Regulatory Toxicology and Pharmacology 70 (2014) S69–S79

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Health assessment of gasoline and fuel oxygenate vapors: Developmental toxicity in rats

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ARTICLE INFO

Article history:
Available online 17 May 2014

Keywords:
Developmental toxicity
Gasoline vapor condensates
Methyl tertiary butyl ether (MTBE)
Ethyl t-butyl ether (ETBE)
t-Amyl methyl ether (TAME)
Diisopropyl ether (DIPE)
Ethanol (EtOH)
t-Butyl alcohol (TBA)
Fuel oxygenates
Evaporative emissions

ABSTRACT

Gasoline-vapor condensate (BGVC) or condensed vapors from gasoline blended with methyl t-butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME) diisopropyl ether (G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA) were evaluated for developmental toxicity in Sprague–Dawley rats exposed via inhalation on gestation days (GD) 5–20 for 6 h/day at levels of 0 (control filtered air), 2000, 10,000, and 20,000 mg/m^3. These exposure durations and levels substantially exceed typical consumer exposure during refueling (<1–7 mg/m^3, 5 min). Dose responsive maternal effects were reduced maternal body weight and/or weight change, and/or reduced food consumption. No significant malformations were seen in any study. Developmental effects occurred at 20,000 mg/m^3 of G/TAME (reduced fetal body weight, increased incidence of stunted fetuses), G/TBA (reduced fetal body weight, increased skeletal variants) and G/DIPE (reduced fetal weight) resulting in developmental NOAEL of 10,000 mg/m^3 for these materials. Developmental NOAELs for other materials were 20,000 mg/m^3 as no developmental toxicity was induced in those studies. Developmental NOAELs were equal to or greater than the concurrent maternal NOAELs which ranged from 2000 to 20,000 mg/m^3. There were no clear cut differences in developmental toxicity between vapors of gasoline and gasoline blended with the ether or alcohol oxygenates.

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1. Introduction

The 1990 amendments to the Clean Air Act (CAA) mandated the use of oxygenates in motor gasoline. In 1994, the U.S. Environmental Protection Agency (EPA) issued a final rule under the Act which added new health effects information and testing requirements to the Agency's existing registration requirements. As described in more detail in a companion paper (Henley et al., 2014), requirements include inhalation exposures to evaporative emissions of the gasoline or additive in question. The health endpoints include assessments for standard subchronic toxicity, neurotoxicity, genotoxicity, immunotoxicity, developmental and reproductive toxicity, and chronic toxicity/carcinogenicity. The results of chronic toxicity testing of gasoline and gasoline combined with MTBE have already been reported (Benson et al., 2011) and reported elsewhere in this issue are the findings for are the findings for subchronic toxicity testing (Clark et al., 2014), genotoxicity (Schreiner et al., 2014), neurotoxicity (O'Callaghan et al., 2014), immunotoxicity (White et al., 2014), reproductive toxicity (Gray et al., 2014), and developmental toxicity testing in mice (Roberts et al., 2014). This paper describes the results of developmental toxicity testing in rats.

2. Materials and methods

Six separate studies were conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI) Mammalian Toxicology Laboratory, Annandale, New Jersey, of a gasoline vapor condensate (BGVC) and vapor

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http://dx.doi.org/10.1016/j.yrtph.2014.05.009
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condensates of gasoline mixed with methyl-t-butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). The gasoline/diisopropyl ether (G/DIPE) study was conducted at Huntingdon Life Sciences Princeton Research Center, East Millstone, NJ. Both of the laboratories are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

2.1. Test material preparation and characterization

Gasoline and gasoline/oxygenate vapor condensates were prepared and supplied in 100 gallon gas cylinders by Chevron Research and Technology Center (Richmond, CA). The test material was dispensed as needed at the testing facility from the 100 gallon cylinders into 5-gallon cylinders using nitrogen pressurization. The methodology for preparation and analytical characterization of the samples is described in a companion paper (Henley et al., 2014).

2.2. Animal selection and care

The test animals were Cesarean-originated Virus Antibody Free (VAF) Crl:CD®(SD)IGSBR outbred albino rats supplied by Charles River Laboratories, Inc, Raleigh, NC. Sexually mature virgin females were allocated to the study groups after confirmation of mating. Sexually mature males were used for mating purposes only in the EMBSI studies and not involved in the actual exposures to test materials. HLS employed timed mated females shipped from Charles River Laboratories to arrive no later than GD 4 for the G/DIPE study.

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, MO) was available without restriction. Analysis of each feed lot used during this study was performed by the manufacturer. Water was available without restriction via an automated watering system. There were no known contaminants in the feed or water expected to interfere with the results of this study. Animals were without food and water while in the exposure chambers.

2.3. Housing and environmental conditions

Animals were housed individually in suspended stainless steel wire mesh cages. During exposure periods, animals were individually housed in stainless steel, wire mesh cages within a 1000 l stainless steel and glass whole-body exposure chamber. A twelve hour light/dark cycle controlled via an automatic timer was provided. For all studies temperature and relative humidity were maintained within the specified range (18–24 °C, and 30–70% relative humidity, respectively). Light (maintained approximately 30–40 foot-candles at 1.0 m above the floor) and noise levels (maintained below 85 dB) in the exposure room were measured pretest and at the beginning, middle and end of the study. Oxygen levels in the exposure chambers were maintained between 19.0 and 20.7%.

2.4. Experimental design

The experimental design is described in Table 1. Untreated animals were mated (1 nulliparous female with 1 male) until sufficient presumed pregnant females were identified by the presence of a copulatory plug in the vagina. Plug positive female rats were distributed by body weight into four different exposure groups (25/group) on gestation day [GD] 0; for the G/DIPE study, timed-pregnant animals were distributed by body weight on GD 4. Presumed pregnant females were exposed to 0 mg/m^3 (air control), 2000 mg/m^3, 10,000 mg/m^3 and 20,000 mg/m^3, 6 h/day from GD 5 to GD 20. The highest exposure level represented approximately 50% of the lower explosive limit (LEL) for each material.

2.5. Administration of test substance and exposure schedule

The experimental and control animals were placed into whole-body inhalation chambers operated under dynamic conditions for at least 6 h per day after target exposure levels were reached from GD 5 through GD 20. The animals remained in the chambers for at least an additional 23 min (theoretical equilibration time) while the test atmosphere cleared.

Females were exposed in 1.0 M^3 stainless steel and glass chambers operated at a flow rate approximately 12–15 air changes/hour. Flow rate and slightly negative pressure were monitored continuously and recorded approximately every 30 min.

The control group was exposed to clean filtered air under conditions identical to those used for groups exposed to the test substance. The test substance was administered fully vaporized in the breathing air of the animals. The chamber concentrations were measured in the breathing zone of the rats by on-line gas chromatography (GC). These chromatographic analyses were used to assess the stability of the test substance over the duration of the study. Analytical concentrations of G/DIPE in the HLS study were determined by infrared spectrometry. Additionally, sorbent tube samples were collected once weekly and stored in a freezer for analysis by a detailed capillary GC method to compare component proportions of the test material atmosphere with the liquid test material.

Distribution samples were drawn from twelve different points within the exposure chambers at each exposure level during the validation of the exposure system to determine homogeneity of exposure concentrations. A particle size determination of the aerosol portion of the test atmosphere was conducted at least once during the chamber trials from the 0 mg/m^3 and 20,000 mg/m^3 concentrations.

2.6. Experimental evaluation

Animals were examined for viability at least twice daily during the study. Body weights were taken prior to selection, and on GD 0 (EMBSI studies), 5, 8, 11, 14, 17, 20 and 21. Food consumption was measured for mated females on GD 5, 8, 11, 14, 17, 20 and 21. A clinical examination was given to each female prior to selection, and daily during gestation. Additionally, group observations of the animals for mortality and obvious toxic signs while in the chambers were recorded at 15, 30, 45 and 60 min after initiation of the exposure and regularly during each exposure.

