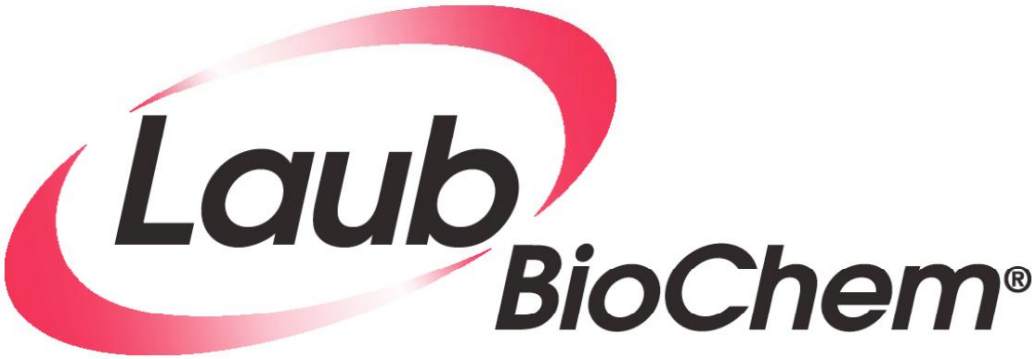
HÜMİ K ASİ T

AKUT Sİ STEMİ K TOKSİ Sİ TE

BIOS EKRANI



Laub BioChemicals Corporation 1401
Quail St., Suite 121 Newport
Beach, CA 92660

Ağ ustos 1998

FRONTISPIECE: Swiss Webster fareleri ile toksisite testi.

İ leri

Bu Rapor, yü rü tü len canlı hayvan toksisite çalış malarını belgelemektedir.
Laub BioChemicals Corp.'un doğ al ü rü nü ve
sentetik humat malzemeleri, BioScreen, Inc., Torrance, CA.
Sentetik (“Hepsyl® ”) humatlar xxx olarak kodlanmış tır , burada
xxx , kullanılan baş langıç malzemesini temsil eder.
sentetik sü reç–CA: kafeik asit; CGA: klorojenik asit;
HGA: homogentisik asit. HA , doğ al ü rü nü ifade eder
hü mik asit.

Doğ al ü rü n ve sentetik humatlar tarafından korunur
ABD patentleri (5,946,445; 6,569,416; 6,524,566; 6,524,567;
6,534,049; 6,576,229) ve diğ er ABD ve uluslararası patentler ve
bekleyen patentler. “Hepsyl® ” Tescillidir
Laub BioChemicals Corp.'un ticari markası (US 2,177,121).

BioScreen® Testing Services, Inc.
3892 Del Amo Boulevard • Suite 705 • Torrance, California 90503
(310) 214-0043 • Fax: (310) 370-3642 • E-Mail: bioscreen@msn.com

August 5, 1998

Dr. Richard Laub
Laub Biochemicals Corporation
P.O. Box 7818
Newport Beach, CA 92660

Dear Dr. Laub:

At your request, BioScreen Testing Services has performed Acute Systemic Toxicity studies on your powder samples labeled:
Hepsyl CA 150K
Hepsyl CGA 150K
Hepsyl HGA 150K
Humic Acid 150K.

For each sample, three concentrations of test article were prepared in sterile, pyrogen-free saline and each injected intravenously into the tail veins of 10 Swiss Webster mice (5 male and 5 female) at dose levels of 50 mg/kg of body weight, 25 mg/kg, and 12.5 mg/kg. Following the IV administration, the mice were examined for symptoms of toxicity for 14 days. All animals survived the tests, and in my opinion, the clinical observations made were not indicative of toxicity.

Complete details of the studies have been provided to you in BioScreen Report Nos. 98300856 through 98300859.

If you need any more information, please let me know.

Sincerely yours,

BIOSCREEN TESTING SERVICES, INC.

Richard J. Schlesinger, Ph.D.

Richard Schlesinger, Ph.D., D.A.B.F.T.*

*Diplomate of the American Board of Forensic Toxicology

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BIOLOGICAL REPORT

Laub BioChemicals Corp.
Attn: Dr. Richard Laub
P.O. Box 7818
Newport Beach, CA 92658

Report Date: 07/17/98
Date Received: 05/13/98
Date Completed: 06/30/98
Project #: 98300856
Reference #: 533VA,1-40

SUMMARY AND EVALUATION OF TEST RESULTS:

An acute intravenous systemic toxicity was performed on Swiss Webster mice at 0 (saline control), 50, 25, and 12.5 mg/kg doses. The animals were observed for any signs of toxicity and general clinical findings were recorded. No mortalities were observed over the fourteen (14) day observation period, and while some clinical findings were observed, they were not indicative of toxicity.

SAMPLE DESCRIPTION:

ACCESSION #	SAMPLE	LOT #	QTY.
98300856	Approximately 110 mg of Hepsyl CGA 150 K powder	N/A	01

TESTS PERFORMED	BTS METHOD:
Acute Intravenous Systemic Toxicity Test (Multiple Dose)	M806R0

SAMPLE PREPARATION:

The sample powder was dissolved in sterile, pyrogen-free 0.9% sodium chloride injection to yield final concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL (See chemistry notebook 475, p. 52)

OBJECTIVE:

To determine the acute intravenous systemic toxicity of the sample doses.

