TOXICOLOGY AND APPLIED PHARMACOLOGY 89, 64–72 (1987)

Time Course of the Tri-*o*-cresyl Phosphate-Induced Testicular Lesion in F-344 Rats: Enzymatic, Hormonal, and Sperm Parameter Studies

STEPHEN G. SOMKUTI, DANIEL M. LAPADULA, ROBERT E. CHAPIN,[1] JAMES C. LAMB IV,[2] AND MOHAMED B. ABOU-DONIA[3]

Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

Received September 8, 1986; accepted January 19, 1987

Time Course of the Tri-*o*-cresyl Phosphate-Induced Testicular Lesion in F-344 Rats: Enzymatic, Hormonal, and Sperm Parameter Studies. SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV, AND ABOU-DONIA, M. B. (1987). *Toxicol. Appl. Pharmacol.* **89**, 64–72. Tri-*o*-cresyl phosphate (TOCP), a known neurotoxic compound, causes testicular toxicity in both leghorn roosters and Fischer 344 rats. The present study was initiated to follow the onset of the testicular lesion through possible changes in sperm numbers and production, serum hormones, and various enzyme activities. Rats were administered TOCP daily (150 mg/kg) for periods of 3, 7, 10, 14, or 21 days. Vehicle-treated animals served as controls. Sperm motility and sperm number per milligram cauda epididymis were both lower in treated animals by Day 10. Testicular weight to body weight ratio was significantly decreased only in the longest treatment duration animals (21 days). Testicular neurotoxic esterase and nonspecific esterase activities were also inhibited, while β-glucuronidase activity was not affected. Luteinizing and follicle-stimulating hormone levels were normal, as were both serum and interstitial fluid testosterone concentrations. Sertoli cell fluid secretion, as measured by testis weight increase after efferent duct ligation, showed no significant changes. Other organs (spleen, liver, kidney, pancreas, small intestine, adrenal and pituitary glands) had no overt signs of pathology as observed by light microscopy in animals treated for 21 days. A separate group of animals was treated for 21 days and subsequently examined after 98 days of observation (two cycles of the rat seminiferous epithelium). No recovery of spermatogenesis was seen, indicating that the toxicity was irreversible at the dose used. The effects noted in these studies further define the testicular lesion produced by TOCP and show that 150 mg/kg/day for 21 days produced irreversible testicular toxicity.

Morphological characterization of the testicular lesion induced by the organophosphate compound tri-*o*-cresyl phosphate (TOCP), at both the light and the electron microscope level, suggested that Sertoli cells and late stage spermatids are initially affected (Somkuti *et al.*, 1986b). Although most of the previous studies involving TOCP have concentrated on the hazards of occupational exposures in regard to its delayed neurotoxic properties (limb paralysis; see Abou-Donia, 1981 for review), we have recently shown TOCP to cause testicular toxicity in leghorn roosters and Fischer 344 rats (Somkuti *et al.*, 1986a–c, 1987a,b). Daily administration of TOCP for a period of 63 days to Fischer 344 rats resulted in seminiferous tubule histopathology

[1] Present address: Fertility and Reproduction Group, National Toxicology Program, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

[2] Present address: United States Environmental Protection Agency, Office of Pesticides and Toxic Substances, Mail Drop TS-788, 401 M Street SW Washington, DC 20460.

[3] To whom correspondence should be addressed at Department of Pharmacology, P.O. Box 3813, Duke University Medical Center, Durham, NC 27710.

0041-008X/87 $3.00

and decreases in sperm numbers and concentrations/cauda epididymides (Somkuti *et al.*, 1986c, 1987a,b).

In this study, the aim was to further characterize the spectrum of toxicity seen after TOCP administration. A preliminary study established 150 mg/kg TOCP as the maximum dose causing testicular toxicity without mortality. The endpoints used for this were sperm motility and number in cauda epididymides, testicular enzyme activities, and serum hormone levels (testosterone, luteinizing hormone and follicle-stimulating hormone), with increasing duration of daily TOCP treatment in Fischer 344 rats. A separate experiment investigated the reversibility of the lesion, wherein animals were allowed to recover for 98 days (two cycles of the seminiferous epithelium) following 21 daily doses of TOCP. A better understanding of the cell types affected and changes occurring in the testis resulting from TOCP exposure may lead to insight regarding the mechanism(s) responsible for the toxicity observed.

