The Effects of Gasoline Vapor Inhalation on Pain Threshold in Male Rats

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Abstract---Studies show that there is association between gasoline vapor inhalation and many aspects of physiological function. This study was exerted to determine the effects of gasoline vapor inhalation on pain threshold in male rats. In our study male Wistar rats were randomly divided into control, and rats exposed to gasoline vapor for 1 h/day, 4h/day, and 8h/day. After 2 weeks pain threshold was measured using tail withdrawal test. The results indicated that pain threshold significantly decreased in all groups of rats exposed to gasoline vapor compared to control group (P<0.001). Our findings show that gasoline vapor inhalation has a stimulatory effect on pain perception.

Keywords---Gasoline Vapor, Pain Threshold, Male Rat

I. INTRODUCTION

Gasoline contains over 500 saturated or unsaturated hydrocarbons having from 3 to 12 carbon atoms. Millions of people are exposed to gasoline constituents in the course of refueling at gasoline stations [1]. Natural gasoline is in the peculiar position of being at the same time a raw material and a finished product. It is seldom heard of in retail transactions involving petroleum products, because it is usually first blended with other fractions to produce motor and aviation fuels, in which it finds its principal outlet. In this case it may be considered a raw material. However, it is usually produced to such stringent specifications that little if any further processing is necessary after it leaves the natural gasoline plant. In this sense it may be considered a finished product.[2] The importance of natural gasoline is evident from the fact that in California the production of this material in recent years has amounted to about one-fifth the net production of refinery gasoline. The ratio in the United States has been smaller, but still it averaged one gallon of natural to about ten gallons of net refinery gasoline prior to the war and has now reached one gallon in six as a result of the increased demand for the volatile natural gasoline fractions in aviation fuel.[3] Natural gasoline is extracted from “wet” gas, which is usually produced simultaneously with crude oil. Wet gas is separated from the oil by stages in field traps and tanks, whence it is collected and delivered to natural gasoline plants. The gas is called “wet” not in the sense iii which the word is customarily used, but because it contains gasoline fractions in an amount sufficient to justify recovering them. Gasoline is a refined product of petroleum consisting of a mixture of hydrocarbons, additives, and blending agents. The composition of gasolines varies widely, depending on the crude oils used, the refinery processes available, the overall balance of product demand, and the product specifications. The typical composition of gasoline hydrocarbons (% volume) is as follows: 4-8% alkanes; 2-5% alkenes; 25-40% isoalkanes; 3-7% cycloalkanes; 1-4% cycloalkenes; and 20-50% total aromatics (0.5-2.5% benzene) (IARC 1989). Additives and blending agents are added to the hydrocarbon mixture to improve the performance and stability of gasoline. [4] These compounds include anti-knock agents, anti-oxidants, metal deactivators, lead scavengers, anti-rust agents, anti-icing agents, upper-cylinder lubricants, detergents, and dyes. At the end of the production process, finished gasoline typically contains more than 150 separate compounds although as many as 1,000 compounds have been identified in some blends[1].

Gasoline is a complex mixture of hydrocarbons and additives; the relative concentration of gasoline components is dependent upon the crude oil source, refinery process and product lines. Gasoline consists principally of paraffins (66 to 69 percent), aromatics (24 to 27 percent), and olefins (6 to 8 percent). Chemicals are added to improve engine performance. Gasoline exists in the environment in four states: as a free-moving liquid, adsorbed into soil, in groundwater, and as an aerosol or vapor. Gasoline components partition in environmental media according to vapor pressure, water solubility and partition coefficients. Because benzene, toluene and xylene have high vapor pressure and water solubility, they may exist in both the vapor phase and water soluble fraction of gasoline [5].

General exposure parameters were employed for adult and infant weight, and corresponding ingestion and inhalation rates. Exposures associated with various exposure durations, expressed as mg/kg/day, are calculated for each exposure scenario in order to compare these exposures to toxicological criteria. Doses are calculated based on total ventilation for pulmonary effects and alveolar ventilation for systemic effects. Using alveolar ventilation as the basis for calculating systemic
doses assumes that only the two-thirds of the inhaled dose that reaches the alveoli is absorbed into the systemic circulation. Non-ingestion exposure to gasoline contaminated water suggests a 2:1 non-ingestion to ingestion ratio; exposure from non-ingestion sources (e.g., showering, dish-washing) are comparable to and may be twice as much as the dose received from ingestion of 2 liters of contaminated water per day [6],[7]. High toxicity of gasoline, benzene, toluene and xylene has been investigated in both short- and long-term exposure studies. The human studies generally involve acute environmental (accidental and deliberate inhalation) and acute and chronic occupational exposures to gasoline or to a mixture of gasoline components (particularly the aromatic compounds). Studies on laboratory animals have focused on the subacute and subchronic effects from exposure to gasoline and its major constituents (benzene, toluene, and xylene) [8],[9].

