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Efficacy of Choice Testing to Predict Chronic Ingestion of Drinking Solutions Adulterated with Chemicals[1]

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KALLMAN, M. J. AND G. L. KAEMPF. *Efficacy of choice testing to predict chronic ingestion of drinking solutions adulterated with chemicals.* PHARMACOL BIOCHEM BEHAV 20(2) 195–200, 1984.—The feasibility of using a measure of palatability in a 2-bottle choice paradigm to determine detriments in fluid intake when unpalatable solutions containing drugs or chemicals were provided as a sole source of fluid was examined. Palatability measures obtained from testing various concentrations of quinine with water in a two-bottle choice paradigm were compared with intake of these same solutions when they were the sole fluid source for 20 consecutive days. Mice were observed to significantly avoid quinine solutions at concentrations as low as 0.0001 mg/ml in a choice situation while fluid intake was reduced in a forced drinking situation only at a concentration of 0.1 mg/ml. Palatability altered forced fluid intake only when quinine solutions comprised 20% or less of total intake in a choice situation. This approach was successfully employed to predict whether various concentrations of halogenated hydrocarbons could be administered in a repetitive forced drinking situation without reducing total fluid intake of mice.

Choice testing Drinking water Palatability Halogenated hydrocarbons Quinine
Single vs. forced exposure Taste Mice

OUR laboratory has been involved in research to predict the potential for behavioral toxicity of selected water contaminants. To provide data which directly addresses the problem of drinking water exposure we have employed either gavage or drinking water exposure, when possible, for the compounds evaluated. Drinking water exposure is preferred since it produces the closest approximation to the normal human exposure, it produces a more graded exposure across a 24-hr period and it produces the least trauma to the test animal.

Exposing animals to potentially hazardous materials via drinking water may produce several problems which interfere with safety assessment. The most overt problem is reduced body weight when a threshold for behavioral toxicity would be desired at exposure levels which do not result in body weight reductions. Another problem is the direct effect of dehydration which may alter behavioral responses such as locomotor activity [4,5] or enhance the toxicity of a compound by increasing absorption from the gut or decreasing elimination of the chemical from the body [4,8].

Other investigators have attempted drinking water exposure to various chemicals and drugs. The most extensive literature exists on the repetitive oral administration of narcotics. Many different procedures have been employed to produce intake levels which would result in dependence. Some investigators have employed saccharin or sucrose [3,10] to camouflage the aversive taste of narcotics while others have resorted to more complicated and time-consuming approaches as limited access to sucrose solutions or condensed milk containing the drug [12, 14, 15, 16] or schedule-induced polydipsia protocols [1]. In all of these investigations the major variable of interest was narcotic exposure level and the resulting dependence rather than a concern for the reduction in normal fluid intake which typically occurred. None of these previously used procedures were applicable to our situation because of the absence of normal fluid intake.

Reductions in fluid intake of drinking water adulterated with a novel substance can result because of two properties of those solutions [1,14]. The first property that is important is palatability or taste of the solution. If a solution is aversive or unpalatable, immediate reductions in fluid intake result with some gradual return toward normal levels observed as dehydration ensues [10]. Kare and Pick [7] have reported that, at least for fowl, relatively severe changes in taste are necessary to reduce forced intake of fluids. The other important variable is the degree of postingestional malaise or toxicosis that is produced. The presence of the latter factor in the absence of palatability problems results in delayed reductions in fluid intake with the onset of reduction delayed less

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when the toxicosis is more severe [14]. This second phenomenon of delayed reduction in intake might be an interesting indicator of general toxicosis in an evaluation but early reductions in fluid consumption and the consequent reduction in exposure level could necessitate termination of an investigation.

This investigation was conducted to determine the utility of two-bottle choice testing [9] to assess the taste quality of a chemical and to define the relationship between degree of aversiveness and fluid intake in forced drinking situations. Choice testing was selected to evaluate palatability since it is a more sensitive indicator of acceptability [7,9] and this procedure would also provide data on the aesthetic quality of contaminants in drinking water which would be useful information in an evaluation of contaminants. To our knowledge, two-bottle choice testing has never been used to predict forced exposure to chemicals or drugs in drinking solutions at concentrations which would not alter fluid intake. The ability to predict prior to the onset of a 90-day subchronic exposure study whether drinking water exposure was feasible without producing severe reduction in total fluid intake during the exposure period would be time saving.