Dams were sacrificed by CO_2 asphyxiation followed by exsanguination on GD 21. A gross necropsy was performed on all confirmed-mated females. Uterine weights with ovaries attached were recorded at the time of necropsy, uterine contents were examined, corpora lutea and the numbers and locations of implantation sites, early and late resorptions, and live and dead (alive or dead *in utero*) fetuses were counted. The uteri of all apparently non-pregnant females were stained with 10% ammonium sulfide to confirm non-gravid status. Evaluations of dams during necropsy and subsequent fetal evaluations were conducted without knowledge of treatment group in order to minimize bias.

Fetuses were counted, weighed and examined externally for gross malformations and variations. Fetal sex was determined by external examination and confirmed internally only on those fetuses receiving visceral examinations. Fetuses were euthanized by CO_2 asphyxiation in the EMBSI studies and by intraperitoneal sodium pentobarbitol in the HLS study.

The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection (Staples, 1974; Stuckhardt

Table 1
Experimental design.

Laboratory	ExxonMobil Biomedical Sciences, NJ	Huntingdon Life Sciences, NJ
Species/strain/source	VAF Crl: CD®(SD)IGSBR; Charles River; NC	VAF Crl: CD®(SD)IGSBR; Charles River; NC
Test substance(s)	BGVC, G/MTBE, G/ETBE, G/TAME, G/EtOH, G/TBA	G/DIPE
Wt./age at mating	224–325 g; 12–13, 13–14, 14–15 weeks	216–280 g; 10–12 weeks
Group sizes	24–25 mated females	24 mated females
Exposure levels [mg/m^3]	0, 2000, 10,000, 20,000	0, 2000, 10,000, 20,000
Exposure days [6 h/d]	GD 5–20	GD 5–20
Necropsy	GD 21	GD 21

Age at mating in EMBSI studies varied by 1 week with youngest animals at 12–13 weeks and oldest at 14–15 weeks depending on the study.

and Poppe, 1984) prior to decapitation of the fetus. The heads were preserved in Bouin's solution for at least two weeks, then rinsed and subsequently stored in 70% ethanol. Free-hand razor blade sections of the Bouin's-fixed fetal heads were examined for the presence of abnormalities. The remaining live fetuses (alive *in utero*) were euthanized and eviscerated, processed by double staining with Alizarin red and Alcian blue, and examined for the presence of bone and cartilage malformations and ossification variations. The fetal skeletons were preserved in glycerine with thymol after they were processed and stained.

2.7. Statistical analysis

All statistical analyses were performed with comparison to concurrent control data. Statistical methods in the EMBSI studies included evaluation of equality of means done by an appropriate one way analysis of variance and a test for ordered response in the dose groups. Bartlett's test was performed to determine if the dose groups had equal variance followed by standard one way analysis of variance (Snedecor and Cochran, 1989). If the variances were equal, subsequent testing was done using parametric methods, otherwise nonparametric techniques were used. Continuous data were tested for statistical significance as follows: Where applicable, percentages were calculated and transformed by Cochran's transformation, followed by the arc sine transformation (Snedecor and Cochran, 1989). Both the raw percentages and the transformed percentages were tested for statistical significance.

For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used (Snedecor and Cochran, 1989). If significant differences among the means were indicated, Dunnett's test was used to determine which treatment groups differed significantly from control (Dunnett, 1964). A standard regression analysis for linear response in the dose groups was performed, which also tested for linear lack of fit in the model.

For the nonparametric procedures, the test of equality of means was performed using the Kruskal–Wallis test (Hollander and Wolfe, 1973). If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment groups differed significantly from the control (Hollander and Wolfe, 1973). In addition to the Kruskal–Wallis test, Jonckheere's Test for monotonic trend in the dose response was performed (Hollander and Wolfe, 1973). Bartlett's test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance. Body weight and food consumption data were not analyzed for non-pregnant females.

Means and standard deviations were calculated for animal, exposure and chamber environmental data. The coefficient of variation also was calculated when considered relevant for the exposure data. Fetal body weight was analyzed by a mixed model analysis of variance that used the number of litters as the basis for analysis and effectively used the litter size as a covariate. The model considered dose group, litter size, and fetal sex as explanatory variables. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups means were tested pairwise vs. the control group using least squares means. The least squares means allowed comparisons that accounted for differences in litter size and sex. The mathematical model was based on a paper by Chen et al. (1996). The analysis was run using SAS with code suggested in Little et al. (1997).

The analysis of anomalies (malformations or variations) was based on a Generalized Estimating Equation (GEE) application of the linearized model (Ryan, 1992). The model used the litter as the basis for analysis and considered correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups were tested pairwise vs. the control group using least squares means. In addition to the developmental category-specific anomalies tested, a series of combined analyses were performed within each category as applicable: These categories were Combined Malformations and Variations for All Fetuses; for Alive Fetuses; for Dead Fetuses; Malformations for All Fetuses; for Alive Fetuses; for Dead Fetuses; Variations for All Fetuses; for Alive Fetuses; for Dead Fetuses

Statistical analyses in the Huntingdon study were comparable to the methods employed at EMBSI and were performed at Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, England.

2.8. Compliance

These studies were conducted in accordance with the United States Environmental Protection Agency's (EPA) Good Laboratory Practice Standards (US EPA, 1994), and complied with all appropriate parts of the Animal Welfare Act Regulations (USDA, 1989, 1991). The studies also met the requirements of US EPA OPPTS 870.3700 guidelines for prenatal developmental toxicity studies (US EPA, 1998).

3. Results

3.1. Chamber monitoring

The analytically measured exposure levels of the airborne test materials were at least 99% of the targeted exposure levels (Table 2). Chamber environmental conditions averaged 23–24 °C and 43% relative humidity. Particle sizing results indicated that the atmospheres were essentially vapor only (data not presented).

Table 3 provides a profile of the major components in the starting test materials. Analysis of the major components in the test materials and the chamber atmospheres showed an acceptably close comparison between the starting condensates and the chamber vaporized test material sampled weekly throughout the studies (data not presented). The data was consistent from week-to-week during the study indicating stability of the test material and the atmosphere generation techniques.

Table 2
Measured exposure concentrations compared to target concentrations.

Exposure levels	Exposure chamber concentrations – mg/m^3 ± standard deviation						
	BGVC	G/MTBE	G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
2000 mg/m^3	1979 ± 98	2101 ± 97	2073 ± 66	1988 ± 88	1982 ± 93	2017 ± 75	2020 ± 106
10,000 mg/m^3	10,676 ± 310	10,725 ± 541	10,149 ± 441	10,327 ± 280	10,072 ± 546	10,198 ± 285	10,395 ± 229
20,000 mg/m^3	20,638 ± 452	20,409 ± 1038	20,303 ± 642	20,541 ± 518	19,776 ± 961	20,755 ± 398	20,777 ± 394
Molecular Wt.[a]	73.8	73.7	65.9	77.1	76.5	65.9	72.1

[a] Average molecular weight of hydrocarbons in condensate samples.

Table 3
Representative hydrocarbon distribution in vapor condensate test materials.

	Hydrocarbons detected in test material (area percent)[a]						
	BGVC	B/MTBE	G/EtOH	G/TAME	G/ETBE	G/DIPE	G/TBA
Isobutane	3.6	2.2	2.2	1.9	2.0	2.0	3.0
n-Butane	15.2	11.1	11.6	10.4	10.6	11.5	9.9
Isopentane	35.1	31.0	34.0	33.6	32.5	32.2	25.2
n-Pentane	13.2	9.1	10.2	10.3	9.8	9.6	11.6
trans-2-Pentene	2.5	2.0	2.1	2.3	2.1	2.1	2.1
2-Methyl-2-butene	3.8	2.9	3.1	3.4	3.2	3.1	3.2
2,3-Dimethylbutane	1.6	0.9	2.2	1.5	1.4	1.3	1.6
2-Methylpentane	6.3	4.5	5.1	5.6	5.1	4.5	6.1
3-Methylpentane	3.6	2.6	2.9	3.2	2.9	2.7	3.8
n-Hexane	3.0	2.1	2.4	2.6	2.4	1.8	3.4
Methylcyclopentane	1.5	1.1	1.2	1.4	1.3	1.0	1.6
2,4-Dimethylpentane	1.0	0.9	1.0	1.2	1.0	1.0	1.0
Benzene	2.1	1.5	1.6	2.0	1.8	1.8	2.0
2-Methylhexane	1.1	1.0	1.1	1.2	1.1	1.1	1.3
2,3-Dimethylpentane	1.1	1.0	1.1	1.3	1.1	1.1	1.3
3-Methylhexane	1.3	1.1	1.2	1.5	1.3	1.3	1.5
Isooctane	1.3	1.2	1.3	1.5	1.4	1.4	1.5
Toluene	3.0	2.5	2.4	3.2	2.7	2.6	3.4
MTBE		21.3					
EtOH			13.3				
TAME				11.9			
ETBE					16.3		
DIPE						17.8	
TBA							16.5

[a] Values for these 18 reference hydrocarbons were derived pre-study (Henley et al., 2014). A total of 131 peaks were separated and identified for the BGVC study. The reference hydrocarbons comprised over 81% of the total mass but are normalized to 100% to ease comparison between laboratories.

