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# Effects of an ethanol–gasoline mixture: results of a 4-week inhalation study in rats

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**ABSTRACT: The inhalation toxicity of an ethanol–gasoline mixture was investigated in rats. Groups of 15 male and 15 female rats were exposed by inhalation to 6130 ppm ethanol, 500 ppm gasoline or a mixture of 85% ethanol and 15% gasoline (by volume, 6130 ppm ethanol and 500 ppm gasoline), 6 h a day, 5 days per week for 4 weeks. Control rats of both genders received HEPA/charcoal-filtered room air. Ten males and ten females from each group were killed after 4 weeks of treatment and the remaining rats were exposed to filtered room air for an additional 4 weeks to determine the reversibility of toxic injuries. Female rats treated with the mixture showed growth suppression, which was reversed after 4 weeks of recovery. Increased kidney weight and elevated liver microsomal ethoxyresorufin-*O*-deethylase (EROD) activity, urinary ascorbic acid, hippuric acid and blood lymphocytes were observed and most of the effects were associated with gasoline exposure. Combined exposure to ethanol and gasoline appeared to exert an additive effect on growth suppression. Inflammation of the upper respiratory tract was observed only in the ethanol–gasoline mixture groups, and exposure to either ethanol and gasoline had no effect on the organ, suggesting that an irritating effect was produced when the two liquids were mixed. Morphology in the adrenal gland was characterized by vacuolation of the cortical area. Although histological changes were generally mild in male and female rats and were reversed after 4 weeks, the changes tended to be more severe in male rats. Brain biogenic amine levels were altered in ethanol- and gasoline-treated groups; their levels varied with respect to gender and brain region. Although no general interactions were observed in the brain neurotransmitters, gasoline appeared to suppress dopamine concentrations in the nucleus accumbens region co-exposed to ethanol. It was concluded that treatment with ethanol and gasoline, at the levels studied, produced mild, reversible biochemical hematological and histological effects, with some indications of interactions when they were co-administered.** 

KEY WORDS: inhalation toxicity; ethanol–gasoline mixture; brain biogenic amines; rats

## Introduction

Use of ethanol-blended gasoline as an alternative transportation fuel has increased in recent years. In Canada, an estimated 929 retail outlets offer ethanol-blended fuels (CRFA, 1999). In the USA more than 170 refuelling stations with blended fuels (85% ethanol, 15% unleaded gasoline) have been accessible to the public since 1999 (Clean Cities Network and the Alternative Fuels Center, 1991). Because of their increased use, there is a need to assess the health effects of these blended fuels. A review of the literature has revealed the existence of many toxicity studies on gasoline or ethanol alone but no information on the toxic effects of ethanol–gasoline mixtures. Neurobehavioral, neurochemical and developmental effects following both subchronic and chronic exposure to ethanol were investigated by Nelson *et al*. (1984a,b, 1988, 1990). These authors reported minor effects at 16 000 ppm ethanol or above, but no effects at lower levels. Effects of gasoline inhalation have been studied extensively and most studies reported renal effects such as protein casts, regenerative changes and tubular dilation (MacFarland, 1982; Kuna and Ulrich, 1984; MacFarland *et al*., 1984). Neurological effects of gasoline and other hydrocarbons also have been reviewed (Burbacher, 1993). No overt signs of neurotoxicity were observed at 1500 ppm or lower doses, and minor neurochemical changes including decreased hypothalamic noradrenaline were noted at 650 ppm (Vyskocil *et al*., 1988).

Several studies have examined the interactive effects of ethanol and industrial chemicals. Snyder and co-workers (1981) reported increased hematotoxic effects of inhaled benzene in mice given ethanol in their drinking water. This work was extended and confirmed by Baarson *et al*. (1982). Ethanol consumption also was found to potentiate benzene-induced hematopoietic effects in rats (Nakajima *et al*., 1985). Research on the effects of repeated exposure to low levels during routine handling of ethanol–gasoline

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mixtures is lacking. The present study therefore was conducted to investigate the toxicological and neurochemical effects in rats following a 4-week inhalation to gasoline and ethanol alone or in combination at levels relevant to exposure at refuelling stations.

