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Malathion: Published Study

Ehrich, M., Shell, L., Rozum, M., and Jortner, B.S. (1993) Short-term clinical and neuropathologic effects of cholinesterase inhibitors in rats. *J. Am. Coll. Toxicol.* 12(1), 55-68 (MRID 45045001). TXR0050532

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OFFICE OF
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In the course of my review of cholinesterase literature, I have identified a publication [Ehrich, M., Shell, L., Roxum, M. and Jortner, B. (1993) Short-term Clinical and Neuropathologic Effects of Cholinesterase Inhibitors in Rats. *J. Am. Coll. Toxicol., 12, 55-68*] (copy appended) which provides important information on the neurotoxicology of a number of cholinesterase inhibiting compounds, *malathion* included. The findings in this publication contrast with the essentially negative FOB parameter findings in the Guideline acute neurotoxicity study (MRID 43146701) of record on malathion as tested at similar doses. This study should be introduced into the record of literature publications pertaining to the neurotoxicology of malathion.

By way of summary (this is not a review of the study), I shall focus my comments on the malathion assessment within this publication. Malathion was evaluated in an acute neurotoxicity study that employed EPA's most recent neurotoxicity functional observational battery (FOB) screening procedure guidelines. Accordingly, malathion (American Cyanamid, 88%) was administered orally to *male* Long Evans rats at the single dose levels of 0, 600, 1000 and 2000 mg/kg, and monitored for clinical signs, FOB parameters (several end points), brain and spinal cord cholinesterase inhibition and neurohistopathology. The FOB was used to screen for neurotoxic effects at days 7, 14 and 21 post-dosing.

In the case of malathion, among FOB parameters examined, increased *activity* was seen at 21 days for the 600 and 1000 mg/kg doe groups, while the same effect was seen by day 14 in the 2000 mg/kg dose group. Exaggerated *response to touch* was evident in the 600 and 1000 mg/kg groups, but "no reaction" (a below normal response) was observed in the 2000 mg/kg group. Difficulty in *ease of removal from cage* was noted for the 1000 mg/kg group at 21 days and in the 2000 mg/kg group by day 14. Exaggerated *reactivity to handling* was noted in the 1000 mg/kg group at day 21, and by day 14 in the 2000 mg/kg dose group. Abnormal *gate* was observed at 21 days in the case of the 2000 mg/kg dose group.

Cholinergic clinical signs were noted only at 2000 mg/kg, but evidently dissipated early and were not present when the FOBs were done on days 7, 14 and 21. Atropine was administered in this study.

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Cholinesterase inhibition in both brain and spinal cord was observed at all dose levels, being most remarkable at 2000 mg/kg. There were no neuropathologic lesions seen in neural tissues examined.

The study report claims in its Discussion that: "However, even after signs of cholinergic poisoning were no longer evident, each of the 7 cholinesterase inhibitors (malathion included) used in the present study caused rats to exhibit alterations in one or more of the parameters of the FOB categorized as indicative of changes in behavior or central nervous system excitability during examination 1 to 3 weeks later."

It should be noted that in the case of malathion, a NOEL was not identified for FOB findings, and that this contrasts with the NOEL/LOEL = 1000/2000 mg/kg (based on increased motor activity) in the malathion Guideline acute neurotoxicity of record. In this latter study, malathion (Cheminova 96.5%) was evaluated at the single oral dosage levels of 0, 500, 1000 and 2000 mg/kg at days 1, 7 and 14 post-dosing. There were no effects on guideline FOB parameters at any dose.

It is my understanding this publication was not included among those literature references under review by the FQPA Safety Factor Committee, nor the HIARC, in determining whether further behavioral effects testing, e.g. developmental neurotoxicity, should be required for malathion. It is my recommendation the reference be included among the other references, for future referral.

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Attachments (1)

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Short-term Clinical and Neuropathologic Effects of Cholinesterase Inhibitors in Rats

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ABSTRACT

Adult male Long Evans rats were given a single administration of 3 dosage levels of the organophosphorus compounds tri-ortho-tolyl phosphate (TOTP), diisopropyl fluorophosphate (DFP), phenyl saligenin phosphate (PSP), mipafox, malathion, and dichlorvos or the carbamate carbaryl. Acetylcholinesterase and neurotoxic esterase activities were inhibited in a dose-dependent manner, with the highest dosages of all of these compounds inhibiting activities of these enzymes in brain by at least 37% and 64%, respectively, at 4 and 48 hours after administration. Rats given the high doses of TOTP (1000 mg/kg), DFP (3 mg/kg), malathion (2000 mg/kg), and carbaryl (160 mg/kg) weighed significantly less than control rats 14 days after administration. A functional observational battery (FOB) was used to screen for neurotoxic effects 1, 2, and 3 weeks after exposure. All 7 test compounds were capable of causing changes in parameters indicative of behavioral and central nervous system excitability. In addition, dose-related alterations in response to approach were seen in rats given DFP, malathion, dichlorvos, and carbaryl. Mild to moderate myelinated fiber degeneration was seen in the rostral levels of the fasciculus gracilis in rats given TOTP, DFP, PSP and mipafox, but no significant neuropathologic lesions were noted in rats given dichlorvos, malathion, or carbaryl.

INTRODUCTION

Cholinesterase inhibitors (organophosphorus esters and carbamates) are widely used as insecticides. These compounds are neurotoxicants, due to their capability to inhibit the activity of neural cholinesterase shortly after man and animals are exposed, resulting in muscarinic, neuromuscular, and centrally mediated clinical signs. Overt manifestations of cholinergic poisoning due to cholinesterase inhibition are of relatively short duration, with most animals seemingly normal within 24 hours[1]. Longer-term effects, however, have been reported to occur in man after the acute crisis has passed[2-5]. These effects were unrelated to the property of some organophosphorus compounds of causing a delayed neuropathy. Neurotoxicity screening in rodents, using guidelines recently recommended by the Environmental Protection Agency U.S. (E.P.A.)[6], could have value in identifying the potential of cholinesterase inhibitors to have longer-term effects. The present study used a functional observational battery (FOB) recommended in the recent guidelines[6] and neuropathology to determine if neurotoxicity of cholinesterase inhibitors could be detected at time points after rodents had recovered from overt clinical signs of cholinergic poisoning.

