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Brain Acetylcholinesterase Activity Recovery Following Acute Methyl Parathion Intoxication in Two Feral Rodent Species: Comparison to Laboratory Rodents

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Widespread use of organophosphorous insecticides (OPs) has produced both acute and chronic intoxication among nontarget organisms. Most such studies have included fish and birds as opposed to mammals. However, numerous OP toxicity studies have been conducted on laboratory rodents creating a temptation to apply this data to feral rodents. Laboratory rodent OP toxicity studies have reported behavioral abnormalities (Crowder et al. 1980), liver enzyme alterations (Madhukar and Matsumura 1979), reduced liver lipid and vitamin A levels (Cecil et al. 1967), and embryo toxicity associated with reduced maternal food intake (Tanimura et al. 1967). In addition, chronic OP exposure has been reported to produce cholinergic adaptation which in turn lowers mortality rates following a subsequent acute anticholinesterase exposure (Costa et al. 1982). The relevance that these laboratory rodent studies have on feral rodents is subject to debate.

Field studies involving OP exposure among nontarget feral mammals have produced contradictory results. Several studies have reported no significant impact on populations (Buckner and Sarrazin 1975; Westlake et al. 1980). In contrast, other studies have reported limited mortalities (Giles 1970), population shifts (Barrett 1968), and inhibition of brain and blood acetylcholinesterase (AChE) activity (Smithson and Sanders 1978; Westlake et al. 1980; Zinkl et al. 1980). Increased mortality as a result of repeated OP application has also been reported (Montz and Kirkpatrick 1985). This observation may be of considerable importance to nontarget feral rodent populations due to the repetitive nature of OP application protocols. The ability of feral rodents to recover brain AChE activity (BAA) between OP application intervals undoubtedly promotes their survival. This study investigated and compared BAA recovery following acute oral methyl parathion intoxication among 2 feral rodent species and among 2 common laboratory rodent species.

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MATERIALS AND METHODS

Brain AChE activity recovery following acute methyl parathion intoxication was determined for both sexes among Sprague-Dawley laboratory rats, (Rattus norvegicus), ICR laboratory mice (Mus musculus) (Harlan Sprague Dawley; Houston, TX), cotton rats (Sigmodon hispidus), and house mice (Mus musculus). Both feral species were colony reared from captive feral stock and they were 1 or 2 generations removed from the wild. Feral rodents were sexually segregated at weaning and age standardized for dosage purposes. At dosage, cotton rats and house mice had mean ages of 65 and 52 days, respectively. All animals were provided water and food (Purina Rodent Chow; St. Louis, MO) ad libitum. All rats were housed 1 or 2 per cage (polycarbonate; 27 x 48 x 20 cm) and all mice were housed 5 per cage (polycarbonate; 27 x 48 x 15 cm). Housing conditions consisted of a 12 hr photoperiod, temperature of 22 $\pm$ 0.5^{o}C, relative humidity of 55 $\pm$ 5%, and 12 filtered ($\geq$0.05 µm) fresh air changes per hr.

Animals were randomly assigned to either a test group or its paired control group (4 to 6 surviving animals per group). For dosage purposes, crystalline methyl parathion (99.9% w/w) was pulverized and seived through a 60 mesh screen. A sufficient quantity was then suspended in corn oil to allow for a vehicle dosage rate of 10 ml/kg body weight. Control animals received vehicle only. Based on previous studies, animals were dosed at levels expected to yield approximately 20% mortality and 100% morbidity. Following dosage on day 0, paired test and control groups were euthanized by CO_2 asphyxiation on days 1, 3, 7, 14, and 28. Brain tissue was excised and excess blood was removed by blotting. Brains were halved, sealed in plastic specimen bags, transported on ice, and then frozen at -20^{o}C until BAA activity was ascertained.