Tables 4–8 summarize the comparative results of all the studies. To facilitate comparisons of extensive data across test materials the data in Tables 4–6 are presented for the 20,000 mg/m^3 groups only. Tables 7 and 8, which address specific endpoints (stunted fetuses and total skeletal variations), present results of all dose levels. Text describes specific effects within studies for individual dose groups.

3.2. Maternal clinical in life observations, survival and pregnancies

All females survived to study termination. No significant clinical signs were observed during any of the studies. Low incidences of alopecia were seen in the abdominal areas and limbs among treated and control animals in each study. Red nasal discharge and chromodacryorrhea were observed at higher doses in various animals in all studies and were considered a common reaction to inhalation exposure and mild stress in rats.

The number of pregnant animals was similar in all studies. Non-pregnant females were identified at terminal sacrifice by the absence of implantation sites in the uterus. With the exception of the G/ETBE 2000 mg/m^3 group which contained 3 non-pregnant rats, only one or two females were non-pregnant in any group of the other test materials. The incidence of three non-pregnant animals in the lowest dose group of G/ETBE was not considered test material significant as the 10,000 and 20,000 mg/m^3 groups contained only one non-pregnant animal each. Although all females in the G/TAME 20,000 mg/m^3 group were pregnant, one female delivered early on GD 20 and data from this litter were not included in the calculations.

3.3. Gestation body weight and food consumption

No significant body weight changes (Table 4) or food consumption effects (Table 5) were observed in females exposed to BGVC. For G/oxygenate blends maternal toxicity was reflected in decreased body weight (G/TBA), decreased body weight gain (usually during the first days of exposure), and/or decreased food consumption at 20,000 mg/m^3 in all studies and also at 10,000 mg/m^3 for G/DIPE and G/TBA.

Animals in the 20,000 mg/m^3 G/MTBE and G/ETBE studies showed statistically significant decreases in body weight gain and food consumption for the GD 8–11 interval. Although G/ETBE also had decreased food consumption in the GD 11–14 interval which contributed to a decrease in food consumption in the GD 5–20 interval, neither corrected body weight nor total body weight gain were significantly different from control.

For animals exposed to G/EtOH slight maternal toxicity was indicated by statistical significant decreases in body weight changes at the GD 20–21 and GD 5–21 intervals at 20,000 mg/m^3 and decreasing linear trends in food consumption over all doses at GD 5–20 interval. Only part of the difference in weight gain late in gestation could be attributed to differences in uterine weight.

