

Developmental toxicity evaluation of unleaded gasoline vapor in the rat

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Abstract

To evaluate the potential of unleaded gasoline vapor for developmental toxicity, a sample was prepared by slowly heating API 94–02 (1990 industry average gasoline) and condensing the vapor. The composition of this vapor condensate, which comprises 10.4% by volume of the starting gasoline, is representative of real-world exposure to gasoline vapor encountered at service stations and other occupational settings and consists primarily of volatile short chain (C4–C6) aliphatic hydrocarbons (i.e. paraffins) with small amounts of cycloparaffins and aromatic hydrocarbons. A preliminary study in rats and mice resulted in no developmental toxicity in either species. However, a slight reduction in maternal body weight gain in rats led to the selection of rats for this guideline study. Groups of pregnant rats ($n = 24/\text{group}$) were exposed to unleaded gasoline vapor at concentrations of 0, 1000, 3000, or 9000 (75% lower explosive limit) ppm equivalent to 0, 2653, 7960, or 23900 mg/m^3 , for 6 h/day on gestation days 6–19. All rats were sacrificed on gestation day 20. No maternal toxicity was observed. Developmentally, there were no differences between treated and control groups in malformations, total variations, resorptions, fetal body weight, or viability. The maternal and developmental NOAEL is 9000 ppm. Under conditions of this study, unleaded gasoline vapors did not produce evidence of developmental toxicity. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Gasoline is the primary product of petroleum refining and is perhaps the most widely used energy source in the world. In addition to industrial applications, the ready availability of gasoline to power automotive engines has made the automobile an indispensable part of modern life and commerce. Gasoline also fuels other equipment used by the public including lawnmowers, snowblowers, small generators, motorcycles, and boats. In Europe, annual consumption of gasoline for industrial and domestic purposes exceeds 100 million tons per year [1]. US total gasoline production for 1999 was approximately 125 billion gallons [2]. Because of widespread use and exposure, unleaded gasoline and unleaded gasoline containing selected oxygenates are the subjects of the Clean Air Act 211(b) test rule [3] to evaluate the toxicity of the evaporative emission fraction of these fuel and fuel-oxygenate blends.

Typical gasoline contains more than 300 individual hydrocarbons consisting primarily of paraffins (30–90% volume), cycloparaffins (1–35% volume), olefins (0–20% volume), and aromatics (5–55% volume), distilling in the approximate range of 86°F–428°F (30°C–220°C). Composition of gasoline varies with source of the crude oil, refinery processes and conditions, and the blending of refinery streams in the gasoline boiling range to meet performance criteria as well as regulatory requirements. Performance criteria may change depending on countries and regions of use, and seasons of the year.

Acute toxicity studies of whole unleaded gasoline demonstrate minimal toxicity by oral ingestion [$\text{LD}_{50} = 14.6 \text{ g}/\text{kg}$, rats] [4], or by dermal exposure ($\text{LC}_{50} > 3.75 \text{ g}/\text{kg}$, rabbits), and no eye irritation [5]. Gasoline causes moderate dermal irritation in the rabbit [6] but is not a skin sensitizer in the guinea pig [5]. Whole unleaded gasoline is not mutagenic to bacterial or mammalian cells in culture and does not cause chromosome damage to rat bone marrow cells after treatment in vivo [7,8]. The only significant effect of repeated inhalation exposure of rats and monkeys to wholly vaporized unleaded gasoline at concentrations of 400 or

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Table 1
Exposure to gasoline vapors

Type of Exposure or Study	Gasoline Vapor ^a (ppm)	References
Gasoline odor detection	0.50	28
Gasoline odor recognition	0.76	28
Gas station perimeter (4 hour average)	0.26	29
Self service fill-up (2 minutes)	10–100	30
Refueling attendants (15 minutes)	0.5–48	31
(6 hours)	0.1–31	31
Mechanics (15 minutes)	0.4–138	31
(6 hours)	0.1–17	31
Occupational exposure standard (8 hours)	300	OSHA

^a Total hydrocarbons as parts per million (ppm) hexane

1500 ppm, 6 h/day, 5 days/week for 90 days was a change in the kidneys of male rats [9]. This finding was subsequently attributed to light hydrocarbon nephropathy, a species and sex-related syndrome observed in male rats that is not toxicologically relevant to humans (10). When rats and mice were exposed to wholly vaporized unleaded gasoline by inhalation for two years at actual concentrations of 67, 292, or 2056 ppm, male rats exhibited occurrences of light hydrocarbon nephropathy and kidney tumors and female mice had liver tumors. However, reproductive organs appeared normal under microscopic examination [11].