## Materials and Methods

### Materials

Ethanol of >99% purity was procured from Fisher Scientific (Ottawa, Ontario, Canada). The purity and identity of the ethanol were verified by gas chromatography. Unleaded gasoline (Indoline) was obtained from the Vehicle Testing Laboratory of Environment Canada (Ottawa, Ontario, Canada) and had the following composition: aromatic hydrocarbons, 27 vol.%; olefines, 0.3 vol.%; sulfur, 0.001 wt.%; lead, <1 mg $l^{-1}$; saturated hydrocarbons, balance. Unless specified otherwise, chemicals and reagents used in this study were purchased from Fisher Scientific (Ottawa, Ontario, Canada).

### Animal Treatment

The study was conducted in accordance with the Guidelines of Canadian Council on Animal Care. Sixty each of male and female specific-pathogen-free Sprague-Dawley rats (body weight 80–100 g) were purchased from Charles River Laboratories (St. Constant, Quebec). After acclimatization to laboratory conditions for 1 week, 15 rats per group of each gender were randomly assigned to one of the following four exposure groups: HEPA/charcoal-filtered room air (control animals); 6130 ppm ethanol; 500 ppm gasoline; and 6130 ppm ethanol + 500 ppm gasoline (ethanol–gasoline vapor concentrations were calculated based on 85% ethanol and 15% gasoline by volume, E-85). Rats were housed individually in polycarbonate cages prior to exposure in the inhalation chamber. The temperature and relative humidity in the animal room were maintained at 22 ± 1 °C and 50 ± 10%, respectively. Lighting (fluorescent daylight) was set on a 12-h light/dark cycle using an automated timing device. During exposure, rats were transferred to stainless-steel cages housed in 2.5 $m^3$ stainless-steel chambers supplied with HEPA/charcoal-filtered air at a flow rate of 500 l $min^{-1}$. The test atmosphere was generated by injecting (with a syringe pump) appropriate quantities of gasoline, ethanol or ethanol–gasoline mixture into a modified j-tube heated at 60 °C. The vapor of ethanol and gasoline was carried by an air stream (20 l $min^{-1}$) into the chamber inlet and mixed with incoming air. Details of chamber set-up and measurement of the vapor concentrations were described by Kumarathasan *et al*. (1996). Rats were placed in the inhalation chambers for 6 h (7.30 a.m.–1.30 p.m.) a day, 5 days per week for 4 weeks. Urine specimens were collected overnight from each rat 1 day prior to the termination, and analyzed for ascorbic acid and hippuric acid (normalized to urine creatinine) using the HPLC procedure described previously (Poon *et al*., 1994). At the end of the treatment period 10 rats from each dose group were anesthetized with an i.p. injection of a barbiturate-based anesthetic at 3.5 ml $kg^{-1}$ body weight and exsanguinated from the abdominal aorta. Each 100 ml of the anesthetic contains 4.04 g of chloral hydrate, 0.97 g of pentobarbital, 2.13 g of magnesium sulfate pentahydrate, 14.8 g of 95% ethanol and 40 g of propylene glycol. The remaining rats from each group were exposed to filtered room air for an additional 4 weeks and then killed to determine the reversibility of effects.

### Laboratory Analysis

Following exsanguination, a 2-ml blood sample in EDTA was removed for evaluation of hematological parameters as described previously (Chu *et al*., 1998). The remaining blood was used to prepare serum for the determination of clinical chemistry endpoints. The brain was removed, rinsed with ice-cold saline, weighed and sectioned into two halves along the midline on an ice-chilled block. The right hemisphere of the brain was frozen rapidly on dry ice and stored at −80 °C for neurochemical analysis. The remaining half of the brain was fixed in 10% buffered formalin (pH 7.4) for histopathological examination. The following tissues were excised and weighed: heart, liver, spleen, kidney, brain, testes and thymus. A 5-g piece of fresh liver was removed and homogenized in 2.5 volumes of ice-cold 0.05 M TRIS–1.15% KCl buffer (pH 7.4) and centrifuged at 10 000 *g*. The supernatant was separated and assayed for ethoxyresorufin-*O*-deethylase (EROD) and pentoxyresorufin-*O*-dealkylase (PROD) activities using the method of Lubet *et al*. (1985). The tissues were excised, fixed in 10% buffered formalin (pH 7.4) and examined histologically as described by Chu *et al*. (1998).