Table 4
Body weights and weight changes in pregnant rats exposed to vapor condensates of gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Gestation days (GD)	BGVC[a] N[h] = 24 EMBSI	G/MTBE[a] N[h] = 24 EMBSI	G/TAME[a] N[h] = 25 EMBSI	G/ETBE[a] N[h] = 24 EMBSI	G/DIPE[a] N[h] = 22 Huntingdon	G/EtOH[a] N[h] = 24 EMBSI	G/TBA[a] N[h] = 23 EMBSI
Group mean maternal body weights (grams ± std. deviation)[b]							
GD 0	272.8 ± 16.5	266.5 ± 14.8	270.3 ± 14.8	269.9 ± 13.7	ND[f]	265.2 ± 13.4	260.4 ± 14.3
	(273.3 ± 17)	(265.9 ± 13)	(269.7 ± 17.1)	(270 ± 14.6)		(265.2 ± 13.3)	(260.6 ± 16.7)
GD 5	302.7 ± 15.8	291.8 ± 24.3	300.7 ± 17.2	295.5 ± 19.1	245 ± 15.2	296.4 ± 18.2	288.9 ± 14.7
	(303.3 ± 18.3)	(297.8 ± 12.7)	(299.5 ± 19.4)	(299.2 ± 16.7)	(244 ± 14.6)	(297.6 ± 16.0)	(293.5 ± 18.8)
GD 8	308.2 ± 15.3	303.5 ± 17.2	302.7 ± 14.1	298.9 ± 18.5	256 ± 16.3	303.1 ± 20.1	288.9 ± 14.7[d]
	(311.6 ± 19.7)	(305.4 ± 12.2)	(306.5 ± 18.9)	(305.4 ± 18.1)	(259 ± 12.8)	(305.8 ± 18.0)	(302.9 ± 20.3)
GD 11	319.8 ± 15.8	312.9 ± 16.4	310.8 ± 16.8	307.3 ± 19.1	275 ± 19.5	314.6 ± 21.6	298.2 ± 15.2[c]
	(323.7 ± 20.9)	(320.1 ± 12.6)	(316.0 ± 22.6)	(317.2 ± 19.3)	(281 ± 14.9)	(320 ± 19.1)	(316.7 ± 21.5)
GD 14	332.0 ± 16.3	325.7 ± 18.0	318.8 ± 18.8	317.6 ± 19.3	290 ± 19.5	327.9 ± 25.1	309.9 ± 15.9[c]
	(336.7 ± 22.8)	(334.3 ± 14.8)	(328.9 ± 25.3)	(330.6 ± 21.4)	(301 ± 17.0)	(334.2 ± 19.3)	(330 ± 23.7)
GD 17	365.8 ± 17.3	358.1 ± 22.4	348.5 ± 20.2	347.5 ± 20.8	321 ± 24.9	358.6 ± 27.7	340.0 ± 18.0[d]
	(367.0 ± 24.4)	(363.3 ± 16.9)	(356.6 ± 28.0)	(358.8 ± 27.1)	(332 ± 22.2)	(366.5 ± 20.5)	(360.1 ± 25.8)
GD 20	416.4 ± 20.5	409.1 ± 28.8	395.3 ± 22.3	394.8 ± 22.9	365 ± 27.1	410.1 ± 32.9	385.5 ± 20.7[d]
	(416.8 ± 29.5)	(410.4 ± 20.3)	(405.0 ± 33.6)	(404.2 ± 33.1)	(378 ± 26.3)	(418.8 ± 26.4)	(409.4 ± 30.9)
GD 21	439.5 ± 20.5	427.3 ± 29.0	414.3 ± 25.3	413.5 ± 26.9	374 ± 27.4	423.7 ± 29.7	404.3 ± 22.4[d]
	(439.1 ± 33.3)	(430. 3 ± 22.1)	(422.6 ± 34.7)	(420.9 ± 35.7)	(388 ± 28.6)	(441 ± 27.7)	(428.4 ± 33.2)
Uterus wt.	118.8 ± 11.4	114.4 ± 14.8	108.3 ± 12.3	104.8 ± 14.9	96 ± 15.7	112.9 ± 22.3	104.1 ± 10.7[c]
	(115.5 ± 19.9)	(111.6 ± 12.7)	(110.9 ± 21.4)	(104.8 ± 21.9)	(101 ± 19.4)	(119.5 ± 21.4)	(114.3 ± 13.0)
GD 21C[e]	320.7 ± 16.0	312.9 ± 18.3	306.0 ± 21.2	308.8 ± 20.7	273 ± 15.9	310.8 ± 23.8	300.2 ± 19.1
	(323.6 ± 21.4)	(318.7 ± 16.1)	(311.7 ± 25.7)	(315.5 ± 20.8)	(286 ± 18.1)	(321.5 ± 18.8)	(314.2 ± 23.0)
Group mean maternal body weight changes (grams ± std. deviation)[b]							
GD 0–5	29.9 ± 7.7	25.3 ± 18.9	30.5 ± 8.5	25.6 ± 9.9	ND[f]	31.2 ± 7.7	28.5 ± 6.1
	(30.0 ± 7.2)	(31.9 ± 8.5)	(29.8 ± 7.1)	(29.2 ± 6.5)		(32.4 ± 7.1)	(32.5 ± 8.5)
GD 5–8	5.5 ± 6.1	11.7 ± 14.7	2.0 ± 7.9[d]	3.4 ± 7.7	11 ± 4.7[c]	6.8 ± 5.6	0 ± 5.9[c]
	(8.3 ± 5.5)	(7.6 ± 5.7)	(7.0 ± 5.1)	(6.2 ± 5.2)	(15 ± 4.5)	(8.2 ± 5)	(9.8 ± 6.1)
GD 8–11	11.7 ± 5.2	9.4 ± 6.7[d]	8.1 ± 4.2	8.4 ± 3.4[d]	20 ± 5.5	11.5 ± 5.1	9.3 ± 3.6[c]
	(12.1 ± 4.2)	(14.8 ± 4.6)	(9.6 ± 6.2)	(11.8 ± 4.6)	(22 ± 4.7)	(14.3 ± 4.7)	(13.8 ± 4.4)
GD 11–14	12.2 ± 5.1	12.8 ± 4.4	8.0 ± 6.6[c]	10.4 ± 3.8	15 ± 4.0[c]	13.3 ± 4.7	11.8 ± 5.4
	(13.0 ± 4.7)	(14.2 ± 5.4)	(12.9 ± 5.1)	(13.4 ± 4.7)	(20 ± 4.2)	(14.2 ± 4.3)	(13.2 ± 4.1)
GD 14–17	33.8 ± 6.4	32.4 ± 6.3	29.7 ± 5.5	29.9 ± 6.4	31 ± 6.8	30.7 ± 6.1	30.1 ± 5.7
	(30.3 ± 5.8)	(29.0 ± 5.5)	(27.7 ± 7.4)	(28.2 ± 9)	(31 ± 7.3)	(32.2 ± 7.8)	(30.2 ± 5.3)
GD 17–20	50.5 ± 8.2	51.0 ± 8.0	46.7 ± 11.0	47.3 ± 6.2	44 ± 7.7	51.5 ± 8.9	45.5 ± 6.4
	(49.7 ± 9.0)	(47.1 ± 8.7)	(48.4 ± 8.6)	(45.4 ± 10)	(46.6 ± 7.6)	(52.4 ± 9.6)	(49.3 ± 6.9)
GD 20–21	23.1 ± 6.3	18.1 ± 4.6	19.0 ± 7.0	18.8 ± 5.8	8.0 ± 5.1	17.7 ± 6.6[d]	18.8 ± 4.6
	(22.4 ± 8.3)	(19.9 ± 8.6)	(17.6 ± 6.3)	(17.4 ± 9.5)	(10.0 ± 5.2)	(22.2 ± 5.6)	(19.0 ± 4.4)
GD 5–21	136.8 ± 11.4	135.5 ± 21.0	113.1 ± 20.1	118 ± 17.1	129.0 ± 16.0	129.2 ± 21.3[d]	115.4 ± 15.7[c]
	(135.8 ± 21.0)	(132.5 ± 17.5)	(123.1 ± 21.8)	(104.5 ± 91.0)	(144 ± 20.6)	(143.4 ± 21.1)	(135.3 ± 20.3)
GD 0–21	166.6 ± 12.4	160.8 ± 21.4	144.0 ± 20.0	143.6 ± 18.8	ND[f]	159.4 ± 20.8	143.8 ± 15.4[c]
	(165.9 ± 22.9)	(164.4 ± 18.1)	(152.9 ± 2.5)	(151.3 ± 24.9)		(175.8 ± 23.9)	(167.8 ± 23.2)
GD 0–21C[e]	47.9 ± 12.6	46.4 ± 13.5	35.8 ± 14.3	38.9 ± 24	ND[f]	46.5 ± 15.9	39.8 ± 13.9[c]
	(50.4 ± 11.5)	(52.8 ± 13.0)	(42 ± 15.2)	(46 ± 11.7)		(56.3 ± 11.7)	(53.5 ± 15.6)
GD 5–21C[e]	NC[g]	NC[g]	NC[g]	NC[g]	29 ± 10.6[c]	NC[g]	NC[g]
					(43 + 11.1)		

[a] Concurrent study control value for each endpoint in parenthesis.
[b] Exposure period is GD 5–20.
[c] Statistically significantly lower compared to concurrent controls at $p < 0.01$.
[d] Statistically significantly lower compared to concurrent controls at $p < 0.05$.
[e] GD 21 C (day 21 corrected) – day 21 body weight minus uterine content.
[f] ND – data not collected for study performed at Huntingdon Life Sciences.
[g] NC – values not calculated in EMBSI studies.
[h] N – Mean number of females in 20,000 mg/m^3 group through most of the study.

For G/TAME animals, significant decreases in body weight change occurred at 20,000 mg/m^3 at the GD 5–8 and GD 11–14 intervals and food consumption intervals of GD 5–8, GD 8–11, GD 11–14 and GD 5–20. At 10,000 mg/m^3, a statistically significant decrease in food consumption at GD 5–8 was not considered evidence of maternal toxicity since no other time interval was affected and no effects were seen on maternal weight or weight gain.

In the G/TBA study, maternal toxicity during the first half (GD 5–14) of the exposure period was seen in decreased food consumption in the 10,000 and 20,000 mg/m^3 groups and reduced weight gain and maternal body weights throughout gestation at the 10,000 and 20,000 mg/m^3 exposure levels. The gravid uterine weight at 20,000 mg/m^3 was statistically significantly lower than the concurrent control. However the lower gravid uterine weight may have been due to the non-significant reduction in average litter size performance for G/TBA animals. In the G/DIPE study, transient reduction in maternal weight gain during the early days of the exposure period at 20,000 mg/m^3 was partially resolved by GD 21. Reduction in food consumption, although statistically significant intermittently, did not exceed 10% during the exposure period. A decrease in uterine weight to 96% of concurrent control value was not statistically significant but likely contributed to the decrease in the overall GD 5–21 body weight interval at 20,000 mg/m^3.

3.4. Reproductive and fetal effects

No treatment related statistically significant differences between test material treated groups and concurrent controls were seen for uterine data (Table 6). Implantation sites, resorptions, mean litter size, fetal number of viable fetuses and fetuses per litter were comparable to concurrent controls in each study. Corpora lutea, established before exposure began, were comparable to concurrent controls with the exception of G/TBA. For this material a