GENERAL METHOD

Subjects

Male, CD-1 mice weighing 26 to 28 g, obtained from Charles River Laboratories (Wilmington, MA), served as subjects for these experiments. All mice were group housed 4/cage in plastic cages (28.5×16.5×12.0 cm) with sawdust bedding. Selection of a 4 mice/cage housing unit was due to our interest in predicting chemical solution intakes in subchronic toxicity evaluations where housing was under these same conditions. Unpublished observations in our laboratory indicated that absolute fluid intakes vary depending on whether mice are housed singly or in groups. Purina lab chow and tap water were available ad lib. except where specified. Illumination in the colony room was varied on a 12-hr light/dark cycle and temperature was maintained at 22.5±1°C. Mice were allowed a minimum of one week to adapt to the colony environment prior to initiation of the experiments.

Chemicals

The quinine sulfate (Lot No. 792199) used as a standard for taste testing was obtained from Fisher Scientific (Pittsburgh, PA). All quinine concentrations were calculated on the salt. The 1,2-dichloroethane (DCE-1,2), Lot No. 120487, purity 99+%; 1,1,2-trichloroethane (TCE-1,2), Lot No. 061197, purity 95%; and 1,1,1-trichloroethane (TCE-1,1), Lot No. 5708DE, purity 97% (inhibited with 3% p-dioxane) were obtained from Aldrich Chemical Corporation (Milwaukee, WI) and the chloral (CHL), Lot No. 925086, purity 99+%, was obtained from J. T. Baker Chemicals (Phillipsburg, NJ). Quinine, CHL and DCE-2 were found to be stable for 3–4 days when dissolved in deionized water while the less soluble compounds, DCE-1,2, TCE-1,2, and TCE-1,1, were suspended in a vehicle of 1 part Emulphor (EL-620, GAF, NY, NY) to 99 parts deionized water.

Solutions for taste comparisons and forced drinking experiments were prepared twice a week and placed in amber water bottles (250 ml) with rubber or cork stoppers and stainless steel drinking tubes. No corrections for spillage were made since mice in each study were always housed on one rack; therefore spillage was assumed to be relatively constant.

EXPERIMENT 1—AVERSIVE PROPERTIES OF QUININE

Taste preference curves for quinine have not been well defined in CD-1 mice, therefore Experiment 1 examined taste thresholds for quinine in a two-bottle choice paradigm. Since quinine is a widely used bitter substance in investigations on taste psychophysics, it was selected as a model for predicting intake difficulties.

Procedure

Eighty mice, housed 4/cage, served as subjects for taste threshold determinations for quinine. For choice testing, two bottles with rubber stoppers fitted with stainless steel drinking spouts were placed on the wire cage top with food available between the bottles. All the mice were given a minimum of two weeks to adapt to the colony and the exposure to tap water availability from two bottles. Twenty-four hr fluid intakes were measured (to the nearest 0.1 ml) at the same time daily (about 11 a.m.) and the bottles were refilled and replaced on the cage. Mice were exposed to two-bottle choice comparisons between deionized water and seven concentrations of quinine: 0.10, 0.03, 0.01, 0.003, 0.001 mg/ml. The order of choice presentations were determined from a Latin square. Each cage of animals was tested on each of the seven comparisons twice, once with the quinine on the left and once with the quinine concentration on the right, to control for position preference. On any given day, the quinine solution was presented on the left for half of the animals and on the right for the other half of the animals. Between each two days of choice testing was interspersed two non-choice days when deionized water was available in both bottles to decrease carryover effects from the prior taste comparison. Body weights were recorded daily. Total volume consumed from each bottle was recorded daily and quinine aversion values were calculated as (quinine intake/quinine intake + water intake) × 100%. Quinine aversion values were analyzed by a one-way analysis of variance to determine the significance ($p \leq 0.05$) of quinine concentration of fluid intake. The concentration effect was further examined by Dunnett's multiple comparisons to determine quinine concentrations which significantly reduced intake when compared to control intake levels determined when two bottles of water were available immediately prior to quinine comparisons.

