

STUDY NO. 03-6141

LIQUIFIED PETROLEUM GAS:

A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN THE RATS

WITH NEUROTOXICITY ASSESSMENTS AND *IN VIVO*

GENOTOXICITY ASSESSMENTS

Final Report

Volume IV of IV

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0511950-3
 Lot Code: FEB 05 05 1C
 Entered: 2/21/2005

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.68	%
FIBER (CRUDE)	4.67	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0751	PPM
CALCIUM	0.893	%
LEAD	0.228	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.755	%
SELENIUM	0.344	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:

- 1) Customer Service at (314) 982-1310 -- for assay methodology
- 2) Dr. Dorrance Haught at (314) 317-5178 -- for nutritional interpretation
- 3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0512460-2
 Lot Code: FEB 16 05 3B
 Entered: 3/3/2005

Assay	Analysis	Units
PROTEIN	20.7	%
FAT ACID (HYDRO.)	5.79	%
FIBER (CRUDE)	4.42	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0843	PPM
CALCIUM	0.898	PPM
LEAD	0.164	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.711	PPM
SELENIUM	0.250	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.03
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:

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- 2) Dr. Dorrance Haught at (314) 317-5178 -- for nutritional interpretation
- 3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

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Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0513237-3
 Lot Code: MAR 02 05 3C
 Entered: 3/29/2005

Assay	Analysis	Units
PROTEIN	20.9	%
FAT ACID (HYDRO.)	5.44	%
FIBER (CRUDE)	5.13	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.100	PPM
CALCIUM	0.838	%
LEAD	0.227	ppm
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.599	%
SELENIUM	0.241	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:

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Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0513676-3
 Lot Code: MAR 11 05 1C
 Entered: 3/31/2005

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	6.05	%
FIBER (CRUDE)	3.83	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0932	PPM
CALCIUM	0.838	%
LEAD	0.209	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.636	%
SELENIUM	0.274	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.05
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0513237-2
 Lot Code: MAR 02 05 3B
 Entered: 3/29/2005

Assay	Analysis	Units
PROTEIN	20.8	%
FAT ACID (HYDRO.)	5.71	%
FIBER (CRUDE)	4.88	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.102	PPM
CALCIUM	0.874	%
LEAD	0.227	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.619	%
SELENIUM	0.220	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0513676-3
 Lot Code: MAR 11 05 1C
 Entered: 3/31/2005

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	6.05	%
FIBER (CRUDE)	3.83	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0932	PPM
CALCIUM	0.838	%
LEAD	0.209	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.636	%
SELENIUM	0.274	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.05
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0515797-3
 Lot Code: APR 22 05 1C
 Entered: 5/12/2005

Assay	Analysis	Units
PROTEIN	21.4	%
FAT ACID (HYDRO.)	5.64	%
FIBER (CRUDE)	4.03	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.123	PPM
CALCIUM	0.859	%
LEAD	0.189	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.633	%
SELENIUM	0.359	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.03
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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- 2) Dr. Dorrance Haught at (314) 317-5178 – for nutritional interpretation
- 3) Richmond, IN Manufacturing Plant at (765) 962-9561 – all other questions

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0515797-2
 Lot Code: APR 22 05 1B
 Entered: 5/12/2005

Assay	Analysis	Units
PROTEIN	20.8	%
FAT ACID (HYDRO.)	5.55	%
FIBER (CRUDE)	4.16	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.12	PPM
CALCIUM	0.857	%
LEAD	0.190	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.642	%
SELENIUM	0.364	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0515797-1
 Lot Code: APR 22 05 1A
 Entered: 5/11/2005

Assay	Analysis	Units
PROTEIN	20.8	%
FAT ACID (HYDRO.)	5.6	%
FIBER (CRUDE)	4.34	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.121	PPM
CALCIUM	0.857	%
LEAD	0.185	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.636	%
SELENIUM	0.38	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.03
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0516825-3
 Lot Code: MAY 12 05 1C
 Entered: 5/31/2005

Assay	Analysis	Units
PROTEIN	20.5	%
FAT ACID (HYDRO.)	6.16	%
FIBER (CRUDE)	3.97	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	LESS THAN 0.05	PPM
CALCIUM	0.901	%
LEAD	0.179	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.666	%
SELENIUM	0.213	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

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Return to Certified Analysis Retrieval

Product Code: 5002M
 Product Desc: CERTIFIED RODENT DIET MEAL
 Lab Number: L0516825-2
 Lot Code: MAY 12 05 1B
 Entered: 5/31/2005

Assay	Analysis	Units	
PROTEIN	20.8	%	
FAT ACID (HYDRO.)	6.34	%	
FIBER (CRUDE)	3.64	%	
ARSENIC	LESS THAN 0.2	PPM	
CADMIUM	LESS THAN 0.05	PPM	
CALCIUM	0.943	%	
LEAD	0.170	PPM	
MERCURY	LESS THAN 0.025	PPM	
PHOSPHORUS	0.698	%	
SELENIUM	0.214	PPM	
ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		
PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:

- 1) Customer Service at (314) 982-1310 -- for assay methodology
- 2) Dr. Dorrance Haught at (314) 317-5178 -- for nutritional interpretation
- 3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

Elizabethtown Water Company									
Physical & Chemical Analyses									
								Date	April 12, 2005
General Source	Raritan-Millstone Plant								
Sample No. 1	Plant Delivered Water - 7:30 a.m. 4-12-05 DD								
Sample No. 2									
Sample No. 3									
Sample No. 4									
Sample No. 5									
Sample No. 6									
Sample No. 7									
Sample No. 8									
Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	54							
Turbidity (NTU)	0.3 NTU	0.09							
Color	10 *	1.0							
Threshold Odor 40 ° C	3 TON *	2.9/1cc							
Threshold Taste	3 TTN *	2.9							
Conductivity (micromhos / cm)	-	322							
Hardness, Total (as mg / l CaCO ₃)	250 mg / l *	92							
Alkalinity	-	42							
pH	6.5-8.5 *	7.0							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/0.92							
Calcium (as mg / l CaCO ₃)	-	64							
Magnesium (as mg / l CaCO ₃)	-	28							
Iron, Total (mg / l Fe)	0.3 mg / l *	nd							
Sulfates (mg / l SO ₄)	250 mg / l *	34.6							
Chlorides (mg / l Cl)	250 mg / l *	39.4							
Fluoride (mg / l F)	2 mg / l *	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	278							
Total Suspended Solids (mg / l)	-	0.2							
Ammonia Nitrogen (mg / l N)	-	0.050							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.10							
Dissolved Oxygen (mg / l O ₂)	-	10.3							
BOD 5 (mg / l O ₂)	-	-							
Langelier Index	+/- 1.0	-1.72							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H ₂ S as S)	-	0							
Nitrite Nitrogen (mg / l N)	1 mg / l	<.01							
Phosphate (mg / l PO ₃)	-	0.09							
Manganese (mg / l MN)	0.05 mg / l *	nd							
Zinc	5 mg / l *	0.20							
T.O.C. (mg / l)	5 mg / l *	1.2							
						Laboratory Analyst: KK			
* Secondary or Recommended MCL									
Any questions please call Water Quality Specialist- (908) 301-3163									

REVIEWED
m 18 Jly 05

Elizabethtown Water Company									
Physical & Chemical Analyses									
								Date	May 3, 2005
General Source	Raritan-Millstone Plant								
Sample No. 1	Plant Delivered Water - 7:30 a.m. 3-03-05 KK								
Sample No. 2									
Sample No. 3									
Sample No. 4									
Sample No. 5									
Sample No. 6									
Sample No. 7									
Sample No. 8									
Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	58							
Turbidity (NTU)	0.3 NTU	0.09							
Color	10 *	1.0							
Threshold Odor 40 ° C	3 TON *	3.0/2cc							
Threshold Taste ①	3 TTN *	3.1							
Conductivity (micromhos / cm)	-	357							
Hardness, Total (as mg / l CaCO ₃)	250 mg / l *	104							
Alkalinity	-	45							
pH	6.5-8.5 *	7.0							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/1.11							
Calcium (as mg / l CaCO ₃)	-	72							
Magnesium (as mg / l CaCO ₃)	-	32							
Iron, Total (mg / l Fe)	0.3 mg / l *	nd							
Sulfates (mg / l SO ₄)	250 mg / l *	45.5							
Chlorides (mg / l Cl)	250 mg / l *	41.8							
Fluoride (mg / l F)	2 mg / l *	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	182							
Total Suspended Solids (mg / l)	-	0.0							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.03							
Dissolved Oxygen (mg / l O ₂)	-	10.2							
BOD 5 (mg / l O ₂)	-	-							
Langelier Index	+/- 1.0	-1.70							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H ₂ S as S)	-	0							
Nitrite Nitrogen (mg / l N)	1 mg / l	<0.1							
Phosphate (mg / l PO ₃)	-	0.16							
Manganese (mg / l MN)	0.05 mg / l *	nd							
Zinc	5 mg / l *	0.29							
T.O.C. (mg / l)	5 mg / l *	1.5							
						Laboratory Analyst: MT			
* Secondary or Recommended MCL									
Any questions please call Water Quality Specialist- (908) 301-3163									

① Taste is not a regulated parameter
 ~ 18 July 05

REVIEWED
 ~ 18 July 05

Elizabethtown Water Company Physical & Chemical Analyses									
							Date	June 14, 2005	
General Source		Raritan-Millstone Plant							
Sample No. 1		Plant Delivered Water - 9:30 a.m. 6-14-05 KK/AC							
Sample No. 2									
Sample No. 3									
Sample No. 4									
Sample No. 5									
Sample No. 6									
Sample No. 7									
Sample No. 8									
Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	78							
Turbidity (NTU)	0.3 NTU	0.16							
Color	10 *	2.0							
Threshold Odor 40 ° C	3 TON *	2.4/2cc							
Threshold Taste ①	3 TTN *	3.2							
Conductivity (micromhos / cm)	-	347							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	100							
Alkalinity	-	39							
pH	6.5-8.5 *	6.6							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/.81							
Calcium (as mg / l CaCO3)	-	66							
Magnesium (as mg / l CaCO3)	-	34							
Iron, Total (mg / l Fe)	0.3 mg / l *	nd							
Sulfates (mg / l SO4)	250 mg / l *	46.7							
Chlorides (mg / l Cl)	250 mg / l *	40.9							
Fluoride (mg / l F)	2 mg / l *	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	246							
Total Suspended Solids (mg / l)	-	0.1							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.01							
Dissolved Oxygen (mg / l O2)	-	6.9							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-2.20							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	1 mg / l	<.01							
Phosphate (mg / l PO3)	-	0.10							
Manganese (mg / l MN)	0.05 mg / l *	nd							
Zinc	5 mg/l *	0.32							
T.O.C. (mg / l)	5 mg/l *	1.9							
							Laboratory Analyst: MT/AC		
* Secondary or Recommended MCL									
Any questions please call Water Quality Specialist- (908) 301-3163									

① Taste is not a regulated parameter
by Elizabethtown Water ~ 27 July

REVIEWED
~ 27 July

Elizabethtown Water Company									
Physical & Chemical Analyses									
							Date	July 19, 2005	
General Source	Raritan-Millstone Plant								
Sample No. 1	Plant Delivered Water - 7:30 a.m. 7-19-05 KK								
Sample No. 2									
Sample No. 3									
Sample No. 4									
Sample No. 5									
Sample No. 6									
Sample No. 7									
Sample No. 8									
Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	80							
Turbidity (NTU)	0.3 NTU	0.10							
Color	10 *	1.0							
Threshold / Odor 40 ° C	3 TON *	3.7/2cc							
Threshold Taste	3 TTN *	3.6							
Conductivity (micromhos / cm)	-	322							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	80							
Alkalinity	-	35							
pH	6.5-8.5 *	6.6							
Chlorine, Free / Total (mg / l Cl)	-	0.06/.81							
Calcium (as mg / l CaCO3)	-	60							
Magnesium (as mg / l CaCO3)	-	20							
Iron, Total (mg / l Fe)	0.3 mg / l *	ND							
Sulfates (mg / l SO4)	250 mg / l *	44.1							
Chlorides (mg / l Cl)	250 mg / l *	35.8							
Fluoride (mg / l F)	2 mg / l *	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	258							
Total Suspended Solids (mg / l)	-	0.1							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	0.94							
Dissolved Oxygen (mg / l O2)	-	7.2							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-2.30							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	1 mg / l	<.01							
Phosphate (mg / l PO3)	-	<.05							
Manganese (mg / l MN)	0.05 mg / l *	ND							
Zinc	5 mg/l *	0.32							
T.O.C. (mg / l)	5 mg/l *	1.8							
							Laboratory Analyst: MT		
* Secondary or Recommended MCL									
Any questions please call Water Quality Specialist- (732) 302-3192									

Odor/odor are not regulated parameters
M 17 Aug 05

REVIEWED

M 17 Aug 05

Huntingdon Life Sciences (ERC) Internal Reference No: APT 012/063480

Study Title

SATELLITE PROCEDURE
LIQUIFIED PETROLEUM GAS
RAT MICRONUCLEUS TEST

TEST GUIDELINES: OECD Guidelines for Testing of Chemicals 474.
Mammalian Erythrocyte Micronucleus Test, (1998).
US EPA OPPTS Health Effects Test Guidelines; 870.5395
Mammalian Erythrocyte Micronucleus Test. (1998)

AUTHOR: Lincoln Pritchard, B.Sc. (Hons.)

