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Behavioral Effects of Acrylamide in the Mouse[1]

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Behavioral Effects of Acrylamide in the Mouse. TEAL, J. J., AND EVANS, H. L. (1982). *Toxicol. Appl. Pharmacol.* **63,** 470–480. The behavioral toxicity of acrylamide was characterized in the mouse by comparing standard measures of toxicity such as body weight loss and mortality with measures of hindlimb grip strength, locomotor activity, and appetitive behaviors including episodic milk-licking. In the latter test, the mouse received 15-min access daily to a highly palatable, nonessential food substance (10% sweetened milk) that was not available in the maintenance diet. Two strains of mice, CD-1 and C57BL/6J, were injected ip five times weekly with either saline, 20, 60, or 100 mg/kg of acrylamide. Subchronic, but not acute, administration of 100 mg/kg produced weight loss, a severe neuropathy within 3 days, and 100% mortality within 2 weeks. Mice receiving 60 mg/kg subchronically had only a slight loss of body weight but developed a neuropathy within 3 weeks of dosing at which time there was a 50% mortality. Preceding these signs of toxicity, there was a highly significant increase in episodic milk-licking; this increase was significant in both strains of mice by Day 2 and remained elevated throughout the dosing period. The 20 mg/kg dose of acrylamide decreased hindlimb grip strength after 5 weeks of dosing but did not affect milk-licking or body weight even after 7 weeks of dosing. The new use of this test of appetitive behavior has documented a robust effect of acrylamide which preceded other signs of toxicity. The appearance of the various toxic signs was better predicted by the magnitude of the daily dose than the cumulative dose. This study also demonstrated the feasibility of using the mouse in behavioral toxicology.

Acrylamide monomer has found many uses in the mining, paper and polymer industries but data from occupational accidents have shown that acrylamide is also a neurotoxicant. In addition to general signs of toxicity such as weight loss, humans exposed to acrylamide show signs of neurotoxicity such as fatigue, mental confusion, ataxia, numbness, and profuse sweating of the extremities (Garland and Patterson, 1967; Auld and Bedwell, 1967). Acrylamide-induced neuropathy begins with the involvement of the distal limbs and slowly progresses to the proximal regions of the body. Histologically, acrylamide causes a distal to proximal dying-back axonopathy of both sensory and motor nerves (Hopkins, 1975; Davenport *et al.*, 1976; Schaumburg *et al.*, 1974); degeneration has also been observed in the central nervous system (Schaumburg, 1979). The neuropathy is usually reversible after the cessation of acrylamide exposure with the dose and duration of exposure being the limiting factors.

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Neuropathy has also been found in rats exposed to acrylamide as documented by a decrease in hindlimb grip strength (Meyer *et al.*, 1979) and an inability to maintain balance on a revolving bar, i.e., rotarod test (Kaplan and Murphy, 1972). Acrylamide-exposed rats also exhibit severe signs of toxicity such as tremor, ataxia, weakness, and bladder distention (LeQuesne, 1980; McCollister *et al.*, 1964; Jolicoeur *et al.*, 1979).

Histological and neurological effects of acrylamide are well known but behavioral effects in animals have received less investigation. Behavioral measures may provide noninvasive evidence of acrylamide toxicity before the occurrence of overt toxic signs. Evidence for this condition is provided by an extensive behavioral toxicological study in rats indicating body weight as the most sensitive measure of subchronic exposure to acrylamide (Tilson *et al.*, 1979a). These investigators predicted that changes in appetitive behavior may precede changes in body weight and, therefore, may be an even more sensitive indicator of acrylamide toxicity.

The objectives of this study were twofold: first, to evaluate the effects of acrylamide (20 and 60 mg/kg) on a test of appetitive behavior (i.e., milk-licking) against standard measures of toxicity such as locomotor activity, body weight, and hindlimb grip strength. Both time-effect and dose-response functions were used in comparisons between tests. The second objective was to determine the suitability of using the mouse for evaluation of behavioral toxicity both acutely and subchronically and to characterize the behavioral effects of acrylamide in this species.

