



Study code: PCDL-0602

FINAL REPORT

Acute oral toxicity study of Pueraria mirifica, PE with 14-day post-treatment observation period in the rat (Limit test)

Initiation of the study: March 6, 2006

Experimental period: from March 9 to March 28, 2006

Sponsor:

COFOPEX Ltd.
H-1022 Budapest,
Bimbó út 92.

Contact Person:
István Bara

Study was performed at:

Pharmaceutical Control and
Development Laboratory Co. Ltd.
H-1149 Budapest, Mexikói út 9.

Contact Person:
Susan Somfai-Relle, M.D.

This Final Report consists of 43 pages plus 2 attachments.

2006

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)
(Study code: PCDL-0602)**

SUMMARY

General information:

Single oral doses of 5,000 mg/kg or 2,300 mg/kg body weight of Pueraria mirifica, PE (Lot number: 031615) were applied to groups of 5 male and 5 female rats by gavage. Animals were weighed, observed for lethality and toxic symptoms for 14 days. Gross pathological examination was carried out on the 15th day.

Lethality, Clinical symptoms and Body weight:

No lethality, adverse clinical symptoms, body weight effects were noted at single oral limit dose up to 5,000 mg/kg Pueraria mirifica, PE in male and female rats.

Gross pathology

All animals survived until the scheduled autopsy on Day 15. All organs of all male and female rats proved to be free of treatment related gross pathological changes. The absolute weight of the prostates decreased slightly in the high dose male group (-17%), this may be in connection with the phytoestrogenic effect of the test article. No significant change in the weight of selected other organs was observed.

Evaluation:

Single oral LD₅₀ and “no adverse effect dose” are higher than 5,000 mg/kg which corresponds to 2,200 fold of the planned human daily dose of Pueraria mirifica, PE as dietary supplement.

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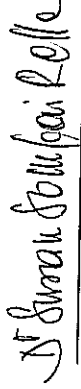
Attachments:

Copy of Analytical Report of Pueraria mirifica, PE
Copy of Statement of GLP Compliance (PCDL)

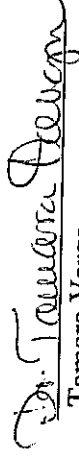
Staff in Charge

Signature _____ Date _____

Director of the Laboratory:

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Study Director:Susan Somfai-Relle
M.D., toxicologist23 May, 2006

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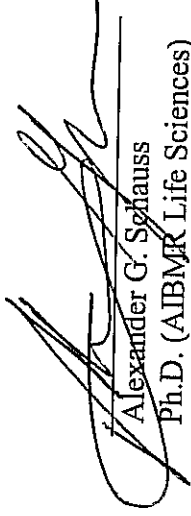
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for Anikó Kövér
M. Sc. Bioengineering24 May, 2006.

Sponsor:

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Managing Director
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Alexander G. Schauss
Ph.D. (AIBMR Life Sciences)June 1, 2006

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)
(Study code: PCDL-0602)**

Study Director's Statement

I hereby certify that this study report provides a true and complete record of the data generated and that the study was conducted in accordance with the Principles of Good Laboratory Practice as set forth in the following documents:

1. US Food and Drug Administration Title 21, Code of Federal Regulations, Part 58 Good Laboratory Practice Regulations for Nonclinical Laboratory Studies
2. Good Laboratory Practice Regulations (National GLP, Joint Decree 9/2001.(III.30) EüM-FVM)
3. OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17 as revised in 1997)
4. Hungarian Act 1998: XXVIII. and Governmental Regulation 243/1998 "Rules of animal experimentation" modified by Governmental Regulation 103/2002, regulating animal protection

Date: 23 May, 2006

Signature:



Susan Somfai-Relle, M.D.
Study Director

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)
(Study code: PCDL-0602)**

Statement of the Quality Assurance Unit

This study has been inspected and the report audited by the Quality Assurance Unit of PCDL in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established, the methods described and the results incorporated in the report accurately and completely reflect the raw data produced during this study.

Inspections concerning adherence to the protocol were performed:

Date of Inspection / Audit	Type of Inspection	Study Director	Date of Report to the Management
Feb 28, 2006	Protocol audit	Feb 28, 2006	Feb 28, 2006
March 14, 2006	Formulation of the test article, sampling of the test suspension for concentration and homogeneity check by gravimetry, treatment, observation of clinical symptoms	March 14, 2006	March 16, 2006
March 28, 2006	Autopsy	March 29, 2006	March 29, 2006
May 5, 2006	Draft report audit	May 8, 2006	May 9, 2006

Date: *May 24, 2006.*

Signature:

Pinku Mishra

for Anikó Kövér
M. Sc. Bioengineering
Quality Assurance Unit at
PCDL

1. GENERAL INFORMATION

1.1. Title of the study

Acute oral toxicity study of Pueraria mirifica, PE with 14-day post-treatment observation period in the rat (Limit test)

Initiation of the study: March 6, 2006

Experimental period: from March 9 to March 28, 2006

1.2. Objective of the study

To develop data on the potential toxicological effects of single oral administration of Pueraria mirifica, PE in the rat. The test article is a powdered alcoholic extract of the root of the plant Pueraria mirifica containing phytoestrogens⁽¹⁾. It is intended to be used as dietary supplement⁽²⁾.