STUDY COMPLETED ON: 15 March 2010

SUBCONTRACTOR: Huntingdon Life Sciences Ltd.,
Eye Research Centre (ERC)
Eye, Suffolk
IP23 7PX
ENGLAND.

**HUNTINGDON LIFE SCIENCES
LTD (PRC) STUDY No.:** 03-6141

**HUNTINGDON LIFE SCIENCES
LTD (ERC) INTERNAL
REFERENCE No.:** APT/012

SUBCONTRACTOR'S SPONSOR: Huntingdon Life Sciences
Princeton Research Center (PRC)
Mettlers Road
East Millstone, NJ 08875-2360
USA

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Huntingdon Life Sciences (ERC) Internal Reference No: APT 012/063480

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The slide evaluation phase of the study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid:

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

US EPA Good Laboratory Practices 40 CFR 792 (TSCA).

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L50/44).

No compliance is claimed for work presented in the Experimental Procedure – In-life phase or Appendix 2 of this report.

[REDACTED]

15 March 2010
Date

Huntingdon Life Sciences Ltd., ERC.

.....
I am claiming compliance for the whole study with the following exceptions:

1. The identity, strength, purity and composition or other characteristics to define the test substance along with storage stability was not determined in a GLP compliant laboratory.
2. The identity, strength, purity and composition or other characteristics to define the positive control article has not been determined by the Testing Facility. The positive control article has been characterized as per the Certificate of Analysis on file with the Testing Facility. The stability of the positive control article has not been determined by the Testing Facility. Analyses to determine the uniformity (as applicable) or concentration of the positive control mixture were not performed by the Testing Facility. The stability of the positive control article mixture has not been determined by the Testing Facility.

[REDACTED]

23 APR 10
Date

Huntingdon Life Sciences Ltd., PRC.

Huntingdon Life Sciences (ERC) Internal Reference No: APT 012/063480

ERC - QUALITY ASSURANCE STATEMENT

The following inspection and audit have been carried out in relation to the slide evaluation phase of this study:

Study Phase	Date of Inspection	Date of Reporting to Principal Investigator and Test Site Management	Date of Reporting to Study Director, Test Facility Management and Lead QA
Process Based			
Slide scoring	1 July 2005	1 July 2005	-
Report Audit	22-27 September 2006 12 March 2010	27 September 2006 12 March 2010	28 September 2006 12 March 2010

Process Based Inspection: At or about the time this phase of the study was in progress, inspections of routine and repetitive procedures employed on this type of study were carried out. The slide scoring inspection was conducted and reported to appropriate Company Management as indicated above.

Report Audit: This appendix has been audited by the test site Quality Assurance Department. This audit was conducted and reported to the Principal Investigator, test site Management, Study Director, test facility Management and lead Quality Assurance Department as indicated above.

Study based inspections were not performed on this phase of the study.

The methods, procedures and observations were found to be accurately described and the reported results of this appendix to reflect the raw data.

[Redacted Signature]

[Redacted Name]

Department of Quality Assurance
Huntingdon Life Sciences Ltd

15 March 2010
Date

Huntingdon Life Sciences Study No: APT 012/03-6141

PRC - QUALITY ASSURANCE STATEMENT

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Huntingdon Life Sciences, East Millstone, New Jersey, and the dates that findings were reported to the Study Director and Management. This report reflects the raw data as far as can be reasonably established.

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
GLP Protocol Review	22-25 Mar 05	25 Mar 05
Exposure Monitoring and Equipment Records	20 Apr 05	28 Apr 05
Body Weight and Feeder Weight Data Collection	8 Jun 05	8 Jun 05
Exposure Monitoring and Equipment Records	24 Jun 05	28 Jun 05
Terminal Necropsy, Sperm Analysis and Genotoxicity Necropsy	20 & 21 Jul 05	21 Jul 05
Protocol Amendments 1 - 3	17 Apr 06	17 Apr 06
Protocol Amendments 4 & 5	29 Jan & 1 Feb 10	1 Feb 10




23 Apr 10
Date

Huntingdon Life Sciences (ERC) Internal Reference No: APT 012/063480

RESPONSIBLE PERSONNEL AND SCIENTIFIC APPROVAL

[Redacted]
[Redacted]
[Redacted]

Department of Safety Assessment, PRC.

23 APR 10
Date

[Redacted]
[Redacted]
[Redacted]

Department of Genetic Toxicology, ERC

15 March 2010
Date

[Redacted]
[Redacted]
[Redacted]

Department of Statistics, HRC

15 March 2010
Date

SUMMARY

This satellite micronucleus study was designed to assess the potential induction of micronuclei by Liquified Petroleum Gas in bone marrow cells of the rat. Animals were exposed for 6 hours daily for 13 weeks (5 days per week) by whole-body inhalation administration of the test substance at exposure levels of 1000, 5000 and 10000 ppm.

The test substance and negative control were administered by whole-body inhalation. The negative control group received clean air. A positive control group was dosed on one occasion by intraperitoneal injection, with cyclophosphamide at 40 mg/kg bodyweight.

Bone marrow smears were obtained from five male and five female animals in the negative control and each of the test substance groups approximately 18 hours after the final exposure and from the positive control group within 24 hours of dosing. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. The proportion of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated mature erythrocytes was also kept.

No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the proportion of immature erythrocytes were observed in rats treated with Liquified Petroleum Gas compared to negative control values ($P > 0.01$ in each case).

The positive control compound, Cyclophosphamide, produced significant increases in the frequency of micronucleated immature erythrocytes and a decrease in the proportion of immature erythrocytes ($P < 0.001$ and $P < 0.01$, respectively).

It is concluded that Liquified Petroleum Gas did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered by whole-body inhalation exposure in this *in vivo* test procedure.

INTRODUCTION

The purpose of this satellite micronucleus study was to assess the potential of Liquified Petroleum Gas to induce mutagenic effects in rats following inhalation administration using an *in vivo* cytogenetic system (Boller and Schmid 1970, MacGregor *et al* 1987, Mavournin *et al* 1990). The inhalation route was selected for use in this test as the most likely route of human exposure.

The procedures used were based on the recommendations of the following guidelines:

- OECD Guidelines for Testing of Chemicals 474. Adopted 21 July 1997, Mammalian Erythrocyte Micronucleus Test, August 1998.
- US EPA OPPTS Health Effects Test Guidelines 870.5395; Mammalian Erythrocyte Micronucleus Test, August 1998.

The bone marrow micronucleus test, originally developed by Matter and Schmid (1971), is a widely employed and internationally accepted short-term assay for identification of genotoxic effects (chromosome damage and aneuploidy) associated with mutagens and carcinogens (Mavournin *et al* 1990). This *in vivo* system allows consideration of various factors including pharmacokinetics, metabolism and DNA repair which cannot be accurately modelled in an *in vitro* system. Young adult rats are chosen for use because of the high rate of cell division in the bone marrow, the wealth of background data on this species, and their general suitability for toxicological investigations.

In mitotic cells in which chromosomal breakage has been caused by the test substance or its metabolites, acentric fragments of the chromosomes do not separate at the anaphase stage of cell division. After telophase these fragments may not be included in the nuclei of the daughter cells and hence will form single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. Micronuclei are seen in a wide variety of cells, but erythrocytes are chosen for examination since micronuclei are not obscured by the main nucleus and are therefore easily detected in this cell type (Boller and Schmid 1970).

Micronucleated immature erythrocytes appear in the bone marrow approximately 24 hours after induction of chromosome damage. These immature erythrocytes can be differentiated by a variety of staining techniques which rely on their relatively high content of residual RNA. An increased incidence of micronucleated immature erythrocytes is indicative of recent exposure to a chromosome-damaging agent. A simultaneous marked increase in the incidence of micronucleated mature erythrocytes is not expected and may be indicative of micronucleus-like artifacts (Schmid 1976).

Substances which interfere with the mitotic spindle apparatus will cause non-disjunction (unequal separation of the chromosomes at anaphase resulting in aneuploidy) or lagging chromosomes at anaphase which may not be incorporated into the daughter nuclei. These lagging chromosomes are not excluded from the erythroblast with the main nucleus and hence also give rise to micronuclei.

Any toxic effects of the test substance on the nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in the number of nucleated cells and immature erythrocytes; to compensate for this, peripheral blood is shunted into the bone marrow (von Ledebur and Schmid 1973). If the proportion of immature erythrocytes is found to be significantly less than the control value, this is taken as being indicative of toxicity. A very large decrease in the proportion would be indicative of a cytostatic or cytotoxic effect.

The slide evaluation phase of the satellite micronucleus study was performed at the Department of Genetic Toxicology, Huntingdon Life Sciences (ERC), Eye, Suffolk, IP23 7PX, England. Statistical analysis for the slide evaluation phase was performed at the Department of Statistics, Huntingdon Life Sciences (HRC), Huntingdon, Cambridgeshire, PE28 4HS, England.

The experimental start and completion dates of the slide evaluation phase of the study were 15 August 2005 and 1 September 2005, respectively.

EXPERIMENTAL PROCEDURE

In-life phase

The in-life phase of the study was carried out at the Princeton Research Center starting on 5 April 2005 and was completed on 27 July 2005.

All animals in the negative control and test substance groups were exposed for 13 weeks (5 days per week) by whole-body inhalation. The non-exposed positive control group was dosed with Cyclophosphamide administered on one occasion by intraperitoneal injection at a volume dosage of 10 mL/kg bodyweight. Cyclophosphamide (CP, CAS # 6055-19-2, lot number (084K1328) received (26 June 2005), expiration (August 2007), white powder, storage 2-8°C, purity 99.7%), was obtained from the Sigma Chemical Company (responsible for its characterization), and was dissolved and diluted in sterile distilled water at Huntingdon Life Sciences to stock concentrations of 4.0 mg/mL for use as the positive control for the micronucleus study.

The experimental design is shown below:

Group	Treatment	Exposure Level (ppm)	Animal Numbers	
			Male	Female
1	Air control	-	1316 - 1320	1816 - 1820
2	Liquified Petroleum Gas	1000	2316 - 2320	2816 - 2820
3	Liquified Petroleum Gas	5000	3316 - 3320	3816 - 3820
4	Liquified Petroleum Gas	10000	4316 - 4320	4816 - 4820
5	Cyclophosphamide	40 (mg/kg)	5311 - 5315	5811 - 5815

Five males and five females from the negative control and each of the test substance groups were sacrificed approximately 18 hours after the final exposure by exsanguination following carbon dioxide inhalation. Five males and five females from the positive control group were sacrificed, within 24 hours of CP dosing, by exsanguination following carbon dioxide inhalation. The right femur was exposed, cut just above the knee and the bone marrow was aspirated into a syringe containing a small volume (about 0.5 mL) of serum. The cells were then flushed into a centrifuge tube of cold serum. The tubes were identified by labels containing the study, group number and animal number.

The bone marrow cells were pelleted by centrifugation at about 150 x g for about 5 min and the supernatant drawn off, leaving a small amount of serum with the cell pellet. The cells were resuspended by aspiration with a pasteur pipette and a small drop of cells was spread onto a clean glass slide. Four slides were prepared from each animal. The slides were allowed to air dry, fixed by dipping for about 3 minutes in methanol, and aged overnight or longer prior to staining. Slides were labelled with experiment and animal number using a lead pencil.

Two slides from each animal were despatched to Huntingdon Life Sciences (ERC), Eye, Suffolk, IP23 7PX, England for slide staining and analysis. The remaining 2 smears and the cell pellet (refrigerated) were held in reserve at PRC in case of technical problems with the first 2 smears.

Fixation and staining of slides

One slide from each animal was stained as follows:

1. Rinsed in purified water.
2. Stained in acridine orange solution (0.01 mg/mL using purified water) for 3 minutes.
3. Washed in purified water for 5 minutes.
4. Rinsed in cold tap water for 2 minutes.
5. Stored in the dark at *ca* 4°C for a minimum of 1 hour until required.
6. Immediately prior to scoring, slides are wet mounted with glass coverslips using purified water.

Microscopic examination

The stained smears were examined (under code) by fluorescent microscopy to determine the incidence of micronucleated cells per 2000 immature erythrocytes per animal. One smear per animal was examined. The remaining smears were held temporarily in reserve in case of technical problems with the first smear.

The proportion of immature erythrocytes for each animal was assessed by examination of at least 1000 erythrocytes. A record of the number of micronucleated mature erythrocytes observed during assessment of this proportion was also kept as recommended by Schmid (1976). The number of micronuclei in mature erythrocytes was then extrapolated to 2000 cells (see Table I).