Results

Figure 1 illustrates the preference values obtained when each of the 7 concentrations of quinine was presented with water for 48 hr in the two-bottle choice situation. Each data point represents the mean of two data points for 20 cages of mice (4/cage) ±S.E.M. As was expected, mice responded to the typically aversive quality of quinine solution by significantly reducing the proportion of total intake consumed from quinine solutions as the concentration of quinine increased, $F(7,133)=50.64$, $p \leq 0.01$. Dunnett's multiple comparisons indicated that all concentrations of quinine significantly ($p \leq 0.05$) altered fluid ingestion from control levels.

EXPERIMENT 2—FORCED EXPOSURE TO QUININE SOLUTIONS

Experiment 1 demonstrated that CD-1 mice could taste

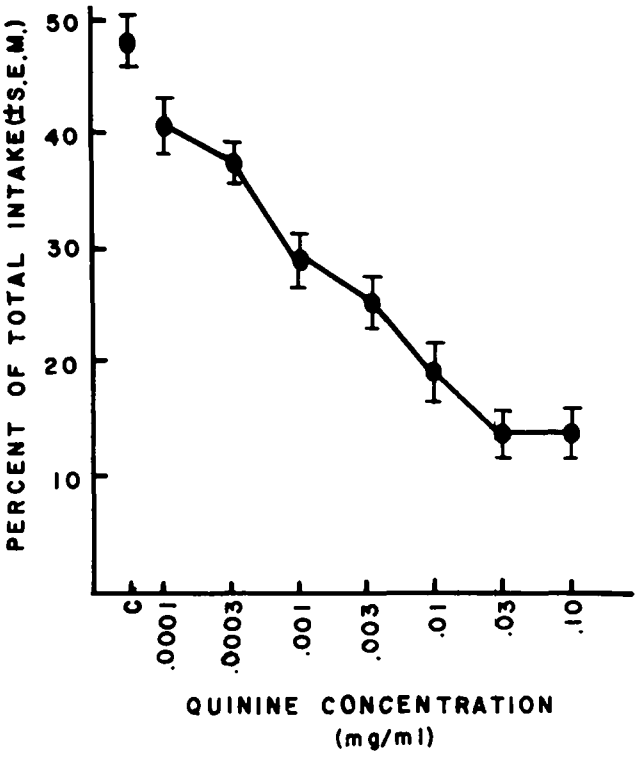

FIG. 1. The effect of varying quinine concentrations on percent of total intake consumed of quinine when mice were presented with one bottle containing quinine and one bottle containing tap water. Each data point is a mean for 20 cages of mice (4/cage) tested for 2 consecutive days on the comparison. All mice were adapted to fluid availability from 2 bottles prior to choice testing. Vertical bars represent ±S.E.M. for each data point. The data point labeled C represents the water – water control value.

quinine when the quinine was presented in various concentrations and they responded by lowering quinine intake as a function of concentration. We were then interested in determining the highest percentage decrease in quinine drinking in a two-bottle test that would result in no effect in a single bottle, repeated-forced drinking situation. Thus, Experiment 2 examined the effect of forced exposure to various concentrations of quinine on total fluid intake.

Procedure

One hundred and forty mice, housed 4/cage (N=35 cages) served as subjects for this experiment. All mice were given 2 weeks to adapt to the colony and to establish stable fluid intake levels of about 9 ml/cage of 4 mice. Mice were randomly assigned to one of the following forced drinking conditions (n=7 cages/treatment): deionized water, 0.10, 0.01, 0.001 or 0.0001 mg/ml quinine for 20 consecutive days. Fluid bottles were removed from the cage at approximately the same time daily (11 a.m.) and the volume of fluid consumed was recorded. Bottles were then refilled and replaced on the cage. The data for each cage were divided by the number of subjects housed per cage [4] to provide fluid intake per animal. These values were analyzed by a mixed factor analysis of variance [13] to determine the effect of quinine concentration and length of exposure. Significant effects ($p \leq 0.05$) were further examined by Dunnett's individual

