TOXICOLOGY AND APPLIED PHARMACOLOGY 79, 83–90 (1985)

# Characterization of Delayed Neurotoxicity in the Mouse following Chronic Oral Administration of Tri-*o*-cresyl Phosphate[1]

DANIEL M. LAPADULA, SUZANNE E. PATTON,[2] GERALD A. CAMPBELL,[3] AND MOHAMED B. ABOU-DONIA[4]

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

*Received August 27, 1984; accepted January 11, 1985*

Characterization of Delayed Neurotoxicity in the Mouse following Chronic Oral Administration of Tri-*o*-cresyl Phosphate. LAPADULA, D. M., PATTON, S. E., CAMPBELL, G. A., AND ABOU-DONIA, M. B. (1985). *Toxicol. Appl. Pharmacol.* **79**, 83–90. The sensitivity of the mouse to organophosphorus-induced delayed neurotoxicity (OPIDN) has been investigated. One group of five mice received two single 1000-mg/kg po doses of tri-*o*-cresyl phosphate (TOCP) at a 21-day interval (on Days 1 and 21 of the study); a second group of five mice was given 225 mg/kg of TOCP daily for 270 days. A third group of five animals served as an untreated control. All animals were killed 270 days after the start of the experiment. Daily po dosing of 225 mg/kg TOCP caused a decrease in body weight gain, muscle wasting, weakness, and ataxia which progressed to severe hindlimb paralysis at termination. On the other hand, po administration of two single 1000-mg/kg doses of TOCP at a 21-day interval produced no observable adverse effects. Brain acetylcholinesterase (AChE) and neurotoxic esterase (NTE) activity were 35 and 10% of the control, respectively, in daily dosed animals while AChE and NTE in mice receiving two single 1000-mg/kg doses of TOCP were not significantly altered from the control group. Plasma butyrylcholinesterase activity was 12% of the control group in daily dosed animals. Hepatic microsomal enzyme activities of aniline hydroxylase and *p*-chloro-*N*-methylaniline demethylase and NADPH–cytochrome *P*-450 content in daily dosed animals were increased (141 to 161% of the control group) when compared to controls and mice receiving two single 1000-mg/kg doses of TOCP; the latter being not significantly different from each other. Degeneration of the axon and myelin of the spinal cord and sciatic fasicle were observed and were consistent with OPIDN. This study demonstrates that chronic dosing of TOCP produces OPIDN and induces hepatic microsomal enzyme activity in mice. It is concluded that while the mouse is susceptible to OPIDN, it is a less sensitive and a less appropriate test animal for studying this effect when compared to the adult hen. 

Tri-*o*-cresyl phosphate (TOCP) induces distal, central–peripheral axonopathy in humans and certain animal species (e.g., chickens, cats, dogs, and farm animals) (Smith *et al.*, 1930; Abou-Donia, 1981). Rodents and some primates are not considered sensitive to organophosphorus-induced delayed neurotoxicity (OPIDN). The adult chicken is the experimental animal of choice to study OPIDN (EPA, 1978). Clinical signs are characterized by a delay period (the length of which depends on the species) followed by ataxia which may progress to paralysis. Histopathologic changes consist of Wallerian-type de-

[1] A preliminary account of this work has been presented (Lapadula *et al.*, 1983).

[2] Present address: National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709.

[3] Present address: Department of Pathology, Duke University Medical Center, Durham, N.C. 27710.

[4] To whom requests for reprints should be addressed.



0041-008X/85 $3.00

generation of the axon and myelin of the central and peripheral nervous systems.

A recent study demonstrated that TOCP produces central and peripheral neuropathologic lesions in Sprague–Dawley rats following repeated exposure to large doses of TOCP (Veronesi and Abou-Donia, 1982). These rats, however, did not exhibit clinical signs characteristic of OPIDN. The present study was designed to examine the sensitivity of another rodent species, the mouse, to OPIDN following two single, po 1000 mg/kg doses of TOCP at a 21-day interval or continuous daily po doses of 225 mg/kg of TOCP for 270 days.