PROCEDURE:

Testing was performed according to the above references and is summarized as follows:

For each Hepsyl CGA 150K dose listed below, ten viral antibody-free Swiss Webster mice (five males and five females) were administered the test material intravenously at the specified dosage; and thereafter, offered a balanced Teklad diet and water *ad libitum* for the duration of the study. Ten (10) additional mice were similarly administered 0.9% Sodium Chloride Injection (the solvent vehicle) as a zero dose. All mice were examined for viability for fourteen (14) days. Zero time, day seven (7) and day fourteen (14) weights and toxic symptoms were recorded (See Table I, Checklist for Clinical Observations).

DOSAGE:

Hepsyl CGA 150K (1 mg/mL), Hepsyl CGA 150K (0.5 mg/mL), Hepsyl CGA 150K (0.25 mg/mL) and 0.9% sodium chloride injection were injected at 50 mL per kilogram. This resulted in the following Hepsyl CGA 150K doses: 50 mg/kg of body weight from 1 mg/mL, 25 mg/kg from 0.5 mg/mL, 12.5 mg/kg from 0.25 mg/mL, and 0 mg/kg from the 0.9% sodium chloride injection.

IACUC:

This toxicity study procedure has been evaluated and approved by the Institutional Animal Care and Use Committee in accordance with the regulations in 9 CFR 2.31.

ANIMAL DATA:

The animals were Swiss Webster mice, which were supplied by Simonsen Laboratories, Inc. They weighed in the range of 17-23 g at the time of testing. A balanced Teklad diet and water were fed *ad libitum* during the acclimation and testing periods. All test animals were quarantined and checked for signs of disease prior to testing.

ENVIRONMENTAL CONDITIONS:

All test animals were group-housed 5 per cage in plastic cages with stainless steel suspended lids.

DISPOSITION OF SAMPLE AND AVAILABILITY OF RAW DATA AND FINAL REPORT:

The remainder of the sample has been stored at BioScreen Testing Services, Inc. The raw data and the final report will be retained in the archives of BioScreen Testing Services, Inc.

RESULTS:

No deaths occurred at any dose.

Zero Dose (0.9% Sodium chloride)

During the fourteen (14) day observation period, no toxic effects or deaths were observed in the five (5) male and five (5) female mice.

Hepsyl CGA 150K (1 mg/mL to yield 50 mg/kg)

Days 1 and 2; Malaise, ptosis, piloerection, and cyanosis were observed in three of the five male mice.

Days 1,2, and 3; Ptosis, piloerection, and cyanosis were observed in two of the five female mice.

After the first three days of the observation period, no signs of toxic effects occurred for the rest of the study period.

BIOLOGICAL REPORT

RESULTS (cont'd):

Hepsyl CGA 150K (0.5 mg/mL to yield 25 mg/kg)

Days 1 and 2; Malaise, ptosis, and cyanosis were observed in one of the five male mice.

Days 1 and 2; Malaise, ptosis, piloerection, and cyanosis were observed in one of the five female mice.

After the first three days of the observation period, no signs of toxic effects occurred for the rest of the study period.

Hepsyl CGA 150K (0.25 mg/mL to yield 12.5 mg/kg)

Day 1; Ptosis, piloerection, cyanosis, and aggressive behavior were observed in two of the five male mice.

No toxic effects were observed in any of the five female mice at this concentration level.

After the first three days of the observation period, no signs of toxic effects occurred for the rest of the study period.

DISCUSSION AND CONCLUSION:

Malaise, ptosis, and piloerection were observed in as many as three (3) of the five (5) male mice. Ptosis and piloerection were observed in two (2) of the five (5) female mice at the highest concentration of 1 mg/mL and in slightly decreasing numbers as the concentration was decreased. The ptosis is probably due to a transient sedative effect. The symptoms disappeared after the initial days of observation, indicating that the effect was a transient, circulatory, mainly peripheral vascular effect. The cyanosis observation was in fact not cyanosis but attributable to the color of the injected sample. No toxic or other effect of the administration was evident over the balance of the period of observation. As the concentration of Hepsyl CGA 150K decreased, the transient effect was evident in fewer animals. The overall findings did not reveal any toxic effects.

Joseph R. Rocha

Joseph R. Rocha, B.S.
Supervisor, Biology
rv

TABLE I
CHECKLIST FOR CLINICAL OBSERVATIONS

1) Mortality/Morbidity:	Record whether the animal is Alive (AL) or Expired (EX).
2) Moribundity:	Is the animal moribund ? Record yes (+) or no (-) for general malaise. Describe under item 12 below General Observations and Comments.
3) Weight:	Record the weight in grams upon selection of the animals, at Zero time, Day 1, Day 7 and Day 14 (or within 24 hours of the post injection dates).
4) Behavior:	Aggressive [animal attacks and bites any object placed in cage] (A), Fearful [irritable, animal jumps when cage is opened and retreats to corner] (B), Malaise [lethargy] (C), or Normal (N).
5) Ocular Effects:	Animal shows Nystagmus [involuntary rapid eye movement and/or rapid rotation of eyes] (E), Ptosis [drooping eye lids] (F) or Lacrimation (tearing or moisture around the eyes(G), or none of the above, or eyes are Normal (N).
6) Skin and Fur:	Fur shows Piloerection [hairs standing up on the back] (D), or [hair and skin are Normal, smooth and unruffled] (N)
7) Respiratory effects:	dyspnea [difficult or painful breathing] (Y), apnea [temporary suspension of breathing] (Z). Normal (N).
8) Motor Effects:	Animal shows Tremors [shaking, shivering] (H), Fasciculations [Involuntary twitching or contractions of muscles] (I), Clonic Convulsions [Alternating contraction and relaxation of muscles of muscles occurring in rapid succession] (J), Tonic Convulsions [sustained muscle contraction] (K), Ataxia [motor incoordination characterized by staggering or lack of righting reflex] (L), or none of the above, Normal (N)
9) Autonomic Effects:	Excessive Salivation (M) or Normal (N).
10) Reactivity to Handling:	See (4) Behavior above. If motor activity and behavior appear Normal (N).
11) Stereotypic Behavior:	Self Mutilation (P) or Walking Backwards (Q) or Absence of stereotypic behavior (-).
12) General Observations:	Muscular Weakness (R), Micturition [abnormal frequency of urination] (S), Diarrhea [describe feces under comments] (T) , Writhing,[pain induced twisting of body movements] (U), Cyanosis [Bluish tint to skin caused by lack of availability of circulating oxygen] (V), Phonation [Vocal noises, may a noxious stimulus] (W), or Absence of any of the above (-).

