DATA EVALUATION RECORD

GASOLINE DIPE VAPOR CONDENSATE

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY [OPPTS870.5395(§84-2)]
Prepared for

American Petroleum Institute
1220 L Street, Northwest
Washington, DC 20005-4070

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

Primary Reviewer:
Gary A. Sega, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Robert H. Ross, M.S. Signature: _____
Date: _____

Po-Yung Lu, Ph.D. Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: William Boyes, Ph.D. **Signature**: _____
Office of Research and Development **Date**_____
EPA Work Assignment Manager: Joe Sopada **Signature**: _____
Office of Transportation and Air Quality **Date**_____

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in the rat; [OPPTS 870.5395 (§84-2) OECD 474.

TEST MATERIAL (PURITY): Gasoline DIPE Vapor Condensate (100% purity)

SYNONYMS: For the DIPE component: diisopropyl ether; 2,2'-oxybispropane; 2-isopropoxypropane; diisopropyl oxide; isopropyl ether.

CITATION: Mason, C. E. (2002) Satellite procedure gasoline DIPE vapor condensate rat micronucleus test. Huntingdon Life Sciences Ltd., Eye Research Centre (ERC), Eye, Suffolk, IP23 7PX, England. ERC Study No. APT/009, July 30, 2002. Unpublished.

SPONSOR: Huntingdon Life Sciences, Princeton Research Center, Mettlers Road, East Millstone, NJ 08875-2360.

EXECUTIVE SUMMARY: In a Crl: CD® IGS BR albino rat bone marrow micronucleus assay (ERC Study No. APT/009), five rats/sex/dose were treated with Gasoline DIPE vapor condensate (100 % a.i., lot # API 01-06) via whole-body inhalation exposures for 4 weeks (6 hours per day and 5 days per week) at target exposure concentrations of 0, 2000, 10,000, or 20,000 mg/m^3. Analytically determined values for test material concentrations were, on average, close to the target concentrations. Bone marrow cells were harvested 24 hours after the final (20th) exposure. The vehicle was air.

No sign of toxicity of the test substance was observed during the study. Gasoline DIPE vapor condensate was tested at an adequate dose based on the highest exposure of 20,000 mg/m^3 (20 mg/mL) which is 20 times the EPA OPPTS Guideline limit value for subchronic inhalation toxicity studies. The vehicle and positive controls induced the appropriate responses. **There was not a significant increase in the frequency of micronucleated immature erythrocytes in bone marrow at any exposure concentration.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Electronic copies of the GLP and Quality Assurance statements were not signed or dated. It is assumed that the original copies had been signed and dated. The identity, strength, purity, composition and other characteristics of the positive control material were not determined by the testing facility. No Data Confidentiality statement was provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Gasoline DIPE (diisopropyl ether) Vapor Condensate

Description:	Colorless liquid, a mixture of 18 organic components + DIPE
Lot/Batch #:	API 01-06
Purity:	100% Gasoline DIPE Vapor Condensate
Compound Stability:	Unstable. Peroxides may form when diisopropyl ether (DIPE) is in contact with the air for a long time. It may then explode by itself or when heated or subjected to shock.
CAS # of TGAI:	DIPE: [108-20-3]
Solvent Used:	None, test material given by inhalation

2. Control materials:

Negative control (if not vehicle) :	Air only	**Final Volume:** NA	**Route:** inhalation
Vehicle:	None	**Final Volume:** NA	**Route:** NA
Positive control :	Cyclophosphamide in water	**Single Dose:** 40 mg/kg	**Route:** i.p.