### Neurochemistry Analysis

Brain biogenic amines analysis is essentially as described by Seegal *et al*. (1997) and Poon *et al*. (2002). Briefly, frozen brains were mounted onto a movable aluminum stage and allowed to warm to approximately −10 °C before cutting 750-μm sections using a microtome. Using the brain dissection guide of Palkovits and Brownstein (1988), tissue punches of specific brain regions (e.g. frontal cortex, caudate nucleus, nucleus accumbens, substantia nigra, hippocampus) were obtained from the 750-μm sections. These tissue punches were homogenized in 150 μl

of ice-cold 0.2 M perchloric acid containing 100 mg $l^{-1}$ EGTA and centrifuged at 4000 *g* to form the pellets of protein. The supernatant was analyzed using HPLC with a Supelco column (LC-18-DB, 5 μm, 25 cm length) and an electrochemical detector (ESA, model Coulochem II) for dihydroxyphenylalanine, dopamine and its metabolites (3-methoxytyramine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid), norepinephrine, serotonin (5-HT) and its metabolite 5-hydroxy indoleacetic acid (5-HIAA). The protein content of each punch was determined using a modified Lowry assay and the results were reported as ng neurotransmitter $mg^{-1}$ protein.

### Statistical Analysis

Except for neurochemical data, all results were analyzed by one-way ANOVA followed by Duncan's multiple-range test. Data in Tables 1–3 are presented as means ± standard deviation of the mean. All data were prescreened for normality and equivalence of variance.

Neurochemical data were analyzed with a 2 × 2 × 2 multivariate analysis of variance (MANOVA) for each brain region. Where the overall MANOVA revealed significant effects or interactions with Hotelling's *T* test, the data were analyzed using Roy-Bargman's Stepdown *F* test and simple effects test (Tabachnick and Fidell, 1989). The analytical strategy provided protection against the considerable increase in Type I error rates that occur when MANOVA was conducted on each neurotransmitter measured in each brain region.

## Results

### Growth

Exposure to either ethanol or gasoline alone had no effects on the growth rate of female rats, but in combination the mixture produced significant reductions in weight gain. After 4 weeks of recovery, the growth rate returned to normal. The growth rate of male rats was not altered significantly by exposure (Table 1).

### Cage-side Observation and Organ Weight

No clinical signs of toxicity were observed. Gross pathological examination of the organs showed no abnormalities. There was a decrease in relative liver weight in the males exposed to the mixture (Table 1). Relative kidney weight was increased in males exposed to gasoline and in the recovery group treated with the mixture. The thymic weight of male rats exposed to ethanol was increased significantly but returned to normal after 4 weeks of recovery. Relative heart weight was increased in all females in the recovery groups.

### Biochemical and Hematological Effects

Among the clinical chemistry parameters, only serum phosphate and glucose were altered by treatment (Table 2). Serum phosphate was elevated in male rats exposed to gasoline, whereas serum glucose was elevated