Inhalation exposure to whole unleaded gasoline at 400 and 1600 ppm from days 6 through 15 of gestation did not cause teratogenic effects in rats [12], nor were effects on fertility or offspring observed in a dominant lethal study in which male mice were treated for 8 weeks, the entire spermatogenic cycle, and mated sequentially to untreated females [13]. A series of inhalation developmental/reproductive screens in rats were conducted with vapor distillates of three gasoline-blending streams. Light alkylate naphtha [14], light catalytic cracked naphtha [15], and light catalytic reformed naphtha [16] were administered at exposures up to 7500 ppm daily during premating and gestation. No toxic effects were reported on fertility, reproductive performance, or fetal development. A two generation reproduction study in rats was recently completed using gasoline vapors recovered at a tank truck refueling center in the Netherlands. This study demonstrated that vapor recovery unit (VRU) gasoline, composed primarily of C4–C6 aliphatic hydrocarbons, administered via inhalation at concentrations as high as 20000 mg/m³ (7400 ppm) did not produce adverse reproductive effects on parental generations or offspring. No toxic effects were reported on reproductive indices, gonadal function, or pathology of reproductive tissue of either generation [17].

The composition of vapors to which humans are most likely exposed in a refinery or work place, or when engaged in automobile refueling is the lighter, more volatile fraction of gasoline. This fraction differs from whole gasoline by containing far less aromatics and longer chain (C7 and longer) aliphatic hydrocarbons. Analysis of workplace ex-

posure to gasoline vapors revealed that C4–C5 length hydrocarbons constitute from 67 to 74% by weight of the typical vapor [18]. Typical exposure levels for consumer use, such as filling vehicle fuel tanks at self-service stations, and for occupations associated with gasoline, such as service station attendants and mechanics, are shown in Table 1.

The current study was designed to evaluate the effects of this lighter fraction of unleaded gasoline on pregnant rats exposed throughout the organogenesis phase of pregnancy until just prior to parturition. Exposure on days 6 through 19 of gestation encompasses the time immediately after implantation begins through the period of organ system development and continuing through fetal growth. The vapor condensate used in this study was distilled from a sample of typical marketed unleaded gasoline without oxygenates by a method that produces a light-end gasoline vapor fraction similar to headspace vapor from a vehicle fuel tank at near maximum in-use temperature. Exposures were conducted up to a maximum target concentration equivalent to 75% of the lower explosive limit (LEL) of the vapor condensate.

2. Materials and methods

2.1. Test material

The unleaded gasoline (American Petroleum Institute 94–02) used to prepare the test material was blended by Phillips 66 Specialty Chemicals, Borger, TX, to meet the specifications required by the Clean Air Act, Section 211(b) for fuel and fuel additive registration. The 211(b) baseline gasoline must contain by volume 1.53 ± 0.3% benzene, 32.0 ± 2.7% aromatics, 9.2 ± 2.5% olefins, and 58.8 ± 2.0% saturated compounds [3]. The test material, identified as unleaded gasoline vapor condensate, was prepared by charging a glass-lined Pfauder kettle, operated as a closed system, with 6746 pounds (approximately 1000 gallons) of unleaded gasoline (API 94–02). The sample was slowly heated and stirred as the liquid temperature was raised to 150° F, resulting in a vapor temperature of 130° F. The vapor leaving the kettle was condensed by passing through a series

of two receiving vessels chilled with cold water (200 gallons) and dry ice (50 gallons), respectively, then through additional vapor traps chilled in dry ice/isopropyl alcohol. The vapor condensate collected by this method represented 10.4% of the initial sample weight. The chilled condensate was uniformly mixed, transferred to 5-gallon containers, and shipped to the testing laboratory where it was stored at ambient temperature in a solvent storage building until use. The unleaded gasoline (density = 0.75) and the unleaded gasoline vapor condensate (density = 0.65) were characterized by gas chromatography using a modified ASTM D5134–92 procedure on a Hewlett Packard 5890 instrument. The lower explosive limit (LEL) of the vapor condensate was determined to be approximately 12000 ppm. The vehicle used in the inhalation exposures was 99.98% nitrogen, purchased from MG Industries, Malvern, PA.

