 min) with Karnovsky's fixative (Karnovsky, 1965). Testes and epididymides were excised and stored for 24 hr in Karnovsky's fixative and then transferred (to minimize shrinkage-induced artifact) to 0.1 M sodium phosphate, pH 7.4, until embedding. Sections of testis and epididymis were dehydrated through graded ethanols (50 to 95%), embedded in 2-hydroxyethyl methacrylate (Dupont–Sorvall), and sectioned on a Leitz 1512 microtome set at 3 μm. Cut sections were stained with periodic acid and Schiff's stain (PAS) to allow visualization of the stages of spermatogenesis classified according to Leblond and Clermont (1952). Longitudinal sections of epididymides were embedded and stained as above.

Scanning Electron Microscopy

Cauda epididymal sperm (20-μl aliquot) were immediately fixed with 2.5% glutaraldehyde and 0.5% tannic acid in 0.1 M phosphate buffer, pH 7.4, at 4°C for 1 hr.

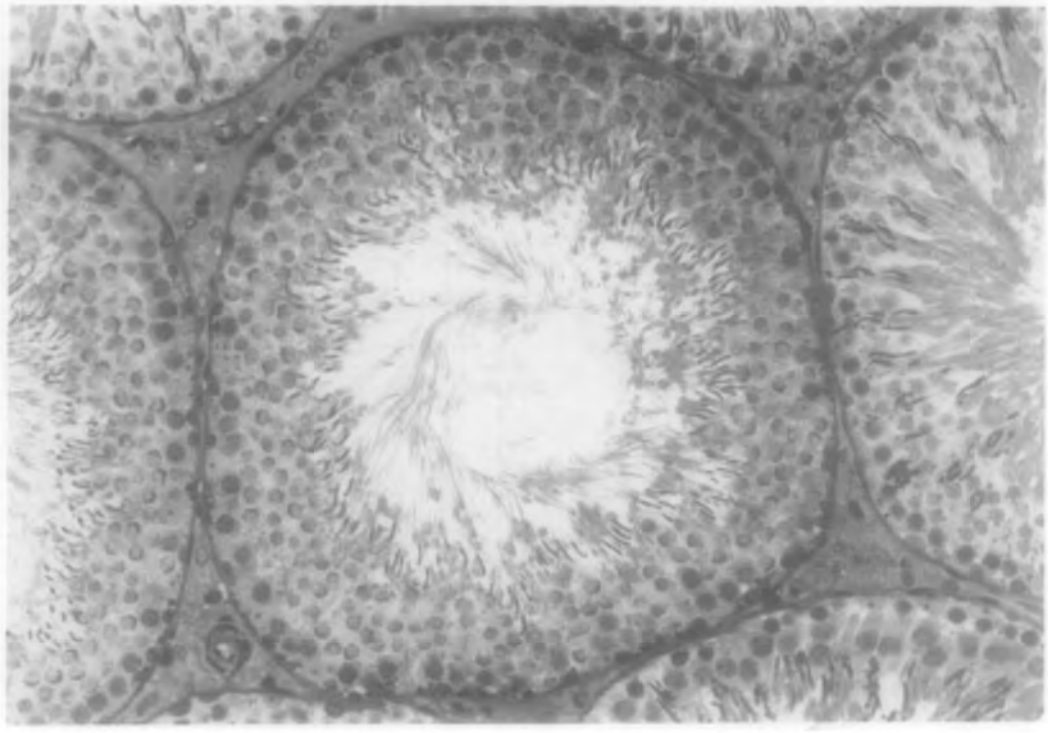

FIG. 5. Control testis cross-section showing no sign of overt pathology (300×).

The samples were rinsed in the buffer and postfixed in 2% osmium tetroxide in the phosphate buffer for 2 hr (Lamb *et al.*, 1978). Following dehydration with ethanol, the samples were dried, sputter-coated with gold palladium, and then observed on a JEOL JSM-35U scanning electron microscope.

Statistics

Significance of the difference between control and treated animals was assessed by a one-way ANOVA and two-tailed Student *t* test. A *p* value of less than 0.05 was considered significant.