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Although it is the signs of cholinergic poisoning that may be life-threatening, prognosis for survival is good if man and animals showing signs of cholinergic poisoning are treated and given time for these signs to subside. Even so, there have been several reports of long-lasting neurotoxicity in humans following a single exposure to cholinesterase inhibitors. Among the neurotoxic effects reported in man some time following recovery from acute clinical manifestations of cholinergic poisoning are incoordination, anxiety, irritability, reduced attention span, impairment of judgment, and memory deficits[2–5]. The relationship between these human complaints and later effects of cholinesterase inhibitors in animals models has not been extensively investigated. Previous studies in laboratory animals have indicated that changes in behavior, locomotion, learning, and peripheral nerve conduction could occur after exposure to cholinesterase inhibitors[7–11]. These have sometimes been associated with morphologic damage in rodents allowed to recover from cholinergic poisoning[11,12]. The present studies were used to examine the possibility that an FOB, such as that recommended in the U.S. EPA Neurotoxicity Screening Guidelines[6,13], would be of use in identifying long-term effects occurring in rodents after recovery from overt signs of cholinergic poisoning. Since the cholinesterase inhibitors used as test compounds in this study included organophosphorus compounds identified as those that could and could not cause delayed neuropathy in other animal models, the present experiments were also used to determine if the rat would be an appropriate experimental model for identification of organophosphorus-induced delayed neuropathy. The studies used both the FOB and neuropathologic examination for identification of organophosphorus-induced delayed neuropathy because previous studies indicated the rat is susceptible to this disorder without the obvious clinical manifestations that are seen in other animal models (hen, cat)[1,2,14,15].

Initial screening procedures for neurotoxicity, which include a FOB, or profile of behavioral effects, use a number of parameters to assess neurotoxicity. In the FOB of Moser et al. (1988), autonomic, sensory, motor, and central nervous system activity of the laboratory rat are assessed as either quantal or quantitative responses. This FOB has been used previously to detect early effects of cholinesterase inhibition, and it has appeared to be a sensitive indicator of neurotoxicity of neurotoxicants belonging to other classes of chemicals that have been tested in our laboratory and others[13,16–21]. The present study used this FOB as a test method for detection of long-lasting behavioral effects associated with acute exposure to cholinesterase inhibitors. Seven cholinesterase inhibitors were tested, including 4 organophosphorus compounds that have potential to cause delayed neuropathic effects in more familiar animal models for this disorder. Neuropathologic examination was used to support studies to distinguish compounds inducing delayed neuropathy.

MATERIALS AND METHODS

Adult male Long Evans rats (> 60 days old, 230 to 360 g at initiation of study) were used for these experiments. They were obtained from Charles River Laboratories, Raleigh, NC. Rats were housed 2 per propylene cage (48 × 27 × 18 cm) and provided with food (Purina 5001 maintenance, Purina Mills, Inc.) and water ad libitum. The animals were kept in a controlled environment (20 to 22°C), with a 12-hour light cycle (on 2200 until 1000). Rats were randomly assigned to control and experimental groups (N = 12–28, with both rats in a cage assigned to the same group). They were allowed to acclimate to their surroundings for 1 week prior to their first examination.

The cholinesterase inhibitors administered to groups of at least 12 rats included the following: 300, 600, and 1000 mg/kg PO tri-ortho-tolyl phosphate (TOTP, Lark Enterprises, Webster, MA, 98% pure); 1, 2, and 3 mg/kg sc diisopropyl phosphorofluoridate (DFP, Aldrich Chemical Co., Milwaukee, 100% pure); 5, 8.5, and 24 mg/kg IM phenyl saligenin phosphate (PSP, Lark Enterprises, 100% pure); 3, 10, and 30 mg/kg IP mipafox (Lark Enterprises, 100% pure); 600, 1000, and 2000 mg/kg PO malathion (American Cyanamid, Pearl River, NY, 88% pure); 5, 10, and 30 mg/kg IP dichlorvos (Fermenta Animal Health, Kansas City, MO, 96% pure); and 30, 70, and 160 mg/kg IP carbaryl (Rhone Poulenc, Research Triangle Park, NC, 99% pure). The highest dosages of the test compounds were based on ability to cause lethality due to cholinergic poisoning or on the ability to inhibit neurotoxic esterase (a predictor of delayed neuropathy) by at least 70%[14,15]. The lowest dosages were based on capability to inhibit either neurotoxic esterase or acetylcholinesterase by at least 25%. Routes of administration were based on previous studies with these compounds[13–15,22–24]. Solutions were prepared in corn oil (TOTP, DFP, malathion, dichlorvos), saline (mipafox), or polyethylene glycol 400 (carbaryl) so that the mg/kg dosage was administered in a volume of 1 mL/kg. PSP was prepared in DMSO and given IM in a volume of 0.25 mL/kg.

Control rats received equivalent volumes of solvent. All rats were pretreated with atropine sulfate (Vetco, St. Joseph, MO), 20 mg/kg IP (solution strength 7.5 mg/mL, pH 7.4) 15 minutes before these compounds were administered. This pretreatment did not affect parameters of the functional observational battery in control rats. Atropine, 10 to 20 mg/kg, was readministered to those showing cholinergic signs (respiratory difficulty) within 1 hour of dosing. All groups of rats were given another 20 mg/kg dose of atropine 2 to 3 hours after treatment with the cholinesterase inhibitors. In addition, rats given TOTP, DFP, and malathion (as well as the control groups for studies with these agents) were given atropine 2 to 3 times daily for the next 24 to 48 hours. Four hours after administration of active toxicants DFP, PSP, mipafox, dichlorvos, and carbaryl and 48 hours after oral administration of protoxicants TOTP and malathion, 4 rats in each group were sacrificed by decapitation and tissues (brain and spinal cord) obtained for determination of esterase inhibition. Surviving rats were used for the FOB evaluation and for neuropathologic assessment.

Acetylcholinesterase and neurotoxic esterase activities were determined spectrophotometrically using the methods of Ellman et al. (1961) and Sprague et al. (1981) using 0.8 and 1.5 mg of brain and spinal cord, respectively, for acetylcholinesterase assays and 13 and 9.4 mg, respectively, for neurotoxic esterase assays.

