

Appendices

1

Appendix A

Contained within are the raw data (section I), transformed data (section II), and graphic representation of the mean data (section III) of MTT toxicity assay for C2C12 cells exposed to various concentrations of either Cre-Ester™, or creatine monohydrate. Cells (5,000/well) were seeded onto 96-well plates and cultured overnight in culture media with fetal bovine serum. Following day, media was removed and replaced with either Cre-Ester™ or creatine monohydrate (0-100 mM) in a physiological assay buffer solution. Cells were incubated at 37^o C for four hours, after which, cells were washed and provided fresh culture media. Cells were then cultured under normal conditions for 72 hours, after which time, cell viability was examined using the MTT assay¹⁷. The assay is based on the metabolic conversion of MTT to a blue formagen dye in living, viable cells.

Appendix A

Section I-Raw Data from MTT Toxicity Study

Absorbance Readings at 550 nm in Fisher BioTech Plate Reader

Cre-Ester™ (mM)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Mean
0	0.664	0.643	0.635	0.663	0.606	0.722	0.587	0.633	0.644
2.5	0.693	0.688	0.753	0.661	0.494	0.584	0.845	0.575	0.661
5.0	0.677	0.856	0.732	0.649	0.583	0.576	0.547	0.573	0.649
10.0	0.704	0.727	0.760	0.817	0.570	0.679	0.564	0.792	0.702
25.0	0.620	0.706	0.698	0.712	0.692	0.695	0.696	0.697	0.690
50.0	0.702	0.702	0.702	0.864	0.678	0.677	0.650	0.644	0.702
100.0	0.609	0.618	0.624	0.651	0.605	0.605	0.545	0.618	0.609

Creatine Mono. (mM)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Mean
0	0.699	0.776	0.775	0.786	0.749	0.783	0.777	0.858	0.775
2.5	0.736	0.755	0.763	0.780	0.676	0.680	0.786	0.713	0.736
5.0	0.746	0.732	0.749	0.780	0.782	0.760	0.775	0.782	0.718
10.0	0.679	0.704	0.704	0.704	0.704	0.659	0.775	0.704	0.704
25.0	0.757	0.859	0.786	0.751	0.680	0.751	0.751	0.673	0.751
50.0	0.723	0.777	0.782	0.798	0.856	0.716	0.762	0.685	0.762
100.0	0.741	0.769	0.745	0.758	0.770	0.784	0.757	0.734	0.713

