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Health assessment of gasoline and fuel oxygenate vapors: Reproductive toxicity assessment

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ABSTRACT

Vapor condensates of baseline gasoline (BGVC), or gasoline-blended with methyl tertiary 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 reproductive toxicity in rats at target concentrations of 2000, 10,000, or 20,000 mg/m^3, 6 h/day, 7 days/week. BGVC and G/MTBE were assessed over two generations, the others for one generation. BGVC and G/MTBE F1 offspring were evaluated for neuropathology and changes in regional brain glial fibrillary acidic protein content. No neurotoxicity was observed. Male kidney weight was increased consistent with light hydrocarbon nephropathy. In adult rats, decreased body weight gain and increased liver weight were seen. Spleen weight decreased in adults and pups exposed to G/TBA. No pathological changes to reproductive organs occurred in any study. Decreased food consumption was seen in G/TAME lactating females. Transient decreases in G/TAME offspring weights were observed during lactation. Except for a minor increase in time to mating in G/TBA which did not affect other reproductive parameters, there were no adverse reproductive findings. The NOAEL for reproductive and offspring parameters was 20,000 mg/m^3 for all vapor condensates except for lower offspring NOAELs of 10,000 mg/m^3 for G/TBA and 2000 mg/m^3 for G/TAME.

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 included inhalation exposures to evaporative emissions of the gasoline or the gasoline-additive blend 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 subchronic toxicity testing (Clark et al., 2014), genotoxicity (Schreiner et al., 2014), neurotoxicity (O'Callaghan et al., 2014), immunotoxicity (White et al., 2014) and developmental toxicity testing in mice and rats (Roberts et al., 2014a,b). This paper describes the results of reproductive toxicity tests performed separately and submitted to EPA.

Test materials evaluated in the reproductive toxicity studies included vapor condensates prepared from an EPA described "baseline gasoline" (without oxygenate) (BGVC), as well as the vapor condensates of gasoline blended with oxygenates (11.3–21.3 wt.%) methyl tertiary butyl ether (G/MTBE), ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME), diisopropyl ether

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(G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). These condensates represent the more easily vaporized fractions of the various gasolines and thus more accurately reproduce human exposure during vehicle fueling and other operations. Baseline gasoline (BGVC) and G/MTBE were evaluated in two-generation studies which included neuropathology assessments and quantitative changes in regional brain glial fibrillary acidic protein (GFAP) content, a measurement of reactive gliosis and an index of underlying neurotoxicity, in F1 offspring. All other vapor condensates were evaluated in one-generation reproductive toxicity studies. The goal of the studies was to provide information on the extent to which the use of oxygenates in gasoline might alter the hazard of evaporative emissions that are encountered during refueling of vehicles, compared to those from gasoline alone.

2. Materials and methods

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). Since only 5-gallon cylinders were practical for use in exposure operations, 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). Test materials included vapor condensates prepared from an EPA described "baseline gasoline" (BGVC), identified as API Lot 99-01, and gasoline blended with methyl tertiary 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).

2.2. Experimental design

The test materials were administered via whole-body inhalation exposures to Sprague Dawley rats (26/sex/dose group) at target concentrations of 2000, 10,000 and 20,000 mg/m^3 for 6 h/day, 7 days/week over two-generations for BGVC and G/MTBE (Table 1) and for one generation for the five other gasoline/oxygenate blends (Table 2).

The control group in each study received air only while in the chamber. Studies were performed at Huntingdon Life Sciences (East Millstone, NJ.) in accordance with US EPA OPPTS 870.3800 reproductive guidelines which address both one-generation and two-generation studies (US EPA, 1998a). In the two-generation studies of BGVC and G/MTBE, F1 pups (5/sex/group/assessment) not selected for F1 mating were evaluated for neurotoxicity by standard Tier 2 neuropathology (per 40 CFR 79.66; US EPA, 1998b) and GFAP (per 40 CFR 79.67; US EPA, 1994b).

2.3. Animal selection, assignment and care

CD (Sprague–Dawley derived) [Crl: CD@ IGS BR] albino rats (approximately 27–29 days of age) were received from Charles River Laboratories (Kingston, NY) for each study. Females were nulliparous and non-pregnant. Animals were acclimated for at least 13 days after receipt and examined to confirm suitability for study. After selection for study (P0 generation) each rat was identified with a metal ear-tag bearing its assigned animal number. In the two-generation studies, selected F1 parental animals were ear-tagged with a unique number at the time of selection.

Animals considered suitable for study on the basis of pretest physical examinations and body weight data were randomly assigned, by sex, to control or treated groups in an attempt to equalize mean group body weights. Individual weights of animals placed on test were within ±20% of the mean weight for each sex for each study. Animals were approximately 40–42 days of age at initiation of exposure. Currently acceptable practices of good animal husbandry were followed (National Academy of Sciences, 1996). Huntingdon Life Sciences, East Millstone, New Jersey is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, Missouri) was available without restriction except during exposure. Water was available without restriction, except during exposures, via an automated watering system. Food and water were analyzed for purity on a regular basis and there were no known contaminants which were expected to interfere with the results of this study.

2.4. Housing and environmental conditions

Animals were individually housed in suspended stainless steel cages with wire mesh fronts and floors with the following exceptions: when mated, one male and one female were co-housed continuously (except during exposure) until mating occurred or for a maximum of 14 days; during lactation, dam and litter were housed together in a solid plastic "shoebox" cage with ground corn cob bedding, (Bed-O-Cobs® ¼ inch irradiated, The Andersons Inc. Maumee, OH), changed at least weekly until weaning.

A 12 h light/dark cycle controlled via an automatic timer was provided. Temperature and relative humidity were monitored in accordance with testing facility SOPs and maintained within the specified range (18–26 °C, and 30–70%, respectively) to the maximum extent possible. Air changes were maintained within a range of 10–15/h. Excursions outside the specified range were not considered to have affected the integrity of the study.