ASSESSMENT OF RESULTS

The results for each treatment group were compared with the results for the concurrent negative control group using non-parametric statistics. Non-parametric statistical methods were chosen for analysis of results because:

- They are suited to analysis of data consisting of discrete/integer values with ties such as the incidence of micronucleated immature erythrocytes.
- The methods make few assumptions about the underlying distribution of data and therefore the values do not require transformation to fit a theoretical distribution (where data can be approximately fitted to a normal distribution, the results of non-parametric analysis and classical analysis of variance are very similar).
- 'Outliers' are frequently found in the proportion of immature erythrocytes for both control and treated animals; non-parametric analysis based on rank does not give these values an undue weighting.

Unless there is a substantial difference in response between sexes, results for the two sexes are combined to facilitate interpretation and maximise the power of statistical analysis.

For incidences of micronucleated immature erythrocytes, exact one-sided p-values are calculated by permutation (StatXact, CYTEL Software Corporation, Cambridge, Massachusetts). Comparison of several dose levels is made with the concurrent control using the Linear by Linear Association test for trend, in a step-down fashion if significance is detected (Agresti *et al.* 1990); for individual inter-group comparisons (*ie* the positive control group) this procedure simplifies to a straightforward permutation test (Gibbons 1985). For assessment of effects on the proportion of immature erythrocytes, equivalent permutation tests based on rank scores are used, *ie* exact versions of Wilcoxon's sum of ranks test and Jonckheere's test for trend.

A positive response is normally indicated by a statistically significant dose-related increase in the incidence of micronucleated immature erythrocytes for the treatment group compared with the concurrent control group ($P < 0.01$); individual and/or group mean values should exceed the laboratory historical control range (Morrison and Ashby 1995).

A negative result is indicated where individual and group mean incidences of micronucleated immature erythrocytes for the group treated with the test substance are not significantly greater than incidences for the concurrent control group ($P > 0.01$) and where these values fall within the historical control range. An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of immature erythrocytes ($P < 0.01$).

MAINTENANCE OF RECORDS

All raw data, samples and specimens arising from the performance of this phase of the study will remain the property of the Sponsor.

Types of sample and specimen that are unsuitable, by reason of instability, for long term retention and archiving may be disposed of.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences PRC in its archive for a period of one year from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and their advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

RESULTS

MICRONUCLEUS TEST

Table 1 gives a summary of the results of the micronucleus test and the results of statistical analysis. The results for individual animals are presented in Table 2.

Micronucleated immature erythrocyte counts (mie)

The test substance did not cause any statistically significant increases in the number of micronucleated immature erythrocytes [$P > 0.01$].

Cyclophosphamide caused significant increases in the frequency of micronucleated immature erythrocytes [$P < 0.001$].

Micronucleated mature erythrocytes (mme)

The test substance did not cause any substantial increases in the incidence of micronucleated mature erythrocytes.

Proportion of immature erythrocytes

The test substance did not cause any significant decreases in the proportion of immature erythrocytes [$P > 0.01$].

Cyclophosphamide caused a statistically significant decrease in the proportion [$P < 0.01$].

CONCLUSION

No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the proportion of immature erythrocytes were observed in rats treated with Liquified Petroleum Gas compared to negative control values ($P > 0.01$ in each case).

It is concluded that Liquified Petroleum Gas did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered by whole-body inhalation in this *in vivo* test procedure.

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TABLE 1

Summary of results and statistical analysis

Sampling time after final exposure	Treatment	Exposure level (ppm)	Proportion of ie (group mean ±SD) †	Incidence mie ^b (group mean ±SD)	Incidence mme ^c (group mean)
18 Hours	Negative control	0	48 ± 4.0	1.4 ± 1.2	0.7
	Liquified Petroleum Gas	1000	46 ± 5.5	2.1 ± 1.3	0.0
	Liquified Petroleum Gas	5000	46 ± 3.7	1.4 ± 1.3	0.3
	Liquified Petroleum Gas	10000	46 ± 5.1	1.5 ± 1.0	0.0
	Cyclophosphamide	40 mg/kg ^a	37 ± 7.1**	8.1 ± 4.7***	0.6

Negative control Air only

^a Positive control dosed via the intraperitoneal route on a single occasion and sacrificed within 24 hours of dosing.

^b Number of micronucleated cells (mie) observed per 2000 immature erythrocytes examined

^c Number of micronucleated mature erythrocytes (mme) observed during scoring of proportion of immature erythrocytes and calculated for 2000 cells:

$$\frac{\text{Sum of group incidence mme scored} \times 2000}{\text{Sum of group me scored}}$$

ie Immature erythrocytes
SD Standard deviation

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

*** P < 0.001 (significant)
** P < 0.01 (significant)
otherwise P > 0.01 (not significant)

† Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table

TABLE 2

Results for individual animals

Treatment	Exposure (ppm)	Animal number	ie	me	Total cells ^a	Incidence mme ^b	Proportion of ie	Incidence mie ^c
Negative control	0 (air only)	M 1316	629	589	1218	0	52	1
		M 1317	728	701	1429	0	51	2
		M 1318	524	543	1067	0	49	1
		M 1319	504	524	1028	0	49	4
		M 1320	505	562	1067	0	47	1
		F 1816	561	564	1125	0	50	1
		F 1817	570	621	1191	1	48	2
		F 1818	459	674	1133	0	41	0
		F 1819	461	674	1135	0	41	0
		F 1820	585	578	1163	1	50	2
Liquified Petroleum Gas	1000	M 2316	569	654	1223	0	47	2
		M 2317	529	716	1245	0	42	4
		M 2318	524	585	1109	0	47	2
		M 2319	628	532	1160	0	54	0
		M 2320	435	699	1134	0	38	2
		F 2816	646	634	1280	0	50	1
		F 2817	401	609	1010	0	40	4
		F 2818	466	550	1016	0	46	3
		F 2819	441	676	1117	0	39	2
		F 2820	569	532	1101	0	52	1
Liquified Petroleum Gas	5000	M 3316	537	557	1094	0	49	1
		M 3317	484	678	1162	0	42	0
		M 3318	592	628	1220	0	49	2
		M 3319	550	656	1206	0	46	2
		M 3320	463	671	1134	1	41	4
		F 3816	502	549	1051	0	48	3
		F 3817	505	541	1046	0	48	1
		F 3818	521	510	1031	0	51	0
		F 3819	547	571	1118	0	49	0
		F 3820	422	601	1023	0	41	1
Liquified Petroleum Gas	10000	M 4316	424	607	1031	0	41	2
		M 4317	572	608	1180	0	48	0
		M 4318	632	655	1287	0	49	1
		M 4319	533	585	1118	0	48	1
		M 4320	421	629	1050	0	40	1
		F 4816	476	547	1023	0	47	1
		F 4817	391	657	1048	0	37	3
		F 4818	500	512	1012	0	49	1
		F 4819	543	536	1079	0	50	2
		F 4820	584	509	1093	0	53	3

^a Number of erythrocytes scored to determine proportion of immature erythrocytes^b Number of micronucleated mature erythrocytes (mme) observed during scoring of proportion of immature erythrocytes (a)^c Number of micronucleated cells (mie) observed per 2000 immature erythrocytes

ie Immature erythrocytes me Mature erythrocytes

TABLE 2 – continued
Results for individual animals

Treatment	Exposure	Animal number	ie	me	Total cells ^a	Incidence mme ^b	Proportion of ie	Incidence mie ^c
CPA	40 (mg/kg)	M 5311	303	703	1006	1	30	12
		M 5312	469	598	1067	0	44	6
		M 5313	428	720	1148	0	37	6
		M 5314	297	830	1127	0	26	12
		M 5315	623	845	1468	0	42	8
		F 5811	325	689	1014	1	32	6
		F 5812	505	503	1008	0	50	10
		F 5813	426	829	1255	0	34	2
		F 5814	474	733	1207	0	39	17
		F 5815	446	732	1178	0	38	2

^a Number of erythrocytes scored to determine proportion of immature erythrocytes

^b Number of micronucleated mature erythrocytes (mme) observed during scoring of proportion of immature erythrocytes (a)

^c Number of micronucleated cells (mie) observed per 2000 immature erythrocytes

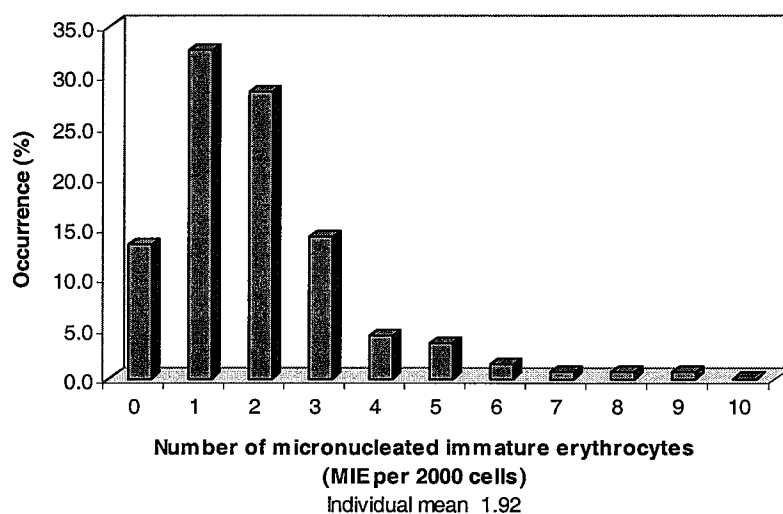
ie Immature erythrocytes

me Mature erythrocytes

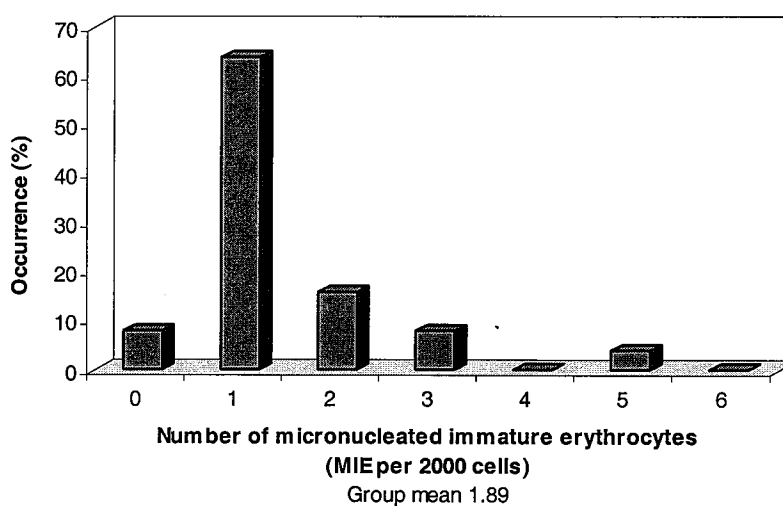
CPA Cyclophosphamide

APPENDIX 1**HISTORICAL CONTROL DATA**

Historical Negative Control Values (November 2003 to March 2005)
Frequency of micronucleated immature erythrocytes (Individual animals)

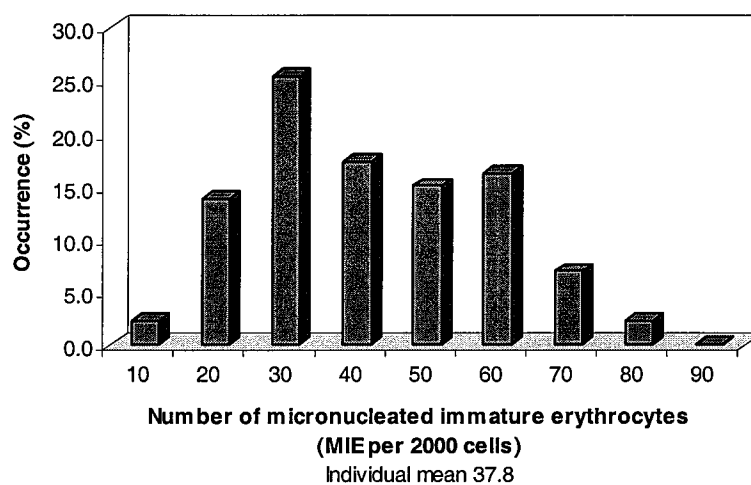


Historical negative control values (November 2003 - March 2005)
Frequency of micronucleated immature erythrocytes (Group mean values)

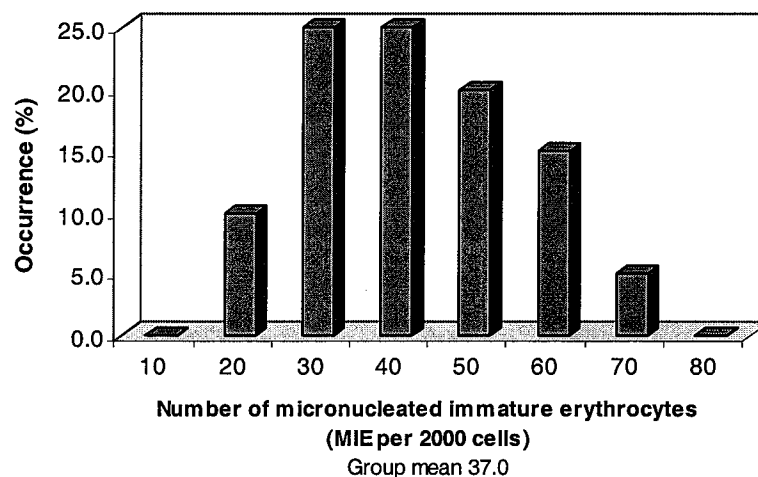


APPENDIX 1: HISTORICAL CONTROL DATA – continued

Historical positive control values (November 2003 - March 2005)
Frequency of micronucleated immature erythrocytes (Individual animals)
Cyclophosphamide



Historical positive control values (November 2003 - March 2005)
Frequency of micronucleated immature erythrocytes (Group mean values)
Cyclophosphamide



APPENDIX 2

GLP COMPLIANCE STATEMENTS

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

TEST TYPE

Huntingdon Life Sciences
Eye Research Centre
Eye
Suffolk
IP23 7PX

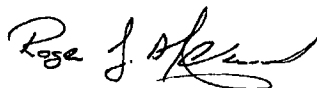
Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

29th January 2001

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


3/4/01

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

LABORATORY

Huntingdon Life Sciences
Huntingdon Research Centre
Wooley Road
Alconbury
Huntingdon
Cambs.
PE28 4HS

TEST TYPE

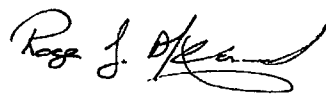
Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

15th January 2001

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


3/4/01

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

LABORATORY	TEST TYPE
Huntingdon Life Sciences Eye Research Centre Occold Eye Suffolk IP23 7PX	Analytical Chemistry Ecosystems Environmental Fate Environmental Toxicity Mutagenicity Toxicology Phys/Chem Tests

DATE OF INSPECTION
22nd April 2003

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to read "Roger G. Alexander", with the date "25/7/03" written below it.