The FOB used to compare behavioral effects was that initially described by Moser et al. (1988) and previously used in our laboratory (1992). In this FOB, parameters for evaluation are placed in the following categories: those indicating behavioral and CNS excitability (including home cage posture, activity, rearing, arousal, ease of handling and ease of removal from the cage, involuntary movements, stereotypy, bizarre behavior); those indicating autonomic effects (including salivation, lacrimation, pupil size, urination, defecation, piloerection); those indicating effects on muscle tone and equilibrium (including gait, fore- and hindlimb grip strengths, foot splay); those indicating effects on motor and sensory systems (including response to approach, touch, sound, and tail pinch); and those indicating effects on the general physiology of the rat (including weight, body temperature, respiration). Additional parameters evaluated and the categories in which they were placed when the FOB was used in this study included the following: oculocardiac reflex (autonomic sign), visual placing ability (motor and sensory system), tail limb reflex (motor and sensory system), righting reflex (muscle tone and equilibrium), and seconds and agility on a rotorod (muscle tone and equilibrium)[19]. These procedures were added to increase the number of parameters for evaluations, especially of muscle tone and equilibrium, and to increase similarity of clinical neurologic examination in the rodent to clinical neurologic examination of veterinary patients. The FOB was performed on each control rat and each rat treated with a cholinesterase inhibitor 1 day prior to toxicant administration and on days 7, 14, and 21 thereafter. Control rats were examined on the same days as the treated rats. The FOB was also performed 1 day after administration of PSP and mipafox and their respective control groups. Two individuals were trained as observers, and they were unaware of dose identification. All data were recorded on standardized data sheets and later entered into a computer for analysis.

As described by Moser et al. (1988), the sequence of the examination of each rat was arranged to progress from the least to the most interactive with the animal, and started with cage observations of posture (described with numbers from 1 to 7, with scores of 1 to 3 for normal postures and 4 to 7 for abnormal postures), activity (graded on a scale from 1 for stupor to 6 for very high activity, with 4 for normal rats), and involuntary movements (described with numbers from 1 to 8 with 1 for normal rats, which had no involuntary movements). The ease of removal of the rat from its cage was next graded from 1 (no resistance, not alert) to 7 (very difficult; normal rats graded as 2 to 3), and the presence or absence of autonomic signs, such as salivation, lacrimation, piloerection, pupil size, palpebral closure, response of the pupil to a small beam of light, and oculocardiac reflex was assessed[27]. Handling reactivity was graded with scores between 1 and 5, with 1 for those rats completely unreactive to handling and 5 for those rats that twisted and attempted to bite the observer (normal rats scored 2 to 3)[13]. Visual placing ability was then evaluated as present or absent by bringing one forelimb of the rat close to the edge of a table to see if the rat responded normally by reaching out a forelimb to place it on the table[28].

The rat was next placed in an open field (90 × 90 cm) for a 3-minute period, during which the presence or absence of involuntary movements were recorded and gait was ranked from 1 (normal) to a 4 (severely abnormal). The level of unprovoked activity and alertness, or arousal, was also ranked from 1 (very low) to 6 (very excited), with normal rats receiving scores of 4. The responses of the rat to the approach of a pen, a touch on its rump, a clicking sound near its ears, and a pinch on its tail were scored from 1 (no reaction) to 5 (exaggerated reaction), with normal rats scored as 2. The righting and tail limb reflexes were then evaluated as described previously[19], followed by weighing of the rat and measuring of its rectal temperature. Forelimb and hindlimb grip strengths were then determined as grams of force needed to pull the rat from a screen attached to a strain gauge. The number of

seconds that the rat could stay on a rotorod apparatus was recorded, and the rat's agility on this rod (35 mm long, 9.5 mm in diameter, 4 revolutions/minute) ranked from 1 (normal) to 4 (severely impaired)[19]. Finally, the lateral hindfeet toe pads were painted, the rat dropped from a height of 30 cm, and the landing foot splay diameter determined. Approximately 10 minutes were required to examine each rat.

Samples for neuropathologic examination were collected from rats anesthetized with pentobarbital and systemically perfused transcardially with saline followed by 5% glutaraldehyde in 0.1 M sodium phosphate buffer. Segments of the medulla, cervical, and lumbar spinal cord and branches of the tibial nerve that supply the gastrocnemius muscle were removed, postfixed in 2% osmium tetroxide, and embedded in Epon. One-μm-thick sections were stained with toluidine blue and safranin and examined with light microscopy. Additionally, portions of the cerebellum were removed, embedded, and 10-μm-thick hematoxylin and eosin (H & E)-stained sections were evaluated by light microscopy. Neuropathologic changes were compared among the rats given the various cholinesterase inhibitors by grading degeneration of myelinated fibers in the rostral fasciculus gracilis. Scoring criteria for fasciculus gracilis degeneration, based on those of Padilla and Veronesi (1985), were as follows: 0 = no lesions, 1 = occasional individual degeneration fibers, 2 = scattered groups or narrow rim of such fibers, 3 = more lateral and wider spread of degeneration, 4 = funnel-shaped region of degeneration involving most of the funiculus. Slides were evaluated by neuropathologists who did not know the treatment the rats had received, with decoding performed after the observations of the pathologists were recorded.

Effects of cholinesterase inhibitors on enzyme activities were analyzed using analyses of variance and Duncan's multiple range tests to distinguish differences among groups of rats in each experiment. $p < 0.05$ was considered statistically significant.

For the FOB study, parameters were categorized as continuous data, descriptive data, rank order data, and quantal data, and analyzed as conducted for previous studies[13,19]. Continuous response variables were analyzed via a general linear model, using SAS, available on the university's mainframe computer. Each rat's day 0 value was used as a covariate. Day 0 adjusted values were then subjected to a 2-way analysis of variance, using a group factor of dose and repeated measures across day. Univariate analyses of dose, comparing dose groups to each other and to the control group for each particular day, were performed when there was a significant overall dose effect. For all statistical tests, $p < 0.05$ was considered significant.

Descriptive (categorical), quantal (present/absent), and rank (ordinal) response variables were analyzed using the categoric data modeling procedure CATMOD on SAS. This procedure fits linear models to functions of response frequencies and can be used for weighted least-squares estimation of parameters for a wide range of general linear models, including repeated measures analyses. Using this system, as described previously[13,19], the descriptive variables were analyzed using marginal probability functions, while the quantal and rank response variables were analyzed using mean score functions.

RESULTS

The compounds tested were capable of causing significant, dose-dependent inhibition of acetylcholinesterase and neurotoxic esterase activities in brain and spinal cord 4 to 48 hours after they were administered (Table 1). Although given atropine before and after administration, rats given 3 mg/kg DFP, 600 mg/kg TOTP, 1000 mg/kg TOTP, 30 mg/kg mipafox, 30 mg/kg dichlorvos, 2000 mg/kg malathion, and 160 mg/kg carbaryl showed cholinergic signs, which resulted in lethality of some of the rats given the test compounds (Table 2). Mortality in those treatment groups occurred within 48 hours of dosing.