TABLE II
CLINICAL OBSERVATIONS
[Hepsyl CGA 150K (50 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
11	M	22	29	34	N	
36	M	20	28	34	N @ initial; C @ 4hr; C,V @ day 1; F @ day 2; N @ day 3 thru 14	Tip of the tail - blue
07	M	19	23	26	N	
08	M	21	29	34	N @ initial; C @ 4hr; C,F @ day 1; F @ day 2; N @ day 3 thru 14	
02	M	23	29	35	N @ initial thru 4hr; F,D @ day 1; D @ day 2; N @ day 3 thru 14	
47	F	19	23	25	N @ initial thru 4hr; F @ day 1 Thru 2; N @ day 3 thru 14	
69	F	20	23	27	N	
70	F	20	24	25	N @ initial; F,D,V @ 4hr thru day 2; F,D @ day 3; N @ day 6 thru 14	Tip of the tail - blue
51	F	19	21	24	N	
56	F	20	23	25	N	

TABLE III
CLINICAL OBSERVATIONS
[Hepsyl CGA 150K (25 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
16	M	20	24	25	N	
25	M	22	29	35	N	
18	M	20	25	27	N @ initial thru 4hr; C,F,V @ day 1 thru 2; N @ day 3 thru 14	
31	M	22	23	28	N	
06	M	21	26	27	N	
59	F	22	24	27	N @ initial; C,D,F,V @ 4hr; A,C,D, F,V @ day 1; C,D,F,V @ day 2; N @ day 3 thru 14	
55	F	19	24	25	N	
53	F	20	22	26	N	
65	F	19	21	24	N	
45	F	20	26	26	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BIOLOGICAL REPORT

TABLE IV
CLINICAL OBSERVATIONS
[Hepsyl CGA 150K (12.5 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
10	M	22	26	31	N @ initial; D @ 4hr; D,F,V @ day 1; N @ day 3 thru 14	
21	M	20	25	30	N	
01	M	23	28	32	N	
19	M	20	29	30	N @ initial; D @ 4hr; A,C,D,F,V @ day 1; N @ day 2 thru 14	
04	M	22	29	32	N	
52	F	21	22	26	N	
56	F	19	20	23	N	
44	F	21	21	24	N	
42	F	20	23	25	N	
60	F	22	23	24	N	

TABLE V
CLINICAL OBSERVATIONS
[Zero Dose]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
37	M	21	28	32	N	
22	M	23	30	34	N	
18	M	18	28	27	N	
26	M	20	27	33	N	
29	M	21	30	34	N	
62	F	22	24	27	N	
51	F	22	24	25	N	
75	F	20	22	23	N	
73	F	20	24	25	N	
43	F	20	23	25	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BioScreen® Testing Services, Inc.

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BIOLOGICAL REPORT

Laub BioChemicals Corp.
Attn: Dr. Richard Laub
P.O. Box 7818
Newport Beach, CA 92658

Report Date: 07/29/98
Date Received: 05/13/98
Date Completed: 07/07/98
Project #: 98300857
Reference #: 541VA,1-40

SUMMARY AND EVALUATION OF TEST RESULTS:

An acute intravenous systemic toxicity was performed on Swiss Webster mice at 0 (saline control), 50, 25, and 12.5 mg/kg doses. The animals were observed for any signs of toxicity and general clinical findings were recorded. No mortalities were observed over the fourteen (14) day observation period, and while some clinical findings were observed, they were not indicative of toxicity.

SAMPLE DESCRIPTION:

ACCESSION #	SAMPLE	LOT #	QTY.
98300857	Approximately 110 mg of Hepsyl CA 150 K powder	N/A	01

TESTS PERFORMED	BTS METHOD:
Acute Intravenous Systemic Toxicity Test (Multiple Dose)	M806R0

SAMPLE PREPARATION:

The sample powder was dissolved in sterile, pyrogen-free 0.9% sodium chloride injection to yield final concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL (See chemistry notebook 475, p. 52)

OBJECTIVE:

To determine the acute intravenous systemic toxicity of the sample doses.

PROCEDURE:

Testing was performed according to the above references and is summarized as follows:

For each Hepsyl CGA 150K dose listed above, ten viral antibody-free Swiss Webster mice (five males and five females) were administered the test material intravenously at the specified dosage; and thereafter, offered a balanced Teklad diet and water *ad libitum* for the duration of the study. Ten (10) additional mice were similarly administered 0.9% Sodium Chloride Injection (the solvent vehicle) as a zero dose. All mice were examined for viability for fourteen (14) days. Zero time, day seven (7), and day fourteen (14) weights and toxic symptoms were recorded (See Table I, Checklist for Clinical Observations).