Table 5
Mean food consumption of pregnant rats exposed to vapor condensates of gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Gestation days (GD)[b]	BGVC[a] N^g = 24 EMBSI	G/MTBE[a] N^g = 24 EMBSI	G/TAME[a] N^g = 24 EMBSI	G/ETBE[a] N^g = 24 EMBSI	G/DIPE[a] N^g = 22 Huntingdon[f]	G/EtOH[a] N^g = 24 EMBSI	G/TBA[a] N^g = 24 EMBSI
Mean food consumption (mean grams/rat/period)							
GD 0–5	122.5- ± 8.7 (120.2 ± 10.4)	118.8 ± 16.2 (121.3 ± 12.6)	121.5 ± 13.3 (121.2 ± 14.7)	117.3 ± 14.5 (119.6 ± 13.1)	ND[e]	122.1 ± 13.2 (123.0 ± 9.2)	114.7 ± 9.4 (117.3 ± 13.0)
GD 5–8	70.5 ± 5.2 (73.5 ± 8.2)	67.9 ± 7.6 (72.1 ± 8.8)	65.8 ± 6.2[c] (72.0 ± 7.0)	64.9 ± 8.5 (70.6 ± 8.4)	79 ± 6.0 (81 ± 6.0)	69.4 ± 8.7 (73.4 ± 7.4)	60.3 ± 4.6[c] (71.6 ± 8.2)
GD 8–11	69.8 ± 5.3 (73.8 ± 6.2)	66.5 ± 7.0[d] (71.9 ± 6.6)	64.2 ± 7.8[d] (70.4 ± 7.8)	61.0 ± 5.8[c] (69.6 ± 7.8)	78 ± 5.7 (85 ± 7.1)	69.2 ± 7.4 (73.6 ± 7.8)	60.0 ± 4.6[c] (71.3 ± 9.3)
GD 11–14	71.5 ± 5.5 (74.5 ± 6.7)	72.1 ± 6.3 (74.1 ± 8.5)	64.2 ± 6.7[c] (70.7 ± 10.4)	64.1 ± 6.5[c] (72.4 ± 7.4)	78 ± 4.0[d] (85 ± 7.1)	71.3 ± 8.2 (75.3 ± 7.0)	63.5 ± 4.6[c] (71.1 ± 8.5)
GD 14–17	77.8 ± 5.6 (78.0 ± 5.8)	77.0 ± 6.9 (77.0 ± 6.3)	69.3 ± 6.3 (72.3 ± 11.2)	68.3 ± 6.7 (71.7 ± 10.7)	79 ± 4.4 (81 ± 4.5)	74.3 ± 8.6 (77.7. ± 5.7)	70.3 ± 6.5 (72.9 ± 7.8)
GD 17–20	81.3 ± 6.0 (81.7 ± 7.6)	82.6 ± 8.0 (80.2 ± 5.8)	75.6 ± 7.6 (76.4 ± 9.1)	74 ± 6.3 (77.3 ± 9.2)	73 ± 4.3 (74 ± 4.5)	80.3 ± 7.8 (82.7 ± 6.1)	76.5 ± 6.7 (77.8 ± 7.5)
GD 20–21	25.1 ± 2.7 (27.5 ± 6.7)	26.9 ± 5.5 (24.3 ± 5.8)	23.4 ± 3.9 (23.8 ± 3.9)	22.9 ± 3.7 (24.5 ± 4.4)	46 ± 7.8[c] (53 ± 8.1)	23.9 ± 3.4 (25.6 ± 3.1)	23.2 ± 3.8 (23.9 ± 3.3)
GD 5–20	371.0 ± 22.3 (380.9 ± 29.8)	366.0 ± 29.1 (375.3 ± 29.7)	338.1 ± 28.8[d] (362.7 ± 37.3)	332.4 ± 29.5[c] (361.5 ± 3.6.)	72 ± 3.8[c] (76 ± 4.7)	364.5 ± 38.2 (381.3 ± 29.4)	330.6 ± 20.8[c] (364.8 ± 37.7)
GD 0–21	518.6 ± 26.8 (528.6 ± 41.6)	513.2 ± 40.4 (520.0.± 38.8)	483.3 ± 39.2 (508.1 ± 51.5)	472.5 ± 43.1 (505.6 ± 49.6)	ND[e]	504.5 ± 41.1 (529.2 ± 38.8)	468.5 ± 23.5[c] (505.9 ± 50.5)

[a] Concurrent study control value for each endpoint in parenthesis.
[b] Exposure period is GD 5–20.
[c] Statistically significantly lower compared to concurrent controls at $p < 0.01$.
[d] Statistically significantly lower compared to concurrent controls at $p < 0.05$.
[e] ND – data not collected for study performed at Huntingdon Life Sciences.
[f] Huntingdon food consumption data calculated as g/kg/day for GD 5–20 duration, not as total for the entire period.
[g] N = Mean number of females in 20,000 mg/m^3 group throughout most of study.

statistically significant non-dose responsive decrease in number of corpora lutea in the 2000 and 20,000 mg/m^3 groups occurred with a subsequent reduction in implantations/litter which was not statistically significant when analyzed with corpora lutea as a covariate. Only one early delivery occurred, this in the 20,000 mg/m^3 G/TAME group for a female who delivered early on GD 20 and data from this litter were not included in the calculations and the early delivery was not associated with exposure. Fetal deaths, which occurred at 20,000 mg/m^3 only in G/ETBE and G/EtOH studies and at low incidence similar to controls, were not associated with exposure.

3.5. Fetal body weights

Mean combined fetal weights of all BGVC – exposed groups were decreased relative to concurrent controls. However, this difference was considered to be a spurious finding because fetal weights in all of the BGVC exposure groups were within the laboratory control group range of 5.3–5.48 g for the other studies in the test program (Fig. 1), no dose–response occurred, and the mean litter size in this control group (15.0) was smaller than the litter sizes for the exposed groups [15.5, 15.6, 16.2 in 2000, 10,000 and 20,000 mg/m^3 groups, respectively]. Smaller litter sizes tend to produce heavier offspring. Exposure to G/TAME and G/TBA resulted in 4% and 3% decreases in fetal body weight respectively at 20,000 mg/m^3. In the G/TBA study fetal body weight did not differ between groups when analyzed with litter size or litter size and fetal sex as covariates but showed statistically significant reduction at 20,000 mg/m^3 when mean corpora lutea count was added as a covariate. Thus, it is not clear whether exposure to G/TBA at 20,000 mg/m^3 is directly linked to decreased fetal body weight. Fetal body weight in G/DIPE 20,000 mg/m^3 litters was statistically significantly lower for females (5%) and combined sexes (3.3%) compared to concurrent controls (Table 6).

In addition to a small reduction in fetal body weight at the highest exposure level, G/TAME 20,000 mg/m^3 and 10,000 mg/m^3 demonstrated a similar higher incidence of stunted fetuses (<4 g) on an affected litter basis compared to concurrent controls (Table 7). Control range for stunted fetuses on all studies was 4–16%.

3.6. Fetal external observations

No significant increases in fetal external variations were seen in any study. Random occurrences of malrotated paw, cleft palate, kinked tail, exencephaly and exophthalmos were reported at various dose levels. In the G/ETBE study a set of conjoined twins was seen in a litter of a dam exposed at 20,000 mg/m^3. None of these findings were related to exposure.

3.7. Fetal visceral observations

Few visceral variations or malformations occurred in any study. Reported incidental findings included retinal folds, hydroureter, hydronephrosis, hydrocephaly, and umbilical artery on left side of bladder (G/TAME, BGVC). None were attributed to the test article exposures.

3.8. Fetal skeletal observations

Exposure did not induce skeletal malformations. No skeletal malformations were reported in any study with the exception of 1 fetus/1 litter at 20,000 mg/m^3 G/DIPE with a vertebral anomaly in which the right exoccipital and cervical arch #1 were fused, and the thoracic centra #13 and lumbar centrum #1 were absent. Skeletal variations (Table 7) included commonly observed findings such as reduced ossification, unossified, asymmetric, bifid or hypoplastic sternebrae, rudimentary lumbar ribs, vertebrae with bifid thoracic centra and dumbbell-shaped thoracic centra, rudimentary 14th vertebrae and cartilaginous variations. Only G/TBA 20,000 mg/m^3 litters showed a statistically significant increase in skeletal variations on a per fetus basis primarily due to increased

Table 6
Developmental toxicity: reproduction and fetal data of rats exposed from GD 5–20 to gasoline or gasoline/oxygenate vapor condensates at 20,000 mg/m^3.