## METHODS

*Chemicals.* TOCP (99%) was obtained from Eastman–Kodak Company (Rochester, N.Y.). Biochemicals used for enzymatic determinations were purchased from Sigma Chemical Company (St. Louis, Mo.).

*Animals.* Three groups of five mice (Harlan Sprague–Dawley, Indianapolis, Ind.) (HSD, ICR, males 22 g) each were used. Group 1 consisted of untreated controls, group 2 was treated with two single 1000-mg/kg po doses of TOCP mg/kg 21 days apart, and group 3 received a 225-mg/kg daily po dose of TOCP for 270 days. Mice had access to feed (Purina Rodent Chow, St. Louis, Mo.) and water *ad libitum.* The animals were kept in a humidity (40 to 60%)- and temperature (21 to 23°C)-controlled room with a 12-hr light/12-hr dark cycle before and during the experiment.

*Histopathologic methods.* After 270 days three animals from each group were killed for enzyme studies, and the remaining two animals from each group were perfused with 2% paraformaldehyde followed by Sorensen's buffered 5% glutaraldehyde. The sciatic nerve and spinal cord were dissected from the perfused animals and stored in buffered glutaraldehyde for 3 days and then switched to buffer alone. Nervous tissues from these animals were embedded in glycol methacrylate. The sciatic, tibial, and peroneal nerves and spinal cords of the three animals used in the enzyme studies were dissected at the time the animals were killed and stored in neutral phosphate-buffered 10% Formalin for 2 weeks and embedded in paraffin. Parasagittal, longitudinal, and cross sections of the spinal cord as well as cross and longitudinal sections of the peripheral nerves were obtained. Methacrylate sections (1 to 2 $\mu$M) were stained with toluidine blue while paraffin sections (8 $\mu$M) were stained with hematoxylin and eosin with luxol fast blue and Holmes' silver stain. Nervous tissues from all animals were examined for histopathology.

*Enzymatic studies.* Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity was measured by procedures previously described (Abou-Donia and Preissig, 1976). Neurotoxic esterase (NTE) activity was determined by the method of Johnson (1977).

Hepatic microsomes were prepared by the method of Schenkman and Cinti (1978). The method of Omura and Sato (1964) was used for cytochrome *P*-450 determination. Aniline hydroxylase activity was measured by the method of Mazel (1971), *p*-chloro-*N*-methylaniline (PCMA) demethylase by the method of Abou-Donia and Dieckert (1971), and NADPH-cytochrome *c* reductase by the method of Guengerich (1982).

*Statistics.* Controls and animals treated with two single 1000-mg/kg doses of TOCP were compared to animals given a 225-mg/kg daily po dose of TOCP by using a one-way ANOVA. All statistical analyses were done on the values of enzymatic activities. The results are expressed as a percentage of control to facilitate comparisons between groups. The control group was assigned the value of 100%, and all other groups and standard errors are expressed as a percentage of control.

## RESULTS

*General observations.* Over the course of this study, the body weight of control mice and of mice treated with two single 1000-mg/kg po doses of TOCP at a 21-day interval, increased by 94 and 111% from their initial weight, respectively. Mice given a daily 225-mg/kg po dose of TOCP for 270 days gained only 38% of their pretreatment weight.

Clinical signs of delayed neurotoxicity developed only in animals chronically treated with TOCP; those given two single 1000-mg/kg po doses of TOCP and the control mice remained normal. The first sign of delayed neurotoxicity was manifested in daily dosed animals as weakness accompanied by a small amount of muscle wasting 8 weeks after the beginning of dosing. The development of neuropathy proceeded very slowly to severe ataxia, which was evident by Day 230 in three of the five chronically treated animals. The clinical condition of all daily dosed animals progressed to severe hindlimb paralysis by the time of termination, 270 days after the beginning of the administration (Fig. 1).