METHODS

Test animals. Fifty-nine male CD-1 mice (Charles River Breeding Co., Wilmington, Mass.) and eighteen male C57 BL/6J mice (Jackson Laboratory, Bar Harbor, Maine) were the subjects in these experiments. All animals were drug-naive at the beginning of the experiment and were housed individually with food (Purina Lab Chow) and water available *ad libitum*. The overhead fluorescent lights in the room were illuminated between 7 AM and 7 PM. The room temperature was 22.2 ± 1°C with a relative humidity of 45 to 75%.

Forty-one CD-1 mice were employed in the first experiment. These mice weighed between 30 and 38 g (52 days old) at the beginning of the experiment and were divided into four groups. Eleven mice were randomly assigned to the group which received 100 mg/kg of acrylamide (i.e., the lethality group). The remaining 30 mice received one week of preliminary testing using the licking paradigm (see below). Data from the last 2 days were used as the basis for dividing the animals into three matched groups of 10 which were counterbalanced for their licking scores. These three groups of mice were designated as the saline (control) group, the 20 mg/kg of acrylamide group, and the 60 mg/kg of acrylamide treatment group, respectively.

The results of the first experiment were replicated (Experiment II) with 18 CD-1 mice with body weights between 28 and 35 g (45 days old) and 18 C57BL/6J mice with body weights between 18 and 23 g (72 days old) at the beginning of the experiment. The mice of each strain were divided into two equal groups counterbalanced for their licking scores obtained during the week of preliminary testing. The two groups were designated as the saline (control) group and the 60 mg/kg acrylamide treatment group.

Licking paradigm. Individual mice were tested for licking in one of six identical test chambers. The chambers were constructed of black Plexiglas and measured 18 cm long by 15.5 cm wide by 15 cm high. A 1-cm by 2-cm hole was located at the end of the chamber 1 cm above the floor. Through this hole the mouse had access to a stainless steel spout containing a 10% solution of sweetened condensed milk in water (Eagle Brand, Borden Co., St. Louis, Mo.). A capacitance operated touch detector (Loveland and Sons, Marblehead, Mass.) and a PDP8 computer with SKED interface recorded the number of licks on the spout for each 15-min session. Results from another study (unpublished data) show that an average of 370 licks is equivalent to 1.0 ml of milk consumed; volume consumed was not routinely measured. Although food deprived mice can be trained to perform complex operant behaviors in order to obtain milk as a reward (Wenger and Dews, 1976), prior results indicated that rats (Evans, 1971) and mice (unpublished data) would readily drink this solution even if they were not food or water deprived.

Locomotor activity was also measured during each 15-min test session (Evans, 1971). Stainless steel bars (3.1 mm in diameter) formed the floor of the chamber described above; two of these bars were connected to a computerized touch detector of the type described above. The total number of times a mouse touched either of the two grids was recorded for each session (i.e., activity counts).

Test sessions of 15-min duration were conducted in the morning after the mice had been weighed. Each animal was always tested in the same chamber, with the assignment of the test chambers being counterbalanced across the three treatment groups. Mice were tested five times weekly during the first 7 weeks of the experiment and twice weekly thereafter (Experiment I). None of the mice in Experiment II were tested for behavioral effects after Day 16 of dosing.

Hindlimb grip strength. Once weekly, hindlimb grip strength of the CD-1 mice in the first experiment was measured by the method of Meyer *et al.* (1979). A mouse was pulled by the tail through a narrow trough where its hind feet encountered and gripped a bar that was positioned parallel to the end of the trough. The mouse continued to be pulled until the hind feet released their grip on the bar. The bar was connected to a force transducer which recorded the force required to break the mouse's grip. The score for each mouse was obtained by averaging the force recorded for three trials in which the mouse firmly gripped the bar with both hind feet. In order to decrease the variability of this test, it was always conducted by the same investigator.

A modification of the "landing foot-spread" method of Edwards and Parker (1977) was used to quantitate the degree of neuropathy in these same mice. Mice were dropped from a height of 33 cm onto a soft, smooth matrix made of flour and water. Their hind paws left an indentation in the matrix which could be used to measure the degree of splaying of the hindlimbs which Edwards and Parker (1977) found to correlate with the grip strength of the hindlimbs. For each mouse, the distance between the third digit on each of the two hind paws was measured in three trials; the average of these trials was the weekly score.

Lethality study. Eleven mice in the first experiment were injected ip with 100 mg/kg of acrylamide for 5 days/week until all members of the group had died. These mice were weighed daily and tested weekly for hindlimb grip strength.