**Table 1.** Body weight gain and organ weights following inhalation exposure to ethanol, gasoline and ethanol–gasoline

| | *n* | Initial body wt. (g) | Body wt. gain (g) | Liver (% body wt.) | Kidney (% body wt.) | Heart (% body wt.) | Thymus (% body wt.) |
|---|---|---|---|---|---|---|---|
| *Male* | | | | | | | |
| Control | 10 | 199 ± 16 | 183 ± 19 | 4.06 ± 0.28 | 0.80 ± 0.44 | 0.32 ± 0.03 | 0.14 ± 0.01 |
| Ethanol | 10 | 199 ± 14 | 187 ± 19 | 3.91 ± 0.16 | 0.76 ± 0.51 | 0.31 ± 0.03 | 0.19 ± 0.04* |
| Gasoline | 10 | 195 ± 13 | 172 ± 22 | 4.00 ± 0.18 | 0.87 ± 0.11* | 0.31 ± 0.02 | 0.16 ± 0.02 |
| Ethanol–gasoline | 10 | 197 ± 14 | 171 ± 18 | 3.77 ± 0.28* | 0.86 ± 0.78 | 0.30 ± 0.02 | 0.17 ± 0.03 |
| *Male (recovery)* | | | | | | | |
| Control | 5 | 190 ± 11 | 275 ± 30 | 3.71 ± 0.30 | 0.72 ± 0.04 | 0.29 ± 0.03 | 0.11 ± 0.02 |
| Ethanol | 5 | 194 ± 10 | 274 ± 28 | 3.54 ± 0.22 | 0.68 ± 0.05 | 0.30 ± 0.03 | 0.10 ± 0.01 |
| Gasoline | 5 | 198 ± 10 | 305 ± 34 | 3.83 ± 0.26 | 0.73 ± 0.04 | 0.30 ± 0.02 | 0.08 ± 0.02 |
| Ethanol–gasoline | 5 | 197 ± 12 | 268 ± 14 | 3.78 ± 0.14 | 0.83 ± 0.06* | 0.31 ± 0.04 | 0.11 ± 0.02 |
| *Female* | | | | | | | |
| Control | 10 | 154 ± 11 | 86 ± 10 | 4.08 ± 0.17 | 0.83 ± 0.07 | 0.35 ± 0.02 | 0.22 ± 0.04 |
| Ethanol | 10 | 150 ± 10 | 85 ± 11 | 3.96 ± 0.20 | 0.81 ± 0.06 | 0.35 ± 0.04 | 0.21 ± 0.03 |
| Gasoline | 10 | 154 ± 10 | 81 ± 17 | 4.08 ± 0.41 | 0.81 ± 0.06 | 0.34 ± 0.02 | 0.20 ± 0.04 |
| Ethanol–gasoline | 10 | 152 ± 12 | 68 ± 5* | 3.86 ± 0.25 | 0.82 ± 0.09 | 0.33 ± 0.02 | 0.21 ± 0.02 |
| *Female (recovery)* | | | | | | | |
| Control | 5 | 151 ± 6 | 123 ± 21 | 3.68 ± 0.25 | 0.73 ± 0.06 | 0.30 ± 0.02 | 0.14 ± 0.03 |
| Ethanol | 5 | 157 ± 12 | 114 ± 10 | 3.94 ± 0.23 | 0.74 ± 0.03 | 0.35 ± 0.03* | 0.16 ± 0.02 |
| Gasoline | 5 | 157 ± 14 | 106 ± 19 | 3.54 ± 0.20 | 0.75 ± 0.08 | 0.35 ± 0.04* | 0.15 ± 0.02 |
| Ethanol–gasoline | 5 | 146 ± 8.9 | 101 ± 19 | 3.90 ± 0.14 | 0.81 ± 0.04 | 0.37 ± 0.03* | 0.15 ± 0.01 |

* Significantly different from controls at $P < 0.05$.

**Table 2.** Changes in hepatic drug-metabolizing enzymes, biochemistry and hematological parameters following inhalation exposure to ethanol, gasoline and ethanol–gasoline