MATERIALS AND METHODS

Chemicals. TOCP (99%) was purchased from Eastman 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, 1-naphthyl acetate, α-naphthol-ASD acetate, Fast Blue BB, and phenolphthalein glucuronide. 4-Nitrophenyl valerate was synthesized by Dr. A. Nomeir.

Animals. Fischer 344 male rats (190–210 g; 10–11 weeks old) were obtained from Harlen Sprague–Dawley (Indianapolis, IN). 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, MO) and tap water *ad libitum.* Animals were handled for 2 min daily during the week prior to TOCP administration to minimize stress-induced hormonal changes during the study.

Experimental design. Animals were randomly assigned to treatment groups consisting of eight rats per group. Animals received 150 mg TOCP/kg in corn oil vehicle via gavage for either 3, 7, 10, 14, or 21 successive days. Treatments were staggered so that all animals were killed on the same day. A span of 2 hr was necessary for the sacrifice which commenced at 8 AM. A separate corn oil-treated group served as vehicle control. Body weights were monitored daily and doses were adjusted accordingly. Daily observations were made to note any clinical signs of acute (tremor, excess salivation, diarrhea) or delayed (ataxia, paralysis) organophosphate toxicity.

A second group of animals (n = 10) was treated for 21 days with 150 mg TOCP/kg/day. Following treatment, two animals were perfused for histopathological examination (see below for method). The remaining eight animals were then allowed to recover for 98 days with no further TOCP treatment and then perfused.

An additional six animals (control or 3 days of 150 mg TOCP/kg; n = 3/group) were used in histochemical localization of nonspecific esterase studies.

Efferent duct ligation was performed (as described below) in a separate group of 32 animals. Rats were gavaged daily with 150 mg TOCP/kg (in corn oil) for 7, 14, or 21 days (n = 8/group). An untreated group served as control.

Enzyme assays. The animals were killed by decapitation 24 hr after the last dose. Immediately following decapitation, brain, plasma (from collected trunk blood), and testes were quickly removed and held at 4°C in appropriate buffers (see below) until assayed. Brain acetylcholinesterase (AChE) and testis-nonspecific esterase (NSE) were measured as previously described (Lapadula *et al.*, 1984). Neurotoxic esterase (NTE) was determined by the method of Johnson (1977). β-Glucuronidase was assayed by the method of Fishman (1974). Proteins were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Evaluation of sperm motility and density. Cauda epididymal sperm samples were used to assess motility (Amann, 1982). A small sample was expressed into egg yolk buffer at 37°C (Turner and Giles, 1981). A sample of this preparation was placed on a 37°C slide and two independent observers assessed motility by counting all sperm in 10 fields (40× magnification); each cell was categorized as either motile or nonmotile. Motility was determined within 5 min after the animal had been killed.

A weighed section of right cauda epididymis (ca. 25 mg) was minced and evenly distributed with a Pasteur pipet in 2 ml of 0.05 M Na-phosphate-buffered saline (PBS), pH 7.4. A 0.5-ml vol of the suspension was diluted to 2 ml with PBS and heated to kill the sperm, and counts were made using a calibrated cell counter (Elzone Model 80 XY, Particle Data, Elmhurst, IL).

Hormone determinations. Trunk blood was centrifuged 5 min at 1000*g* at 4°C. Serum was assayed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone. LH and FSH were measured with radioimmunoassay (RIA) kits kindly provided by

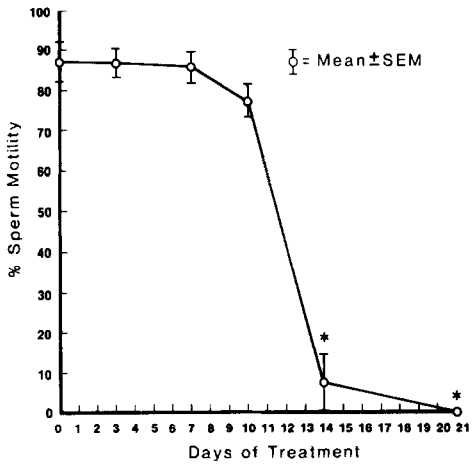

FIG. 1. Time course of sperm motility inhibition. A decrease in cauda epididymal sperm motility was observed following increasing treatment duration with 150 mg TOCP/kg/day (n = 8 animals/time point). *Significantly different from control value.

the NIAMDD, Dr. A. F. Parlow, and Dr. C. M. Kuhn. Testosterone was measured by RIA purchased from Leeco Diagnostics Corp. (Southfield, MI). Interstitial testicular fluid (assayed for testosterone) was collected by the method of Sharpe and Cooper (1983). Intraassay coefficients of variation (CV) for the testosterone, LH, and FSH assays were 1.2, 2.4, and 1.8%, respectively. The interassay CVs were 5.0, 12.3, and 9.8%, respectively.

