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The Effects of Inhaled Isoparaffins on Locomotor Activity and Operant Performance in Mice

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BOWEN, S. E. AND R. L. BALSTER. *The effects of inhaled isoparaffins on locomotor activity and operant performance in mice.* PHARMACOL BIOCHEM BEHAV **61**(3) 271–280, 1998.—Very little is known qualitatively or quantitatively about the acute central nervous system effects of isoparaffin solvents that are widely used in household and commercial applications. Four isoparaffinic hydrocarbon solvent products differing in predominant carbon number and volatility (ISOPAR-C™, -E™ -G™, -H™) were tested for their acute effects on locomotor activity and operant performance after inhalation exposure in mice. For both measures, concentration–effect curves were obtained for 30-min exposures using a within-subject design. The more volatile products, ISOPAR-C™ and -E™, were as easily vaporized under our conditions as vapors such as toluene and TCE, which have acute effects on human behavior and are abused. ISOPAR-G™ was slowly volatilized and ISOPAR-H™ could not be completely volatilized within our 30-min exposures, suggesting that acute human exposures may be less likely and that it may be more difficult to abuse them. ISOPAR-C™, -E™, and -G™ produced reversible increases in locomotor activity of mice at 4000 and 6000 ppm while ISOPAR-C™ and -E™ produced reversible concentration-dependent decreases in rates of schedule-controlled operant behavior in approximately the same concentration range as they affected locomotor activity. The fact that only locomotor activity increases were observed with these isoparaffins provides evidence that they produce a different pattern of effects than those reported for abused solvents such as toluene and TCE. Further research will be needed to determine if this different pattern of effects on animal behavior between isoparaffins and abused solvents is correlated with a different pattern of acute intoxication and abuse potential in humans. © 1998 Elsevier Science Inc.

Solvents Inhalant abuse Isoparaffins Operant behavior Locomotor activity Toxicology

HUMANS have always experimented with ways to achieve intoxication (28). A relatively simple means of altering consciousness is achieved through the inhalation of vapors from selected volatile compounds. Because these compounds are highly lipophilic and volatile at room temperature, their inhalation results in a rapid absorption. This rapid uptake into the brain results in central nervous system (CNS) effects that can include euphoria and intoxication (42). This has resulted in the widespread abuse and sometimes lethal experimentation with chemical solvents used in many industrial and household products (1, 7, 25, 29, 36).

Isoparaffins are branched aliphatic hydrocarbons used in the manufacture of many products, including paints and enamels, polishes, stain removers, inks, adhesives, charcoal lighter fluids, and typewriter correction fluids. They can also be found in some lotions intended for human use. Part of the basis for their increased use stems from the move to replace halogenated hydrocarbon solvents that damage the ozone with less environmentally toxic materials. In general, isoparaffins do not have a strong odor and are considered to have low systemic toxicity in humans (35). Isoparaffin solvent products are typically blends of isomers of branched hydrocarbons differing in carbon number. This composition affects their volatility and hence suitability for various products. For example, the Exxon Corporation manufactures a variety of isoparaffins, named ISOPARs™ (18) including the more volatile ISOPAR-C™ and ISOPAR-E™ and the less volatile ISOPAR-G™ and ISOPAR-H™ (see Table 1). Very little is known

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TABLE 1
CHARACTERISTICS OF SELECTED ISOPAR™ ISOPARAFFINIC HYDROCARBON SOLVENTS*

Product	Carbon Number†	Specific Gravity	Vapor Pressure‡
ISOPAR-C™	7–8	.70	13.1
ISOPAR-E™	8–9	.72	6.9
ISOPAR-G™	10–11	.75	1.9
ISOPAR-H™	11–12	.76	0.8

*Information provided by Exxon Corporation (Exxon, 1984, and Material Safety Data Sheets). Values are representative of current production. All may vary within modest ranges.
†Predominant carbon number of isomers in product.
‡kPa at 38ºC.

about the neurobehavioral effects of acute exposures to isoparaffin solvents nor whether they have properties that could lead them to be abused. We are not aware of any information on the actual abuse of products containing these solvents. Because isoparaffin solvent products, such as the ISOPARs™, are found in many common household products, it is of interest and importance to begin to characterize their acute neurobehavioral effects and potential for abuse. One way to do this is by comparing their effects to those of solvents known to have acute effects on behavior and abuse potential (3).

The acute intoxication produced by solvents is probably the primary basis for why individuals abuse them. Support for this comes from previous investigations which have shown that some solvents share behavioral and pharmacological properties with abused CNS depressant drugs (4,16,17,34). As with barbiturates and alcohol (30,43), acute exposures to abused solvents such as toluene and 1,1,1-trichloroethane (TCE) have been shown to increase locomotor activity with lower exposures and decrease behavior with greater exposures in both rats (24) and mice (8–10,26,27,46). If this biphasic effect of solvents on motor activity represents their depressant drug-like abuse potential, then other solvents that have abuse potential may produce this profile of effects as well. In addition, locomotor activity studies are widely used in neurobehavioral toxicology research for initial characterization of CNS effects and safety assessment of chemicals with potential effects on the brain and behavior (13).

In addition to locomotor activity, operant behavior has also been used in several laboratories to evaluate the acute effects of volatile compounds (16,21,23). For example, studies using fixed-ratio (FR) schedules have shown that various abused solvents produce concentration-dependent and reversible decreases in response rates (5,22,32,33). Few studies have directly compared the effects of inhalants on locomotor activity and operant behavior. We found that rates of locomotor activity and operant behavior may not be affected in the same manner by vapor exposures and that individual chemicals differ in which measure is affected at lowest concentrations (8). Therefore, another objective of this study was to evaluate the effects of isoparaffin solvents on both motor activity and schedule-controlled behavior under comparable conditions.

