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Behavioral Alterations in Male Golden Hamsters Exposed to Chlorodibromomethane

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KORZ, V., AND R. GATTERMANN. *Behavioral alterations in male golden hamsters exposed to chlorodibromomethane.* PHARMACOL BIOCHEM BEHAV **58**(3) 643–647, 1997.—A number of behavioral parameters in male golden hamsters (*Mesocricetus auratus*) exposed to chlorodibromomethane (CDBM) were registered over a 10-day (day −9 to day 0) pretest period and a 14-day (day 1 to day 14) test period beginning at 8 weeks of age. Whereas the subchronically treated group (5 mg/kg body weight) only showed significantly increased water bottle contacts on days 4–7 compared with vehicle controls, the acutely treated group (50 mg/kg body weight) exhibited significantly increased locomotor activity on days 3–6 and decreased wheel running at day 6 until day 9. In the open field, the acutely exposed males displayed significantly more flank-mark movements at days 4 and 7 than did the vehicle control animals. After day 9, the acutely treated males showed no differences in any parameter compared with the control males, indicating recovery. During the social confrontation on day 14, the subchronically exposed males bit and approached the intruder significantly less often than did the control males, indicating an impairment of selected social behavior affected by CDBM. Our results of low-level (5 mg/kg) behavioral effects contribute to the general characterization of CDBM and suggest that tests on social capabilities in behavioral toxicology provide significant results in a field of low-level and sublethal chronic application on small samples of experimental animals. © 1997 Elsevier Science Inc.

Chlorodibromomethane Activity Open field Behavior Social confrontation Aggression Stress Coping

CHLORODIBROMOMETHANE (CDBM) and other trihalomethanes formed during water disinfection by chlorination are present in drinking water supplies. The exposition of CDBM in rats and mice have induced increases in the weights of liver, thymus, kidneys and spleen (7,18) with fatty change, cytoplasmatic changes, necrosis and hepatocytomegaly, hepatocellular adenoma and carcinoma as certain toxic effects (6,10). Furthermore, reduced brain and body weights (7,10) and depressed food consumption (5) have been observed. The humoral immune system can indicate trihalomethane toxicity by the decreased numbers of splenic IgM antibody-forming cells (18). Most of these effects are determinable only in a range of high-dose administration of CDBM (i.e., >100 mg/kg body weight). Very few studies on the behavioral toxicology of trihalomethanes are available. Alterations of operant behavior (lever press for milk presentations) in mice have been described for 60-day administrations of 100 mg/kg body weight of trichloromethane, dichlorobromomethane and tribromomethane and of 400 mg/kg body weight of dibromochloromethane (1). However, nearly half of the animals given dibromochloromethane died on day 25.

The present study aimed to characterize CDBM at lower sublethal dosages that were more comparable to the concentration in the drinking water supply. The chosen parameters of home-cage behavior (wheel-running, locomotor, and licking activity) and open-field behavior in the golden hamster are proven indicators for well-being and intoxication (12,23). The applied sublethal dosages were based on an approximative LD_{50} of 145 mg/kg body weight, with a 95% confidence interval of 118–187 mg/kg body weight, evaluated by a maximum-likelihood calculation from different preliminary tests. Thus, the male golden hamster shows a considerably higher susceptibility to CDBM than male mice and rats: 800 mg/kg and 1186 mg/kg body weight, respectively (2,4).

METHODS

Animals and Housing Conditions

Adult male golden hamsters from a laboratory stock (Zoh: GOHA) were studied. The animals were kept in a windowless indoor room in standard plastic cages (55 × 33 × 20 cm) lined with wood shavings (Altromin; Faser) with a wire mesh on

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top and a running wheel (30 cm in diameter). Food pellets (one part altromin 7014, two parts Altromin 7024) and water were provided ad libitum. At an age of 6 weeks, 31 males were removed from their litters and housed individually. The light/dark conditions were 12/12 h, the light intensity was 200–300 lx (light period) to 5 lx (dark period). The light period lasted from 07.00 to 19.00 h. The room temperature was 24°C ± 2°C, and the relative humidity was 60–65%. The animals were allowed to adapt to this situation for 14 days. They then were put in clean cages with new wood shavings, and the test session was started.