*Histopathologic changes.* Sections of spinal cord and peripheral nerves from all control

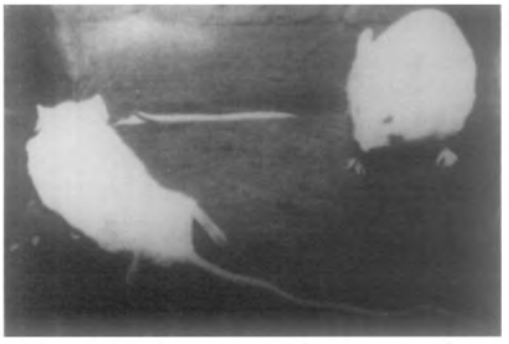

FIG. 1. A control mouse (right) and a mouse after 270 consecutive daily po doses of 225 mg/kg TOCP. The treated mouse could not stand and exhibited splaying of the hindlegs.

and TOCP-treated mice were examined by light microscopy (Table 1). No histopathologic changes were evident in the control group or the animals treated with two single 1000-mg/kg doses of TOCP.

Morphologic examination of sections of the spinal cord of mice chronically treated with TOCP indicated extensive degeneration of the axon and myelin of the ventral columns in the lumbar cord and of the lateral columns in the cervical cord (Fig. 2). Histologic changes in the dorsal columns of the cervical spinal cord were infrequent and were designated as "equivocal changes"; they were characterized by rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining.

Histopathologic lesions in the peripheral nerves of two chronically treated mice showed frank axon and myelin degeneration while sections from the remaining three mice of this group exhibited equivocal changes only (Fig. 3).

*Enzymatic activity.* In none of the enzymes assayed, were control animals and those treated with two single 1000-mg/kg doses of TOCP significantly different from each other (Table 2). By contrast, enzymatic activities of brain AChE and NTE and of plasma BuChE were all significantly inhibited in animals chronically treated with a daily 225-mg/kg dose of TOCP. Also, hepatic microsomal cytochrome *P*-450 content and aniline hydroxylase, PCMA demethylase, and NADPH–cytochrome *c* reductase activity, in daily dosed animals were significantly increased over the activity in the control group.

## DISCUSSION

This study demonstrates the susceptibility of the mouse to TOCP-induced delayed neurotoxicity following chronic daily po administration of 225 mg/kg of TOCP, but not after two single 1000-mg/kg po doses of TOCP at a 21-day interval. The clinical condition was characterized by a delay of 8 weeks before onset of ataxia which progressed to paralysis. The morphology of neuronal lesions is characterized by a Wallerian-type degeneration of the axon and myelin of the central and peripheral nervous systems. The distribution of lesions in the dorsal and lateral columns of the cervical spinal cord and the ventral columns of the lumbar and sacral spinal cords was similar to that reported for other sensitive species (Cavanagh, 1973). Degeneration of the peripheral nerves was more prominent in the tibial and peroneal nerves and their branches than in the sciatic nerve itself.

TABLE 1

HISTOPATHOLOGIC CHANGES[a] IN NERVOUS SYSTEM TISSUES OF MICE AFTER CHRONIC (9 MONTHS) ORAL ADMINISTRATION OF A DAILY 225-mg/kg DOSE OF TOCP

| | Spinal cord | | | |
|---|---|---|---|---|
| Animal number | Anterior columns | Lateral columns | Dorsaal columns | Peripheral nerves |
| 1[b] | + | + | ± | ± |
| 2[b] | + | + | ± | ± |
| 3[b] | ++ | + | – | + |
| 4[c] | ++ | + | ± | + |
| 5[c] | + | + | + | ± |

[a] Abbreviations: +, degenerated axon and myelin; ±, swollen axons without fragmentation of axon or myelin; –, changes absent.

[b] Paraffin sections stained with H&E, LFB (See Methods).

[c] Methacrylate sections stained with toluidine blue (See Methods).