RESULTS

14-Day Study

A data summary of the dose–range pilot study is presented in Table 1. Administration of greater than 400 mg TOCP/kg resulted in near 100% animal mortality within 48 hr. The 200 and 400 mg/kg groups suffered 60 and 70% mortality, respectively (between Days 3–5), while all animals in the 100 mg/kg treatment group survived. Every animal treated with greater than 200 mg TOCP/kg exhibited signs of acute cholinergic toxicity (severity increasing and time to onset decreasing with greater doses). The 100 mg/kg treated animals did not exhibit any such signs.

Cross-sections of testes and epididymides from control animals revealed normal seminiferous tubule cytoarchitecture, germ cell associations, and high density of spermatozoa. Epididymal sperm density, body weight, and seminiferous tubule histology of surviving treated animals were all adversely affected by TOCP. There was a dose-dependent decrease in apparent sperm density per milligram cauda. Seminiferous tubule degeneration and necrosis were observed in all treated

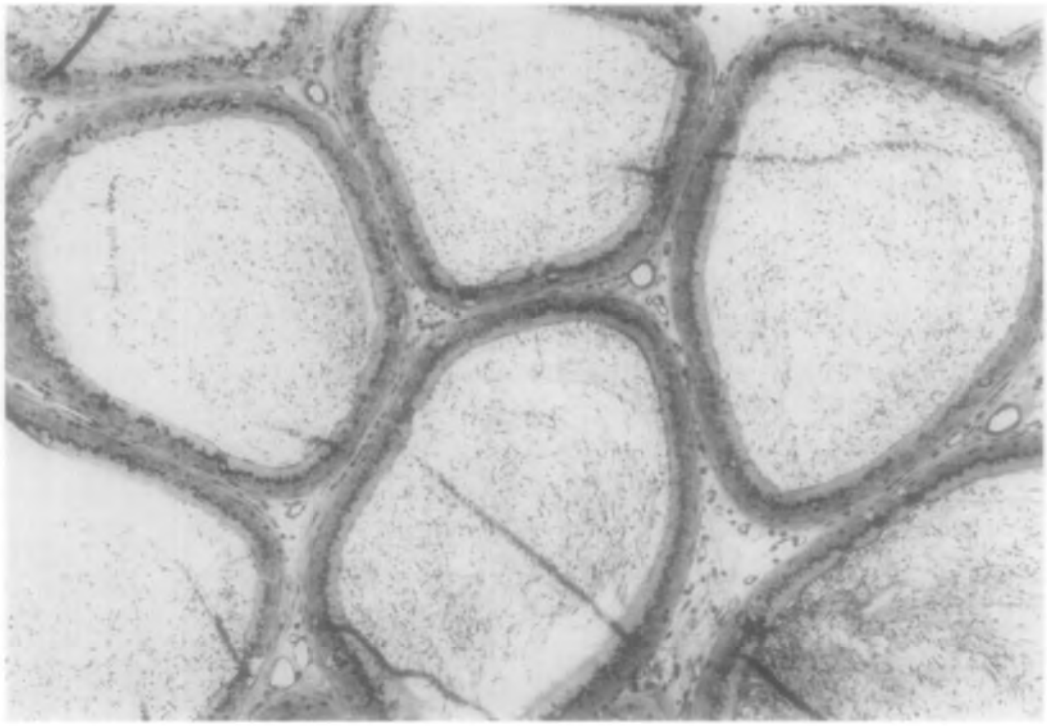

FIG. 6. Control epididymis cross-section presenting with high spermatozoa density (145×).

animals; the severity of the lesions was positively correlated to the increasing dose administered.

63-Day Study

Body and organ weights. A treatment-related decrease in weight gain was seen in the 50, 75, and 100 mg TOCP/kg groups although the average food consumption per animal remained relatively constant throughout the study for all groups (Table 2). No animals died during the study.

Testis to total body weight ratios decreased in the three highest treatment groups (Table 3). This ratio was also decreased in pair-fed animals; however, the decrease was not as great as that associated with the 100 mg TOCP/kg treated animals.