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

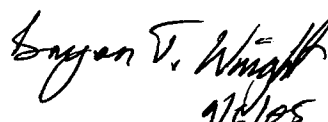
LABORATORY	TEST TYPE
Huntingdon Life Sciences	Analytical Chemistry
Eye Research Centre	Clinical Chemistry
Occold	Ecosystems
Eye	Environmental Fate
Suffolk	Environmental Toxicity
IP23 7PX	Mutagenicity
	Toxicology
	Phys/Chem Testing

DATE OF INSPECTION

12th April 2005

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


Mr. Bryan J. Wright
Head, UK GLP Monitoring Authority



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

28th January 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.


4/03/08

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



MHRA



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Osoold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical/Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology

DATE OF INSPECTION

17-19 February 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to be 'A. Gray', with the date '1/5/09' written below it.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority





**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC**

TEST FACILITY

TEST TYPE

Huntingdon Life Sciences Ltd.
Huntingdon Research Centre
Woolley Road
Alconbury
Cambridgeshire
PE28 4HS

Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Toxicology

DATE OF INSPECTION

2nd November 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

A handwritten signature in black ink, appearing to read 'A. Gray', with the date '11/01/10' written below it.

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority



PROTOCOL

LIQUIFIED PETROLEUM GAS

**A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS
WITH NEUROTOXICITY ASSESSMENTS AND
IN VIVO GENOTOXICITY ASSESSMENTS**

CONFIDENTIAL

HLS Study No.:

03-6141

Protocol No.:

Final

Date:

5 April 2005

Huntingdon Life Sciences
Study No. 03-6141

Page 2
Final Protocol

PROTOCOL SIGNATURES / PREFACE

(Confidential Information – to be distributed on a need-to-know basis)

Study Title: Liquified Petroleum Gas : A 13-Week Whole-Body
 Inhalation Toxicity Study in Rats with Neurotoxicity
 Assessments and *In Vivo* Genotoxicity Assessments

HLS Study No.: 03-6141

This is the Final Protocol. It has been reviewed and approved by:


 7 APR 05
Date

Huntingdon Life Sciences (HLS)
Address: 100 Mettlers Road
 East Millstone, NJ 08875-2360
Phone No.: 732-873-2550 x2920
Fax No.: 732-873-3992
Email: 


 April 6, 2005
Date

American Petroleum Institute (API)
Address: 1220 L Street, Northwest
 Washington, D.C. 20005-4070
Phone No.: 202-682-8480
Fax No.: 202-682-8270
Email: 

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1. INTRODUCTION

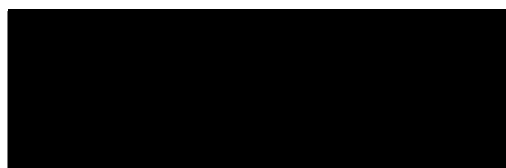
HLS Study No.	03-6141
Study Title	Liquified Petroleum Gas: A 13-Week Whole-Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and <i>In Vivo</i> Genotoxicity Assessments
Testing Facility	Huntingdon Life Sciences 100 Mettlers Road East Millstone, NJ 08875-2360
Purpose	This study is designed to assess the potential inhalation toxicity of a test substance when administered via whole-body exposures to rats for at least 13 weeks. The assessment will include routine toxicology parameters as well as detailed evaluations of neurotoxicity and genotoxicity parameters.

2. STUDY PERSONNEL

Study Director:



Alternate Contact:



Additional personnel will be documented in the project file and presented in the final report.

3. PROPOSED STUDY DATES

Study Initiation Date:	Date Study Director signs protocol
Receipt of Test Animals:	5 April 2005(Experimental Start)*
Initiation of Exposures:	20 April 2005(Experimental Start)**
Termination of Exposures:	26 July 2005
Necropsy:	20-27 July 2005
Submission of Draft Final Report:	30 November 2005
Experimental Termination:	Date of last data collection = date pathologist signs final report
Study Completion Date:	Date final report is signed by Study Director

*as per OECD GLPs; ** as per EPA GLPs

4. EXPERIMENTAL DESIGN

Group	Exposure Level ^a (ppm)	Number of Animals (MAIN STUDY)							
		Initial		Clinical Laboratory Studies		Necropsy ^b		Microscopic Pathology	
				Terminal		Terminal		Terminal	
		M	F	M	F	M	F	M	F
1 (control)	0 (air only)	10	10	10	10	10	10	10	10
2 (low)	1000	10	10	10	10	10	10	A.R.	A.R.
3 (mid)	5000	10	10	10	10	10	10	A.R.	A.R.
4 (high)	10000	10	10	10	10	10	10	10	10

^aExposures will be 6 hours/day, generally 5 days/week for 13 weeks for at least 65 exposures for Main Study. Exposure levels are expressed as ppm of test substance. The exposures will be conducted via whole-body exposure. Exposure levels were determined by a Range-Finding Study 03-6140.

^bComplete postmortem evaluations will also be performed on animals which are found dead or euthanatized in a moribund condition during the course of the study.

A.R. = As Required: 1) Target organs/tissues identified by Group 4 evaluations at Terminal Sacrifice (additional cost); 2) Lungs will be examined microscopically for all animals in all groups at the Terminal sacrifice interval; 3) All tissues from animals found dead or euthanatized in a moribund condition during the course of the study (additional cost); 4) Gross lesions (additional cost).

M = Male; F = Female; The first day of exposure will be Day 0 for the study.

EXPERIMENTAL DESIGN (CONTINUED)

Group	Exposure Level ^a	Number of Animals (SATELLITE STUDIES) ^b			
		Neuropathology		Genotoxicity (Micronucleus)	
		M	F	M	F
1 (control)	0 (air only)	5	5	5	5
2 (low)	1000	5	5	5	5
3 (mid)	5000	5	5	5	5
4 (high)	10000	5	5	5	5
5 (positive control)	40 mg/kg	0	0	5	5

^aExposures will be 6 hours/day, generally 5 days/week for 13 weeks for at least 65 exposures for the Neuropathology and Genotoxicity Studies. Exposure levels are expressed as ppm of test substance. The exposures will be conducted via whole-body exposure. Exposure levels were determined by a Range-Finding Study 03-6140.

^bThis number of animals includes 5/sex/group designated for neuropathology after the Terminal Sacrifice and 5/sex/group designated for Genotoxicity (Micronucleus) evaluations following a 13 week exposure period. **The Genotoxicity animals for Groups 1-4 will not be separate animals but will be selected from the Main Study animals outlined on the prior page.** For the Genotoxicity evaluation, a separate group (Group 5) of animals (5/sex) will be used as positive control animals for these evaluations. These positive control animals will be given a dose of 40 mg/kg Cyclophosphamide (CP) intraperitoneally (IP) within 24 hours prior to sacrifice. These positive control animals will not be chamber exposed prior to dosing and sacrifice. The CP solutions prepared for dosing will **not** be assayed for purity, uniformity and stability as per GLP's.

M = Male; F = Female; The first day of exposure will be Day 0 for the study.

4.1. JUSTIFICATION FOR ROUTE, DURATION AND FREQUENCY OF ADMINISTRATION

The inhalation route is one of the potential routes of human exposure to this test substance. The duration and frequency of the exposures are as recommended in the relevant OECD and EPA guidelines.

4.2. JUSTIFICATION FOR TEST ANIMAL SELECTION

The rat is used as a surrogate for humans in the detection of toxicity and is a species in which known toxicants and mutagens have been detected. This rodent species is commonly used in the conduct of toxicity studies and is that recommended by the relevant OECD and EPA guidelines. Historical control data are also available with this strain of rat for comparative evaluation, if necessary.

4.3. JUSTIFICATION FOR NUMBER OF ANIMALS

The number of animals in this study is considered the minimum necessary to allow for meaningful interpretation of the data as required by OECD and EPA guidelines. This includes a guideline required positive control group for the genotoxicity evaluation.

4.4. EXPOSURE LEVEL SELECTION AND JUSTIFICATION

LPG: Based on results of range-find testing 03-6140 which showed no effects at 10000 and 1000 and 100 ppm. The exposure levels were also selected based on establishing (for safety reasons) the high exposure level as no more than 50% of the lower explosion limit ($LEL = 2.1\% = 21000 \text{ ppm}$) for the test substance.

CP: Based on results of 7 prior studies (e.g. 00-6125) at this Testing Facility, the single IP dose of 40 mg/kg will result in no clinical toxicity prior to sacrifice 1 day later. This IP dose of CP (in the absence of a similarly practical and effective inhalable agent) has been previously shown (e.g. 00-6125) to induce a significant genotoxic effect demonstrating the sensitivity of this colony of test animals to a genotoxic agent.

5. TEST SUBSTANCE

5.1. TEST SUBSTANCE	Liquified Petroleum Gas
Test Substance Category	Industrial gas

Description, lot number, storage, expiration date (if available) and handling procedures, as well as other pertinent information will be documented in the study data and final report. The test substance is supplied by the Sponsor and will be stored (ambient conditions) in an outside solvent shed except when in use in the inhalation laboratory. The test substance will be handled as a flammable gas.

5.2. IDENTIFICATION OF TEST SUBSTANCE

Unless otherwise noted, the identity, strength, composition, stability and method of synthesis, fabrication and/or derivation of each batch of the test substance will be documented by the Sponsor before its use in the study. This documentation will be maintained by the Sponsor at the address indicated on the signature page of this protocol. The storage stability will be the responsibility of the Sponsor but the stability of the test substance under the conditions of use is the responsibility of the Testing Facility (see section 7.6).

5.3. ARCHIVAL SAMPLES

Sponsor will arrange for storage of an archival sample at a contract archival facility (EPL Archives, Inc., 45610 Terminal Drive, Sterling, VA 20166 20166, 703-435-8780 ext 201: Sam Busey). Since multiple studies are conducted with the same test substance, a common archival sample will be taken and appropriately labeled. Laboratory will arrange for safe transportation of the archival sample to the archival facility, in compliance with all applicable regulations.

5.4. UNUSED TEST SUBSTANCE

Following completion of the final report for the final study with this test substance, the Testing Facility will inform the Sponsor of the volume of remaining test substance as well as the number of test substance containers. The Sponsor will then instruct the Testing Facility whether to dispose of the remaining test substance and containers or to ship the test substance and containers to another location. No test substance or container (even empty ones) will be disposed of until the issuance of the final report.

6. TEST ANIMALS

Albino Rats (Outbred) VAF/Plus®

6.1. SPECIES

Sprague Dawley - derived (CD®)
[CrI: CD® IGS BR]

6.2. SUPPLIER

Charles River Laboratories, Kingston, NY.

Documentation of the specific breeding facility will be maintained in the study file and included in the final report.

6.3. ANIMAL REQUIREMENTS/SPECIFICATIONS

6.3.1. NUMBER ON TEST

Total	Males	Females
130	65	65

6.3.2. AGE

Study Phase	Age at Receipt (weeks)	Age at Exposures Initiation (weeks)
Main/Genotoxicity	~6	~8
Neuropathology	~6	~8

Note: Females will be nulliparous and non-pregnant.

6.3.3. WEIGHT

Approximately 225 to 275 grams (males) and 175 to 225 grams (females) at first exposure. Animals outside of this range may be used at the discretion of the Study Director upon Sponsor approval.

6.4. ACCLIMATION PERIOD

Approximately two weeks; all animals will be checked for viability twice daily. Prior to assignment to study all animals will be examined by a staff member to ascertain suitability for study.