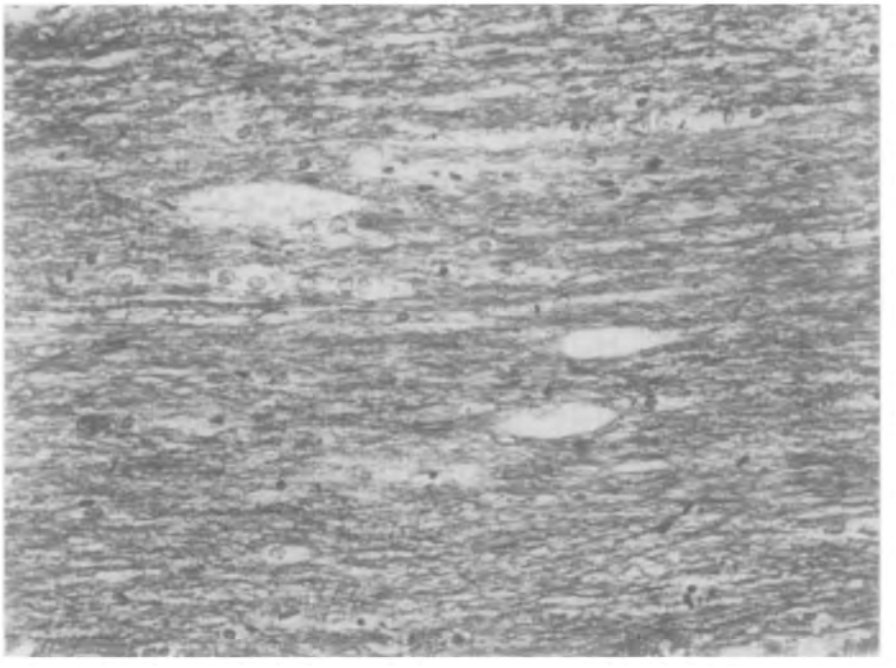

FIG. 2. A longitudinal section of a spinal cord from a mouse treated with a daily 225-mg/kg dose of TOCP for 270 days. Three swollen axons are evident in this section. One of these axons as well as several other axon tracts are surrounded by macrophages, some of which contain myelin debris within their cytoplasm. H&E–LFB, ×250.

When compared to other studies the present data indicate that while the mouse is not as sensitive to OPIDN as the chicken, it is much more susceptible than the rat. The threshold single po dose of TOCP that causes OPIDN in the hen is 250 mg/kg (Abou-Donia *et al.*, 1982). On the other hand, chronic exposure of rats to large po doses of TOCP produced limited neuropathologic changes without being accompanied by neurologic dysfunctions (Veronesi and Abou-Donia, 1982).

The results of this study demonstrate that although chronic po administration of 225 mg/kg of TOCP produced 65% inhibition of acetylcholinesterase no cholinergic signs of toxicity were evident at termination. Both nonspecific esterases, brain neurotoxic esterase and plasma BuChE, exhibited 87 and 88% inhibition, respectively. These results are in agreement with previous findings that correlate the inhibition of NTE with the production of OPIDN (Johnson, 1981, 1982). Earlier studies have also shown that NTE inhibited by an organophosphorus compound must undergo "aging" in order for OPIDN to be manifested. Also, the 11% inhibition of NTE produced in mice treated with two single 1000-mg/kg po doses of TOCP is consistent with the results that no OPIDN was manifested in these animals by this regimen. It is noteworthy that the mouse brain content of NTE is approximately 9% that of hen brain (Lapadula *et al.*, 1984).

It is interesting to speculate on the reasons why the mouse, although susceptible to TOCP-induced delayed neurotoxicity, is less sensitive than the chicken. Factors that may play a role in this reduced sensitivity are (1) the metabolism, disposition, and pharmacokinetics of TOCP and its metabolites (Abou-Donia, 1983), (2) the sensitivity of the nervous tissue to the neurotoxic action of TOCP (Patton *et al.*, 1984a,b), and (3) the ability of