Histopathology. Animals for histology studies 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 for 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, and sectioned on a Leitz 1512 microtome set at 3 μm. Cut sections were stained with periodic acid and Schiff's stain (PAS), a procedure which facilitated visualization of the stages of spermatogenesis. The stages were classified according to Leblond and Clermont (1952). Longitudinal sections of epididymides were embedded and stained as above.

Sections of spleen, liver, kidney, pancreas, pituitary and adrenal glands, and small intestine were embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined for microscopic abnormalities.

Enzyme histochemistry. The right testis from 3-day TOCP-treated and control animals (n = 3/group) was excised and frozen on dry ice, sectioned (8 μm) in a cryostat, and stained with α-naphthol-ASD acetate as substrate coupled to Fast Blue BB for localization of nonspecific esterase activity (Pearse, 1972).

Efferent duct ligation. After 7, 14, or 21 days of daily TOCP treatment, animals were anesthetized with 25 mg/kg of sodium pentobarbital. A suprapubic incision was then made in the abdomen and the left testis was exposed. A round suture needle and surgical thread were used to make two ligatures around the vasa efferentia at the point where they leave the testis. These were placed as close as possible to the origin of the vasa efferentia. The testis was returned to the abdominal cavity and a wound clip was applied to the incision. Sixteen hours postsurgery, both testes were removed, carefully trimmed of the exterior connecting adipose tissue and epididymis, blotted dry, and weighed (Collins and Tsang, 1979).

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

Clinical Observations

At no point in the study did animals display any signs of delayed neurotoxicity. Acute cholinergic toxicity consisting of lacri-

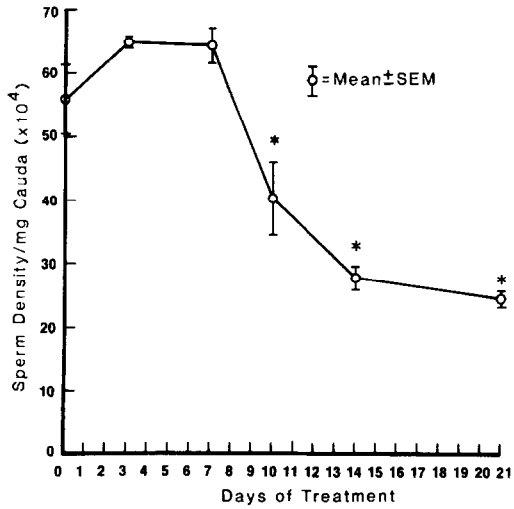

FIG. 2. Time-dependent decrease in sperm density per milligram cauda epididymis following TOCP treatment with 150 mg/kg/day TOCP (n = 8 animals/time point). *Values significantly different from control.

TABLE 1

TESTIS: NONSPECIFIC AND NEUROTOXIC ESTERASE ACTIVITIES[a]

	Nonspecific esterase activity		Neurotoxic esterase activity	
Days of treatment	nm/mg/min	% of control	nm/mg/min	% of control
Control	263.2 ± 4.8[b]	100.0	1.92 ± 0.39	100.0
3	126.3 ± 3.8*	48.0	1.24 ± 0.20*	64.6
7	112.5 ± 6.6*	42.7	0.66 ± 0.13*	34.3
10	131.9 ± 6.2*	50.1	0.47 ± 0.11*	24.5
14	114.3 ± 5.4*	43.4	0.70 ± 0.21*	36.5
21	110.3 ± 4.0*	41.9	0.35 ± 0.15*	18.2

[a] $n = 8$ animals/group.
[b] ±SE.
* Significantly different from control ($p < 0.05$).

mation and diarrhea was seen in 30% of animals during the first 5 days of compound administration.

Sperm Parameter Studies

Daily administration of 150 mg TOCP/kg caused significant reduction of cauda epididymal sperm motility after 14 and 21 days of treatment (Fig. 1). Likewise, sperm number per milligram cauda epididymis was significantly decreased after 10, 14, and 21 days (Fig. 2). The testis to body weight ratio was reduced significantly only in the 21-day group (27% less than control).

