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# Effects of Methomyl and Ethanol On Behavior in the Sprague-Dawley Rat[1]

ODIE L. BRACY, REBECCA S. DOYLE, MAURICE KENNEDY, STEPHEN M. McNALLY
J. DUANE WEED AND B. MICHAEL THORNE[2]

*Mississippi State University, Mississippi State, MS 39762*

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BRACY, O. L., R. S. DOYLE, M. KENNEDY, S. M. McNALLY, J. D. WEED AND B. M. THORNE. *Effects of methomyl and ethanol on behavior in the Sprague-Dawley rat.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 21–25, 1979.—Emotional behavior and activity levels were studied following administration of ethanol and/or a carbamate pesticide, methomyl, to rats via a ground chow diet. Acetylcholinesterase levels were lowered following the experimental diets. The group having the greatest reduction in AChE, the methomyl group, showed less evidence for habituation in an open-field test. No differences relative to control subjects were noted on handling and muricide tests.

Ethanol Carbamate pesticide (methomyl) Muricide Open-field behavior Handling reactivity

THE POSSIBLE deleterious effects of agricultural insecticides on human and animal health and behavior have long been of concern. The concern is particularly true in cases where the toxic effects of over exposure to high concentrations result in apparent physical and behavioral symptoms. For example, Levin, Rodnitsky and Mick [11] reported that the symptoms of acute poisoning ". . . include giddiness, tension, anxiety, restlessness, and emotional lability . . ." while chronic exposure at high concentrations has resulted in ". . . depression, schizophrenic symptoms, impairment of memory, and concentration deficit . . ." (p. 225). Additional evidence for behavioral changes following exposure to insecticides was noted in a review by Clark [2].

The manifestation of aberrant behaviors in cases of over exposure at low concentration over a period of time may be much more subtle but still potentially harmful. The idea that some insecticides may differentially affect behavior when there is interaction of the insecticides with other chemicals (such as ethanol) has not received a great deal of attention.

It has been well established that organophosphate and carbamate insecticides act as cholinesterase (ChE) inhibitors. That is, the insecticides cause a reduction in ChE levels in tissue and blood and subsequent accumulation of acetylcholine (ACh) in both peripheral and central systems [4, 9, 10, 20]. As part of a study on the effects of this action on the behavior of Sprague-Dawley rats, Kurtz [9] reported that IP injection of the carbamate insecticide MOBAM resulted in decreases in spontaneous motor activity in comparison with control rats. Furthermore, increase in the dosage ratio (mg/kg) resulted in greater decreases in spontaneous motor activity. By contrast, Levin and Rodnitzky [10] said that increased anxiety and irritability are often present in organophosphate toxicity. They suggested that this may also be the case in mild ChE inhibition.

In regard to the effects of ethanol on the same body systems, Hunt and Dalton [3] reported a significant reduction of ACh in Sprague-Dawley rats at low blood concentrations of ethanol but an increase in ACh at high blood concentrations. This well-documented biphasic action of ethanol is reported to be responsible for increases in mouse locomotion at low ethanol levels and decreases in locomotion at high levels at least in some strains [14].

Similarly, Buckalew and Cartwright [1] reported increased exploratory activity at low ethanol dosage (0.25 cc/100 g body weight) and decreased activity at high dosage (0.75 cc/100 g body weight). Shock elicited fighting was reported to have increased in hooded rats at low ethanol dosage and decreased at high dosage [21]. Yanai and Ginsburg [22] demonstrated that cricket killing and open-field activity in mice was significantly reduced in mice that received ethanol transplacentally and via the mother's milk.

Since it appears that both the carbamate insecticides and ethanol affect the cholinergic system and it is highly likely that a combination of the two may frequently occur in nature, the combined effects on behavior may be of interest. In one study, Klemm [8] found that ethanol and the ChE inhibitor, physostigmine, were antagonistic in EEG measures since the anticholinesterase appeared to block the effects of ethanol on EEG readings. The physical symptoms of ethanol intoxication were unaffected, however.

Taken singularly or together, carbamate insecticides and ethanol may have harmful effects and cause changes in be-

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[2]Reprint requests should be sent to: Michael Thorne, P.O. Drawer PF, Mississippi State, MS 39762.

havior. While the effects of high dosage exposure are more apparent and are more widely studied, low concentration exposure over time may also result in physical symptoms and behavioral changes. Therefore, the purpose of the present study was to investigate the effects on behavior of low level administration of a carbamate insecticide, Methomyl (Methyl N-[[Methylamino) Carbonyl] oxy]-ethanimidothioate), ethanol, and a combination of the two. The behaviors selected for study were muricide (mouse-killing), handling/emotionality behaviors, and open-field activity.