Acute acrylamide administration. At the end of 81 days, 10 control mice which had received saline injections in the first experiment were used to study the effects of an acute dose of acrylamide. Five of these animals continued to be used as controls and received a saline injection (ip), whereas, the other five mice received one dose of 100 mg/kg of acrylamide (ip). All mice were injected in the late afternoon the day before the first behavioral test; the first milk-licking test occurred 18 to 20 hr after the injection (i.e., Day 1). Body weight and milk-licking were monitored daily. The homecage water and food intake for each individual mouse (one mouse/cage) was determined daily by weighing the water bottles and food cups (powdered food).

Dosing and solutions. Acrylamide (reagent grade, J. T. Baker Chemical Co., Phillipsburg, N.J.) was dissolved in sterile 0.9% saline (Abbott Laboratories, North Chicago, Ill.) to deliver the desired dose (20, 60, or 100 mg/kg) in a volume of 0.1 ml. Solutions were made fresh every other week and were stored in the dark in a refrigerator. Mice were injected ip with either saline or acrylamide in the afternoon 5 days per week with no injections on Saturdays or Sundays. The duration of the dosing differed among the various groups. In the first experiment dosing with acrylamide was discontinued in the 60 mg/kg group after 21 days. Thereafter, these animals received saline injections (as did control mice) up to Day 47, when injections were stopped for all three groups. In the second experiment dosing with 60 mg/kg of acrylamide and saline lasted 18 days; these mice were not studied further after this time. Acrylamide doses were determined on the basis of a pilot study in which tremor and lethality were evaluated in mice receiving similar doses of acrylamide for one week. Behavioral tests were administered in the morning, at least 17 to 21 hr after the most recent injection to minimize the influence of any acute irritation associated with the injection.

Statistical analyses. Data for behavioral tests from mice that died before completing the study were excluded from statistical analyses. For the subchronic behavioral experiment, overall differences between treatment groups and control groups during the dosing period were evaluated by an analysis of variance for repeated measures. Those groups which were significantly different ($p \leq 0.05$) were analyzed further by the Welch statistic for a two-sample t test. The Welch statistic is considered to be a conservative test (Welch, 1947) in which the variance of each group is estimated separately. All calculations were made with BMDP computer programs (Dixon and Brown, 1979).

RESULTS

Subchronic administration of acrylamide produced both a dose-related and a time-related increase in mortality (Fig. 1). The mortality for the mice that received 100 mg/kg of acrylamide reached 100% within 13 days from the beginning of the injections (10 doses) with a cumulative dose between 600 and 700 mg/kg producing a 50% mortality. The group receiving 60 mg/kg of acrylamide in the first experiment reached a 50% mortality after 31 days (16 doses = 960 mg/kg cumulative dose). There were no deaths in the 20 mg/kg group even after 49 days of acrylamide administration (34 doses = 680 mg/kg cumulative dose; data

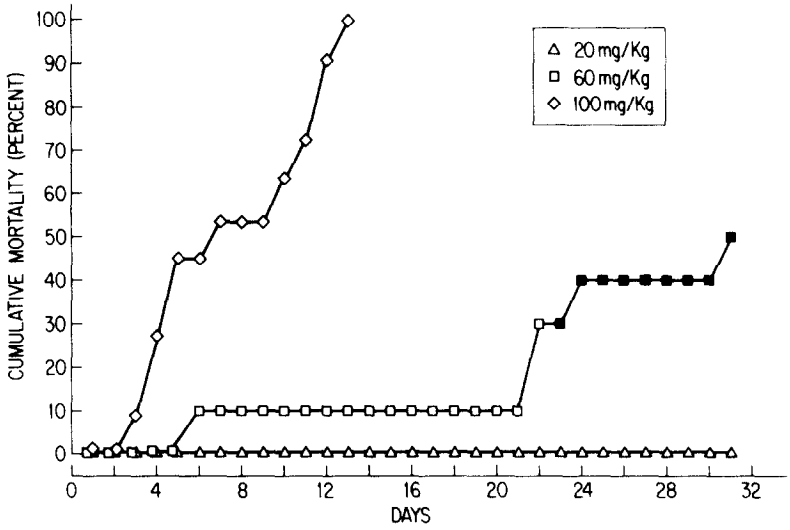

FIG. 1. Daily percentage cumulative mortality of CD-1 mice in Experiment I given acrylamide (ip), five times weekly. The filled symbols signify data obtained following the cessation of acrylamide exposure. Initially there were 11 mice in the 100 mg/kg group and 10 mice in each of the other groups.