In a previous study of an isoparaffin solvent, ISOPAR-E™ was compared to the abused solvent TCE and found to produce some effects similar to those produced by TCE, including concentration-related decreases in schedule-controlled behavior, production of cross dependence in TCE-dependent mice, and substantial levels of ethanol-lever responding in a drug discrimination procedure (6). On the other hand, differences were also obtained between the effects of ISOPAR-E™ and TCE, including differences in observable effects at high concentrations and in the separation of behavioral and toxic effects. The current investigation was conducted to obtain additional information on the commercial isoparaffin solvents ISOPAR-C™, -E™, -G™, and -H™, using both locomotor activity and operant responding in mice.

METHOD

Animals

Experimentally naive male mice (CFW, Charles River Co., Wilmington, MA) were used in these experiments ($n = 10$ for the locomotor activity study, and $n = 10$ for the operant study). Subjects were housed individually in standard mouse cages (18 × 29 × 13 cm) fitted with steel wire tops and containing wood chip bedding. The room in which the animals were housed was maintained at controlled temperatures (22–24°C) on a 12 L:12 D cycle. All testing was done during the light cycle. Animals used in the locomotor activity study were allowed free access to food and water with mice weighing between 30–35 g when testing began. Animals used in the operant study were allowed to gain weight to a maximum of 35 ± 5 g by postsession feeding of 3–4 g/day of rodent chow (Rodent Laboratory Chow, Ralston-Purina Co., St. Louis, MO). These studies were approved by an Animal Care and Use Committee and were carried out consistent with the DHHS guidelines for animal care and use.

Static Exposure Chambers

Vapor exposures for the locomotor activity studies were conducted in 29-l cylindrical jars (47 cm H × 35 cm diameter; total floor space = 962 cm^2), which have been described previously (31). Briefly, vapor generation commenced when liquid solvent was injected through a port onto filter paper suspended below the sealed lid. A fan, mounted on the inside of the lid, was then turned on, which volatilized and distributed the agent within the chamber. Exposure chambers were located in a fume hood. Chamber concentrations were verified by continuous analysis using single wavelength monitoring infrared spectrometry (Miran 1A, Foxboro Analytical, North Haven, CT). A visual record of chamber concentrations over time was obtained by plotting absorbance on an X-Y recorder. These analyses revealed that chamber concentrations did not vary from nominal (calculated) final concentrations by more than 10% and, for each volume of liquid injected, were very repeatable from day to day. After chamber concentrations were verified by chemical analysis, routine monitoring was not necessary. All vapor exposures were limited to 30 min in duration to preclude problems with waste gas accumulation within these sealed chambers.

Dynamic Exposure Chambers

Mouse operant conditioning chambers were modified to allow for solvent vapor exposure (4). The exposure/behavioral chamber was a 4.25-l stainless steel canister (#C-7206-40, Cole-Parmer Instrument Co., Chicago, IL) with a loosely fitting stainless steel lid. Vapor generation occurred by initially directing air flow through a bubbler that was immersed in a 500-ml solvent bath contained in a 1-liter round-bottom flask. Air saturated with vapor exited the bath and was mixed with filtered fresh air from outside the building that was then deliv-

ered to the exposure chamber. Control of the vapor concentrations was accomplished with a Dyna-blender (Model 8219, Matheson, Montgomeryville, PA), which monitored and controlled the air flow rate through two valves—one for fresh air and one for vapor-laden air. An IBM-compatible microcomputer (AT 486, WIN Laboratories, Fairfield, VA) was interfaced with the Dyna-Blender and dictated what flow rates were needed for each test concentration. The total flow entering the chambers was held constant at 10 liters per min. Animals were regularly cycled from one of four air-only chambers on nontest days (Monday, Wednesday, Thursday) to the vapor exposure chambers on test days (Tuesday and Friday). Air-only and vapor-exposure chambers were identical except that the latter were placed in a fume hood. Control tests using air-only exposures were also conducted in the vapor exposure chambers. Vapor concentrations in the chamber exhaust were monitored on line during all sessions using a single wavelength monitoring infrared spectrometer (Miran 1A, Foxboro Analytical, North Haven, CT).

Locomotor activity

Locomotor activity was measured unobtrusively via two sets of photocells (Micro Switch, Freeport, IL) that bisected the static exposure chambers. Each of the two photocells and their respective detectors were mounted on 1-in wooden bases and placed on the sides of the exposure chambers. The second photocell/detector unit was placed at a 90° angle to the first, resulting in a bisection at the center of the exposure tank. Interruptions of these photo beams resulted in an analog signal being delivered by the photocell, which in turn triggered a counter. Mice were placed individually into the same exposure chamber in the same sequence each day. Activity was monitored once daily (Monday–Friday) for 30 min for approximately 5 days prior to solvent exposures. This resulted in stable day to day levels of activity which served as a baseline against which solvent effects could be determined. The same animals were used for all subsequent testing. Solvent exposure tests were conducted on Tuesdays and Fridays, with placement in the exposure chambers with air only exposure occurring between test days (Mondays, Wednesdays, and Thursdays).

Operant Behavior

The subjects were trained to lever press in two-lever mouse operant-conditioning chambers, which also served as exposure chambers as described above. The response levers were located on the front wall 8 cm apart, 2.5 cm above a stainless steel floor, and extended 0.8 cm into the chamber. The response lever was held forward by an electromagnet and when the subject pressed the lever (requiring 4–5 g force) an optoelectronics device detected the movement. Located midway between the levers was a 3-cm diameter opening containing a trough into which 0.02 ml of sweetened-condensed milk (1 part sugar, 1 part condensed milk, and 2 parts water by volume) could be delivered via a calibrated peristaltic infusion pump (Masterflex, Cole-Parmer Instr. Co., Chicago, IL). Illumination of a house light, located above the right lever, signaled that the session was in progress. Mice were trained to press the right lever only during daily (5 days per week), 30-min sessions under a FR-20 schedule. Responses on the left lever were ineffective and not counted. Animals were trained daily for approximately 2 months before entering into the testing phase of the experiment.