3.Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived albinos [Crl: CD® IGS BR]
Age/weight at study initiation:	~6 weeks, both sexes / males: mean weight 123 g, range 108 - 138 g; females: mean weight 113 g, range 95 - 130 g.
Source:	Charles River Laboratories, Kingston, NY 12484
No. animals used per dose	5 Males; 5 Females
Properly Maintained?	Yes

4. Test compound administration:

	Dose Levels mg/m^3	**Final Volume**	**Route**
Preliminary:	not done		
Main Study:	0, 2000, 10,000, 20,000	Not applicable	Inhalation

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:		once		twice (24 hrs apart)			x	Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other: by inhalation for 6 hours per day on 5 days per week for 4 weeks										

b. Negative and/or vehicle control:

Dosing:		once		twice (24 hrs apart)			x	Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other: Air only in chamber. Inhalation for 6 hours per day on 5 days per week for 4 weeks										

c. Positive control:

Dosing:	x	once		twice (24 hrs apart)				Other		
Sampling (after last dose):	x	24 hr		12 hr		24 hr		48 hr		72 hr
Other:										

2. Tissues and cells examined:

Bone marrow	
No. of polychromatic erythrocytes (PCE, mature erythrocytes) examined per animal:	2000
Immature erythrocytes examined per animal:	# In at least 1000 erythrocytes
Other (if other cell types examined, describe):	

3. Details of slide preparation: Bone marrow cells were recovered from the femurs of each animal and spread on 4 slides, 2 of which were held in reserve. Slides were air-dried and fixed in methanol. A modified Feulgen staining procedure was used to specifically stain DNA-containing bodies deep purple while leaving mast cell granules unstained. The method also gives reasonable differentiation of mature and immature erythrocytes and produces permanent preparations. The stained smears were examined under code by light microscopy.

4. Evaluation criteria: Micronuclei from smears examined by light microscopy were identified by meeting the following criteria: (a) being large enough to discern morphological characteristics, (b) having a generally rounded shape with a clearly defined outline, (c) being deeply stained, but not black, and similar in color to the nuclei of other cells, (d) lying in the same focal plane as the cell, (e) lacking internal structure, and (f) lacking micronuclear-like debris in the area surrounding the cell. For each rat, the proportion of immature erythrocytes among at least 1000 erythrocytes (immature (IE) + mature (ME) erythrocytes), the incidence of micronucleated immature erythrocytes (MIE) per 2000 immature erythrocytes, and the incidence of micronucleated mature erythrocytes (MME) per 2000 mature erythrocytes were determined. The group means (both sexes combined) for these parameters were determined for each treatment and control group. Criteria for a positive result were a statistically significant increase ($p<0.01$) in the incidence of any of these parameters in a treatment group in comparison with the vehicle control group. The individual and/or group mean values also should exceed the laboratory historical range.

5. Statistical methods: Differences between the incidences of MIEs in treated and control groups were analyzed for significance using nonparametric statistical methods. Comparison of the treatment groups with the controls was made using a Linear by Linear Association test for trend, in a step-down fashion if significance was detected. For assessment of effects on the proportion of IEs, exact versions of Wilcoxon's sum of ranks test and Jonekheere's test for trend were used. Significance levels with $p<0.01$ were flagged. A positive response is indicated by a statistically significant, dose-related increase in the incidence of MIEs of the treated animals compared to the controls. Bone marrow cell toxicity or depression is

indicated by a substantial and statistically significant dose-related decrease in the proportion of IEs. The statistical methods used were appropriate.

II. REPORTED RESULTS:

A. DOSIMETRY: Dosimetry using an IR analytical procedure was carried out to determine how well the target concentrations of the test substance in air were achieved. By analytical methods, the concentration of the test material vapor in air was determined four times on each of the 20 exposure dates for both males and females. The means ± SDs of these analytical values for each of the target concentrations of 2000, 10,000, and 20,000 mg/m^3 were 2006 ± 150, 9870 ± 607, and 19,850 ± 652 mg/m^3, respectively, for the males and 1997 ± 135, 9927 ± 646, and 20,040 ± 876 mg/m^3, respectively, for the females. Thus, on average, all three of the observed concentrations were close to the target concentrations.

B. PRELIMINARY TOXICITY ASSAY: No preliminary toxicity assay was reported, and no rationale was presented for the dose selection in the micronucleus assay. However, all doses used equaled or exceeded the 1 mg/L limit concentration for an OPPTS Subchronic inhalation toxicity study.