## METHOD

### *Animals*

Thirty-two male, 60–75-day-old Sprague-Dawley rats shipped from Madison, Wisconsin, were used. All animals were received in one shipment and were housed individually in cages measuring 17.78×25.40×17.78 cm. They were allowed seven days to adjust to the new housing and a reversed dark/light cycle. The dark/light cycle was maintained by automatic switching so that from 6 a.m. to 6 p.m. the laboratory was illuminated with dim red light (dark cycle). During the light portion of the cycle the laboratory was illuminated with incandescent lighting. The mean weight of the rats was 242.85 g at the beginning of the study.

### *Apparatus*

The open-field box (76.2×76.2×25.4 cm) used for assessing spontaneous locomotor activity was painted flat black with white grid lines that divided the floor into 25 equal 15.24 cm squares. A door in the wire mesh top provided entry.

A large white asbestos glove was worn on the left hand during the handling test. In addition, a stopwatch was used to time the handling and activity tests.

### *Procedure*

The rats were randomly assigned to groups, their cages numbered, and diets administered by experimenters not participating in the behavioral tests in such a manner as to keep the testers blind to the experimental treatments. After the seven day adjustment period the diets were adjusted according to assignment so that eight rats in each of four groups received partially ground Purina Rat Pellets which were coated with corn syrup containing the experimental substances as follows: (1) Group Control (C) received pellets coated with corn syrup and sucrose; (2) Group Ethanol (E) received pellets coated with corn syrup containing ethanol; (3) Group Methomyl (M) received pellets coated with corn syrup containing methomyl and sucrose; (4) Group Methomyl and Ethanol (ME) received pellets coated with corn syrup containing both methomyl and ethanol. Each animal received the equivalent of 140 cal/day with 100 calories from the coated pellets and the remainder from ethanol or sucrose depending upon the group. This amount of food was sufficient to last the 24 hr feeding period so that the rats had food and water available continuously. Ethanol, 95%, $CH_3CH_2OH$, contributed 25% of the total calories in the diets containing ethanol, and sucrose supplied the additional calories in the control and methomyl diets. The level of methomyl added to the corn syrup was 200 ppm/day.

An analysis of ethanol loss performed by gas chromatography showed a possible 14% loss by evaporation in a 24-hr period. However, since the rats typically consumed the corn syrup coating immediately, the loss of ethanol was probably much less in actual practice. Similar analysis for possible instability and loss of methomyl performed by gas chromatography on chow stored for 7 days showed a recovery rate of 99.6% of methomyl added to the chow.

For each testing session the home cage of a rat was covered by a clipboard and the animal and its cage were carried from the cage rack to the testing table. The handling/emotionality test consisted of a five-component rating scale modified from King [7]. The components, rated on a 4-point scale (0–3), were as follows: (1) reaction to a pencil moved slowly from side to side near the perioral region while the animal was in its home cage; (2) reaction to a pencil tap on the flank; (3) ease of capture from the home cage; (4) resistance to handling following capture; and (5) amount of vocalization during testing. Each animal's daily score was the total of the five component ratings, and the length of the test was approximately one minute.

For assessment of open-field activity each rat was placed on the center square of the open-field box, the door to the box was closed, and a stopwatch was started. The observer counted both the number of squares entered by the hind feet (horizontal activity) and the number of rearings (elevation of the forepaws from the bottom of the test box) during a one-minute interval.

On experimental Day 0 all animals were weighed, administered the first handling and open-field tests and introduced to the experimental diets. The tests were repeated on experimental Days 4, 7, 10 and 13.

Reliability checks were performed on three different test days with the assistance of an independent observer. A correlation coefficient for the handling tests based upon the daily totals for each animal rated by two observers was found to be $r=0.63$, $p<0.01$. The correlation coefficient obtained on the independent counting of squares entered by the hind feet in the 1-min open-field activity test proved to be higher ($r=0.99$) as was the correlation coefficient based on independent counting of rearing while in the open-field box ($r=0.96$).

In addition to the handling and activity tests, on experimental Day 13, a muricidal or mouse-killing test was administered to each rat. For this test, an adult albino mouse was placed into the home cage and each animal was observed continuously for 30 minutes. Latency to kill was recorded if killing occurred. At the end of the test interval, the live mouse or the remains of any dead mice were removed from the cages.