Isoparaffin Exposure

The selection of test concentrations of the isoparaffins was guided by knowledge on the potency of ISOPAR-E™ obtained in a previous study (6) and by the upper limit of their volatility under our exposure conditions. ISOPAR-H™, in particular, was difficult to completely vaporize so it could not be tested at higher concentrations. For locomotor activity testing, the following isoparaffins (with target test concentrations) were tested in the order shown: ISOPAR-C™—1000, 4000, 6000 ppm; ISOPAR-E™—1000, 4000, 6000 ppm; ISOPAR-G™—1000, 4000, 6000 ppm; ISOPAR-H™—500, 1000, 2000 ppm. One of the days before each solvent exposure was selected as an "air" control test for purposes of data analysis. A similar target concentration range of the same products were also tested for effects on operant behavior: ISOPAR-C™—1000, 2000, 4000, 6000 ppm; ISOPAR-E™—1000, 2000, 4000, 6000 ppm; ISOPAR-G™—500, 1000, 2000, 4000 ppm; ISOPAR-H™—500, 1000, 2000, 4000 ppm. A 0 ppm (air only) concentration was tested as one of the test concentrations for each isoparaffin. All of the isoparaffins were provided by the Exxon Chemical Corporation (Houston, TX).

Data Analysis

Concentration–effect curves for each test compound were analyzed using repeated measures analysis of variance (ANOVA) and Tukey post hoc comparisons ($p < 0.05$). When a test of sphericity was unsuccessful, an appropriate adjustment was made to the degrees of freedom for the averaged tests of significance using the Greenhouse-Geisser factor. For the locomotor activity study, the control activity levels were determined by averaging motor activity on one control air-only test session for each animal prior to determination of each of the isoparaffin concentration–effect curves so that each animal served as its own control. For operant behavior, planned comparisons were made with the 0 ppm test concentration. To determine the reversibility of isoparaffin effects on schedule controlled behavior, rates of responding on the first nontest session following each test session were compared to the 0 ppm test session for each compound. For Tuesday tests, the next nontest session was on Wednesday. For Friday tests, the next nontest session was normally on the following Monday except when holidays intervened.

RESULTS

Performance of Isoparaffins in the Exposure Chamber

To validate nominal concentrations of test atmospheres in the static exposure chambers an IR spectrometer was connected in conjunction with a recirculating pump to ports on the lid of the tank, providing an on-line measure of chamber concentration. Further validation of chamber concentration was performed by directly comparing the absorbance reading while monitoring in-line with the exposure tank and those obtained from closed loop calibration (i.e., actual vs. expected). For three different concentrations of each of the isoparaffins the absorbance recorded from the exposure tank never deviated more than 10% from the expected closed loop value. For both ISOPAR-C™ and ISOPAR-E™ test concentrations were rapidly achieved and maintained throughout the 30-min exposure (Fig. 1), whereas the less volatile ISOPAR-G™ and ISOPAR-H™ required longer to completely volatilize. As seen in Fig. 1, ISOPAR-C™ reached a maximal concentration within 0.5 min of injection, while ISOPAR-E™ required approximately 2 min to reach an asymptotic level. For ISOPAR-