6.5. ANIMAL HUSBANDRY/NON-EXPOSURE

6.5.1. HOUSING

Animals will be housed individually in suspended stainless steel wire mesh cages. Cages will be arranged in such a way that possible effects due to placement are minimized. Each cage will be fitted to secure a glass feeder jar with a stainless steel lid. Clean feeder jars and fresh feed will be provided at least weekly. Clean feed jars and fresh feed will be provided at least weekly for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded.

6.5.2. FEED & FEED ANALYSIS

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St. Louis, MO) *ad libitum*.

Analytical certification of batches of feed will be maintained on file at the Testing Facility and included in the final report. There are no known contaminants in the feed that are expected to interfere with the results of this study.

6.5.3. WATER & WATER ANALYSIS

Elizabethtown Water Company, Westfield, NJ; *ad libitum*, via automated watering system.

Monthly water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis, provided by the supplier, will be maintained on file at the Testing Facility and included in the final report. In addition, internal biannual chemical and microbiological water samples collected from representative rooms in this facility are assayed by a subcontract laboratory (Benchmark Analytical, Center Valley, PA). Results are maintained on file at the Testing Facility and included in the final report. There are no known contaminants in the water that are expected to interfere with the results of this study.

6.5.4. VETERINARY CARE

Animals are monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, a staff veterinarian will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of this protocol. **The staff veterinarian will approve the animals for inclusion on test.**

6.5.5. ENVIRONMENTAL CONDITIONS

Light/Dark Cycle

Twelve hour light/dark cycle provided via automatic timer.

Temperature

Temperature will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18-26°C is maintained to the maximum extent possible.

Humidity

Humidity will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 30 to 70% is maintained to the maximum extent possible.

6.6. ANIMAL HUSBANDRY DURING EXPOSURE

6.6.1. HOUSING Individually in stainless steel wire mesh cages.

6.6.2. FEED None.

6.6.3. WATER None.

6.7. SELECTION FOR STUDY

More animals than required for the study will be purchased and acclimated. Animals considered suitable for study on the basis of pretest physical examinations, body weight data and any other pretest evaluations will be 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 shall be within +/- 20% of the mean weight for each sex for each study. Disposition of all animals not utilized in the study will be maintained in the study file.

6.8. ANIMAL IDENTIFICATION

Each animal will be assigned a temporary identification number upon receipt. After selection for study, each animal will be ear-tagged by the Testing Facility with a number assigned by the Testing Facility. The number assigned by the Testing Facility plus the study number will comprise the unique animal number for each animal. If the tag is lost, it will be replaced or the animal will be tattooed for identification. Each cage will be provided with a cage card that will be color-coded for exposure level identification and will contain the study number and animal number.

7. TEST SUBSTANCE ADMINISTRATION

7.1. ROUTE OF ADMINISTRATION

Inhalation via whole-body exposures.

7.2. JUSTIFICATION FOR ROUTE OF ADMINISTRATION

The inhalation route is one of the potential routes of human exposure to this test substance and is the route specified in the referenced guidelines.

7.3. FREQUENCY OF ADMINISTRATION

Once daily, 6 hours/day.

7.4. DURATION OF ADMINISTRATION

The test substance will be administered for 6 hours/day, generally 5 days per week for 13 weeks for at least 65 exposures for Main/Neuropathology/Genotoxicity Studies. Test substance administration will continue through the day prior to necropsy for all animals.

7.5. ADMINISTRATION OF TEST SUBSTANCE

The test substance will be administered as a gas in the breathing air of the animals. The test atmosphere will be generated by an appropriate procedure determined during pre-study trials. The trials will be performed (at least three 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable and uniform atmosphere at the target exposure levels. The method will be described in the raw data of the study and in the report.

The whole-body exposure chambers will each have a volume of approximately 1000 liters. Each chamber will be operated at a minimum flow rate of 200 liters per minute. The final airflow will be set to provide at least one air change in 5.0

minutes (12 air changes/hour) and a T₉₉ equilibrium time of at most 23 minutes. This chamber size and airflow rate is considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of the exposure, all animals will remain in the chamber for a minimum of the T₉₉ equilibrium time. During this time, the chamber will be operated at approximately the same flow rate using clean air only.

7.6. EXPOSURE CONCENTRATION DETERMINATION

A nominal exposure concentration will be calculated daily. The flow of air through the chamber will be monitored using appropriate calibrated equipment (dry test meter). The test substance consumed during the exposure (weight difference of test substance cylinder) will be divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each exposure, measurements of airborne concentrations will be performed in the animals' breathing zone at least 4 times using an appropriate sampling procedure (samples drawn through the IR monitor by a vacuum pump) and infrared spectrophotometric analytical procedure (samples assayed at 10.9 μ m wavelength). Also, one sample (gas tight syringe) per chamber per week (as well as at start and end of the pre-study trials) will be analyzed by gas chromatography (GC) to characterize at least 5 major components (comprising at least 90% by weight of the test substance) to show test substance stability and comparison between the neat test substance and the test atmospheres.

If more than the normal amount of trials is required because of test substance generation or monitoring problems (80 technician hours), the Sponsor will be consulted prior to additional trials (additional cost).

7.7. PARTICLE SIZE DISTRIBUTION ANALYSIS

During each week of exposure, particle size determinations will be performed using a TSI Aerodynamic Particle Sizer to demonstrate the absence of any condensation aerosol.

7.8. CHAMBER AND EXPOSURE ROOM ENVIRONMENT

Chamber temperature, humidity, airflow rate and static pressure will be monitored continuously and recorded every 30 minutes during exposure. Chamber temperature and relative humidity will be maintained, to the maximum extent possible, between 20 to 24°C and 40 to 60%, respectively. Chamber oxygen levels (maintained at least 19%) will be measured pretest and at the beginning, middle and end of the study.

Air samples will be taken in the vapor generation area pretest and at the beginning, middle and end of the study. Light (maintained approximately 30-40 foot-candles at 1.0 meter above the floor) and noise levels (maintained below 85 decibels) in the exposure room will be measured pretest and at the beginning, middle and end of the study.

7.9. SUMMARY OF CHAMBER ACTIVITY

The minimum frequency of chamber activity is summarized below:

Activity	Frequency/chamber
Measured Test Substance Concentration	4X/day
Measured Test Substance Characterization	1X/week
Particle Size	1X/week
Temperature	13X/day
Relative Humidity	13X/day
Airflow Rate	13X/day
Static Pressure	13X/day
Nominal Test Substance Concentration (excluding the air control chamber)	1X/day
Rotation Pattern of Exposure Cages	1X/week
Loading/Unloading Verification	1X/day

8. EXPERIMENTAL EVALUATION

8.1. OBSERVATIONS

8.1.1. VIABILITY CHECKS (IN-CAGE)

Observations for mortality, general appearance and signs of severe toxic or pharmacological effects will be made at least twice daily. Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia.

8.1.2. PHYSICAL EXAMINATIONS

In-Chamber: All animals will be observed as a group at least once during each exposure. This will routinely be performed near the middle of each exposure and may be performed more frequently if significant signs of toxicity are noted. Pertinent behavioral changes and all signs of toxicity, including mortality, should be recorded. These signs should include time of onset, degree and duration.

Out-of-Chamber: Each animal (except Neuropathology animals) will be removed from its cage and examined twice pretest and once weekly during the study period. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) will be recorded as well as evaluations of respiration, palpation for tissue masses, circulatory effects, central nervous system effects, changes in motor activity, and reactivity to handling or sensory stimuli. During the treatment period these evaluations will be performed prior to exposures. Neuropathology animals will be removed from their cage and examined once pretest before selection onto study.

8.2. BODY WEIGHTS

Body weights of the male and female rats will be recorded at the time of randomization into test groups, on the day treatment is initiated and weekly thereafter throughout the study until euthanized (fasted).

8.3. FEED CONSUMPTION

Feed consumption will be measured (weighed) during the week prior to treatment initiation and weekly throughout the study. Feed will be available without restriction 7 days/week except during inhalation exposures and when fasting prior to blood collection. Animals will be presented with weighed feeders at the scheduled intervals. After 7 days, the feeders will be reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal per week. The grams consumed per kilogram of body weight per day will then be calculated for each animal.

8.4. OPHTHALMOSCOPIC EXAMINATION

All animals will be examined pretest and at study termination. Eyelids, lacrimal apparatus and conjunctiva will be examined grossly; cornea, anterior chamber, lens, vitreous humor, retina and optic disc will be examined by indirect ophthalmoscopy. The eyes will be examined after instillation of a mydriatic such as tropicamide.

8.5. NEUROBEHAVIORAL STUDIES

Observations, as outlined below, on male and female rats will be recorded. Testing will be staggered over several sessions and will be conducted on non-exposure days at least 16 hr post-exposure. Each session will consist of approximately equal numbers of animals/sex/exposure group. Testing will be performed on the Neuropathology and 5/sex/group of the Main Study animals. Noise level will be maintained within a level of 55 to 65 decibels by a white noise generator. Temperature, humidity and illumination will be measured and recorded to ensure that variations in environmental conditions are minimal during all evaluations. The functional observational battery will be performed for all animals before evaluation of motor activity.

8.5.1. FUNCTIONAL OBSERVATIONAL BATTERY

The functional observational battery will include assessments in the home cage, open field, and reflex testing. The time of testing will be balanced across treatment groups. All observations will be performed by trained observers who will be unaware of the animals' treatment. Positive historical control data demonstrating the sensitivity of the procedure will be documented in the final report.

Number of Animals/Intervals 10/sex/group pretest and
during the 2nd, 4th, 8th and
13th weeks of exposure

Parameters Evaluated

Evaluations will be performed according to the Testing Facility's Standard Operating Procedures which include defined scales for each function listed below. In addition, descriptive comments can be made during the testing period about behaviors that are not adequately described by the defined scales. Testing will proceed from least to the most interactive with the subject.

1. Assessment of signs of autonomic function:
 - Ranking and degree of lacrimation and salivation
 - Presence or absence of piloerection and exophthalmos
 - Count of urination and defecation
 - Pupillary function and degree of palpebral closure
2. Description, incidence and severity of any convulsions, tremors or abnormal movements both in home cage and the open field
3. Ranking of reactivity to general stimuli, such as removal from cage or handling, with a range of severity scores from no reaction to hyperactivity
4. Ranking of the subjects general level of activity during observations of the unperturbed subject in the open field, with a range of severity scores from unresponsive to hyperactive
5. Description, ranking and incidence of posture and gait evaluations in the home cage and/or open field
6. Forelimb and hindlimb grip strength measurements
7. Quantitative measure of landing foot splay
8. Sensorimotor responses to visual and auditory stimuli and the response to a tail pinch
9. Body weight
10. Description and incidence of any abnormal behaviors, excessive or repetitive actions, altered fur appearance, or red/crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data

11. Ranking of righting ability

12. Body Temperature

8.5.2. MOTOR ACTIVITY

Activity will be monitored using an automated Photobeam Activity System (San Diego Instruments, Inc.). Sessions will be 60 minutes in length; each session will be divided into twelve 5-minute intervals. The time of testing will be balanced across treatment groups. Positive historical control data demonstrating the sensitivity of the procedure will be documented in the final report.

Number of Animals/Intervals	10/sex/group pretest and during the 2nd, 4th, 8th and 13th weeks of exposure
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8.6. ESTROUS CYCLICITY

Daily vaginal smears will be taken at approximately the same time each day, and the stage of estrous will be determined for each female (Main Study animals) for three weeks prior to and on the day of termination. Care will be taken to ensure that pseudo-pregnancy is not induced.

8.7. CLINICAL LABORATORY STUDIES

Clinical pathology procedures and parameters are based on those recommended in guidelines published by the Joint Scientific Committee for International Harmonization of Clinical Pathology Testing in "Harmonization of Animal Clinical Pathology Testing in Toxicity and Safety Studies", *Fund. Appl. Tox.*: 29, 198-201 (1996).

Blood will be obtained from lightly anesthetized (carbon dioxide/oxygen, 60%/40%) animals via puncture of the orbital sinus (retrobulbar). Rats will be fasted overnight prior to blood collection. The Main Study animals will be bled at the Terminal interval. Blood will be collected and studies performed as follows:

8.7.1. HEMATOLOGY STUDIES

Blood (~0.25 mL) for hematology studies will be collected into tubes containing EDTA anticoagulant.

Number of Animals	10/sex/group
Collection Interval	Termination

Tests (If inadequate volume of sample available, these parameters are in priority order)

erythrocyte count
hematocrit
hemoglobin concentration
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
leukocyte count (total and differential)
platelet count
mean platelet volume
red cell distribution width
reticulocyte count
peripheral blood smear (erythrocyte and platelet morphology)

To be evaluated only if deemed necessary by the Study Director and Sponsor, based on results of other hematology and/or pathology results (additional cost):

bone marrow differential count

8.7.2. COAGULATION STUDIES

Blood (~1.0 mL) for coagulation studies will be collected into tubes containing sodium citrate anticoagulant.

Number of Animals 10/sex/group

Collection Interval Termination

Tests (If inadequate volume of sample available, these parameters are in priority order)

prothrombin time
activated partial thromboplastin time

8.7.3. CLINICAL CHEMISTRY

Blood (~1.0 mL for all animals) for clinical chemistry studies will be collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum.

