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NEUROTOXIC EFFECTS OF COMBINED TREATMENT OF 2,5-HEXANEDIONE AND TRIETHYLLEAD CHLORIDE

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Triethyllead chloride and 2,5-hexanedione are known neurotoxicants that apparently work through separate mechanisms. The effect of combined treatment of triethyllead chloride and 2,5-hexanedione for 6 weeks on Fischer 344 rats was investigated. Ten rats were given 0.7 mg/kg triethyllead chloride in a volume of 2 ml/kg by gavage while another group was given 0.5% 2,5-hexanedione in drinking water and vehicle by gavage (2 ml/kg). A third group was given a combination of the two treatments. A fourth group served as controls and was given vehicle by gavage. 2,5-Hexanedione produced a reversible loss of body weight, decreased grip strength, and decreased horizontal motor activity. Triethyllead chloride alone increased hot-plate latencies. Triethyllead chloride and 2,5-hexanedione treated animals recovered 4 weeks after cessation of treatment. Neither treatment alone produced fatalities. In combination (2,5-hexanedione + triethyllead chloride) decreases in body weight appeared additive and there was a 40% mortality by 6 weeks of dosing. Rats given the combined treatment had significant loss of both grip strength and increased hot-plate latencies. Neurobehavioral deficits and neuropathological changes were greater in the combined treatment with 2,5-HD and TEL than when either chemical was used alone; there was little indication of a synergistic interaction between these two types of neurotoxicants.

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INTRODUCTION

Triethyllead chloride and 2,5-hexanedione are known to produce behavioral, pathological, and biochemical alterations which are specific for the individual toxicant (Tilson et al., 1982; Krigman et al., 1980; Abou-Donia 1983; Spencer et al., 1980). Both organolead compounds and aliphatic hexacarbons are widely used in industry, chemistry, and agriculture with the potential for exposure to more than one of these compounds. Although the neurotoxicity produced by either triethyl lead or 2,5-hexanedione alone has been characterized, the toxicological consequences of simultaneous exposure to these compounds is unknown. Neurological effects produced by these compounds are characterized by a Wallerian-type degeneration of axons and myelin in the central and peripheral nervous system; however, these changes differ in both the morphology and distribution of the lesion (Abou-Donia et al., 1985a; Nickolwitz, 1975). Dissimilarities between these two classes of chemicals imply that they produce their neurotoxicity by different mechanisms.

Several mechanisms of aliphatic hexacarbon neurotoxicity have been proposed. The most attractive of these include a direct binding of 2,5-hexanedione to cytoskeletal elements (i.e., neurofilaments, Graham and Abou-Donia, 1980; DeCaprio et al., 1981; Sayre et al., 1985), resulting in crosslinking of neurofilaments, which has previously been demonstrated (Lapadula et al., 1986) or an alteration in protein phosphorylation (Patton et al., 1983; Sayre et al., 1985; Lapadula et al., 1986).

Organoleads have been demonstrated to produce several biochemical effects; however, the mechanism is unknown. An uncoupling of oxidative phosphorylation (Aldridge et al., 1962), inhibition of amino acid transport (Vardanis and Quastel, 1961), interference with the disposition of trace metals (Nickolwitz and Yeager, 1973), and inhibition of microtubule assembly (Roderer and Doenges, 1983), have all been proposed.

Potentiation of neurotoxicity occurs when both substances are neurotoxic and the action of simultaneous exposure produces effects which are greater than what would be expected from a simple summation (Abou-Donia et al., 1985a). This may occur from an interaction at the target site, an interaction with drug metabolizing enzymes, a modification of the absorbtion site changing the body burden, or alteration of the chemical or physical characteristics of the compounds leading to an alteration in biological action.

The present study was designed to investigate the neurotoxic effects of concurrent exposure of triethyllead chloride and 2,5-hexanedione. Specifically, we investigated the: (1) time course of neurotoxicity, (2) extent of neurologic deficit, (3) severity of histopathological lesions, and (4) effects on the drug metabolizing system.

