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Effects of Single or Repeated Dermal Exposure to Methyl Parathion on Behavior and Blood Cholinesterase Activity in Rats

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Key Words
Methyl parathion · Acetylcholinesterase · Dermal exposure · Learning · Memory · Motor function

Abstract
The effects of a single or repeated dermal administration of methyl parathion on motor function, learning and memory were investigated in adult female rats and correlated with blood cholinesterase activity. Exposure to a single dose of 50 mg/kg methyl parathion (75% of the dermal LD_{50}) resulted in an 88% inhibition of blood cholinesterase activity and was associated with severe acute toxicity. Spontaneous locomotor activity and neuromuscular coordination were also depressed. Rats treated with a lower dose of methyl parathion, i.e. 6.25 or 12.5 mg/kg, displayed minimal signs of acute toxicity. Blood cholinesterase activity and motor function, however, were depressed initially but recovered fully within 1–3 weeks. There were no delayed effects of a single dose of methyl parathion on learning acquisition or memory as assessed by a step-down inhibitory avoidance learning task. Repeated treatment with 1 mg/kg/day methyl parathion resulted in a 50% inhibition of blood cholinesterase activity. A decrease in locomotor activity and impairment of memory were also observed after 28 days of repeated treatment. Thus, a single dermal exposure of rats to doses of methyl parathion which are lower than those that elicit acute toxicity can cause decrements in both cholinesterase activity and motor function which are reversible. In contrast, repeated low-dose dermal treatment results in a sustained inhibition of cholinesterase activity and impairment of both motor function and memory.

Introduction

Organophosphorous insecticides produce their toxicity through inhibition of acetylcholinesterase, thereby causing accumulation of acetylcholine at peripheral and central cholinergic synapses and resulting in overstimulation of the cholinergic system [40]. Acute exposure to a high dose of an organophosphorous compound can cause abdominal cramps, nausea, vomiting, diarrhea, increased salivation, sweating, miosis, muscle twitching, convulsion, respiratory failure and death [39, 40]. In addition to these somatic signs and symptoms, high-level acute or repeated exposure to organophosphorous compounds can

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induce behavioral changes in humans, including difficulty in concentrating, decreased cognition, muscular weakness, confusion, anxiety and memory impairment [14, 23]. Changes in motor function and deficits in the performance of learning tasks have also been observed in experimental animals [7–9, 19, 22, 24, 26, 36].

Methyl parathion (O-O-dimethyl-O-*p*-nitrophenyl phosphorothioate), an organophosphorous compound that may only be used lawfully as an insecticide on agricultural crops, has received attention recently as a consequence of its illegal use as a pesticide in private homes and other buildings in several states of the USA [1, 17]. This compound can be absorbed through the skin as well as through both the respiratory system and gastrointestinal tract, although dermal absorption tends to be slow compared to absorption by other routes [12, 15, 38]. Decontamination of skin by common soap/water washing procedures is difficult, so skin absorption may be prolonged after initial exposure [12, 25]. High temperatures increase skin absorption, as does the presence of dermatitis [25]. Dermal absorption has been identified as the primary route of methyl parathion intake as a consequence of occupational exposure [29, 42, 43]. Lack of immediate recognition of exposure to methyl parathion, coupled with poor decontamination, is characteristic of recent widespread human exposure to illegal methyl parathion spray; also, transdermal absorption of methyl parathion may have been a significant feature of these exposures [1, 17]. However, there are limited data documenting the health impact of dermal exposure to methyl parathion that has occurred as a result of illegal domestic spraying or occupational exposure. In the present study, the effects of a single or repeated dermal exposure to methyl parathion on rat spontaneous locomotor activity, neuromuscular coordination, learning and memory were investigated. The examination of motor function was selected because the results of epidemiological studies provide evidence for a link between exposure to organophosphorous insecticides and Parkinson's disease [22]. It has been shown that cholinesterase inhibitors can induce Parkinson's-like motor dysfunction, and parkinsonian symptoms can be exacerbated by cholinergic stimulation [10, 30, 32]. The behavioral tests were also correlated with changes in blood cholinesterase activity. Such changes have proven to be useful in the clinical diagnosis of organophosphorous compound intoxication and in estimating the changes in brain cholinesterase activity following exposure to organophosphorous compounds [34].

Materials and Methods

Animals and Chemicals

Female Sprague-Dawley rats (175–200 g) were purchased from Harlan Sprague Dawley Inc. (Indianapolis, Ind., USA). Rats were housed in groups of 3 before treatment and individually after the onset of treatment. Animals were housed in a room maintained at 21 ± 1 °C with an automatic 12-hour light and dark cycle and free access to food and tap water. Methyl parathion was purchased from Chem Service (West Chester, Pa., USA). Other chemicals were obtained from Sigma Chemicals Co. (St. Louis, Mo., USA).