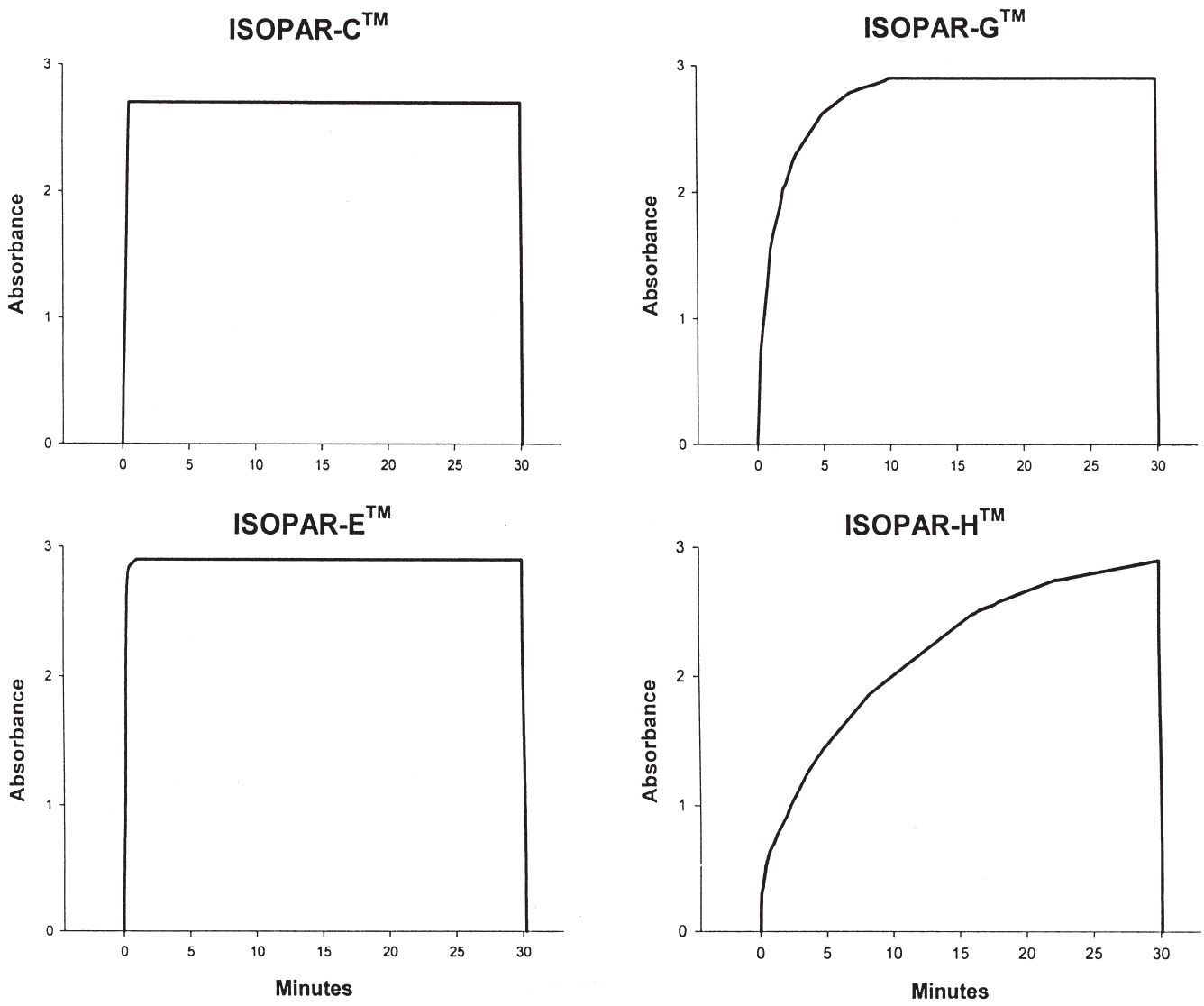

FIG. 1. Time course of static exposure chamber concentrations as determined by IR spectrometry after injections of ISOPAR-C™, -E™, -G™, and -H™. Increases in chamber concentrations are directly reflected in increased absorbance. The fan in the chamber was turned on at the zero time point.

G™, nearly 10 min was required for complete volatilization under these conditions, after which the chamber concentration remained stable.

ISOPAR-H™ was the least volatile of the isoparaffins, with maximal levels not being reached even at 30 min after injection into the chamber.

In the dynamic exposure system, the flow rates through the solvent bath needed to volatilize each of the solvents increased linearly as a function of target concentration (Fig. 2). This relationship illustrates the impact of differences in vapor pressure among these compounds in our exposure systems. For ISOPAR-G™ and -H™, flow rates approximately two- to threefold higher than those of ISOPAR-C™ or -E™ were required to generate the same concentrations.

Effects of Isoparaffins on Locomotor Activity

All mice maintained steady baseline levels of locomotor activity during the study. The mean (±SE) baseline counts/min for the air-only test sessions before solvent testing in the static system was 16.8 ± 4.6. Locomotor activity returned to baseline levels during air-only sessions, which occurred between solvent test sessions. This is evident in the air-control sessions conducted before each concentration–effect determination (Fig. 4). Although there was some apparent habituation because absolute locomotor activity counts were lower during the air-control session before testing the last solvent (ISOPAR-H™) than before the first (ISOPAR-C™), the motor activity increasing effects of the ISOPARs™ were clearly reversed. This reversibility was generally seen in the very next motor activity-control session after each test session (i.e., Wednesday or Monday; data not shown).

As shown in Figure 3, exposure to ISOPAR-C™, -E™, and -G™ produced concentration-dependent increases in locomotor activity [ANOVA for ISOPAR-C™, $F(3, 39) = 22.7$, $p < 0.001$; ISOPAR-E™, $F(3, 39) = 49.4$, $p < 0.001$; ISOPAR-G™, $F(3, 39) = 11.9$, < 0.001] with a minimally effective concentration of 4000 ppm. At 6000 ppm of ISOPAR-C™, lo-

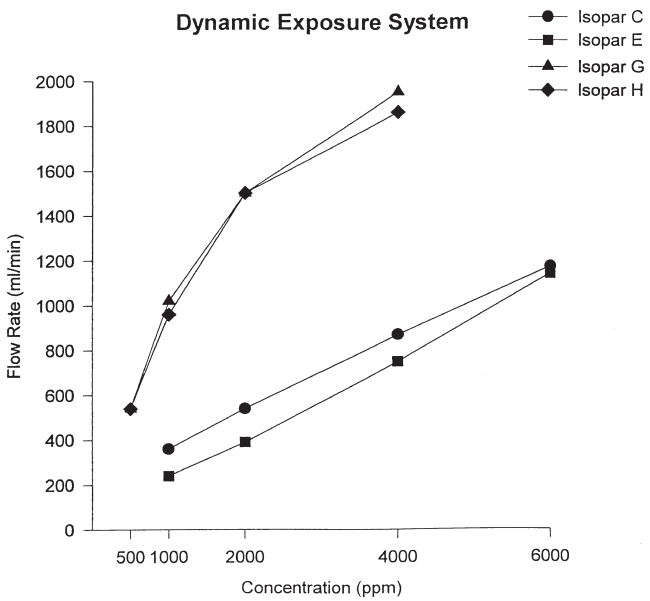

FIG. 2. Flow rates needed in the dynamic exposure system to produce various concentrations of ISOPAR-C™, -E™, -G™, and -H™. Target concentrations were measured by IR spectrometry. Note that about two- to threefold higher flow rates through the liquid solvent were required to volatilize ISOPAR-G™ and -H™ than for ISOPAR-C™ or -E™.

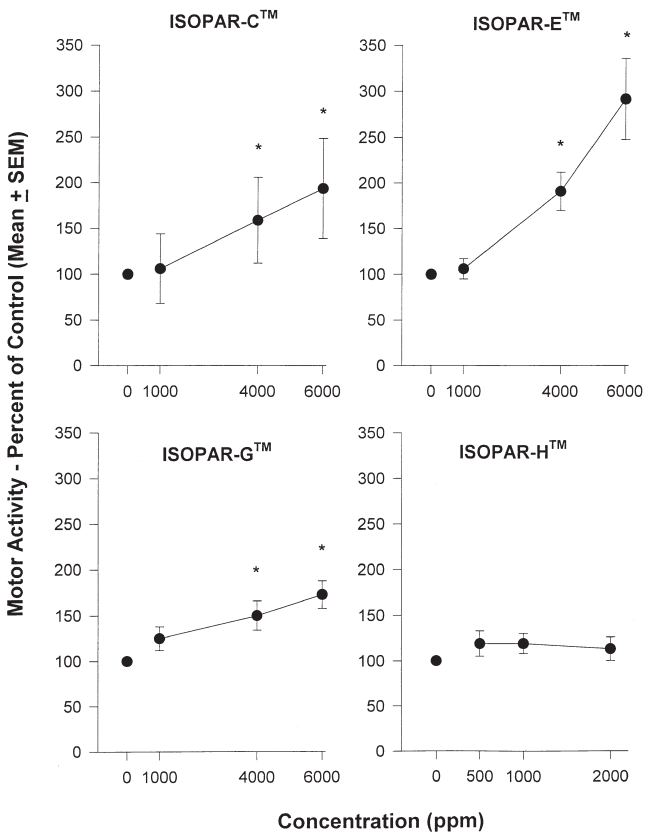

FIG. 3. Concentration–response curves for effects of inhaled ISOPAR-C™, -E™, -G™, and -H™ on mouse locomotor activity. Activity is shown as a percent of control. Shown are the target concentrations for each test. Actual chamber concentrations for higher concentrations of ISOPAR-G™ and ISOPAR-H™ throughout the 30-min exposure were somewhat less due to lower volatility. *Significantly different from 0 ppm ($p < 0.05$). ($n = 10$ mice per concentration).