Sperm measures. Daily administration of TOCP resulted in a dose-dependent decrease in sperm motility, with the three higher (50, 75, and 100 mg TOCP/kg) dose groups having no observable motile sperm (Fig. 1). Indeed, some rats in the 75 and 100 mg/kg groups (two of five and three of five, respectively) lacked any epididymal sperm at all. Corn oil and TPCP administration did not affect sperm motility at the doses used. Pair-fed animals had a lower percentage of motile sperm than controls; however, it was significantly higher than the three highest treatment groups. Likewise, cauda epididymal sperm density was decreased by TOCP in a dose-dependent manner (Fig. 2). Cauda epididymal fluid analysis revealed skewed distribution in sperm size of treated animals when compared to controls (data not shown). Light microscopic examination of sperm revealed significant increases in the numbers of abnormal spermatozoa in TOCP-treated animals. The morphologic abnormalities, observed in the 50, 75, and 100 mg TOCP/kg groups,

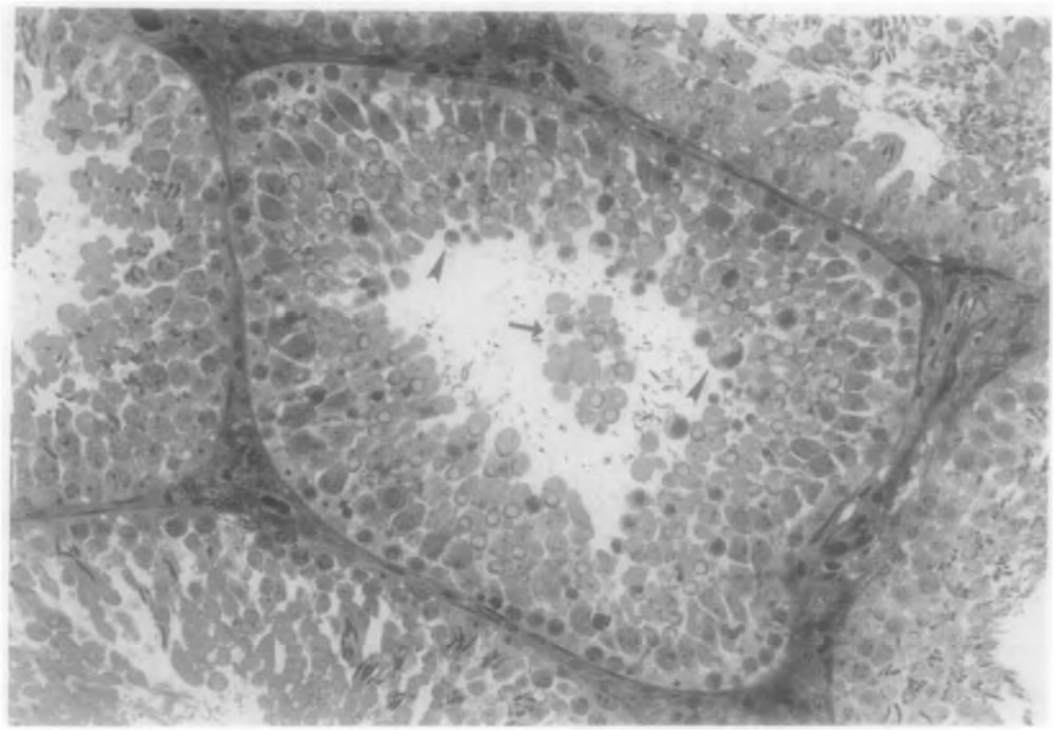

FIG. 7. Testis from animal treated with 25 mg TOCP/kg/day for 63 days. There is intratubular debris (arrow) and many PAS-positive droplets near the edge of the lumen (arrowheads) (300×).

consisted of headless sperm, spermatozoa with no hook, and amorphous heads (Table 4). Scanning electron micrographs of control and amorphous sperm from 100 mg/kg treated animals are shown in Figs. 3 and 4. TPCP treatment resulted in lower cauda epididymal sperm density; however, no abnormal sperm were noted in morphological evaluations.

Histology. Testicular cross-sections from control animals (both untreated and corn oil vehicle controls) contained characteristic developing germ cells associations defining normal epithelial morphology (Fig. 5). The epididymal tubules were full of sperm (Fig. 6). Testicular morphology from animals treated with 10 mg TOCP/kg/day was indistinguishable from controls. The first observable changes at the light microscopic level were seen in three of the five rats in the 25 mg TOCP/kg/day group (Fig. 7). The normal array of germ cells appeared disorganized and there were many PAS-positive "droplets" within the tubule lumena. Pathology was consistently present in all animals of 50, 75, and 100 mg TOCP/kg groups. The presence of immature germ cells, PAS-positive staining bodies, and multinucleated giant cells was also noted within the tubule lumina (Fig. 8). Decreases in germ cell numbers were observed in the 75 and 100 mg/kg animals (Fig. 9). The epididymis likewise showed a marked decrease in sperm concentration; epididymis from 100 mg/kg treated animals were devoid of any sperm (Fig. 10).