Brain AChE activity (BAA) was determined using the colorimetric method of Ellman et al. (1961) as modified and described by Hill and Fleming (1982). Homogenates were prepared from 1 of the brain halves using a Potter Elvehjem teflon on glass tissue grinder at the rate of 1.0 ml 50 mM Tris buffer (pH 8) per 100 mg brain tissue. Homogenates were refrigerated until analyzed within 24 hr after preparation. Assays were run in duplicate using a Beckman Model #35 uv-vis spectrophotometer and the mean change in absorbance per min was determined. A correction was made for spontaneous hydrolysis of the chromogen. Activity was expressed in Units (U = µmole substrate hydrolyzed per min) per g tissue. The mean activity of test groups was expressed as a % of the mean activity of its paired control group (relative activity). The assay procedure was monitored twice daily using known AChE (Sigma Chemicals; St. Louis, MO).

Activity variability of the 5 control groups and the 5 test

groups used in each of the 8 recovery studies was evaluated by analysis of variance (ANOVA). Test group relative activity was also evaluated by ANOVA. Significant increases in activity between ranked test groups were detected using Duncan's multiple range test (MRT). Recovery was presumed to have occurred when a test group's BAA was not significantly different from its paired control group (2 sided, unpaired, t-test; $P>0.05$). Activity patterns were also analyzed by linear regression pitting $\log_{10}$ time following exposure vs. mean test group BAA (expressed as relative activity).

RESULTS AND DISCUSSION

Dosages ranging from 14 to 80 mg/kg produced mortality rates which varied from 16% to 45% (Table 1). Morbidity was 100% among survivors with the exception of one male laboratory mouse. Recovery patterns for rats and mice (Table 2) were found to be essentially logarithmic. The observed logarithmic patterns are in agreement with previous studies (Fleming and Bradbury 1981). However, a recent field study of meadow voles (Microtus pennsylvanicus) exposed to acephate did not report a logarithmic recovery pattern (Jett 1986). The lack of a logarithmic recovery pattern was presumably due to chronic acephate exposure via foodstuff residues.

The recovery patterns of male cotton rats were compared to male laboratory rats (Fig. 1). No significant difference in BAA was observed among the control groups for both rat species ($P>0.05$; ANOVA). However, significant differences were observed within their corresponding test groups ($P<0.05$; ANOVA). A significant increase in BAA was detected for male laboratory rats between days 3 and 7 ($P<0.05$; MRT). In contrast, a similar increase for male cotton rats did not occur until between days 7 and 14 and between days 14 and 28 ($P<0.05$; MRT). Recovery, as previously defined, occurred on day 7 for male laboratory rats ($P>0.05$; t-test). In contrast, male cotton rats did not recover until day 28 ($P>0.05$; t-test). Under the experimental conditions employed in this study, male laboratory rats appeared to recover significant BAA earlier than did male cotton rats.

The recovery pattern for female cotton rats was compared to the pattern for female laboratory rats (Fig. 1). Activity between female laboratory rat control groups was not significantly different ($P>0.05$; ANOVA). However, at least one female cotton rat control group had different activity from the others ($P<0.05$; ANOVA). These differences may have been due to sample size, age, body weight, sex or circadian rhythms. Activity among the respective test groups was significantly different for both species ($P<0.05$; ANOVA). A significant increase in BAA occurred between days 3 and 7 for female laboratory rats and between days 7 and 14 for female cotton rats ($P<0.05$; MRT). Recovery occurred on day 14 for both species ($P>0.05$; t-test). However, day 28 female cotton rats had not recovered ($P<0.05$; t-test). Lack of

day 28 recovery was possibly due to the previously cited variables. Under these experimental conditions, both sexes of cotton rats had similar recovery patterns and both appeared to recover slower when compared to laboratory rats of the same sex.

The recovery pattern for male house mice was compared to male laboratory mice (Fig. 2). For male laboratory mice, BAA was not significantly different between the control groups nor was a difference evident between the test groups ($P>0.05$; ANOVA). The lack of test group variability may have been due to a large increase in BAA prior to sampling on day 1. Male house mice, on the other hand, were observed to have significant BAA differences for both the control groups ($P<0.05$; ANOVA) and the test groups ($P<0.05$; ANOVA). A significant increase in BAA occurred in male laboratory mice between days 1 and 7 ($P<0.05$; MRT) and recovery occurred on day 7 ($P>0.05$; t-test). In contrast, male house mice underwent significant BAA increases between days 7 and 14 ($P<0.05$; MRT). Male house mice recovery occurred on day 14 ($P>0.05$); t-test). Male house mice had significant BAA increases later than their laboratory animal counterparts. They also recovered later than their laboratory animal counterparts.