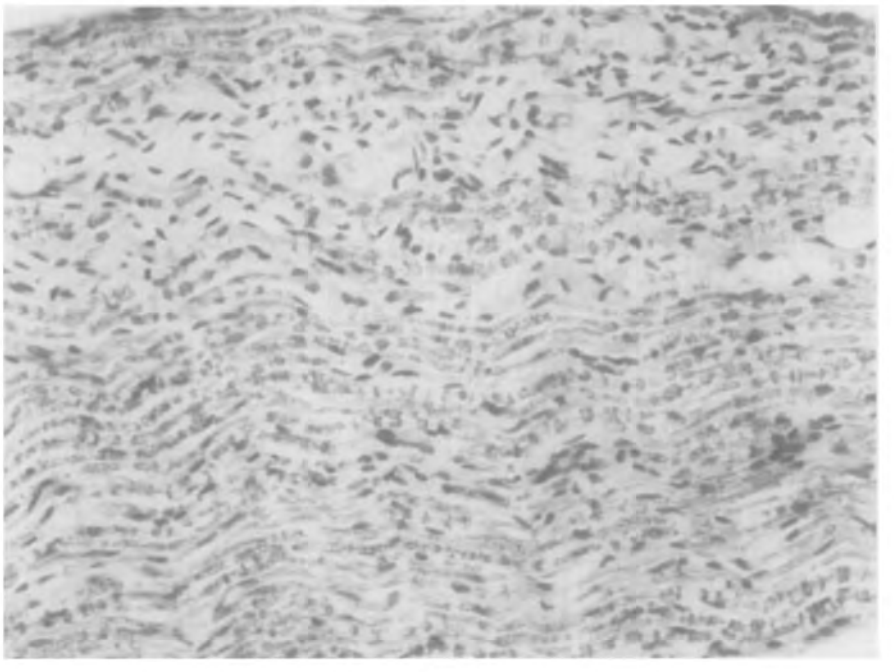

FIG. 3. Longitudinal section of a sciatic nerve from a mouse treated with a daily 225-mg/kg dose of TOCP for 270 days. In the upper portion of the field are axons with focal swellings and extensive demyelination scattered macrophages are present. The nerve in the lower portion of the field is relatively normal. H&E–LFB, ×250.

the nervous tissue to repair itself following initial injury (Richardson, 1984).

*In vivo* and *in vitro,* TOCP undergoes metabolic activation to a more potent neurotoxic agent; saligenin cyclic-*o*-tolyl phosphate (Eto *et al.,* 1962; Nomeir and Abou-Donia, 1984; Nomeir *et al.,* 1984). This metabolic biotransformation proceeds via two reactions (Eto *et al.,* 1967) (1) the hydroxylation of one of the three *ortho*-methyl groups by the hepatic microsomal *P*-450 system to hydroxymethyl TOCP, and (2) the cyclization of this product, mostly by plasma albumin, to yield saligenin cyclic-*o*-tolyl phosphate. It has been suggested that the balance between the two processes, activation and inactivation, influences the production of OPIDN in various species (Abou-Donia, 1979, 1983). The present study indicates that chronic po administration of TOCP induces hepatic microsomal enzymes in the mouse which may result in its metabolic activation. TOCP and/or its active metabolites then accumulate at the neurotoxicity target until they reach a minimum amount of "threshold" that produces delayed neurotoxicity. The threshold level in the mouse could only be obtained from chronic po administration of 225 mg/kg of TOCP and not from two single 1000-mg/kg doses of TOCP at a 21-day interval. These results also suggest that the chronic TOCP treatment may have saturated the detoxification and/or elimination processes of TOCP in the rodent since a single po dose of the neurotoxic organophosphorus compound is metabolized and eliminated rapidly in the urine (Nomeir and Abou-Donia, 1984). It is also possible that the target tissue of OPIDN is species specific. This speculation is in agreement with recent findings that prominent species differences exist in endogenous protein phosphorylation between the hen and rat spinal cord and brain cytosolic and membrane fractions (Patton *et al.,*