Rats given high doses of TOTP, DFP, and carbaryl weighed less than control rats 7 days after treatment, although no clinical signs of cholinergic poisoning were evident. These animals also weighed less than control rats 14 days after initiation of the experiment, but were not different at 21 days (Table 2). Weight was also less in groups of rats given lower doses of DFP and carbaryl 7 days after these test compounds were administered.

No parameters of the FOB considered indicative of autonomic dysfunction were present when the FOBs were done, even in rats given mipafox and PSP 1 day prior to testing. Rats given mipafox, however, did show tremors at 24 hours, a parameter of the FOB considered indicative of behavioral and CNS excitability[13,19]. Although signs of autonomic dysfunction were not present when the FOBs were done on Days 7, 14, 21, administration of each of the organophosphorus esters used in this study was capable of significantly affecting FOB parameters considered

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TABLE 1. EFFECT OF TOTP, DFP, PSP, MIPAFOX, MALATHION, DICHLORVOS, AND CARBARYL ON ESTERASE ACTIVITIES IN RAT BRAIN AND SPINAL CORD

Compound	Dosage (mg/kg)	Acetylcholinesterase (% Inhibition)[1]		Neurotoxic Esterase (% Inhibition)[2]	
		Brain	Spinal Cord	Brain	Spinal Cord
TOTP	0	8.2 ± 0.4[a,b]	8.2 ± 0.7[a]	9.6 ± 0.2[a]	10.2 ± 0.4[a]
	300	10.7 ± 1.4 (0%)[a]	3.8 ± 0.2 (54%)[b]	6.0 ± 0.5 (37%)[b]	5.3 ± 0.5 (48%)[b]
	600	7.3 ± 0.2 (12%)[b]	2.1 ± 0.1 (74%)[c]	4.1 ± 0.5 (57%)[c]	3.3 ± 0.2 (68%)[c]
	1000	1.8 ± 0.2 (78%)[c]	1.7 ± 0.1 (80%)[c]	1.1 ± 0.2 (88%)[d]	1.4 ± 0.3 (86%)[d]
DFP	0	8.3 ± 0.4[a]	5.8 ± 0.8[a]	9.6 ± 1.0[a]	8.7 ± 0.8[a]
	1	2.8 ± 0.1 (66%)[b]	1.8 ± 0.2 (73%)[b]	7.4 ± 1.4 (23%)[a,b]	6.5 ± 0.3 (25%)[b]
	2	1.2 ± 0.2 (86%)[c]	0.4 ± 0.2 (94%)[b,c]	4.8 ± 0.3 (50%)[b]	3.6 ± 0.3 (58%)[b,c]
	3	0.3 ± 0.1 (97%)[d]	0.2 ± 0.1 (97%)[c]	1.6 ± 0.4 (84%)[c]	1.2 ± 0.1 (86%)[c]
PSP	0	17.9 ± 0.2[a]	6.6 ± 0.3[a]	8.1 ± 0.9[a]	12.0 ± 0.9[a]
	5	16.6 ± 0.7 (7%)[a,b]	6.4 ± 0.3 (3%)[c]	5.5 ± 0.2 (32%)[b]	11.3 ± 0.6 (10%)[a]
	8.5	15.0 ± 0.2 (16%)[b]	6.1 ± 0.4 (7%)[a]	5.3 ± 0.5 (35%)[b]	5.9 ± 0.4 (51%)[b]
	24	15.6 ± 0.7 (13%)[b]	4.0 ± 0.1 (39%)[b]	1.9 ± 0.5 (77%)[c]	3.4 ± 0.6 (71%)[c]
Mipafox	0	14.0 ± 0.4[a]	13.4 ± 1.0[a]	8.8 ± 0.5[a]	12.7 ± 0.3[a]
	3	14.3 ± 0.4 (0%)[a]	8.6 ± 0.4 (36%)[b]	6.6 ± 0.3 (24%)[b]	10.8 ± 0.7 (16%)[a]
	10	8.8 ± 0.6 (36%)[b]	7.3 ± 0.1 (46%)[b]	3.3 ± 0.3 (63%)[c]	4.7 ± 0.4 (63%)[b]
	30	2.6 ± 0.3 (81%)[c]	3.1 ± 0.2 (77%)[c]	1.2 ± 0.1 (87%)[d]	1.2 ± 0.2 (90%)[c]

Compound	Dosage (mg/kg)	Acetylcholinesterase (% Inhibition)[1]		Neurotoxic Esterase (% Inhibition)[2]	
		Brain	Spinal Cord	Brain	Spinal Cord
Malathion	0	13.6 ± 1.4[a]	6.2 ± 0.4[a]	7.4 ± 0.4[a]	10.1 ± 1.1[a]
	600	10.0 ± 0.8 (26%)[b]	5.1 ± 0.5 (18%)[b]	6.1 ± 0.8 (19%)[a,b]	5.7 ± 0.5 (44%)[b]
	1000	10.6 ± 0.6 (22%)[a,b]	5.2 ± 0.3 (17%)[a,b]	4.9 ± 0.9 (35%)[b]	4.9 ± 0.6 (52%)[b]
	2000	6.0 ± 0.2 (56%)[c]	3.3 ± 0.3 (47%)[c]	1.9 ± 0.4 (75%)[c]	4.9 ± 0.8 (52%)[b]
Dichlorvos	0	11.9 ± 0.6[a]	6.8 ± 0.3[a]	8.1 ± 0.6[a]	11.5 ± 1.3[a]
	5	11.1 ± 0.9 (6%)[a]	5.1 ± 0.4 (25%)[b]	5.3 ± 0.5 (35%)[b]	7.7 ± 0.9 (33%)[b]
	10	9.4 ± 0.7 (21%)[b,c]	3.9 ± 0.3 (42%)[c]	2.0 ± 0.5 (76%)[c]	4.7 ± 0.9 (59%)[c]
	30	7.5 ± 0.6 (37%)[c]	3.8 ± 0.1 (44%)[c]	1.2 ± 0.1 (85%)[c]	4.9 ± 1.0 (57%)[c]
Carbaryl	0	13.5 ± 1.0[a]	6.4 ± 0.4[a]	10.1 ± 0.3[a]	12.4 ± 0.8[a]
	30	11.1 ± 0.3 (18%)[b]	4.3 ± 0.3 (33%)[b]	6.8 ± 0.6 (33%)[b]	8.4 ± 1.1 (32%)[b]
	70	8.1 ± 0.2 (40%)[c]	3.7 ± 0.1 (42%)[b]	4.1 ± 0.1 (60%)[c]	6.1 ± 0.7 (51%)[b,c]
	160	7.1 ± 0.5 (47%)[c]	2.2 ± 0.1 (65%)[c]	3.7 ± 0.4 (64%)[c]	3.8 ± 1.0 (69%)[c]

[1] Activity expressed as 10^{-8} mol acetylthiocholine hydrolyzed/min/mg protein 48 hr after administration of TOTP and malathion and 4 hr after administration of DFP, PSP, mipafox, dichlorvos and carbaryl. Mean ± SEM, N = 4. Percentage inhibition of activity compared to controls in parenthesis. Different superscripts (a,b,c, and d) in columns representing a single compound indicate activity differences among control and treated groups, ANOVA followed by Duncan's test, $p < 0.05$.