In a manner consistent with the rest of the investigation, female house mice recovery was compared to their laboratory animal counterparts (Fig. 2). The control group's BAA did not differ among female laboratory mice ($P>0.050$; ANOVA). However, at least one test group differed from the others ($P<0.05$; ANOVA). In contrast, both female house mice test groups and control groups had at least one significant difference within their respective groups ($P<0.05$; ANOVA). A significant increase in BAA for female laboratory mice occurred between days 7 and 14 ($P<0.05$; MRT), whereas a similar phenomena occurred for female house mice between days 3 and 7 ($P<0.05$; MRT). Recovery occurred for female laboratory mice on day 14 ($P>0.05$; t-test) and on day 28 for female house mice ($P>0.05$; t-test). Although female house mice experienced significant BAA gains earlier than female laboratory mice, the feral rodents had a later recovery time. The longer recovery period for house mice may have been due to greater initial intoxication as measured by mortality rate (38% vs 20%).

The recovery from acute OP intoxication is an important parameter among nontarget feral mammals due to the repetitive nature of agricultural and forestry pesticide application procedures. However, the response of nontarget feral mammals to this situation has not been clearly defined. Zinkl et al. (1980) reported BAA depression following a single aerial acephate application in both Columbian ground squirrels (Spermophilus columbianus) and in red squirrels (Tamiasurus hudsonicus). Only 1 of 5 ground squirrels had depressed BAA 25-26 days after exposure. None of 3 red squirrels had depressed BAA on those same monitor days. A large percentage of both squirrel species was observed to have approximately 30% BAA depression out to day 6 following exposure. In a similar study, Smithson and

Sanders (1978) were unable to demonstrate a statistically significant reduction in BAA among cottontail rabbits exposed to repetitive applications of methyl parathion and toxaphene. This lack of inhibition may have been due to methyl parathion resistance in cottontails. Westlake et al. (1980) were also unable to detect a reduction in BAA among wood mice (Apodemus sylvaticus) exposed to chlorfenvinphos. This observation may have been due to animal movement between the adjacent control and test areas. A statistically significant BAA depression among nontarget feral mammals as a result of OP exposure appears to depend upon the OP, the application rate, the species, and the sampling technique.

A few laboratory studies have investigated the ability of feral rodents to recover from OP intoxication. Rattner and Hoffman (1984) investigated recovery as a result of chronic acephate exposure in feed (400 ppm). Two feral rodent species: colony reared white-footed mice (Peromyscus leucopus noveboracensis and captive feral meadow voles (Microtus pennsylvanicus), were compared to laboratory mice (Mus musculus). The recovery patterns were similar for all 3 species with each experiencing greater recovery in the first week compared to the second week. It was also observed that laboratory mice gained approximately twice as much BAA as the feral rodents (+34% vs +17%). Montz and Kirkpatrick (1985) investigated recovery following diazinon and parathion intoxication in colony reared feral white-footed mice (Peromyscus leucopus). Diazinon was administered orally at 19 mg/kg to females (6% mortality) and recovery was recorded 2 days later. Oral parathion at 10 mg/kg to males and females (3% and 9% mortality, respectively) also produced recovery in 2 days. In all 3 recovery studies, a considerable degree of variation was observed in the latter portion of the observations.

The recovery patterns in this investigation are similar to reports in the literature. The relative delay in recovery when comparing cotton rats to laboratory rats was not clearly a dosage related phenomena. Despite a 41% mortality rate, male laboratory rats attained recovery on day 7; whereas male cotton rats (20% mortality) did not recover until day 28. Both female laboratory rats and female cotton rats had a 16% mortality rate. Yet, the laboratory animals maintained recovery from days 14 to 28 whereas the cotton rats did not.