Administration of Methyl Parathion

Rats were lightly anesthetized with halothane, and an area of the back between the scapulae was shaved. Methyl parathion was applied to the shaven area in 100 µl of ethanol. For a single dermal exposure, rats were treated with a single dose of 6.25, 12.5 or 50 mg/kg methyl parathion. Control animals were treated with an equal volume of ethanol only. Blood samples were collected before and 2, 7, 14, 21 and 28 days following methyl parathion administration. For repeated dermal exposure, rats received 0.1 or 1 mg/kg/day methyl parathion for 28 days. Blood samples were collected before and 1, 7, 14, 21 and 28 days after the onset of treatment.

Behavioral Tests

For rats given a single dose of methyl parathion, motor function was tested before and 2, 7, 14, 21 and 28 days following methyl parathion administration. Performance of a learning task was evaluated 28 days after treatment. For rats dosed repeatedly with methyl parathion, behavioral tests were performed 28 days after methyl parathion administration. All the behavioral tests were performed between 9.00 and 12.00 a.m.

To test the effect of methyl parathion on spontaneous locomotor activity, an open field test was performed in a cardboard box (50 × 50 × 46 cm high) with clear Plexiglas on the inner surface. The floor of the box was divided into 25 equal squares. An animal was placed in the box and allowed to walk freely for 3 min. Then, the number of squares crossed with all paws during the next 2 min was recorded. A 90% alcohol solution and soapy water were used to clean the inner surface of the box between trials to remove interfering odors left by the previous animal.

Neuromuscular coordination was assessed using a rota-rod. Each animal was placed on a rota-rod treadmill (Rotamex V-EE/85, Columbus Instruments, Columbus, Ohio, USA), and the speed was accelerated from 0 to 80 rpm over a period of 4 min. The maximum speed obtained before an animal slipped from the treadmill was recorded. Rats were trained for 2 days before data recording and were considered trained when the standard deviation of the mean of 3 measurements was not more than 10 rpm.

The effects of methyl parathion on learning and memory were tested using a step-down inhibitory avoidance learning task. The apparatus used was a 40 × 25 × 25 cm acrylic chamber consisting of a floor made of parallel 2-mm-caliber stainless steel bars spaced 1 cm apart. An electric shock was administered through the floor bars. A 2.5-cm-high, 8 × 25 cm wooden platform was placed on the left extreme of the chamber. Each animal was gently placed on the platform. Upon stepping down, the animal immediately received a single 80-volt footshock. If the animal did not return to the platform, the footshock was repeated every 5 s. An animal was considered to have learned the avoidance task if it remained on the platform for more

than 2 min. The number of footshocks was recorded as an index of learning acquisition. To assess memory, the animal was again placed gently on the platform after 24 h. The time an animal remained on the platform was recorded as an index of memory retention. Staying on the platform for 2 min was counted as maximum memory retention (ceiling response).

Blood Cholinesterase Assay

Blood samples (50 µl) were obtained from the tail vein and diluted in an equal volume of ice-cold heparinized (20 units/ml) saline. Total cholinesterase activity was measured using a modified version [31] of the traditional spectrophotometric assay described by Ellman et al. [16]. Blood samples were diluted in cold (0–4 °C) 20 m*M* Na_2PO_4 buffer (pH 8.0) and incubated with 0.27 m*M* 5,5′-dithio-bis(2-nitrobenzoic acid) in 0.1 *M* Na_2PO_4, pH 7.0, containing 1.5 mg/ml $NaHCO_3$. Incubations were allowed to proceed at room temperature in 96-well microtiter plates for 10 min. Then, acetylthiocholine iodide (0.83 m*M* in 0.1 *M* Na_2PO_4 buffer, pH 8.0) was added to each well, and absorbance at 405 nm was measured continuously for 30 min. Cholinesterase activity was calculated on the basis of a standard curve generated using increasing concentrations of glutathione and expressed as nanomoles of acetylthiocholine iodide hydrolyzed per minute after normalization to blood hemoglobin content. The hemoglobin concentration was determined as described by van Kampen and Zijlstra [41].

Statistical Analysis

One-way repeated-measures analysis of variance was used to analyze the effects of methyl parathion on blood cholinesterase activity and body weight. Friedman repeated-measures analysis of variance on ranks or the Mann-Whitney rank sum test was used to analyze the open field test results. The data from the rota-rod test were analyzed by one-way repeated-measures analysis of variance or t test. The data from the step-down avoidance learning task were analyzed by Kruskal-Wallis one-way analysis of variance on ranks, one-way analysis of variance or t test. Differences were deemed significant at $p < 0.05$.