METHODS

Chemicals

Triethyllead chloride was obtained from Ventron Corporation (Alfa Products, Danvers, Mass.) and cold washed in ether solution before usage. 2,5-Hexanedione (98%) was obtained from Eastman Kodak Co. (Rochester, N.Y.).

Subjects

Forty male Fischer-344 rats (Harlan Laboratories, Indianapolis, Ind.) approximately 8–10 weeks of age were used as subjects. Animals were housed in groups of 5 in plastic cages in a temperature controlled room (21–23°C) with a 12 h light/dark cycle before and during the experiment. They were supplied with feed (Purina Rat Chow, Ralston-Purina Co., St. Louis, Missouri) and water ad libitum unless otherwise specified.

Treatment of Animals

Each treatment group consisted of 10 rats. One group was dosed by gavage with triethyllead chloride for five days per week with 0.7 mg/kg in a volume of 2 ml (Tilson et al., 1982). A second group was treated with 0.5% 2,5-hexanedione in their drinking water for 7 d/wk and 2 ml/kg of distilled water by gavage. A third group was exposed to both triethyllead chloride and 2,5-hexanedione, while a fourth group (controls) received 2 ml/kg distilled water and freely available tap water. All animals were treated for six weeks and tested behaviorally each week. Behavioral tests were conducted prior to the dose given on the day of testing. Five animals from control, 2,5-hexanedione, and triethyllead chloride groups were then sacrificed for histopathology and enzyme assays. Surviving animals receiving vehicle, triethyllead or 2,5-hexanedione were retested behaviorally four weeks after cessation of dosing. There was not a sufficient number of surviving rats in the combination group to test behaviorally. After the last behavioral test during the recovery period, the remaining rats from the triethyllead and 2,5-hexanedione treatment groups were sacrificed for histopathology.

Procedures

Neurological and neurobehavioral testing Four days prior to dosing, rats were assessed for neurological and behavioral functioning. Briefly, in addition to body weight, a battery of tests were used in this experiment and are described elsewhere (Tilson et al., 1982). The battery consisted of measurements of grip strength, sensitivity to noxious stimuli, and motor activity. Forelimb and hindlimb grip strength were measured by commercially available units (Meyer et al., 1979). Hot-plate latencies were measured by placing the rats on a commercially avail-

able unit (Techni-Lab Instruments, Pequannock, N.J.) set at a nominal 60°C. The time for the rat to elevate one of its hindlimbs and lick its paw was recorded. Horizontal and vertical movements occurring over a 3 min period were measured automatically using a commercially available activity monitor (Automex, Columbus Instruments, Columbus, Ohio).

Histopathology and enzyme assays Livers were removed and a microsomal fraction prepared (Schenkman and Cinti, 1978). Cytochrome P-450 content was measured by the method of Omura and Sato (1964). Cytochrome *c* reductase activity was measured by the method of Guengerich (1982). Brain, spinal cord, and sciatic nerve were fixed with buffered formalin. Cross sections and parasagittal longitudinal sections near the midline were prepared from cervical, thoracic, and lumbar regions of the spinal cord. Peripheral nerves were prepared in cross and longitudinal sections. Tissues were dehydrated in graded ethanol and imbedded in paraffin or glycol methacrylate. Paraffin sections (8 μm) from spinal cord were stained with hematoxylin and eosin (H and E) combined with luxol fast blue (LFB) or Glees stain. Sections from peripheral nerves also were stained with Holmes stain. Glycol methacrylate sections of spinal cord and peripheral nerve (1 to 2 μm) were stained with Glees stain.

Statistical Analysis

Data involving more than two groups were analyzed for overall treatment effects using Analysis of Variance (ANOVA). If repeated testing on the same animals was done, data were assessed using a repeated measures ANOVA (Winer, 1962). If an overall treatment effect or significant interaction with treatment was observed, then differences between groups were assessed using Fisher's Least Significant Difference Test (LSD) (Miller, 1966). The accepted level of significance was $p < 0.05$ in all experiments.