BIOLOGICAL REPORT

DOSAGE:

Hepsyl CA 150K (1 mg/mL), Hepsyl CA 150K (0.5 mg/mL), Hepsyl CA 150K (0.25 mg/mL) and 0.9% sodium chloride injection were injected at 50 mL per kilogram. This resulted in the following Hepsyl CA 150K doses: 50 mg/kg of body weight from 1 mg/mL, 25 mg/kg from 0.5 mg/mL, 12.5 mg/kg from 0.25 mg/mL, and 0 mg/kg from the 0.9% sodium chloride injection.

IACUC:

This toxicity study procedure has been evaluated and approved by the Institutional Animal Care and Use Committee in accordance with the regulations in 9 CFR 2.31.

ANIMAL DATA:

The animals were Swiss Webster mice, which were supplied by Simonsen Laboratories, Inc. They weighed in the range of 17-23 g at the time of testing. A balanced Teklad diet and water were fed *ad libitum* during the acclimation and testing periods. All test animals were quarantined and checked for signs of disease prior to testing.

ENVIRONMENTAL CONDITIONS:

All test animals were group-housed 5 per cage in plastic cages with stainless steel suspended lids.

DISPOSITION OF SAMPLE AND AVAILABILITY OF RAW DATA AND FINAL REPORT:

The remainder of the sample has been stored at BioScreen Testing Services, Inc. The raw data and the final report will be retained in the archives of BioScreen Testing Services, Inc.

RESULTS:

No deaths occurred at any dose.

Zero Dose (0.9% Sodium chloride)

During the fourteen (14) day observation period, no toxic effects or deaths were observed in the five (5) male and five (5) female mice.

Hepsyl CA 150K (1 mg/mL to yield 50 mg/kg)

Zero Time and 4 Hours; Malaise, piloerection, and cyanosis were observed in one of the five male mice.

Zero Time and 4 Hours; Malaise, ptosis, piloerection, and cyanosis were observed in one of the five female mice. Piloerection and cyanosis were observed in a second female mice.

After the first day of the observation period, no signs of toxic effects occurred for the rest of the study period.

BIOLOGICAL REPORT

RESULTS (cont'd):

Hepsyl CA 150K (0.5 mg/mL to yield 25 mg/kg)

No toxic effects were observed in any of the five male mice at this concentration level.

Zero Time, 4 Hours, and Day 1; Malaise, piloerection, and cyanosis were observed in one of the five female mice.

After the first day of the observation period, no signs of toxic effects occurred for the rest of the study period.

Hepsyl CA 150K (0.25 mg/mL to yield 12.5 mg/kg)

No toxic effects were observed in any of the five male or five female mice at this concentration level.

DISCUSSION AND CONCLUSION:

At the time of injection and at four (4) hours, malaise, piloerection, and cyanosis was observed in a single male mouse while malaise, piloerection, cyanosis, and ptosis were observed in two (2) of the five (5) female mice for the 50 mg/kg group only. The others were showing no effects of the injection at all with the exception of a single female in the 25 mg/kg group, which showed similar symptoms. The observation described as cyanosis was in fact simply the accumulation of the pigment in the test article. The malaise or ptosis was mainly a transient sedative effect. The overall clinical examination did not reveal any toxic effects.

Joseph R. Rocha, B.S.
Supervisor, Biology
rv

TABLE I
CHECKLIST FOR CLINICAL OBSERVATIONS

1) Mortality/Morbidity:	Record whether the animal is Alive (AL) or Expired (EX).
2) Moribundity:	Is the animal moribund ? Record yes (+) or no (-) for general malaise. Describe under item 12 below General Observations and Comments.
3) Weight:	Record the weight in grams upon selection of the animals, at Zero time, Day 1, Day 7 and Day 14 (or within 24 hours of the post injection dates).
4) Behavior:	Aggressive [animal attacks and bites any object placed in cage] (A), Fearful [irritable, animal jumps when cage is opened and retreats to corner] (B), Malaise [lethargy] (C), or Normal (N).
5) Ocular Effects:	Animal shows Nystagmus [involuntary rapid eye movement and/or rapid rotation of eyes] (E), Ptosis [drooping eye lids] (F) or Lacrimation (tearing or moisture around the eyes(G), or none of the above, or eyes are Normal (N).
6) Skin and Fur:	Fur shows Piloerection [hairs standing up on the back] (D), or [hair and skin are Normal, smooth and unruffled] (N)
7) Respiratory effects:	dyspnea [difficult or painful breathing] (Y), apnea [temporary suspension of breathing] (Z). Normal (N).
8) Motor Effects:	Animal shows Tremors [shaking, shivering] (H), Fasciculations [Involuntary twitching or contractions of muscles] (I), Clonic Convulsions [Alternating contraction and relaxation of muscles of muscles occurring in rapid succession] (J), Tonic Convulsions [sustained muscle contraction] (K), Ataxia [motor incoordination characterized by staggering or lack of righting reflex] (L), or none of the above, Normal (N)
9) Autonomic Effects:	Excessive Salivation (M) or Normal (N).
10) Reactivity to Handling:	See (4) Behavior above. If motor activity and behavior appear Normal (N).
11) Stereotypic Behavior:	Self Mutilation (P) or Walking Backwards (Q) or Absence of stereotypic behavior (-).
12) General Observations:	Muscular Weakness (R), Micturition [abnormal frequency of urination] (S), Diarrhea [describe feces under comments] (T) , Writhing,[pain induced twisting of body movements] (U), Cyanosis [Bluish tint to skin caused by lack of availability of circulating oxygen] (V), Phonation [Vocal noises, may a noxious stimulus] (W), or Absence of any of the above (-).