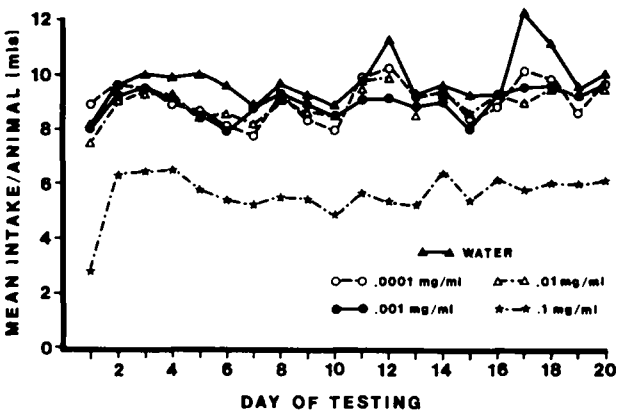

FIG. 2. The effects of various concentrations of quinine on ad lib. fluid consumption when quinine solutions were the sole source for fluid consumption. Each data point is a mean for 7 cages of mice (4/cage).

comparisons to determine significant ($p \leq 0.05$) shifts in fluid intake from control intake levels.

Results

Mean fluid intake for each group across the 20 days of forced exposure is presented in Fig. 2. When mice were exposed to varying concentrations of quinine as their sole fluid source, total fluid consumed was affected by the concentration administered, $F(4,30)=28.20$; $p \leq 0.01$. A Dunnett's multiple comparison was used to compare fluid intake levels of each quinine exposure group to intake levels in the deionized water group. Only the highest quinine concentration tested, 0.10 mg/ml, significantly ($p \leq 0.05$) reduced fluid intake from normal levels. The reduction in intake was greatest on the first day of forced quinine exposure as indicated by a decrease in fluid intake to about 30% of normal levels. Some return toward normal intake levels was observed by day 2 of forced exposure but fluid consumption remained at about 70% of normal levels for the remainder of the 20 days of quinine forced exposure.

EXPERIMENT 3—PREFERENCE TESTING WITH HALOGENATED HYDROCARBONS

Experiment 2 suggested that concentrations of quinine which constituted only 20% or less of total intake when presented in a choice-test situation would produce a reduction in total fluid intake when the same quinine concentration was available as the sole source for fluid intake. Quinine concentrations which were ingested more (>20% total consumption) in choice testing did not alter total fluid consumption when these were the sole fluid source. These findings suggested that short-term choice testing might be used to predict chemical concentrations which would reduce total-fluid intake in forced-drinking exposure. Moreover, these data would provide information about the aesthetic quality of a specific chemical solution which would have utility in setting standards for acceptable drinking water levels. Therefore, several halogenated hydrocarbons which were scheduled for subchronic toxicity evaluation were also tested in the 2-bottle choice procedure. The dosage levels for the subchronic studies were not based upon palatability although no chemical concentration above a daily exposure of 1/10 of the

TABLE 1
PREFERENCE VALUES FOR 2-BOTTLE COMPARISONS*

Compound	Concentration	% of Total Consumption† (Mean ± S.E.M.)
CHL	0.70 mg/ml	35.9 ± 3.2
DCE-1,2	0.20 mg/ml	15.6 ± 1.9
TCE-1,2	0.20 mg/ml	42.3 ± 1.9
TCE-1,1	0.50 mg/ml	41.7 ± 2.4
	5.00 mg/ml	33.3 ± 1.6

*All mice were adapted to drinking from 2 drinking tubes prior to initiation of 4 days of choice testing.
†Percent of total intake consumed from contaminant solution across 4 days of exposure.

LD_{50} would be evaluated in subchronic toxicity studies. Ultimately, our question was whether daily exposure by drinking water at a daily level as high as 1/10 of the LD_{50} was feasible without seriously decreasing fluid intakes. Several problems were encountered because of the compounds that were to be evaluated. First, some of the compounds were insoluble in water and therefore were suspended in a 1% Emulphor and water vehicle. Also, all of these compounds are solvents and thus would dissolve rubber stoppers and they could be degraded by light. These problems necessitated the use of amber drinking bottles fitted with cork rather than rubber stoppers.