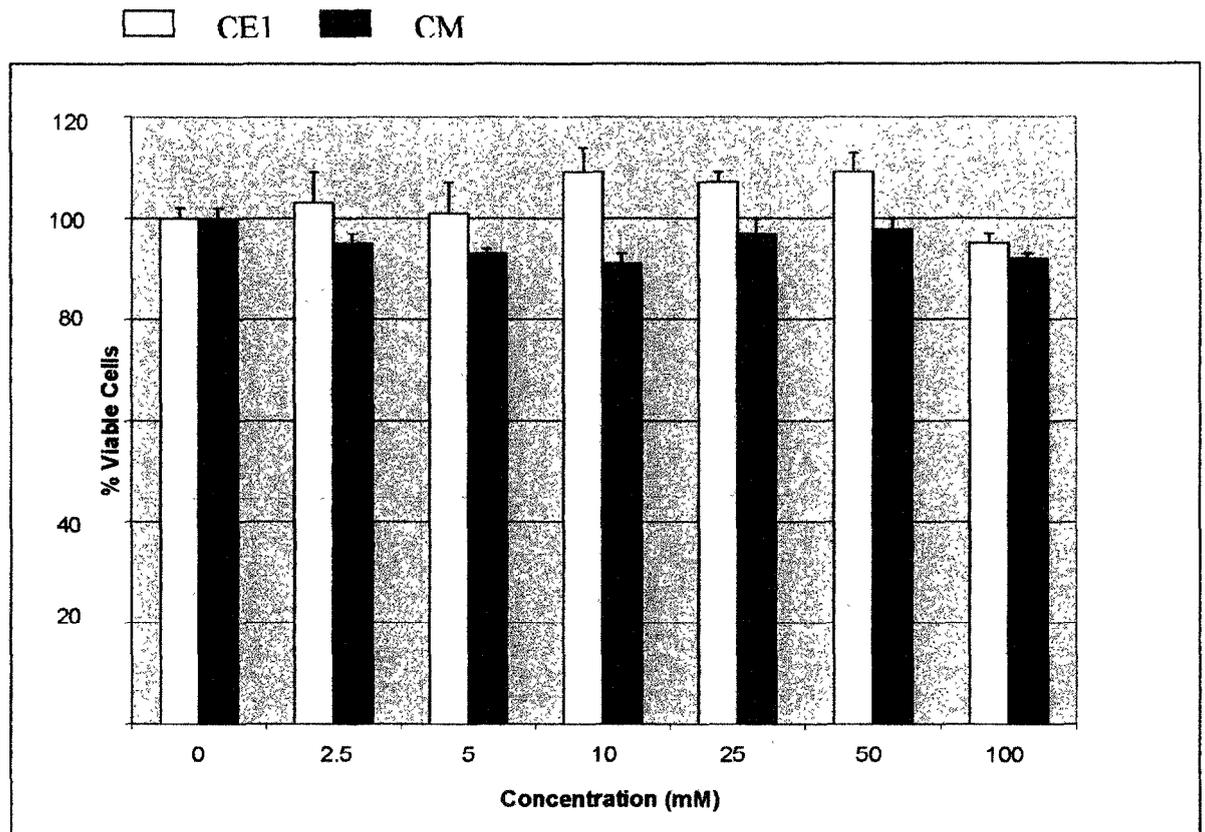
Section II-Toxicity Data Expressed as Percent of Control (i.e. no creatine present)

Cre-Ester™ (mM)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Mean
0	103	100	99	103	94	112	91	98	100
2.5	108	107	116	103	77	91	131	89	103
5.0	105	133	114	101	91	89	85	89	101
10.0	109	113	118	127	88	105	88	123	109
25.0	96	110	108	111	107	108	108	108	107
50.0	109	109	109	134	105	105	101	100	109
100.0	95	96	97	101	94	94	85	96	95

Creatine Mono. (mM)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Mean
0	90	100	100	101	97	101	100	111	100
2.5	95	97	98	101	87	88	101	92	95
5.0	96	94	97	93	89	92	87	93	93
10.0	88	91	91	91	91	85	100	91	91
25.0	98	111	101	97	88	97	97	87	97
50.0	93	100	101	103	110	92	98	88	98
100.0	92	92	92	101	88	90	91	90	92

Section III-Graphic Representation of Toxicity Data for Cre-Ester™ and Creatine Monohydrate

Section III-Graphic Representation of Toxicity Data for Cre-Ester™ (CE1) and Creatine Monohydrate (CM). Values represent the mean \pm SEM of eight observations.



Appendix B

In vivo toxicology study for creatine ethyl ester hydrochloride salt (Cre-Ester™) in adult, male Sprague-Dawley rats. Rats were treated with 430 mg/kg of Cre-Ester™ or the vehicle control (physiological saline solution; 2 ml/kg) for a period of seven consecutive days. The rats were dosed once a day via oral gavage. The dose selected represented a 30 g daily dose equivalent in humans. A total of 6 rats were selected for the Cre-Ester™ treatment and vehicle control groups. Weight changes were recorded for all rats during the course of the experiment. Blood samples were collected from all rats at the conclusion of the study for a complete metabolic panel of clinical tests. Finally, two rats from each group were randomly selected for comprehensive necropsy by an independent contract veterinary research facility. Results of these studies are provided in Appendix B and have been grouped into the following:

Section I-Weight Gain During Time Period of Experiment

Section II-Blood Chemistry Profiles*

Section III-Necropsy reports from the University of Missouri

*the following abbreviations are used in the blood analysis:

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

Alk Phos: Alkaline Phosphatase

BUN: Blood urea nitrogen

CRN: Creatinine

BUN/CRN: Blood urea nitrogen/creatinine ratio

Appendix B

Rat Toxicology Study for Creatine Ethyl Ester Hydrochloride

Section I. Weight Gain

Day 1 weight in grams

Control #1	Control #2	Control #3	Control #4	Control #5	Control #6	Mean	SEM (standard error of mean)
347	417	395	391	399	384	389	10

CEE #1	CEE #2	CEE #3	CEE #4	CEE #5	CEE #6	Mean	SEM
419	388	364	371	389	364	383	9

Day 7 weight in grams

Control #1	Control #2	Control #3	Control #4	Control #5	Control #6	Mean	SEM
358	421	409	397	398	392	396	9

CEE #1	CEE #2	CEE #3	CEE #4	CEE #5	CEE #6	Mean	SEM
421	388	373	374	399	373	388	8

Change in Weight (From Day1 to Day7)

Control #1	Control #2	Control #3	Control #4	Control #5	Control #6	Mean	SEM
11	4	14	6	-1	8	7	2

CEE #1	CEE #2	CEE #3	CEE #4	CEE #5	CEE #6	Mean	SEM
2	0	9	3	10	9	6	2

Section II. Blood Chemistry Profile (at conclusion of 7 day study)

Control Rats Receiving 2 ml/kg Physiological Saline Solution via oral gavage

	Control #1	Control #2	Control #3	Control #4	Control #5	Control #6	Mean	SEM
AST	58	55	89	59	78	97	73	7
ALT	87	55	60	52	58	52	61	5
Alk Phos.	nd	154	181	152	nd	176	166	6
Protein	7.4	6.5	6.5	6.8	7.2	6.2	6.8	0.2
Albumin	3.6	3	3.2	3.4	3.6	3	3.3	0.1
Glucose	141	294	262	197	155	178	205	23
BUN	19	18	21	18	16	19	19	0.6
CRN	1.2	0.4	0.5	0.5	0.6	0.5	0.6	0.1
BUN/CRN	16	45	42	36	27	38	34	4
Sodium	152	144	145	145	155	145	148	2
Potassium	6	3.9	3.9	3.5	5.4	4.1	4.5	0.4
Chloride	103	101	101	102	100	103	102	0.5
Calcium	10.8	9.6	9.7	9.7	10.9	9.1	10.0	0.3

Treatment Rats Receiving 2 ml/kg of 215 mg/ml solution of Creatine Ethyl Ester via oral gavage

	CEE #1	CEE #2	CEE #3	CEE #4	CEE #5	CEE #6	Mean	SEM	statistical difference compared to control
AST	53	70	65	75	60	63	64	3	NONE
ALT	58	77	64	72	57	61	65	3	NONE
Alk Phos.	169	nd	190	nd	162	159	170	6	NONE
Protein	6.5	7.5	6.9	7.6	6.3	6.4	6.9	0.2	NONE
Albumin	2.9	3.7	3.3	3.9	2.9	3	3.3	0.2	NONE
Glucose	275	136	115	174	259	208	195	24	NONE
BUN	12	18	20	17	15	16	16	1	NONE
CRN	0.4	0.6	0.5	1.1	0.4	0.4	0.6	0.1	NONE
BUN/CRN	30	30	40	15	38	40	32	4	NONE
Sodium	145	153	145	152	143	145	147	2	NONE
Potassium	3.4	4.5	4.3	7.3	3.9	3.6	4.5	0.5	NONE
Chloride	105	98	104	102	101	104	102	1	NONE
Calcium	9.7	10.8	9.9	10.8	9.4	9.5	10.0	0.2	NONE

Appendix B

Section III

Necropsy report from RADIL, University of Missouri:

Note: The blue band rats are control (i.e. received saline solution), and the red band rats are treated (i.e. received creatine ethyl ester).