All animals were tested between 8:00 and 11:00 a.m. or from 2 to 5 hr into the dark portion of the dark/light cycle.

On experimental Day 14 the animals were sacrificed and a 30 $\mu$l blood sample was taken, placed on ice, and analyzed immediately for acetylcholinesterase levels.

## RESULTS

### *Biochemical Analysis*

Analysis of the blood samples for plasma and erythrocyte cholinesterase levels was performed by the Voss and Sachsse procedure, a colorimetric method which is modified for micro-blood samples [19]. Since the plasma and erythrocyte assays revealed identical between-group relationships, only the results of the erythrocyte analysis are reported. A one-way analysis of variance performed on the acetylcholinesterase levels revealed a significant F ratio,

TABLE 1

ACETYLCHOLINESTERASE LEVELS*
(μMOLE/ML WHOLE BLOOD)
ERYTHROCYTE ANALYSIS

| | Group C | Group E | Group M | Group ME |
|---|---|---|---|---|
| Mean | 0.44 | 0.22 | 0.11 | 0.181 |
| SD | ± 0.03 | ± 0.0397 | ± 0.0158 | ± 0.026 |

*All groups are significantly different from all others at the 0.01 level except Groups E and ME which differ at the 0.05 level.

$F(3,28)=167.08$, $p<0.01$. All pairwise comparisons were made with the Tukey HSD test and all were found to be significant. That is, all treated groups had acetylcholinesterase levels lower than the values found for Group C and each of the treated groups differed from the others with Group M showing the greatest reduction. The actual group means and standard deviations are shown in Table 1.

*Weight Data Analysis*

The animals were weighed on each test day and the average weights/group are plotted in Fig. 1 relative to a normal weight gain curve [13]. It is evident that the rate of weight gain was very similar to the normal growth curve.

A one-way analysis of variance comparing the weights on the last test day resulted in a significant F-ratio, $F(3,28)=5.5$, $p<0.01$. Individual comparisons with the Tukey HSD test revealed that Group ME animals gained less weight than rats in either Group C or Group M.

*Handling/Emotionality Tests*

There were no significant differences between groups on total handling/emotionality ratings and all scores were quite low. The group means for the 5-day total ratings were: Group C, 2.9; Group E, 2.9; Group M, 2.2; Group ME, 2.8.

*Open-Field Tests*

There were no significant differences between groups in either total horizontal open-field activity or in total rearing scores across the 5-day test period. The group means for total horizontal activity were: Group C, 172.8; Group E, 185.1; Group M, 186.4; Group ME, 184.2. The means for total rearing scores were: Group C, 26.6; Group E, 24.6; Group M, 26.8; Group ME, 26.4.

The average daily horizontal activity per group is shown in Fig. 2. Inspection of Fig. 2 suggests that Group M animals had higher activity levels on the last three days of testing than did animals in the other groups. However, the difference is not significant except when the combined three day scores are compared. Comparison of the means for the total of the last three days of testing resulted in a significant F-ratio, $F(3,28)=8.396$, $p<0.01$. Individual comparisons with the Tukey HSD test revealed that Group M animals had a higher rate of horizontal activity on the last three test days compared to all other groups.

The Spearman rank order correlation coefficient between horizontal activity on the last day of testing and acetylcholinesterase levels across all subjects was found to be significant ($r_s=-0.32$, $p<0.05$, one-tailed test). Thus, there was

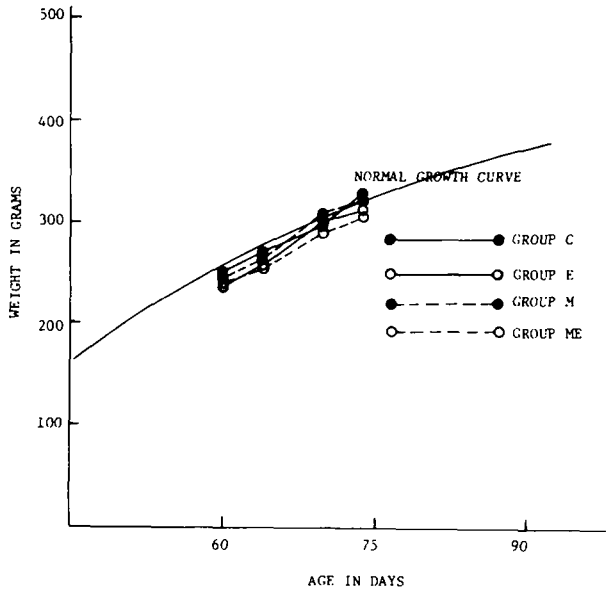


FIG. 1. The average group weights at each test session are plotted relative to a normal growth curve [13].