Enzyme Activities

Testicular activities of NSE and NTE were significantly inhibited at all time points by TOCP (Table 1). Brain activities of AChE and NTE were also inhibited (Table 2). β-Glucuronidase, a Sertoli cell-specific enzyme activity localized to lysosomal membranes, was not affected by TOCP.

Histochemistry of NSE

NSE activity, as visualized by diazo dye reaction product deposits in frozen testis sections, was present within the seminiferous tu-

TABLE 2

BRAIN: ACETYLCHOLINESTERASE AND NEUROTOXIC ESTERASE ACTIVITIES[a]

	Acetylcholinesterase activity		Neurotoxic esterase activity	
Days of treatment	μm/min/mg	% of control	nm/mg/min	% of control
Control	10.9 ± 1.3[b]	100.0	1.90 ± .18	100.0
3	10.6 ± 1.0	97.2	1.70 ± .17	89.5
7	5.5 ± 1.0*	50.5	0.93 ± .18*	48.9
10	5.8 ± 0.7*	53.2	0.86 ± .12*	45.2
14	7.6 ± 0.6*	69.7	1.11 ± .05*	58.4
21	5.1 ± 1.1*	46.8	0.84 ± .12*	44.2

[a] $n = 8$ animals/group.
[b] ±SE.
* Significantly different from control ($p < 0.05$).

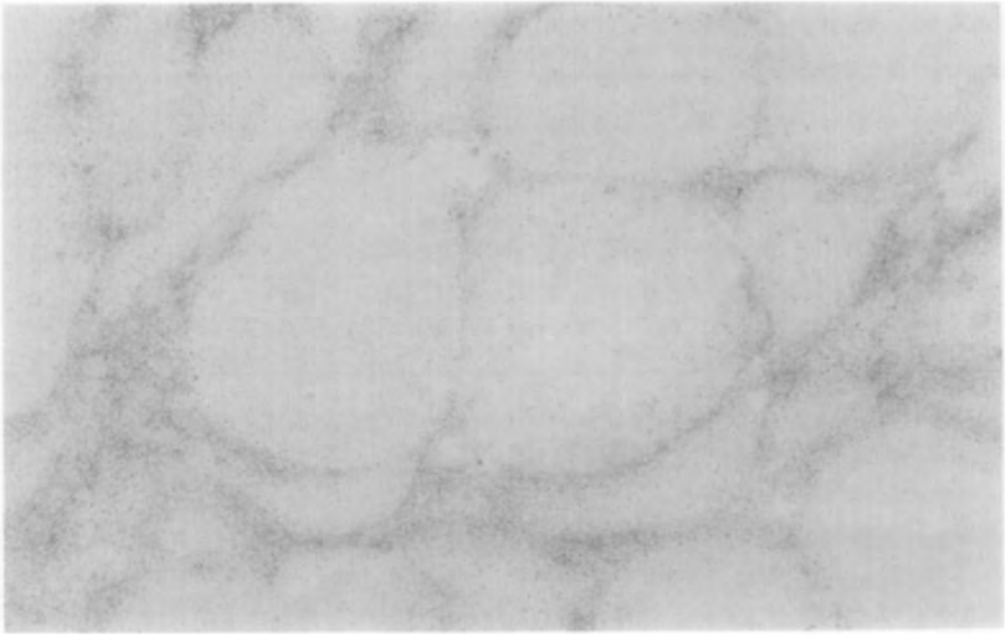

FIG. 3. Nonspecific esterase histochemistry staining pattern from control frozen testis section. Note that most of the dye product is localized to the interstitial spaces surrounding the tubules (270×).

bules, although the majority of activity was localized to the interstitial spaces surrounding the tubules (Fig. 3). Animals treated for 3 days had a marked decrease in NSE activity, as visualized by the sharp reduction in the intensity of the stain reaction product (Fig. 4).

Hormones and Efferent Duct Ligation

There were no decreases in serum levels of LH, FSH, testosterone, or interstitial fluid testosterone when compared to concurrent control values. Despite a wide fluctuation of serum values observed between the various lengths of TOCP treatment in both control and treated populations, the differences between groups at each time point were not statistically significant (with the exception of one LH point; Tables 3 and 4).

A general decreasing trend in ligated testis weight was observed with increasing duration of treatment; however, no significant differences were observed between control and treated animals (data not shown).

Organ Pathology

TOCP did not induce any observable pathology in the spleen, liver, kidney, pancreas, small intestinal wall, and adrenal or pituitary glands.