FINAL REPORT OF LABORATORY EXAMINATION
MU Research Animal Diagnostic Laboratory
 1600 East Rollins, Columbia MO 65211 1-800-669-0825 1-573-882-5983
 radil@missouri.edu http://www.radil.missouri.edu

CASE NUMBER: 6735-2002

RECEIVED ON: 12/18/2002
COMPLETED ON: 1/9/2003

SUBMITTED BY:

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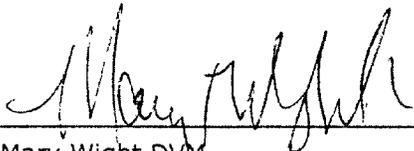
SPECIMEN DESCRIPTION:

SPECIES: rat
NUMBER OF SPECIMENS: 4

<u>RADIL ID</u>	<u>Client ID</u>	<u>Strain</u>	<u>Gender</u>	
1	blue tail, 1 band	sprague	M	303 grams
2	blue tail, 2 bands	sprague	M	344 grams
3	red tail, 1 band	sprague	M	322 grams
4	red tail, 2 bands	sprague	M	333 grams

PROFILE/EXAM REQUESTED: CBC with liver and kidney function panel on blood test; Histopathology

SUMMARY: Animal 3 had a mild focal adenitis of the coagulating gland. Animal 4 had a prostatitis. Animals 1 and 2 had evidence of mild myocardial degeneration. All other histologic findings reported are considered incidental findings. Please see the report for details.

Pathologist: 
 Mary Wight DVM
 If you'd like to discuss the results, please contact me
 at (573) 882-4472 or CarterMW@missouri.edu

NECROPSY:

No gross lesions were detected in any of the body systems and organs examined.

HISTOPATHOLOGY:

ANIMAL: 1

brain	no significant lesions
cecum	no significant lesions
coagulating gland	no significant lesions
colon	no significant lesions
duodenum	no significant lesions
heart	moderate focal subendocardial myocardial necrosis of right ventricle with associated lymphocytic infiltrate; mild focal subepicardial myocardial necrosis with associated lymphocytic infiltrate of right ventricle
ileum	no significant lesions
jejunum	no significant lesions
kidney	no significant lesions
liver	microgranulomas; focal hematopoiesis
lung	peribronchiolar lymphoid infiltrates
penis	no significant lesions
prostate gland	no significant lesions
quadriceps muscle	no significant lesions
salivary gland(s)	no significant lesions
seminal vesicle	no significant lesions
spleen	mild diffuse distribution of hemosiderin in macrophages of the red pulp
stomach	no significant lesions
testes	no significant lesions
urinary bladder	focal proteinaceous concretion

ANIMAL: 2

brain	no significant lesions
cecum	no significant lesions
coagulating gland	no significant lesions
colon	pinworm profile
duodenum	no significant lesions
heart	2 small foci subendocardial myocardial necrosis of left ventricle with occasional lymphocyte
ileum	no significant lesions
jejunum	no significant lesions
kidney	no significant lesions
liver	microgranulomas; focal hematopoiesis
lung	focal peribronchiolar lymphoid aggregates with transmucosal lymphocyte migration; multifocal accumulations of foamy alveolar macrophages; foci of pigment laden macrophages

Histopathology (continued)

penis	no significant lesions
prostate gland	no significant lesions
quadriceps muscle	no significant lesions
salivary gland(s)	no significant lesions
seminal vesicle	no significant lesions
spleen	mild diffuse distribution of hemosiderin in macrophages of the red pulp
stomach	no significant lesions
testes	no significant lesions
urinary bladder	no significant lesions

ANIMAL: 3

brain	no significant lesions
cecum	no significant lesions
coagulating gland	focal adenitis characterized by degenerating neutrophils and debris within a gland lumen
colon	no significant lesions
duodenum	no significant lesions
heart	no significant lesions
ileum	no significant lesions
jejunum	no significant lesions
kidney	small foci of mineralization
liver	microgranulomas; focal hematopoiesis
lung	mild multifocal perivascular lymphoid aggregates; focal accumulation of foamy alveolar macrophages; bronchiole containing a few mucosal cells with proteinaceous inclusions
penis	no significant lesions
prostate gland	no significant lesions
quadriceps muscle	no significant lesions
salivary gland(s)	no significant lesions
seminal vesicle	no significant lesions
spleen	mild diffuse distribution of hemosiderin in macrophages of the red pulp
stomach	no significant lesions
testes	no significant lesions
urinary bladder	focal proteinaceous concretion