	BGVC[a] EMBSI	G/MTBE[a] EMBSI	G/TAME[a] EMBSI	G/ETBE[a] EMBSI	G/DIPE[a,b] Huntingdon	G/EtOH[a] EMBSI	G/TBA[a] EMBSI
No. of females mated	25 (25)	25 (25)	25 (25)	25 (25)	24 (24)	25 (25)	25 (25)
No. of pregnant females	24 (24)	24 (24)	24[c] (25)	24 (25)	22 (23)	24 (25)	24 (25)
No. pregnancies aborted	0	0	0	0	0	0	0
No. early deliveries	0	0	1[c]	0	0	0	0
No. litters with viable fetuses	24 (24)	24 (24)	25 (25)	24 (25)	22 (23)	24 (25)	24 (25)
Corpora lutea	17.6 ± 2.6 (16.4 ± 3.4)	16.7 ± 3.2 (16.0 ± 1.9)	16.3 ± 1.8 (16.0 ± 3.2)	15.1 ± 2.2 (15.9 ± 1.5)	15.2 ± 2.6 (14.9 ± 2.8)	16.1 ± 2.4 (16.4 ± 3.0)	15.1 ± 2.7[e] (16.3 ± 2.0)
Implantation sites	16.7 ± 1.8 (15.5 ± 3.2)	15.6 ± 2.2 (15.5 ± 1.8)	15.9 ± 1.8 (15.7 ± 3.1)	14.6 ± 2.3 (14.6 ± 3.0)	14.0 ± 2.4 (13.5 ± 2.7)	15.5 ± 3.3 (15.9 ± 2.9)	14.7 ± 2.1 (15.7 ± 1.8)
Preimplantation loss – %	4.6 ± 6.9 (5.1 ± 12.6)	5.1 ± 10.5 (3.0 ± 3.9)	2.2 ± 3.9 (1.6 ± 2.7)	3.2 ± 4.4 (7.9 ± 17.7)	7.6 ± 9.1 (9.3 ± 13.5)	4.6 ± 15.8 (3.0 ± 3.9)	2.3 ± 4.1 (3.7 ± 5.3)
Resorptions mean ± S.D.	0.42 ± 0.72 (0.58 ± 0.83)	0.33 ± 0.56 (0.63 ± 0.77)	0.67 ± 1.13 (0.72 ± 0.84)	0.33 ± 0.48 (0.4 ± 0.58)	0.7 ± 0.6 (0.5 ± 0.7)	0.29 ± 0.46 (0.32 ± 0.56)	0.29 ± 0.55 (0.28 ± 0.6)
Number of viable fetuses	390 (359)	366 (358)	366 (374)	342 (353)	292 (300)	365 (389)	345 (385)
Mean litter size	16.2 ± 1.8 (15.0 ± 3.1)	15.2 ± 2.1 (14.9 ± 2.2)	15.2 ± 2.1 (15.0 ± 3.1)	14.2 ± 2.3 (14.1 ± 3.1)	13.3 ± 2.6 (13.0 ± 2.6)	15.2 ± 3.3 (15.6 ± 2.9)	14.4 ± 2.1 (15.4 ± 1.8)
Mean number of fetuses/ litter							
Males ± S.D	8.6 ± 1.6 (7.5 ± 2.9)	7.0 ± 2.6 (7.2 ± 2.6)	7.4 ± 2.6 (7.4 ± 2.5)	7.1 ± 2.1 (6.9 ± 2.4)	6.1 ± 2.1 (6.3 ± 1.7)	7.3 ± 3.1 (7.9 ± 2.6)	6.4 ± 1.5 (7.8 ± 2.1)
Females ± S.D	7.7 ± 2.3 (7.4 ± 2.6)	8.3 ± 2.6 (7.8 ± 2.3)	7.8 ± 2.3 (7.6 ± 2.4)	7.1 ± 2.1 (7.2 ± 2.2)	7.1 ± 2.5 (6.7 ± 2.0)	7.9 ± 2.9 (7.6 ± 2.4)	8.0 ± 2.1 (7.5 ± 2.2)
Fetuses/implantation	0.98 ± 0.04 (0.97 ± 0.05)	0.98 ± 0.04 (0.96 ± 0.05)	0.96 ± 0.07 (0.95 ± 0.07)	0.98 ± 0.03 (0.95 ± 0.1)	0.95 ± 0.06 (0.96 ± 0.1)	0.98 ± 0.03 (0.98 ± 0.04)	0.98 ± 0.04 (0.98 ± 0.04)
Resorptions/implantation	0.02 ± 0.04 (0.04 ± 0.05)	0.02 ± 0.04 (0.04 ± 0.05)	0.04 ± 0.07 (0.05 ± 0.07)	0.02 ± 0.03 (0.04 ± 0.1)	0.05 ± 0.03 (0.04 ± 0.05)	0.02 ± 0.03 (0.02 ± 0.03)	0.02 ± 0.04 (0.02 ± 0.04)
Postimplantation loss – %	2.5 ± 4.1 (3.3 ± 5.4)	2.1 ± 3.6 (4.2 ± 5.4)	4.2 ± 7.0 (5.1 ± 6.7)	2.5 ± 3.3 (4.6 ± 10.2)	5.2 ± 5.2 (3.4 ± 4.7)	2.1 ± 3.0 (2.0 ± 3.4)	2.3 ± 3.8 (1.9 ± 3.8)
Fetal deaths mean ± S.D	0	0	0	0.04 ± 0.2 (0.04 ± 0.2)	0	0.04 ± 0.2 (0)	0 (0.04 ± 0.2)
Mean body weight ± S.D							
Male fetuses	5.5 ± 0.4 (5.8 ± 0.4)	5.6 ± 0.5 (5.5 ± 0.4)	5.2 ± 0.5 (5.4 ± 0.3)	5.4 ± 0.5 (5.5 ± 0.4)	5.7 ± 0.3 (5.9 ± 0.3)	5.5 ± 0.4 (5.6 ± 0.4)	5.3 ± 0.5 (5.4 ± 0.4)
Female fetuses	5.2 ± 0.4 (5.5 ± 0.3)	5.3 ± 0.4 (5.2 ± 0.4)	5.0 ± 0.5 (5.2 ± 0.4)	5.1 ± 0.4 (5.2 ± 0.4)	5.4 ± 0.3c (5.6 ± 0.3)	5.2 ± 0.4 (5.4 ± 0.3)	5.0 ± 0.3 (5.2 ± 0.4)
Combined weights[f]	5.36[d,g] (5.62)	5.42 (5.38)	5.10[d] (5.31)	5.25 (5.33)	5.54[d] (5.71)	5.37 (5.48)	5.16 (5.32)

Preimplantation loss = (corpora lutea minus implants)/corpora lutea.
Postimplantation loss = (implants minus live fetuses)/implants.
[a] Concurrent study control value for each endpoint in parenthesis.
[b] Study performed at Huntingdon Life Sciences; 24 presumed pregnant animals at study initiation.
[c] G/TAME early delivery GD20, not included in calculations.
[d] statistically significantly lower compared to concurrent controls at $p < 0.01$].
[e] statistically significantly lower compared to concurrent controls at $p < 0.05$.
[f] Combined fetal body weight is least squares mean fetal weight adjusted for litter size in individual studies.
[g] BGVC all dose levels showed similar changes from controls and effect was not considered biologically significant.

Table 7
Incidence of stunted fetuses [weight < 4 g] in litters of rats exposed from GD 5 to 20 to vapor condensates of gasoline or gasoline/oxygenate blends.

Exposure level	BGVC EMBSI	G/MTBE EMBSI	G/TAME EMBSI	G/ETBE EMBSI	G/DIPE Huntingdon	G/ETOH EMBSI	G/TBA EMBSI
20,000 mg/m^3							
Litters affected[a]	2/24	3/24	5/24	5/24	0/22	4/24	4/24
% Affected[b]	8.3	12.5	20.8	20.8	0	16.7	16.7
10,000 mg/m^3							
Litters affected[a]	3/24	1/24	5/24	1/24	2/24	2/24	3/23
% Affected[b]	12.5	4.1	20.8	4.1	8.3	8.3	13
2000 mg/m^3							
Litters affected[a]	3/24	3/24	2/23	1/23	0/23	1/22	2/23
% Affected[b]	12.5	12.5	8.6	4.3	0	4.5	8.7
Control							
Litters affected[a]	0/24	2/24	1/25	4/25	1/23	1/25	2/25
% Affected[b]	0	8.3	4	16	4.3	4	8

[a] Litters affected = litters with stunted fetuses/total litters.
[b] % Affected = affected litters/total litters.

rudimentary ribs (16/24 litters, 67% compared to control 12/25 litters, 48%). An increase in skeletal variations identified at 10,000 mg/m^3 G/ETOH was attributed to chance rather than exposure, as no increase occurred at 20,000 mg/m^3.

