

Study Title

Mouse Lymphoma Protocol (Rotenone)

Data Requirement

Guideline No. 84-2

Authors

See below

Study Completed On

November 27, 1984

Performing Laboratory

Performed by IRI for
National Toxicology Program
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Laboratory Project ID

28037

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Agency U.S. Fish and Wildlife Service

Agency Agent: Fred P. Meyer

Date: April 24, 1987

Director
National Fisheries Research Center
La Crosse, Wisconsin

Fred P. Meyer
Signature

MOUSE LYMPHOMA PROTOCOL

A detailed protocol of the L5178Y mouse lymphoma forward mutation assay is presented in Myhr et al., Progress in Mutation Research 5:555-568 (1985). All chemicals are tested as coded compounds and all results are confirmed with repeat tests. In summary, 6×10^6 L5178Y mouse lymphoma cells are treated with the test chemical for 4 hours, then washed, resuspended in media, and maintained in log phase for 2 days to allow for expression of the mutants. The cells are then plated for both cloning efficiency (600 cells) and mutant counts (3×10^6 cells). Those cloned for mutant counts are treated with trifluorothymidine at the time of plating. All plates are incubated at 37°C in 5% CO₂ and colonies are counted after 10-12 days. The mutant count is divided by the product of the cloning efficiency and the number of cells at risk (3×10^6 cells) to yield the mutant fraction. The top dose treated is determined by solubility or toxicity, not to exceed 5 mg/ml. Appropriate solvent and positive controls are run concurrently. The assay is initially conducted without exogenous metabolic activation; however, if a clear positive response is not indicated, the test is repeated with Aroclor 1254-induced S9. All data are evaluated statistically for both trend and peak response. An experiment is considered positive if $p < 0.05$ for at least 1 of the 3 highest dose sets (peak response) and if there is a significant trend ($p < 0.05$). If there is only a peak or a trend response the call is "questionable"; it is "negative" if there is neither a trend or peak response. A chemical is considered "positive" only if the positive response is confirmed in a repeat test.

GOOD LABORATORY PRACTICE STATEMENT

The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

Submitter U.S. Fish and Wildlife Service

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
 MOUSE LYMPHOMA TESTING RESULTS

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7/13/84

EVALUATION:
 NOT ACTIVATED

EXPERIMENT
 DATE 07/01/84

LAB
 IRI

CONTRACT
 26037

DOSE LEVEL	CELL DENSITY		RELATIVE SUSPENSION GROWTH		CLONING EFFICIENCY	RELATIVE TOTAL GROWTH	TOTAL MUTANT COUNT	MUTANT FREQUENCY PER 10E6	RELATIVE	P-VALUE
	1	2	1	2						
.0000	9.6	9.6	102	113	59	89	117	56		
	9.4	7.9	100	93	86	125	136	54		
	9.2	8.0	98	94	82	106	153	58		
Q3	9.5	7.8	97	91	91	97	527	07		
CTRL			(100)	(100)	(78)	(100)		(59)		
.0000	7.1	7.0	75	82	38	31	274	236		
	7.6	7.3	74	86	38	32	267	231		
CTRL			(75)	(84)	(38)	(31)		(236)	330.5	0.0001
.5000	5.3	4.2	56	49	55	26	401	241		
	5.7	3.7	61	44	60	22	339	174		
			(53)	(42)	(51)	(21)		(208)	351.5	0.0001
.1	4.6	3.6	49	45	53	15	637	483		
	4.9	3.3	43	45	56	14	423	242		
			(42)	(45)	(55)	(15)		(323)	546.0	0.0000
.0000	3.2	3.8	34	33	49	7	753	516		
	3.1	3.7	33	42	31	5	1946	2184		
			(34)	(33)	(40)	(6)		(1310)	2215.5	0.0000
.0000	2.6	3.0	30	36	13	2	1397	3652		
	2.8	3.6	39	36	17	3	1342	2521		
			(30)	(33)	(15)	(2)		(3142)	5314.5	0.0000
Q4	1.6	2.1	0r	0r	0r	0r	0r	0r		
Q4	1.4	1.5	0r	0r	0r	0r	0r	0r		
.0000	1.8	1.4	0r	0r	0r	0r	0r	0r		
Q4	1.3	0.8	0r	0r	0r	0r	0r	0r		