Esterase activities/testosterone/vitamin E. Specific activities of esterases known to be sensitive to delayed neurotoxicants are shown in Table 5. While enzyme activities from animals in the corn oil, pair-fed, and TPCP groups were affected only slightly or not at all, TOCP treatment decreased the spe-

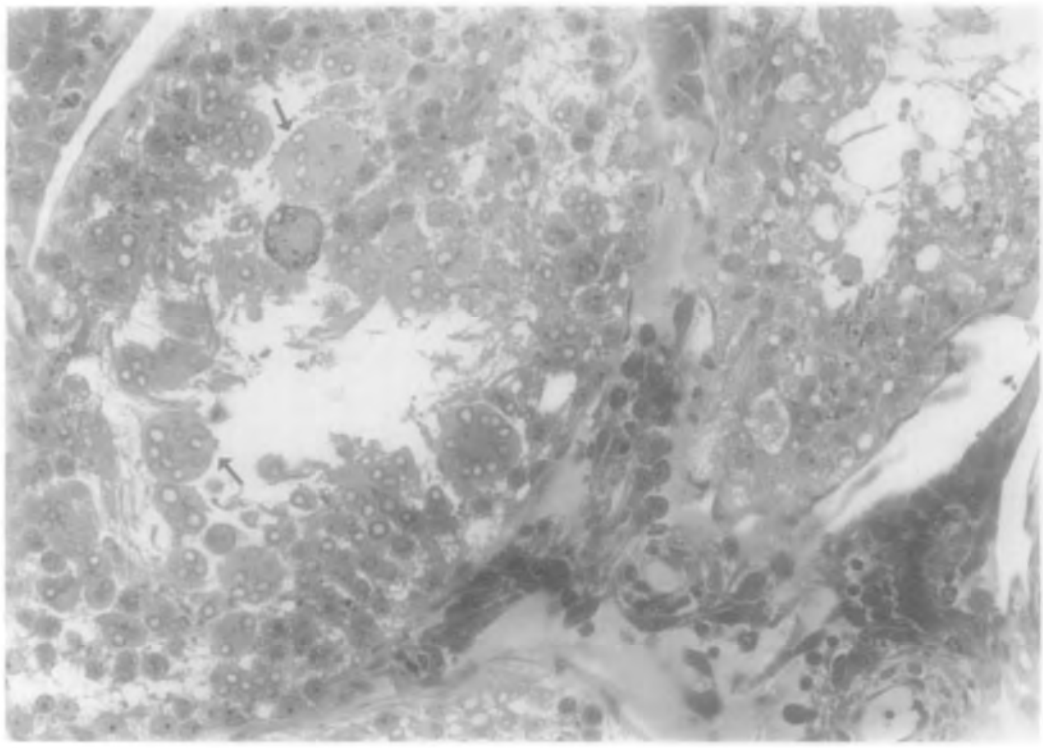

FIG. 8. Testis cross-section from animal receiving 75 mg TOCP/kg/day for 63 days. There is a dramatic loss of normal seminiferous tubule germ cell associations and a large number of multinucleated giant cells evident (arrows) (300×).

cific activities of NSE and NTE in a dose-dependent manner. AChE assays performed on testicular homogenates yielded no assayable activity. There was no significant change in testosterone among TOCP-treated animals. There was, however, a slight decrease (25% lower than control) seen in the TPCP-treated rats. Plasma vitamin E assayed in the highest dose treatment animals (0.87 μg/ml $\pm$ 0.07 standard error; $n = 5$) revealed no significant changes from control values (0.96 μg/ml $\pm$ 0.06; $n = 10$).

DISCUSSION

In the pilot study, rats tolerated no greater than 100 mg TOCP/kg/day without substantial mortality. Epididymal spermatid data and epididymal and testicular histopathology showed that the male Fischer 344 rat was sensitive to reproductive tract toxicity following TOCP.