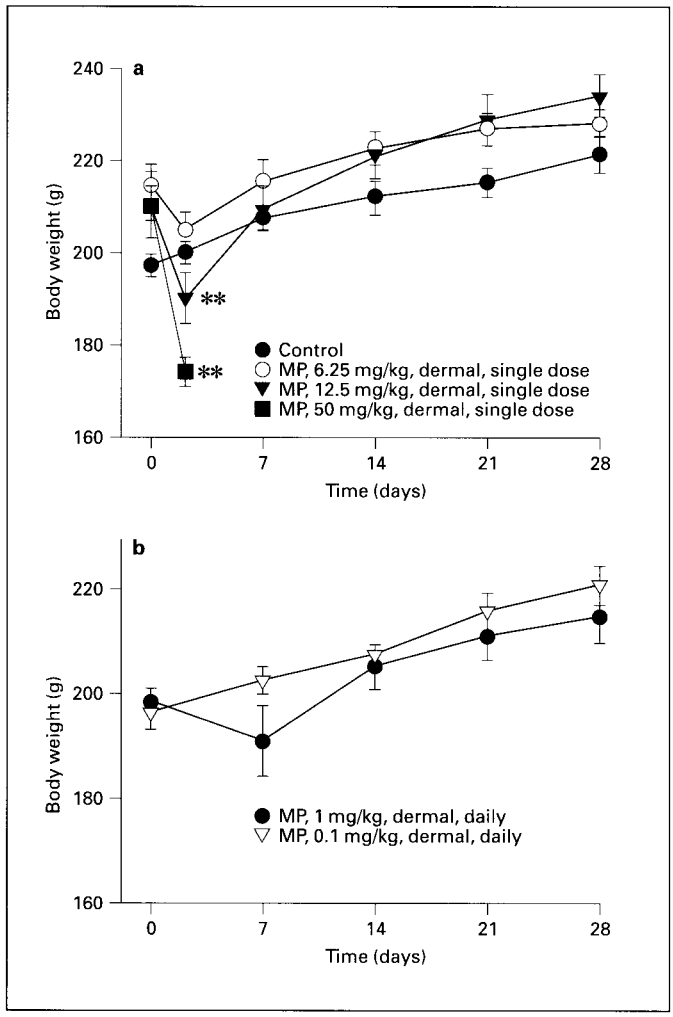


Fig. 1. Effects of a single (**a**) or repeated (**b**) dermal treatment with methyl parathion (MP) on rat body weight. The body weight was measured every 7 days. Each symbol represents the mean ± SEM of 6–8 animals. ** $p < 0.01$ compared to the body weight at 0 days (one-way repeated-measures analysis of variance).

Results

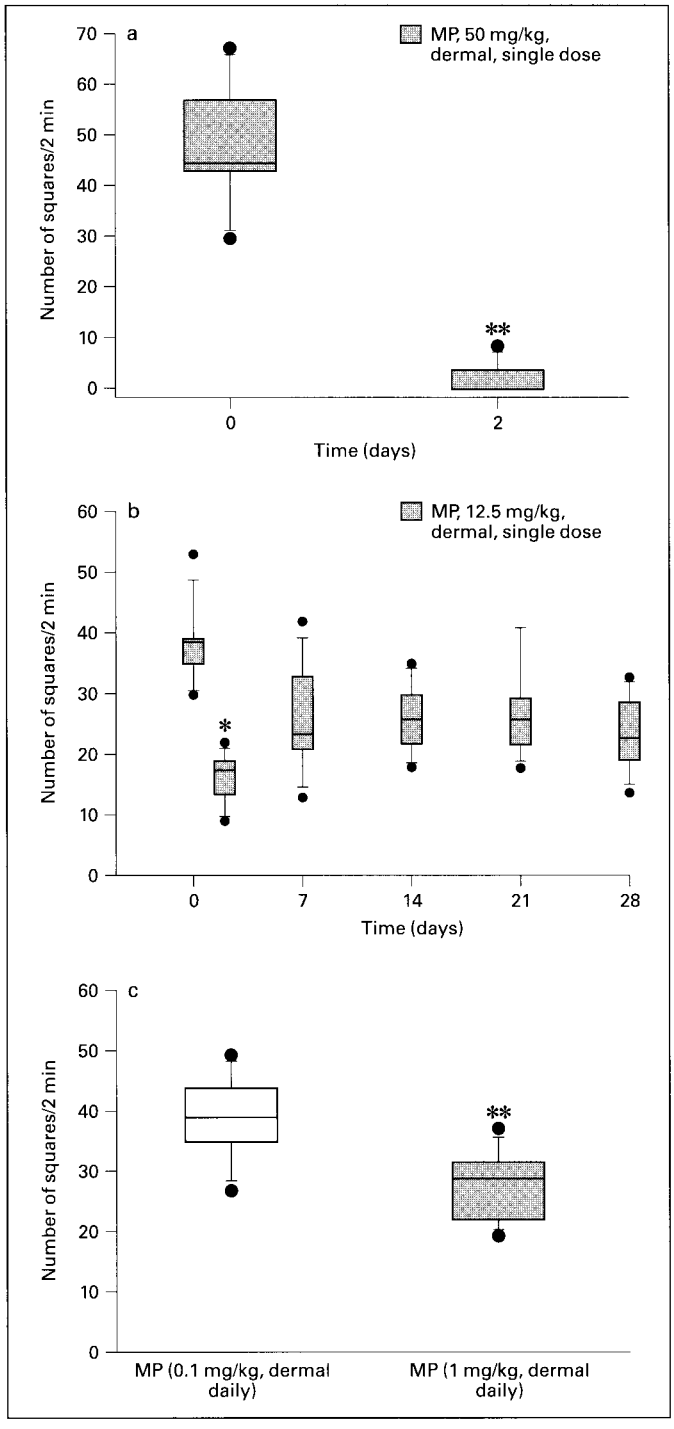
General Toxicity of a Single or Repeated Dermal Administration of Methyl Parathion

Animals exposed to a single dose of 50 mg/kg methyl parathion showed severe signs of acute toxicity (e.g. tremors, purposeless chewing and lacrimation) within 24 h of dosing and died within 72 h. Body weight declined about 17% during the 48 h after dosing (fig. 1a). Animals which were treated with a lower dose of methyl parathion, i.e. 6.25 or 12.5 mg/kg, showed little or no signs of toxicity. However, animals that received 12.5 mg/kg methyl parathion lost 9.6% of their body weight, a loss which was recovered 1 week following treatment. The body weights of animals treated with 6.25 mg/kg methyl parathion or vehicle were not affected.