Table 1
Experimental design: two generation studies (BGVC, G/MTBE).

Exposure levels (mg/m^3)[a]	Number of animals									
	Mated adults				Microscopic pathology adult generations				Macroscopic postmortem exams – pups[b]	
	P0		F1		P0		F1		F1	F2
	M	F	M	F	M	F	M	F		
0 (air only)	26	26	26	26	10	10	10	10	3/sex/litter	3/sex/litter
2000	26	26	26	26	–	–	–	–	3/sex/litter	3/sex/litter
10,000	26	26	26	26	–	–	–	–	3/sex/litter	3/sex/litter
20,000	26	26	26	26	10	10	10	10	3/sex/litter	3/sex/litter

[a] Exposures daily, 7 days/week for 6 h per day.
[b] F1 pups (5/sex/group/assessment) not selected for F1 mating were evaluated for standard Tier 2 Neuropathology (as per 40 CFR 79.66) and GFAP assessments (as per 40 CFR 79.67) on Postpartum Day 27 or 28.

Table 2
Experimental design – one generation studies (G/TAME, G/ETBE, G/DIPE, G/ETOH, G/TBA).

Exposure level (mg/m^3)[a]	Number of animals				
	Mated adults P0		Microscopic pathology adult generations P0		Microscopic postmortem examinations – pups F1
	M	F	M	F	
0 (air only)	26	26	10	10	3/sex/litter
2,000	26	26	–	–	3/sex/litter
10,000	26	26	–	–	3/sex/litter
20,000	26	26	10	10	3/sex/litter

[a] Exposures daily (7 days/week) for 6 h per day.

Table 3
Exposure schedule.

	One Generation		Second generation	
Study Phase	**P0 Females**	**P0 Males**		
Premating 70 days (10 weeks)				
Mating 14 days				
Gestation GD 0-19 exposure				
GD20 - LD 4			**F1 Females**	**F1 Males**
Lactation days 5-28				
F1 weaning at LD28	P0 dams and F1 pups not mating	Sacrificed	5/sex/litter for F2 mating.	5/sex/litter for F2 mating
Premating 70 days (10 weeks)				
Mating 14 days				
Gestation days 0-19 exposure				
GD20 - LD 4				
Lactation days 5-28				
F2 weaning at LD-28			F1 dams and F2 pups	F1 sires and F2 pups
Shaded areas indicate exposure periods (6hrs/day, 7 days/week)				

2.5. Test material administration

The test material was administered as a vapor in the breathing air of the animals as described in Clark et al. (2014). Maximum exposure levels were 50% of the lower flammable limits of these test materials.

The exposure schedules are summarized on Table 3. P0 males and females received 70 consecutive days (10 weeks) of exposure

prior to mating for 6 h/day, 7 days/week and continued to be exposed during the 14-day mating period. Mated females were exposed daily from Gestation day 0 (GD0) through GD19.

Females were not exposed after GD19 through lactation day 4 (LD4). Beginning on LD5, nursing P0 females were exposed daily until weaning on LD28. P0 females with no confirmed day of mating continued exposure for 25 days following completion of the mating period. P0 females with no confirmed day of mating but with evidence of pregnancy (weight gain) were exposed until presumed GD19 and females with a confirmed day of mating that did not deliver were sacrificed on presumed GD25. P0 males were exposed daily and sacrificed on the date proximate to the date of the first litter weaning or after the last day F1 pups were delivered (approximately 16–20 weeks of exposure).

Two generation studies: Selected F1 males and females (26 mating pairs/group) started exposure at weaning on LD28 and continued treatment for 10 weeks prior to pairing to produce the F2 generation. Exposure continued through the 14 day mating period. Mated F1 females were exposed daily from GD0 through GD19. F1 females were not exposed after GD19 through lactation day 4 (LD4). Beginning on LD5, nursing F1 females were exposed daily until weaning of the F2 generation on LD28. F1 males were exposed daily and sacrificed on the date proximate to the date of the first F2 litter weaning.

2.6. Exposure procedures

Details of exposure procedures and chamber operation and diagram of the exposure chambers are described in Clark et al. (2014). The flow of air through the chamber was monitored using appropriate calibrated equipment. Exposure levels were analyzed using an infra-red spectrophotometer 4 times per chamber per day. The test material's major components were assayed once per chamber per week. Particle size distribution measurements were also made once per chamber per week using a TSI aerodynamic particle sizer.

2.7. Study performance

Viability checks were performed twice daily for mortality and signs of severe health effects. Physical observations and body weights were recorded twice pretest (P0 generation) and at least weekly during the study. Food consumption was measured beginning the week prior to treatment initiation (P0 generation) and at least weekly during the study. For P0 and F1 dams, body weight and food consumption were measured on Gestation Days [GD] 0, 7, 14, 20 and on Lactation Days [LD] 1, 4, 7, 14, 21 and 28. All parental male animals were sacrificed during the lactation period for a total of 16–20 weeks of exposure and all parental females (P0 and F1) were sacrificed on their respective LD28. Females that failed to mate were sacrificed 25 days after the end of the mating period and females with confirmed mating but without delivery were sacrificed on presumed GD25. Non-pregnant status was confirmed by staining with ammonium sulfide for implantation sites. Selected organs [adrenals, brain, heart, liver, lungs, kidneys, spleen, thymus, ovaries, uterus, testes, seminal vesicles, prostate, epididymides] were weighed and organ/body weight and organ/brain weight ratios calculated. Macroscopic examinations were performed on all parental rats and histological evaluations of the tissue samples from the weighed organs of 10 randomly selected rats in the control and 20,000 mg/m^3 groups were performed. Details of general histopathology procedures are found in Clark et al. (2014). Reproductive organs from all male and bred female rats in control and high dose groups were evaluated. Examination of all parental females included a vaginal smear at time of necropsy to determine stage of estrus and a count of uterine implantation scars if present. Ovary histopathology included evaluation of the primordial follicle population, number of growing follicles and corpora lutea. Right testes and right epididymis from each animal were removed intact, weighed (testes weighed together and separately) and fixed in modified Davidson's solution for 48 h prior to permanent storage in 10% neutral buffered formalin for possible histopathology at Huntingdon Life Sciences. Sperm evaluations included motility, testicular homogenization-resistant sperm and cauda epididymal sperm count and sperm morphology. Sperm evaluations were performed on specimens shipped frozen on dry ice to Pathology Associates International, Frederick MD (PAI). Analyses were performed on the left epididymis, vas deferens and left testis.