| | | Liver | Serum | | Blood | |
|---|---|---|---|---|---|---|
| | *n* | EROD (nmol $min^{-1}$ $mg^{-1}$ protein) | Inorganic phosphate (mg $dl^{-1}$) | Glucose (mg $dl^{-1}$) | Hemoglobin (g $dl^{-1}$) | Lymphocytes (%WBC)[a] |
| *Male* | | | | | | |
| Control | 10 | 0.14 ± 0.05 | 7.60 ± 0.66 | 243 ± 42 | 15.4 ± 0.8 | 80.4 ± 6.3 |
| Ethanol | 10 | 0.13 ± 0.04 | 8.05 ± 0.59 | 232 ± 37 | 15.8 ± 0.9 | 84.2 ± 3.7 |
| Gasoline | 10 | 0.19 ± 0.07* | 8.23 ± 0.67* | 254 ± 39 | 15.3 ± 0.8 | 85.9 ± 4.1* |
| Ethanol–gasoline | 10 | 0.18 ± 0.03 | 7.95 ± 0.44 | 253 ± 28 | 15.1 ± 0.7 | 85.2 ± 3.0* |
| *Male (recovery)* | | | | | | |
| Control | 5 | 0.34 ± 0.05 | 6.34 ± 0.51 | 291 ± 31 | 14.7 ± 0.9 | 82.9 ± 3.5 |
| Ethanol | 5 | 0.27 ± 0.08 | 6.04 ± 0.29 | 271 ± 26 | 14.9 ± 0.6 | 82.2 ± 1.8 |
| Gasoline | 5 | 0.33 ± 0.04 | 7.18 ± 0.63* | 285 ± 52 | 14.4 ± 0.5 | 84.6 ± 3.1 |
| Ethanol–gasoline | 5 | 0.25 ± 0.07 | 6.18 ± 0.37 | 295 ± 34 | 14.3 ± 1.1 | 82.8 ± 2.0 |
| *Female* | | | | | | |
| Control | 10 | 0.12 ± 0.04 | 7.14 ± 0.56 | 280 ± 77 | 15.1 ± 0.9 | 83.5 ± 2.8 |
| Ethanol | 10 | 0.10 ± 0.03 | 7.77 ± 1.57 | 290 ± 78 | 14.2 ± 1.4 | 86.1 ± 4.6 |
| Gasoline | 10 | 0.16 ± 0.04 | 7.51 ± 0.67 | 260 ± 58 | 14.3 ± 0.7* | 88.5 ± 2.6* |
| Ethanol–gasoline | 10 | 0.15 ± 0.04 | 7.64 ± 0.55 | 280 ± 41 | 15.1 ± 0.4 | 87.9 ± 3.1* |
| *Female (recovery)* | | | | | | |
| Control | 5 | 0.31 ± 0.10 | 6.02 ± 0.78 | 243 ± 29 | 13.8 ± 0.7 | 87.5 ± 3.2 |
| Ethanol | 5 | 0.25 ± 0.06 | 6.30 ± 0.69 | 320 ± 50* | 14.6 ± 0.8 | 87.2 ± 1.7 |
| Gasoline | 5 | 0.28 ± 0.10 | 6.06 ± 0.38 | 302 ± 55* | 14.5 ± 0.8 | 87.2 ± 4.9 |
| Ethanol–gasoline | 5 | 0.31 ± 0.11 | 6.36 ± 0.44 | 277 ± 24 | 14.5 ± 0.3 | 83.4 ± 2.2 |

[a] WBC, white blood cells.
* Significantly different from controls at $P < 0.05$.

only in the recovery group of gasoline-treated female rats. Male rats exposed to gasoline showed elevated hepatic microsomal EROD activity (Table 2). After cessation of exposure, EROD activity of gasoline-exposed male rats returned to normal. In the female, treatment with gasoline alone or in combination with ethanol appeared to increase liver EROD activity, but the increase was not statistically significant.

Decreased hemoglobin was observed in gasoline-treated females whereas elevated lymphocyte counts were noted in both male and female rats treated with gasoline and the mixture. These hematological effects were reversed in the recovery groups.

Both male and female rats exposed to gasoline or the mixture showed increased urinary excretion of ascorbic acid and hippuric acid (Table 3). Four weeks after cessation of exposure, urinary excretion of the acids returned to normal.