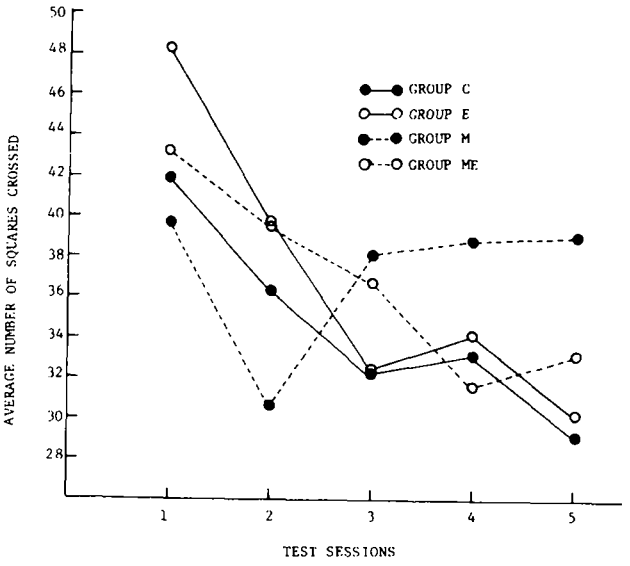


FIG. 2. The average group horizontal activity in the open-field box is plotted over test sessions.

an inverse relationship between activity and acetylcholinesterase level.

*Muricidal Behavior*

In the muricide test, 3 of 8 rats killed in each group except Group M, while no animals in that group killed. This yields an overall 28% rate of killing which is only slightly above previously reported control animal rates [5, 6, 17, 18]. No differences were found in comparisons between groups.

## DISCUSSION

A significant reduction in acetylcholinesterase was found in both ethanol and methomyl groups in the present experiment. The reduction in the methomyl groups supports the idea that carbamate insecticides act as cholinesterase inhibitors [4, 9, 10, 20].

Additionally, the acetylcholinesterase reduction in the ethanol groups is in line with the increased ACh levels seen by Hunt and Dalton [3] in rats with high blood concentrations of ethanol. Animals in the ethanol groups received about 25% of their total daily caloric intake in the form of ethanol. Although blood concentration of ethanol was not measured, the level of ethanol in the diet could have resulted in high concentrations.

Although both ethanol and methomyl resulted in decreases in acetylcholinesterase, the combination of the two substances was not additive. Thus, treatment with methomyl alone resulted in a greater reduction in AChE than was seen in Group ME. One possible explanation for this finding is that the mechanism of action of the ethanol is at least partially antagonistic to the anticholinesterase effects of methomyl. In a possibly related finding, Klemm [8] observed that the effects of ethanol and the ChE inhibitor physostigmine on EEG measures were antagonistic.

Despite the very dramatic changes in AChE noted, only very minor behavioral changes were seen. Specifically, Group M rats had higher open-field horizontal activity scores over the last three test days than did rats in the other groups. This finding could be interpreted as a failure to habituate to the open-field situation and is possibly related to the finding by Russell [15] that lowering of acetylcholinesterase level by organophosphate insecticides results in failure to extinguish a conditioned response.

It is perhaps worthy of note that the actual difference observed involved Group M animals who, as mentioned earlier, showed the greatest AChE reduction. The finding of a small, but significant, negative correlation between AChE level and horizontal open-field activity is also relevant here. Thus, the behavioral effects seem to be related to reduction of AChE but only in the most extreme form, i.e., Group M. Again, this finding may provide support for Russell's [15] idea of a critical level of acetylcholinesterase reduction. Thus, it is possible that behavior is unchanged until a certain level of reduction is reached, and beyond this "critical" level behavioral changes such as reduced habituation and extinction occur.

A number of experimenters have reported that either cholinergic stimulation or a reduction of AChE by injection of cholinesterase inhibitors results in an increase in mouse killing [12,16]. We found no evidence for cholinergic involvement in muricide in the present study. There were no group differences in muricide and no animals killed in the group having the greatest AChE reduction, Group M.

There are, of course, many methodological differences between our study and the ones in which changes in muricide have been found. One major difference is that in many of the studies reporting changes in muricide the cholinergic stimulant or the cholinesterase inhibitor was injected directly into the brain undoubtedly resulting in a more dramatic effect than with oral administration.

In the analysis of the weight-gain data, a significant difference was found on the last test day in that Group ME animals gained significantly less weight than either Group C or Group M rats. Since the animals in Group ME did not differ from the control subjects on any behavioral measure, the significant difference in weight gain does not appear to be meaningful.