2.8. Mating, gestation and lactation procedures for one-generation and two-generation studies

2.8.1. Mating

Vaginal smears were taken daily for each female beginning 3 weeks prior to cohabitation for P0 and F1 rats and continuing until there was evidence of mating or until the 14-day mating period was ended. Following 10 weeks premating exposure, one male and one female from the same group were mated overnight until evidence of mating was observed or 14 days had elapsed. The day evidence of mating was observed (a copulation plug in the vagina and/or microscopic observation of sperm in a vaginal smear) was defined as Day 0 of gestation (GD0).

Animals were not paired during the daily exposure period. During mating of F1 generation, male and female littermates were never paired together. At weaning of each F1 litter on Lactation day (LD) 28, one pup/sex/litter was chosen at random to continue with exposure to BGVC or G/MTBE as the F1 parental generation. When less than 26 litters were available in a group, additional pups from other litters within the group were selected at random to make up 26 mating pairs/group.

2.8.2. Parturition and lactation

On GD18, exposure was ended and each female was transferred to a plastic shoebox with bedding material and observed for evidence of parturition twice daily. The day on which parturition was observed was LD0. These females were not exposed from GD19 [P0 and F1 dams] until exposure was resumed on LD5 to weaning at LD28.

2.8.3. Offspring

Pups of the F1 and F2 generations were observed as soon as possible after delivery for sex, number of live and dead pups and pup abnormalities. All pups were uniquely identified within the litter by toe tattoo. Pups dead at delivery were identified as stillborn or liveborn found dead based on lung floatation (air in the lungs) evaluation. Thereafter litters were observed twice daily and litter size was recorded daily from LD1 to LD28. On LD4, F1 litters with more than 10 pups were randomly culled to 10 pups with sex distribution equalized if possible. Pups were examined and weighed on LD1 (delivery day), 4 (preculled), 7, 14, 21 and 28.

2.8.4. One-generation studies (G/EtOH, G/TAME, G/ETBE, G/DIPE and G/TBA)

Pups from one-generation studies were terminated on LD28. Macroscopic examinations were performed on up to 3 randomly selected pups/sex/litter on LD28 including identification of any structural abnormalities or pathological changes. All remaining F1 pups were examined for external abnormalities and sacrificed. Pups with abnormalities were preserved intact in 10% neutral buffered formalin. Brain, spleen and thymus gland were weighed from one randomly selected pup/sex/litter on LD28.

2.8.5. Two-generation studies: (BGVC and G/MTBE)

At weaning one pup/sex/group was selected for mating to produce the F2 generation. F1 pups [5/sex/group/assessment] not selected for F1 mating were evaluated for standard Tier 2 neuropathology (40 CFR79.66; US EPA, 1998b) or for GFAP assessments (40 CFR79.67; US EPA, 1994b) on postpartum day 28. Methods employed for both procedures are provided in O'Callaghan et al. (2014). The standard Tier 2 neuropathologic evaluation was performed at Huntingdon Life Sciences. For GFAP analyses, brains were removed, weighed and processed, then shipped on dry ice to the US Centers for Disease Control and Prevention, Health Effects Laboratory Division, Morgantown, WV for analysis by Dr. James O'Callaghan. The remaining pups were examined for external abnormalities and sacrificed. Pups with abnormalities were preserved intact in 10% neutral buffered formalin. Three pups/sex/litter in each group (F1 and F2) were selected for macroscopic examination and selected organs [brain, spleen, thymus] were weighed from one pup /sex/litter.

2.9. Statistical methods

For continuous data [body weights, body weight change, food consumption, organ weight data, gestation length, pup body weights, number of pups (live, dead, total), mean age-to-criteria for vaginal opening and preputial separation], mean values of all exposure groups were compared to the mean value for the concurrent control group at each time interval. The litter was considered the operative unit for offspring data (e.g., pups/litter). Evaluation of equality of group means was made with standard one-way analysis of variance (ANOVA) using the F ratio followed by Dunnett's test (Dunnett, 1955, 1964; Dunlap and Duffy, 1975) if needed.

For sperm and ovary data the following parameters were analyzed statistically: mean sperm count (testicular sperm count and caudal epididymal sperm count), sperm morphology, and motility data and numbers of primordial and growing follicles by ovary and total. If a significant difference occurred ($p < 0.05$) between groups using the nonparametric Kruskal–Wallis test, the Wilcoxon (Mann–Whitney *U*) test was used for pair-wise comparisons of each treated group to the vehicle control group (Games and Howell, 1976; Kruskal and Wallis, 1952, 1953; Siegel, 1956).

Incidence data [mortality, mating indices, pregnancy rates, male fertility indices, live birth indices, and pup viability indices (Days 0–4) and lactation indices (Days 4–28)] were analyzed using the Chi-square test (2 x n). If Chi-square analysis was not significant, no additional analyses were performed (Mantel, 1963; Dunlap et al., 1981). If Chi-square was significant, a Fisher Exact Test with Bonferroni correction was performed to identify differences between the groups.