The administration of TOCP for 63 days established 25 mg/kg as the lowest dose which produced testicular toxicity and 10 mg/kg as a no-observable effect dose under these conditions. The toxicity was expressed as an increase in abnormal sperm forms in the epididymis, as well as the presence of numerous hematoxylin-positive bodies (residual bodies) within and near the seminiferous tubular lumen; this was first seen in the 25 mg/kg treated animals. Residual bodies are thought to be the cytoplasm shed by developing stage 19 spermatids (Russell, 1979) and normally become phagocytosed by the Sertoli cell. Several investigators have suggested that Sertoli cells resorb these residual bodies

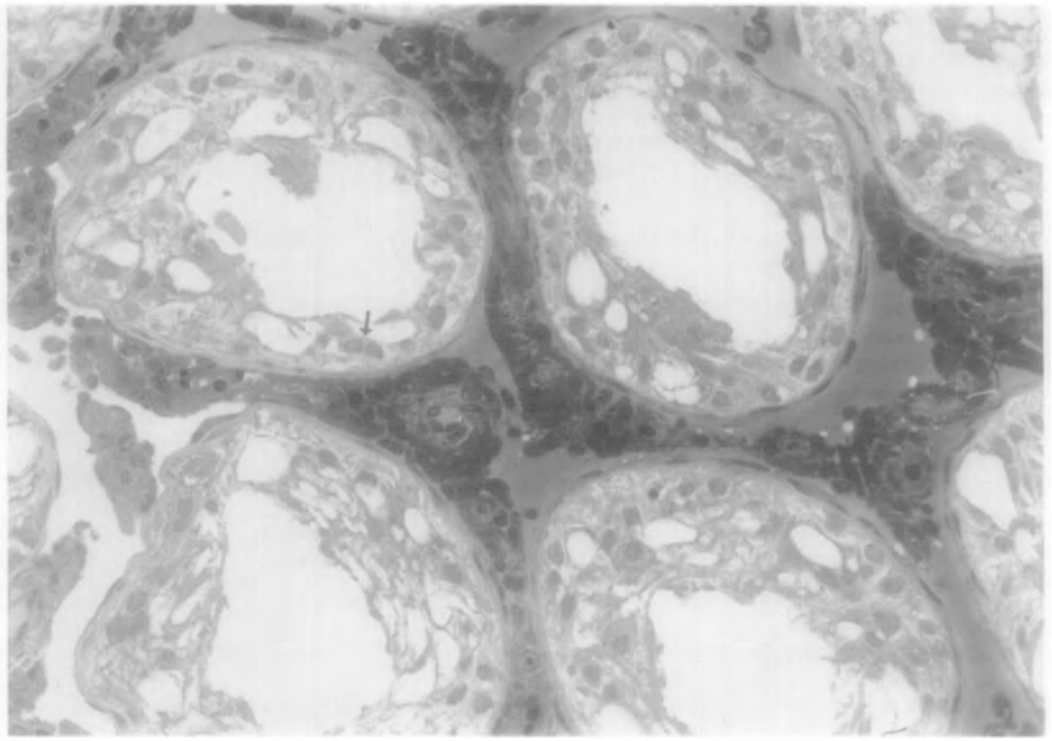

FIG. 9. Severe loss of germ cells associated with spermatogenesis (100 mg TOCP/kg/day). Only outlines of Sertoli cell nuclei are apparent (arrow) (300×).

cast off by spermatids (Dietert, 1966; Nicander, 1967). The abnormal presence of such residual bodies suggests the Sertoli cell capacity to ingest them may be affected. TPCP, control, and pair-fed controls did not exhibit any such lesions.

Weight loss and prolonged restricted food intake have been correlated with atrophy of the testis (Mason, 1933). The pair-fed animals in these studies were matched to the highest treatment group (100 mg TOCP/kg) to evaluate the effects of weight loss in this group. The pair-fed animals gained an average of 67 g over the duration of the 63 day study, versus the 112 g per animal in the controls. No testicular lesions were observed in pair-fed animals, indicating that weight-gain depression alone was not responsible for the testicular pathology observed in the TOCP-treated animals. In addition, all enzyme parameters were normal in the pair-fed rats. The sperm motility and density measures in the pair-fed animals were lower (25 and 40%, respectively) than those in controls (both untreated and corn oil treated). However, the 50, 75, and 100 mg TOCP/kg groups all had 0% motile sperm and much lower sperm density (59, 63, and 62% of control, respectively). Thus, although weight-gain depression may have contributed to the effects on epididymal spermatid number and motility, it did not have a role in TOCP-induced changes in testicular histology.