Unike the situation in rats, the relative delay in recovery for feral vs laboratory mice may have been dosage related. Laboratory vs feral mortality rates were 31% vs 45% for males and 20% vs 37% for females. In both instances, feral house mice had greater initial BAA depression. Initial BAA values for laboratory vs feral mice were 63% vs 54% for males and 69% vs 57% for females. The laboratory mice vs feral mice recovery times were 7 vs 14 days for males and 14 vs 28 days for females. Among feral mice, the higher mortality rates were reflected by greater initial BAA depression as well as by longer recovery periods.

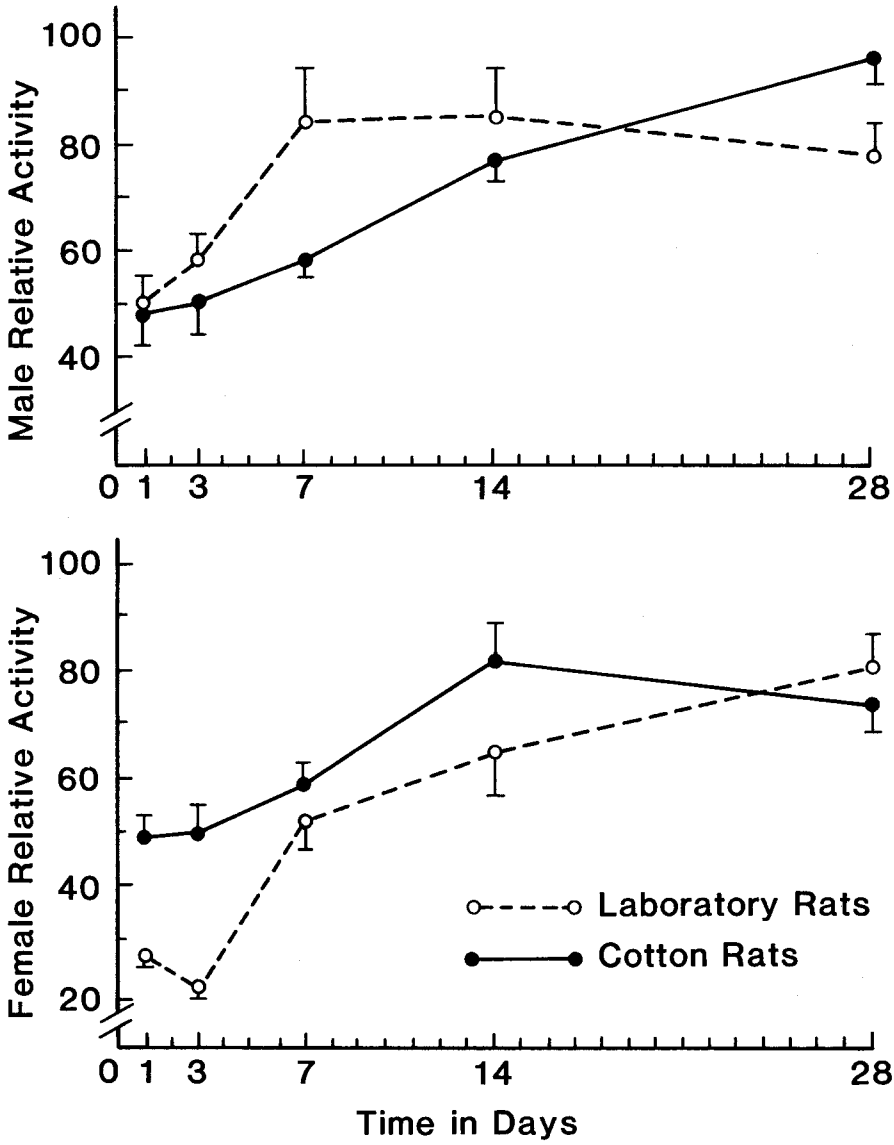

Figure 1. Brain AChE activity in laboratory rats and colony reared feral cotton rats following acute nonlethal oral methyl parathion intoxication. Relative activity is the mean test group activity (U/g of tissue) expressed as a percentage of the mean activity of its paired control group. Exposure was on day 0.