TABLE 2

EFFECT OF TOCP ON PLASMA, BRAIN, AND HEPATIC ENZYME ACTIVITIES[a]

| | Control $\bar{x} \pm$ SE | Two doses[b] $\bar{x} \pm$ SE | Daily doses[c] $\bar{x} \pm$ SE |
|---|---|---|---|
| Plasma | | | |
| BuChE | 100 ± 11 | 126 ± 10 | 12 ± 5[d] |
| Brain | | | |
| AChE | 100 ± 2 | 105 ± 10 | 35 ± 3[d] |
| NTE | 100 ± 9 | 89 ± 18 | 10 ± 6[d] |
| Liver | | | |
| Cytochrome *P*-450 | 100 ± 5 | 113 ± 6 | 144 ± 18[d] |
| AH | 100 ± 16 | 78 ± 9 | 151 ± 12[d] |
| PCMA demethylase | 100 ± 16 | 71 ± 8 | 145 ± 17[d] |
| Cytochrome *c* reductase | 100 ± 17 | 80 ± 7 | 141 ± 14[d] |

[a] Results are expressed as percentage of control. Control values were 36.36 ± 4.04 μmol BuCh hydrolyzed per min/mg protein; 22.53 ± 0.47 μmol AtCh hydrolyzed per min/mg protein; 2.583 ± 0.248 nmol phenylvalerate hydrolyzed per min/mg protein; 0.6494 ± 0.171 nmol cytochrome *P*-450/mg protein; 0.1040 ± 0.0173 nmol *p*-aminophenol formed per min/mg protein; 0.0799 ± 0.0129 nmol *p*-chloroaniline formed per min/mg protein; 51.08 ± 8.92 nmol cytochrome *c* reduced per min/mg protein.

[b] Mice were given two single po doses of 1000 mg/kg TOCP at a 21-day interval. These animals were killed after 270 days.

[c] Mice were given daily po doses of 225 mg/kg for 270 days.

[d] Significantly different from control and two-dose groups using one-way ANOVA.

1985a,b). Recent studies demonstrate increased *in vitro* $Ca^{2+}$-calmodulin department protein phosphorylation in the brains of adult hens which developed OPIDN as the result of the administration of TOCP (Patton *et al.*, 1983; Abou-Donia *et al.*, 1984). Similarly treated rats did not show an increase in protein phosphorylation.

Since studies on delayed neurotoxicity can only be performed using experimental animals, it is essential to use the animal model from which neurotoxicity data can be best extrapolated to humans. Many organophosphorus compounds, including TOCP require metabolic activation to more potent neurotoxicants and undergo metabolic detoxification. The active neurotoxic form binds to specific molecular and cellular targets in the nervous system. Resulting changes are limited by repair mechanisms. All of these processes are strain- and species dependent. It is, therefore, expected that the response to any delayed neurotoxic organophosphorus compound will be a function of strain and species. Thus, it seems that rodents are not good model animals to screen organophosphorus compounds for OPIDN. In descending order the relative species sensitivity to OPIDN is chicken, cat, mouse, and then rat. The absence of clinical manifestation in the rat and the long time necessary to treat the mouse before developing OPIDN make the rodent a poor paradigm compared to the adult chicken which develops neurologic dysfunction accompanied by histopathologic lesions following a single dose of the delayed neurotoxic organophosphorus compound (Smith *et al.*, 1930; Abou-Donia, 1981). Of interest are the recent electrophysiologic (Lapadula *et al.*, 1982; Trofatter and Abou-Donia, 1981), neurologic (Abou-Donia *et al.*, 1983b), histopathologic (Abou-Donia *et al.*, 1983a), and metabolism studies (Abou-Donia *et al.*, 1983c,d) of TOCP and *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN) which show that although the cat is less sensitive

than the chicken to OPIDN it may be a more appropriate test animal to be compared to the human, than is the chicken.

## ACKNOWLEDGMENTS

The authors thank Mrs. Erna S. Daniel for her secretarial help in the preparation of this manuscript. This study was supported by NIEHS Grant ESO2717 and NIOSH Grant OHO2003.

## REFERENCES

ABOU-DONIA, M. B. (1979). Pharmacokinetics and metabolism of a topically applied dose of *O*-4-bromo-2,5-dichlorophenyl *O*-methyl phenylphosphonothioate in hens. *Toxicol. Appl. Pharmacol.* **51,** 311–328.