Statistical methods for the GFAP assay employed separate one-way analysis of variance (ANOVA) for each of the brain areas from male and female rats (JMP®, SAS Institute, 1995). The significance level was $p < 0.05$ and, to ensure detection of between group treatment effects, the Least Significance-Difference test (Keppel, 1973) was used for post hoc analyses.

2.10. Compliance

These studies were conducted in accordance with the United States Environmental Protection Agency's (EPA) Good Laboratory Practice Standards (US EPA, 1994a), 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 870.3800 guidelines for one and two-generation reproductive toxicity studies (US EPA, 1998a).

3. Results

3.1. Chamber monitoring

The analytically measured exposure levels of the airborne test material are shown in Table 4. Measured concentrations were acceptable for this type of vapor exposure. The average molecular weights of the hydrocarbons contained in the different condensate samples can be used to calculate the exposure levels in approximate part per million (ppm) concentrations. Chamber environmental conditions averaged 24 °C and 43% relative humidity. Particle sizing results indicated that the atmospheres were essentially vapor only (data not presented).

Table 5 provides a profile of the major components in the starting test materials. Analysis of the major components in the starting test material and the test material atmospheres (20 measurements throughout the two-generation studies and 10 measurements for the one-generation studies) showed an acceptably close comparison between the starting test material and the chamber vaporized test material (data not presented). These data demonstrate that the test animals were exposed, as expected, to all of the major components of the test material in their proper proportions. The data were consistent from week-to-week during the study indicating stability of the test material and the atmosphere generation techniques.

3.2. Systemic Parental Effects

3.2.1. Mortality

There were no significant effects of treatment on survival in any study. All animals in the G/EtOH study survived to termination. Mortality among parental animals in other studies was incidental to mishandling or self-inflicted injuries (broken limbs or tooth damage). Litter survival results are presented in Section 3.3.

3.2.2. Parental data

Parental animal data are summarized in Table 6a (Females) and Table 6b (Males). To facilitate comparisons across test materials the data are presented for the 20,000 mg/m^3 groups only. Statistical significance was determined by comparison with concurrent air

Table 4
Measured exposure concentrations compared to target concentrations.

Exposure levels (mg/m^3)		Exposure chamber concentrations – mg/m^3 ± standard deviation[a]						
		BGVC	G/MTBE	G/EtOH	G/TAME	G/ETBE	G/DIPE	G/TBA
2000	Analytical	2014 ± 96	2015 ± 120	2068 ± 224	2060 ± 239	2040 ± 109	2034 ± 128	2015 ± 176
10,000	Analytical	10,140 ± 369	10,240 ± 622	10,130 ± 320	10,500 ± 516	10,450 ± 507	10,260 ± 621	10,254 ± 355
20,000	Analytical	20,010 ± 599	20,120 ± 776	20,300 ± 1042	20,500 ± 1184	20,420 ± 815	20,230 ± 775	20,382 ± 928
Molecular Wt.[b]		73.8	73.7	65.9	77.2	77.1	76.5	72.1

[a] Total hydrocarbon concentrations as determined by infrared spectroscopy.
[b] Average molecular weight of hydrocarbons in condensate sample.

Table 5
Representative hydrocarbon distribution in vapor condensate test materials.[a]

	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 labs.

Table 6a
Effects on female rats (P0 and F1) from exposure to vapor condensates of baseline gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Endpoints	Control range	BGVC		G/MTBE		G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
		P0	F1	P0	F1	P0	P0	P0	P0	P0
Females										
Premating body weight gain (g)	108–144 (P0) 175–200 (F1)	125**	189	129**	154**	103	111**	126	131	110*
% of control		89.3%	94.5%	89.6%	96.0%	95.4%	88.1%	97.7%	91.0%	90.2%
Gestation day 0–20 weight gain (g)	113–129 (P0) – 120, 121 (F1)	125	122	129	121	129	125	122	129	128
% of control		101.6%	100.8%	95.3%	115.4%	104.0%	98.4%	107.9%	102.4%	103.2%
Lactation day 21–28 weight gain (g)	−3 to 13 (P0) 7–15 (F1)	−1	7	15	8*	10	11	8	17	19**a
% of control		(control-3)	100.0%	115.4%	53.3%	100.0%	110.0%	(control -1)	100.0%	380.0%
Lung, discolored foci (macroscopic)	0/26–4/26	1/26	5/26	5/26	15/26*	5/26	2/23	10/26	4/26	14/26**
% of rats	0–15.4%	3.8%	19.2%	19.2%	57.7%	20.0%	8.7%	38.5%	15.4%	53.8%
Relevant organ weight changes		↑ kidney	NE	↑ liver	NE	↑ kidney	NE	NE	NE	↓ spleen

Statistical significance based upon comparison to each study's concurrent control.
NE = no effect.
* $p < 0.05$.
** $p < 0.01$.

Table 6b
Effects on male rats (P0 and F1) from exposure to vapor condensates of baseline gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Endpoints	Control range	BGVC		G/MTBE		G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
		P0	F1	P0	F1	P0	P0	P0	P0	P0
Males										
Premating body weight gain (g)	225–313 (P0) 350–379 (F1)	250	364	273	321*	213	239**	284	285	287*
% of control		95.8%	96.0%	98.9%	91.7%	94.7%	85.7%	97.3%	82.2%	91.7%
Lung, discolored foci (macroscopic)	0/26–4/26	2/26	1/26	3/26	1/26	1/16	6/26	6/26	3/26	12/26
% of rats	0–15.4%	7.7%	3.8%	11.5%	3.8%	3.8%	23.1%	23.1%	11.5%	46.2%
Relevant organ weight changes		↑ kidney	↑ kidney	↑ kidney, liver	↑ kidney, liver	↑ kidney, liver	↑ kidney, liver	↑ kidney, liver	↑ kidney	↑ kidney, ↓ spleen

Statistical significance based upon comparison to each study's concurrent control.
* $p < 0.05$.
** $p < 0.01$.

control values in each study. Text describes specific effects within studies at individual dose groups.