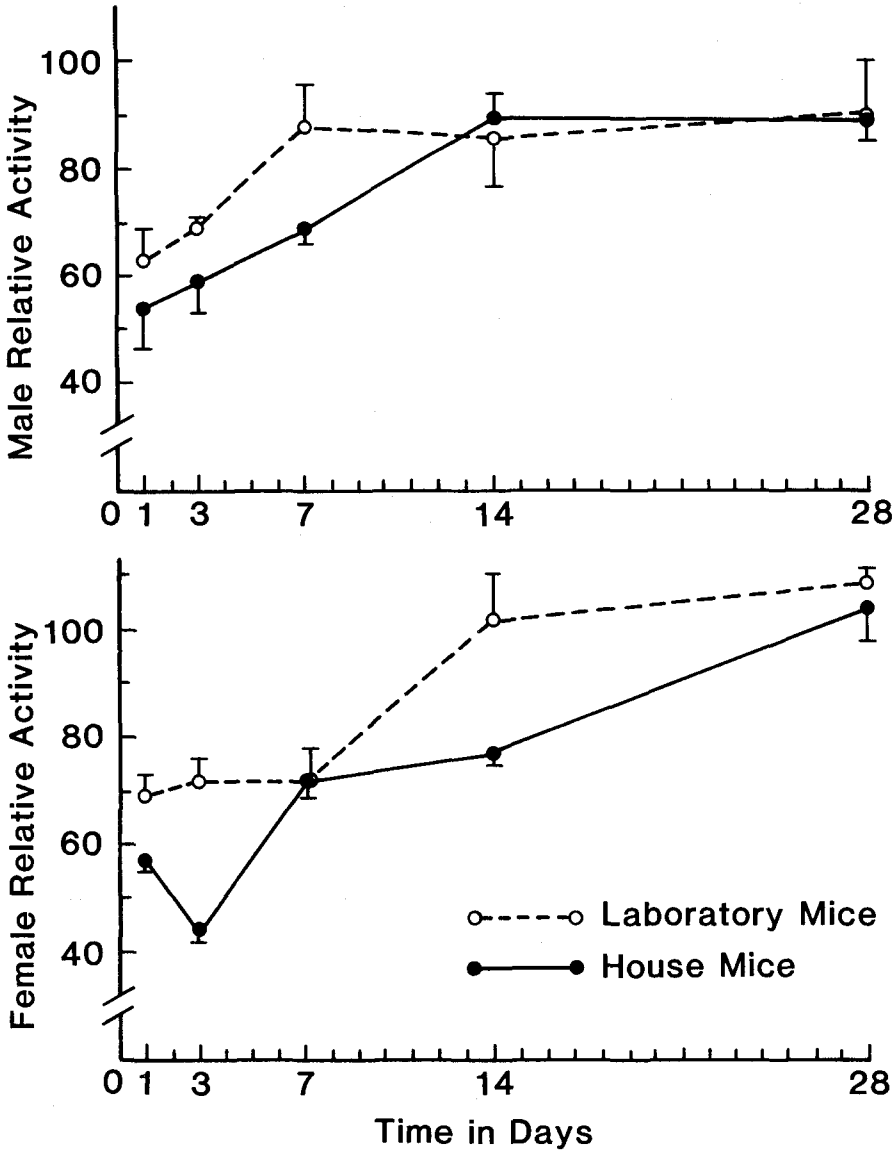

Figure 2. Brain AChE activity in laboratory mice and colony reared feral house mice following acute nonlethal oral methyl parathion intoxication. Relative activity is the mean test group activity (U/g of tissue) expressed as a percentage of the mean activity of its paired control group. Exposure was on day 0.

Feral rodents serve as an important link between lower and upper strata in the biosphere. Their ability to survive agricultural and forestry applications of OPs is an important environmental concern. Under these situations, their survival is dependent upon the degree of intoxication which in turn depends upon the particular OP, the application rate, the OP's persistence in the environment, the type of application, and the conditions during application. For persistent OPs, and under conditions of rapid reapplication, exposure may approximate chronic. Under these situations, cholinergic adaption (Costa et al. 1982) may play a role in assisting survival. However, if the OP is relatively nonpersistent and if the application rates are spaced at periods greater than the persistence of the OP, the conditions of exposure would approximate acute rather than chronic. Under acute exposure conditions, the ability of feral rodents to recover BAA depression would greatly contribute to their probability of surviving a subsequent OP application.