ABOU-DONIA, M. B. (1981). Organophosphorus ester induced delayed neurotoxicity. *Annu. Rev. Pharmacol. Toxicol.* **21,** 511–548.

ABOU-DONIA, M. B. (1983). Toxicokinetics and metabolism of delayed neurotoxic organophosphorus esters. *Neurotoxicology* **4,** 113–130.

ABOU-DONIA, M. B., AND DIECKERT, J. W. (1971). Gossypol: Subcellular localization and stimulation of rat liver microsomal oxidases. *Toxicol. Appl. Pharmacol.* **18,** 507–516.

ABOU-DONIA, M. B., GRAHAM, D. G., AND KINNES, C. G. (1983a). Sensitivity of the cat to delayed neurotoxicity induced by *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate. *Toxicol. Appl. Pharmacol.* **68,** 54–65.

ABOU-DONIA, M. B., JENSEN, D. N., AND LAPADULA, D. M. (1983b). Neurological manifestations of tri-*o*-cresyl phosphate delayed neurotoxicity in cats. *Neurobehav. Toxicol. Teratol* **5**(4), 431–442.

ABOU-DONIA, M. B., KINNES, C. G., ABDO, K. M., AND BJORNSSON, T. D. (1983c). Physiological disposition and metabolism of *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate in male cats following a single dermal administration. *Drug Metab. Dispos.* **11,** 31–36.

ABOU-DONIA, M. B., MAKKAWY, H. M., SALAMA, A. E., AND GRAHAM, D. G. (1982). Effect of age of hens on their sensitivity to delayed neurotoxicity induced by a single oral dose of tri-*o*-cresyl phosphate. *Toxicologist* **2,** 178.

ABOU-DONIA, M. B., PATTON, S. E., AND LAPADULA, D. M. (1984). Possible role of endogenous phosphorylation in organophosphorus compound-induced delayed neurotoxicity. *Proceedings of the Symposium on Cellular and Molecular Basis of Neurotoxicity of Environmental Agents, San Diego, Calif.* (in press).

ABOU-DONIA, M. B., AND PREISSIG, S. H. (1976). Delayed neurotoxicity of leptophos: Toxic effects on nervous systems of hens. *Toxicol. Appl. Pharmacol.* **35,** 269–282.

ABOU-DONIA, M. B., SIVARAJAH, K., AND ABOU-DONIA, S. A. (1983d). Disposition, elimination, and metabolism of *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate after subchronic dermal administration in male cats. *Toxicology* **26,** 93–111.

CAVANAGH, J. B. (1973). Peripheral neuropathy caused by chemical agents. *CRC Crit. Rev. Toxicol.* **2,** 365–417.

Environmental Protection Agency (1978). *Pesticide Programs. Proposed Guidelines for Registering Pesticides in the U.S.: Hazard Evaluation. Health and Domestic Animals. Acute Delayed Neurotoxicity Study. Fed. Regist.* **43,** 37362–37363.

ETO, M., CASIDA, J. E., AND ETO, T. (1962). Hydroxylation and cyclization reactions involved in the metabolism of tri-*o*-cresyl phosphate. *Biochem. Pharmacol.* **11,** 337–352.

ETO, M., OSHIMA, Y., AND CASIDA, J. E. (1967). Plasma albumin as a catalyse in cyclization of diaryl *O*-(α-hydroxyl)tolyl phosphates. *Biochem. Pharmacol.* **16,** 295–308.

GUENGERICH, F. P. (1982). Microsomal enzymes involved in toxicology—Analysis and separation. In *Principles and Methods of Toxicology.* (A. E. Hayes, ed.), pp. 609–635. Raven Press, New York.

JOHNSON, M. K. (1977). Improved assay for neurotoxic esterase of screening organophosphates for delayed neurotoxicity potential. *Arch. Toxicol.* **37,** 113–115.