[2] Activity in mmol/min/mg protein. Tissue collected and analysis of data done as described for acetylcholinesterase.

TABLE 2. EFFECT OF SINGLE ADMINISTRATION OF TOTP, DFP, PSP, MIPAFOX, MALATHION, DICHLORVOS AND CARBARYL ON RAT BODY WEIGHTS

Compound	*Dosage (mg/kg)*	*# Surviving/ # Dosed*	*Weight Before Compound Administration (g)*[a]	*Weight After Compound Administration*		
				7 Days	*14 Days*	*21 Days*
TOTP	0	12/12	345 ± 10	370 ± 13	389 ± 14	414 ± 19
	300	12/12	333 ± 14	331 ± 12	362 ± 13	383 ± 14
	600	11/12	378 ± 8	358 ± 10	395 ± 10	425 ± 10
	1000	11/14	356 ± 13	312 ± 19[b]	348 ± 18[b]	378 ± 18
DFP	0	10/10	291 ± 4	316 ± 3	349 ± 4	374 ± 4
	1	12/12	286 ± 5	303 ± 7	336 ± 9	364 ± 10
	2	12/12	285 ± 5	282 ± 10[b]	326 ± 10	352 ± 9
	3	9/21	288 ± 10	247 ± 6[b]	295 ± 5[b]	350 ± 10
PSP	0	12/12	284 ± 6	319 ± 8	349 ± 3	378 ± 10
	5	12/12	257 ± 7	285 ± 6	309 ± 7	334 ± 9
	8.5	12/12	263 ± 6	285 ± 6	312 ± 8	338 ± 10
	24	12/12	279 ± 4	294 ± 4	322 ± 5	345 ± 7
Mipafox	0	12/12	302 ± 5	328 ± 6	354 ± 8	380 ± 10
	3	12/12	303 ± 5	330 ± 6	365 ± 7	398 ± 10
	10	12/12	293 ± 6	302 ± 10	334 ± 10	363 ± 11
	30	9/12	295 ± 2	308 ± 6	345 ± 4	378 ± 4

Compound	Dosage (mg/kg)	# Surviving/ # Dosed	Weight Before Compound Administration (g)[a]	Weight After Compound Administration		
				7 Days	14 Days	21 Days
Malathion	0	12/12	300 ± 7	313 ± 12	343 ± 12	365 ± 13
	600	12/12	293 ± 7	305 ± 9	341 ± 11	368 ± 17
	1000	12/12	292 ± 5	315 ± 5	344 ± 8	369 ± 17
	2000	11/28	298 ± 8	307 ± 5	327 ± 5[b]	348 ± 6
Dichlorvos	0	12/12	284 ± 6	324 ± 12	336 ± 9	360 ± 10
	5	12/12	285 ± 5	317 ± 8	336 ± 10	362 ± 14
	10	12/12	282 ± 5	317 ± 7	338 ± 9	358 ± 8
	30	9/12	272 ± 13	314 ± 10	336 ± 11	361 ± 14
Carbaryl	0	12/12	295 ± 7	324 ± 7	355 ± 8	384 ± 10
	30	12/12	284 ± 6	289 ± 9[b]	321 ± 6	347 ± 8
	70	12/12	290 ± 6	288 ± 8[b]	316 ± 6	342 ± 7
	160	11/12	292 ± 5	297 ± 8[b]	307 ± 10[b]	329 ± 12[b]

[a]Weight expressed as mean ± SEM.

[b]Significant difference from control group, analysis using a general linear model on SAS with each rat's weight before compound administration as a covariate. Weights of rats on days 7–21 were surviving rats not sacrificed for assay of esterase activities.

indicative of toxicant effects on behavioral and central nervous system excitability at one or more of these times (Table 3). These include changes in activity (TOTP, PSP, malathion, dichlorvos, carbaryl), ease of removal from cage (TOTP, DFP, malathion), reactivity when handled (TOTP, DFP, malathion), home cage posture (PSP, mipafox), arousal (mipafox), and involuntary movements (mipafox). Other parameters in the category of behavioral and CNS excitability approached significance (reactivity when handled, $p = 0.06$ in rats given mipafox, $p = 0.1$ in rats given TOTP, malathion, and carbaryl; activity, $p = 0.15$ in rats given dichlorvos; and arousal, $p = 0.16$ in rats given DFP). Parameters of Table 3 giving a dose response include the following: (1) TOTP effects on day 14 activity ($p < 0.01$) and gait score ($p < 0.02$); (2) DFP effects on day 7 and day 14 response to approach ($p < 0.02$); (3) PSP effects on activity and home cage posture on days 7 to 21 ($p < 0.05$); (4) mipafox effects on all parameters altered ($p < 0.05$); (5) malathion effects on activity ($p \leq 0.001$), response to touch ($p < 0.01$), and ease of removal from cage ($p < 0.05$); (6) dichlorvos response to approach ($p < 0.05$); and (7) carbaryl response to approach ($p < 0.01$).