There were no remarkable clinical observations reported in any of the studies. Statistically significant reduction in weight gain during the premating period was observed in females exposed to 20,000 mg/m^3 BGVC (P0) G/MTBE (P0 and F1), G/ETBE and G/TBA. No effects on maternal body weight gains occurred during gestation (GD0–20) and lactation (LD1–28) with two exceptions. The average body weight gain of G/MTBE F1 20,000 mg/m^3 dams during lactation was 53% of the control value. The G/MTBE LD1–28 F1 control value of 15 g was considered comparatively high in relation to other lactation weight gains. The lactation weight gain of G/TBA dams of 19 g was significantly higher than the concurrent control of 5 g and the highest concurrent control value range of 13 g in P0 dams. Neither of these weight gain changes during lactation was considered biologically significant.

Among males, decreased body weight changes were seen in parental animals in G/ETBE and G/TBA studies and in the F1 generation animals exposed to G/MTBE.

Food consumption (data not shown) was comparable to concurrent controls for BGVC, G/MTBE (P0), G/ETBE, G/DIPE, G/EtOH and G/TBA. G/MTBE exposed rats had decreased food consumption (5–10%) in all F1 animals (especially males) during the first 7 weeks of the 10 week premating period. Food consumption of G/TAME exposed rats was comparable to controls through premating, mating and gestation but was decreased in all female dose groups throughout the lactation period. Decreased food consumption ranged from 10% to less than 15% below controls especially during the mid-lactation period LD7–14 and LD14–21 ($p < 0.01$ at 20,000 mg/m^3; $p < 0.01$ or 0.05 at 10,000 and 2000 mg/m^3).

All test material exposure caused a statistically significant increase in male kidney weights in the 10,000 and 20,000 mg/m^3 groups consistent with light hydrocarbon nephropathy (data not shown). Slight increases in female kidney weights were observed at 20,000 mg/m^3 in BGVC P0 and G/TAME studies. Other organ weight changes included increased liver weights in G/MTBE 20,000 mg/m^3 P0 males and females and in F1 males, and in males exposed to G/TAME, G/ETBE, and G/DIPE. Statistically significantly decreased spleen weights were seen in G/TBA male rats at the mid and high dose and in females at all dose levels. Increases in epididymal absolute weights at 10,000 and 20,000 mg/m^3 and seminal vesicles/coagulating gland absolute weight and relative to body and brain weights at 20,000 mg/m^3 were present in G/DIPE males and increased prostate weight, absolute and relative to body weight at 20,000 mg/m^3 was seen in G/EtOH animals. None of these organ weight increases were accompanied by adverse histopathology findings. The minor differences to male organ weights in the G/EtOH and G/DIPE studies did not occur in a dose-responsive manner and were interpreted as unlikely to be due to exposure or of toxicological (adverse) relevance.

In the 20,000 mg/m^3 G/ETBE group statistically significant increases in epididymis, seminal vesicle and ovary weights relative to body weights (10%, 12%, and 13%, respectively) were considered spurious. The absolute organ weights were comparable to controls and the relative weights did not correlate with body weights as confirmed by supplementary covariance analysis.

Discolored (tan) foci were observed macroscopically in greater incidence in the lungs of adult animals, most significantly in G/MTBE F1 females, G/TBA both sexes and G/DIPE females. These observations were not consistent across generations exposed to G/MTBE and were not accompanied by changes in lung weight or histopathology.

No remarkable histopathologic changes were reported in any study with the exception of minimal increases in aggregates of intraalveolar macrophages in G/MTBE exposed rats, most apparent in P0 20,000 mg/m^3 males, P0 2000 mg/m^3 females and F1 females at all doses. Light hydrocarbon nephropathy was strongly indicated in all studies by the presence of hyaline droplets in kidneys of 20,000 mg/m^3 male rats (Alden, 1986). No treatment related macroscopic or microscopic changes were seen in male or female reproductive organs.

3.3. Offspring observations

Offspring observations are summarized in Table 7. There were no effects attributable to test material exposure on litter size (pups/litter, pups born dead/litter), number of implantation sites/litter, pup birth weight, offspring survival, or sex ratio. Postnatal growth of pups was transiently reduced by exposure to G/TAME or G/TBA. Statistically significant decreases in G/TAME pup weight

Table 7
Effects on offspring (F1 and F2) from exposure of parents to vapor condensate of gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Endpoints	Control range	BGVC		G/MTBE		G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
		F1	F2	F1	F2	F1	F1	F1	F1	F1
Litter size (Pups delivered)	12.1–14.5	13.7	13.2	14	13	14.3	13.7	13.1	14.1	14.1
Pup weights (g) sexes combined										
LD 1	6.8–7.4	7	7	7.2	6.9	6.9	7	7.3	7	7
LD 4	9.7–11.4	10.1	10.2	10.1	10.1	9.6	9.9	10.5*	10.1	9.9
LD 7	13.5–14.7	13.8	13.9	13.8	14	13.5	13.5	14.3	13.9	13.1
LD 14	23.7–26.0	23.3	24.7	24.2	24.3	23.7**	23.5	24.3	24.1	22.7*
LD 21	38.7–42.5	38.8	41.2	40.6	38.5	38.5*	38.9	41.2	39.9	37.9
LD 28	69.5–78.8	66.1	75.4	74.8	72.1	73.6	71.9	76.9	75.4	71.9
Pup survival sexes combined										
LD 0–4	93.1–99.6%	93.8%	95.4%	98.9%	97.1%	96.6%	97.4%	98.6%**[a]	98.3%	96.1%*
LD 5–21	98.7–100%	100.0%	99.6%	99.6%	99.6%	99.5%	100.0%	95.5%	99.2%	99.5%
Other endpoints										
Spleen weight (g)	0.263–0.327	0.232*	0.306	0.312	0.311	0.290	0.298	0.299	0.321	0.278**
GFAP assay[b]		Negative	NE	Negative	NE	NE	NE	NE	NE	NE
Vaginal opening (day)	35.3–37.5	35	NE	36.3	NE	NE	NE	NE	NE	NE
Preputial separation (day)	46.2–46.4	48	NE	46.8	NE	NE	NE	NE	NE	NE

Statistical significance based upon comparison to each study's concurrent control.
NE = not evaluated.
* $p < 0.05$.
** $p < 0.01$.
[a] Significantly better survival than concurrent control (93.1%).
[b] GFAP details in Tables 9 and 10.