only shown for the first 31 days). There were also no deaths in the saline-injected group (data not shown). In the replication study two out of nine CD-1 mice died within 18 days of acrylamide administration (60 mg/kg) whereas none of the C57BL/6J mice in the 60 mg/kg group died during the same dosing period (data not shown). All of the mice that died exhibited tremor and hindlimb weakness; several had distended bladders at necropsy, a sign of loss of peripheral nervous system function also reported in rats (McCollister *et al.*, 1964; Edwards, 1975) and humans (Garland and Patterson, 1967).

The severity of the toxicity of 100 mg/kg of acrylamide given subchronically was further demonstrated by the accelerated loss of body weight in this group compared to the weight loss of the mice in the 60 mg/kg group (Fig. 2). There was a large rebound in body weight of the 60 mg/kg group over the weekends, when no injections were given (see Days 7 and 14, which were Mondays, in Fig. 2). This weekend rebound in body weight allowed these mice to stay within the range of the mean body weight of the control group. Both CD-1 and C57BL/6J mice in the replication study had similar acrylamide-induced changes in their body weights (data not shown). After acrylamide was discontinued in the 60 mg/kg group (Week 4), body weights gradually increased and remained slightly higher than those of the control group. The mice in the 20 mg/kg group did not exhibit any weight loss but maintained their initial status of being 3 to 8% heavier than the control group.

Chronic administration of 60 mg/kg of acrylamide caused a significant ($p \leq 0.01$) increase in the number of licks of a solution of sweetened milk (Fig. 3). The milk-licking data for the CD-1 mice in experiments I and II were pooled since there was no significant difference between the two groups. The increase in milk-licking by mice given 60 mg/kg became significant on Day 2 and remained significantly different from saline controls for the duration of the dosing period. Over the course of 16 days of dosing there was a gradual increase in the number of licks by the control mice. The 60 mg/kg acrylamide-treated mice showed a within-

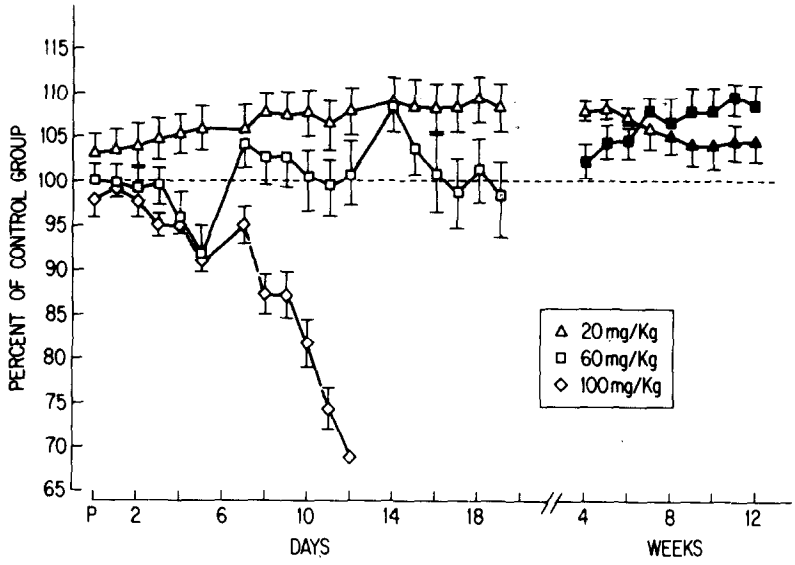

FIG. 2. Mean (±SEM) body weights of CD-1 mice in Experiment I receiving acrylamide (ip), five times weekly expressed as the percentage of the saline group's mean. Body weights were measured on a Sartorius 3716 electronic balance to an accuracy of ± 0.1 g. The filled symbols signify data obtained following the cessation of acrylamide exposure. The P on the abscissa denotes the pretreatment mean of the control group (34.2 g); the control group averaged 38.7 g on Week 12. The abscissa is defined in terms of days for the first 19 days (days 1, 7, and 14 are Mondays) and thereafter in weeks. Data expressed in terms of weeks represent the mean weight for each group during the week. For clarity, only one side of the SEM bars is shown in some cases. Other details are the same as in Fig. 1.

week pattern of increasing their licking as the week progressed. Licking was elevated less following the weekends, when dosing was suspended, although licking was still significantly higher than controls. Control mice did not show this within-week pattern.