JOHNSON, M. K. (1981). Initiation of organophosphate neurotoxicity. *Toxicol. Appl. Pharmacol.* **61,** 480–481.

JOHNSON, M. K. (1982). The target for initiation of delayed neurotoxicity by organophosphorus esters. Biochemical studies and toxicological applications. *Rev. Biochem. Toxicol.* **4,** 141–202.

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.

LAPADULA, D. M., KINNES, C. G., SOMJEN, G. G., AND ABOU-DONIA, M. B. (1982). Monosynaptic reflex depression in cats with organophosphorus neuropathy: Effects of tri-*o*-cresyl phosphate. *Neurotoxicology* **3,** 51–62.

LAPADULA, D. M., PATTON, S. E., AND ABOU-DONIA, M. B. (1983). Characterization of delayed neurotoxicity and induction of hepatic microsomal enzymes in the mouse following chronic administration of tri-*o*-cresyl phosphate (TOCP). The Third International Congress on Toxicology, San Diego, Calif., Aug. 28–Sept. 3, 1983. *Toxicol. Lett.* **18** (Suppl. I), 4.

MAZEL, P. (1971). Experiments illustrating drug metabolism *in vitro.* In *Fundamentals of Drug Metabolism and Drug Disposition* (D. N. LaDu, H. D. Mandel, and E. L. Way, eds.), pp. 546–592. William and Wilkins, Baltimore, Md.

NOMEIR, A. A., AND ABOU-DONIA, M. B. (1984). Disposition of tri-*o*-cresyl phosphate (TOCP) and its metabolites in various tissues of the male cat following a single dermal application of [$^{14}$C]TOCP. *Drug Metab. Disp.* **12,** 705–711.

NOMEIR, A. A., H. A. MAKKAWY, J. H. BOWER, AND M. B. ABOU-DONIA (1984). Distribution and metabolism of [$^{14}$C]-tri-*o*-cresyl phosphate (TOCP) in male rat following oral administration. *Toxicologist* **4,** 90.

OMURA, T., AND SATO, R. (1964). The carbon-monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239,** 2370–2378.

PATTON, S. E., LAPADULA, D. M., AND ABOU-DONIA, M. B. (1985a). Comparison of endogenous phosphorylation of the rat spinal cord proteins and partial characterization of optimal phosphorylation conditions for hen spinal cord. *Neurochem. Int.*, in press.

PATTON, S. E., LAPADULA, D. M., AND ABOU-DONIA, M. B. (1985b). Partial characterization of endogenous phosphorylation conditions for hen brain cytosolic and membrane proteins. *Brain Res.* **328,** 1–14.

PATTON, S. E., O'CALLAGHAN, J. P., MILLER, D. B., AND ABOU-DONIA, M. B. (1983). The effect of oral administration of tri-*o*-cresyl phosphate on *in vitro* phosphorylation of membrane and cytosolic proteins from chicken brain. *J. Neurochem.* **41,** 897–901.

RICHARDSON, R. (1984). The role of neurotoxic esterase in normal and pathological conditions. *Proceedings of the symposium on Cellular and Molecular Basis of Neurotoxicity of Environmental Agents, San Diego, Calif.*

SCHENKMAN, J. B., AND CINTI, D. L. (1978). Preparation of microsomes with calcium. In *Methods in Enzymology* (S. Fleischer and L. Packer, eds.), Vol. 52, pp. 83–89. Academic Press, New York.

SMITH, M. I., ELVOVE, E., AND FRAZIER, W. H. (1930). The pharmacological action of certain phenol esters, with special reference to the etiology of so-called ginger paralysis. *Public Health Rep.* **45,** 2509–2524.

TROFATTER, L. P., AND ABOU-DONIA, M. B. (1981). Electromyographic abnormalities in cats treated with tri-*o*-cresyl phosphate (TOCP). *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **40,** 477.

VERONESI, B., AND ABOU-DONIA, M. D. (1982). Central and peripheral neuropathology induced in rats by tri-*o*-cresyl phosphate (TOCP). *Vet. Hum. Toxicol.* **24**(3), 222.