Reproductive and developmental effects are among the most sensitive non-cancer toxic endpoints for benzene, toluene, and xylene exposures. These effects include increased resorptions, reduced fetal body weight, and delayed skeletal development, and in the case of benzene, induced bone marrow suppression in offspring. Benzene and xylene have been shown to be teratogenic in rats at maternally toxic doses after inhalation and oral exposure [10].

Unleaded gasoline, benzene, toluene and xylene have been evaluated for genotoxic effects in a variety of test systems. Generally, unleaded gasoline is not mutagenic in bacterial systems while positive results have been recorded for sex-linked mutations in Drosophila melanogaster, forward mutations with mouse lymphoma cells and induction of unscheduled DNA synthesis [11].

One adequate carcinogen bioassay has been conducted with gasoline vapors. In that study, statistically significant increases in kidney tumors in male Fischer 344 rats and hepatocellular tumors in B6C3F1 mice were observed. Major uncertainties are (1) the vapor composition in this study was different from the ambient human environment and (2) the kidney tumors observed in male rats may be the result of a mechanism specific to the male rat and not female rats or other species. The male rat appears to selectively distribute the hydrocarbons (e.g., 2,2,4-trimethylpentane) believed responsible for the nephrotoxicity to the kidney [12].

II. MATERIAL AND METHODS

A. Animals

Adult Wistar rats weighting 200±30g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran). The temperature was at 23±2 °C and animals kept under a schedule of 12h light:12h darkness (light on at: 08: 00 a.m.) with free access to water and standard laboratory chow.

B. Protocol of Study

Male Wistar rats were randomly divided into control, and rats exposed to gasoline vapor for 1 h/day, 2h/day, and 3h/day. After 2 weeks blood samples were collected and pain threshold was measured using Tail Withdrawal Test. Briefly, in the classic radiant heat test, a heat source is targeted onto a small area of the tail, and the latency to withdraw the tail away from the heat source is measured. In the tail-immersion test, a container of liquid is heated or cooled to a nociceptive temperature – normally 50–55°C or below 0°C. The animal subject is then placed with its tail immersed in the liquid, and the latency to withdraw the tail from the liquid is measured. .

C. Statistical Analysis

All values are presented as mean ± S.D. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19.

III. RESULTS

Figure I represents pain threshold in rats exposed to gasoline vapor for 1h/day, 4h/day and 8h/day for a period of 2 weeks.

![Pain threshold in rats exposed to gasoline vapor for 1h/day, 4h/day and 8h/day for a period of 2 weeks.](image)

Fig. 1 Pain threshold in rats exposed to gasoline vapor for 1h/day, 4h/day and 8h/day for a period of 2 weeks. * indicates significant difference compared with control animals.

Our results show that inhalation of gasoline vapor resulted in decreased pain threshold (P<0.001) in all experimental groups compared with control animals.

IV. DISCUSSION

In our study, we reported the dose gasoline vapor inhalation results in decreased pain threshold in male rats indicating the stimulatory effects of gasoline vapor on nociception response in male rats.

In accordance with our findings, studies indicate that some components of gasoline - in particular Ethyl Tertiary-Butyl Ether (ETBT) - appear to be capable to negatively alter the reproductive steroid levels, and since steroids influence pain perception [13], [14], so, the association between gasoline vapor and alteration in pain perception can be explained by alteration occurred in steroids levels after exposure to gasoline vapor. The studies also show that odorant substances trigger pain [15]. There is also association between gasoline and DNA damage [16], which in turn, may bring about impairments in neural system resulting in sensitivity to pain.
V. CONCLUSION

We have shown that gasoline vapor inhalation results in increased response to thermal pain stimulus indicating that gasoline vapor inhalation can reduce pain threshold.

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