Data from Fig. 3 are replotted in Fig. 4 as weekly means to illustrate recovery after dosing was discontinued. The increase in milk licking was qualitatively similar in the CD-1 and C57BL/6J mice receiving 60 mg/kg. Licking remained elevated through week 3 for the CD-1 mice but was no longer significantly elevated for the C57BL/6J mice at 3 weeks. Thereafter, individual CD-1 mice differed considerably in the number of licks per session with 40% of the mice having a high number of licks and others returning to control levels. None of the data points after cessation of dosing were significantly different from controls due to this high variability between individual mice, and the smaller number of subjects used. The mice given 20 mg/kg did not differ from control mice.

Mice receiving 100 or 60 mg/kg of acrylamide exhibited hindlimb neuropathy which could be quantified by measuring the grip strength of the mouse's hindlimbs (Fig. 5). Mice exposed to 100 mg/kg showed a significant ($p \leq 0.01$) decrease in hindlimb grip strength within the first week of dosing (300 mg/kg cumulative dose). By Week 2 the grip strength was 30% of that of the control group (cumulative dose of 800 mg/kg). Mice in the 60 mg/kg group had a significant ($p \leq 0.05$) loss of grip strength starting in the third week of exposure (780 mg/kg cumulative dose). Their grip strength decreased further to 11% of control on Week 4, following cessation of acrylamide administration. Thereafter, these mice showed a time-related recovery which was significantly complete by Week 7. The 20 mg/kg group

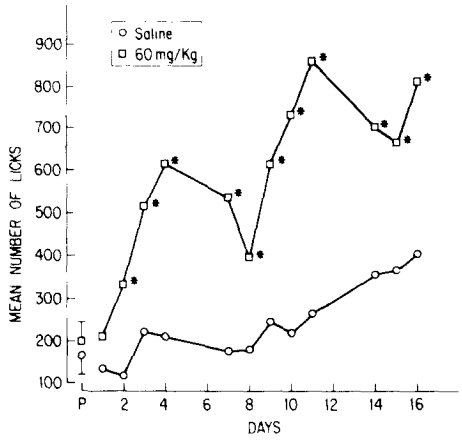

FIG. 3. The daily mean number of licks of sweetened milk by CD-1 mice from both Experiments I and II. Mice were injected with either 60 mg/kg of acrylamide ($n = 17$) or saline ($n = 19$) Monday through Friday throughout the 16 days shown (Days 4 and 11 are Fridays). The points above P on the abscissa indicate the mean from two pretreatment sessions ± SEM. Points with asterisks indicate significant ($p \leq 0.05$) difference from saline-treated mice. Saline-treated mice averaged 243 licks and the acrylamide-treated mice averaged 582 licks per 15-min session which was equal to consuming approximately 0.7 and 1.6 ml of milk, respectively.

showed a smaller and more gradual decrease in hindlimb grip strength which became significant ($p \leq 0.01$) on Week 5 (460 mg/kg cumulative dose). These animals also completely recovered after termination of the acrylamide injections (Week 10). At Week 7 these mice had received a total cumulative dose of 660 mg/kg.

The landing foot-spread technique also provided evidence of acrylamide-induced neuropathy (Table 1) with foot spread being affected by acrylamide in two ways. At low doses (20 mg/kg) and early in exposure to higher doses (60 and 100 mg/kg), hindlimb splaying produced an increase in landing foot spread. As the severity of hindlimb weakness progresses with continued exposure to high doses (60 mg/kg), the mice become unable to extend their legs when dropped and therefore the foot spread is lower than control values. In the 60 mg/kg group, the smallest foot spread measurement was observed when the grip strength was the lowest (Week 4). However, due to the high variability, the differences were not significant.