Table 8
Mean GFAP levels on specific regions of rat brains of F1 generation offspring following whole body inhalation exposure of maternal rats to gasoline (BGVC) vapor condensate.

Brain area	Control	2000 mg/m^3	10,000 mg/m^3	20,0000 mg/m^3
Males ($N = 5$)				
Striatum	0.33 ± 0.01[a]	0.38 ± 0.02	0.40 ± 0.04	0.33 ± 0.05
Hippocampus	2.01 ± 0.14	2.36 ± 0.22	2.48 ± 0.20	1.75 ± 0.20
Cortex	0.61 ± 0.05	0.72 ± 0.08	0.76 ± 0.07	0.54 ± 0.07
Olfactory Bulb	1.34 ± 0.08	1.19 ± 0.07	1.28 ± 0.08	1.13 ± 0.09
Thalamus	0.86 ± 0.07	0.88 ± 0.07	0.94 ± 0.07	0.65 ± 0.05[b]
Hypothalamus	1.81 ± 0.18	1.99 ± 0.20	1.73 ± 0.14	1.36 ± 0.16
Cerebellum	3.04 ± 0.07	3.34 ± 0.26	3.13 ± 0.18	2.47 ± 0.27
Rest of Brain	2.56 ± 0.25	3.00 ± 0.22	3.07 ± 0.27	2.41 ± 0.39
Females ($N = 5$)				
Striatum	0.34 ± 0.04	0.42 ± 0.02	0.37 ± 0.04	0.35 ± 0.03
Hippocampus	2.04 ± 0.09	2.18 ± 0.09	2.26 ± 0.14	2.06 ± 0.09
Cortex	0.60 ± 0.02	0.67 ± 0.04	0.69 ± 0.04	0.63 ± 0.07
Olfactory Bulb	1.29 ± 0.11	1.37 ± 0.11	1.22 ± 0.09	1.12 ± 0.07
Thalamus	0.77 ± 0.03	0.86 ± 0.06	0.93 ± 0.06	0.73 ± 0.07
Hypothalamus	1.89 ± 0.12	1.68 ± 0.09	1.75 ± 0.18	1.68 ± 0.20
Cerebellum	2.84 ± 0.18	3.42 ± 0.30	2.94 ± 0.23	2.44 ± 0.27
Rest of Brain	2.96 ± 0.52	2.94 ± 0.20	3.32 ± 0.42	2.64 ± 0.22

[a] Each value represents the mean ± SEM for the concentration of GFAP (μg/mg total protein).
[b] Statistically different from control ($p < 0.05$).

Table 9
Mean GFAP levels on specific regions of rat brains of F1 generation offspring following whole body inhalation exposure of maternal rats to gasoline MTBE (G/MTBE) Vapor Condensate.

Brain area	Control	2000 mg/m^3	10,000 mg/m^3	20,0000 mg/m^3
Males ($N = 5$)				
Striatum	0.35 ± 0.02[a]	0.41 ± 0.02	0.39 ± 0.03	0.42 ± 0.05
Hippocampus	2.31 ± 0.15	2.06 ± 0.13	2.28 ± 0.20	2.28 ± 0.24
Cortex	0.78 ± 0.07	0.75 ± 0.05	0.69 ± 0.03	0.78 ± 0.04
Olfactory Bulb	1.43 ± 0.11	1.36 ± 0.12	1.26 ± 0.09	1.36 ± 0.20
Thalamus	0.79 ± 0.08	0.83 ± 0.03	0.89 ± 0.10	0.91 ± 0.11
Hypothalamus	1.81 ± 0.09	1.87 ± 0.28	1.56 ± 0.13	2.11 ± 0.27
Cerebellum	2.72 ± 0.07	2.66 ± 0.14	2.70 ± 0.16	2.95 ± 0.39
Rest of Brain	2.86 ± 0.24	2.60 ± 0.10	2.75 ± 0.24	3.17 ± 0.47
Females ($N = 5$)				
Striatum	0.36 ± 0.05	0.38 ± 0.06	0.43 ± 0.04	0.39 ± 0.03
Hippocampus	2.36 ± 0.24	2.37 ± 0.08	2.16 ± 0.30	2.26 ± 0.12
Cortex	0.68 ± 0.06	0.77 ± 0.06	0.78 ± 0.04	0.79 ± 0.10
Olfactory Bulb	1.22 ± 0.09	1.34 ± 0.08	1.51 ± 0.10	1.27 ± 0.03
Thalamus	0.81 ± 0.07	0.91 ± 0.05	0.89 ± 0.05	0.82 ± 0.05
Hypothalamus	1.61 ± 0.14	2.10 ± 0.21	1.90 ± 0.18	1.69 ± 0.17
Cerebellum	2.78 ± 0.29	2.72 ± 0.10	2.91 ± 0.15	2.81 ± 0.18
Rest of Brain	3.08 ± 0.27	3.08 ± 0.16	3.45 ± 0.36	2.90 ± 0.21

No values were statistically different from control ($p < 0.05$).
[a] Each value represents the mean ± SEM for the concentration of GFAP (μg/mg Total protein).

gain over LD7–14 occurred at 10,000 and 20,000 mg/m^3 and continued to be statistically smaller for male pups and combined sexes in the 20,000 mg/m^3 group on LD21. At 2000 mg/m^3, pup weight was reduced on LD21 for male and combined sexes as was weight gain for intervals LD4–21 and 14–21. However, weights of mid-dose (10,000 mg/m^3) G/TAME exposed males and all F1 females were not statistically different from controls at LD21 and there were no statistically significant differences from controls for any exposure group on postnatal day 28. In offspring of females exposed to G/TBA there was a slight but statistically significant decrease in pup weight gains early in lactation up to LD14 but weights were comparable to controls by LD21 and postnatal day 28.