Animals dosed repeatedly with 0.1 or 1 mg/kg methyl parathion showed little or no signs of acute toxicity. Repeated dermal administration of 1 mg/kg methyl parathion, however, caused a slight transient decrease in body weight 7 days after treatment, after which, body weight returned to control levels over the next 21 days (fig. 1b).

Behavioral Effects of Methyl Parathion

Animals treated with a single dose of 50 mg/kg methyl parathion showed a total loss of spontaneous locomotor activity as assessed by the open field test (fig. 2a). Neuromuscular coordination (rota-rod test) was also reduced by 90% (fig. 3). Spontaneous locomotor activity (fig. 2b) and neuromuscular coordination (fig. 3) of rats treated with



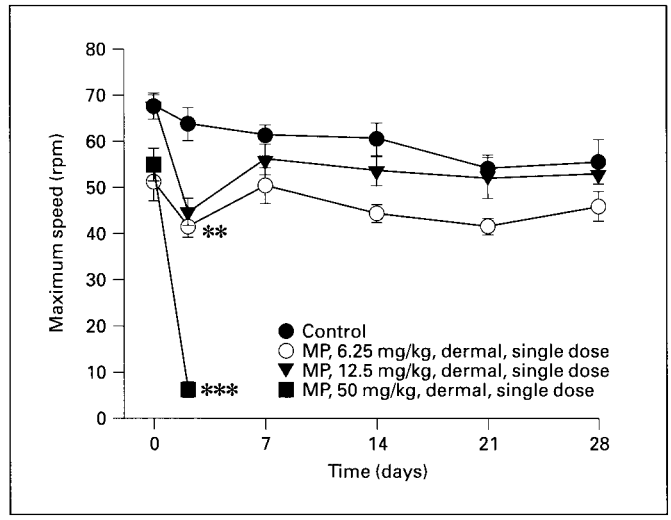


Fig. 3. Effects of a single dermal exposure to methyl parathion (MP) on neuromuscular coordination (rota-rod test). The rota-rod test was performed before and 2, 7, 14, 21 and 28 days after methyl parathion exposure as described in Materials and Methods. Data are expressed as the speed at which the animals fell from the rota-rod. Each symbol represents the mean ± SEM of 6–8 rats. ** $p < 0.01$, *** $p < 0.001$ compared to the maximum speed at 0 days in the rats exposed to 12.5 mg/kg methyl parathion (one-way repeated-measures analysis of variance).

Fig. 2. Effects of a single (**a**, **b**) or repeated (**c**) dermal exposure to methyl parathion (MP) on spontaneous locomotor activity (open field test), expressed as the number of squares the rats crossed within 2 min. For rats that received a single dose of methyl parathion, the open field tests were performed before and 2, 7, 14, 21 and 28 days after the exposure. For rats that received repeated exposures, the open field tests were performed 28 days after treatment. Each symbol represents the median, quartile (25 and 75%), maximum and minimum of 6–8 rats. * $p < 0.05$, ** $p < 0.01$ compared to the spontaneous locomotor activity at 0 days (Friedman repeated-measures analysis of variance on ranks followed by Dunnett's multiple comparisons or the Mann-Whitney rank sum test).

12.5 mg/kg methyl parathion were significantly decreased 2 days after dosing and recovered by day 7. Neither locomotor activity nor neuromuscular coordination was affected by treatment with 6.25 mg/kg methyl parathion. A single dermal administration of 6.25 or 12.5 mg/kg methyl parathion had no effect on learning acquisition or memory assessed 28 days after treatment (data not shown).

Repeated administration of methyl parathion (1 mg/kg/day) decreased spontaneous locomotor activity