Method

Thirty-two male mice, housed 4/cage, served as subjects for each taste comparison study. All mice were exposed to the availability of water from two bottles for two weeks prior to initiating choice testing. Since we hoped to work with a smaller number of mice, we decided to measure preferences across 4 days rather than two days as was assessed in Experiment 1. Therefore, each preference comparison was made on naive mice across 4 days of exposure to the choice situation. The cage position of the bottle containing the contaminant was counterbalanced in an ABBA order for each cage across the 4 days of exposure to control for side preferences. The following taste comparisons were made: (1) 0.70 mg/ml CHL vs. deionized-distilled water; (2) 0.20 mg/ml DCE-1,2 vs. deionized-distilled water; (3) 0.20 mg/ml TCE-1,2 vs. Emulphor vehicle; (4) 5.0 or 0.50 mg/ml TCE-1,1 vs. Emulphor vehicle. TCE-1,1 was tested initially at two concentrations because the concentration which produced a daily exposure at a level 1/10 of the LD_{50} was higher than all other concentrations of interest. Actual concentrations for chemical solutions were based on average body weights (39 g) and previously determined daily fluid intakes for mice housed 4/cage (9 ml/day).

Total fluid consumed from each bottle was recorded daily for the four days of baseline and the four days of comparison testing. A preference measure was computed for preference test days as (total contaminant intake/total intake) × 100%.

Results

Preference scores for each two-bottle comparison were computed as the mean for all cages tested across the 4 days when the two bottles of fluid were available for 24 hr ad lib consumption. Table 1 illustrates the preference values that were observed for each of the comparisons made. The concentrations tested in the two-bottle choice paradigm produced little aversion with the exception of DCE-1,2. A mean preference score of 15.6% was observed when mice were exposed to 0.2 mg/ml DCE-1,2 solutions. The observed preference value was significantly less than the level of random intake measured during the 4-day baseline prior to DCE-1,2 exposure, $t(7)=13.0$; $p \leq 0.01$. On the basis of the data collected in Experiments 1 and 2, a mean preference value of 15.6% indicated that this concentration of DCE-1,2 would reduce fluid intake in the forced-drinking situation since this value was below the 20% cut-off observed to reduce fluid consumption in forced-drinking of quinine. Fluid consumption was predicted to be normal for forced-drinking exposure to all of the other chemicals at or below the concentrations tested.

EXPERIMENT 4—FORCED FLUID INTAKE OF HALOGENATED HYDROCARBONS

All of these halogenated hydrocarbons were evaluated for subchronic toxicity with drinking water as the route of exposure. Fluid intakes were measured throughout the subchronic exposure period. Other measures of toxicity were also made on these mice after exposure was terminated, but those data are reported elsewhere. The measure of interest (total fluid intake) was examined to ascertain the efficacy of preference testing to identify difficulties with fluid intake when mice were forced to drink contaminant solutions.

Method

Naive, CD-1 mice were used to examine the effects of forced fluid intake of adulterated solutions on total fluid consumption. Mice were housed 4/cage and adapted to the lab colony for at least one week prior to exposure to the contaminant solutions. Mice were then exposed to one concentration of a chemical continuously for 90 days. Different groups of mice (N=8 cages, 4 mice/cage) were exposed to each of the following solutions: 0.7 or 0.70 mg/ml CHL; 0.02 and 0.20 mg/ml TCE-1,2; 0.5 and 5.0 mg/ml TCE-1,1 and 0.02 and 0.20 mg/ml DCE-1,2. All compounds were suspended with the same vehicle as described for two-bottle choice testing. Total fluid intake was measured biweekly when fresh solutions were provided. CHL stability as determined by GC with head space analyses indicated that all of these chemical concentrations were stable at a level >90% when contained in amber water bottles with cork stoppers for 4 days. Mouse body weights were also determined weekly at the time drinking solutions were changed during the 90-day exposure period.