1.3. Type of the study

Preclinical toxicological study in compliance with the principles of the

- Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration, (21 CFR 58)
- Good Laboratory Practice Regulations (National GLP, Joint Decree 9/2001.(III.30) EüM-FVM) and
- OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17 as revised in 1997);
- as well as the Hungarian Act 1998: XXVIII. and Governmental Regulation 243/1998 "Rules of animal experimentation" modified by Governmental Regulation 103/2002, regulating animal protection.

The study was set up according to the

- US-FDA, Center for Food Safety and Applied Nutrition: Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food; "Redbook II" - DRAFT, Acute oral Toxicity Tests. 1993⁽³⁾
- OECD GUIDELINES FOR TESTING OF CHEMICALS (Guideline No.: 423, adopted: 17th December, 2001, Acute Oral Toxicity - Acute Toxic Class Method)⁽⁴⁾.

2. MATERIALS**2.1. Test article**

Name: Pueraria mirifica, PE
(proprietary cold alcoholic extract⁽¹⁾)
Manufacturer: Bio-Botanica® Inc., 75 Commerce Drive,
Hauppauge, NY 11788
Lot number: 031615
Identification number at PCDL: 2006/01892
Appearance: light beige colored, free-flowing powder⁽¹⁾
Package form: 500 g plastic bag
Aroma: characteristic, aromatic⁽¹⁾
Taste: aromatic, characteristic, slightly sweet⁽¹⁾
Active ingredients: each 100 g contains: Miroestrol, 34.14 mg;
Daidzin, 11.71 mg; Puerarin, 23.72 mg;
Genistin, 1.27 mg; Daidzein, 1.67 mg;
Genistein, 1.39 mg. ⁽¹⁾
Solubility: miscible with water⁽¹⁾
pH (in water 200 mg/ml): 5.10 (measured at PCDL Feb.22, 2006)
Storage conditions: cool dry place⁽²⁾
Expiration date: 02/02/07⁽²⁾

2.1.1. Chemical analysis

Certificate of Analysis (dated 02/02/06) provided by the Sponsor is attached to this Study Report ⁽¹⁾. Composition of the mixture and the analytical control are the Sponsor's responsibility.

2.1.2. Stability control of the test article

Stability control of the test article is the Sponsor's responsibility.

2.1.3. Formulation of the test article

The necessary amount of the test article was weighed and mixed in with distilled water not earlier than 20 min before administration.

Dose 5,000 mg/kg:

12.5 g Pueraria mirifica, PE + distilled water ad 50 ml
(concentration of the suspension: 250 mg/ml)

Dose 2,300 mg/kg:

11.50 g Pueraria mirifica, PE + distilled water ad 50 ml
(concentration of the suspension: 230 mg/ml)

2.1.4. Concentration and homogeneity check of the formulated test article

Concentration and homogeneity of the test suspensions were checked by gravimetry. Samples were taken from both concentrations of the formulated test suspension immediately before the dosing procedure: 3 samples of 0.5 ml each were taken from the top, middle as well as bottom regions.

Results of the concentration and homogeneity check

Nominal concentration mg/ml	Sample	Actual concentration mg/ml	Standard deviation	Difference %	Date of sampling / measurement
500	top	256	3.67	+ 2.27	March 14-15, 2006
	middle	257	3.29	+ 2.80	
	bottom	251	3.72	+ 0.267	
230	top	233	2.73	+ 1.45	March 14-15, 2006
	middle	234	1.79	+ 1.74	
	bottom	236	4.73	+ 2.61	

The homogeneity and the actual concentration were within the acceptable limits of $\pm 5\%$.

2.2. Characteristics of article used for formulation of the test article

Name: distilled water
 Manufactured by: PCDL Tox. Group
 Batch number: A 001 1005
 Storage conditions: at room temperature
 Expiration date: 10.2006

2.3. Characteristics of article used for over-anesthesia before necropsy

Name:	T 61
Ingredients:	0.2 g embutramide, 0.005 g tetracaine hydrochloride, and 0.05 g mébézonium iodide per ml
Manufacturer:	Intervet International
Batch number:	12 D 012
Storage conditions:	at room temperature, in safety box for poisonous drugs
Expiration date:	11. 2009
Dose:	0.1 ml / 100 g body weight
Administration:	i.v.