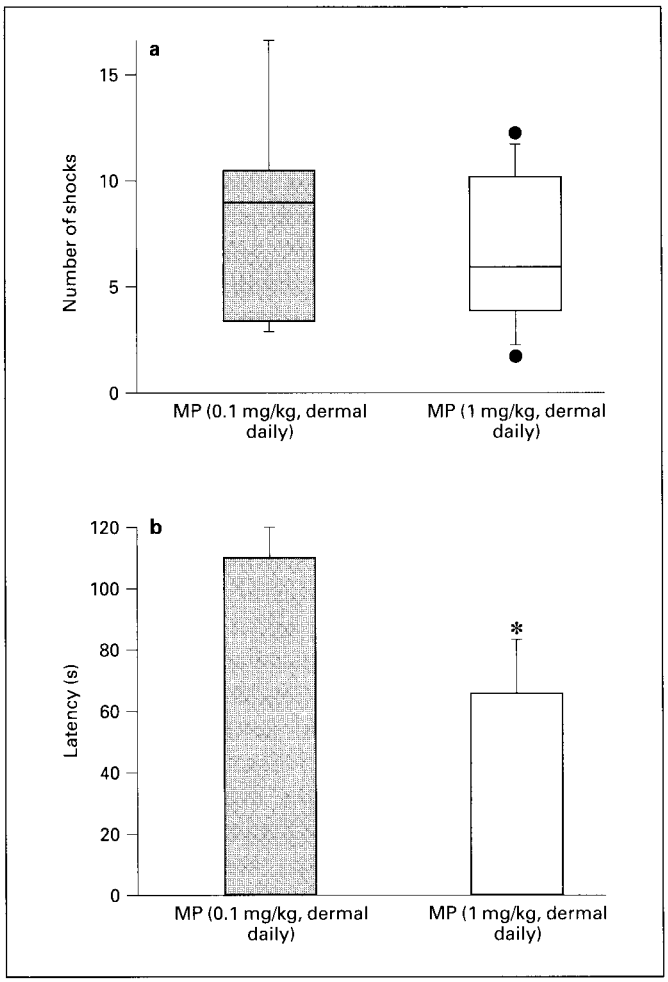


Fig. 4. Effects of repeated dermal exposure to methyl parathion (MP) on learning and memory. The step-down inhibitory avoidance learning task was performed 28 days after exposure. **a** Results of learning acquisition tests. Data are expressed as the number of foot-shocks required before each rat learned to remain on the platform for longer than 2 min. Each symbol represents the median, quartile (25 and 75%), maximum and minimum of 7–8 rats. There were no significant differences between the two groups (Mann-Whitney rank sum test). **b** Results of memory retention tests. The latency to step down by placing four paws on the grid was measured 24 h after the training. Staying on the platform for 2 min was regarded as the ceiling effect. Data are expressed as mean ± SEM of 7–8 rats. The rats exposed to 1 mg/kg methyl parathion showed a significant decrease in latency compared to those exposed to 0.1 mg/kg methyl parathion (* $p < 0.05$, t test).

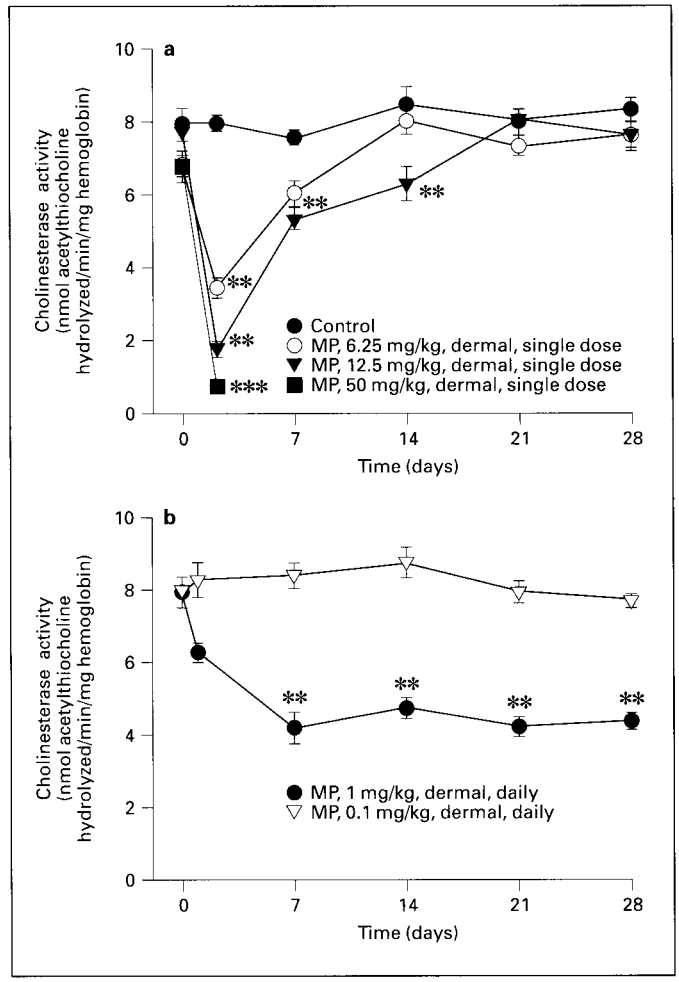


Fig. 5. Effects of a single (**a**) or repeated (**b**) dermal treatment with methyl parathion (MP) on blood cholinesterase activity. The blood cholinesterase activity was measured as described in Materials and Methods. Each symbol represents the mean ± SEM of 6–8 animals. ** $p < 0.01$, *** $p < 0.001$ compared to the blood cholinesterase activity measured before treatment in the same animal (one-way repeated-measures analysis of variance).

(fig. 2c) and impaired memory (decrease in latency; fig. 4b) in comparison to animals treated with 0.1 mg/kg/day methyl parathion. There were no differences in neuromuscular coordination (data not shown) or learning acquisition (fig. 4a) between the two groups.