No adverse effects were seen on offspring organ weights with the exception of reduced spleen weights in both sexes from the G/TBA exposed group. Spleen weights were also reduced in the G/TBA parental rats. Lower spleen weights were seen in F1 offspring of BGVC exposed rats; however the effect was not expressed to the F2 offspring and was unlikely to be a toxicologically significant adverse finding. Expressions of sexual maturation (vaginal opening and preputial gland separation) were not altered by exposure to BGVC or G/MTBE. Additionally there were no decrements in reproductive performance when the F1 animals (1/sex/litter) were bred to produce the second generation.

Neuropathology and GFAP assessments were performed on randomly selected F1 pups from BGVC and G/MTBE exposed rats. No adverse neuropathology was observed and there were no differences between control and BGVC or G/MTBE F1 offspring in brain length or width. GFAP results are presented in Tables 8 and 9. Exposure to either test material did not cause changes in GFAP levels in any brain region examined with the exception of a single decrease in the BGVC F1 male thalamus at 20,000 mg/m^3 that was not considered biologically significant by the investigator. Overall, GFAP results indicated that none of these substances induced gliosis in the brain regions examined.

3.4. Reproductive parameters

Reproductive parameters are summarized in Table 10. There were no differences in male and female fertility or reproductive performance with exposure to any test material. Estrus cyclicity and semen parameters were comparable between exposed and concurrent control groups. A slight non-significant lengthening in the number of days of pairing until mating occurred in animals exposed to 20,000 mg/m^3 G/TBA, due to 7 of 23 pairs that failed to mate during the first or second estrus opportunity. However, there were no differences in acyclics, persistent estrus or irregular cycles and the lengthening did not translate to observed effects on endpoints of reproductive performance. There was a slight increase in abnormal sperm (2%) in 20,000 mg/m^3 G/MTBE P0 males that was not seen in F1 males. A statistically significant increase in epididymal sperm count of G/MTBE P0 males was not considered biologically significant due to a low concurrent control value and the absence of a similar response in F1 males.

4. Discussion

Table 11 presents No Observed Adverse Effects Levels (NOAEL) and rationales for parental, reproductive and offspring results. Kidney weight changes in male rats consistent with light hydrocarbon nephropathy are excluded from the parental NOAEL determinations because this finding has been generally accepted scientifically as not relevant to the assessment of human health risk assessment (US EPA, 1991). No neuropathology or neurotoxicity expressed as GFAP changes were observed in F1 offspring in BGVC or G/MTBE studies.

4.1. Parental assessments

The BGVC parental NOAEL was based on decreased body weight gains during the premating period in P0 females and F1 males and increased P0 female kidney weight which had no histopathologic correlate. The G/ETOH parental NOAEL was also due to decreased body weight gain in 20,000 mg/m^3 animals, during the initial 3 weeks of the premating period. The G/MTBE parental NOAEL could not be determined due to minimal increased incidence of intraalveolar macrophages most apparent in P0 high dose males, P0 low dose females and all F1 animals. Decreased food consumption was seen in all F1 animals during the 7 week premating period when inhalation exposure to G/MTBE was initiated beginning at weaning on postnatal day 28.

Table 10
Effects on reproductive parameters on parental animals (P0 and F1) from exposure to vapor condensates of gasoline or gasoline/oxygenate blends at 20,000 mg/m^3.

Endpoint	Control range	BGVC		G/MTBE		G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
		P0	F1	P0	F1	P0	P0	P0	P0	P0
Male Fertility	20/25–25/26	25/26	24/25	26/26	24/25	24/26	23/25	23/26	25/26	22/25
	80–96.2%	96.2%	96.0%	100.0%	96.0%	92.3%	92.0%	88.5%	96.2%	88.0%
Female Fertility	21/24–25/25	25/25	25/25	26/26	24/24	23/25	23/25	23/23	25/26	22/22
	87.5–100%	100.0%	100.0%	100.0%	100.0%	92.0%	92.0%	100.0%	96.2%	100.0%
Number of Litters	20–25	25	25	26	24	23	23	23	25	22
Days to Mating	2.4–3.4	2.6	2.7	3.2	3.3	2.9	2.8	3.1	2.8	4
Estrus Cycle Length (days)	4.2–5.2, 5.7	4.5	4.1	5.3	4.3	5	4.2	4.3	4.3	4.1
Semen parameters										
Sperm Count, testis ($\times 10^6$/g)	81.2–124.1	115.4	100.6	108.5	102.2	93.5	106.5	103.9	104.5	108.3
Motility (%)	89–96%	94%	95%	91%	91%	88%	94%	93%	95%	90%
Morphology (% abnormal)	0.6–1.7%[a]	1.20%	0.50%	2.0%*	0.60%	1.90%	0.60%	0.4%*[b]	0.70%	0.50%
Epididymal sperm Count (x 10^6/g)	753.6–956.7	955.3	933.5	891.2*[c]	933.1	855.7	1053	927.9	832.4	793.9

Statistical significance based upon comparison to each study's concurrent control.
** $p < 0.01$.
* $p < 0.05$.
[a] Did not include control value of 7.4% from the G/DIPE study, which was influenced strongly by two males with abnormally high percentages (90.5% and 84%) of amorphous sperm heads.
[b] Significantly fewer abnormal sperm than concurrent control (7.4% abnormal).
[c] Significantly higher sperm count than low concurrent control value of 753.6 [PAI historical control average = 869.9].