BIOLOGICAL REPORT

Laub BioChemicals Corp. **Project #:** 98300857

TABLE II
CLINICAL OBSERVATIONS
[Hepsyl CA 150K (50 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
85	M	22	31	35	C,D,V @ initial thru 4hr; N @ day 1 thru 14	Tip of the tail - blue
93	M	18	29	31	N	
82	M	20	31	33	N	
87	M	23	30	36	N	
102	M	23	31	35	N	
114	F	22	27	29	C,D,F,V @ initial thru 4hr; V @ day 1; N @ day 2 thru 14	Tip of the tail - blue
117	F	18	21	22	N	
118	F	18	19	23	N	
101	F	23	25	27	D,V @ initial thru 4hr; N @ day 1 thru 14	Tip of the tail - blue
103	F	22	25	27	N	

TABLE III
CLINICAL OBSERVATIONS
[Hepsyl CA 150K (25 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
80	M	20	30	35	N	
115	M	19	29	33	N	
119	M	22	32	Not Recorded	N	
144	M	18	27	32	N	
140	M	22	31	32	N	
151	F	19	22	32	C,D,V @ initial thru day 1; N @ day 2 thru 14	
115	F	19	20	26	N	
126	F	20	24	26	N	
99	F	22	25	27	N	
84	F	19	22	25	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BIOLOGICAL REPORT

Laub BioChemicals Corp. **Project #:** 98300857

TABLE IV
CLINICAL OBSERVATIONS
[Hepsyl CA 150K (12.5 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
116	M	21	32	36	N	
96	M	21	30	35	N	
111	M	22	31	34	N	
81	M	21	27	32	N	
86	M	20	21	32	N	
89	F	17	20	24	N	
142	F	20	21	24	N	
92	F	18	21	24	N	
79	F	20	21	23	N	
104	F	19	22	23	N	

TABLE V
CLINICAL OBSERVATIONS
[Zero Dose]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
80	M	22	31	33	N	
79	M	21	30	36	N	
94	M	23	33	39	N	
90	M	21	31	34	N	
91	M	19	30	33	N	
98	F	19	23	24	N	
97	F	21	24	28	N	
78	F	20	23	24	N	
95	F	21	24	28	N	
88	F	21	25	28	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BioScreen® Testing Services, Inc.
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BIOLOGICAL REPORT

Laub BioChemicals Corp.
Attn: Dr. Richard Laub
P.O. Box 7818
Newport Beach, CA 92658

Report Date: 07/29/98
Date Received: 05/13/98
Date Completed: 07/07/98
Project #: 98300858
Reference #: 541VA,41-70

SUMMARY AND EVALUATION OF TEST RESULTS:

An acute intravenous systemic toxicity was performed on Swiss Webster mice at 0 (saline control), 50, 25, and 12.5 mg/kg doses. The animals were observed for any signs of toxicity and general clinical findings were recorded. No mortalities were observed over the fourteen (14) day observation period, and while some clinical findings were observed, they were not indicative of toxicity.

SAMPLE DESCRIPTION:

ACCESSION #	SAMPLE	LOT #	QTY.
98300858	Approximately 110 mg of Hepsyl HGA 150 K powder	N/A	01

TESTS PERFORMED	BTS METHOD:
Acute Intravenous Systemic Toxicity Test (Multiple Dose)	M806R0

SAMPLE PREPARATION:

The sample powder was dissolved in sterile, pyrogen-free 0.9% sodium chloride injection to yield final concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL (See chemistry notebook 475, p. 52)

OBJECTIVE:

To determine the acute intravenous systemic toxicity of the sample doses.

PROCEDURE:

Testing was performed according to the above references and is summarized as follows:

For each Hepsyl CGA 150K dose listed above, ten viral antibody-free Swiss Webster mice (five males and five females) were administered the test material intravenously at the specified dosage; and thereafter, offered a balanced Teklad diet and water *ad libitum* for the duration of the study. Ten (10) additional mice were similarly administered 0.9% Sodium Chloride Injection (the solvent vehicle) as a zero dose. All mice were examined for viability for fourteen (14) days. Zero time, day seven (7), and day fourteen (14) weights and toxic symptoms were recorded (See Table I, Checklist for Clinical Observations).

DOSAGE:

Hepsyl HGA 150K (1 mg/mL), Hepsyl HGA 150K (0.5 mg/mL), Hepsyl HGA 150K (0.25 mg/mL) and 0.9% sodium chloride injection were injected at 50 mL per kilogram. This resulted in the following Hepsyl HGA 150K doses: 50 mg/kg of body weight from 1 mg/mL, 25 mg/kg from 0.5 mg/mL, 12.5 mg/kg from 0.25 mg/mL, and 0 mg/kg from the 0.9% sodium chloride injection.

IACUC:

This toxicity study procedure has been evaluated and approved by the Institutional Animal Care and Use Committee in accordance with the regulations in 9 CFR 2.31.