Treatment

The study lasted over a 10-day (day −9 to day 0) pretest period and a 14-day (days 1–14) test period. On and after day 0, CDBM (98%, Aldrich-Chemie, Steinheim, 20,632-6) in olive oil was administered subchronically to 9 males by gavage at 5 mg (0.024 mmol)/kg body weight (9 males were used as vehicle controls); on day 10, a dosage of 50 mg (0.24 mmol)/kg body weight was administered acutely to 6 males (7 males were used as vehicle controls). Dose mixtures were prepared fresh each day and applied with an inflexible probang 1–1.5 h after lights on. Subchronic 14-day exposure is more suitable than long-term studies in determining the toxicity of halomethanes (18).

Test Procedure

During the study, the home-cage activities, wheel-running, locomotion (by infrared detectors; Wizard Guardall Limited, Edinburgh, Scotland) and contacts with water bottle teats (licking) were counted continually by the Chronobiology Kit system (Stanford Software Systems, Stanford, CA). On day −9 until day 0, behavioral parameters were registered to obtain scores for individual untreated males. On days −6, −3, 1, 4, 7, 10 and 13, the males were exposed to an open field. Within 1–1.5 h after lights on, the males were removed from their home cages via a plastic cup, weighed and individually placed by hand into a plastic cage of the same size as their home cage, cleaned each time with 3% acetic acid and covered with a holey plastic top. The light intensity within the test cage, placed in the housing room, was 75 lx. The animals were observed for 5 min and then placed back into their home cages. Application always took place after the open-field test. Behavioral occurrences and duration were fed by direct observation into a laptop computer with the Observer program package (Noldus, version 3.0). Locomotion was registered automatically with a commercial recording system (TBS 402, G/P-Elektronik, Berlin) and given as traveled distance (meters) during observation time. On day 7, the males were put in clean cages with fresh wood shavings. On day 14, without previous application of CDBM, the males were confronted in their home cages with a same-sexed intruder for 10 min, and the behavior of both males was recorded by direct observation. Intruders, randomly taken from the laboratory stock, were weight matched; mean body weights of the intruders were 140.8 ± 27.3 g for the treated males and 134.2 ± 27.3 g for the vehicle controls. Confrontations took place within 1–1.5 h after lights on. Open-field and intruder test data of only 6 treated and 5 control males for the acutely dosed group and 7 males each for the subchronically treated group were available.

Behavioral Elements

The following behavioral elements in the open field were recorded: defecation, locomotion, rearing, urination, and flank marking (Fig. 2). Flank marking is when the animal arches its back toward the cage wall, rubbing its side with the flank gland against the wall. Scrape is defined as the animal scraping the ground alternately with both forefeet. Self-grooming is defined as the occurrence of at least one of these elements: washing the face, scratching with hind feets and nibbling the fur.

The number of occurrences (in the case of short and distinct acts such as flank marking) or the total duration of executed behavioral elements (those lasting more than 1 s, e.g., self-grooming) of individual males were computed.

The following behavioral elements were recorded during the intruder test: chase, follow, retreat, sniff, and upright stance (Fig. 3). Approach is defined as the action of one animal approaching the other. Bite is defined as the action of one male biting the other.

Nonbehavioral Parameters

At the end of the study (on day 14), the males were killed by decapitation, and the kidneys, adrenal glands, testes and seminal vesicles were removed and weighed. Hematocrit and hemoglobin values from blood samples were determined. Food and water consumption in the pretest phase and in the first and second halves of the test phase were determined by reweighing.

Statistics

Daily activity of treated males and vehicle controls in wheel running, locomotor activity and licking in the home cages, the behavioral scores in the open-field and during the intruder tests and the scores for the nonbehavioral parameters were compared with the Mann–Whitney *U*-test. Differences in locomotor activity scores before and after cage change were evaluated by the Wilcoxon sign-rank test. The level of significance in all tests was set at $p \leqslant 0.05$. A pretest value, the arithmetic mean of the last six per day values before first application, was calculated to exclude the effect on the mean value of the samples caused by differences in individual dispositions toward executing a specific behavior. The test values were given as the deviation from the pretest value. Values were presented as arithmetic means and standard deviations. For the intruder tests, values are presented as median with interquartile ranges and minimum/maximum values.