Effects of a Single or Repeated Dermal Administration of Methyl Parathion on Blood Cholinesterase Activity

Inhibition of blood cholinesterase activity by a single dermal administration of methyl parathion was dose-dependent (fig. 5a). Forty-eight hours following treatment, cholinesterase activity was inhibited by 51 and 77%, respectively, by 6.25 and 12.5 mg/kg methyl parathion. Similarly, cholinesterase activity was decreased by 88% with a methyl parathion dose of 50 mg/kg. In animals treated with lower doses of methyl parathion, i.e. 6.25 or 12.5 mg/kg, cholinesterase activity recovered fully by day 14–21.

The effects of repeated dermal methyl parathion treatment on blood cholinesterase activity are shown in figure 5b. Cholinesterase activity in rats treated with 1 mg/kg/day methyl parathion for 28 days was reduced by 50% within the first 7 days, and inhibition was sustained over the course of treatment. In contrast, there was no significant reduction in cholinesterase activity in rats treated with 0.1 mg/kg methyl parathion.

Discussion

The difference between the effects of a single and repeated dermal exposure to methyl parathion on motor function, learning and memory were investigated in adult female rats and related to changes in blood cholinesterase activity. Inhibition of blood cholinesterase activity by a single dermal treatment with 6.25 or 12.5 mg/kg methyl parathion (corresponding to about 10 and 20% of the dermal LD_{50}, respectively [18]) reversed completely within 21 days. The cholinesterase activity showed a rapid recovery during the first 7 days following exposure and a slower phase of recovery during the next 14 days. However, the high dose of methyl parathion (50 mg/kg, 75% of the LD_{50}) resulted in an 88% inhibition of cholinesterase activity that had not changed within 48 h, and all of the treated animals were dead within 3 days following this methyl parathion treatment. These results indicate that cholinesterase activity is not irreversibly inhibited by acute exposure to lower doses of methyl parathion. Rather, normal enzyme activity may be restored after a single dermal exposure to a low dose of methyl parathion. The early rapid recovery phase could be due to simple hydrolysis of the inhibitor-acetylcholinesterase complex [35]. Synthesis of new enzyme may contribute to the late phase of recovery [4, 13]. At high doses (i.e. 50 mg/kg), methyl parathion may form a depot on or in the epidermis, which can prolong both its presence and the resulting inhibition of acetylcholinesterase. Pope et al. [34] reported a more rapid recovery of brain and plasma cholinesterase in rats exposed to a single subcutaneous injection of methyl parathion (18 mg/kg) than that observed following exposure to parathion or chlorpyrifos. The more rapid recovery could be due to the more rapid hydrolysis of dimethylphosphorylated cholinesterase relative to the diethylphosphorylated enzyme [34]. Since the recovery of brain cholinesterase activity is correlated with recovery of plasma cholinesterase [34], the change in brain cholinesterase activity after methyl parathion treatment could be estimated by the change in blood cholinesterase activity.

In contrast, daily dermal exposure to a low dose of methyl parathion, i.e. 1 mg/kg, which corresponds to 1.5% of the dermal LD_{50}, resulted in a sustained inhibition (50%) of blood cholinesterase activity that persisted throughout the 28-day treatment period. Continued dermal exposure to methyl parathion and sustained formation of the active paraoxon may favor aging or nearly irreversible inhibition of the enzyme [5, 6, 21].

Methyl parathion-induced inhibition of cholinesterase activity is accompanied by behavioral changes. However, the enzyme inhibition did not correlate linearly with functional changes. The 88% inhibition of cholinesterase activity produced by a single dose of 50 mg/kg methyl parathion was accompanied by severe signs of acute toxicity, total loss of spontaneous locomotor activity and a 90% loss of neuromuscular coordination. However, the rats treated with 12.5 mg/kg methyl parathion (77% inhibition) showed little or no signs of acute toxicity, only a 50% decrease in locomotor activity and a 30% loss of neuromuscular coordination. No signs of acute toxicity and no changes in motor activity were seen in the rats treated with 6.25 mg/kg methyl parathion, whereas the cholinesterase activity was reduced to 50% of control values. The rats treated daily with 1 mg/kg methyl parathion displayed little or no signs of cholinergic toxicity, but blood cholinesterase activity was decreased by 50% in this group of animals. In the study by Nemec et al. [29] of dermal exposure to methyl parathion in humans, no noticeable toxic symptoms were observed when blood cholinesterase activity dropped to 60% of preexposure levels. However, rats receiving 5 mg/kg methyl parathion orally showed signs of cholinergic toxicity 7 min after dosing, with convulsions beginning within 16 min [44]. The plasma cholinesterase activity was reduced to 43.6% of control levels in these animals. These data indicate that rapid absorption of methyl parathion through oral administration results in a rapid decline in cholinesterase activity that is accompanied by signs of toxicity, while slow absorption from the skin may cause a gradual decline in the enzyme activity. These results agree with the speculation that a sudden loss in cholinesterase activity is more important than the absolute amount of the loss in the manifestation of cholinergic signs [27]. In this study, the full recovery of blood cholinesterase activity was accompanied by a full recovery of motor function as assessed by the open field and rota-rod test. Thus, there is no delayed effect on rat motor function after a single dose of methyl parathion.