ANIMAL DATA:

The animals were Swiss Webster mice, which were supplied by Simonsen Laboratories, Inc. They weighed in the range of 17-23 g at the time of testing. A balanced Teklad diet and water were fed *ad libitum* during the acclimation and testing periods. All test animals were quarantined and checked for signs of disease prior to testing.

ENVIRONMENTAL CONDITIONS:

All test animals were group-housed 5 per cage in plastic cages with stainless steel suspended lids.

DISPOSITION OF SAMPLE AND AVAILABILITY OF RAW DATA AND FINAL REPORT:

The remainder of the sample has been stored at BioScreen Testing Services, Inc. The raw data and the final report will be retained in the archives of BioScreen Testing Services, Inc.

RESULTS:

No deaths occurred at any dose.

Zero Dose (0.9% Sodium chloride)

During the fourteen (14) day observation period, no toxic effects or deaths were observed in the five (5) male and five (5) female mice.

Hepsyl HGA 150K (1 mg/mL to yield 50 mg/kg)

Zero Time, 4 Hours, and Day 1; Piloerection was observed in one of the five male mice.

No toxic effects were observed in any of the five female mice at this concentration level.

After the first day of the observation period, no signs of toxic effects occurred for the rest of the study period.

BIOLOGICAL REPORT

RESULTS (cont'd):

Hepsyl HGA 150K (0.5 mg/mL to yield 25 mg/kg)

No toxic effects were observed in any of the five male or five female mice at this concentration level.

Hepsyl HGA 150K (0.25 mg/mL to yield 12.5 mg/kg)

No toxic effects were observed in any of the five male or five female mice at this concentration level.

DISCUSSION AND CONCLUSION:

At the time of injection, four (4) hours, and at day one (1) only piloerection was observed in a single male mouse for the 50 mg/kg dose only. This finding is not significant and the general conclusion is that no toxic effects were observed.

Joseph R. Rocha, B.S.
Supervisor, Biology
rv

TABLE I
CHECKLIST FOR CLINICAL OBSERVATIONS

1) Mortality/Morbidity:	Record whether the animal is Alive (AL) or Expired (EX).
2) Moribundity:	Is the animal moribund ? Record yes (+) or no (-) for general malaise. Describe under item 12 below General Observations and Comments.
3) Weight:	Record the weight in grams upon selection of the animals, at Zero time, Day 1, Day 7 and Day 14 (or within 24 hours of the post injection dates).
4) Behavior:	Aggressive [animal attacks and bites any object placed in cage] (A), Fearful [irritable, animal jumps when cage is opened and retreats to corner] (B), Malaise [lethargy] (C), or Normal (N).
5) Ocular Effects:	Animal shows Nystagmus [involuntary rapid eye movement and/or rapid rotation of eyes] (E), Ptosis [drooping eye lids] (F) or Lacrimation (tearing or moisture around the eyes(G), or none of the above, or eyes are Normal (N).
6) Skin and Fur:	Fur shows Piloerection [hairs standing up on the back] (D), or [hair and skin are Normal, smooth and unruffled] (N)
7) Respiratory effects:	dyspnea [difficult or painful breathing] (Y), apnea [temporary suspension of breathing] (Z). Normal (N).
8) Motor Effects:	Animal shows Tremors [shaking, shivering] (H), Fasciculations [Involuntary twitching or contractions of muscles] (I), Clonic Convulsions [Alternating contraction and relaxation of muscles of muscles occurring in rapid succession] (J), Tonic Convulsions [sustained muscle contraction] (K), Ataxia [motor incoordination characterized by staggering or lack of righting reflex] (L), or none of the above, Normal (N)
9) Autonomic Effects:	Excessive Salivation (M) or Normal (N).
10) Reactivity to Handling:	See (4) Behavior above. If motor activity and behavior appear Normal (N).
11) Stereotypic Behavior:	Self Mutilation (P) or Walking Backwards (Q) or Absence of stereotypic behavior (-).
12) General Observations:	Muscular Weakness (R), Micturition [abnormal frequency of urination] (S), Diarrhea [describe feces under comments] (T) , Writhing,[pain induced twisting of body movements] (U), Cyanosis [Bluish tint to skin caused by lack of availability of circulating oxygen] (V), Phonation [Vocal noises, may a noxious stimulus] (W), or Absence of any of the above (-).

TABLE II
CLINICAL OBSERVATIONS
[Hepsyl HGA 150K (50 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
76	M	21	30	33	D @ initial thru day 1; N @ day 2 thru 14	
83	M	22	31	33	N	
148	M	22	not recorded	35	N	
120	M	23	33	35	N	
89	M	18	29	32	N	
113	F	20	25	27	N	
88	F	22	28	29	N	
132	F	19	24	26	N	
135	F	18	20	23	N	
78	F	20	25	27	N	

TABLE III
CLINICAL OBSERVATIONS
[Hepsyl HGA 150K (25 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
130	M	23	32	36	N	
129	M	23	31	37	N	
149	M	22	32	36	N	
134	M	23	33	35	N	
137	M	21	30	33	N	
107	F	19	24	28	N	
91	F	18	21	25	N	
149	F	20	27	28	N	
93	F	20	23	25	N	
128	F	20	23	25	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BIOLOGICAL REPORT

Laub BioChemicals Corp. **Project #:** 98300858

TABLE IV
CLINICAL OBSERVATIONS
[Hepsyl HGA 150K (12.5 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
110	M	23	31	36	N	
77	M	21	27	34	N	
129	M	23	29	36	N	
143	M	20	29	34	N	
131	M	23	31	37	N	
145	F	21	24	32	N	
105	F	19	23	24	N	
121	F	19	23	26	N	
119	F	20	25	28	N	
104	F	22	24	28	N	

TABLE V
CLINICAL OBSERVATIONS
[Zero Dose]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
80	M	22	31	33	N	
79	M	21	30	36	N	
94	M	23	33	39	N	
90	M	21	31	34	N	
91	M	19	30	33	N	
98	F	19	23	24	N	
97	F	21	24	28	N	
78	F	20	23	24	N	
95	F	21	24	28	N	
88	F	21	25	28	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BioScreen® Testing Services, Inc.