Pathologic studies, using samples of the medulla, spinal cord, and tibial nerve branches collected 21 days after toxicant administration, indicated that TOTP, DFP, PSP and mipafox were capable of causing morphologic changes. In rats given TOTP, there was primary involvement of the rostral extent of the fasciculus gracilis at the medullary level. Large, pale-staining single or multiloculated, vacuolated, swollen myelinated axons were noted. In addition, some swollen and degenerating axons with more densely stained contents were observed. Some

TABLE 3. FOB PARAMETERS AFFECTED BY ADMINISTRATION OF TOTP, DFP, PSP, MIPAFOX, MALATHION, DICHLORVOS, OR CARBARYL TO MALE RATS

Chemical	*Dose (mg/kg)*	*Parameter Affected*[a]	*Effect Compared to Control*	*Days*			
				1	*7*	*14*	*21*
TOTP	300	Activity[b]	↓ Activity		X	—	—
	300	Ease of removal from cage[c]	↓ Resistance		X	—	—
	300	Reactivity when handled[d]	↓ Response		X	—	—
	600	Activity[b]	↓ Activity		—	X	—
	600	Ease of removal from cage[c]	↓ Resistance		X	X	—
	600	Reactivity when handled[d]	↓ Response		X	—	—
	600	Gait[e]	Abnormal		—	X	—
	1000	Activity[b]	↓ Activity		—	X	—
	1000	Gait[e]	Abnormal		—	X	—
DFP	1	Ease of removal from cage[c]	↑ Difficulty		—	—	X
	1	Reactivity when handled[d]	Exaggerated		—	—	X
	2	Ease of removal from cage[c]	↑ Difficulty		—	—	X
	2	Reactivity when handled[d]	Exaggerated		—	—	X
	2	Response to approach[f]	No Reaction		X	—	—
	3	Response to approach[f]	No reaction		X	—	—
	3	Response to approach[f]	Exaggerated		—	X	—
PSP	8.5	Activity[b]	↑ Activity	X	X	—	—
	24	Activity[b]	↑ Activity	—	X	X	X
	24	Home cage posture[g]	Abnormal	—	—	—	X
Mipafox	3	Home cage posture[g]	Abnormal	—	—	—	X
	30	Home cage posture[g]	Abnormal	X	—	—	X
	30	Arousal[n]	Increased	X	—	—	—
	30	Involuntary movement[i]	Tremors	X	—	—	—
	30	Response to touch[j]	No reaction	X	—	—	—

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affected fibers evolved to myelin ovoids, likely representing progression to Wallerian-like degeneration. Lesion scores in the rostral fasciculus gracilis of individual controls and treated rats are presented in Table 4.

The other neuropathy-inducing organophosphorus esters, DFP, PSP, and mipafox, mainly ellicited pale, swollen, vacuolated myelinated axonopathy in the rostral fasciculus gracilis 21 days after toxicant administration. Lesion scores of individual rats given these compounds are provided in Table 4. Mipafox elicited the most profound change in the fasciculus gracilis. Two of 4 rats given the high dosage of DFP also had Wallerian-like degeneration in the tibial nerve branches to the gastrocnemius muscle.

DISCUSSION

Results obtained in this study, which used 6 organophosphorus esters and a carbamate as test agents, indicated that an FOB and neuropathologic examination included in the neurotoxicity screening battery described in the recently revised federal guidelines for neurotoxicity testing[6] had value in identifying these compounds as neurotoxicants, even after overt clinical signs of cholinergic poisoning were no longer present. The use of the FOB to detect effects of cholinesterase inhibitors 7 to 21 days after exposure was, however, less persuasive as an indicator of neurotoxicity than when rats given the cholinesterase inhibitors were experiencing signs of cholinergic poisoning[13]. At that time, parameters indicative of effects on the autonomic nervous system were significantly affected by the test compound. However, even after signs of cholinergic poisoning were no longer evident, each of the 7 cholinesterase inhibitors used in the present study caused rats to exhibit alterations in one or more of the parameters of the FOB categorized as indicative of changes in behavioral or central nervous system excitability during examination 1 to 3 weeks later. Reactivity when handled appeared to be the one parameter most likely to be altered by all 7 of the test compounds ($p = 0.1$). All 4 of the neuropathy-inducing organophosphorus esters were identified on neuropathologic examination, although they could not be distinguished by the FOB alone. This may have been because both organophosphorus compounds that did and did not cause neuropathy (with the exception of PSP) caused sufficient inhibition of acetylcholinesterase to cause overt clinical signs of cholinergic poisoning and lethality.

Although neurotoxicity was indicated in rats given cholinesterase inhibitors, there is contrast with studies using some other toxicants[13,16–21] because alteration of a single parameter at a particular time point could not be specifically identified as indicative of delayed effects resulting from exposure to all of the cholinesterase inhibitors used as test compounds. Some investigators have suggested that changes of individual parameters within the 4 broad categories of the FOB used for studies such as this (autonomic, muscle tone and equilibrium, sensorimotor, and central nervous system) be pooled and a single index for each of the categories be used to identify

TABLE 4. LESION SCORES OF INDIVIDUAL RATS GIVEN NEUROPATHY-INDUCING ORGANOPHOSPHORUS ESTERS

		Toxicant Dosage[a]		
Toxicant	*Control*	*Low*	*Medium*	*High*
TOTP	1,1,1,2	1,1,2,2,2	1,1,1,2,4	1,1.5,2.5,3,4
DFP	0,0,0,0,0	0,0,0,1,2	0,1,1,1,1,1	1,1,1,3
PSP	0,0,1,1,2	1,1,1,2,2	1,1,2,2,2	1,1,1,2,2
Mipafox	0,0,1,1,1	0,0,1,1,1	0,1,1,1,3	3,3,3

[a]Dosages were 300, 600, and 1000 mg/kg for TOTP; 1, 2, and 3 mg/kg for DFP; 5, 8.5, and 24 mg/kg for PSP; and 3, 10, and 30 mg/kg for mipafox. Lesions of the fasciculus gracilis were evaluated without knowledge of the treatment. The scoring system ranged from 0 (no lesions) to 4 (involvement of most of the fasciculus). Scores are presented for each rat examined as the average from evaluations done by two neuropathologists.

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neurotoxicants[29]. If the FOB is, indeed, to be a screen for neurotoxicity, this approach may need to be considered for detection of the capability of cholinesterase inhibitors to cause long-term neurotoxic effects.

In this study, all 7 of the test compounds were classified as cholinesterase inhibitors. Even so, there are factors that differ among them that would contribute to temporal differences in expression of neurotoxic effects. These include the contributions of lipid solubility and route of administration to absorption, and differences in type and efficiency of pathways responsible for clearance. These could contribute to differences in time points at which neurotoxic signs were noted among the test compounds. Recovery from neurotoxicity is another factor to consider, as changes may be reversible. Esterases, for example, can reactivate and be resynthesized[11], and morphologic changes associated with organophosphate-induced delayed neuropathy in rats can regress[30].