RESULTS

Repeated exposure to 2,5-hexanedione resulted in a significant decrease in body weight evident as early as 1 wk after initiation of exposure (Fig 1). Repeated exposure to triethyllead also decreased body weight beginning 2 weeks after initiation of exposure and for the remainder of the experiment. The combination of triethyllead and 2,5-hexanedione resulted in a greater loss of body weight than either treatment given alone. In addition, rats receiving the combined treatment showed mortality (3 died by wk 5 of exposure with an additional animal dying by wk 6 of treatment) that was not evident in the other groups. Assessment of the animals receiving either triethyllead or 2,5-hexanedione 4 wk after cessation of exposure indicated no residual loss of

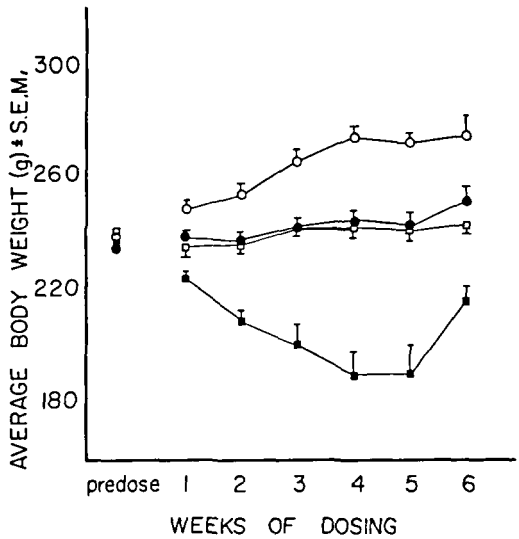

FIGURE 1. Effect of treatment of animals with 2,5-hexanedione and triethyllead chloride on body weights. Open circles, control; closed circles, triethyllead chloride; open squares, 2,5-hexanedione; closed squares, combined treatment.

body weight; in fact, the rats treated with triethyllead weighed significantly more than controls 4 wk after cessation of dosing (Table 1).

Figure 2 shows that repeated exposure to 2,5-hexanedione decreased both fore- and hindlimb grip strength, while triethyllead had no significant effect on grip strength. Rats receiving the combined treatment also had decreased grip strength following repeated exposure. After 5 wk of exposure, rats receiving both triethyllead and 2,5-hexanedione had significantly lower scores than animals receiving only 2,5-hexanedione. Four wk after cessation of exposure, rats having received either triethyllead or 2,5-hexanedione did not differ significantly from vehicle controls (Table 1).

TABLE 1. Body Weights and Neurobehavioral Measurements 4 Weeks After Cessation of Dosing

	Average Measurement (x ± SE)[a] Treatment		
	Vehicle	TEL	2,5-HD
Body Weights	229 ± 8	322 ± 5[b]	295 ± 8
Horizontal Activity (Counts/3 min)	390 ± 49	410 ± 97	373 ± 112
Vertical Activity (Counts/3 min)	5 ± 1.1	3 ± 0.4	1 ± 0.2[b]
Forelimb Grip (G)	720 ± 15	734 ± 25	722 ± 20
Hindlimb Grip (G)	390 ± 33	396 ± 25	348 ± 15
Hot-Plate Latency (Sec)	8.4 ± 1.1	9.5 ± 0.4	9.4 ± 0.9

[a] Data are for 5 rats per treatment group.
[b] Significantly different from vehicle ($p < 0.05$ following one way ANOVA).