2.2. Animals

Nulliparous, nonpregnant female Sprague Dawley derived (CD®)[CrI:CD®BR] rats, 71 days old at receipt, were purchased from Charles River Laboratories, Portage, MI and were acclimated 14 days prior to mating at 85 days of age. Mated females were ear-tagged with an individual number on day 0 of gestation when they were assigned to test groups.

2.3. Mating

Females were placed with males nightly in a 1:1 ratio. Females were considered to have mated if sperm were seen microscopically in a vaginal rinse, taken each morning, and/or a plug was observed in the vaginal opening. The day on which evidence of mating occurred was defined as day 0 of gestation.

Mated females were randomly assigned to groups daily to provide an equal distribution among groups and equalize to the extent possible the gestation day 0 mean body weights between groups. The mean weight of mated females per group on day 0 of gestation was 261–264 g.

2.4. Husbandry

Except during mating, rats were housed individually in suspended stainless-steel cages with wire mesh floors and fronts in an air-conditioned room with a 12 h light-dark cycle, a monitored temperature range of 20°–24°C and a humidity range of 38–74%. Certified Rodent Diet No. 5002 Meal (PMI Feeds, Inc. St. Louis, MO) and water from an automated water delivery system were available ad libitum except during exposure.

2.5. Experimental design

The test procedures employed in this study were performed in accordance with US EPA TSCA Test Guideline

No. 798-4350: Inhalation Developmental Toxicity Study [20], except that the exposure period was extended to encompass the phase of fetal development. The study complied with US EPA Good Laboratory Practices (40 CFR Part 792) and appropriate parts of the Animal Welfare Act Regulations, 9 CFR parts 1 and 2 (Final rules FR54, No. 168, 8/31/89) and 9 CFR part 3 (Final rule FR56, No. 32, 2/15/91).

Species of rodent and dose levels used in this study were selected based on the results of an inhalation range finding study in which unleaded gasoline vapor condensate was administered to pregnant Sprague Dawley rats or pregnant CD-1 mice (Charles River Laboratories, Portage, MI), 10/group/species, at levels of 0 (controls), 300, 1000, 3000, or 9000 ppm from day 6 through 19 of gestation or day 6 through 17 of gestation, respectively. Dams were observed for signs of systemic toxicity. At sacrifice, uterine contents were evaluated for litter loss, viable litter size, fetal weight, and external fetal appearance. No maternal or developmental toxicity was observed in mice at any dose levels. In the rat, no developmental toxicity was observed; however, reduced maternal body weights and body weight gains over day 6 through 20 of gestation, and some reduced food consumption on day 6 through 9 appeared to occur in the 9000 ppm group. Thus, the rat was selected as the potentially more sensitive species for the guideline developmental toxicity study.

Unleaded gasoline (API 94–02) vapor condensate was administered as a vapor, via whole body inhalation, to 72 mated female rats (24/group) for 6 h/day during days 6 through 19 of gestation at target exposure levels of 1000, 3000, and 9000 ppm (2653, 7960, or 23900 mg/m³). Twenty-four mated rats were sham exposed to houseline nitrogen mixed with filtered room air only under the same inhalation chamber conditions for the same treatment interval. Chamber exposure concentrations were evaluated six times each day using infrared spectrophotometry and once daily using gas chromatography (GC). GC samples were analyzed for the major airborne components of the test vapor.

Mated rats were sacrificed on day 20 of gestation. The gravid uterus with ovaries was removed, weighed intact, and examined for the number of fetuses and resorption sites. Corpora lutea were counted in the ovaries. Fetuses were removed from the uterus, weighed, sexed externally, and evaluated. One half of the viable fetuses in each litter was processed for soft tissue evaluation; the remaining half was processed for skeletal evaluation.

3. Exposure

3.1. Chamber operation

Mated female rats were housed individually in wire-mesh, stainless-steel cages within a 1000-L glass and stainless steel exposure chamber (Wahlmann Mfg. Co., Timo-

nium, MD). Chamber temperature and humidity were monitored every half hour during exposure and maintained, to the extent possible, within the actual ranges of 20°–25°C and 34–70% relative humidity. Animals did not receive food or water during the 6-h exposure period. Exposure chambers were operated dynamically at a calibrated airflow rate of approximately 200 L/min with a complete air change in 5 min. Chamber size and airflow rates were adequate for an animal-loading factor below 5% and an oxygen level above 19%. Recordings of airflow and static pressure were made every half hour. All animals remained in the chamber for a minimum of 30 min at the end of each exposure period while the chamber was operated using clean air only. Chambers were exhausted through a system of a coarse filter, a HEPA filter, and a charcoal filter.