RESULTS

The results of the nonbehavioral measures investigated are given in Table 1. None of these parameters, generally used in toxicology, offered significant differences between males exposed to CDBM and vehicle control males. Figure 1 shows the mean daily test values of the wheel-running, locomotor and licking activities of the males. Only the acutely treated males displayed significantly higher locomotor activity from day 3 to day 6 and a decreased wheel-running activity from day 6 to day 9 as compared with the controls. The reason for the decline on day 1 of both the acutely treated and control males is still unclear.

The subchronically treated males exhibited significantly greater licking activity on days 4–7 than did the controls. The water intake, measured by reweighing the water bottles, did not significantly differ between groups (11.1 g ± 2.0 g/day vs. 9.9 g ± 0.5 g/day; $n_1 = 9$, $n_2 = 9$; $U = 27$, $p = 0.231$; values for the first half of the test phase; for the overall intake, see Table 1). This result suggests that treated males gnawed the teats of water bottles even without consuming water, which may indicate stereotypic behavior.

TABLE 1

NON-BEHAVIORAL PARAMETERS IN MALE GOLDEN HAMSTERS SUBCHRONICALLY (14 DAYS) AND ACUTELY EXPOSED TO CHLORODIBROMOMETHANE

	Subchronic		Acute	
	5 mg/kg Body Weight	Vehicle Control	50 mg/kg Body Weight	Vehicle Control
Body weight [g]*	9.90 ± 4.62	13.38 ± 4.59	14.56 ± 1.41	10.13 ± 4.54
Water intake [g/day]	9.71 ± 2.10	10.32 ± 0.43	11.19 ± 0.58	10.90 ± 0.63
Food intake [g/day]	8.03 ± 1.36	8.81 ± 1.36	9.69 ± 0.94	10.23 ± 2.71
Kidneys weight				
(absolute) [mg]	924.9 ± 43.3	962.0 ± 71.4	906.7 ± 52.0	955.0 ± 92.1
(relative) [%]	0.72 ± 0.04	0.68 ± 0.04	0.73 ± 0.04	0.75 ± 0.04
Adrenal glands weight				
(absolute) [mg]	27.57 ± 3.10	28.57 ± 1.13	30.83 ± 2.23	31.80 ± 2.17
(relative) [%}	0.021 ± 0.002	0.020 ± 0.001	0.025 ± 0.001	0.025 ± 0.001
Testes weight				
(absolute) [g]	3.34 ± 0.60	3.15 ± 1.00	3.25 ± 0.34	3.46 ± 0.50
(relative) [%]	2.60 ± 0.57	2.26 ± 0.80	2.63 ± 0.27	2.73 ± 0.47
Seminal vesicles weight				
(absolute) [mg]	594.1 ± 126.0	476.7 ± 259.9	637.2 ± 87.3	636.4 ± 82.3
(relative [%]	0.46 ± 0.11	0.35 ± 0.20	0.51 ± 0.06	0.50 ± 0.08
Hematocrit [%]	48.3 ± 0.6	47.7 ± 2.1	48.5 ± 1.3	51.0 ± 1.4
Hemoglobin [g/100ml]	20.64 ± 3.58	20.93 ± 3.59	22.33 ± 2.96	21.17 ± 5.15

The arithmetic means and standard deviations are given. Relative weights are given as percentage of body weight.
*Body weights are given as deviation from pretest va'lue on day 14. Water and food intakes refer to the test phase. There are no significant differences between CDBM-exposed males and vehicle control males. Mann–Whitney *U*-test, $p > 0.05$, two-tailed.