In summary, we found that both ethanol and a carbamate insecticide, methomyl, resulted in significant reduction in AChE levels when administered as a coating on ground rat chow. Some evidence was found for lack of habituation to the open-field test in Group M animals while no changes in muricide were seen.

## REFERENCES


1. Buckalew, L. W. and G. M. Cartwright. General and differential behavioral effects of five ethanol dosages on the albino rat. *Psychol Rep.* **23:** 1151–1154, 1968.
2. Clark, G. Organophosphate insecticides and behavior, a review. *Aerospace Med.* **42:** 735–750, 1971.
3. Hunt, W. A. and T. K. Dalton. Regional brain acetylcholine levels in rats acutely treated with ethanol or rendered ethanol-dependent. *Brain Res.* **109:** 628–631, 1976.
4. Kaplan, A. M. and H. Sherman. Toxicity studies with Methyl N-[[(Methylamino) carbonyl]oxy]-ethanemedothiate. *Toxic. appl. Pharmac.* **40:** 1–17, 1977.
5. Karli, P. The Norway rat's killing response to the white mouse: An experimental analysis. *Behaviour* **10:** 81–103, 1956.
6. Karli, P., M. Vergnes and F. Didiergeorges. Rat-mouse interspecific aggressive behavior and its manipulation by brain ablation and by brain stimulation. In: *Aggressive Behavior*, edited by S. Garattini and E. B. Sigg. New York: Wiley, 1969.
7. King, F. A. Effects of septal and amygdaloid lesions on emotional behavior and conditioned avoidance responses in the rat. *J. nerv. ment. Dis.* **16:** 57–63, 1958.
8. Klemm, W. R. Dissociation of EEG and behavioral effects of ethanol provide evidence for a noncholinergic basis of intoxication. *Nature* **251:** 234–236, 1974.
9. Kurtz, P. J. Behavioral and biochemical effects of the carbamate insecticide, MOBAM. *Pharmac. Biochem. Behav.* **6:** 303–310, 1977.
10. Levin, H. S. and R. L. Rodnitzky. Behavioral effects of organophosphate pesticides in man. *Clin. Toxic.* **9:** 391–405, 1976.
11. Levin, H. S., R. L. Rodnitzky and D. L. Mick. Anxiety associated with exposure to organophosphate compounds. *Archs gen. Psychiat.* **33:** 225–228, 1976.
12. Lonowski, D. J., R. A. Levitt and W. A. Dickinson. Carbachol-elicited mouse killing by rats: Circadian rhythm and dose response. *Bull. Psychon. Soc.* **6:** 601–604, 1975.
13. National Academy of Sciences. *Nutrient Requirements of Laboratory Animals*, 1972.
14. Randall, C. L., J. A. Carpenter, D. Lester and H. J. Friedman. Ethanol-induced mouse strain differences in locomotor activity. *Pharmac. Biochem. Behav.* **3:** 533–535, 1975.

15. Russell, R. W. Behavioral aspects of cholinergic transmission. *Fedn Proc.* **28:** 121–131, 1969.
16. Smith, D. E., M. B. King and B. G. Hoebel. Lateral hypothalamic control of killing: Evidence for a cholinoceptive mechanism. *Science* **167:** 900–901, 1970.
17. Thorne, B. M., M. Aaron and E. E. Latham. Effects of olfactory bulb ablation upon emotionality and muricidal behavior in four rat strains. *J. comp. physiol. Psychol.* **84:** 339–344, 1973.
18. Thorne, B. M., M. Aaron and E. E. Latham. Olfactory system damage in rats and emotional, muricidal, and rat pup killing behavior. *Physiol. Psychol.* **2:** 157–163, 1974.
19. Voss, G. and K. Sachsse. Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by modified acetylthiocholine/DTNB procedure. *Toxic. appl. Pharmac.* **16:** 764–772, 1970.
20. Wecker, L., P. L. Mobley and W. D. Dettbarn. Central cholinergic mechanisms underlying adaptation to reduced cholinesterase activity. *Biochem. Pharmac.* **26:** 633–637, 1977.
21. Weitz, M. K. Effects of ethanol on shock-elicited fighting behavior in rats. *Q. Jl Stud. Alcohol* **35:** 953–958, 1974.
22. Yanai, J. and B. E. Ginsburg. Long-term effects of early ethanol on predatory behavior in in-bred mice. *Physiol. Psychol.* **4:** 409–411, 1976.