ANIMAL: 4

brain	no significant lesions
cecum	no significant lesions
coagulating gland	no significant lesions
colon	no significant lesions

Histopathology (continued)

duodenum	no significant lesions
heart	no significant lesions
ileum	no significant lesions
jejunum	no significant lesions
kidney	no significant lesions
liver	microgranulomas; focal hematopoiesis; mild diffuse microvesicular lipidosis
lung	mild multifocal perivascular lymphoid aggregates; focal accumulation of foamy alveolar macrophages; focal peribronchiolar lymphoid aggregates with transmucosal lymphocyte migration
penis	no significant lesions
prostate gland	lymphocytic prostatitis characterized by moderate diffuse infiltration of the prostate interstitium with lymphocytes and occasional mast cell with mild hyperplasia of the epithelium and occasional apoptotic cell within the lumen
quadriceps muscle	no significant lesions
salivary gland(s)	no significant lesions
seminal vesicle	no significant lesions
spleen	mild diffuse distribution of hemosiderin in macrophages of the red pulp
stomach	no significant lesions
testes	no significant lesions
urinary bladder	mild focal perivascular lymphoid infiltrate; focal proteinaceous concretion

Comments: Animal 1 had 6 occurrences of hematopoiesis and 1 granuloma in median liver lobe. Animal 2 had 12 areas of hematopoiesis and 6 microgranulomas; animal 3 had 7 areas of hematopoiesis and 2 microgranulomas and animal 4 had 3 areas of hematopoiesis and 2 microgranulomas in the corresponding liver lobe. These lesions are considered incidental findings.

CLINICAL PATHOLOGY:Hematology

	Normal Range	1	2	3	4
WBC (x10 ³ /μl)	2.9 - 20.9	11.50	17.16	16.16	14.90
Segmented Neutrophils (x10 ³ /μl)	0.3 - 8.5	0.92	0.85	1.29	0.59
Band Neutrophils (x10 ³ /μl)	0 - 0.1	0	0	0	0
Lymphocytes (x10 ³ /μl)	3.8 - 15.3	9.66	15.44	13.89	12.36
Monocytes (x10 ³ /μl)	0.0 - 1.4	0.92	0.85	0.80	1.93
Eosinophils (x10 ³ /μl)	0.0 - 0.3	0	0	0.16	0
Basophils (x10 ³ /μl)	0.0 - 0.1	0	0	0	0
Segmented Neutrophils (%)	5.3 - 38.1	8	5	8	4
Band Neutrophils (%)		0	0	0	0
Lymphocytes (%)	56.7 - 93.1	84	90	86	83
Monocytes (%)	0.0 - 7.7	8	5	5	13
Eosinophils (%)	0.0 - 3.4	0	0	1	0
Basophils (%)	0.0 - 0.4	0	0	0	0

Clinical Pathology (continued)

	Normal Range	1	2	3	4
Nucleated RBCx (/100RBC)	rare	2	1	0	0
RBC (x10 ⁶ /μl)	4.60 - 9.19	14.18	13.10	12.15	14.06
Hemoglobin (g/dL)	10.0 - 16.7	24.3	22.7	22.4	23.1
Hematocrit (%)	34.0 - 53.0	69.8	65.8	63.5	66.2
MCV (fL)	50.0 - 77.8	49.2	50.2	52.3	47.1
MCH (pg)	16.0 - 23.1	49.2	17.3	18.4	16.4
MCHC (g/dL)	28.2 - 34.1	34.8	34.5	35.3	34.9
Platelets (x10 ³ /μl)	685 - 1436	1516.	1609.	1529.	1707.
MPV (fL)	5.0 - 20.0	6.8	6.9	6.3	6.9

All red blood cell and platelet parameters were measured on a HemaVet 850 (CDC Technologies, Inc., Oxford, CT) analyzer. White blood cell counts were also measured on the HemaVet. White blood cell differentials were obtained by manual counting of 100 leukocytes on stained blood smears. Normal range values were established by CDC Technologies. Our laboratory has not established hematology reference values for our equipment. The age, gender, and genotype of laboratory animals can influence the mean or range of reference values. Therefore, these reference values should be interpreted as approximations.