After the cage change at day 7, an overall increased locomotor activity in both samples was registered (Fig. 1). Comparing the means of days 5 and 6 with those of days 8 and 9 provide the following results: acute group before cage change, −27.2 counts ± 137.1 counts and thereafter 177.4 counts ± 245.1 counts, $t = 11$, $p = 0.01$, $n = 13$; subchronic group before cage change, 17.7 counts ± 111.0 counts and thereafter 159.3 counts ± 196.8 counts, $t = 31$, $p = 0.02$, $n = 18$. This induced

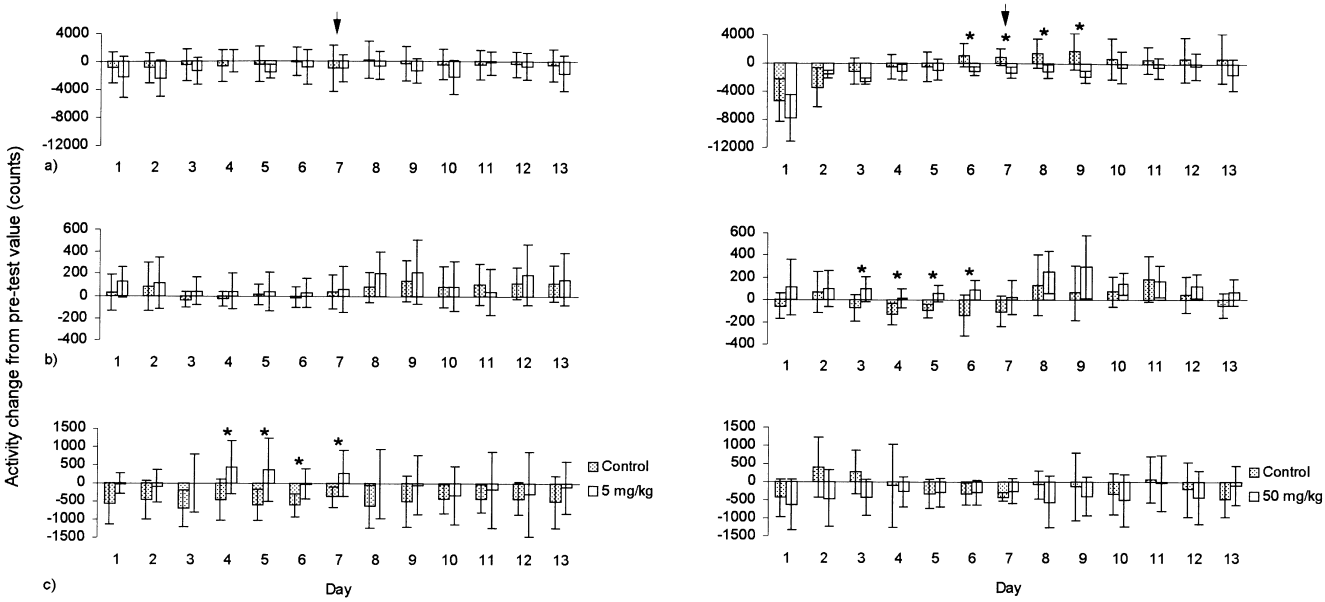

FIG. 1. Wheel-running (a), locomotor (b) and water bottle contact (c) activities of subchronically (left) CDBM-exposed (5 mg/kg body weight, $n = 9$) and vehicle control ($n = 9$) and of acutely (right) CDBM-exposed (50 mg/kg body weight, $n = 6$) and vehicle control ($n = 7$) male golden hamsters in their home cages. The arithmetic averages and standard deviations of test values are given (as deviation from the arithmetic average of the counts of the last six days before the first application: prevalue). The arrow indicates the day of cage changes. *Significant differences, $p <$ 0.05, two-tailed, Mann–Whitney *U*-tests.

