DATA EVALUATION RECORD

BASELINE GASOLINE VAPOR CONDENSATE
OPPTS § 870.7800

STUDY TYPE: IMMUNOTOXICITY - RAT

Prepared for

Office of Transportation and Air Quality
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, D.C. 20460

Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity inhalation exposure-rat; OPPTS 870.7800.

TEST MATERIAL (PURITY): Baseline Gasoline Vapor Condensate (100%)

SYNONYMS: None

CITATION: White, K.L. (2001). 3rd Draft Report: Immunological evaluation of baseline gasoline vapor condensate in female Sprague Dawley rats. ImmunoTox, Inc., Virginia Bio•Technology Research Park, 800 East Leigh St., Suite 209, Richmond, VA 23219-1534. Project No. ITI 900, July 31, 2001. Unpublished.

SPONSOR: American Petroleum Institute, 1200 L St., NW, Washington, DC 20005

EXECUTIVE SUMMARY: In an immunotoxicity study (ITI 900), groups of 10 young adult female Sprague Dawley rats were exposed by inhalation to baseline vapor gasoline condensate 6 hours/day, 5 days/week for 4 weeks (20 exposures) at concentrations of 0, 2, 10, or 20 mg/L. Four days before sacrifice, all rats were sensitized with sheep red blood cells by tail vein injection. After sacrifice, the spleens of all rats were removed and the humoral-mediated primary response to sheep erythrocytes was measured using a modified hemolytic plaque assay. Cell counts were done and the number of cells/spleen, antibody forming cells (AFC)/spleen and AFC/10^6 spleen cells were determined.

There were no treatment-related effects on body, spleen, or thymus weights, spleen cell number or IgM antibody production. Under the conditions of the study, baseline gasoline vapor condensate did not adversely affect the humoral immune response of female rats.

A LOAEL for baseline gasoline vapor condensate immunotoxicity in rats was not identified. The NOAEL for baseline gasoline vapor condensate was the highest concentration tested, 20 mg/L.

This immunotoxicity study is Classified **Acceptable/Guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in female rats.

COMPLIANCE: The electronic version of the report contained GLP, Quality Assurance, and Data Confidentiality statements, but these were not signed or dated. The reviewer assumes the hard copy report contains the signed and dated forms.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Baseline Gasoline Vapor Condensate

Description:	Clear liquid (data from Huntingdon Life Sciences Report 00-6125)
Lot/Batch #:	API 99-01 (data from Huntingdon Life Sciences Report 00-6125)
Purity:	100% (data from Huntingdon Life Sciences Report 00-6125)
Compound Stability:	Stable at room temperature (data from Huntingdon Life Sciences Report 00-6125)
CAS # of TGAI:	Not applicable
Structure:	Not applicable

2. Vehicle and/or positive control: cyclophosphamide (CPS), 50 mg/kg

3. Test animals: (Information from Huntingdon Life Sciences Report 00-6125)

Species:	Rat
Strain:	Sprague Dawley [Crl: CD®IGS BR]
Age/weight at study initiation:	Females ~6 weeks, 140-176 g
Source:	Charles River Laboratories, Kingston, NY
Housing:	Individually in stainless steel cages with wire mesh floors
Diet:	Certified Rodent Diet No. 5002, PMI Nutrition International, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	15-26°C
Humidity:	12-79%
Air changes:	Not reported
Photoperiod:	12 hrs light/dark
Acclimation period:	≥16 days

B. STUDY DESIGN:

1. In life dates: Start: December 13, 2000; End: January 9, 2001. The in-life portion of the study was done by the contracting sponsor Huntingdon Life Sciences Princeton Research Center. The immunotoxicity study was conducted at ImmunoTox, Inc..

2. Animal assignment: The female rats were randomly assigned to the test groups noted in Table 1 based on body weight. The rats were exposed in two 1000 L stainless steel and glass whole-body exposure chambers 6 hours/day, 5 days/week, for 4 weeks (20 exposures). The CPS positive control rats were not chamber exposed.