3. TEST SYSTEM

3.1. Animals

Species / Strain:	rat, Cri:CD (SD) of Sprague Dawley origin
Age at arrival:	approx. 7 weeks
Body weight at arrival:	males: 201.9 - 219.2 g females: 152.8 - 170.9 g
Number of animals ordered:	30 (15 males, 15 females)
Number of animals involved in the study:	30 (15 males, 15 females)

3.1.1. Breeder/Supplier

Charles River Laboratories Hungary Ltd. Isaszeg, Ady Endre u. 47., H-2117, Hungary

3.1.2. Hygienic class

SPF at arrival, kept in good conventional environment during the study.

3.2. Reason for the selection of species

The rat is commonly used for toxicological studies in accordance with international recommendations. The Sprague Dawley strain is a well-known laboratory model with sufficient historical data.

3.3. Identification and housing of animals

The animals were identified by ear numbering technique and housed in cages by one. The cages were labeled with tags indicating the I.D. numbers of the rats, the study code, the group identification, sex, route of administration, and the starting and ending dates of the experimental period.

3.4. Housing conditions

Hygienic level: good conventional

Type of animal cages: polypropylene bottoms with stainless steel lids

Size of cage: H x W x D : 17.5 cm x 36.5 cm x 40.5 cm

Cleaning: by changing the bedding material containing

bottom of the cages two times a week

Number of animals per cage: 1

Number of animal keeping room: 122

3.4.1. Environmental conditions

Air exchange: approximately 15 times/hour

Temperature: 22 ± 3°C

Relative humidity: 30 - 70 %

Lighting: artificial, 12-hour light-dark cycles.

Environmental conditions were maintained by a regulated air-conditioning system, temperature and relative humidity were continuously recorded. (Results are kept in the study file.)

3.4.2. Feed

Free access to standardized rat and mouse diet sniff SM R/M-Z+H, 15 mm, autoclavable except for the overnight fasting period prior to treatment, during treatment as well as for the first two hours of the post-treatment observation.

The composition of the diet and the acceptable level of contaminants were controlled by the Manufacturer sniff Spezialdiäten GmbH; D-59494 Soest, Germany. The diet was identified by lot number: 4935450 and the date of manufacturing (December 15, 2005), expiry date: June, 2006.

3.4.3. Drinking

Rats had free access to tap water via drinking bottles. Drinking water is checked monthly by the Microbiological Department of PCDL.

3.5. Acclimatization period

The animals were observed for 5 days prior to the treatment. Only healthy animals, free from any clinical symptoms were used in the study.

3.6. Randomization

The animals were assigned to groups on the basis of their body weight so that their individual body weights fell in an interval within $\pm 20\%$ of the mean weight of the group at treatment.

4. EXPERIMENTAL DESIGN

4.1. Dose levels, group division

The following doses and animals were used:

Treatment	Group	Dose mg/kg po.	Volume ml/kg	Males		Females	
				Number of animals	Identification No's	Number of animals	Identification No's
Pueraria mirifica, PE	1	5,000	20	5	#181 - #185	-	-
	2	5,000	20	-	-	5	#191 - #195
	3	2,300	10	5	#186 - #190	-	-
	4	2,300	10	-	-	5	#196 - #200
Control*	5	0	0	5	#201 - #205	-	-
	6	0	0	-	-	5	#211 - #215

*As no clinical symptoms or mortality occurred, the five male and five female animals purchased together with and handled similarly to the main groups however remained untreated, were decided to serve as controls to the organ weights of the treated animals (Amendment to the Protocol No 1).

4.2. Reason for dose selection

2,000 or 5,000 mg/kg are the limit doses recommended in the OECD Guideline 423 for acute oral toxicity testing of chemicals⁽⁴⁾ and 5,000 mg/kg is suggested to be given as limit dose of a food additive by the FDA guideline⁽³⁾. A food additive for use by man will not be granted a tolerance that will exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals.⁽⁵⁾

Considering that the test article Pueraria mirifica, PE is a plant extract without known toxicity, it is not expected to produce mortality when administered in high dose to animals, therefore, 5,000 mg/kg has been selected as limit dose. Pueraria mirifica, PE is foreseen to be used as dietary supplement in an oral dose of 80 mg taken twice a day i.e. 160 mg per day⁽¹⁾. If the suggested 5,000 mg/kg limit dose results in no toxicity, this will correspond to a safety factor of approx. 2,200.

The 2,300 mg/kg (secondary) limit dose applied in this study to rats corresponds to 1,000 fold the about 2.3 mg/kg bw. daily dose consumed by an adult of 70 kg body weight. In case any kind of toxicity occur after the 2,300 mg/kg dose, a further reduced dose, e.g. 230 mg/kg, applied to additional male and female groups might still represent a safety factor of 100.

The procedure is carried out on rats of both sexes as no information is available which sex is more sensitive to the test article.