Number of Animals 10/sex/group

Collection Interval Termination

Tests (If inadequate volume of sample available, these parameters are in priority order)

alanine aminotransferase
alkaline phosphatase
aspartate aminotransferase
creatine kinase
gamma-glutamyl transferase
lactate dehydrogenase
urea nitrogen
bilirubin (total)
bilirubin conjugated (direct)
bilirubin unconjugated (indirect)
glucose
total protein
albumin
globulin (calculated as total protein - albumin = globulin)
albumin/globulin ratio (calculated)
creatinine
sodium
potassium
chloride
calcium
phosphorus, inorganic
cholesterol (total)
triglycerides

8.7.4. RETENTION/STORAGE OF SPECIMENS

Any remaining (frozen) serum, which may have limited storage stability, will be stored for up to six months after completion of assays and will then be discarded. Peripheral blood smears will be retained and archived with the study.

8.8. GENOTOXICITY EVALUATIONS

Note: The cyclophosphamide will be received from Sigma Chemical Company. The identity, strength, purity and composition or other characteristics to define the positive control article will not be determined by the Testing Facility. The positive control article will be characterized as per the Certificate of Analysis on file with the Testing Facility. The stability of the positive control article will not be determined by the Testing Facility. Analyses to determine the uniformity (as applicable) or concentration of the positive control mixture will not be performed by the Testing Facility. The stability of the positive control article mixture will not be determined by the Testing Facility.

Sprague Dawley-derived (CD[®]) rats, 5/sex/group, will be exposed to Liquefied Petroleum Gas via inhalation at exposure levels of 0, 1000, 5000 or 10000 ppm for a 13 week (5 days per week) exposure period. A group of non-exposed (5/sex) positive control animals (40 mg/kg cyclophosphamide, IP with a 4.0 mg/mL solution @ 10 mL/kg, within 24 hours prior to sacrifice) will also be dosed. The test animals will be sacrificed under carbon dioxide anesthesia. The time between last exposure and tissue harvest will be approximately 18 hours. The right femurs will be removed and fixed. Unstained slides (4 per animal – 2 for shipment and 2 for retention) will be prepared and shipped to HLS Eye, Suffolk UK Research Centre (attn: Principal Investigator, Christine Mason). Upon receipt, slides will be stained (Acridine orange) and evaluated using a fluorescent microscope for determination of micronucleus response as detailed in the referenced guidelines (see section 13.1). The Testing Facility will be responsible for the GLP compliance of this subcontractor.

9. POSTMORTEM

9.1. MACROSCOPIC POSTMORTEM EXAMINATIONS

Complete macroscopic postmortem examinations will be performed on all Main/Neuropathology animals including animals euthanatized in a moribund condition or for humane reasons or found dead; all abnormal observations will be recorded. The necropsy will include examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Examination of all Main Study females will include a vaginal smear examined to determine the stage of the estrous cycle.

9.2. TIME OF NECROPSY

9.2.1. MORIBUND AND HUMANELY SACRIFICED ANIMALS

Animals showing signs of severe debility, particularly if death appears imminent, will be euthanized to prevent loss of tissues through autolysis.

9.2.2. TERMINAL NECROPSY

Necropsy of all animals surviving to study termination will be performed after animals have been treated and/or held for the appropriate duration. Necropsy schedules will be established in order to assure that approximately equal numbers of males and females from each group will be examined on each day of necropsy and that examinations of animals of both sexes from each group will be performed at similar times of the day throughout the necropsy period.

9.3. METHOD OF EUTHANASIA

Exsanguination following carbon dioxide inhalation for Main Study animals.
See Section 8.8 for methods for Genotoxicity animals.
See Section 9.9 for methods for Neuropathology animals.

9.4. ORGAN WEIGHTS

Organs indicated below will be taken from all Main Study animals at the scheduled necropsy, weighed, recorded and organ/body and organ/brain weight ratios calculated. Organs will not be weighed for animals found dead or euthanatized in a moribund condition or for humane reasons during the course of the study. Prior to weighing, all organs will be carefully dissected and properly trimmed to remove fat and other contiguous tissue in a uniform manner. Organs will be weighed as soon as possible after dissection to avoid drying except the thyroid/parathyroids which will be weighed after fixation. Paired organs will be weighed together:

adrenals	kidneys	pituitary	testes
brain with brainstem	liver	prostate	thymus
epididymides	lungs	seminal vesicles	thyroid/parathyroids
heart	ovaries	spleen	uterus

9.5. TISSUES PRESERVED

Tissues listed in Appendix A will be obtained at necropsy for all Main Study rats from each exposure group and preserved in 10% neutral buffered formalin with the exception of eyes and testes which will be preserved in a modified Davidson's fixative for at least 24 hours and then stored in 10% neutral buffered formalin. Lungs (gravity method) and will be infused with 10% neutral buffered formalin for optimal preservation.

9.6. SPERM COUNT, MOTILITY AND MORPHOLOGY ASSESSMENTS

All of the sperm count, motility and morphology assessments are to be done at the Testing Facility using a Hamilton Thorne IVOS Sperm Analyzer.

All Main Study males euthanized at termination in each group will have the following sperm evaluations available for analysis: 1) motility; 2) a count of homogenization-resistant testicular sperm; 3) a count of caudal epididymal sperm; and 4) sperm morphology (cauda epididymis). Only samples for the high-exposure and control group will be analyzed if there is no effect in the high-exposure group. Evaluations will be performed as follows:

- The right testis from each animal will be removed intact, weighed (right and left testes weighed together) and preserved in modified Davidson's solution for at least 24 hrs prior to permanent storage in neutral buffered formalin for histopathological evaluation. The right epididymis from each animal will be removed intact, weighed (right and left epididymides weighed together) and preserved in neutral buffered formalin for histopathological evaluation.
- The left epididymis will be removed intact, weighed, and frozen on dry ice. The epididymides will be stored frozen at -70° C until evaluation for caudal sperm count. Each epididymis will be thawed and the caudal portion removed and weighed. A homogenized sample of the caudal epididymis will be stained and examined using the sperm analyzer. For each stained preparation, 20 fields will be counted. The total number of sperm in the caudal epididymis will be calculated and adjusted for the caudal epididymal weight. Additionally, for each male, two sperm morphology slides will be prepared, stained with Eosin and evaluated for morphological development. If a macroscopic abnormality is noted on the left testis or left epididymis, then the right testis and right epididymis will be evaluated and the left testis and left epididymis will be preserved as indicated for possible histopathology.
- The left vas deferens will be excised and placed in a prewarmed solution of phosphate buffered saline and 1% Bovine Serum Albumin. After a minimum three minute "swimout" period, a sample will be placed into a Hamilton Thorne IVOS sperm analyzer. Five fields will be selected and stored as digital images. These images will be analyzed for percent motility and transferred to optical media for permanent storage.
- The left testis will be removed and frozen on dry ice. The testis will be stored frozen at -20°C until processed for counting of homogenization-resistant sperm.

Tissues retained from these evaluations will be discarded following issuance of the final report following consultation with the Sponsor.

9.7. MICROSCOPIC PATHOLOGY EVALUATIONS

Slides of tissues listed in Appendix A (under Microscopic Examination) will be prepared and examined microscopically for all Main Study animals from the Terminal Sacrifice as indicated. Target organs will be designated by the Study Director, pathologist and/or Sponsor based on experimental findings; authorization will be obtained from the Sponsor prior to performing examinations. Note: Any abnormalities not noted during macroscopic postmortem examinations which are seen during histological processing will be recorded. Gross lesions will be examined at additional cost.

Histopathological examinations of the designated testes (see 9.6) will be conducted to identify potential treatment-related effects such as retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of spermatogenic cells into the lumen. The examination of the intact epididymis will be of a longitudinal section that will permit examination of the caput, corpus and cauda regions. These examinations will identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or the absence of clear cells in the cauda epididymal epithelium.

Histopathological examination of the ovary will include evaluation of five sections taken at least 100 μm apart from the inner third of each ovary. These examinations can detect depletion of the primordial follicle population and enumerate the total number of primordial follicles for comparison with the ovaries from control animals. These examinations can also confirm the presence or absence of growing follicles and corpora lutea in comparison to control ovaries. Qualitative evaluations will be performed by the Testing Facility. If significant test substance related findings are noted, then quantitative evaluations (Sponsor consulted, additional cost) may be performed by Pathology Associates International, Frederick, MD (Principal Investigator, Michael D. Mercieca). The Testing Facility will be responsible for the GLP compliance of this subcontractor.

9.8. STAINS

Standard stains used: hematoxylin and eosin on paraffin sections, toluidine blue on epoxy resin sections. Special stains may be employed on selected tissues to aid in making a diagnosis at the discretion of the Study Pathologist. Special stains may be employed at the request of the Sponsor (additional cost).

9.9. NEUROPATHOLOGY SACRIFICE

Animals (5/sex/group) will be anesthetized with an intraperitoneal injection (~1.5 mL/kg) of sodium pentobarbital and transcardially perfused with phosphate

buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer. After perfusion, the required tissues will be dissected out. Measurement of the size (length and width) and weight of the whole brain (cerebrum, cerebellum and pons-medulla) shall be made. All tissues will then be placed into a fresh solution of the same fixative prior to processing. Tissues listed below will be preserved for all designated Neuropathology animals (5/sex/group) after 13 weeks of treatment:

Tissue	Preserved (Groups)	Microscopic Examination (Groups)	
		1 and 4	2 and 3
brain (forebrain, central cerebrum, hippocampus, basal ganglia, midbrain, cerebellum and pons, medulla)	X	X	
eye with optic nerve	X	X	
spinal cord (cervical, thoracic, lumbar, cross and longitudinal sections)	X	X	
sciatic nerve (cross and longitudinal sections)	X	X	
tibial nerve (cross and longitudinal sections)	X	X	
sural nerve (cross and longitudinal sections)	X	X	
trigeminal ganglia	X	X	
dorsal root ganglia (from C ₃ -C ₆ and L ₄ -L ₆)	X	X	
dorsal root fibers (from C ₃ -C ₆ and L ₄ -L ₆)	X	X	
ventral root fibers (from C ₃ -C ₆ and L ₄ -L ₆)	X	X	
lungs and trachea	X	X	X
tissues with macroscopic findings	X	X	X

Peripheral nerves will be post-fixed in 1% osmium tetroxide, processed and embedded in epoxy resin and microtomed at approximately 2 microns. Sections will be stained with toluidine blue. All other tissues, including the brain, eye with optic nerve, spinal cord, trigeminal ganglia, dorsal root ganglia, dorsal and ventral root fibers, lungs and trachea will be processed by standard techniques, embedded in paraffin, microtomed at approximately 6 microns and stained with hematoxylin and eosin.

The tissues listed above will be examined microscopically for all animals as indicated. Tissues with macroscopic lesions will be examined in all animals (additional cost). Any abnormalities not noted during macroscopic postmortem examinations which are seen during histological processing will be recorded. If microscopic findings indicative of an effect of test substance administration are seen in high-exposure animals, then examinations will be made of these tissues/organs for low- and mid-exposure animals (additional cost). Additional examinations will be made only after consultation with the Sponsor and receipt of authorization from the Sponsor.

If any evidence of neuropathology is found, then the following procedure will be employed to obtain a more objective diagnosis of the exposure-response relationships (additional cost): All regions of the nervous system exhibiting any evidence of neuropathological changes will be included in the analysis. Sections from all exposure groups from each region will be coded and examined in a randomized order without knowledge of the code. The frequency and severity of each lesion will be recorded. After all samples have been rated, the code will be broken and statistical analysis performed to evaluate exposure-response relationships.

10. PRESERVATION OF RECORDS AND SPECIMENS

All data documenting experimental details and study procedures and observations will be recorded and maintained as raw data. At the completion of the study, all reports, raw data, preserved specimens and retained samples (including those of subcontractors) will be maintained in the Testing Facility's Archives for a period of one year after submission of the signed final report. Biological samples collected for clinical pathology will be discarded as described elsewhere in this protocol (section 8.7.4).

The Sponsor will be contacted in order to determine the final disposition of these materials. The Sponsor is responsible for all costs associated with the storage of these materials beyond one year from the issuance of the final report and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.

11. STATISTICAL EVALUATIONS

The following items will be analyzed statistically in the final report:

- mean body weight values and body weight changes (from pretest)
- mean feed consumption values (presented as grams of feed/kg of body weight/day)
- mean clinical laboratory values
- mean terminal organ weights, organ/body weight ratios and organ/brain weight ratios
- mean motor activity counts
- mean FOB data including forelimb and hindlimb grip strength measurements,

mean landing foot splay measurements and body temperatures
micronucleus counts
sperm analysis

11.1 The following mean measures will be analysed as described below:

- mean body weight values and body weight changes (from pretest)
- mean feed consumption values (presented as grams of feed/kg of body weight/day)
- mean clinical laboratory values
- mean terminal organ weights, organ/body weight ratios and organ/brain weight ratios
- sperm analysis

Mean values of all exposure groups will be compared to the mean value for the control group at each time interval. Evaluation of equality of group means will be made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Bartlett, 1937; Sokal and Rohlf, 1995; Snedecor and Cochran, 1967) will be performed to determine if groups have equal variances. For all parameters except organ weights, if the variances are equal, parametric procedures will be used; if not, nonparametric procedures will be used. Organ weight data will be analyzed only by parametric methods. The parametric method will be the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means are indicated, additional tests will be used to determine which means are significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method will be the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953) and if differences are indicated, Shirley's test (Shirley, 1977), Steel's test (Steel, 1959) or Pairwise Comparison with Bonferroni Correction (Games and Howell, 1976) will be used to determine which means differ from control. Bartlett's test for equality of variance will be conducted at the 1% significance level; all other statistical tests will be conducted at the 5% and 1% significance levels.