comotor activity was significantly increased nearly twofold above air control, whereas an identical concentration of ISOPAR-E™ increased behavior almost threefold as compared to air-only exposures. For ISOPAR-G™, maximal increases were approximately one and half times the level of air-only exposures. In contrast, ISOPAR-H™ was without significant effects on locomotor activity, and higher concentrations could not be obtained under these test conditions. Even the 4000 ppm test concentration of ISOPAR-H™ was not completely volatilized within the 30-min exposure (Fig. 1), possibly accounting for its lack of effects.

Figure 4 shows the time course of effects of the four isoparaffins on locomotor activity. For ISOPAR-H™, these within-session data confirm that this isoparaffin was without effects under these exposure conditions and that the average session data shown in Fig. 3 do not obscure more momentary increases or decreases in activity. For ISOPAR-C™, ISOPAR-E™, and ISOPAR-G™, increases in locomotor activity were observed within 6-min of the initiation of exposure and lasted for the duration of the exposure. Indeed, for ISOPAR-E™, locomotor activity increased steadily over the duration of the exposure. At no concentrations were decreases in activity observed at any time during exposures, providing no evidence of a biphasic effect. Convulsions were occasionally observed at 6000 ppm of ISOPAR-C™ and ISOPAR-E™, although subjects recovered quickly and did not die. For both ISOPAR-G™ and ISOPAR-H™, higher concentrations could not be completely volatilized within 30-min and, hence, could not be tested.

Effects of Isoparaffins on Operant Performance

All mice maintained steady baseline rates of responding throughout all studies. In addition, responding always returned to baseline rates between solvent test sessions showing that all effects were reversible. Table 2 shows the rates of responding on the first nontest session following each vapor test compared with the rates of responding for the 0 ppm control test session for each isoparaffin. It can be seen that these values did not differ by very much except following the test with 4000 ppm ISOPAR-E™, where the lower response rate on the next session likely was the result of a 2–3-week holiday period when sessions were not conducted. The effects of exposure to the isoparaffins on overall response rates are presented in Fig. 5. ISOPAR-C™ and -E™ [ANOVA for ISOPAR-C™, $F(4, 36) = 10.2, p < 0.0001$; ISOPAR-E™, $F(4, 36) = 4.2, p < 0.01$] produced concentration-dependent decreases in response rates with the highest testable concentration of ISOPAR-C™, suppressing response rates to approximately 40% of control values. The maximal effects of ISOPAR-E™ were less. Both compounds produced increases in rates of responding at 1000 ppm in 6 of the 10 subjects, but only response rate-decreasing effects were statistically significant. Higher concentrations of ISOPAR-C™ and -E™ were not tested because several seizures were observed at the 6000 ppm concentration. ISOPAR-C™ was about twice as potent as ISOPAR-E™ with minimally effective concentrations of 2000 and 4000, respectively. Neither ISOPAR -G™ or ISO-

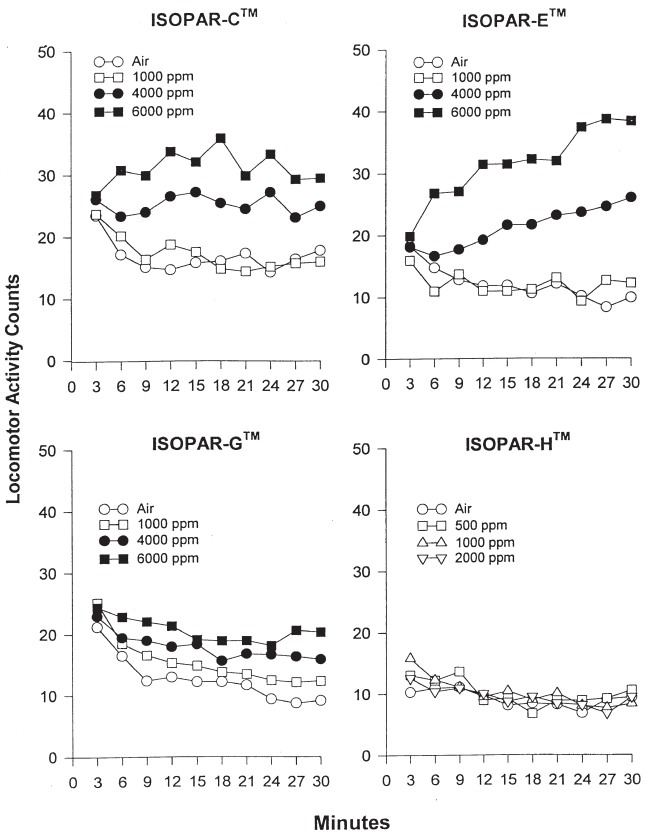

FIG. 4. Time course for the effects of inhaled ISOPAR-C™, -E™, -G™, and -H™ on locomotor activity in mice. Activity counts are shown for successive 3-min segments of a 30-min exposure. Shown are the target concentrations for each test. Actual chamber concentrations for higher concentrations of ISOPAR-G™ and ISOPAR-H™ throughout the 30-min exposure were somewhat less due to lower volatility. Concentrations that produced effects significantly different from the air control in the analysis of the total session activity are represented by filled symbols.