Recovery Study

At the conclusion of the 98-day recovery period, testes from recovery animals were de-

TABLE 3

SERUM LUTEINIZING HORMONE CONCENTRATIONS

Days	Control (ng/ml)	Treated[a] (ng/ml)
3	20.3 ± 7.6	21.9 ± 6.2
7	18.5 ± 3.8	13.8 ± 7.4
10	33.7 ± 4.9	21.6 ± 6.9
14	53.9 ± 8.8	29.7 ± 7.4
21	55.1 ± 13.7	51.2 ± 8.5

[a] Animals received 150 mg TOCP/kg daily for the indicated times; $n = 8$ animals/group.

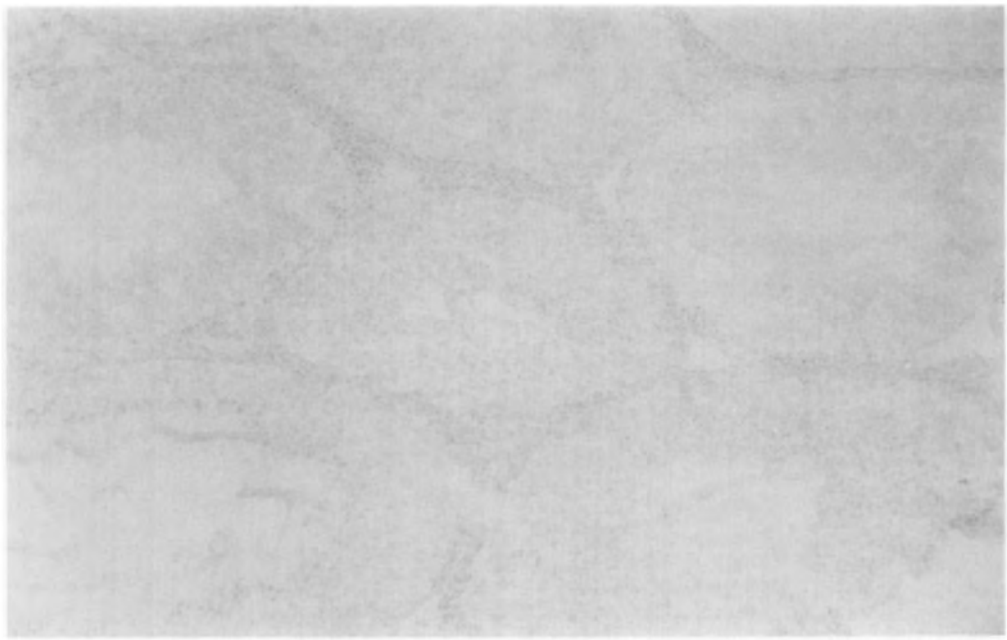

FIG. 4. Nonspecific esterase staining pattern from animal treated 3 days with 150 mg TOCP/kg/day. There is a marked decrease in the deposition of the dye reaction product (270×).

creased in size compared to controls. Testis cross-sections from untreated animals examined under the light microscope revealed the characteristic seminiferous tubule morphology associated with controls (Fig. 5). A complete absence of germinal cells was seen in all treated animals. Only Sertoli cells and Sertoli cell processes were visible intratubularly (Fig. 6). The tubules appeared to have decreased in diameter. Epididymides (both proximal and distal sections) were devoid of any sperm.

TABLE 4

SERUM FOLLICLE-STIMULATING HORMONE CONCENTRATIONS

Days	Control (ng/ml)	Treated[a] (ng/ml)
3	145.6 ± 33.7	188.2 ± 51.3
7	167.3 ± 38.6	161.5 ± 38.4
10	187.7 ± 25.9	200.6 ± 41.6
14	158.3 ± 30.4	128.0 ± 30.8
21	139.6 ± 43.3	162.8 ± 33.7

[a] Animals received 150 mg TOCP/kg daily for the indicated times; $n = 8$ animals/group.

DISCUSSION

The data presented above support the conclusion that TOCP-mediated male reproductive toxicity occurs via a direct mechanism of action on testicular targets.

The lack of observable overt pathology of the spleen, liver, pancreas, lung, kidney, small intestine, and adrenal and pituitary glands after 21 days of treatment suggests that TOCP toxicity is relatively specific for testis in the rat. Circulating concentrations of the pituitary gonadotropins LH and FSH, and the testicular androgen testosterone (in both serum and interstitial fluid), did not vary from control values at all times examined after chemical administration, suggesting that TOCP may act directly on the testis and not through hormonal alterations.