Many behavioral changes have been reported in humans exposed to cholinesterase inhibitors even after cholinesterase activity was no longer depressed (i.e., insommia, anxiety, loss of concentration, impairment of judgment, fatigue[2–5]). A recent study also indicated that neuropsychologic performance was altered in a group of OP-poisoned individuals even as long as 2 years after a single toxic exposure[5]. Not all of the signs appeared in every individual and, when they did, the signs were unrelated to the intensity or duration of exposure. The signs also did not appear at any predictable time after exposure. Furthermore, some of these signs were reversible, while others were relatively persistent. Similarly sporadic signs in rats may contribute to difficulty in obtaining dose- and time-response relationships on the FOB in animals exposed to this class of compounds, although dose-response effects were noted on at least 1 parameter of the FOB for each of the 7 cholinesterase inhibitors used as test compounds for the present study. It is, however, difficult to extrapolate between the behavior of humans and rats, especially since human performance is often indicated by verbal or written responses. To be more useful for detecting behavior reported in humans after acute effects of cholinesterase inhibitors have passed, an FOB may need determinants not curently included in the FOB used here.

Weight as well as behavioral changes were monitored during this study. Decreases in weight gain after administration of high doses of organophosphorus esters and carbaryl appeared notable even weeks after exposure. Significance weight loss, therefore, appeared to be a parameter included with the FOB that was affected by administration of the majority (4 of 7) of the cholinesterase inhibitors. This may be related to the decreased activity that follows exposure to these substances and early gastrointestinal effects of cholinergic poisoning.

In addition to inhibition of acetylcholinesterase activities, this study indicated that a single administration of TOTP, DFP, PSP, mipafox, dichlorvos, malathion, and carbaryl could cause significant inhibition of neurotoxic esterase activity in the rat. Inhibition of neurotoxic esterase activity by 70% or more within 48 hours following a single administration of organophosphorus esters to rats and hens has been shown to be predictive for development of neuropathy[14,15,31]. In this study, inhibition of neurotoxic esterase was greater than 70% in brain and spinal cord samples taken from rats administered high doses of TOTP, DFP, PSP, and mipafox, and these 4 compounds were capable of causing lesions detected in neural tissue 21 days after their administration. In contrast to results noted in chickens when neurotoxic esterase inhibition was this high[24,32], lesions in rats were restricted both in region and severity. Furthermore, in contrast to hens, lesions in rats, particularly vacuolated myelinated axons, reflect an increase in quantity of lesions not morphologically distinct from background lesions in the rodent species[33]. The extent of lesions in rats and chickens has been compared with another organophosphorus ester, mipafox, and the tendency for these lesions to regress noted in the rat, but not in the chicken[31,33]. The regression of neuropathy in the rat could explain why some changes noted at 14 days (i.e., gait in rats given TOTP) were not noted at 21 days.

No neuropathologic lesions were seen in neural tissue from rats given malathion, dichlorvos, and carbaryl, even though inhibition of neurotoxic esterase activity in either brain or spinal cord approached or surpassed 70%. In hens, inhibition of neurotoxic esterase activity must be followed by "aging," a process whereby the inhibiting compound is irreversibly attached to the enzyme, before delayed neuropathy develops[30]. Not all organophosphorus compounds will interact with the enzyme in this manner, and carbamates cannot age; thus, such compounds are not expected to cause pathologic changes[30]. Although the capability of the organophosphorus esters and carbaryl to irreversibly inhibit rat brain and spinal cord neurotoxic esterase was not examined in this study, it is likely that this process would not be significant after administration of malathion, dichlorvos or carbaryl, as administration of these compounds did not cause neuropathy.

The results of this study with 7 cholinesterase inhibitors indicated that effects of the organophosphorus esters and carbaryl on muscle tone and equilibrium were minimal. This was true even of organophosphorus esters such as TOTP, DFP, PSP, and mipafox, which were shown to cause delayed peripheral neuropathy. The lack of notable effect on parameters indicative of muscle tone and equilibrium after TOTP and DFP contrasts with effects of other

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substances that cause peripheral neuropathy, such as acrylamide, 2,5-hexanedione and 3',3'-iminodiproprionitrile[16,17,19]. Muscle damage has been noted when cholinesterase inhibition is severe for at least 6 hours[34,35], but, if such damage did occur, it did not affect the FOBs done 1 to 21 days after toxicant administration in this study.

It appears from this study that behavioral effects determined using the FOB after administration of cholinesterase inhibitors to rats may have some value in the initial screening of this class of chemicals for potential acute and delayed neurotoxicity. The screening procedures recommended for neurotoxicity testing include use of both the FOB and neuropathologic evaluation[6]. It appears that neuropathologic evaluation is especially important if the rat is to be used to distinguish between organophosphorus compounds that cause delayed neuropathy and organophosphorus compounds that do not. This is because all but 1 of the test compounds used in this study caused cholinergic poisoning whether or not they were capable of inducing delayed neuropathy, and because no set of specific parameters of the FOB could be used to distinguish these two types of organophosphorus compounds. Further investigations both in animals and humans are needed to determine if changes in the FOB would indicate potential for long-term effects in people receiving toxic doses of cholinesterase inhibitors.

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REFERENCES

1. MURPHY, S.D. (1986). Toxic effects of pesticides, in *Casarett and Doull's Toxicology, the Basic Science of Poisons*. 3rd Ed. C.D. Klaassen, M.O. Amdur, and J. Doull (eds.). Macmillan: New York, pp. 519–581.
2. ECOBICHON, D.J., DAVIES, J.E., DOULL, J., EHRICH, M., JOY, R., MCMILLAN, D., MACPHAIL, R., REITER, L.W., SLIKKER, W., JR., and TILSON, H. (1990). Neurotoxic effects of pesticides, in *The Effect of Pesticides on Human Health*. S.R. Baker, and C.F. Wilkinson (eds.). Princeton Scientific Publishing: Princeton, NJ, pp. 131–199.
3. ECOBICHON, D.J., and JOY, R.M. (1982). Pesticides and Neurological Diseases. CRC Press: Boca Raton, FL.
4. SAVAGE, E.P., KEEFE, T.J., MOUNCE, L.M., HEATON, R.K., LEWIS, J.A., and BURCAR, P.J. (1988). Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch. Environ. Hlth. 43:38–45.
5. ROSENSTOCK, R., KEIFER, M., DANIEL, W.E., MCCONNELL, R., CLAYPOOLE, K., and The Pesticide Health Effects Study Group (1991). Chronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 338:223–227.
6. U.S. ENVIRONMENTAL PROTECTION AGENCY (1991). Pesticide assessment guidelines, subdivision E. Hazard evaluation: Human and domestic animals. Addendum 10: Neurotoxicity, series 81, 82 and 83. Office of Prevention, Pesticides and Toxic Substances, Washington, D.C. EPA 540/09-91-123. Available from: NTIS, Springfield, VA PB91-154617.
7. LEHOTZKY, K. (1982). Effect of pesticides on central and peripheral nervous system function in rats. Neurobehav. Toxicol. Teratol. 4:665–669.
8. KARCZMAR, A.G. (1981). Acute and long lasting central actions of organophosphorus agents. Fundam. Appl. Toxicol. 4:S1–S17.
9. GIARDINI, V., MENEGUZ, A., AMORICO, L., DE ACETIS, L., and BIGNAMI, G. (1982). Behaviorally augmented tolerance during chronic cholinesterase reduction by paraoxon. Neurobehav. Toxicol. Teratol. 4:335–345.
10. BACCAFUSCO, J.J., GRAHAM, J.H., VAN LINGEN, J., and ARONSTAM, R.S. (1989). Protection afforded by clonidine from the acute and chronic behavioral toxicity produced by the cholinesterase inhibitor soman. Neurotoxicol. Teratol. 2:39–44.
11. RAFFAELE, K., HUGHEY, D., WENK, G., OLTON, D., MODROW, H., and MCDONOUGH, J. (1987). Long-term behavioral changes in rats following organophosphonate exposure. Pharmacol. Biochem. Behav. 27:407–412.