PAR-H™ [ANOVA for ISOPAR-G™, $F(4, 36) = 8.9$, $p < 0.001$; ISOPAR-H™, $F(4, 24) = 1.6$, $p = 0.20$] had reliable effects on overall rates of responding under these test conditions. Inspection of individual subject data revealed that only one ISOPAR-H™-exposed subject and none of the ISOPAR-G™-exposed subjects showed possible evidence of effects.

The within-session time course for isoparaffin effects on operant behavior is shown in Fig. 6. The average rates of responding as a function of concentration are shown for successive 3-min segments of the exposure. In general, both ISOPAR-C™ and -E™ exhibited effects by the second 3 min of exposure, with ISOPAR-C™ producing a pronounced progressive disruption with continued exposure. At 6000 ppm of ISOPAR-C™, subjects were maximally effected after 12–15 min of exposure. A similar pattern was observed at 2000 and 4000 ppm, in which a progressive decrease in response rates was obtained after 6–9 min of exposure. For ISOPAR-E™, each of the higher concentrations (4000 and 6000 ppm) exhibited effects by the second 3 min of exposure without a pronounced progressive disruption with continued exposure. At 2000 ppm, some effects were apparent early in the session, with rates becoming progressively more similar to control

TABLE 2

RECOVERY FROM EFFECTS OF ISOPARIFFINS ON OPERANT BEHAVIOR

Isoparaffins Concentration	Control Response Rate (Responses/s ± SEM)[a]	Next Session Response Rate (Responses/s ± SEM)[b]
ISOPAR-C™		
1000 ppm	1.10 (0.14)	0.97 (0.18)
2000 ppm	1.10 (0.14)	1.21 (0.15)
4000 ppm	1.10 (0.14)	0.97 (0.17)[c]
6000 ppm	1.10 (0.14)	1.16 (0.14)
ISOPAR-E™		
1000 ppm	1.01 (0.16)	1.12 (0.22)
2000 ppm	1.01 (0.16)	1.04 (0.18)
4000 ppm	1.01 (0.16)	0.69 (0.18)[c]
1000 ppm	1.01 (0.16)	1.09 (0.18)
ISOPAR-G™		
500 ppm	1.27 (0.18)	0.97 (0.21)
1000 ppm	1.27 (0.18)	0.89 (0.20)
2000 ppm	1.27 (0.18)	0.89 (0.22)
4000 ppm	1.27 (0.18)	0.89 (0.23)
ISOPAR-H™		
500 ppm	1.20 (0.22)	1.17 (0.28)
1000 ppm	1.20 (0.22)	1.26 (0.20)
2000 ppm	1.20 (0.22)	0.97 (0.30)
4000 ppm	1.20 (0.22)	1.23 (0.25)

[a]Rate of responding on control test sessions proceeding tests of each isoparaffins.

[b]Rate of responding on the first nonexposure session following the test session.

[c]Includes data from subjects whose first nonexposure session following this test occurred after a 2–3 week period of suspended training.

over the session. Thus, it is likely that 2000 ppm ISOPAR-E™ was an effective concentration even though the analysis of session total data failed to produce a significant effect. For ISOPAR-G™, there is a possibility that some concentrations produced response-rate decreasing effects early in the session followed by higher rates than air control by session end. A similar pattern is evident in the ISOPAR-H™ results with possible response-rate decreasing effects toward the middle of the session, diminishing in magnitude as the session progressed. However, the large variability between subjects in the magnitude of response rate disruption and the concentrations at which they occurred resulted in no significant differences from air control rates.

DISCUSSION

The primary purpose of the present studies was to begin to characterize the acute behavioral effects of a series of isoparaffinic hydrocarbon solvents that are used in industry and household products. Included in this study were the commercial solvents ISOPAR-C™, -E™, -G™, and -H™, which differ in predominant carbon number and partial pressures, among other features. Other chemical companies make isoparaffin solvents similar to the ISOPARs™ that would be expected to have similar effects to the corresponding ISOPARs™. The procedures used to study these ISOPARs™ have been used previously to study the effects of various abused solvents, so a second goal was to compare the results obtained with the isoparaffins to those reported previously for these more

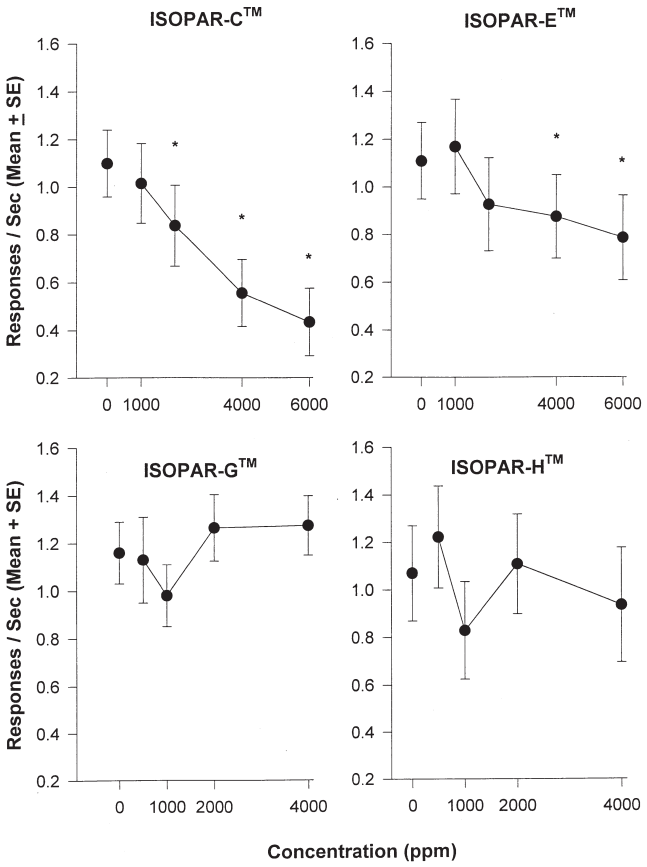

FIG. 5. Effects of inhaled ISOPAR-C™, -E™, -G™, and -H™ on rates of lever pressing under a fixed-ratio schedule of milk reinforcement in mice. *Significantly different from 0 ppm ($p < .05$) ($N = 10$ mice per concentration).