Clinical Chemistry

	Normal Range	1	2	3	4
Glucose (mg/dL)	85 - 132	141	155	136	174
Urea Nitrogen (mg/dL)	32 - 54	19	16	18	17
Creatinine (mg/dL)	0.53 - 0.86	1.2	0.6	0.6	1.1
Sodium (mmol/L)	141 - 150	152	155	153	152
Potassium (mmol/L)	5.2 - 7.8	6.0	5.4	4.5	7.3
Chloride (mmol/L)	99 - 114	103	100	98	102
Total Protein (g/dL)	6.3 - 8.6	7.4	7.2	7.5	7.6
Albumin (g/dL)	3.3 - 4.9	3.6	3.6	3.7	3.9
Globulin (g/dL)	2.4 - 3.9	3.8	3.6	3.8	3.7
Calcium (mg/dL)	10.7 - 13.7	10.8	10.9	10.8	10.8
Phosphorus (mg/dL)	6.2 - 11.7	8.8	8.4	7.8	9.5
Cholesterol (mg/dL)	46 - 92	126	75	101	76
Total Billi (mg/dL)	0.08 - 0.20	0.3	0.2	0.1	0.3
ALT (U/L)	17 - 50	58	78	77	72
ALP (U/L)	39 - 216	87	58	70	75
Amylase (U/L)		2425	2320	1590	2234
Total CO2 (mmol/L)		41	42	41	42
Anion Gap (mmol/L)		14	18	18	15

Normal range values are from Hillyer, E.V. and K.E. Quesenberry; Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery; W.B. Saunders: Philadelphia, 1997. Our laboratory has not established hematology and serum chemistry reference values for our equipment. The age, gender, and genotype of laboratory animals can influence the mean or range of reference values. Therefore, these reference values should be interpreted as approximations.

Appendix C

Blood and urine chemistry test result from five adult males ranging in age from 39 years to 64 years are listed according to subject number in Appendix C. The daily dosage and length of exposure to the supplement ranged from 1.0 to 3.0 grams and 238 to 414 days respectively. No significant abnormalities in blood or urine chemistries were noted. The only abnormal finding was a slightly elevated serum creatinine of 1.7 mg/dL.

Subject:	Age in years	Amount in grams of daily supplementation	Duration of supplementation in days
1	64	1.5	238
2	59	1.5	414
3	52	3.0	414
4	47	1.5	379
5	39	1.0	379

HUMAN EXPERIENCE

Subject 1

64 y/o M

Creatine Ethyl Ester Daily Dose: 1.5 gm
Length of Supplementation: 238 days

Blood	Test Results	
AST	24	U/L
ALT	25	U/L
Alk Phos.	52	U/L
Albumin	4.1	g/dL
BUN	21	mg/dL
creatinine	1.5	mg/dL
Bilirubin Direct	0	mg/dL
Bilirubin Total	0.4	mg/dL
gamma gt	31	U/L

Urinalysis

Color	Yellow	
Appearance	clear	
Glucose	neg	mg/dL
Bilirubin	neg	
Ketone	neg	mg/dL
Specific Gravity	1.005	
Blood/urine	neg	
pH/Urine	6	
Protein	neg	mg/dL
Urobilinogen	0.2	EU/dL
Nitrite	neg	
Leukocyte esteras	neg	
Albumin/ Creatinine ratio	4.63 (mcg ALB/mg Crea)	