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12. SIKORA-VANMETER, K.C., WIERWILLE, R.C., WILLETTS, E.C., and VANMETER, W.G. (1987). Morphological evidence for increased protein synthesis in CNS neurons after soman exposure. Fundam. Appl. Toxicol. **8**:23–38.
13. MOSER, V.C., MCCORMICK, J.P., CREASON, J.P., and MACPHAIL, R.C. (1988). Comparison of chlordimeform and carbaryl using a functional observational battery. Fundam. Appl. Toxicol. **11**:189–206.
14. PADILLA, S. and VERONESI, B. (1985). The relationship between neurological damage and neurotoxic esterase inhibition in rats acutely exposed to tri-ortho-cresyl phosphate. Toxicol. Appl. Pharmacol. **78**:78–87.
15. VERONESI, B., PADILLA, S., and LYERLY, D. (1986). The correlation between neurotoxic esterase inhibition and mipafox-induced neuropathic damage in rats. Neurotoxicology **7**:207–216.
16. MOSER, V.C. (1989). Screening approaches to neurotoxicity: A Functional Observational Battery. J. Am. Coll. Toxicol. **8**:85–93.
17. MOSER, V.C. (1991). Prolonged effects in rats of short–term exposure to 3',3'-iminodiproprionitrile (IDPN) using a neurobehavioral screening battery. Toxicologist **11**:167.
18. MOSER, V.C., SUMRELL, B.M., and MACPHAIL, R.C. (1992). Profiles of chemical effects using a neurobehavioral screening battery. Toxicologist **12**:349.
19. SHELL, L., ROZUM, M., JORTNER, B.S., and EHRICH, M. (1992). Neurotoxicity of acrylamide and 2,5-hexanedione in rats evaluated using a functional observational battery and pathological examination. Neurotoxicol. Teratol., in press.
20. PETTERSEN, J.C., CHOW, E., and LEAHY, C.L. (1992). A neurobehavioral validation study in the CD rat. Toxicologist **12**:349.
21. BEYROUTY, P.C., ROBINSON, K.J., and BROXUP, B.R. (1992). Positive control neurotoxicity studies for EPA (FIFRA) guidelines. Toxicologist **12**:349.
22. COHEN, S.D. and EHRICH, M. (1976). Cholinesterase and carboxylesterase inhibition by dichlorvos and interactions with malathion and tri-ortho-tolyl phosphate. Toxicol. Appl. Pharmacol. **37**:39–48.
23. BROWN, C., GROSS, W.B., and EHRICH, M. (1986). Effects of social stress on the toxicity of malathion in young chickens. Avian Dis. **30**:679–682.
24. EHRICH, M., JORTNER, B.S., and GROSS, W.B. (1988). Types of adrenocorticoids and their effect on organophosphorus-induced delayed neuropathy in chickens. Toxicol. Appl. Pharmacol. **92**:214–223.
25. ELLMAN, G., COURTNEY, K.D., ANDRES, V., JR., and FEATHERSTONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. **7**:88–95.
26. SPRAGUE, G.L., SANDVIK, L.L., BROOKINS-HENDRICKS, M.J., and BICKFORD, A.A. (1981). Neurotoxicity of two organophosphorus ester flame retardants in hens. J. Toxicol. Environ. Health **8**:507–518.
27. DEJONG, R.M. (1979). *The Neurological Examination*. 4th Ed. Harper & Row: New York.
28. O'DONOGHUE, J.L. (1989). Screening for neurotoxicity using a neurologically based examination and neuropathology. J. Am. Coll. Toxicol. **8**:97–115.
29. HOE, C.L., MORRISSEY, R.E., and SOPER, K.A. (1992). Functional indices simplify statistical analysis of the functional observational battery (FOB). Toxicologist **12**:350.
30. CARBONI, D., EHRICH, M., DYER, K., and JORTNER, B.S. (1992). Comparative evolution of lesions in mipafox-induced delayed neuropathy in rats and hens. Neurotoxicology, in press.
31. JOHNSON, M.K. (1982). The target of initiation of delayed neurotoxicity by organophosphorus esters: Biochemical studies and toxicological applications, in *Reviews in Biochemical Toxicology*. Vol. 4. E. Hodgson, J.R. Bend, and R. Philpot (eds.). Elsevier: New York, pp. 141–212.
32. DYER, K., JORTNER, B.S., SHELL, L., and EHRICH, M. (1992). Comparative dose-response studies of organophosphorus ester-induced delayed neuropathy in rats and hens administered mipafox. Neurotoxicology, in press.
33. EISENBRANDT, D.L., MATTSSON, J.L., ALBEE, R.R., *et al.* (1990). Spontaneous lesions in subchronic neurotoxicity testing of rats. Toxicol. Pathol. **18**:154–164.
34. DETTBARN, W.D. (1984). Pesticide induced muscle necrosis: Mechanisms and prevention. Fundam. Appl. Toxicol. **4**:S18–S26.
35. PATERSON, G.T., GUPTA, R.C., MISULIS, K.E., and DETTBARN, W.D. (1988). Prevention of diisopropylphosphorofluoridate (DFP)-induced skeletal muscle fiber lesions in rats. Toxicology **48**:237–244.

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