Sertoli cells secrete a number of products, including intratubular fluid (Waites and Gladwell, 1982), and Sharpe and Cooper (1983) have used fluid secretion as a general index of Sertoli cell function. In this study, the weight increase of the efferent duct ligated

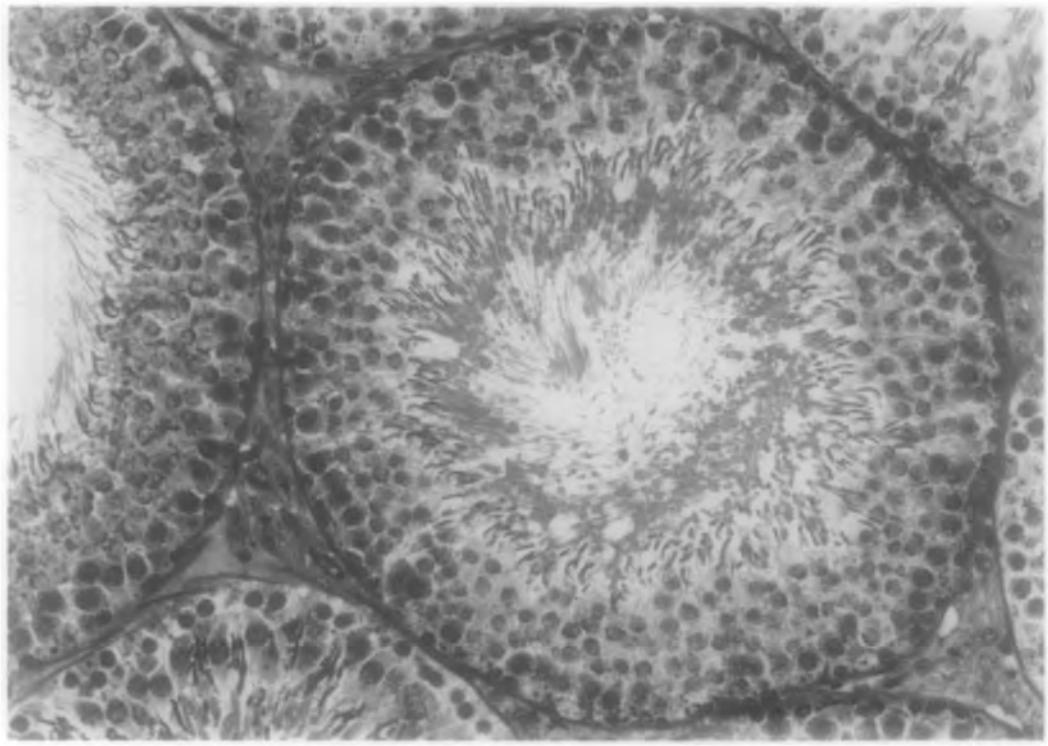

FIG. 5. Control testis revealing the characteristic densely populated germ cell associations of the seminiferous epithelium (290×).

testis (16 hr postsurgery) vs the nonligated contralateral testis was used as a measure of Sertoli cell fluid-secreting ability. That TOCP treatment did not impair the weight increase in the ligated testis may be due in part to total numbers of Sertoli cells damaged (i.e., not enough cells were affected to demonstrate perceptible changes). Alternatively, despite TOCP treatment, Sertoli cells may have retained their secretory ability at the times examined. This observation is in contrast to the effect of phthalate esters (mono-2-ethylhexyl phthalate; Gray *et al.*, 1982), which markedly inhibit Sertoli cell fluid secretion.

Testis esterase activities (NTE and NSE) were found sensitive to TOCP administration. Brain activities of AChE and NTE were also inhibited, although no clinical signs of delayed neurotoxicity usually associated with TOCP administration to sensitive species were seen. Histochemical staining of testis NSE demonstrated that the majority of the NSE activity was localized to the interstitium. Both Cameron and Snydle (1985) and Molenaar *et al.*, (1985) have localized NSE to Leydig cells *in vitro*. It is interesting to note that following 3 days of treatment, a dramatic inhibition of NSE was seen both by histochemical and by biochemical assay in homogenate. However, no histopathology of Leydig cells (Somkuti *et al.*, 1986a) or alterations in testosterone concentrations were noted. The Leydig cells maintained the ability to produce and secrete testosterone despite NSE inhibition. Despite the lack of a defined physiological role for NSE, its intense concentrations in Leydig cells may serve as an indicator of organophosphate compound exposure. Cameron and Snydle (1985) have additionally localized NSE activity to Sertoli cells in culture. Our histochemical results likewise showed a NSE activity intratubu-