Memory deficiency has been reported in workers after acute or chronic exposure to organophosphorous com-

pounds [14, 23]. Impairment of learning acquisition, assessed by the Hebbs-Williams maze, has also been observed in rats 3 weeks following a single oral exposure to a near-lethal dose of methyl parathion [19]. In the present study, the delayed effects of a single dermal treatment with methyl parathion on learning and memory were examined using a step-down inhibitory avoidance learning task. No significant deficiency in either learning acquisition or memory retention was detected 28 days after a single dose of methyl parathion. Thus, a single dermal dose of methyl parathion has no delayed effects on learning and memory retention. However, impairment of memory retention was observed in the rats that were treated with 1 mg/kg/day methyl parathion dermally for 28 days. These data suggest that daily dermal exposure to low doses of methyl parathion, as might occur in domestic and occupational settings, can induce memory impairment without noticeable signs of cholinergic toxicity. The decreased latency observed in the memory retention test was considered not to be due to the change in spontaneous motor function, as motor slowing should tend to prolong rather than shorten the latency. The central cholinergic system has been linked to synaptic plasticity, learning and memory processes by many studies [2, 3, 33, 37, 45]. It is believed that organophosphorous compounds play a role in memory loss by producing cholinergic dysfunction at the level of the synapse [9]. Overstimulation followed by depression of cholinergic receptors, downregulation of muscarinic receptors [11, 20] or decreased white matter of the brain [28] following repeated treatment may be involved in the impairment of memory processes after subacute dermal methyl parathion exposure.

In summary, a single dermal exposure to doses of methyl parathion lower than those that elicit acute toxic symptoms can cause both blood cholinesterase inhibition and motor function changes that are reversible in rats. In contrast, repeated low-dose dermal exposure results in sustained inhibition of cholinesterase activity and causes impairment of both motor function and memory retention.

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References

1 Anonymous: Methyl parathion comes inside. Environ Health Perspect 105:690–691;1997.
2 Bakin JS, Weinberger NM. Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. Proc Natl Acad Sci USA 93:11219–11224;1996.
3 Baskerville KA, Schweitzer JB, Herron P. Effects of cholinergic depletion on experience-dependent plasticity in the cortex of the rat. Neuroscience 80:1159–1169;1997.
4 Benke GM, Murphy SD. Anticholinesterase action of methyl parathion, parathion and azinphosmethyl in mice and fish: Onset and recovery of inhibition. Bull Environ Contam Toxicol 12:117–122;1974.
5 Blaber JC, Creasey NH. The mode of recovery of cholinesterase activity in vivo after organophosphorus poisoning: 1. Erythrocyte cholinesterase. Biochem J 77:591–596;1960.
6 Blaber JC, Creasey NH. The mode of recovery of cholinesterase activity in vivo after organophosphorus poisoning: 2. Brain cholinesterase. Biochem J 77:597–604;1960.
7 Bushnell PJ, Kelly KL, Ward TR. Repeated inhibition of cholinesterase by chlorpyrifos in rats: Behavioral, neurochemical and pharmacological indices of tolerance. J Pharmacol Exp Ther 271:15–25;1994.
8 Bushnell PJ, Padilla SS, Ward TR, Pope CN, Olszyk VB. Behavioral and neurochemical changes in rats dosed repeatedly with diisopropylfluorophosphate. J Pharmacol Exp Ther 256:741–750;1991.
9 Carr RL, Chambers JE. Acute effects of the organophosphate paraoxon on schedule-controlled behavior and esterase activity in rats: Dose-response relationships. Pharmacol Biochem Behav 40:929–936;1991.
10 Carriero DL, Outslay G, Mayorga AJ, Aberman J, Gianutsos G, Salamone JD. Motor dysfunction produced by tacrine administration in rats. Pharmacol Biochem Behav 58:851–858; 1997.
11 Chaudhuri J, Chakraborti TK, Chanda S, Pope CN. Differential modulation of organophosphate-sensitive muscarinic receptors in rat brain by parathion and chlorpyrifos. J Biochem Toxicol 8:207–216;1993.
12 Clifford NJ, Nies AS. Organophosphate poisoning from wearing a laundered uniform previously contaminated with parathion. JAMA 262:3035–3036;1989.
13 Crisson CM, Wilson BW. Recovery of acetylcholinesterase in cultured chick embryo muscle treated with paraoxon. Biochem Pharmacol 26: 1955–1960;1977.
14 D'Mello GD. Behavioral toxicity of anticholinesterases in humans and animals – a review. Hum Exp Toxicol 12:3–7;1993.
15 Durham WF, Wolfe HR, Elliot JW. Absorption and excretion of parathion by spraymen. Arch Environ Health 24:381–387;1972.
16 Ellman GC, Courtney KO, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95;1961.
17 Environmental Protection Agency: Illegal Indoor Use of Methyl Parathion 2000.
18 Gaines TB. The acute toxicology of pesticides to rats. Toxicol Appl Pharmacol 2:88–99; 1960.
19 George J, Andrade C, Joseph T. Delayed effects of acute oral and chronic inhalational exposure to methyl parathion on learning and memory in rats. Indian J Exp Biol 30:819–822; 1992.
20 Gupta RC, Rech RH, Lovell KL, Welsch F, Thornburg JE. Brain cholinergic, behavioral, and morphological development in rats exposed in utero to methyl parathion. Toxicol Appl Pharmacol 77:405–413;1985.
21 Hobbiger FW. Effect of nicotinhydroxamic acid methiodide on human plasma cholinesterase inhibited by organophosphates containing a dialkylphosphate group. Br J Pharmacol 10: 356–362;1955.