Subject 2

59 y/o M

Creatine Ethyl Ester Daily Dose: 1.5 gm
Length of Supplementation: 414 days

Blood	Test Results		Normal	
AST	27	U/L	15-46	U/L
ALT	25	U/L	11-66	U/L
Alk Phos.	80	U/L	38-126	U/L
Albumin	4.1	g/dL	3.6-5.0	g/dL
BUN	16	mg/dL	7-20	mg/dL
creatinine	1.3	mg/dL	0.8-1.5	mg/dL
Bilirubin Direct	0	mg/dL	0-0.4	mg/dL
Bilirubin Total	0.3	mg/dL	0.2-1.4	mg/dL
gamma gt	38	U/L	8-78	U/L

Urinalysis

Color	Yellow	
Appearance	clear	
Glucose	neg	mg/dL
Bilirubin	neg	
Ketone	neg	mg/dL
Specific Gravity	1.005	1.003-1.035
Blood/urine	neg	
pH/Urine	6	4.5-8.0
Protein	neg	mg/dL
Urobilinogen	0.2	EU/dL
Nitrite	neg	
Leukocyte esteras	neg	
Albumin/ Creatinine ratio	5.2 (mcg ALB/mg Crea)	<30

Subject 3

52 y/o M

Creatine Ethyl Ester Daily Dose: 3.0gms
Length of Supplementation: 414 days

Blood	Test Results	
AST	37	U/L
ALT	38	U/L
Alk Phos.	55	U/L
Albumin	4	g/dL
BUN	21	mg/dL
creatinine	1.1	mg/dL
Bilirubin Direct	0	mg/dL
Bilirubin Total	0.3	mg/dL
gamma gt	69	U/L

Urinalysis

Color	Yellow
Appearance	clear
Glucose	neg mg/dL
Bilirubin	neg
Ketone	neg mg/dL
Specific Gravity	1.025
Blood/urine	neg
pH/Urine	6
Protein	neg mg/dL
Urobilinogen	0.2 EU/dL
Nitrite	neg
Leukocyte esteras	neg

Albumin/
Creatinine ratio 3.04
(mcg ALB/mg Crea)

Subject 4

47 y/o M

Creatine Ethyl Ester Daily Dose: 1.5 gm
Length of Supplementation: 379 days

Blood	Test Results		Normal	
AST	36	U/L	15-46	U/L
ALT	35	U/L	11-66	U/L
Alk Phos.	65	U/L	38-126	U/L
Albumin	4.2	g/dL	3.6-5.0	g/dL
BUN	9	mg/dL	7-20	mg/dL
creatinine	1.7*	mg/dL	0.8-1.5	mg/dL
Bilirubin Direct	0	mg/dL	0-0.4	mg/dL
Bilirubin Total	0.4	mg/dL	0.2-1.4	mg/dL
gamma gt	56	U/L	8-78	U/L

Urinalysis

Color	Yellow		
Appearance	clear		
Glucose	neg mg/dL	neg	mg/dL
Bilirubin	neg	neg	
Ketone	neg mg/dL	neg	mg/dL
Specific Gravity	1.03	1.003-1.035	
Blood/urine	neg	neg	
pH/Urine	5	4.5-8.0	
Protein	neg mg/dL	neg	mg/dL
Urobilinogen	0.2 EU/dL	0.2-1.0	EU/dL
Nitrite	neg		
Leukocyte esteras	neg		

Albumin/
Creatinine ratio 2.79 <30
(mcg ALB/mg Crea)

Appendix D

Reports of analysis for metals and chemical carcinogens

A Cre-Ester™ sample was analyzed using toxicity characteristic leaching procedure (TCLP) and the results of this analysis are reported. Of the metals examined arsenic, mercury, selenium, silver, chromium, lead, cadmium, nickel, and copper were below the limits of detection of the assay. The batch sample contained a small amount of barium (0.32 mg/L) and zinc (0.17 mg/L). However, these amounts were well below the maximum permissible level for the metals examined. The Cre-Ester™ sample was also shown to be free of all chemical carcinogens examined. These included benzene, carbon tetrachloride, chlorobenzene, chloroform, 1,4-dichlorobenzene, 1,2-dichloroethane, 1,1-dichloroethene, methyl ethyl ketone, tetrachlorethene, trichloroethene, and vinyl chloride.