3.2. Atmosphere generation

Unleaded gasoline (API 94–02) vapor condensate was pumped directly from the storage container, refrigerated to minimize volatilization during transfer, into a countercurrent volatilization chamber. Gasoline vapor laden nitrogen flowed through the top of the volatilization chamber into the turret of the 1000 L exposure chamber, where it mixed with room air to the appropriate exposure concentration as it was drawn into the chamber. Prior to the initiation of animal exposures, infrared spectrophotometric samples were taken to assure uniform distribution of the test material in the chambers.

3.3. Exposure chamber monitoring

Total hydrocarbon levels were measured hourly for the 1000, 3000, and 9000 ppm chambers and once daily for the control chamber using a Miran Ambient Air Analyzer with strip chart recorder and multimeter. Exposure levels were determined by comparison of the measured absorbance to a calibrated response curve. The limit of detection under these operating conditions was an absorbance of 0.01, equivalent to 7 ppm. Nominal concentrations (ppm) were calculated daily for each exposure group by weight difference in storage container before and after exposure divided by measured chamber air flow

One sample per chamber per week was collected by syringe grab sampling and analyzed by gas chromatography (Hewlett Packard 5890II with flame ionization detector) to characterize 12 major components of the test atmosphere to demonstrate stability of test vapor over the course of the study. Composition and stability of the test material were evaluated by characterizing the liquid unleaded gasoline vapor condensate and comparing the major components with the generated atmospheres throughout the study.

To verify the absence of a significant aerosol phase of the test vapor, measurement of any background aerosol was performed once during each exposure (20 s sample at 5

L/min.) for chambers and room air using a TSI Aerodynamic Particle Sizer.

3.4. Clinical observations

Observations for mortality, general appearance and signs of toxic or pharmacologic effects were made twice daily. Detailed physical examination of each rat was performed on days 0 and 6 through 20 of gestation. During days 6 through 19, animals were evaluated pre- and post-exposure when animals were removed from the inhalation chambers

3.5. Body weights and food consumption

Each mated rat was weighed on days 0, 3, 6, 9, 12, 15, 18, and 20 of gestation. Day 20 gestation body weights were presented as actual and corrected (day 20 body weight minus weight of the gravid uterus). Food consumption was recorded during the same intervals. Mean maternal food consumption (g/kg/day) was calculated by dividing the grams of food consumed per kilogram of body weight from the first day's weighing of each interval by the number of days in the interval (e.g. days 0–3 = 3 day interval using day 0 body weight).

4. Pathology

4.1. Maternal postmortem examination

Adult animals were sacrificed by anesthesia with inhaled carbon dioxide followed by exsanguinations on day 20 of gestation. Complete macroscopic postmortem examinations were performed on all test animals. The intact uterus with ovaries attached was removed and weighed. Ovaries were dissected free and the presence and number of corpora lutea were recorded. Uteri were dissected longitudinally and the number and location of contents recorded for each horn: number of live fetuses, number of dead fetuses (absence of movement when touched with no visible degeneration), number of late resorptions (degenerating recognizable dead fetuses), number of early resorptions (implantation site but no recognizable fetus), and total implantation sites. Where no uterine implants were grossly apparent, the uterus was stained with ammonium sulfide to visualize any uterine foci. If no foci were observed, the female was considered not pregnant. All carcasses of adult females were discarded.

4.2. Fetal evaluations

All fetuses were weighed, sexed externally, and given a macroscopic external examination. Approximately one half of the fetuses from each litter were evaluated for soft tissue malformations/variations using a microdissection technique similar to Staples [21] performed on fresh fetal specimens shortly after removal from the uterus. Fetuses were decap-

itated and heads were fixed in Bouin's solution for later evaluation. Decapitated fetuses were dissected under a microscope. Tissues in the thoracic, abdominal and pelvic cavities were evaluated and fetuses were then stored in 10% neutral buffered formalin. Razor-cut, transverse serial sections of Bouin's-fixed fetal heads were examined for malformations of palate, eyes and brain under a dissecting microscope. Head sections were placed in plastic cassettes and stored in 70% ethanol solution.