22 Le Couteur DG, McLean AJ, Taylor MC, Woodham BL, Board PG. Pesticides and Parkinson's disease. Biomed Pharmacother 53: 122–130;1999.
23 Levin HL, Rodnitzky LR. Behavioral effects of organophosphate pesticides in man. Clin Toxicol 9:391–405;1976.
24 Llorens J, Crofton KM, Tilson HA, Ali SF, Mundy WR. Characterization of disulfoton-induced behavioral and neurochemical effects following repeated exposure. Fundam Appl Toxicol 20:163–169;1993.
25 Matsumura F: Toxicology of Insecticides, ed 2. New York, Plenum, 497–498;1985.
26 McDonald BE, Costa LG, Murphy SD. Spatial memory impairment and central muscarinic receptor loss following prolonged treatment with organophosphates. Toxicol Lett 40:47–56; 1988.
27 Midtling JE, Barnett PG, Coye MJ, Velasco AR, Romero P, Clements CL, O'Malley MA, Tobin MW, Rose TG, Monosson IH. Clinical management of field worker organophosphate poisoning. West J Med 142:514–518;1985.
28 Nagymajtenyi L, Desi I, Lorencz R. Neurophysiological markers as early signs of organophosphate neurotoxicity. Neurotoxicol Teratol 10:4429–4434;1988.
29 Nemec SJ, Adkisson PL, Dorough HW. Methyl parathion absorbed on the skin and blood cholinesterase levels of persons checking cotton treated with ultra-low-volume sprays. J Econ Entomol 61:1740–1742;1968.
30 Noring U, Povlsen UJ, Casey DE, Gerlach J. Effect of a cholinomimetic drug (RS 86) in tardive dyskinesia and drug-related parkinsonism. Psychopharmacology (Berl) 84:569–571; 1984.
31 Nostrandt AC, Duncan JA, Padilla S. A modified spectrophotometric method appropriate for measuring cholinesterase activity in tissue from carbaryl-treated animals. Fundam Appl Toxicol 21:196–203;1993.
32 Ott BR, Lannon MC. Exacerbation of parkinsonism by tacrine. Clin Neuropharmacol 15: 322–325;1992.
33 Overstreet DH. Behavioral plasticity and the cholinergic system. Prog Neuropsychopharmacol Biol Psychiatry 8:133–151;1984.
34 Pope CN, Chakraborti TK, Chapman ML, Farrar JD, Arthun D. Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. Toxicology 68:51–61;1991.
35 Reiner E. Spontaneous reactivation of phosphorylated and carbamylated cholinesterases. Bull World Health Organ 44:109–112;1971.
36 Romano JA Jr, Landauer MR. Effects of the organophosphorus compound, O-ethyl-N-dimethyl-phosphoramidocyanidate (tabun), on flavor aversions, locomotor activity, and rotarod performance in rats. Fundam Appl Toxicol 6:62–68;1986.
37 Sachdev R, Lu S, Wiley R, Ebner F. Role of the basal forebrain cholinergic projection in somatosensory cortical plasticity. J Neurophysiol 79:3216–3228;1998.
38 Simpson GR. Exposure to orchard pesticides. Dermal and inhalation exposures. Arch Environ Health 10:884–885;1965.
39 Tafuri J, Roberts J. Organophosphate poisoning. Ann Emerg Med 16:193–202;1987.
40 Taylor P. Anticholinesterase agents. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed 9. New York, McGraw-Hill, 177–197; 1996.
41 van Kampen EJ, Zijlstra WG. Spectrophotometry of hemoglobin and hemoglobin derivatives. Adv Clin Chem 23:199–255;1983.
42 Ware GW, Morgan DP, Estesen BJ, Cahill WP. Establishment of reentry intervals for organophosphate-treated cotton fields based on human data. I. Ethyl and methyl parathion. Arch Environ Contam Toxicol 1:48–49;1973.
43 Ware GW, Morgan DP, Estesen BJ, Cahill WP. Establishment of reentry intervals for organophosphate-treated cotton fields based on human data. III. 12 to 17 hours post-treatment exposure to monocrotophos, ethyl and methyl parathion. Arch Environ Contam Toxicol 3: 289–306;1975.
44 Youssef SHA, El-Sayed MGA, Atef M. Influence of gentamicin and rifamycin on toxicity and biotransformation of methyl parathione in rats. Dtsch Tierärztl Wochenschr 94:203–205; 1987.
45 Zhu XO, Waite PME. Cholinergic depletion reduces plasticity of barrel field cortex. Cereb Cortex 8:63–72;1998.