Treatment with ethanol or gasoline resulted in altered levels of neurotransmitters that were brain- and gender-specific. Female rats appeared to be affected more by ethanol treatment than the males. For instance, ethanol exposure decreased mediodorsal thalamus 5-hydroxyindoleacetic acid (5-HIACC) and hippocampal 5-HT in female rats (Fig. 1). Similarly, treatment with gasoline increased entorhinal 5-HT in female rats but the males were not affected. In the frontal cortex, the effect of gasoline on dopamine and DOPAC was opposite in males and females, with an increase in males and a decrease in females. Although there is no generalized interaction with neurotransmitters by co-exposure to ethanol and gasoline, gasoline decreased dopamine in the nucleus accumbens of ethanol-treated rats of both genders (Fig. 1).

The upper respiratory tract and adrenal gland were the target organs of ethanol–gasoline treatment and the effects appeared to be more severe in the male rats. Morphological changes in the respiratory tract were characterized by minor non-suppurative inflammation of the nasal turbinate and were observed only in the mixture-treated groups but not in the gasoline or ethanol treatment alone (Table 4). Morphological alteration in the adrenal gland was characterized by cytoplasmic vacuolation in the cortical area. Combined exposure to ethanol and gasoline appeared to have an additive effect on the adrenal gland of male and female rats because the histological scores were the highest in the mixture-treated group.

## Discussion

Inhalation exposure of rats to ethanol, gasoline or a combination resulted in a broad range of effects: growth suppression, increased kidney weight and biochemical, neurochemical, hematological and morphological changes. With the exception of neurochemical effects, most were attributed to gasoline treatment.

**Table 3.** Changes in urinary ascorbic acid and hippuric acid following inhalation exposure to ethanol, gasoline and ethanol–gasoline

| | *n* | Urinary ascorbic acid (mg $g^{-1}$ creatinine) | Urinary hippuric acid (mg $g^{-1}$ creatinine) |
|---|---|---|---|
| *Male* | | | |
| Control | 10 | 80 ± 47 | 1.03 ± 1.06 |
| Ethanol | 10 | 117 ± 126 | 1.38 ± 1.75 |
| Gasoline | 10 | 528 ± 237* | 2.10 ± 1.24* |
| Ethanol–gasoline | 10 | 684 ± 316* | 2.04 ± 0.96* |
| *Male (recovery)* | | | |
| Control | 5 | 167 ± 58 | 1.64 ± 0.55 |
| Ethanol | 5 | 97 ± 81 | 1.12 ± 0.58 |
| Gasoline | 5 | 138 ± 128 | 1.48 ± 0.61 |
| Ethanol–gasoline | 5 | 160 ± 120 | 1.79 ± 0.28 |
| *Female* | | | |
| Control | 10 | 41 ± 41 | 1.01 ± 0.54 |
| Ethanol | 10 | 68 ± 52 | 1.41 ± 0.69 |
| Gasoline | 10 | 235 ± 68* | 1.92 ± 0.56* |
| Ethanol–gasoline | 10 | 252 ± 85* | 1.73 ± 0.80* |
| *Female (recovery)* | | | |
| Control | 5 | 91 ± 82 | 1.75 ± 0.97 |
| Ethanol | 5 | 93 ± 54 | 1.24 ± 0.54 |
| Gasoline | 5 | 112 ± 42 | 1.84 ± 0.47 |
| Ethanol–gasoline | 5 | 102 ± 58 | 1.47 ± 0.33 |

* Significantly different from controls at $P < 0.05$.