References for these procedures are:

Armitage, P. 1971. *Statistical Methods in Medical Research*. Oxford, UK: Blackwell Scientific Publications; Bartlett, M.S. 1937. Properties of sufficiency and statistical tests. *Proceedings of the Royal Society, Series A*, 160: 268-282; Cochran, W.G. and Cox, G.M. 1959. *Experimental Designs*, New York: John Wiley, pp. 100-102; Dunlap, W.P. and Duffy, J.A. 1975. Fortran IV Functions for Calculating Exact Probabilities Associated with Z, Chi-Square, T and F Values. *Behav. Res. Methods and Instrumentations* 7:59-60; Dunlap, W.P., Marx, M.S. and Agamy, G.G. 1981. Fortran IV functions for calculating probabilities associated with Dunnett's test. *Behav. Res. Methods and Instrumentation* 13: 363-366; Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatments

with a control. *Journal of the American Statistical Association* 50: 1096-1121; Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20-3: 482-491; Games, P.A. and Howell, J.F. 1976. Pairwise multiple comparison procedures with unequal n's and/or variances: a monte-carlo study. *Journal of Educational Statistics* 1: 113-125; Kruskal, W.H. and Wallis, W.A. 1952. Use of Ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 47: 583-621; Kruskal, W.H. and Wallis, W.A. 1953. Errata for Kruskal-Wallis (1952) *Journal of the American Statistical Association* 48: 907-911; Shirley, E.A.C. 1977. A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* 33: 386-389; Snedecor, G.W. and Cochran, W.G. 1967. *Statistical Methods*. 6th edition. Ames: Iowa State University Press; Sokal, R.R. and Rohlf, F.J. 1995. *Biometry*. 3rd Edition. San Francisco: W.H. Freeman pp. 369-371; Steel, R.G.D. 1959. A multiple comparison rank sum test: treatment versus control. *Biometrics* 15: 560-572.; Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27: 103-117; Williams, D.A. 1972. The comparison of several dose levels with a zero dose control. *Biometrics* 28: 519-531.

11.2 Motor Activity and Functional Observational Battery (FOB) Data:

All analyses will include sex as an independent variable. In those instances where there are significant effects of sex separate analyses by sex may be done to explain the nature of the effect. The analyses will be performed by Graham Healey, M.Sc. of Huntingdon Life Sciences, Cambridgeshire, England. The Testing Facility will be responsible for the Subcontractor's GLP compliance. After submission of the final report, all of the records of the Subcontractor will be shipped to the Testing Facility to be archived.

Motor Activity Data: The analysis of the motor activity will use a mixed model analysis of covariance with an unstructured error relationship among the 5 minute periods, and a first order autoregressive error structure on weeks. The pretest response will be used as the covariate. The residuals from the model will be tested for normality at the $p < 0.01$ level of significance by the Kolmogorov-Smirnov test for normality. If the residuals are not normally distributed the dependent variable (number of beam breaks) will be converted to a function of its percentile rank, then transformed by the inverse normal distribution and reanalyzed as above. The transformation is known as Blom's transformation where the rank order is replaced by $[\text{rank} - 0.375] / [n + 0.25]$, and reanalyzed [Blom, 1958].

FOB Data: Some of the sensorimotor and observational battery variables are measured on a continuous scale and some are nominal or count scale. All measures will be classified as either continuous or nominal. The analysis of the continuous variables will be conducted by a mixed model analysis of covariance with a first order autoregressive error structure on the time points. The pretest response will be used as the covariate. The residuals from the model will be tested for normality at

the $p < 0.01$ level of significance. Those variables that do not exhibit normally distributed residuals at the 0.01 level will be transformed by Blom's normalized rank transformation and reanalyzed [Blom, 1958]. The nominal data of the functional observational battery will be analyzed by a cumulative logit repeated measures analysis with contrast terms to test for differences between control and dosed groups [Agresti, 1989]. For the measures that are count data (such as number of rears or number of urinations), the count responses may be grouped into several data ranges to allow sufficient data in the cells. For example, the count of urinations might be separated into three groups of 0, 1 to 4, 5 or more, and analyzed. If these methods are not feasible due, for example, to excessive ties or zeroes, then suitable alternative methods will be considered.

References for these procedures are:

Agresti, Alan, "A survey of models for repeated ordered categorical response data", *Statistics in Medicine*, vol 8, 1989, pg 1209-1224. Blom, G., *Statistical Estimates and Transformed Beta Variables*, J. Wiley and Sons, NY, 1958. Galecki, AT, "General class of covariance structures for two or more repeated factors in longitudinal data analysis," *Communications in Statistics-Theory and Methods*, 23(11), 1994 pg 3105 -3119. Pinheiro, JC and Bates, DM, *Mixed-Effects Models in S and S-PLUS*, Springer-Verlag, NY, 2002. Shapiro, S.S. and Wilk, M.B., "An analysis of variance test for normality (complete samples)", *Biometrika*, 52, 1965, pg 591-611. (Shapiro-Wilk test). Stephens, M.A., 'EDF statistics for goodness of fit and some comparisons', *Journal of the American Statistical Association*, vol 69, 1974, pg 730-737 (Kolmogorov-Smirnov test).

11.3 Micronucleus Analysis:

The results obtained for each treatment group will be compared with the results obtained for the concurrent air control group using non-parametric statistical methods. Unless there is a substantial difference (subjective evaluation by Principal Investigator) in response between sexes (which occurs only rarely) results for the two sexes are combined to facilitate interpretation and maximize the power of statistical analysis. For incidences of micronucleated immature erythrocytes, exact one-sided p-values are calculated by permutation (StatXact, CYTEL Software Corporation, NC, USA). Comparison of several dose levels is made with the concurrent control using the Linear by Linear Association test for trend in a step-down fashion if significance is detected (Agresti et al. 1990); for individual inter-group comparisons (eg the positive control group) this procedure simplifies to a straightforward permutation test (Gibbons 1985). For assessment of effects on the proportion of immature erythrocytes, equivalent permutation tests based on rank scores are used, ie exact versions of Wilcoxon's sum of ranks test and Jonckheere's test for trend.

References for these procedures are:

AGRESTI, A., MEHTA, C.R. and PATEL, N.R. (1990) Exact inference for contingency tables with ordered categories. *Journal of the American Statistical Association*, **85**, 453. CYTEL (1995) *StatXact 3 for Windows: Statistical Software for Exact Nonparametric Inference*. Cytel Software Corporation, NC, USA. GIBBONS, J.D. (1985) *Nonparametric Statistical Inference*, 2nd edition, Marcel Dekker, New York.

12. REPORTING

12.1. STATUS REPORTS

Periodic verbal and written updates on study progress will be provided by the Study Director. In general, written status reports will be submitted weekly and at termination of the study.

12.2. FINAL REPORT

One unbound hard copy and one electronic copy of an audited draft report will be submitted following termination of the study. After receipt and review of the Sponsor's comments, appropriate changes will be made and an unbound hard copy and electronic copy of the revised report will be forwarded to the Sponsor for a final review. Upon receipt of the Sponsor's final comments, appropriate changes will be made and two hard copies and one electronic copy of a signed, final report will be issued. (Additional copies will be provided at additional cost). The report will minimally include:

12.2.1. Body of Report

- Compliance Statement (including Sponsor signature line)
- Quality Assurance Statement
- Summary
- Introduction
- Experimental Design (including justifications for exposure levels)
- Materials and Methods
- Results and Discussion
- Conclusion and No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) Statement
- Statistical Procedures
- Protocol Deviations and Study Impact Statements

12.2.2. Summary Tables

- Exposure data
- Mortality data
- Summary of physical in-life observations
- Mean ophthalmology data
- Mean body weight data (and graphs)
- Mean animal weight gain data
- Mean feed consumption data

- Mean hematology and coagulation data and blood cell counts
- Mean clinical chemistry data
- Mean functional observational battery data
- Mean positive control neurobehavioral studies
- Mean motor activity counts
- Sperm analysis results
- Mean organ weight and organ weight to body weight and brain weight ratios
- Summary of macroscopic pathology
- Summary of microscopic pathology
- Summary of neuropathology
- Summary of estrous cyclicity

12.2.1. Appendix tables (individual data)

- Individual animal termination history
- Individual physical in-life observations
- Individual body weight data and weight change data
- Individual feed consumption data
- Individual estrous cyclicity data
- Individual hematology and coagulation data and blood cell counts
- Individual clinical chemistry data
- Individual FOB data
- Individual motor activity counts
- Sperm analysis results
- Individual organ weight and organ weight to body weight and brain weight ratios
- Individual macroscopic pathology data, including female stage of estrous at necropsy
- Individual microscopic pathology data
- Individual neuropathology data

12.2.2. APPENDICES

- Analytical results
- Feed and Water analyses
- Genotoxicity Report
- References for experimental methodology
- Personnel involved in the study
- Protocol and Amendment(s)

12.3. ROBUST SUMMARY

This report will be separately provided by the Testing Facility's UK office in both IUCLID and WORD® electronic formats.

13. REGULATORY REFERENCES

13.1. TEST GUIDELINE

This study is designed to meet or exceed the pertinent requirements of:

OECD Guidelines for Testing of Chemicals 413, Adopted 12 May 1981, Subchronic Inhalation Toxicity: 90 Day Study.

US EPA OPPTS Health Effects Test Guidelines 870.3465, 90-Day Inhalation Toxicity, August 1998.

OECD Guidelines for Testing of Chemicals 474, Adopted 21 July 1997, Mammalian Erythrocyte Micronucleus Test, August 1998.

US EPA OPPTS Health Effects Test Guidelines 870.5395, Mammalian Erythrocyte Micronucleus Test, August 1998.

13.2. GOOD LABORATORY PRACTICES

This study will be conducted in compliance with Organization for Economic Cooperation and Development (OECD) Good Laboratory Practices as set forth in ENV/MC/CHEM(98)17 and EPA Good Laboratory Practices as set forth in 40 CFR Part 792 (TSCA). The micronucleus evaluation will *also* be conducted in compliance with the UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

13.3. FACILITIES MANAGEMENT/ANIMAL HUSBANDRY

Currently acceptable practices of good animal husbandry will be followed, e.g., *Guide for the Care and Use of Laboratory Animals*; National Academy Press, 1996. Huntingdon Life Sciences, Inc. is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

13.4. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991. The Sponsor should make particular note of the following:

1. The Sponsor's signature on this protocol documents for the study described, there are no generally accepted non-animal alternatives and the study does not unnecessarily duplicate previous experiments.
2. All procedures used in this study have been designed to avoid discomfort, distress and pain to the animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
3. Any procedures outlined in this study protocol which are expected to cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing, by the Sponsor and the Study Director and approved by the IACUC; in which case the procedure will continue for the minimum time necessary. Documentation of the justification for withholding treatment for pain or distress and IACUC approval of the procedures will be made prior to study initiation on the IACUC Protocol Review form.
4. Animals experiencing more than momentary or slight pain or distress due to test substance or emergency situations such as injury or illness will be treated by the Testing Facility's veterinarian staff with approved analgesics or agents to relieve pain. If possible, the Study Director will be consulted prior to treatment; however, the veterinary staff is authorized to administer emergency treatment as necessary. Any subsequent treatment or euthanasia will be administered after consultation with the Study Director. The Sponsor will be advised by the Study Director of all emergency situations in as timely a manner as possible.
5. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

13.5. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

The IACUC Protocol Review Subcommittee has reviewed this protocol and found it to be in compliance with all appropriate regulations.

14. QUALITY ASSURANCE MONITORING

The Huntingdon Life Sciences Quality Assurance Unit will monitor the facilities, equipment, personnel, methods, practices, records and controls used in this study to assure that they are in conformance with this protocol, company SOP's, and the appropriate Good Laboratory Practice regulations. The Testing Facility QA will be the Lead QA for this multi-site study.

15. ALTERATION OF DESIGN

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such changes will be honored by the Testing Facility and will be followed by a written verification. All protocol modifications will be signed by the Study Director and a Sponsor representative. Any modifications potentially affecting animal welfare will also be signed by two members of the Institutional Animal Care and Use Committee prior to the modification's implementation.