Midwest Laboratories, Inc.SM

Report Number
02-364-2089

13611 "B" Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121

www.midwestlabs.com

REPORT OF ANALYSIS

For: (14523) PRONUTRIENT TECHNOLOGIES INC
(402)573-6500

Date Reported: 12/30/02

Date Received: 12/18/02

Mail to: **PRONUTRIENT TECHNOLOGIES INC
SAMUEL
11515 N 84TH ST
OMAHA NE 68122-**

DECEMBER 15TH PRODUCTION

Lab number: 812939 Sample ID: CREATINE ETHYL ESTER HCL

Analysis	Level		Detection		Analyst- Date
	Found	Units	Limit	Method	
Arsenic (TCLP)	n.d.	mg/L	0.5	EPA 6010	ccm-12/30
Barium (TCLP)	0.32	mg/L	0.005	EPA 6010	ccm-12/30
Mercury (TCLP)	n.d.	mg/L	0.001	EPA 7470	cvs-12/30
Selenium (TCLP)	n.d.	mg/L	0.10	EPA 6010	ccm-12/30
Silver (TCLP)	n.d.	mg/L	0.01	EPA 6010	ccm-12/30
Chromium (TCLP)	n.d.	mg/L	0.01	EPA 6010	ccm-12/30
Lead (TCLP)	n.d.	mg/L	0.05	EPA 6010	ccm-12/30
Cadmium (TCLP)	n.d.	mg/L	0.005	EPA 6010	ccm-12/30
Nickel (TCLP)	n.d.	mg/L	0.01	EPA 6010	ccm-12/30
Copper (TCLP)	n.d.	mg/L	0.01	EPA 6010	ccm-12/30
Zinc (TCLP)	0.17	mg/L	0.01	EPA 6010	ccm-12/30

Notes:

n.d. - Not Detected.

add'l report (DUAL)

MAXIMUM PERMISSIBLE LEVELS FOR TCLP EXTRACTS (in mg/L): Arsenic- 5.0

barium- 100, cadmium- 1.0, chromium- 5.0, lead- 5.0, mercury- 0.2,

selenium- 1.0, silver- 5.0 .

Respectfully Submitted



Heather Ramig/Sue Ann Seitz
Client Services

The above analytical results apply to the sample(s) submitted.

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Midwest Laboratories, Inc.SM

Report Number
02-364-2089

13611 "B" Street • Omaha, Nebraska 68144-3693 • (402) 334-7770 • FAX (402) 334-9121

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REPORT OF ANALYSIS

For: (14523) PRONUTRIENT TECHNOLOGIES INC
(402)573-6500

Date Reported: 12/30/02
Date Received: 12/18/02

Mail to: PRONUTRIENT TECHNOLOGIES INC
SAMUEL
11515 N 84TH ST
OMAHA NE 68122-

DECEMBER 15TH PRODUCTION

Lab Number: 812939 Sample ID: CREATINE ETHYL ESTER HCL

Analysis	Level Found	Detection Limit	Analysis	Level Found	Detection Limit
Method: ZERO HEADSPACE TCLP 1311 Units: $\mu\text{g/L}$ Analyst: sde Date: 12/20/02					
25 Benzene	n.d.	5	1,1-Dichloroethene	n.d.	5
Carbon Tetrachloride	n.d.	5	2-Butanone (Methyl Ethyl Ketone)	n.d.	40
Chlorobenzene	n.d.	5	Tetrachloroethene	n.d.	5
Chloroform	n.d.	5	Trichloroethene	n.d.	5
1,4-Dichlorobenzene	n.d.	5	Vinyl Chloride	n.d.	5
1,2-Dichloroethane	n.d.	5			

Notes:
n.d. - Not Detected.
add'l report (DFT).