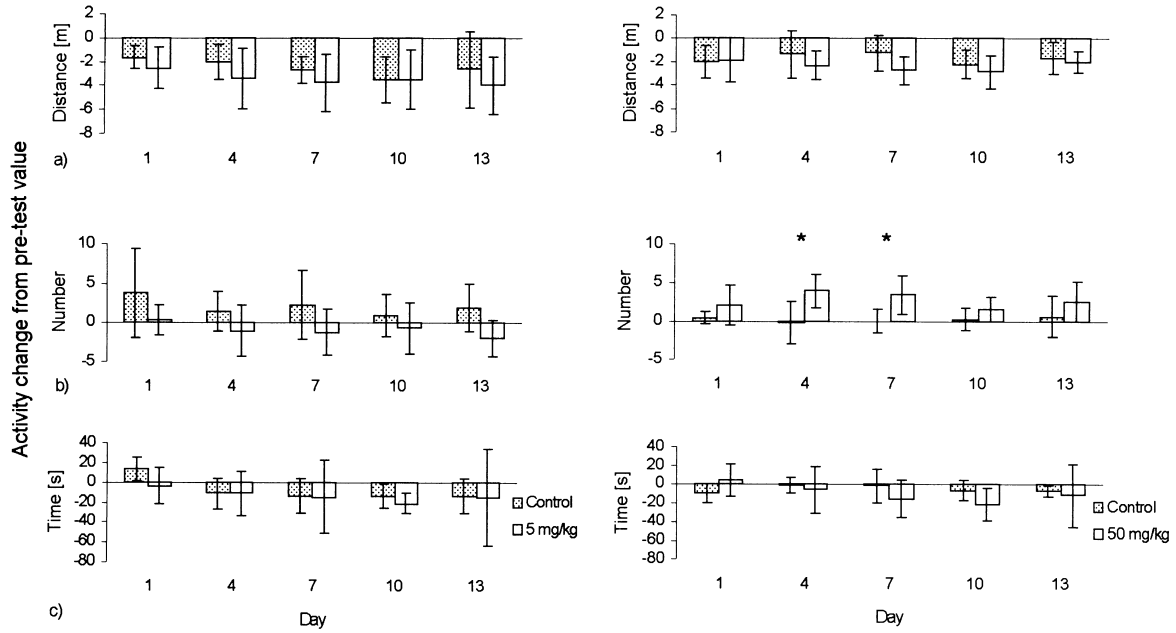

FIG. 2. Open-field behavior (a: locomotion, b: flank marking, c: scraping) of subchronically (left) CDBM-exposed (5 mg/kg body weight, $n = 7$) and vehicle control ($n = 7$) and of acutely (right) CDBM-exposed (50 mg/kg body weight, $n = 6$) and vehicle control ($n = 5$) male golden hamsters. The arithmetic averages and standard deviations of test values are given (as deviation from the arithmetic average of the scores of two tests before the first application: prevalue). The test took 5 min. *Significant differences, $p < 0.05$, two-tailed, Mann–Whitney *U*-tests.

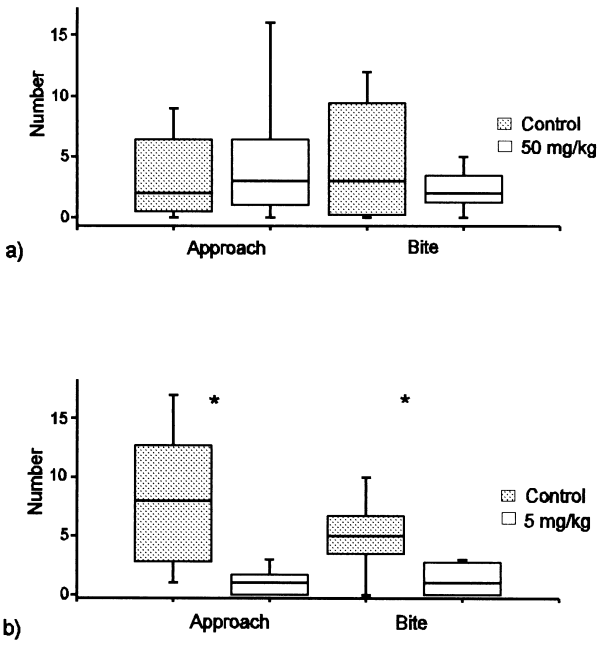

FIG. 3. Behavior during the intruder tests (10 min) of acutely (a) CDBM-exposed (50 mg/kg body weight, $n = 6$) and vehicle control ($n = 5$) and of subchronically (b) CDBM-exposed (5 mg/kg body weight, $n = 6$; one test had to be stopped because of severe wounds on the treated male) and vehicle control ($n = 7$) male golden hamsters. The medians with interquartile ranges and minimum and maximum values are given. *Significant differences, $U = 6$, $p = 0.03$, two-tailed, Mann–Whitney *U*-tests.

activity increase was slightly but not significantly higher in the treated groups. Along with this finding, the differences in licking behavior disappeared.