APPENDIX A
Tissues Preserved/Examined Microscopically

Tissue ^a	Preserved	Microscopic Examination (Groups)	
		1, 4	2, 3
adrenal gland	X	X	
aorta (thoracic)	X	X	
bone (sternum, left femur)	X	X	
bone marrow (rib) ^b	X	X ^c	
brain (medulla/pons, cerebrum and cerebellum)	X	X	
epididymides	X	X	
esophagus	X	X	
eye	X	X ^c	
heart	X	X	
kidneys	X	X	
large intestine (cecum, colon, and rectum)	X	X	
lacrimal gland	X	X ^c	
larynx ^d	X	X	
liver	X	X	
lungs (with mainstem bronchi)	X	X	X
lymph node (mediastinal and mesenteric)	X	X	
mammary gland	X	X ^c	
muscle (biceps femoris)	X	X ^c	
nasopharyngeal tissue ^e	X	X	
nerve (sciatic)	X	X	
optic nerve	X	X ^c	
ovaries	X	X	
pancreas	X	X	
pituitary	X	X ^c	
prostate	X	X	
salivary gland with submandibular lymph node	X	X	

Tissue ^a	Preserved	Microscopic Examination (Groups)	
		1, 4	2, 3
seminal vesicles	X	X	
skin	X	X ^c	
small intestine (duodenum, jejunum, ileum)	X	X	
spinal cord (cervical, thoracic, lumbar)	X	X	
spleen	X	X	
stomach	X	X	
testes	X	X	
thymic region	X	X	
thyroid (with parathyroids)	X	X	
trachea	X	X	
uninary bladder	X	X	
uterus (body/horns with cervix)	X	X	
Zymbal's gland	X	X ^c	
gross lesions ^f	X	X	X
target organs ^{f,g}	X	X	X

^a These tissues will also be examined for animals in Groups 2 and 3 which die prior to study termination (at additional cost).

^b Qualitative examination (no differential count). Bone marrow smears will be prepared and archived. They will only be evaluated (Sponsor approval, additional cost) if needed.

^c Tissues need only be examined microscopically if indicated by signs of toxicity or target organ involvement (additional cost).

^d The laryngeal mucosa shall be examined. Sections of the larynx to be examined include the epithelium covering the base of the epiglottis, the ventral pouch and the medial surfaces of the vocal processes of the arytenoid cartilages.

^e Four sections of the nasopharyngeal tissue shall be examined. This shall include sections through the nasal cavity and examinations of the squamous, transitional, respiratory and olfactory epithelia.

^f Examinations performed at additional cost.

^g Target organs will be designated by the Study Director, Pathologist and/or Sponsor based on experimental finding.

Protocol Amendment No. 1

Study Title: Liquified Petroleum Gas: A 13-Week Inhalation
Toxicity Study in the Rat via Whole-Body Exposures

Changes

1. Positive Control Substance, page 12:

Add: Cyclophosphamide monohydrate (CP); Description, lot number, storage, expiration date (if available) and handling procedures, as well as other pertinent information will be documented in the study data and final report; Unless otherwise noted, the identity, strength, composition, stability and method of synthesis, fabrication and/or derivation of each batch of the positive control substance will be documented by the Supplier before its use in the study. This documentation will be maintained by the Supplier; Testing Facility will arrange for storage of an archival sample; The unused positive control substance will be retained by the Testing Facility for possible future use or disposed upon submission of the final report.

2. Genotoxicity Evaluations, page 26:

Revise:The time between last exposure and tissue harvest will be approximately 18-24 hours. The right femurs will be removed and ~~sampled~~* fixed.... The Testing Facility will be responsible for the GLP compliance of this subcontractor. The Subcontractor will be responsible for the QA inspection and audit of the conduct and reporting of the evaluations phase according to their own procedures. The Testing Facility's Quality Assurance department will act as Lead QA to ensure that there is adequate quality assurance inspection coverage at the test site throughout the study.

3. Tissues Preserved, page 27:

Revise:Lungs (gravity method) and urinary bladder will be infused with 10% neutral buffered formalin for optimal preservation.

4. Sperm Count, Motility and Morphology Assessments, page 27:

Revise:

All Main Study males euthanized at termination in each group will have the following sperm evaluations available for analysis: 1) motility; 2) a count of homogenization-resistant testicular sperm; 3) a count of caudal epididymal sperm; and 4) sperm morphology (cauda epididymis). Only samples for the high-exposure and control group will be analyzed if there is no effect in the high-exposure group. Evaluations will be performed as follows:

The right testis from each animal will be removed intact, weighed (right and left testes weighed together **and separately) and preserved in modified Davidson's solution for at least 24 hrs prior to permanent storage in neutral buffered formalin for histopathological evaluation. The right epididymis from each animal will be removed intact, weighed (right**

Protocol Amendment No. 1

and left epididymides weighed together **and separately**) and preserved in neutral buffered formalin for histopathological evaluation.

If a macroscopic abnormality is noted on the left testis or left epididymis, then the right testis and right epididymis will be evaluated and the left testis and left epididymis will be preserved as indicated for possible histopathology.

The left epididymis will be removed intact **and the caudal portion removed and weighed; the remainder of the tissue will be discarded.** ~~weighed, and frozen on dry ice.~~ The **caudal portion of the** epididymides will be stored frozen at -70° C until evaluation for caudal sperm count. Each **caudal portion of the** epididymis will be thawed and ~~the caudal portion removed and weighed.~~ A homogenized sample of the caudal epididymis will be stained (**Ident**) and examined using the sperm analyzer. For each stained preparation, **at least 10* 20** fields will be counted. The total number of sperm in the caudal epididymis will be calculated and adjusted for the caudal epididymal weight.

The left vas deferens will be excised and placed in a prewarmed solution of **Medium 199*** ~~phosphate-buffered-saline~~ and 1% Bovine Serum Albumin. After a ~~minimum three minute~~ "swimout" period, a sample will be placed into a Hamilton Thorne IVOS sperm analyzer. **At least five fields (at least 200 sperm)** will be selected and stored as digital images. These images will be analyzed for percent motility and transferred to optical media for permanent storage.

Additionally, for each male, two sperm morphology slides will be prepared **from fixed (10% NBF) vas deferens samples (Medium 199/BSA), stained with Eosin and Nigrosine** and evaluated for morphological development (**sample aliquots will be shipped to HLS UK; Clare Bowden = Principal Investigator; the Subcontractor will be responsible for the QA inspection and audit of the conduct and reporting of the evaluations phase according to their own procedures; the Testing Facility's Quality Assurance department will act as Lead QA to ensure that there is adequate quality assurance inspection coverage at the test site throughout the study).**

The left testis will be removed and frozen on dry ice. The testis will be stored frozen at **-70 ± 20 °C** until processed for counting of homogenization-resistant sperm. **A homogenized sample of the thawed testis will be stained (Ident) and examined using the sperm analyzer. For each stained preparation, 10 fields will be counted. The total number of sperm in the testis will be calculated and adjusted for the testis weight.**

Tissues retained from these evaluations (**except homogenized testis samples for sperm count which will be discarded, due to limited viability, after all samples have been**

Protocol Amendment No. 1

analyzed and the digital images stored) will be discarded following acceptance issuance of the final report following consultation with by the Sponsor.

5. Neuropathology Sacrifice, page 29:

Revise: Animals (5/sex/group) will be anesthetized with an intraperitoneal injection (~1.0* 1.5 mL/kg) of 26% sodium pentobarbital and transcardially perfused with phosphate buffered saline followed by 2* 1% glutaraldehyde and 2* 4% paraformaldehyde in the same buffer. The carcass will be placed in the same fixative as above for approximately 24 hours, followed by 10% NBF prior to dissection of tissues. Postmortem examination will be limited to the tissues designated for microscopic evaluation....

6. Statistical Evaluation, page 31:

Revise: The following items will be analyzed statistically in the final report:
mean body weight values and body weight changes (interval-to-interval changes and overall change from pretest)

Reasons for Changes

1. Oversight in original protocol.
2. Clarification of procedures.
3. Clarification of procedures.
4. Clarification of procedures.
5. Clarification of procedures.
6. Clarification of procedures.

*these items will also be identified in the data and the final report as protocol deviations.

Amendment approved by:

[Redacted Signature]

[Redacted Signature]

[Redacted Signature]

[Redacted Signature]

14 Feb 06
Date

2/14/2006
Date

Huntingdon Life Sciences
Study No. 03-6141

Page 1 of 1
Final Protocol

Protocol Amendment No. 2

Study Title: Liquified Petroleum Gas: A 13-Week Inhalation
Toxicity Study in the Rat via Whole-Body Exposures

Changes

Sperm Count, Motility and Morphology Assessments, page 27:

Add: All Main Study males euthanized at termination in Groups 2 and 3 will have sperm morphology (cauda epididymis) evaluated.

Reasons for Changes

Evaluation of the Groups 1 and 4 sperm morphology revealed an increase in the incidence of 'mid-tail blob' (cytoplasmic droplet) in the Group 4 animals, a finding that suggests a delay in maturation of the sperm. Evaluation of Groups 2 and 3, to determine if there is an exposure level response, will assist with the interpretation of this finding and determination of a NOAEL, if appropriate.

Amendment approved by:

[Redacted Signature]

[Redacted Signature]

[Redacted Signature]

[Redacted Signature]

S Jun 06

Date

7/5/06

Date

Protocol Amendment No. 3

Study Title: Liquified Petroleum Gas: A 13-Week Inhalation
Toxicity Study in the Rat via Whole-Body Exposures.

Changes

Sperm Count, Motility and Morphology Assessments, page 27:

Add: Remaining fixed sperm samples will be used to prepare a new set of slides for the Group 1 and Group 4 exposure groups, with a target of 400 sperm per animal evaluated. Slide preparations and sperm morphology evaluations will be performed at the HLS UK laboratory (see protocol amendment #1).

Reasons for Changes

Initial evaluation of the Groups 1 and 4 sperm morphology revealed an increase in the incidence of 'mid-tail blob' (cytoplasmic droplet) in the Group 4 animals, a finding that suggests a delay in maturation of the sperm. Evaluation of Groups 2 and 3, to determine if there is an exposure level response, was also performed (see protocol amendment #2) and the results indicated that Groups 2 and 3 were unaffected. This additional evaluation may permit a more robust conclusion regarding the apparent effect among Group 4 (high exposure level) animals.

Amendment approved by:

[Redacted signature]

[Redacted signature]

[Redacted signature]

11 APR 06
Date

April 10, 2006
Date

Protocol Amendment No. 4

Study Title: Liquified Petroleum Gas: A 13-Week Inhalation
Toxicity Study in the Rat via Whole-Body Exposures

Changes

Sperm Count, Motility and Morphology Assessments, page 27:

Add: Hematoxylin and eosin stained sections of testes for the Group 1 and Group 4 exposure groups will be used to conduct a stage aware qualitative examination of spermatogenesis. The examination will be conducted to identify changes such as spermatid retention, stage specific germ cell degeneration, and the presence or absence of germ cells in inappropriate stages of the cycle in accordance with the recommendations of the Society of Toxicologic Pathology (Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ, Regan KS and Goodman DG. 2002 Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol 30, 518-531). The evaluation will be performed by [REDACTED], Ph.D, Dip RCPATH (tox), FRCPath who has specific expertise in staging and will be completed by 31 August 2006 and will be reported (audited) by 15 September 2006. Any abnormalities will be recorded in the Xybion Data Capture system and the results of the examination will be specifically mentioned in the microscopic pathology section of the study report.

Reasons for Changes

Evaluation (initial and repeated) of the Groups 1 and 4 sperm morphology revealed an increase in the incidence of 'mid-tail blob' (cytoplasmic droplet) in the Group 4 animals, a finding that suggests a delay in maturation of the sperm. Evaluation of Groups 2 and 3, to determine if there is an exposure level response, was also performed and the results indicated that Groups 2 and 3 were unaffected. This additional evaluation of the testes may permit a more robust conclusion regarding the apparent effect among Group 4 (high exposure level) animals.

Amendment approved by:

[REDACTED]

[REDACTED]

[REDACTED]

28/06

Date

8/25/06

Date

Protocol Amendment No. 5

Study Title: Liquified Petroleum Gas: A 13-Week Inhalation
Toxicity Study in the Rat via Whole-Body Exposures

Changes

1. Micronucleus Test, page 26:

Revise: Principal Investigator: [REDACTED]

2. Statistical Evaluations (MA and FOB and MN), pages 33-34:

Revise.....Analysis will be performed by [REDACTED]
(Principal Investigator) of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury,
Huntingdon, Cambridgeshire, PE28 4HS, England. The Testing Facility will be
responsible for the GLP compliance and the archiving of the raw data and original final
report.....**This phase of the study was *also* conducted in compliance with principles of
Good Laboratory Practice Standards as set forth in: The UK Good Laboratory
Practice Regulations (Statutory Instrument 1999 No 3106 as amended by Statutory
Instrument 2004 No. 994) and EC Commission Directive 2004/10/EC of 11 February
2004 (Official Journal No L 50/44).**

Reasons for Changes

1. [REDACTED] has left the employ of HLS and [REDACTED] will assume the PI
responsibilities for finalizing the report.

2. [REDACTED] has retired from HLS and this study was reassigned to [REDACTED]
to assume the responsibilities of Principal Investigator that had belonged to [REDACTED]
[REDACTED]. Also, clarification of the GLP regulations relevant to the statistical evaluations.

Amendment approved by: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

8 Dec 09

Date

12/08/2009

Date

	2-Week Range-Finding Study Report	Appendix BB
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