C. MICRONUCLEUS ASSAY: Results of the micronucleus assay are summarized in Table 1. No cellular toxicity, as measured by a lowering of the proportion of immature/mature erythrocytes, was seen at any exposure concentration in either sex. No statistically significant increase in the percentage of MIEs over vehicle control values was seen at any exposure concentration in either sex or in the pooled data from both sexes. The group means for the incidence of MIEs in experimental groups (sexes combined) ranged from 0.7-0.9 MIEs per 2000 cells, which was in the range of the historical control data (reported graphically) of 0-2.6 MIEs per 2000 IEs. The vehicle and positive control values were appropriate. The route of exposure was appropriate in view of the most likely means of human exposure. While no rationale was presented for the choice of doses, the doses used were equal to or exceeded the EPA Guideline for a limit concentration of 2mg/L for an inhalation toxicity study.

TABLE 1 Summary of micronucleus assay

Exposure level (mg/m^3)	Proportion of immature erythrocytes (%) among total erythrocytes ± SD [a]	Incidence of micronucleated cells per 2000 immature erythrocytes ± SD	Incidence of micronucleated cells per 2000 mature erythrocytes
0 (air control)			
Males [b]	51.4 ± 3.8	0.8 ± 0.4	0.0
Females [b]	50.8 ± 2.4	0.8 ± 0.8	0.0
Pooled	51.1 ± 3.0	0.8 ± 0.6	0.0
2000			
Males	52.6 ± 2.0	1.0 ± 1.0	0.0
Females	51.2 ± 1.6	0.6 ± 0.6	2.25
Pooled	51.9 ± 1.8	0.8 ± 0.8	1.12
10000			
Males	49.0 ± 2.0	0.8 ± 0.4	0.0
Females	49.0 ± 4.2	1.0 ± 0.7	2.13
Pooled	49.0 ± 3.1	0.9 ± 0.6	1.06
20000			
Males	52.6 ± 3.6	0.6 ± 0.6	0.0
Females	53.2 ± 6.4	0.8 ± 0.4	1.55
Pooled	52.9 ± 4.9	0.7 ± 0.5	0.77
Cyclophosphamide (40 mg/kg)	44.4 ± 2.8	9.8 ± 3.1	0.0
Males	41.4 ± 2.6	20.4 ± 6.8	0.0
Females	42.9 ± 3.0***	15.1 ± 7.5***	0.0
Pooled			

Data summarized from Tables 1 and 2 (C. Mason, 2002. ERC Study No. APT /009), pp.18-20.
[a] SDs calculated by the reviewer.
[b] Male and female averages were calculated by the reviewer.
*** Statistically significant, $p < 0.001$.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The investigator concluded that gasoline DIPE vapor condensate did not increase the incidence of MIEs in rat bone marrow. They also concluded that the test substance did not cause a statistically significant decrease in the proportion of MIEs among the total erythrocytes. Thus, the gasoline DIPE vapor condensate showed no evidence of causing chromosome damage or bone marrow cell toxicity.

B. REVIEWER COMMENTS: The reviewer agrees with the investigator's conclusions. Gasoline DIPE vapor condensate did not increase the incidence of micronucleated immature erythrocytes over vehicle control values at any test concentration in either sex, thus indicating no clastogenic or aneugenic activity. The test material also did not cause any significant reduction in the proportion of immature erythrocytes to total erythrocytes and was, therefore, not considered to be toxic to bone marrow cells. The test material was tested to a concentration of 20,000 mg/m^3 for 6 hours per day on 5 days per week for 4 weeks. This maximum concentration of the test material was 20 times the limit concentration of 1mg/L set by EPA Guidelines for an OPPTS Subchronic inhalation toxicity study.

C. STUDY DEFICIENCIES: There were no major deficiencies. A minor deficiency was that there was no signed and dated Data Confidentiality statement provided. This minor deficiency would not alter the outcome of this study.