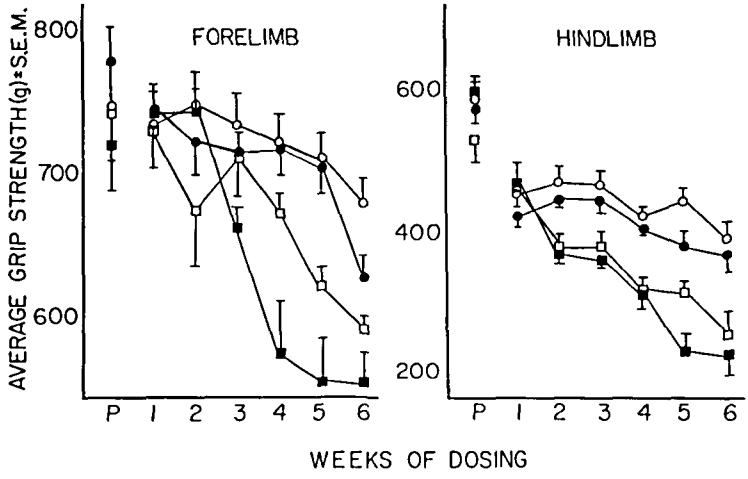

FIGURE 2. Average grip strength scores of animals treated with triethyllead chloride or 2,5-hexanedione. Symbols are identical to Fig. 1.

Figure 3 shows that 2,5-hexanedione did not significantly alter latencies to respond to a noxious thermal stimulus, while triethyllead significantly increased hot-plate latencies. Animals receiving the combined treatment did not differ from those animals receiving only triethyllead. The effects of triethyllead on hot-plate latencies were not evident 4 wk after cessation of dosing (Table 1).

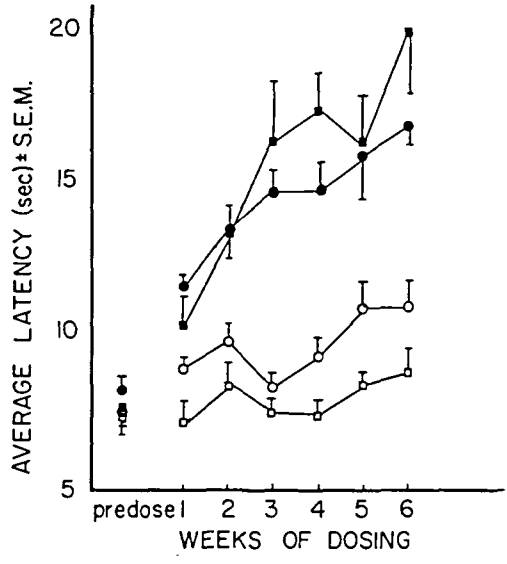

FIGURE 3. Hot-plate latencies of animals treated with triethyllead or 2,5-hexanedione. Symbols are identical to Fig. 1.

Repeated exposure to 2,5-hexanedione significantly decreased horizontal motor activity beginning 2 wk after initiation of dosing, while rats receiving triethyllead or the combined treatment did not differ from controls (Fig 4). Analysis of variance indicated no significant treatment or interaction of treatment with day of testing on vertical motor activity. Table 1 shows that decreases in locomotor activity observed in 2,5-hexanedione were not evident 4 wk after cessation of dosing.

Histopathology failed to show any overt damage in the brains or spinal cords of treated animals. Equivocal changes occurred in the spinal cords of two animals receiving triethyllead and in one animal receiving the combined treatment. Histological damage was seen in the sciatic nerves of three animals receiving the combined treatment which consisted of Wallerian-type degeneration and swollen axons (Fig 5). The degree of damage observed in the sciatic nerves of animals receiving the combined was also greater in severity than in those receiving either treatment alone. Only one animal showed histological damage in 2,5-hexanedione or triethyllead chloride (Table 2).

Cytochrome P-450 content and cytochrome *c* reductase activity were not significantly altered by any of the treatments.

DISCUSSION

Potentiation of neurotoxicity has been previously shown to occur between different neurotoxicants (Abou-Donia et al., 1985a,b). This study was undertaken to investigate the possible interactions, if any, between triethyllead chloride and 2,5-hexanedione. In the current