Fetuses designated for skeletal evaluations were killed with an overdose of inhaled carbon dioxide. Intact fetuses were internally sexed by inspection of the gonads, eviscerated, and processed for staining of skeletal structures with Alizarin Red S. Specimens were stored in 100% glycerin containing several crystals of thymol and evaluated under a dissecting microscope for malformations and ossification variations.

Late resorptions, if present, were weighed, examined macroscopically for external malformations, and discarded unless external malformations were observed. Early resorptions were noted and discarded.

4.3. Statistical evaluations

Statistical analyses of interval data (i.e. body weight data, food consumption, litter information) began by first evaluating equal variance of means using Bartlett's test at 1% significance, two-sided risk level [22]. All other tests were conducted at 5% and 1% significance, two-sided risk level. If equal, parametric procedures (one-way ANOVA) using the F distribution were employed to assess significance. When significant differences were indicated, Dunnett's test was used to determine which means were significantly different from control. If Bartlett's test indicated heterogeneity of variances, a nonparametric method, the Kruskal-Wallis test [23], was used to detect differences between groups, followed by Dunn's summed rank test to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed, using standard regression techniques with a test for trend and lack of fit where variances were equal [24], or Jonckheere's test for monotonic trend in nonparametric cases [23].

Statistical analyses of incidence data (i.e. females with resorptions, pregnancy rates, litters with resorptions, incidence of fetuses with malformations/variations, and incidence of litters containing fetuses with malformations/variations) were performed using contingency tables. All ratios such as pre- and postimplantation loss indices were transformed via Bartlett's transformation followed by arc sine transformation [24] prior to analysis. Standard χ^2 analysis determined if the proportion of indices differed between the groups tested. A 2×2 Fisher Exact Test was used to compare each treatment group to control; the significance level was corrected by Bonferroni inequality [25] to assure an overall test of the stated significance level. Armitage's

Table 2
Composition of unleaded gasoline (API 94-02) and unleaded gasoline vapor condensate

SAMPLE	API 94-02 (liquid)	API 94-02 (vapor)
Composition (Volume %)		
N-Paraffins	12.3	28.5
Total Paraffins	48.4	79.5
Naphthenes	5.6	2.3
Total Olefins	9.7	14.1
Total Aromatics	34.4	4.0
Benzene	1.4	1.0
Toluene	8.0	2.0
Carbon Number (Volume %)		
4	5.1	18.9
5	16.8	46.4
6	18.6	23.6
7	19.4	7.8
8	20.7	3.1
9	11.0	0.2
10	5.2	0.0
11	2.1	0.0
12+	1.0	0.0
Total (Volume %)	99.9	100

test for linear trend [26] in the treatment groups was also performed. All tests were reported at the 5% and 1% levels of significance.

5. Results

5.1. Analytical characterization

Table 2 presents the composition of the parent Unleaded Gasoline (API 94–02) liquid and the Unleaded Gasoline Vapor Condensate, both by carbon chain length and hydrocarbon class, characterized by gas chromatography. The starting gasoline met the specifications set in the Clean Air Act 211(b) final rule. Components in the C4–C5 range make up 65% by volume of the vapor condensate while these components comprise only 22% by volume of the parent liquid. The vapor condensate contains substantially less total aromatics and toluene than the parent liquid.

5.2. Chamber monitoring

Achieved mean exposure concentrations of 0, 1015, 2984, and 8993 ppm were close to the respective target [0, 1000, 3000, 9000 ppm] and nominal concentrations [0, 1018, 3227, 7187 ppm]. Particle mass distribution measurements were similar for all exposure groups including the control group, demonstrating there was no measurable test material present as aerosol.

Results of GC syringe sample fingerprints of the exposure atmosphere confirmed the stability and uniformity of the test material under the exposure conditions employed.