TABLE 1. Study design

Test group	Nominal conc. (mg/L)	Analytical conc. (mg/L)		MMAD (μm)		GSD		No. Rats
		Chamber 1	Chamber 2	Chamber 1	Chamber 2	Chamber 1	Chamber 2	
Control	0	0	0	4.855	4.424	2.457	2.371	10
Low (LCT)	2.563	2.078	2.091	2.426	3.236	2.150	2.112	10
Mid (MCT)	10.080	10.080	10.140	4.937	4.901	2.419	2.451	10
High (HCT)	20.520	20.660	20.330	5.416	2.720	2.645	2.297	10
Pos. Cont.	NA	NA		NA		NA		10

NA = Not applicable
Data from Appendix B, Table A of ITI 900

3. **Dose selection rationale:** A dose selection rationale was not provided.

4. **Generation of the test atmosphere/chamber description:** For both exposure chambers, nitrogen was delivered to a metering valve attached to a back pressure gauge and into the vapor inlet valve of the test material cylinder. The metering valve was used to adjust and maintain the pressure within the cylinder. From the cylinder, the test material flowed to a flowmeter regulated by a metering valve to adjust chamber concentration. The exposure atmosphere was then directed to the exposure chambers.

   Chamber Humidity: 21-61%
   Chamber Temperature: 19-28°C
   Chamber Oxygen: ~19%
   T_{99}: ~23 minutes.
   Air flow: 200 L/min (12 air changes/hour)

   Analytical Chemistry: Samples from the animal breathing area of the exposure chambers were analyzed by infrared spectrophotometry at least four times during each exposure. Also samples were collected in charcoal tubes once per week and analyzed by gas chromatography to characterize 10 major components to verify stability and for comparison between the neat and vaporized material.

   Test atmosphere concentration: Results are in Table 1 above.

   Particle size determination: Particle size was determined using a TSI Aerodynamic Particle Sizer. Samples were drawn for 20 seconds at a flow rate of 5 LPM and the MMAD and GSD were calculated based on the amount of particles collected. No further description was provided. Results are in Table 1 above.

5. **Statistics:** The mean and standard deviation were calculated for each parameter and submitted to Bartlett's Test for homogeneity. If homogenous, the data were analyzed by ANOVA and significant differences between groups determined by Dunnett's Test. Heterogenous data were analyzed by nonparametric ANOVA and significant differences between groups determined by Gehan-Wilcoxon Rank Sum Test. The Jonckheere's Test was used to test for exposure-related trends. The positive control was compared to the vehicle control group

using the Student's t Test where statistical significance was the criteria for accepting the study results. The statistical methods were appropriate.

C. METHODS:

1. **Observations:** With the exception of a pretest physical examination, no other examinations were done. The rats were observed for morbidity and moribundity twice daily throughout the study.

2. **Body weight:** Animals were weighed weekly.

3. **Food/water consumption and compound intake:** Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/kg body weight/day.

4. **Sacrifice and pathology:** One day following the last exposure (day 27), the rats were sacrificed by CO_2 inhalation following an overnight fast.

   a. **Gross necropsy:** Gross necropsy was not done.

   b. **Tissue preparation/histopathology:** At necropsy, the thymus of each rat was removed, weighed, and preserved in 10% buffered formalin for possible histopathology. The spleen of each rat was removed, weighed, placed in Earle's Balanced Salt Solution and shipped on wet ice to the study laboratory, ImmunoTox, Inc..

5. **Immunotoxicity:**

   a. **Antibody plaque-forming cell (AFC) assay, day 4 response:** With the exception of the positive control and vehicle control rats, the animals were exposed to the test material 20 out of 26 days. The positive control rats received a 50 mg/kg IP injection of CPS once per day for 4 days before sacrifice. Four days before sacrifice, all rats were sensitized with sheep red blood cells (sRBC, 2×10^8) by tail vein injection. The primary response to sheep erythrocytes was measured using a modified hemolytic plaque assay (Jerne, N.K., et al., Plaque forming cells: Methodology and Theory. Transpl. Rev. 18:130-191, 1974). Cell counts were performed and the number of cells/spleen, AFC/spleen and AFC/10^6 spleen cells were determined.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

2. **Mortality:** None of the rats died during the study.

B. <u>BODY WEIGHT, BODY WEIGHT GAIN, AND FOOD CONSUMPTION</u>: There were no treatment-related effects on body weight or body weight gain (Table 2) and there were no treatment-related effects on food consumption.