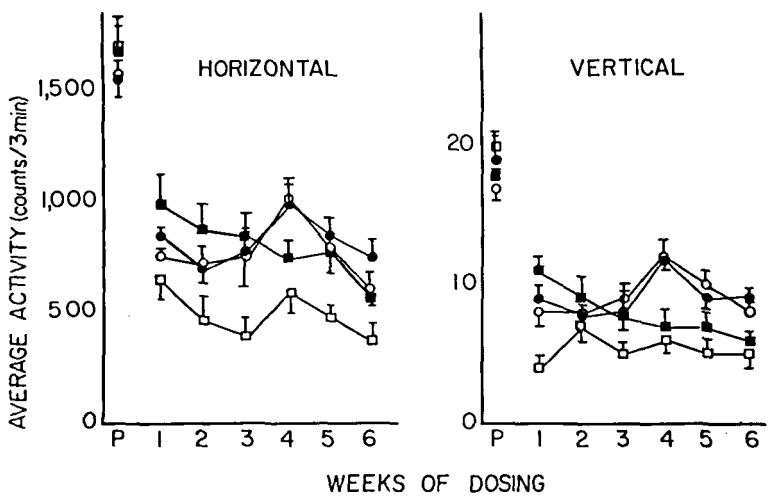

FIGURE 4. Vertical and horizontal activity of animals treated with triethyllead and 2,5-hexanedione. Symbols are identical to Fig. 1.

FIGURE 5. Wallerian degeneration in the sciatic nerve of an animal with the combined treatment. (Glees, plastic embedded; ×440).

study a number of neurological, neurobehavioral, and biochemical tests were undertaken to assess the interaction between these compounds. In addition, neuropathological examination of the animals was undertaken to determine the contribution of each neurotoxicant to the development of these alterations.

TABLE 2. Histological Damage Produced by 2,5-Hexanedione and Triethyllead

Treatment		Spinal Cord						Sciatic Nerve					
		Wallerian degeneration			Swollen axons			Wallerian degeneration			Swollen axons		
HD	TEL	++	+	±	++	+	±	++	+	±	++	+	±
0	0	–	–	–	–	–	–	–	–	–	–	–	–
+	0	–	–	–	–	–	–	–	1	–	–	1	–
0	+	–	–	2	–	–	1	–	–	1	–	–	–
+	+	–	–	1	–	–	–	3	–	–	–	1	–
Recovery													
0	0	–	–	–	–	–	–	–	–	–	–	–	–
+	0	–	–	–	–	–	1	–	–	1	–	–	–
0	+	–	–	–	–	–	1	–	–	–	–	–	1

HD indicate 2,5-hexanedione and TEL indicates triethyllead. There were 5 rats per group.
– = no changes.
± = equivocal changes in less than 2 axons.
+ = definite changes in less than 2 axons.
++ = definite changes in 3 or more axons.

Neurobehavioral testing indicated the treatment of animals with triethyllead chloride or 2,5-hexanedione resulted in specific neurological alterations. Triethyllead chloride had its most dramatic effect on hot-plate latencies, which were increased, (Walsh et al., 1984) while 2,5-hexanedione decreased grip strength (Gerber and O'Shaughnessy, 1986). These effects were reversible as demonstrated by the recovery of the animals 4 wk after cessation of the dosing. There was little evidence of a synergistic interaction between triethyllead and 2,5-hexanedione at the behavioral level. Animals receiving both triethyllead and 2,5-hexanedione were significantly more affected on the grip strength measure after 5 wk of dosing than animals receiving only 2,5-hexanedione. This may be associated with the increased incidence of severe Wallerian degeneration in rats receiving the combined treatment, as compared to either treatment alone. Body weight measurements indicated an additive effect of the combined treatment with the mortality rate in the combined treatment due to the severe loss in body weight. The absence of biochemical alterations in the drug metabolizing enzymes demonstrate, that at least at the doses administered in this study, there were no interactions of the chemicals at this level.

Although other neurotoxicants have been demonstrated to produce synergistic or potentiating effects (Abou-Donia et al., 1985a,b), there was only marginal evidence of it occurring in the present study. These data support the hypothesis that these neurotoxicants produce their toxicity through two separate and independent mechanisms, thus leading to the finding of an additive effect of their toxicity. However, the potential interaction between triethyllead and 2,5-hexanedione on sciatic nerve pathology and grip strength deserves additional research.

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