Although the hindlimbs of the mice in the 60 mg/kg group were very weak, the mice were able to use their forelimbs for locomotion. Evidence of this continued mobility of the mice was that the locomotor activity scores of mice of the 60 mg/kg acrylamide group were not significantly different from control mice. The mean (±SEM) locomotor activity scores for CD-1 mice (Expt II) for Weeks 1, 2, and 3 of dosing were as follows: Control group = 105.1 ± 17, 111.0 ± 18, and 77.2 ± 21, respectively, and 60 mg/kg acrylamide group = 95.5 ± 12, 116.1 ± 27, and 91.6 ± 19, respectively.

In order to compare the effects of acrylamide administered acutely versus subchronically, appetitive behaviors were evaluated in mice treated acutely with either 100

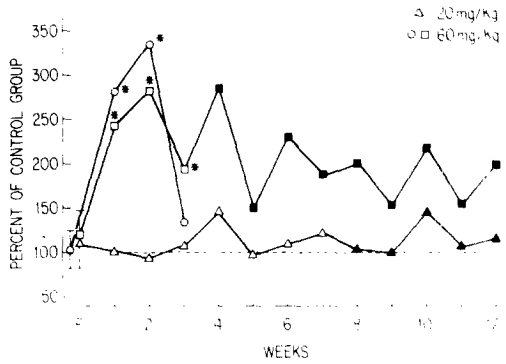

FIG. 4. The weekly mean licking of sweetened milk by acrylamide-treated mice expressed as the percentage of the licking by saline-injected mice. Each point represents the weekly mean number of licks determined in two to five sessions. The points above P on the abscissa indicate the data from two pretreatment sessions ± SEM. CD-1 mice from both Experiments I and II combined (△, □) and the C57BL/6J mice (○) in Experiment II are shown. The saline-treated controls averaged 243 and 256 licks for the CD-1 and C57BL/6J mice, respectively. The filled symbols signify data obtained following the cessation of acrylamide exposure. Asterisks denote a significant difference from saline-treated mice ($p \leq 0.05$). Other details are the same as in Fig. 2.

mg/kg of acrylamide or saline. Acute injection of acrylamide had no effect upon body weight and only slightly increased milk-licking (Fig. 6). In contrast, two other appetitive behaviors, homecage food and water consumption, were markedly decreased on Day 1 (Fig. 6). Thereafter, all appetitive behaviors increased above the control level before starting to return to pretreatment levels. No deaths occurred with the acute 100 mg/kg dose of acrylamide.

TABLE 1

LANDING FOOT SPREAD (mm ± SEM)

Week	Control	20 mg/kg	60 mg/kg	100 mg/kg
1	38 ± 6	40 ± 5	41 ± 6	46 ± 4
2	42 ± 5	46 ± 4	46 ± 4	55 ± 5
3	42 ± 4	45 ± 3	41 ± 4	
4	42 ± 6	44 ± 4	31 ± 7	
5	40 ± 5	45 ± 5	34 ± 4	
6	38 ± 11	46 ± 5	36 ± 2	

Note. Weekly mean landing foot spread (±SEM) for mice receiving saline (control), 20, 60, or 100 mg/kg of acrylamide (ip). Mice were dropped from a height of 33 cm onto a soft, horizontal surface, and the distance between their two hind paws after landing was measured. Each group initially consisted of 11 (100 mg/kg) or 10 (other three groups) mice. The data from the 100 mg/kg group are shown only for Weeks 1 and 2 because all of the mice died after the second week.

DISCUSSION

Toxicants are generally reported to decrease appetitive behaviors such as homecage food and water consumption (Berthoud *et al.*, 1976; Tilson *et al.*, 1979a; Squibb *et al.*, 1980). Our data showed that subchronic administration of 60 but not 20 mg/kg of acrylamide produced a robust increase in episodic milk-licking even in severely intoxicated mice. Furthermore, this test of appetitive behavior revealed effects of acrylamide earlier than other tests of toxicity. These results were replicated in two experiments with CD-1 mice and in one experiment with C57BL/6J mice.