Results

Table 2 summarizes the effects of exposure to each chemical concentration for 90 days on total fluid consumed. As can be seen in the table, most of the chemicals did not decrease fluid consumption from the volume of tap water consumed by control mice. The highest concentration of DCE-1,2, 0.20 mg/ml, did reduce fluid intake as was predicted from two-bottle choice testing with this concentration of DCE-1,2. Total fluid consumption was reduced to about 75% of control volumes. Actual DCE-1,2 exposure was calculated from intake values and these mice received about half of the intended DEC-1,2 exposure (49.0 vs. 24.0 mg/kg/day).

Figure 3 illustrates the effect of forced exposure to solutions adulterated with DCE-1,2 across three 30-day intervals

TABLE 2

EFFECT OF ADULTERATING DRINKING WATER WITH HALOGENATED HYDROCARBONS ON FLUID INTAKE*

Compound	Exposure Concentration†	Intended Exposure‡	Fluid Intake§	Actual Exposure‡	Body Weight (g)
CHL	0	0	8.5 ± 0.3	0	39.8 ± 0.8
	0.07	14.4	7.3 ± 0.3	15.7 ± 0.6	38.0 ± 0.7
	0.70	144.0	7.7 ± 0.2	159.8 ± 3.8	37.5 ± 0.8
TCE-1,2	0	0	8.2 ± 0.1	0	36.7 ± 0.6
	0.02	3.8	7.8 ± 0.1	4.4 ± 0.1	36.5 ± 0.8
	0.20	38.0	7.6 ± 0.2	45.6 ± 1.2	37.1 ± 0.6
TCE-1,1	0	0	7.7 ± 0.1	0	41.4 ± 0.6
	0.5	100	7.7 ± 0.1	103 ± 2.0	41.2 ± 0.5
	5.0	1000	8.0 ± 0.1	1041 ± 16.0	41.2 ± 0.6
DCE-1,2	0	0	5.1 ± 0.1	0	40.2 ± 0.5
	0.02	4.9	5.5 ± 0.1	3.5 ± 0.1	41.1 ± 0.6
	0.20	49.0	3.9 ± 0.1	24.0 ± 0.7	38.9 ± 0.5

*Tabled values represent means ± S.E.M. for 8 cages of mice housed 4/cage.
†mg/ml.
‡mg/kg/day.
§ml/mouse.

of the 90-day study. Control mice consumed more fluid during the first 30 days of exposure than during the remaining 60 days. This observed shift in normal fluid intake is typical of mice of this age which are developing rapidly and require more fluid and food during this period. Adulteration of drinking solutions with 0.02 mg/ml DCE-1,2 produced no decrease in fluid consumed or a slight increase while adulteration with the higher concentration of 0.20 mg/ml reduced fluid intake across the entire 90 days of exposure as was predicted from choice testing in Experiment 3. The greatest reduction in fluid intake occurred during the first 30 days of exposure to 0.2 mg/ml DCE-1,2 when fluid consumption was reduced to 81% of the normal level. In no case did reductions in fluid consumed compromise body weight but these reductions did produce concomitant reductions in daily exposure to DCE-1,2. Reduction in DCE-1,2 exposure ranged from 71–34% of the intended exposure of 49 mg/kg/day.

GENERAL DISCUSSION

Exposure to aversive substances does alter total fluid intake in a forced exposure situation. The taste quality of quinine concentrations as determined by two-bottle choice testing indicated concentrations which would produce reduction in total fluid intake when quinine solutions were the only available source of drinking fluid. Quinine concentrations which were ingested less than 20% in a choice situation were observed to reduce forced intake to about 70% of normal levels. Observed sensitivities to quinine concentrations were within the same range as those observed for the rat [9]. The relationship between palatability and forced ingestion for a bitter solution (quinine) was also observed with the halogenated hydrocarbons tested, suggesting that this relationship is not peculiar to quinine but rather is characteristic of unpalatable solutions in general.

