Neurochemical Effects on Rats of \( n \)-Heptane Inhalation Exposure

H. Savolainen and P. Pfäffli
Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, Haartmaninkatu 1, SF-00290 Helsinki 29, Finland

Abstract. Inhalation exposure of male rats at three dose levels (4.2, 21, 62 mol/L) to \( n \)-heptane vapor caused a dose-dependent brain and body solvent burden, which increased during two weeks of exposure. Initial neurochemical effects included reduced RNA concentration and increased NADPH-diaphorase in brain at the lowest dose. Increased proteolysis was detected in the cerebral samples in the second week at all doses, and the NADPH-diaphorase returned to the control range. Brain RNA content tended to be larger than in the controls. All biochemical effects were abolished after two weeks of withdrawal from the two-week exposure, with the exception of reduced glutathione at the lowest dose. None of the rats presented clinical signs of neuropathy, which indicates that heptane and its metabolites do not share the specific neurotoxicity of hexane and its metabolites.

Mixtures of aliphatic hydrocarbons of short to medium chain lengths are, in general, used as thinners, degreasants, and dry-cleaning agents. Common formulas contain hexanes to decanes. Except for the established neurotoxicity of \( n \)-hexane and its oxidized metabolites (Perbellini et al. 1978; Spencer et al. 1978) very little is known about the toxicity of aliphatics with longer carbon chains. However, nonane and decane accumulate in marine birds living in contaminated areas (Lawler et al. 1978). Mallard ducklings fed crude oil-contaminated food displayed a dose-dependent lag in growth as well as degenerative liver and kidney disease (Szaro et al. 1978).

\( N \)-heptane was chosen as representative of the aliphatic low-molecular-weight hydrocarbons, because it is actively metabolized in mammals (Frommer et al. 1972). The initial breakdown products are various alcohols with 2-heptanol as a major metabolite (Frommer et al. 1972). By analogy to \( n \)-hexane, \( n \)-heptane has been suspected to cause similar chronic neurotoxicity (Bruckner and Peterson 1977). Despite the fact that it is volatile with a vapor pressure of 35 mm Hg at 20°C (Verschueren 1977) no clinical cases of overt \( n \)-heptane neuropathy have been reported in the literature.

The aim of the present study was to show the possible neural effects of \( n \)-heptane in a short-term inhalation exposure at three different dose levels, and
Table 1. Brain and perirenal fat n-heptane concentrations after inhalation exposure to 4.2, 21 or 62 μmol n-heptane/L

| Weeks exposure | Brain* | | | | | Perirenal fat |
|----------------|--------|--------|--------|--------|--------|--------|--------|
|                | 4.2    | 21     | 62     | 4.2    | 21     | 62     | 4.2    | 21     | 62     |
| 1              | 11 ± 3 | 18 ± 5 | 35 ± 2 | 22 ± 5 | 341 ± 71 | 1257 ± 308 | 14 ± 2 | 44 ± 16 | 135 ± 43 |
| 2              | 14 ± 2 | 44 ± 16 | 135 ± 43 | 34 ± 6 | 474 ± 69 | 1814 ± 393 | 22 ± 5 | 341 ± 71 | 1257 ± 308 |

* Each figure is the mean of five animals ± S.D.

also to examine the biochemical recovery of rats withdrawn from exposure for two weeks.

Materials and Methods

Forty-five male Wistar rats with an average body weight of 357 ± 12 g (± S.D.) were divided into three groups and exposed to n-heptane vapor at concentrations of 4.2 (100 ppm), 21 (500 ppm) or 62 μmol/L (1,500 ppm) in dynamic exposure chambers of one m³. The exposures were performed in the dark with an inverted daylight rhythm of 12 hr. The vapor concentrations were monitored continuously by an infra-red spectrophotometer (Miran 1, Wilks Scientific Corp., U.S.A.) which automatically controlled the vapor generator. The deviations in the exposure levels were less than 2% as calculated from the graphic recordings. No food was given during the exposure periods which lasted six hr daily, 5 days a week, for one or two weeks. Control animals with body weights of 354 ± 13 g (± S.D.) were sham-exposed simultaneously. Water was available ad libitum.

Rats were killed by decapitation after one or two weeks of exposure in groups of five, and five rats in each group were allowed to recover from the effects of the two-week exposure for another two-week period. Brain and a perirenal fat sample were taken at necropsy, and the right cerebral hemisphere and the fat specimen were analyzed for n-heptane as follows: The samples were homogenized in ten volumes (w/v) of dimethylformamide. The liquid phase was separated by centrifugation at 3,500 g and 4°C for 15 min, an aliquot (2 ml) was analyzed by a Perkin-Elmer F-11 gas-chromatograph with a back-flush technique. The apparatus was equipped with a 30 cm pre-separation column and a 2 m x 4 mm analytical column packed with 10% Carbowax 20 M on Chromosorb W. Carrier gas was nitrogen; column temperature was 100°C. Back flushing of the pre-separation column was begun 30 sec after the injection; this prevented dimethylformamide from entering the longer column. A flame ionization detector equipped with an automatic integrator was used for detection and calculation. The mean error for extraction and analysis was 6.4% with a recovery of 92% at a concentration of 24.5 nmol/g (N = 10). The respective figures for higher concentrations were even better.

The left hemispheres were homogenized in ten volumes of 0.1 M phosphate buffer at pH 7.2 for the determination of RNA (Morimoto et al. 1974) and for glutathione (Hissin and Hilf 1976). Acid proteinase (Marks et al. 1975), NADPH-diaphorase (Elovaara et al. 1977) and superoxide dismutase (Marshall and Worsfold 1978) were assayed in the same samples.

Statistical analyses were made with the aid of Student's t-test.

Results

No n-heptane was found in rats withdrawn from the exposure for two weeks, whereas cerebral solvent concentration was in a linear relationship to the exposure level in the first week of exposure (y = 0.42 x + 9.27 where y is brain n-heptane (nmol/g) and x atmospheric n-heptane (μmol/L), r² = 0.99) (Table 1). This relationship changed during the second week to y = 2.12 x + 2.80 (r² = 0.99), because of n-heptane accumulation. Similar linear relationships between