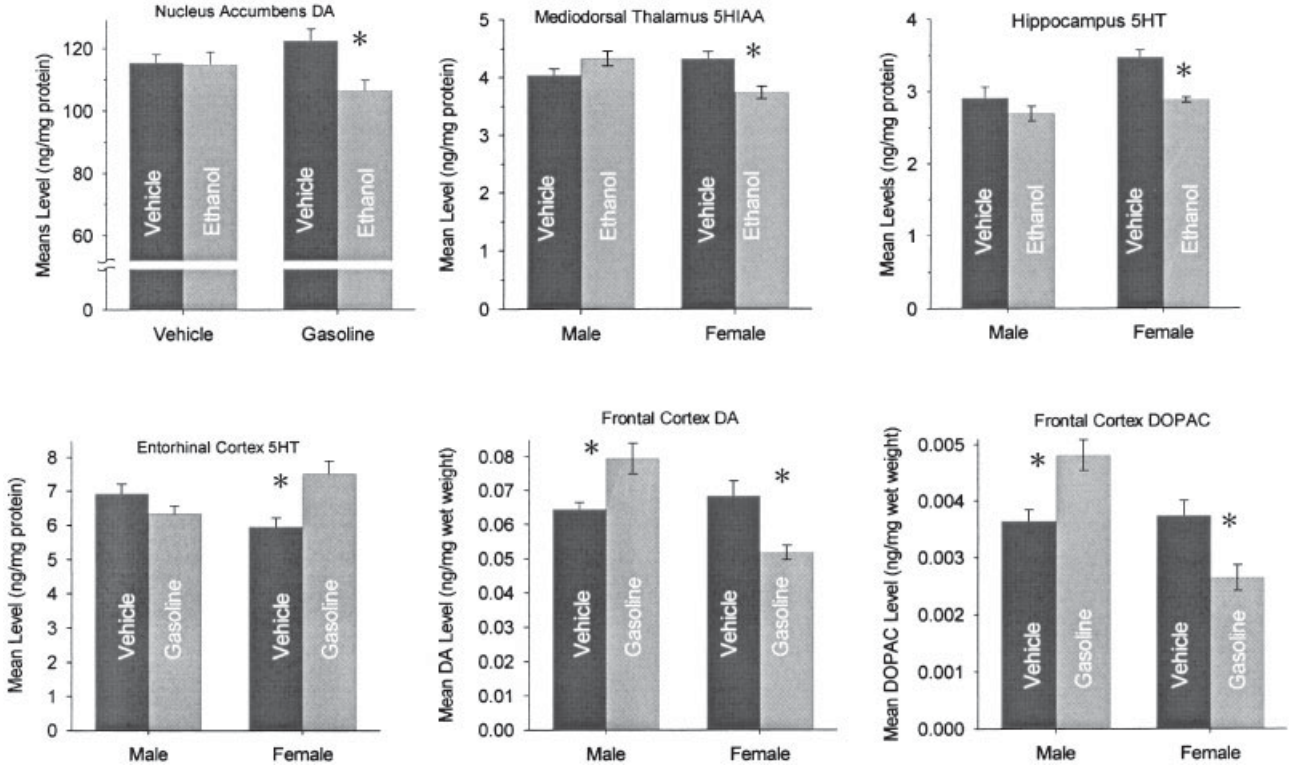


**Figure 1.** Neurochemical changes of rats exposed by inhalation to ethanol, gasoline or a mixture (* $P < 0.05$)

Increased levels of serum phosphate and glucose were observed in treated animals but these changes were not considered biologically significant because they were not associated with any target organ effects. A mild elevation in liver microsomal EROD activity and increased levels of urinary hippuric acid and ascorbic acid reflect adaptative responses by the exposed rats to increase drug-metabolizing enzyme activities in order

**Table 4.** Histological changes following inhalation exposure to ethanol, gasoline and ethanol–gasoline

| | Non-suppurative epithelial inflammation of nasolacrimal duct B | Cytoplasmic vacuolation of adrenal cortex |
|---|---|---|
| *Male* | | |
| Control | 0/10 | 10/10 (2.1) |
| Ethanol | 0/10 | 10/10 (2.6) |
| Gasoline | 0/10 | 10/10 (2.5) |
| Ethanol–gasoline | 7/10 (1.03) | 10/10 (2.8) |
| *Male (recovery)* | | |
| Control | 1/5 (0.38) | 5/5 (2.0) |
| Ethanol | 0/5 | 5/5 (2.6) |
| Gasoline | 0/5 | 5/5 (3.2) |
| Ethanol–gasoline | 5/5 (1.00) | 5/5 (3.0) |
| *Female* | | |
| Control | 4/10 (0.28) | 10/10 (1.3) |
| Ethanol | 0/10 | 10/10 (1.0) |
| Gasoline | 0/10 | 10/10 (1.3) |
| Ethanol–gasoline | 8/10 (0.73) | 10/10 (1.8) |
| *Female (recovery)* | | |
| Control | 4/5 (0.40) | 5/5 (1.0) |
| Ethanol | 0/5 | 5/5 (1.0) |
| Gasoline | 0/5 | 5/5 (1.2) |
| Ethanol–gasoline | 5/5 (0.80) | 5/5 (1.4) |