Disruption of normal androgen status can produce a characteristic testicular lesion, in which some spermatogonia, spermatocytes, and cap phase spermatids are visibly necrotic (Russell *et al.*, 1981). TOCP did not affect testicular testosterone concentrations in our experiment, supporting the hypothesis that Sertoli cells are the target for TOCP toxic effects. TPCP-treated rats had 25% lower testoster-

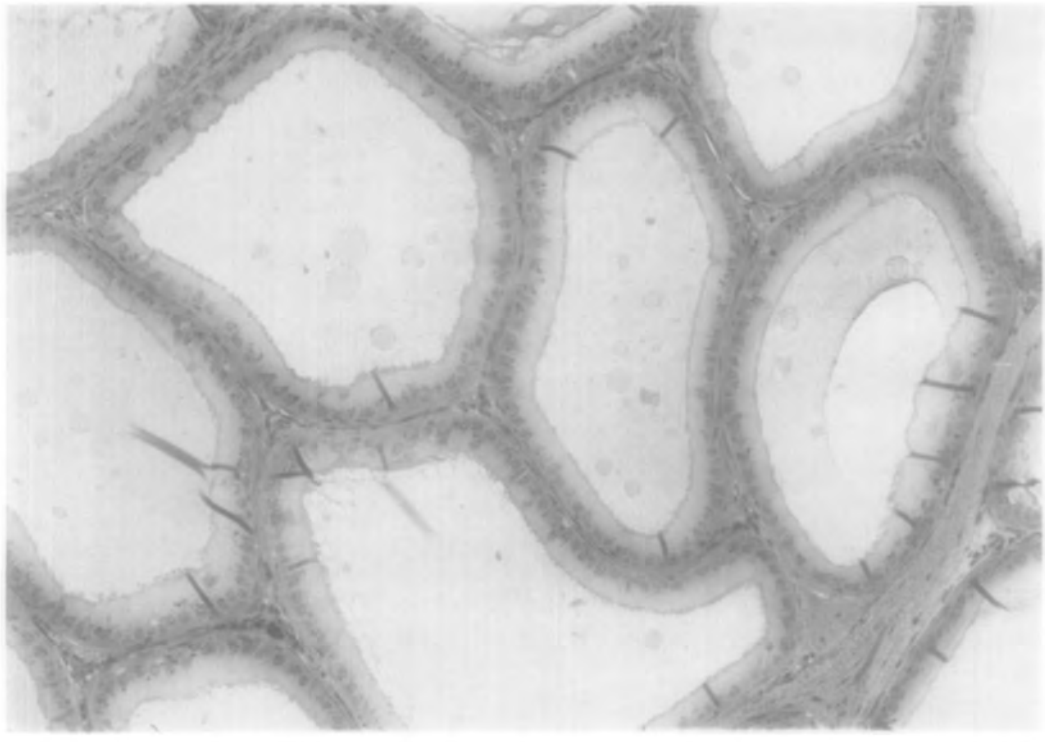

FIG. 10. Epididymis from 100 mg TOCP/kg/day treated animal lacking any sperm (145×).

one values. Despite this decrease, they exhibited normal testicular morphology, demonstrating that this magnitude of depression of testosterone at least is ineffective at producing pathology.

TOCP treatment is reported to decrease plasma vitamin E (Meyers and Mulder, 1953; Meyers *et al.*, 1953); however, no such decrease was observed in our study. Meyers *et al.* administered in excess of 10 times the present dose of TOCP. α-Tocopherol supplemented into the diet was found in much lower plasma concentrations of TOCP-treated animals than in controls. The lack of an effect on circulating vitamin E levels in our study excludes this as a cause for the reproductive toxicity of TOCP at the doses used.

The TOCP-related increases in the percentage of abnormal sperm and decreases in motility and sperm density in the epididymis are consistent with earlier observations in roosters (Somkuti *et al.*, 1987a). The skew to the left seen in the size distribution histograms corresponded to tailless heads as confirmed in previous analyses (Lamb and Dutton, unpublished observations).