4. Discussion

The test program was conducted to evaluate the potential for developmental toxicity from exposure to vapors of gasoline or

Table 8
Incidence of total skeletal variations in litter of rats exposed from GD 5 to 20 to vapor condensates of gasoline or gasoline/oxygenate blends.

Exposure level	Litters with fetal skeletal variations						
	BGVC EMBSI	G/MTBE EMBSI	G/TAME EMBSI	G/ETBE EMBSI	G/DIPE Huntingdon	G/ETOH EMBSI	G/TBA EMBSI
20,000 mg/m^3							
Litters affected[a]	14/24	14/24	16/24	18/24	12/22	15/24	20/24[c]
% Affected[b]	58.3	66.7	66.7	75	54.5	62.5	83.3
10,000 mg/m^3							
Litters affected[a]	11/24	15/24	16/24	18/24	14/24	21/24[c]	13/23
% Affected[b]	45.8	62.5	66.7	75	58.3	87.5	56.5
2000 mg/m^3							
Litters affected[a]	16/24	16/24	16/23	16/23	8/23	12/22	13/23
% Affected[b]	66.7	66.7	69.6	69.6	34.8	54.5	56.5
Control							
Litters affected[a]	12/24	13/24	19/25	17/25	13/23	15/25	15/25
% Affected[b]	50	54.2	76	70.8	56.5	60	60

[a] Litters affected = litters with affected fetuses/total litters.
[b] % Affected = affected litters/total litter.
[c] Statistically significant at $p < 0.05$ on a per fetus basis.

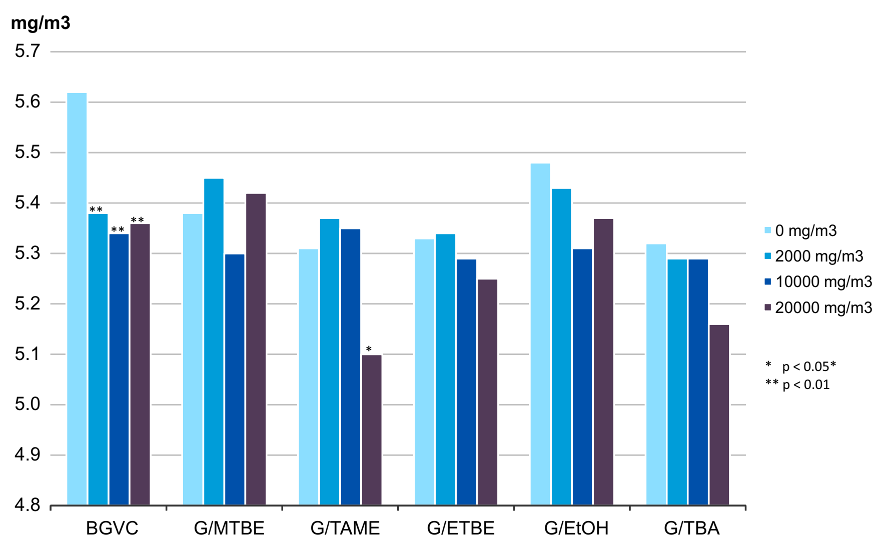

Fig. 1. Fetal body weights for offspring of rats exposed in EMBSI studies.

gasoline blended with oxygenates. The composition of the vapor condensates used as test materials represent the real-world exposure that occurs during vehicle refueling. Typical refueling exposures are <1.0 mg/m^3 but can reach 7.0 mg/m^3 under extreme conditions (Clayton, 1993; NATLSCO, 1995). The highest exposure concentrations in this collection of studies were approximately 2800-fold higher than measured “extreme” exposure levels during refueling. The exposure duration in these tests, six hours per day, is similar to occupational exposure and about 72-fold longer than typical refueling exposures that last approximately five minutes.

Table 9 summarizes the NOAEL values for all studies. Maternal NOAELs were based primarily on reduced body weight, body weight changes and food consumption, most of which occurred in the GD 5–8, GD 8–11, and/or GD 11–14 intervals which sometimes carried through the entire exposure period and were reflected in the GD 5–21 body weight interval and/or the GD 5–20 food consumption interval. These effects were relatively mild, resulting in significant differences in maternal body weight only for G/TBA, for which the decrements were less than 10% from control values. Although decreased body weight gain was seen in pregnant rats exposed to G/MTBE at 20,000 mg/m^3, no decreased body weight or body weight gains were seen in the companion mouse study (Roberts et al., 2014).

Developmental NOAELs were 20,000 mg/m^3 for BGVC, G/MTBE, G/ETBE, and G/EtOH. Although mean combined fetal weight in all BGVC-exposed groups was decreased relative to the concurrent control, these changes did not occur in a dose-responsive manner, all groups had values similar to the range of control groups within the testing program, and no such effect was observed upon birth weight in the reproduction study with BGVC (Gray et al., 2014). The difference was attributed to the smaller litter size of the control group [mean 15.0 vs. 16.2 in the 20,000 mg/m^3 group]. In smaller litters, individual fetal weights tend to be heavier (Chen, 1993). In mice, average fetal body weight/litter was reduced at BGVC 10,000 and 20,000 mg/m^3 levels (Roberts et al., 2014). In both rats and mice exposed to BGVC in our test program, reduced ossification, which often accompanies true reductions in prenatal growth, did not occur. Collectively, the weight of evidence from data of reproduction and developmental studies conducted with unleaded gasoline vapor do not support specific or selective harm to prenatal development. NOAELs of 10,000 mg/m^3 for G/TAME, G/DIPE and G/TBA were based on reduced fetal body weights and

Table 9
No observed adverse effect levels.

NOAELS (mg/m^3)	BGVC EMBSI	G/MTBE EMBSI	G/TAME EMBSI	G/ETBE EMBSI	G/DIPE Huntingdon	G/ETOH EMBSI	G/TBA EMBSI
Maternal	20,000 No adverse effects	10,000 Reduced body wt gain and food consumption over GD 8–11 interval	10,000 Reduced body wt gain and food consumption reflected in GD 5–21 and GD 5–20 intervals, respectively	10,000 Reduced food consumption at GD 8–11, 11–14 intervals reflected in GD 5–20 interval	2000 Reduced body weight gain at GD 11–14 interval for 10,000 and 20,000mg/m^3 reflected in GD 5–21	10,000 Reduced body wt changes, food consumption at GD 5–21 interval, decreased linear trends	2000 Reduced body wt and wt gains reflected in GD5-21 and GD0-21 at 10,000 and 20,000mg/m^3
Developmental	20,000 No adverse effects	20,000 No adverse effects	10,000 Reduced fetal body weights, increased stunted fetuses	20,000 No adverse effects	10,000 Reduced fetal body weights	20,000 No adverse effects	10,000 Reduced fetal body weights; increased skeletal variations

increased number of stunted pups (G/TAME) and increased skeletal variations (G/TBA).

Developmental effects resulting from gasoline abuse in humans have been reported. Mental retardation, hypertonia, scaphocephaly (premature fusion of the sagittal suture), poor postnatal head growth and other developmental anomalies were identified in a Canadian Amerindian community where sniffing of leaded gasoline and alcohol abuse were widespread (Hunter et al., 1979). However, the specific role of gasoline and its hydrocarbon components could not be separated from the impact of lead and alcohol. Developmental studies in rats of whole unleaded gasoline (API, 1978) and unleaded gasoline vapor containing 10% of the starting fuel (Roberts et al., 2001) have demonstrated the absence of developmental toxicity at doses up to 23,881 mg/m^3 consistent with the NOAEL of 20,000 mg/m^3 BGVC reported here.