Table 3
Summary of reproduction and mean fetal weight data

	Exposure Levels (ppm)			
	0	1000	3000	9000
Number of Females Mated	24	24	24	24
No. Pregnant (%)	24 (100.0)	22 (91.7)	22 (91.7)	21 (87.5)
No. Pregnancies Aborted	0	0	0	0
No. Premature Births	0	0	0	0
No. Litters with Viable Fetuses	24	22	22	21
Female Mortality No.	0	0	0	0
Corpora Lutea				
Mean \pm SD	16.7 \pm 3.9	18.1 \pm 2.1	17.3 \pm 2.9	17.3 \pm 2.0
Implantation Sites				
Mean \pm SD	14.8 \pm 4.5	16.1 \pm 2.7	16.1 \pm 3.1	16.1 \pm 2.4
Preimplantation Loss Index				
Mean \pm SD	0.121 \pm 0.193	0.104 \pm 0.131	0.071 \pm 0.118	0.063 \pm 0.107
Number of Viable Fetuses	327	334	336	325
Number of Dead Fetuses	0	0	0	0
Mean Litter Size \pm SD	13.6 \pm 5.0	15.2 \pm 2.6	15.3 \pm 3.4	15.5 \pm 2.3
Mean No. Males \pm SD	7.0 \pm 3.2	7.5 \pm 2.6	8.0 \pm 2.7	7.6 \pm 2.7
Mean No. Female \pm SD	6.6 \pm 3.0	7.7 \pm 2.3	7.3 \pm 3.3	7.9 \pm 2.0
Resorptions				
Mean \pm SD	1.2 \pm 2.7	1.0 \pm 0.8	0.8 \pm 1.0	0.7 \pm 0.7
Resorptions/Implants Ratio				
Mean \pm SD	0.075 \pm 0.017	0.058 \pm 0.054	0.055 \pm 0.063	0.041 \pm 0.044
No. of Litters with Resorptions (%)	10 (41.7)	14 (63.6)	11 (50.0)	11 (52.4)
Mean Body Weight (g) of Viable Fetuses \pm SD	3.66 \pm 0.33	3.71 \pm 0.30	3.68 \pm 0.17	3.66 \pm 0.32
Male Fetuses	3.75 \pm 0.36	3.80 \pm 0.28	3.78 \pm 0.20	3.82 \pm 0.34
Female Fetuses	3.61 \pm 0.24	3.64 \pm 0.36	3.54 \pm 0.20	3.54 \pm 0.32
Sex Ratio of Viable Fetuses				
Total Males/Total Females	1.1	1.0	1.1	1.0

Note: Preimplantation Loss = $\frac{\text{Corpora lutea} - \text{implants}}{\text{Corpora lutea}}$

No statistically significant differences

5.3. Maternal evaluations

All mated females survived to scheduled sacrifice. Physical examinations performed pre- and post-exposure did not indicate any adverse effect from exposure to unleaded gasoline vapor condensate. Mean maternal body weight and weight gain during gestation were not adversely affected by treatment. Mean weight gains over days 6 through 20 of gestation using the corrected day 20 gestation weights were comparable to controls: 31.2 g, 30.5 g, 31.6 g, and 28.1 g for controls, 1000, 3000, and 9000 ppm groups, respectively. Mean food consumption during the pretreatment (days 0–6) and treatment period (days 6–20) for unleaded gasoline vapor condensate treated groups was comparable to control data. No adverse effects of treatment to maternal rats were indicated from any of these parameters.

5.4. Reproductive/fertility evaluation

Results are summarized in Table 3. Pregnancy rates in treated groups were statistically indistinguishable from the sham treated control group. No adverse effects of treatment were evident from uterine implantation data. There were no

aborted pregnancies or premature deliveries in any group. Mean number of corpora lutea, uterine implantation sites, live fetuses, resorptions per female, and mean pre- and post-implantation loss indices for all treated groups were comparable to control data. There were no dead fetuses in any litter from the control or treated groups. No macroscopic abnormalities related to test material exposure were observed in postmortem examination of maternal animals.

5.5. Fetal evaluations

Mean fetal weights, by sex or combined, were comparable to controls for all treatment groups. Mean number of male and female fetuses per pregnant female and the ratio of total male to female fetuses were also comparable to controls for all treatment groups (Table 3).

No external malformations or variations were recorded among fetuses from control or treated groups.

Results from the fetal examinations for soft tissue and skeletal malformations and variations are summarized by litter incidence in Table 4. The only soft tissue malformation was microphthalmia of the left eye observed in one fetus among 172 (0.6%) in the 1000 ppm treatment group.