Table 11
No observed adverse effect levels[a].

Study	BGVC	G/MTBE	G/TAME	G/ETBE	G/DIPE	G/EtOH	G/TBA
Parental	10,000 Decreased body weight gain P0 females, F1 males. Increased P0 female kidney weight	<2000 Increased intra-alveolar macrophages in some P0 and all F1 rats. Decreased food consumption in F1 rats	<2000 Decreased food consumption in all lactating females	2000 Increased liver weights	2000 Increased liver weights	10,000 Decreased body weight gain	<2000 Decreased spleen weights at all dose levels
Reproductive	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects
Offspring	20,000 No adverse effects	20,000 No adverse effects	2000 Decreased pup body weight	20,000 No adverse effects	20,000 No adverse effects	20,000 No adverse effects	10,000 Decreased spleen weights

[a] NOAEL determination excluded male rat kidney hydrocarbon nephropathy as not relevant to human risk assessment.

The G/ETBE and G/DIPE parental NOAELs were based on increased liver weights (absolute and relative to body weight) in the mid and high dose groups. The G/TBA parental NOAEL could not be determined based on statistically significant decreased spleen weights at all dose levels in females and at the mid and high dose levels in males. Although similar differences were not seen in the 13 week inhalation dose study (Clark et al., 2014) that study involved smaller group sizes and only 65 exposures on a 5 day/week regime whereas this study involved at least 112 consecutive daily exposures. G/TAME parental males had increased liver weights (absolute and relative to body weight) at 10,000 and 20,000 mg/m^3 with no histologic correlates. Increased kidney weights were seen in males at all doses reflective of light hydrocarbon nephropathy and 20,000 mg/m^3 females with no microscopic correlate. However a parental NOAEL could not be determined due to decreased food consumption in lactating females at all doses which while not reflected in decreased maternal body weights, may have contributed to decreased pup weights during lactation.

4.2. Reproductive and offspring assessments

The NOAEL was the highest exposure tested for BGVC, G/MTBE, G/ETBE, G/DIPE, G/TBA and G/ETOH as no differences from controls were seen for fertility, days to mating, estrus cycle length, sperm counts or morphology or developmental parameters in pups.

The offspring NOAEL for G/TBA was based on decreased spleen weight in pups from 20,000 mg/m^3 exposed dams. Decreased spleen weight was also seen in parental animals.

The G/TAME reproductive NOAEL was 20,000 mg/m^3. The offspring NOAEL of 2000 mg/m^3 was based on transient statistically significant reductions in pup weight gains seen over LD7–14 at mid and high dose levels and continued in male pups and combined sexes in the 20,000 mg/m^3 group at LD21. However all groups were comparable to controls by weaning at postnatal day 28. At the lowest exposure level (2000 mg/m^3) although pup weights were statistically significantly lower at LD21 for males and combined sex pups resulting in lower weight gains in postnatal intervals 5–21 and 14–21, weights recovered by postnatal day 28. Reduced pup weights during lactation was previously reported in a two generation study with inhalation exposure of TAME alone at concentrations of 1040, 2250 and 12,500 mg/m^3 (0, 250, 1500, 3000 ppm; Tyl et al., 2003).

Developmental toxicity studies were performed in pregnant rats using the same gasoline blended test materials at exposure concentrations of 2000, 10,000, 20,000 mg/m^3 over GD5–20. Developmental NOAELs were 20,000 mg/m^3 for BGVC, G/MTBE, G/ETBE, G/EtOH and G/DIPE. For G/TAME and G/TBA, developmental NOAELs were 10,000 mg/m^3 due to decreased fetal body weight and increased incidence of stunted fetuses at 20,000 mg/m^3 in the G/TAME study, and increased skeletal variations, possibly related to maternal stress (decreased body weight gain and food consump-

tion) at 20,000 mg/m^3 in the G/TBA study (Roberts et al., 2014b). Developmental studies at the same doses performed with BGVC and G/MTBE only in pregnant mice in which exposure lasted from GD5–17 resulted in developmental NOAEL values of 2000 and 20,000 mg/m^3, respectively (Roberts et al., 2014a). The NOAEL for development in the BGVC study was conservatively based upon a decrease in fetal weight, non-dose-responsive, that was unaccompanied by other indicators of delayed or reduced prenatal growth. As with the reproduction and developmental studies in rats, there were no findings of malformations. These studies support the findings of the reproductive studies in illustrating the absence or minimal effect of exposure to gasoline vapor or vapors of gasoline/oxygenate blends on developmental parameters.

The fuel condensates were not selective toxicants to reproduction. The decreased body weights in offspring of G/TAME exposed females correlated with the decreased food consumption of the dams throughout lactation. The decreased spleen weights in parental animals exposed to G/TBA were also seen in offspring.

5. Conclusions

Reproductive and Offspring NOAEL values of 10,000–20,000 mg/m^3 were similar among all gasoline and gasoline/oxygenate blend vapor condensates tested with the exception of G/TAME where transient decreased offspring weights resulted in a NOAEL = 2000 mg/m^3, still a very high exposure level. Exposures of rats to concentrations of up to 50% of the lower flammable limits of evaporative emissions from these condensates indicate minimal impact on reproductive parameters at concentrations well in excess of potential human exposure at refueling, which typically measure less than 1.0 mg/m^3 but excursions could reach 7.0 mg/m^3 (Clayton, 1993; NATLSCO, 1995). Further, inclusion of oxygenates with gasoline does not appear to alter the minimal effects of exposure to gasoline evaporative emissions alone.

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