Increased thirst and/or hunger are two physiological mechanisms that might account for the observed increase in milk-licking. Acrylamide produces disturbances in water balance (Gipon *et al.*, 1977) and pro-

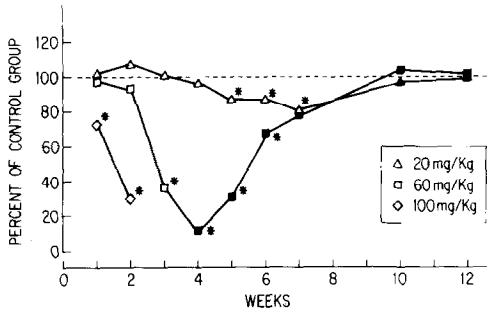

FIG. 5. The mean hindlimb grip-strength of CD-1 mice injected with acrylamide expressed as the percentage of the mean grip-strength of saline-treated mice. Mice were given three trials at each weekly test. Asterisks indicate significant difference from saline-treated mice. The average score for the saline-treated mice was 112 g. Filled symbols represent data obtained after dosing stopped. Other details are the same as in Fig. 2.

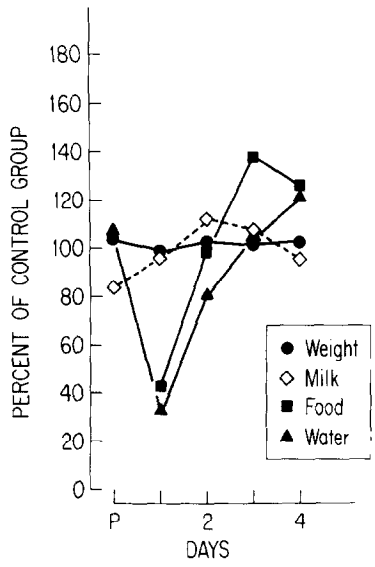

FIG. 6. Mean body weight (●), milk licking (◇), home cage food consumption (■), and home cage water intake (▲) in five mice tested before (P) and on each of 4 days after an acute ip injection of 100 mg/kg of acrylamide. Results are shown as the percentage of saline-injected mice which averaged 37.5 g for body weight, 796 licks for milk licking, 4.6 g for daily home cage food consumption and 6.7 ml for home cage water consumption.

duces lesions in the hypothalamus (Schaumburg and Spencer, 1978), an important center in thirst and hunger regulation (Lytle and Campbell, 1975; Lytle and Messing, 1976). In addition, other toxicants have produced "antidotal thirst," a behavioral adaptation by which an animal increases its water load to increase the urinary excretion of water-soluble toxicants, thereby lessening their toxic effects (Smith *et al.*, 1970; Mineka *et al.*, 1972).

Several reasons argue against any of the above mechanisms as the cause of the acrylamide-induced increase in milk-licking. If central nervous system lesions had occurred, the recovery of milk-licking to control levels would have been much slower than observed (Fig. 4); the gradual recovery of grip strength (Fig. 5) was more typical of nerve regeneration. On the other hand, the recovery of milk-licking was not fast enough to be attributed to an osmotic effect of acrylamide. Note that the elevated milk-licking persisted on Mondays after acrylamide administration had been discontinued for 2 days (Fig. 3). Furthermore, elevated licking occurred when body weight was equal to or above control weight (e.g. Mondays in Fig. 2), ruling out hunger due to weight loss as the driving force behind milk-licking. The calories obtained by our mice from the episodic consumption of milk did not greatly affect body weight since the average milk intake never exceeded 5% of the mouse's daily requirement of 20 kilocalories (Purina, 1980). Since our nondeprived, acrylamide-treated mice drank 35% of their normal daily fluid intake during the brief 15-min daily access to milk, the taste of the milk appears to be an important factor.

Although we have not identified the physiological mechanism of acrylamide-induced licking, these data can be interpreted at the behavioral level. Animals can be conditioned to eat or drink in the presence of certain stimuli, sometimes referred to as "stressors." Such stimuli preferentially increase the consumption of palatable substances by both animals (Rowland and Antelman, 1976; Morley and Levine, 1980) and humans, where "snacking" is increased. Rats stressed by tail-pinch drank more sweetened milk but not more water than controls; the milk was preferred over three other sweetened solutions (Antelman *et al.*, 1976). The increase in milk-licking due to tail-pinch is believed to be due to a facilitation of nigrostriatal dopamine's action (Antelman *et al.*, 1976; Rowland and Antelman, 1976). Acrylamide increases both the affinity and number of binding sites of striatal dopamine receptors (Agrawal *et al.*, 1981); these effects are completely reversed 8 days after the termination of dosing. Therefore, an injection of acrylamide may function as these other stressful stimuli by increasing the consumption of highly palatable food. This idea is compatable with the increased consumption of food pellets (Evans *et al.*, 1981) and the preferential increase in episodic milk-licking by benzene-exposed mice (unpublished data). If toxicants are increasing milk-licking by serving as stressors, our episodic milk-licking procedure may be able to document early effects induced by a variety of toxicants.