Figure 2 shows the test values of locomotion, flank marking and scraping in the open field. Only in the frequencies of flank marking did a significant difference occur between acutely treated and control males. The first marked more frequently on days 4 and 7. In all other open-field parameters, there were no significant differences between controls and CDBM-exposed males in both treatment groups (data not shown).

The results of the intruder tests are given in Fig. 3. There were no significant differences in the behavior of the acutely dosed and control group toward the intruders. In contrast, there was a distinct and significant difference in the numbers of approaching and biting between the subchronically treated males and the controls, i.e., the treated males approached the intruder less and bit it less often than did the control males. This behavior was not caused by the corresponding behavior of the intruder because it did not differ between the dosed and control groups: approach 1/1.25 and bite 0.0/0.0 toward treated males; approach 1.0/1.0 and bite 0.0/0.0 toward control males ($U = 16$, $p = 0.459$ and $U = 21$, $p = 1.0$; $n_1 = 6$, $n_2 = 7$ each; the medians/mean interquartile intervals are given, Mann–Whitney *U*-tests, each two tailed). This result also holds true for the behavior of the intruders toward the acutely dosed and control groups. In a cross comparison, there were no significant differences in the behavioral scores of all untreated animals, i.e., the control and intruder groups. Therefore, the alteration of this social behavior was an effect of the CDBM application.

DISCUSSION

The present study aimed to characterize CDBM at sublethal dosages by different behavioral indicators. The open-field

method and the chosen parameters of home-cage behavior such as wheel-running and locomotor activity are frequently employed in behavioral toxicology, whereas tests on social behavior such as intruder tests are rarely used in that context (9,11,17).

In consequence of the acute gavage (50 mg/kg), we found differences in the home-cage activities of wheel running and locomotion and in the open-field activity of increased flank marking compared with the vehicle controls. The causation of the latter finding is unclear, and for a clarification we need more detailed studies on this phenomenon. During the first half of the subchronic treatment period (5 mg/kg), the males showed more licking activity than the controls. However, because the males did not differ in their water intake, this behavior can be assumed to be stereotypic gnawing.

This CDBM-induced behavior diminished when the cages were changed. The exposition to the new environment has been correlated with different stress responses [e.g., heart rate and core body temperature increase (13)] that were accompanied in the present study by a switch from licking to locomotor activity. Such a change from stereotypic wire gnawing to other active behavior in response to variations in the external conditions also has been found in laboratory mice (24). Stereotypic behavior in general can be regarded as a strategy to cope with increased stress levels (3,16,20).

The applied intruder tests trigger social stress [e.g., in golden hamsters, guinea pigs and rats (13,15,21,22)] and thus require other behavioral abilities for coping. In untreated male golden hamsters, the consequence of such a confrontation is an immediate attack on the intruder by the resident (8,14,19). In contrast, the subchronically treated animals in the present study were less aggressive, indicating an impact of CDBM. However, the acutely dosed males did not differ from controls. Because there were no more differences as compared with the controls in either test value after day 9 of the postapplication period (Figs. 1, 2), the acutely treated males seemed to have recovered by the time of the intruder confrontation (day 14). Social confrontation tests also are suitable to obtain clear differences between young male rats and mice chronically exposed to low levels of lead (9,11,17). Although in rats aggression decreases toward same-sexed intruders (11), it increases in mice with a shorter latency to fight (9).

Our results of low-level (5 mg/kg) behavioral effects contribute to the general characterization of CDBM. Tests on selected social behavior in toxicology provide significant results in a field of low-level and sublethal chronic application on small samples of experimental animals.

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