Acetyl- and butyryl (nonspecific)-cholinesterase activities have been histochemically localized in mice and rats to Sertoli cells, spermatids, and spermatozoa (Chakraborty and Nelson, 1974, 1976). Specifically, the majority of activity in fixed testes appeared to be within the Sertoli cell smooth endoplasmic reticulum, at the Sertoli–spermatid junctional interface, and within residual bodies. However, testis AChE assays suggested that this enzyme is not present in testis homogenates of F344 rats (Somkuti, unpublished observations). Recently, Davenport and Heindel (1986) have used binding studies to demonstrate the presence of acetylcholine receptors in Sertoli cell cultures; they have yet to establish the presence of acetylcholinester-

TABLE 5

TESTIS: NONSPECIFIC AND NEUROTOXIC ESTERASE-SPECIFIC ACTIVITIES

Treatment group[a]	Nonspecific esterase activity		Neurotoxic esterase activity	
	nm/mg/min	Percentage of control	nm/mg/min	Percentage of control
Control	612.4 ± 16.2[b]	100.0	3.05 ± 0.36	100.0
Corn oil control	558.4 ± 21.6	91.2	3.04 ± 0.32	99.7
10 mg TOCP/kg/day	405.1 ± 29.7*	66.1	2.79 ± 0.27	91.5
25 mg TOCP/kg/day	334.2 ± 13.9*	54.6	2.49 ± 0.23	81.6
50 mg TOCP/kg/day	301.8 ± 11.0*	49.3	1.32 ± 0.21*	43.3
75 mg TOCP/kg/day	304.3 ± 27.7*	49.7	1.40 ± 0.11*	45.9
100 mg TOCP/kg/day	261.6 ± 9.4*	42.7	0.84 ± 0.13*	27.5
Pair-fed control	394.2 ± 15.5*	64.4	3.27 ± 0.42	107.2
100 mg TPCP/kg/day	532.3 ± 13.5	86.9	3.23 ± 0.10	104.9

[a] $n = 5$ animals/group; animals were dosed daily for 63 days.

[b] ±SE.

* Significantly different from control ($p < 0.05$).

ase. Our findings of a 57% inhibition of testis NSE by TOCP occurred at a dose (100 mg/kg) that increased numbers of residual bodies and vacuolations of the seminiferous epithelium. NTE, also inhibited, may prove a useful marker for exposure of testes to neurotoxic compounds.

The appearance of the AChE and NSE enzyme in the plasma membrane of early spermatids may eventually contribute to the generation of impulses responsible for the slow undulatory movements seen in epididymal spermatids (Chakraborty and Nelson, 1974). With further development of the spermatid, the increased amount of the enzyme may underlie the rhythmic beat. However, the mechanisms supporting sperm motility remain uncertain. Previous reports by Harbison *et al.* (1976) proposed that the decrease of sperm motility seen after the organophosphate compound trimethylphosphate (TMP) was due to spermatozoa choline acetyltransferase inhibition. More recent reports from Goodman *et al.* (1984) have noted that carnitine acetyltransferase activity was responsible for maintenance of sperm motility. They showed evidence that estimates of spermatozoan choline acetyltransferase actually measured carnitine acetyltransferase activity. The presence of a true choline acetyltransferase has yet to be established in mammalian sperm (Goodman and Harbison, 1981). It appears that much work remains to be done in regards to defining the mechanisms responsible for sperm motility and the events surrounding toxic compound-induced changes.

TOCP in chicken brain alters protein phosphorylation patterns of axonal microtubule and cytoskeletal elements (Patton *et al.*, 1983). It may be that the mechanisms causing the TOCP-induced testicular histopathology observed in the current study are related to the mechanisms inducing nervous system damage following TOCP. Data in the literature suggest that many delayed neurotoxicants are able to induce structural neuronal damage (Seifert and Casida, 1982), as opposed to acute neurotoxicants that are limited to more transient effects. This relationship is being investigated in ongoing studies.

The data presented in this study demonstrate toxic effects of TOCP on the male rat reproductive tract using biochemical, morphologic, and epididymal spermatid endpoints. The time course of development of

these effects are examined in the following paper (Somkuti *et al.*, 1987b).

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