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BIOLOGICAL REPORT

Laub BioChemicals Corp.
Attn: Dr. Richard Laub
P.O. Box 7818
Newport Beach, CA 92658

Report Date: 07/17/98
Date Received: 05/13/98
Date Completed: 06/30/98
Project #: 98300859
Reference #: 533VA,41-70

SUMMARY AND EVALUATION OF TEST RESULTS:

An acute intravenous systemic toxicity was performed on Swiss Webster mice at 0 (saline control), 50, 25, and 12.5 mg/kg doses. The animals were observed for any signs of toxicity and general clinical findings were recorded. No mortalities were observed over the fourteen (14) day observation period, and while some clinical findings were observed, they were not indicative of toxicity.

SAMPLE DESCRIPTION:

ACCESSION #	SAMPLE	LOT #	QTY.
98300859	Approximately 110 mg of Humic Acid 150 K powder	N/A	01

TESTS PERFORMED	BTS METHOD:
Acute Intravenous Systemic Toxicity Test (Multiple Dose)	M806R0

SAMPLE PREPARATION:

The sample powder was dissolved in sterile, pyrogen-free 0.9% sodium chloride injection to yield final concentrations of 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL (See chemistry notebook 475, p. 52)

OBJECTIVE:

To determine the acute intravenous systemic toxicity of the sample doses.

PROCEDURE:

Testing was performed according to the above references and is summarized as follows:

For each Hepsyl CGA 150K dose listed below, ten viral antibody-free Swiss Webster mice (five males and five females) were administered the test material intravenously at the specified dosage; and thereafter, offered a balanced Teklad diet and water *ad libitum* for the duration of the study. Ten (10) additional mice were similarly administered 0.9% Sodium Chloride Injection (the solvent vehicle) as a zero dose. All mice were examined for viability for fourteen (14) days. Zero time, day seven (7), and day fourteen (14) weights and toxic symptoms were recorded (See Table I, Checklist for Clinical Observations).

DOSAGE:

Humic Acid 150K (1 mg/mL), Humic Acid 150K (0.5 mg/mL), Humic Acid 150K (0.25 mg/mL), and 0.9% sodium chloride injection were injected at 50 mL per kilogram. This resulted in the following Humic Acid 150K doses: 50 mg/kg of body weight from 1 mg/mL, 25 mg/kg from 0.5 mg/mL, 12.5 mg/kg from 0.25 mg/mL, and 0 mg/kg from the 0.9% sodium chloride injection.

IACUC:

This toxicity study procedure has been evaluated and approved by the Institutional Animal Care and Use Committee in accordance with the regulations in 9 CFR 2.31.

ANIMAL DATA:

The animals were Swiss Webster mice, which were supplied by Simonsen Laboratories, Inc. They weighed in the range of 17-23 g at the time of testing. A balanced Teklad diet and water were fed *ad libitum* during the acclimation and testing periods. All test animals were quarantined and checked for signs of disease prior to testing.

ENVIRONMENTAL CONDITIONS:

All test animals were group-housed 5 per cage in plastic cages with stainless steel suspended lids.

DISPOSITION OF SAMPLE AND AVAILABILITY OF RAW DATA AND FINAL REPORT:

The remainder of the sample has been stored at BioScreen Testing Services, Inc. The raw data and the final report will be retained in the archives of BioScreen Testing Services, Inc.

RESULTS:

No deaths occurred at any dose.

Zero Dose (Sodium chloride 0.9%)

During the fourteen (14) day observation period, no toxic effects or deaths were observed in the five (5) male and five (5) female mice.

Humic Acid 150K (1 mg/mL to yield 50 mg/kg)

Days 1,2, and 3; Ptosis, piloerection, and cyanosis were observed in one of the five male mice.

Days 1,2 and 3; Ptosis and piloerection were observed in one of the five female mice. Cyanosis was observed in two of the five female mice.

After the first three days of the observation period, no signs of toxic effects occurred for the rest of the study period.

BIOLOGICAL REPORT

RESULTS (cont'd):

Humic Acid 150K (0.5 mg/mL to yield 25 mg/kg)

No toxic effects were observed in any of the five male mice at this concentration level.

Days 1 and 2; Ptosis, piloerection, and cyanosis were observed in one of the five female mice.

After the first two days of the observation period, no signs of toxic effects occurred for the rest of the study period.

Humic Acid 150K (0.25 mg/mL to yield 12.5 mg/kg)

Day 1; Piloerection and aggressive behavior were observed in one of the five male mice.

Initial time and 4 hours; Piloerection was observed in one of the five female mice.

After the first day of the observation period, no signs of toxic effects occurred for the rest of the study period.