The within-week loss of body weight was not a particularly robust indicator of acrylamide toxicity in the mouse (Figs. 2 and 6). In contrast, Tilson *et al.* (1979a) found a significant decrease in the body weight of rats after 4 weeks of receiving 20 mg/kg of acrylamide by gavage three times weekly. This po administration of acrylamide probably has a more direct toxic effect on the gastrointestinal tract than the intraperitoneal administration.

Hindlimb grip strength provided as good a model of acrylamide-induced neuropathy in the mouse as in the rat; results were time- and dose-related and reversible (Fig. 5). The development of neuropathy with 20 mg/kg of acrylamide was slower in the mouse (5 weeks) than has been reported for the rat (2 weeks; Tilson and Cabe, 1979), although both species recovered completely within 5 weeks after dosing stopped.

Several other tests appear to be less useful in documenting hindlimb neuropathy. Our data from the foot spread test and those of others (Edwards and Parker, 1977) revealed an inverted "U-shaped" dose–response relationship which complicates the interpretation of grouped means. We found, as did Tilson *et al.* (1979b), that locomotor activity was a less sensitive and specific indicator of acrylamide neuropathy than grip-strength. The rotarod technique has been able to document acrylamide-induced neuropathy in both rats (Kaplan and Murphy, 1972; Gipon *et al.* (1977) and mice (Hashimoto *et al.*, 1981). Therefore, the rotarod and grip-strength techniques seem equally capable of documenting acrylamide-induced neuropathy.

The development of neuropathy produced by acrylamide was said to depend solely on the total cumulative dose regardless of the spacing between doses or their magnitude (Kuperman, 1958). Neither our data nor those of Tilson *et al.* (1979a) support this idea. The time to develop neuropathy was inversely related to the magnitude of the daily dose with no clear correlation with the cumulative dose at which neuropathy developed (i.e., 300, 780, and 460 mg/kg cumulative dose for developing neuropathy in the 100, 60, and 20 mg/kg groups). The cumulative dose was also not the critical determinant in increasing milk-licking. Neither a single dose of 100 mg/kg nor 7 weeks administration of 20 mg/kg of acrylamide (680 mg/kg cumulative dose) increased milk-licking to the extent produced by two doses of 60 mg/kg of acrylamide.

The lethality of acrylamide was both dose and duration dependent. The lethality of 100 mg/kg, half the acute LD50 (Shiraishi, 1978), was 100% over a 2-week period, 50% for 60 mg/kg over 4 weeks, and 0% for 20 mg/kg over 7 weeks (Fig. 1). There was no correlation with cumulative dose and mortality since the cumulative LD50 in the 100 mg/kg group was between 600 and 700 mg/kg, and this cumulative dose in the 20 mg/kg group produced no deaths. Thus, the severity of intoxication increased and the latent period decreased as the daily dose increased.

A detailed examination of the time–effect relationships may shed new light upon the classical picture of acrylamide toxicity. Subchronic administration of 60 mg/kg of acrylamide produced changes in appetitive behavior which preceded by 3 weeks changes in hindlimb strength, a measure of the classical sign of subchronic acrylamide neuropathy. Licking of milk increased significantly on Day 2 after administration of 60 mg/kg, whereas body weight did not decline until Day 4, and the first death did not occur until Day 6. Acute administration of acrylamide also produced changes in appetitive behavior within one day. Clearly, the appetitive behaviors studied in this experiment are exquisitely sensitive to acrylamide, and their mechanisms are not identical to those mediating the slower-onset neuropathies.

The second objective of this study was also met since the mouse was found to be an appropriate species for evaluating the behavioral effects of acrylamide. The time course for the development of neuropathy and the qualitative aspects of the neuropathy in the two strains of mice were similar to those observed in the rat (Tilson and Cabe, 1979).

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