BASED ON SOLVENT
 .0000 EXCLUDING DOSES WITH AVG RTG < 10.0%
 .0000 EXCLUDING DOSES WITH AVG RTG < 7.5%
 .0000 EXCLUDING DOSES WITH AVG RTG < 5.0%
 ZT P-VALUE FOR TREND AGAINST TOXICITY
 0.0000

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
HOUSE LYMPHOMA TESTING RESULTS

CULTURE NUMBER	SELECTIVE AGENT COMPOUND	CONCENTRATION	TREATMENT (HRS)	PARTICLES CLONED FOR VIABLE COUNT	MUTANT COUNT
0684	TFT	993.00 UG/ML	04	600	3000000

* THIS EXPERIMENT WAS REJECTED BECAUSE IT FAILED *
* TO MEET QUALITY CONTROL CRITERIA. *

CELLULAR AND GENETIC TOXICOLOGY RESEARCH UNIT
 MOUSE LYMPHOMA TESTING RESULTS

REFERENCE
 CAS #: 83-79-4
 QUOT: 542524

EVALUATION:
 NOT ACTIVATED

EXPERIMENT
 DATE 11/27/84
 LAB ISI
 CONTI 280

SOL: ACET	DOSE LEVEL	CELL DENSITY		RELATIVE SUSPENSION GROWTH		CLONING EFFICIENCY	RELATIVE TOTAL GROWTH	TOTAL MUTANT COUNT	MUTANT FREQUENCY PER 10 ⁶	RELATIVE	P-VALUE
		1	2	1	2						
	0.0000	12.5	7.7	101	91	91	109	209	77		
		14.2	7.6	115	90	72	97	186	87		
		11.8	9.1	95	108	77	103	141	61		
		11.0	9.4	89	111	71	91	125	59		
AVERAGE SOLVENT CONTROL				(109)	(100)	(77)	(100)		(71)		
FOS: FMS	15.0000	7.8	7.3	63	96	35	25	242	232		
		9.2	7.7	74	91	35	31	159	151		
AVERAGE POSITIVE CONTROL				(69)	(89)	(35)	(28)		(191)	269.2	0.0011
TREATED DOSES											
	0.2500	6.8	4.8	55	54	67	26	236	117		
		5.3	6.1	43	72	61	24	209	115		
				(49)	(63)	(54)	(25)		(116)	163.4	0.0334
	0.5000	6.5	6.6	53	86	50	28	216	146		
		7.4	5.0	60	71	66	37	182	92		
				(56)	(76)	(58)	(32)		(119)	167.4	0.0311
	1.0000	4.3	4.6	40	57	56	17	492	292		
		5.3	4.6	43	54	74	23	411	184		
				(41)	(56)	(65)	(20)		(238)	335.4	0.0002
	2.0000	5.1	5.0	41	59	56	19	412	238		
		5.1	5.2	41	62	50	16	443	298		
				(41)	(66)	(54)	(17)		(268)	377.6	0.0001
01	4.0000	04 2.5	04 2.0	0r	0r	0r	0r	0r	0r		
		03 4.6	03 3.6	0r	0r	0r	0r	0r	0r		
01	8.0000	04 3.3	04 2.2	0r	0r	0r	0r	0r	0r		
		04 2.8	04 2.1	0r	0r	0r	0r	0r	0r		
01	16.0000	04 2.0	04 1.6	0r	0r	0r	0r	0r	0r		
		04 2.5	04 2.4	0r	0r	0r	0r	0r	0r		

ZT P-VALUE BASED ON SOLVENT
 0.0000

ZT P-VALUE FOR TREND AGAINST TOXICITY
 0.0000

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
MOUSE LYMPHOMA TESTING RESULTS

 CULTURE NUMBER 0884	SELECTIVE AGENT COMPOUND TFT	CONCENTRATION 003.00 US/ML	TREATMENT (HRS) 04	PARTICLES CLOVED FOR VIABLE COUNT 600	MUTANT COUNT 3000000
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