Concentrations of both quinine and the four halogenated hydrocarbons administered in forced exposure were ac-

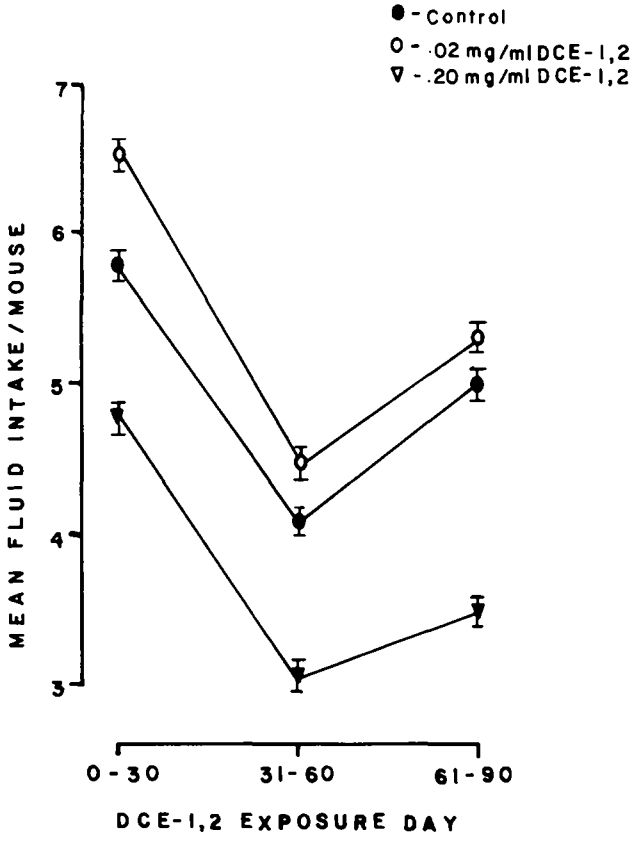

FIG. 3. The effects of various concentrations of DCE-1,2 in the drinking water on total fluid intake when the contaminant solutions were the sole source of fluid. Data are presented for each 30-day segment of the total 90-day exposure period. Fluid intakes are based on biweekly measures for 8 cages of mice housed 4/cage. Vertical bars represent ±S.E.M. for each data point.

cepted by the mice when preference scores were above 20%. When preference values were below 20%, fluid intakes were reduced when mice were exposed to the concentration in a forced drinking situation. Thus, conducting 2-bottle choice testing can be used to predict concentrations for forced drinking water studies which will not result in significantly lowered fluid intake. When greater than 20% of total intake is of the flavored solution in the choice test, then these concentrations should not lower overall fluid intake during a 90-day forced drinking study. Conversely, concentrations of a test compound which do result in less than 20% consumption in a 2-bottle choice situation may not be readily administered in drinking water for 90-day studies. Forced exposure to adulterated solutions appears to be relatively insensitive to palatibility factors as determined in choice situations.

The reported aversion to DCE-1,2 at a concentration of 0.20 mg/ml seems to result from an innate taste aversion. When DCE-1,2 and other halogenated hydrocarbons were examined to determine their ability to produce learned aversions in the classical conditioned taste aversion paradigm, DCE-1,2 did produce conditioned aversions but a bolus gavage of 300 mg/kg was required to produce a significant effect [6]. The data reported are based on a 24-hr exposure to DCE-1,2 or approximately 24 mg/kg, therefore it would seem unlikely that acquired aversions were responsible for the rejection of forced exposure to the DCE-1,2 solution. Moreover, none of the drinking solutions accepted in the forced drinking situation have been observed to produce acquired aversions [6] at the exposure levels tested in this investigation. Although these data support the lack of learned aversions, reduction in fluid intake in other forced drinking situations might result from innate and/or learned aversions.