Some of the aromatic hydrocarbons contained in gasoline produce developmental effects when tested alone. Toluene and benzene have been identified as developmental toxicants by regulatory agencies such as the European Chemicals Bureau and California Environmental Protection Agency. However the percentages of aromatic compounds in general and of toluene in particular in the vapor of gasoline and gasoline blended with oxygenates are well below those in whole gasoline. Benzene levels represented 1.5–2.0% of the total vapor, while toluene levels were 2.4–3.4% of the total vapor and 7.6% of the liquid. Xylene levels were not measured in this study but eight-carbon aromatics represented less than 1% of any of the test substances. Although the test program's highest exposure levels represented 50% of the Lower Explosive Limits, the resulting attainable atmospheric concentrations of aromatic constituents were below those reported in the literature to produce developmental effects.

Case reports indicate that toluene abuse during pregnancy causes congenital malformations. Actual exposure levels for toluene abuse have been estimated at 5000 ppm [18,842 mg/m^3] for a glue-soaked cloth in a paper bag (Cavender, 1993). ACGIH (1991) estimated that women who deliberately concentrate and inhale toluene may experience exposure of 10,000 ppm [>36,000 mg/m^3] (Bukowski, 2001). Spontaneous abortion is the most common reproductive effect associated with occupational exposure to toluene and other chemical solvents, but most regulatory agencies have considered the evidence to be inconclusive (ATSDR, 2000; European Commission, 2001). In animal studies, toluene does not induce malformations but has caused lower birth and postnatal weights and postnatal developmental delays, primarily skeletal. Behavioral effects in offspring have been observed when toluene is administered at high doses (1200–1500 ppm) during periods of fetal brain development. Generally, inhalation NOAEL for toluene for developmental effects range from 400 to 750 ppm [1500–2812 mg/m^3] and LOAEL from doses of 1000 to 2000 ppm [3750–7500 mg/m^3] with exposure during periods of organogenesis and major growth (US EPA, 2006b).

According to the European Risk Assessment of Benzene (ECB, 2003) epidemiology studies implicating benzene as a developmental toxicant have many limitations and there are insufficient data to assess the effects of benzene on the human fetus. Studies are limited largely because of concomitant exposure to other chemicals, inadequate sample size and lack of quantification of exposure levels. In animal studies by the inhalation route, no specific teratogenic effects have been demonstrated with exposure to benzene during organogenesis but fetotoxicity expressed as decreased fetal body weight, delayed skeletal development and increased resorptions associated with maternal stress have been reported (Kuna and Kapp, 1981; Coate et al., 1984; Kuna et al., 1992; US EPA, 2006a). The issue of additivity of solvents was explored by Medinsky et al., 1994 using physiologically-based pharmacokinetic modeling. It was demonstrated that gasoline components can inhibit benzene metabolism and thus expression of benzene toxicity. The extent of inhibition depends on gasoline vapor concentration and inhaled concentrations.

There are limited data addressing the effects of inhalation exposure xylene on pregnancy outcomes (US EPA, 2005). In animal studies by the inhalation route, developmental effects on fetal weights and skeletal variations have occurred primarily at dose levels high enough to cause maternal stress in the range of 1000–2000 ppm (Saillenfait et al., 2003). No regulatory agencies identify xylene as a developmental toxicant.

The oxygenates used in this test program, except for TBA, have been evaluated individually for developmental toxicity potential. The most studied of these is ethanol. Most of the reproductive and developmental toxicity reports for ethanol deal with oral abuse during pregnancy and the resulting fetal alcohol syndrome (EU SIDS, 2005). No fertility or developmental effects were seen at inhalation exposures up to 16,000 ppm (30,400 mg/m^3). The lowest reported NOAEL for fertility by the oral route was 2000 mg/kg body weight in rats, equivalent to a blood alcohol concentration of 1320 mg/l, although this was based on a significant increase in the number of small pups rather than a direct effect on fertility; such direct effects are not seen until much higher doses. Physiologically-based pharmacokinetic modeling for ethanol indicates that very high exposure levels are required to produce blood ethanol concentrations associated with developmental harm in rodents (Martin et al., 2012) The collective weight of evidence is that the NOAEL for developmental effects in animals is high, typically $\geqslant$6400 mg/kg body weight, compared to maternally toxic effects at 3600 mg/kg body weight. The potential for reproductive and developmental toxicity exists in humans from deliberate over-consumption of ethanol. Blood ethanol concentrations resulting from ethanol exposure by any other route are unlikely to produce reproductive or developmental effects.

Developmental studies conducted with the ether oxygenates indicate a potential to produce adverse effects to prenatal development at maternally toxic exposure levels. For MTBE, a

developmental test on mice and rabbits indicated that exposure at 14,400 mg/m^3 on gestation days 6–15 resulted in reduced pup viability and an increased incidence of cleft palate in mice at 28,800 mg/m^3 but no developmental effects were seen in rabbits in this study (Bevan et al., 1997).

Testing of TAME alone in a developmental toxicity study in rats resulted in a no-observable-adverse-effect level (NOAEL) of 250 ppm for maternal toxicity and 1500 ppm for developmental toxicity in rats based on near-term fetal body weights. In mice, TAME exposure resulted in reduced fetal body weight, cleft palate, prenatal mortality, and increased variations along with reduced maternal weights; the NOAEL values for maternal and developmental toxicity were 250 ppm (Welsch et al., 2003).

Studies with ETBE were primarily performed by the oral route. ETBE does not appear to be selectively toxic to reproduction or embryofetal development in the absence of other manifestations of general toxicity. No embryofetal effects were observed in rabbits. Early postnatal rat pup deaths show no clear dose–response and have largely been attributed to total litter losses with accompanying evidence of maternal neglect or frank maternal morbidity (dePeyser, 2010).

Exposure of pregnant Sprague–Dawley rats to DIPE by inhalation at concentrations of 430, 3095, or 6745 ppm for 6 h/day on GD 6–15 resulted in a slight reduction in body weight gain and a significant decrease in food consumption for dams in the 6745 ppm group. A concentration-related increase in the incidence of rudimentary 14th ribs was observed, but its significance was uncertain. DIPE induced only a low order of toxicity in developmental effects (Dalbey and Feuston, 1996).

No specific studies of TBA by the inhalation route were found.

Exposure to oxygenates alone seemed to produce minimal developmental effects in laboratory animals at exposure concentrations higher than or equal to levels producing maternal toxicity. Mice appeared to be more sensitive than rats or rabbits. Exposure to vapor condensate of gasoline blended with these oxygenates demonstrated either no developmental toxicity or effects only at the highest dose tested in rats.

5. Conclusions

Exposure of pregnant rats to vapors of gasoline alone, or gasoline blended with various ethers or alcohols affected maternal body weight gain and food consumption during gestation. Developmental effects occurred for G/DIPE, G/TAME, and G/TBA at exposure levels greater than or equal to those that affected the dams. Thus the components in evaporative emissions from vapors of gasoline or gasoline-oxygenate blends do not produce selective developmental toxicity in rats. Indeed the minimal effects to the general health of the adult animals and fewer effects to prenatal development suggest that evaporative emissions of gasoline and gasoline/oxygenate blends are not selective developmental toxicants and pose minimal risk to human prenatal development.

Conflicts of interest

Dr. Roberts reports she is employed by the petroleum industry.

Dr. Gray reports that he was the API sponsor representative for the conduct of this study by the laboratory. He is now retired from API and received no compensation for his efforts in preparing this article.

Mr. Trimmer has nothing to disclose.

Dr. Parker has nothing to disclose.

Dr. Murray reports personal fees from American Petroleum Institute, during the conduct of the study; personal fees from American Petroleum Institute, outside the submitted work.

Dr. Schreiner reports that while employed at Mobil Oil Corp. she was involved in the API technical workgroup that oversaw the design and conduct of the studies. The American Petroleum Institute employed her after retirement as a consultant to assist in preparing manuscripts from the original laboratory study reports. There was no influence exerted on evaluation of the scientific data and manuscript content.

Dr. Clark reports that while employed at Phillips 66 Company, he was involved in the API technical work group that designed and oversaw the conduct of the studies. The American Petroleum Institute employed him after retirement as a consultant to assist in preparing manuscripts from the original laboratory study reports.

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