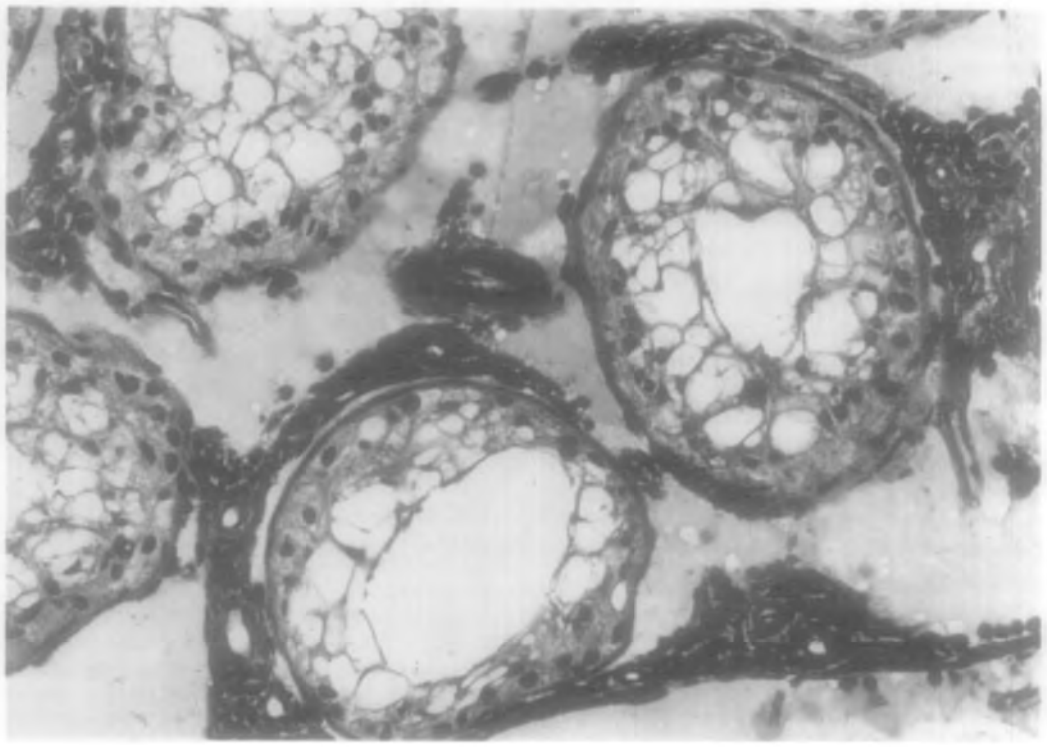

FIG. 6. Seminiferous tubules from recovery animals showing Sertoli cell nuclei; no germ cells are evident (290×).

larly, corresponding to Sertoli cells (albeit a much weaker reaction than in the interstitium), which was also decreased by TOCP treatment. Lysosomal (and to a lesser extent, mitochondrial) β-glucuronidase is a specific marker enzyme for Sertoli cells and developing spermatogonia (Males and Turkington, 1971). The lack of inhibition after TOCP treatment suggests that this enzyme is not a target of TOCP or an active metabolite of the chemical. The testicular toxicant 2,5-hexanedione, on the other hand, dramatically inhibits β-glucuronidase (Chapin *et al.*, 1982).

Recovery from a compound-induced testicular lesion has been defined for the purposes of this study as a reinitiation of spermatogenesis following a recovery period corresponding to two cycles of the seminiferous epithelium (in rat: 49 days × 2 cycles = 98 days; Amann, 1982). There was no evidence of seminiferous tubule regeneration or of reinitiation of spermatogenesis in animals after this 98-day observation period. Thus, this dose schedule of TOCP produced an irreversible testicular lesion. Whether the target cell for TOCP is the Sertoli cell or the developing germ cells is still unclear. While the previous studies observed changes in Sertoli cells (Somkuti *et al.*, 1986c, 1987a,b), the biochemical endpoints used above also indicated changes in Leydig cell enzymatic activities.

ACKNOWLEDGMENT

The authors thank Ms. Monica D. Ross for her expert technical assistance in the skillful preparation of the frozen testis sections. This work was supported in part by Grant OHO-0823 from the National Institute of Occupational Safety and Health of the Centers for Disease Control.

REFERENCES

ABOU-DONIA, M. B. (1981). Organophosphorous ester-induced delayed neurotoxicity. *Annu. Rev. Pharmacol. Toxicol.* **21**, 511–48.