DISCUSSION AND CONCLUSION:

Ptosis, piloerection, and cyanosis or blue color effect were observed in one (1) of the five (5) female and one (1) of the five (5) male mice tested for the first three days observation at the highest concentration tested. The number of days of these observations and the number of animals showing these symptoms decrease as the concentration is decreased. The cyanosis was not in fact a true cyanosis but the effect of the pigment in the test article. The ptosis observed was probably due to a slight transient sedative effect. No toxic effect of the administration was evident over the balance of the observation period. The overall clinical findings did not reveal any toxic effects.

Joseph R. Rocha

Joseph R. Rocha, B.S.
Supervisor, Biology
rv

TABLE I
CHECKLIST FOR CLINICAL OBSERVATIONS

1) Mortality/Morbidity:	Record whether the animal is Alive (AL) or Expired (EX).
2) Moribundity:	Is the animal moribund ? Record yes (+) or no (-) for general malaise. Describe under item 12 below General Observations and Comments.
3) Weight:	Record the weight in grams upon selection of the animals, at Zero time, Day 1, Day 7 and Day 14 (or within 24 hours of the post injection dates).
4) Behavior:	Aggressive [animal attacks and bites any object placed in cage] (A), Fearful [irritable, animal jumps when cage is opened and retreats to corner] (B), Malaise [lethargy] (C), or Normal (N).
5) Ocular Effects:	Animal shows Nystagmus [involuntary rapid eye movement and/or rapid rotation of eyes] (E), Ptosis [drooping eye lids] (F) or Lacrimation (tearing or moisture around the eyes(G), or none of the above, or eyes are Normal (N).
6) Skin and Fur:	Fur shows Piloerection [hairs standing up on the back] (D), or [hair and skin are Normal, smooth and unruffled] (N)
7) Respiratory effects:	dyspnea [difficult or painful breathing] (Y), apnea [temporary suspension of breathing] (Z). Normal (N).
8) Motor Effects:	Animal shows Tremors [shaking, shivering] (H), Fasciculations [Involuntary twitching or contractions of muscles] (I), Clonic Convulsions [Alternating contraction and relaxation of muscles of muscles occurring in rapid succession] (J), Tonic Convulsions [sustained muscle contraction] (K), Ataxia [motor incoordination characterized by staggering or lack of righting reflex] (L), or none of the above, Normal (N)
9) Autonomic Effects:	Excessive Salivation (M) or Normal (N).
10) Reactivity to Handling:	See (4) Behavior above. If motor activity and behavior appear Normal (N).
11) Stereotypic Behavior:	Self Mutilation (P) or Walking Backwards (Q) or Absence of stereotypic behavior (-).
12) General Observations:	Muscular Weakness (R), Micturition [abnormal frequency of urination] (S), Diarrhea [describe feces under comments] (T) , Writhing,[pain induced twisting of body movements] (U), Cyanosis [Bluish tint to skin caused by lack of availability of circulating oxygen] (V), Phonation [Vocal noises, may a noxious stimulus] (W), or Absence of any of the above (-).

TABLE II
CLINICAL OBSERVATIONS
[Humic Acid 150K (50 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
12	M	22	28	32	N @ initial; D,F,V @ 4hr thru day 3; N @ day 6 thru 14	Tip of the tail - blue
23	M	20	26	29	N	
17	M	21	28	35	N	
26	M	22	30	35	N	
28	M	19	25	26	N	
63	F	21	23	Not Recorded	N	Tip of the tail - blue
72	F	19	21	23	N @ initial thru 4hr; V @ day 1; F,D,V @ day 2; F,D @ day 3; N @ day 6 thru 14	
68	F	19	22	24	N	
66	F	18	22	24	N	
74	F	18	22	23	N	

TABLE III
CLINICAL OBSERVATIONS
[Humic Acid 150K (25 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
20	M	23	30	32	N	
09	M	21	27	32	N	
15	M	20	28	32	N	
33	M	21	29	33	N	
35	M	22	28	30	N	
57	F	18	22	23	N @ initial thru 4hr; D,F,V @ day 1 thru 2; N @ day 6 thru 14	
61	F	20	22	25	N	
46	F	22	25	27	N	
49	F	21	24	27	N	
54	F	19	22	23	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none

BIOLOGICAL REPORT

Laub BioChemicals Corp. **Project #:** 98300859

TABLE IV
CLINICAL OBSERVATIONS
[Humic Acid 150K (12.5 mg/kg)]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
30	M	21	29	36	N @ initial thru 4hr; A,D @ day 1; N @ day 2 thru 14	
34	M	19	24	31	N	
24	M	21	28	33	N	
36	M	18	25	26	N	
27	M	21	28	33	N	
41	F	19	22	23	N	
67	F	22	25	26	N	
58	F	19	22	24	N	
62	F	19	22	24	N	
38	F	21	23	24	D @ initial thru 4hr; N @ day 1 thru 14	

TABLE V
CLINICAL OBSERVATIONS
[Zero Dose]

		Animal Weights (grams)				
Animal No.	Sex	Initial	7 Days	Final	Toxicity Summary	Comments
37	M	21	28	32	N	
22	M	23	30	34	N	
18	M	18	28	27	N	
26	M	20	27	33	N	
29	M	21	30	34	N	
62	F	22	24	27	N	
51	F	22	24	25	N	
75	F	20	22	23	N	
73	F	20	24	25	N	
43	F	20	23	25	N	

Toxicity Key:
N=Normal AL=Alive EX=Expired M=Moribund (+)=Yes or present (-)=No or none