This study suggests that at comparable levels of methyl parathion intoxication, laboratory rats tend to recover more rapidly than colony reared cotton rats. Although laboratory mice also tended to underestimate the same phenomena in house mice, the situation in mice may have been related to the level of initial intoxication. The use of common laboratory rodents to predict recovery from methyl parathion intoxication in feral cotton rats and house mice may be acceptable in that laboratory rodents do not appear to overestimate the time interval required for recovery.

Table 1. Brain AChE activity recovery following acute oral methyl parathion intoxication: Dosage and mortality data

Species	Sex	n	Deaths	Mortality Rate	Dosage (mg/kg)
Rattus norvegicus	M	49	20	41%	14
(laboratory)	F	25	4	16%	27
Sigmodon hispidus	M	30	6	20%	65
(feral)	F	29	7	24%	80
Mus musculus	M	45	14	31%	29
(laboratory)	F	35	9	20%	34
Mus musculus	M	47	21	45%	24
(feral)	F	51	19	37%	55

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Table 2. Brain AChE activity recovery[a] in laboratory and feral rats (and mice) following acute oral methyl parathion intoxication

Species	Sex	Gp[b]	Day after exposure (day 0) and corresponding number (n) of test animals									
			1	n	3	n	7	n	14	n	28	n
Rattus norvegicus (laboratory)	M	C	5.16±0.70	5	5.07±0.84	4	4.84±0.31	5	4.91±0.81	5	5.81±0.62	4
	M	T	2.60±0.28	4	2.95±0.26	5	4.06±0.50	4	4.16±0.46	5	4.53±0.34	5
	F	C	5.99±0.58	5	5.92±0.89	5	5.65±0.19	5	5.81±0.71	5	6.31±0.89	5
	F	T	1.64±0.12	4	1.25±0.14	5	2.95±0.28	4	3.75±0.48	4	5.11±0.38	4
Sigmodon hispidus (feral)	M	C	7.65±0.57	5	6.58±0.60	6	7.45±0.51	5	7.51±0.54	6	7.34±0.51	6
	M	T	3.70±0.43	5	3.29±0.40	4	4.31±0.23	5	5.79±0.28	5	7.04±0.34	5
	F	C	5.88±0.68	5	7.29±0.45	5	8.91±0.42	6	7.34±0.34	6	8.24±0.57	5
	F	T	2.88±0.22	5	3.64±0.34	4	5.28±0.35	4	6.04±0.53	5	6.13±0.44	4
Mus musculus (laboratory)	M	C	5.99±0.73	5	6.72±0.39	5	6.10±0.47	5	5.20±0.40	5	5.93±0.57	5
	M	T	3.75±0.33	5	4.67±0.15	5	5.38±0.50	5	4.49±0.47	5	5.38±0.62	5
	F	C	5.79±0.66	5	6.22±0.30	5	7.08±0.68	5	5.20±0.68	5	5.88±0.47	5
	F	T	4.00±0.24	5	4.49±0.25	5	5.11±0.41	5	5.31±0.45	5	6.38±0.15	5
Mus musculus (feral)	M	C	6.66±0.35	6	8.15±1.02	5	8.67±0.47	5	6.75±0.53	6	8.58±0.19	5
	M	T	3.58±0.56	6	4.83±0.48	4	5.96±0.22	4	6.07±0.27	4	7.72±0.39	4
	F	C	8.08±0.31	6	9.06±0.35	5	8.02±0.16	6	8.31±0.36	6	6.65±0.34	5
	F	T	4.64±0.18	6	3.99±0.15	6	5.79±0.26	4	6.42±0.18	5	6.90±0.37	5

[a] Activity is in Units [μmole substrate hydrolyzed per min per g of tissue (wet weight)]. Data is expressed as $\bar{x} \pm$ SE.

[b] Group: C = control, T = test.

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