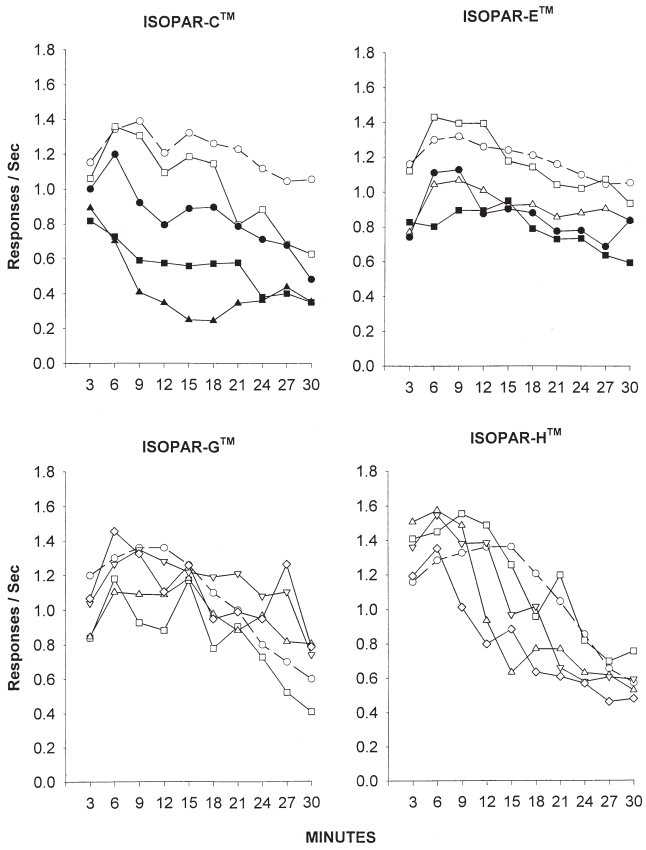

FIG. 6. Time course for the effects of inhaled ISOPAR-C™, -E™, -G™, and -H™ on rates of schedule-controlled responding in mice. Effects are shown for successive 3-min segments of a 30-min exposure. Concentrations that were determined to be significantly different from the air control in the analysis of the total session responding are represented by filled symbols.

widely studied inhalants. It may be possible to obtain some information from animal studies relevant to predicting the abuse potential of newer solvents by determining the degree of overlap in their acute CNS effects with those of known solvents of abuse (3). To our knowledge, this represents the first reported study of the acute behavioral effects of ISOPAR-C™, -G™, and -H™, although we have reported previously some other studies with ISOPAR-E™ (6).

Our first major finding was that the reported differences in the partial pressures of these ISOPARs™ greatly affected their volatility in our exposure systems. The two most volatile products, ISOPAR-C™ and ISOPAR-E™, were readily vaporized under both conditions in which we studied them, and their volatility under these conditions was similar to what we have previously observed with abused solvents such as toluene and TCE (5,6,31,47). It is not possible to directly compare the ease of volatility for our exposure chambers and actual abuse conditions, where liquids are typically placed on rags or in paper bags for vaporization. Nonetheless, it is very likely that ISOPAR-C™ and ISOPAR-E™ would both lend themselves to easy vaporization if they were contained in products subject to abuse. ISOPAR-H™, on the other hand, which has a low vapor pressure compared to the other ISOPAR™ products, could not be easily volatilized in our exposure systems, even when air was bubbled directly through the liquid solvent. Indeed, its volatility was so low that we could not administer concentrations with reliable behavioral activity. It is likely that a similar problem would occur were someone trying to self-administer ISOPAR-H™, probably diminishing its abuse potential. Direct studies have not been done comparing the volatility of solvents with their attractiveness for abuse; however, from a practical point of view, abusers would need to be able to easily and rapidly volatilize liquid solvents to effectively use them. ISOPAR-G™ behaved in our exposure systems more similar to ISOPAR-H™ than to ISOPAR-C™ and -E™. However, under the static exposure system consisting of a glass jar with an enclosed fan placed above the liquid solvent, complete volatility of behaviorally active concentrations could be obtained within 10 min. More research would be needed to determine if this degree of volatility would be sufficient to make the abuse of ISOPAR-G™ practical under actual conditions of use. It should be remembered that the volatility of these products greatly affects their suitability for use in different applications. For many products, rapid volatilization is a key reason why certain solvents are more desirable than others.

The difficulty of volatilizing ISOPAR-G™ and -H™ means that the animals were not exposed to the target concentrations throughout the 30-min exposures for the motor activity testing because vapor concentrations were only slowly rising

over that time period. This could result in an under estimation of what the effects of higher concentrations would have been had we been able to obtain a full 30 min of exposure to these actual concentrations. This was not a problem for the operant study, however, where flow rates through the bubbler were adjusted to compensate for the lower volatility. With 10 liters/min flow rates into these 4.25-liter chambers, target concentrations would achieve 95% of asymptotic values within 2–4 min.

Our second major finding was that ISOPAR-C™, -E™, and -G™ all had reversible acute effects on mouse behavior, with minimally effective concentrations in the 2000 to 4000 ppm range. In this respect, they resemble the effects of many psychoactive drugs and abused solvents (16). In the case of ISOPAR-E™, similar results have been reported previously using somewhat different behavioral measures (6). The effects observed were changes in locomotor activity and rates of schedule-controlled operant performance, two measures widely used to study drug and toxicant effects on behavior. A within-subjects design was used with control sessions interspersed among solvent exposure test sessions, allowing us to observe recovery of baseline behavioral performance between successive exposures. This basic approach to behavioral pharmacology and toxicology research with drugs has proven useful in the past for studying volatile solvents (16,19–21,37–39), and here we extend that observation to the isoparaffins.