AMANN, R. P. (1982). A critical review of methods for evaluation of spermatogenesis from seminal characteristics. *J. Androl.* **2,** 37–58.

CAMERON, D. F., AND SNYDLE, F. E. (1985). Selected enzyme histochemistry of Sertoli cells *in vitro*. *Andrologia* **17,** 64–71.

CHAPIN, R. E., NORTON, R. M., POPP, J. A., AND BUS, J. S. (1982). The effects of 2,5-hexanedione on reproductive hormones and testicular enzyme activities in the F-344 rat. *Toxicol. Appl. Pharmacol.* **62,** 262–272.

COLLINS, P. M., AND TSANG, W. N. (1979). An assessment of the function of the seminiferous tubules and interstitium of the rat testis following ligation of the vasa efferentia. *Biol. Reprod.* **20,** 671–680.

FISHMAN, W. H. (1974). β-Glucuronidase. In *Methods of Enzymatic Analysis* (H. U. Bergmeyer, Ed.), Vol. 2, pp. 929–943. Academic Press, New York.

GRAY, T. J. B., BEEMAND, J. A., AND GANGOLLI, S. D. (1982). Effects of phthalate esters on rat testicular cultures and on Sertoli cell function. *Toxicologist* **2,** 78.

JOHNSON, M. K. (1977). Improved assay of NTE for screening organophosphates for delayed neurotoxicity potential. *Arch. Toxicol.* **37,** 113.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27,** 138A.

LAPADULA, D. M., CARRINGTON, C. D., AND ABOU-DONIA, M. B. (1984). Induction of hepatic microsomal cytochrome P-450 and inhibition of brain, liver, and plasma esterases by an acute dose of *S,S,S*-tri-*n*-butyl phosphorotrithioate (DEF) in the adult hen. *Toxicol. Appl. Pharmacol.* **73,** 300–310.

LEBLOND, C. P., AND CLERMONT, Y. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. N.Y. Acad. Sci.* **55,** 548–573.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

MALES, J. L., AND TURKINGTON, R. W. (1971). Hormonal control of lysosomal enzymes during spermatogenesis in the rat. *Endocrinology* **88,** 579–588.

MOLENAAR, R., ROMMERTS, F. F. G., AND VAN DER MOLEN, H. J. (1985). Non-specific esterase: a specific and useful marker enzyme for Leydig cells from mature rats. *J. Endocrinol.* **108,** 329–334.

PEARSE, A. G. E. (1972). *Histochemistry: Theoretical and Applied,* 3rd ed., Vol. 2. Churchill and Livingstone, New York.

SHARPE, R. M., AND COOPER, I. (1983). Testicular interstitial fluid as a monitor for changes in the intratesticular environment in the rat. *J. Reprod. Fertil.* **69,** 125–135.

SOMKUTI, S. G., CHAPIN, R. E., LAPADULA, D. M., ROSS, M. D., AND ABOU-DONIA, M. B. (1986a). The spectrum of tri-*o*-cresyl phosphate's effects on the male reproductive system: Effects on multiple cell types. *J. Androl.* **7,** 30-P.

SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV., AND ABOU-DONIA, M. B. (1986b). The morphogenesis and ultrastructural changes of the TOCP-induced testicular lesion in F344 rats. *Toxicologist* **6,** 290.

SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV., AND ABOU-DONIA, M. B. (1986c). Testicular toxicity of TOCP in F344 rats. *Toxicologist* **6,** 290.

SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV, AND ABOU-DONIA, M. B. (1987a). Reproductive tract lesions resulting from subchronic administration (63 Days) of tri-*o*-cresyl phosphate in male rats. *Toxicol. Appl. Pharmacol.* **89,** 49–63.

SOMKUTI, S. G., LAPADULA, D. M., CHAPIN, R. E., LAMB, J. C., IV., AND ABOU-DONIA, M. B. (1987b). Testicular toxicity following oral administration of TOCP in roosters. *Toxicol. Lett.*, in press.

TURNER, T. T., AND GILES, R. D. (1981). The effects of carnitine, glycerol phosphor choline, caffeine, and egg yolk on the motility of rat epididymal spermatozoa. *Gamete Res.* **4,** 283–295.

WAITES, G. M. H., AND GLADWELL, R. T. (1982). Physiological significance of fluid secretion in the testis and blood-testis barrier. *Physiol. Rev.* **62,** 624–671.