Data denote number of animals showing changes out of the number of animals examined. The severity of histology grading, given in parentheses, is as follows: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, severe. The scores are obtained by dividing the sum of total scores by the number of tissues examined. For tissue changes that are focal, locally extensive and multifocal, a score of less than an integer is assigned. The scores are: minimal focal, 0.25; minimal locally extensive, 0.50; minimal multifocal, 0.75; mild focal, 1.25; mild locally extensive, 1.50; mild multifocal, 1.75, etc.

to detoxify gasoline. Upon cessation of exposure, the enzyme activities returned to normal. Similarly, mild biochemical effects were observed in our previous study where rats were exposed to gasoline and methanol (Poon *et al*., 1995, 1998). Hematological effects were generally very mild, and increased lymphocytes in gasoline- and mixture-treated rats were most likely a secondary effect due to inflammation of the nasolacrimal duct.

Exposure of rats to gasoline at 1552 ppm but not 380 ppm has been reported to produce renal lesions consisting of increased regenerative epithelium and dilated tubules (Kuna and Ulrich, 1984). In our study no renal lesions were observed in the group treated with 500 ppm gasoline. Instead, mild histological changes were seen in the respiratory tract and adrenal gland. These results would suggest that the dose of gasoline used in our study is below the minimal effective dose required to produce renal effects. Adrenal vacuolation usually occurs when rats are under stress. Vacuolation in the adrenal gland of treated rats suggests that exposure to the test materials caused stress in these animals.

Gasoline is known to consist of 27% aromatic hydrocarbons. In a previous study, the hematotoxicity of benzene (an aromatic hydrocarbon) was potentiated by co-exposure with ethanol (Baarson *et al*., 1982). The potentiation was attributed to the increased microsomal enzyme activity induced by ethanol treatment, which in turn increased the production of toxic metabolites of benzene. Similarly, Nakajima *et al.* (1985) reported that ethanol consumption enhanced the hematopoietic disorders of benzene in rats. In our study, the toxicity of gasoline was not affected by co-administration with ethanol. The lack of increased gasoline hematotoxicity in rats co-exposed to ethanol is probably related to the route of administration, dose levels and/or chemical-specificity. Because ethanol at the dose level studied had no effects on the liver microsomal enzyme activities, it would preclude any potentiative effects due to the metabolism of gasoline. Some indications of additive/interactive effects have been observed in our study. For example, although treatment with either ethanol or gasoline alone had no effects on the growth of animals, exposure to the mixture appeared to exert growth suppression in female rats (Table 1). Additive or possibly synergistic effects were also noted in the upper respiratory tract because exposure to either gasoline or ethanol alone caused no irritation to this organ. When given as a mixture, inflammation of the nasolacrimal duct was observed in both male and female rats (Table 4). An interaction with neurochemicals in the brain was also noted. Treatment with ethanol alone had no effects on dopamine but in the presence of gasoline the dopamine level in the nucleus accumbens was decreased. It is not clear why the levels of dopamine and DOPAC in the frontal cortex area increased in males

but decreased in females. However, these neurochemical results suggest that the effects of ethanol and gasoline are gender-specific.

In conclusion, co-exposure of rats to gasoline (500 ppm) and ethanol (6130 ppm) showed additive and possibly some synergistic effects on growth, neurochemistry and histopathology in the adrenal gland and respiratory tract, as noted above. Effects observed following exposure to these two compounds were generally mild and adaptative in nature, and after cessation of exposure they return to normal.

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