Little is yet known about the nature of these acute behavioral effects of isoparaffins nor the mechanisms for producing them. Although substantially less odiforous than abused solvents such as toluene and TCE, isoparaffins at high concentrations do smell and can produce irritation to the eyes and upper airways (35), so it is possible that the behavioral changes observed were secondary to these peripheral effects. Neither locomotor activity nor operant behavior are selective for agents affecting only the CNS (2,12,41); nonetheless, there are some data that would suggest that these isoparaffins do alter behavior through effects on the brain. First, convulsions were observed in some animals at 6000 ppm of ISOPAR-C™, -E™, and -G™. This is almost certainly a CNS effect, and it occurs at concentrations only somewhat higher than threshold concentrations for behavioral effects. Second, in a previous study of ISOPAR-E (6), behavioral effects were observed that were probably mediated by effects on the CNS. These include ethanol-like discriminative stimulus effects and cross dependence with TCE, where exposure to ISOPAR-E™ reduced withdrawal convulsions caused by discontinuing repeated TCE exposure. These effects of ISOPAR-E™ occurred at the same concentrations (2000 to 4000 ppm) as were effective in changing motor activity and operant behavior in the present study. Third, effects of ISOPAR-C™, -E™, and -G™ were not typically apparent immediately upon initiation of exposure, but required a few minutes to appear, and then, in many cases intensified over the period of exposure. Odor and irritant effects would be expected to occur immediately, whereas effects mediated by the CNS would be expected to require a few minutes for biodistribution of the material to the brain, depending on the partition coefficients of the individual chemicals involved. Indeed, even with continued exposure to highly lipophilic chemicals such as toluene and TCE, brain concentrations can continue to rise over the duration of the exposure (14,15,40, 45), possibly accounting for an effect of exposure duration on behavior. Thus, we are quite convinced that the observed behavioral effects of ISOPAR-E™ are mediated by direct actions on the CNS. We cannot be as confident that this is true for ISOPAR-C™ and -G™, but this seems likely considering their similar potency to ISOPAR-E™ and the other data mentioned.

ISOPAR-C™, -E™, and -G™ produced only concentration-dependent increases in locomotor activity up to a concentration that was convulsant in some animals. This is clearly different from what is found with abused vapors such as TCE and toluene. TCE and toluene generally produce biphasic effects on activity, with increases at intermediate concentrations or with shorter exposures and then decreases at high concentrations or with long exposures (9,24,26,27,46). At very high concentrations of TCE and toluene, anesthetic-like effects are observed (10,44). This is similar to the biphasic pattern of effects observed with abused depressant drugs such as the barbiturates and ethanol (17) as well as with classical anesthetic vapors such as ether and methoxyflurane (10, 11). The pattern of results with the ISOPARs™ also differs from what has been found with the convulsant vapor flurothyl, which does produce convulsions without evidence of anesthetic-like effects (11), but fails to produce locomotor activity increases under conditions identical to what were used here to evaluate the isoparaffins (8). Another somewhat distinctive pattern of acute behavioral effects has also been observed for benzene (44). In short, what is emerging from animal behavioral pharmacology research with inhalants is the general finding that there may be different groups of chemicals with distinctive effects on behavior, differing as much as different classes of psychoactive drugs differ from one another. This presents a challenge to categorize these inhalants, identify their mechanisms of action, and determine the relevance of these effects to practical issues of human exposure and abuse potential.

Only ISOPAR-C™ and ISOPAR-E™ produced statistically reliable changes in overall rates of operant behavior during 30-min exposures. The minimally effective concentration of ISOPAR-C™ was 2000 ppm. Effects were apparent with 3–6 min of initiating the exposure and tended to intensify with continued exposure. The minimally-effective concentration of ISOPAR-E™ for altering overall rates of operant behavior was 4000 ppm; however, inspection of the within-session data suggests that 2000 ppm may also have had effects early in the exposure. In a previous study of the effects of 5- and 20-min exposures of ISOPAR-E™ on FR responding in mice (6), effects were also observed at 2000 ppm and the concentration–effect curve from that study was nearly identical to the one obtained here. Although ISOPAR-C™ and ISOPAR-E™ produced increases in motor activity, only response-rate decreases were obtained in schedule-controlled responding, but this is not surprising considering the high baseline rates of behavior maintained under the FR schedule. Other inhalants, such as toluene and TCE, can also produce increases in locomotor activity at the same concentrations that produce decreases in rates of schedule-controlled behavior (8). Although ISOPAR-G™ and ISOPAR-H™ did not produce reliable effects on our overall measure of rate of responding, inspection of the within-session response rate data leave unresolved the possibility that effects may have been apparent during early portions of the exposures. Further research would be needed to completely rule out the possibility of effects on schedule-controlled behavior for these isoparaffin products.

In conclusion, we have provided data on the acute behavioral effects of four isoparaffinic hydrocarbon solvent products differing in predominant carbon number and volatility. The more volatile products, ISOPAR-C™ and ISOPAR-E™, are as easily vaporized under our conditions as vapors such as toluene and TCE, which have acute effects on human behavior and are abused. ISOPAR-G™ was slowly volatilized, and

ISOPAR-H™ could not be completely volatilized within our 30-min when allowed to evaporate from a piece of filter paper, suggesting that acute human exposures may be less likely and it may be more difficult to abuse them. ISOPAR-C™, -E™, and -G™ produced reversible increases in locomotor activity of mice, and ISOPAR-C™ and -E™ produced reversible concentration-dependent decreases in rates of schedule-controlled operant behavior. The fact that only locomotor activity increases were observed with these isoparaffins provides evidence that they produce a different pattern of effects than abused solvents such as toluene and TCE. Further research will be needed to determine if this different pattern of effects on animal behavior between isoparaffins and abused solvents is correlated with a different pattern of acute intoxication and abuse potential in humans. Of particular importance will be information on actual abuse of products in which isoparaffinic hydrocarbon solvents predominate. As of yet, no such reports have been published.

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