TABLE 2. Average body weights and body weight gains of rats exposed to baseline vapor gasoline condensate [a]

Exposure conc. (mg/L)	Body weights (g±SD)		Total weight gain	
	Week 0	Week 3	g	% of control
0	161 ± 7.5	236 ± 8.8	75 ± 7.7	–
2	163 ± 10.1	240 ± 15.1	77 ± 10.6	103
10	163 ± 9.8	237 ± 16.0	74 ± 10.9	99
20	163 ± 11.1	233 ± 10.7	71 ± 9.6	95
CPS (50 mg/kg)	162 ± 10.3	233 ± 17.5	71 ± 9.8	95

[a] Data from Appendix B, Tables C and D of ITI 900

C. <u>ORGAN WEIGHTS</u>: As shown in Table 3, treatment with baseline gasoline vapor condensate did not significantly affect the spleen or thymus weights of treated rats. The positive control, CPS, decreased the spleen weight by 57% and the thymus weight by 75% (Table 3) relative to vehicle control rats.

TABLE 3. Absolute (mg) and relative to body weight (%) spleen and thymus weights of female rats exposed to baseline gasoline vapor condensate.

Parameter	Vehicle	Baseline gasoline vapor condensate (mg/L)			CPS (50 mg/kg)
		2	10	20	
Spleen					
Absolute	615 ± 38	647 ± 26	600 ± 23	675 ± 102	265** ± 10
Relative	0.248 ± 0.014	0.252 ± 0.012	0.239 ± 0.010	0.276 ± 0.041	0.119** ± 0.004
Thymus					
Absolute	808 ± 33	761 ± 38	758 ± 44	724 ± 34	205** ± 14
Relative	0.327 ± 0.013	0.297 ± 0.018	0.303 ± 0.018	0.293 ± 0.013	0.093** ± 0.006

Data from Table 1, page 19 of ITI 900
**=$p \leq 0.01$

D. <u>IMMUNOTOXICITY TESTS</u>: Antibody plaque-forming cell (AFC) assay: Immunotoxicity findings for the antibody plaque-forming cell assay are summarized in Table 4. Under the conditions of this study, baseline gasoline vapor condensate did not suppress the humoral immune response in rats. Exposure did not alter the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. In contrast, the positive control CPS eliminated the humoral immune response.

TABLE 4: Results of antibody plaque-forming cell assay of rats exposed to baseline gasoline vapor condensate[a]

Test group (n = 10)	Spleen cells (x 10^7)	IgM AFC/10^6 Spleen cells	IgM AFC/Spleen (x 10^3)
Vehicle control	53.18 ± 2.15	1639 ± 408	880 ± 209
2 mg/L	62.26 ± 2.35	1540 ± 194	980 ± 143
10 mg/L	55.82 ± 4.02	1687 ± 235	903 ± 120
20 mg/L	57.96 ± 3.33	1175 ± 111[b]	685 ± 77[b]
CPS, 50 mg/kg	9.69** ± 0.41	3** ± 3	0** ± 0

[a]Data obtained from Table 2, page 20 of ITI 900
[b]One outlier removed from calculation
** =p <0.01

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that inhalation exposure of female rats to baseline gasoline vapor condensate 6 hours/day, 5 days/week for 4 weeks did not alter the humoral immune response as evaluated by the IgM antibody-forming cell response to the T-dependent antigen sRBC. There were no treatment-related effects on body, spleen, or thymus weights, spleen cell number or IgM antibody production. Under the conditions of the study, baseline gasoline vapor condensate did not adversely affect the immune response of female rats.

B. REVIEWER COMMENTS: The reviewer concurs with the study author that baseline vapor gasoline condensate did not alter the humoral response of female rats. **A LOAEL for baseline gasoline vapor condensate immunotoxicity in female rats was not identified. The NOAEL for baseline gasoline vapor condensate immunotoxicity in female rats was the highest concentration tested, 20 mg/L.**

C. STUDY DEFICIENCIES: As provided, the immunotoxicity report does not contain sufficient information concerning the in-life and exposure portions of the study. However, these were contained in the subchronic toxicity report, Huntingdon Life Sciences Report 00-6125. This would not affect the integrity of the study results, but these details should be included in the immunotoxicity report for completeness.