Table 4
Summary of fetal examinations by litter

	Exposure Levels (ppm)			
	0	1000	3000	9000
No. of Litters Evaluated	24	22	22	21
Soft Tissue Observations				
No. Fetuses Evaluated	168 ^a	172	174	169
Visceral Malformations				
Microphthalmia	0	1 (4.5%)	0	0
Soft Tissue Variations				
Ureter(s)-Tortuous		1 (4.5%)	1 (4.5%)	0
Skeletal Observations				
No. Fetuses Evaluated	160 ^a	162	162	156
Skeletal Malformations	2 (8.3%)	0	1 (4.5%)	1 (4.8%)
Total Skeletal Variations	23 (95.8%)	22 (100%)	21 (95.5%)	21 (100%)
Rudimentary Ribs	9 (37.5%)	15 (68.2%)	13 (59.1%)	16 (76.2%)

^a One fetus from one control group litter was evaluated for both soft tissue and skeletal anomalies

Given the low incidence of this finding and the absence of similar malformations in the 3000 and 9000 ppm groups, this occurrence of microphthalmia is not considered a treatment related response.

Soft tissue variations are defined as subtle changes in size, shape, or appearance of visceral organs/tissues. The only soft tissue variation seen in this study was tortuous ureters in one fetus each from the 1000 and 3000 ppm groups with an incidence of 0.6% (1/172 fetuses, 1/174 fetuses, respectively). The incidence of this variation in the low and mid treatment groups was within the range of historical control data for this laboratory and is not considered an adverse effect of treatment.

Skeletal malformations included the presence of a fourteenth rib or rib pair and 27 presacral vertebrae in one fetus each from the control (0.6%; 1/160 fetuses) and 3000 ppm (0.6%; 1/162 fetuses) groups and two fetuses from one litter in the 9000 ppm group (1.3%; 2/156 fetuses). These incidences, both on a per fetus and per litter basis for the treatment groups were considered similar to control data and not an adverse effect of treatment. The only other skeletal malformation was the presence of 5 lumbar vertebrae in one control fetus.

Ossification variations represent delays or irregularities in the ossification process, which may or may not be present in the adult animal. The overall incidence of ossification variations on a per fetus and per litter basis in groups exposed to unleaded gasoline vapor condensate was comparable to controls.

The only ossification variation noted with increased incidence in treated groups was rudimentary rib(s), a finding seen frequently in rat fetuses. The incidence of this variation was compared with historical data from this laboratory (litter average 21.2%; maximum 63.6%) and published control data on the CrI:CD rat from MARTA [Mid-Atlantic Reproduction and Teratology Association] and MTA [Midwest Teratology Association - litter average 4%; maximum

55%] [27]. Although the frequency of rudimentary first lumbar rib(s) in the exposed groups is slightly elevated from the range of historical control values, the frequency in these groups did not increase significantly with substantial increases in dose. When reviewed in conjunction with all ossification data for this study, the slight increase in rudimentary ribs among the treated groups is not biologically or toxicologically significant.

6. Discussion

Exposure of pregnant rats to unleaded gasoline vapor condensate at concentrations up to 75% LEL from day 6 through 19 of gestation did not induce maternal toxicity, adverse reproductive effects, or developmental toxicity in fetuses at any exposure level. Exposure had no measurable effect upon development of the viscera. No significant soft tissue or skeletal malformations were observed. Although there was a slight increase in the incidence of rudimentary ribs in the vapor condensate exposed groups, there was no statistically significant difference between control and treated groups in overall ossification variations, either on a per fetus or per litter basis. The maternal and fetal no-observed-adverse-effects level (NOAEL) for unleaded gasoline [94–02] vapor condensate is 9000 ppm.

These findings are consistent with the absence of developmental and reproductive toxic effects in the recent two generation reproduction study in rats employing vapor recovery unit gasoline vapor [17] and also with the series of inhalation developmental/reproductive screens in rats exposed to distillates of gasoline blending streams, which also showed no toxicity in parental animals or offspring [14–16]. All distillates contained the same classes of components as the unleaded gasoline vapor condensate in different concentrations but consistently greater than 50% hydrocarbons in the C4–C6 range and low aromatic content.

The results of all these studies on the lighter fractions of gasoline and refinery blending streams demonstrate that exposure to hydrocarbon vapors in the C4-C6 range does not induce reproductive or developmental toxicity. Additionally, actual consumer and occupational exposure levels are far below the exposure levels tested in this study. It can reasonably be concluded that the unleaded gasoline vapor to which humans are exposed when filling the gas tank of their automobile or lawn mower is not a reproductive hazard.

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