Choice testing with water contaminants can provide other information in addition to the feasibility of subchronic drinking water exposure. In the event of palatability problems, choice testing could be used to access potential camouflage approaches. Secondly, choice testing provides information about the aesthetic quality of drinking supplies contaminated with a specific chemical. Certainly, contaminants which reduce fluid consumption because of smell and taste factors should be identified. Finally, choice testing might also identify highly palatable solutions which would cause increases in fluid intake. Although the latter situation has a low probability in most situations, this event could lead to elevated exposure of the population solely as a function of increased intake. Enhancement of fluid intake can be produced in the lab by providing sapid drinking solutions. Rats have been reported to increase their intakes 3–4 fold when preferred solutions are freely available for ingestion [2, 11, 12]. Alternatively, a third approach that might be considered is one used by Zenick and his colleagues [15,16] to simulate oral exposure to alleviate the problems associated with gavage. Their approach has been to maintain rats on limited fluid availability. Typically, the chemical solution is available from 6 p.m.–8 a.m. daily in a restricted volume and then unrestricted tap water is available for the next four hr to prevent fluid deprivation. This approach has been successful in producing total ingestion of an adulterated solution within a limited time period. As Zenick *et al.* [16] have pointed out, circadian behaviors may be altered by disrupting the normal drinking pattern. Moreover, by isolating the environmental variables associated with chemical exposure, one may increase the likelihood of avoidance of the adultereated solution, especially when the chemical exposure produces toxic consequences.

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REFERENCES

1. Dews, P. B. Introduction: Schedule-induced polydipsia and oral intake of drugs. *Pharmacol Rev* **27:** 447, 1976.
2. Ermits, T. and J. D. Corbit. Taste as a dipsogenic stimulus. *J Comp Physiol Psychol* **83:** 27–31, 1973.
3. Fuentes, N. O., W. B. Hunt and J. Crossland. The production of morphine tolerance and physical dependence by the oral route in the rat. *Psychopharmacology (Berlin)* **59:** 65–69, 1978.
4. Grossman, S. P. Physiological mechanisms in thirst (Chapter 7). *A Textbook of Physiological Psychology*. New York: J. Wiley and Sons, 1967. p. 395.
5. Hokanson, J. E. *The Physiological Basis of Motivation*. New York: J. Wiley and Sons, 1969, pp. 12–32.
6. Kallman, M. J., M. R. Lynch and M. R. Landauer. Taste aversions to several halogenated hydrocarbons. *Neurobehav Toxicol Teratol* **5:** 23–27, 1983.
7. Kare, M. R. and H. L. Pick, Jr. The influence of the sense of taste on feed and fluid consumption. *Poultry Sci* **39:** 697–706, 1960.
8. Mayer, S. E., K. L. Malmos and A. G. Gilman. Introduction: The dynamics of drug absorption, distribution, and elimination. In: *The Pharmacological Basis of Therapeutics*, (6th Ed.), edited by A. G. Gilman, L. S. Goodman and A. Gilman. New York: MacMillan Publishing Co., 1980, pp. 1–27.
9. Pfaffman, C. The pleasures of sensation. *Psychol Rev* **4:** 253–268, 1960.
10. Risner, M. E. and K. A. Khavari. Morphine dependence in rats produced after five days of ingestion. *Psychopharmacologia* **28:** 51–62, 1973.
11. Rolls, B. J., R. J. Wood and R. M. Stevens. Effects of palatability on body fluid homeostasis. *Physiol Behav* **20:** 15–19, 1978.
12. Shuster, L., R. V. Hannom and W. E. Boyle, Jr. A simple method for producing tolerance to dihydromorphinane in mice. *J Pharmacol Exp Ther* **440:** 149–154, 1963.
13. Winer, B. J. *Statistical Principles in Experimental Design* (2nd Ed.). New York: McGraw-Hill Book Co., 1971.
14. Wolf, G., Y. Jacquet and M. Carol. Test for oral and postingestational factors mediating differential acceptability of morphine, methamphetamine and chlordiazepoxide drinking solutions. *Psychopharmacology (Berlin)* **60:** 101–102, 1978.
15. Zenick, H., R. Padick, T. Tokarek and P. Aragon. Influence of prenatal and postnatal lead exposure on discrimination learning in rats. *Pharmacol Biochem Behav* **8:** 347–350, 1978.
16. Zenick, H., F. Pecorraro, D. Price, K. Saez and J. Ward. Maternal behavior during chronic lead exposure and measures of offspring development. *Neurobehav Toxicol* **1:** 65–71, 1979.