5. ADMINISTRATION

5.1. Route of administration and reason for the selection

Application was oral by gavage. The route of application was selected in compliance with international guidelines. The oral route is the anticipated route of human exposure to the test article.

5.2. Frequency and duration of application

Single dose.

5.3. Volume of application

The test article was administered in volumes of 10 or 20 ml/kg body weight (see Table in § 4.1.)

5.4. Duration of the experimental period

Five days of acclimatization, treatment's day, 14 days post-treatment observation period including the treatment's day, and the 15th day: autopsy.

6. OBSERVATIONS, EXAMINATIONS

6.1. Lethality

Observations were made twice daily at the beginning and end of the working day and at least once on weekend days.

6.2. General state, external appearance, behavior, and clinical symptoms

Careful clinical observation of the rats was carried out once before the exposure then, after the treatment for 4 hours continuously, and during the subsequent period, animals were checked twice daily for physical signs of toxicity. On week-ends, animals were checked at least once daily. Signs to be observed included changes in skin, fur, eyes and visible mucous membranes; occurrence of secretions and excretions and autonomic activity (e.g.

lacrimation, piloerection, diarrhea, pupil size, unusual respiratory pattern). Furthermore, potential changes in gait, posture and response to handling as well as the presence of somnolence, trembling, clonic or tonic movements, stereotypes or bizarre behavior were recorded.

6.3. Body weight

Animals were weighed at arrival in the laboratory, on the day of randomization, on the day of treatment before the treatment, as well as on the 2nd, 8th days, and on the 15th day of the experiment prior to autopsy.

7. PATHOLOGY

7.1. Autopsy

On completion of the post-treatment observation period, all rats were exterminated under T61 over-anesthesia and autopsied. All external and internal organs were carefully observed for toxic signs and the results recorded. As no clinical symptoms or mortality occurred during the study, the five male and five female animals purchased together with and handled similarly to the main groups however remained untreated, were decided to serve as controls to the organ weights of the treated animals (Amendment to the Protocol No 1) . Considering the fact that the test article contains phytoestrogens, organs listed below were weighed from all animals (paired organs together): thymus, adrenals, prostate, testicles, epididymides, ovaries and uterus. The relative organ weights were calculated (g/100g or mg/100g body weight). As no toxic lesions occurred, no tissue samples were preserved for histological examination

8. EVALUATION, STATISTICAL ANALYSIS

Groups of males and females were evaluated separately. Individual changes from body weights weighed on Days 1 and 2, Days 2 and 8, Days 8 and 15 as well as Days 1 and 15 were calculated and tabulated (body weight gain). Mean values and standard deviations from the individual body weights, body weight changes, absolute and body weight related organ weights were calculated.

8.1. Parametric values

Body and organ weights were evaluated by STATISTICA™ Version 5.5 (Edition 99, StatSoft Inc., 2300 East 14th Street, Tulsa OK, USA) using standard statistical methods: Bartlett's test, ANOVA, Tukey test, Kruskal-

Wallis nonparametric one-way analysis, Kolmogorov-Smirnov test
Measured and calculated values were rounded if rational.

8.2. Non parametric values (lethality and clinical symptoms)

The incidence of lethality and clinical symptoms were tabulated.

9. PROCEDURES

The experiments were performed according to the current Standard Operating Procedures of the Department of Toxicology of the Pharmaceutical Control and Development Laboratory Co. Ltd.

10. ANIMAL PROTECTION

In the interests of animal welfare, the unnecessary use of animals was avoided. To order the mild extermination of unambiguously moribund animals would have been the responsibility of the study director. The present method (limit test) is using a reduced number of experimental animals in comparison to other known and acknowledged acute toxicity tests.

11. DATA RECORDING AND ARCHIVATION

All original data are maintained, as dictated by the Standard Operating Procedures, on appropriate forms as follows:

- Test Compound weighing
- Animal room logbook
- Body weight logbooks
- Lethality and Clinical observations logbooks
- Postmortem records

The data obtained in the course of the study were collected in a Study File. The Study Protocol, all data generated during and as a result of the study, the documents and all information in connection with the study, a control sample of the test article, and the Final Report will be stored at least for 15 years in the Archives of the PCDL then offered to the Sponsor.

12. SCHEDULE OF THE STUDY

Arrival of the animals:	March 09, 2006
Randomization:	March 13, 2006
Treatment's day:	March 14, 2006
Autopsy:	March 28, 2006

13. RESULTS

13.1. Lethality

(see Table 1. and Appendices 1.1.-1.2.)

No death occurred up to 5,000 mg/kg single oral dose of *Pueraria mirifica*, PE. All males and females survived until the end of the 14-day observation period.

13.2. Clinical symptoms

(see Table 2. and Appendices 2.1.-2.4.)

No treatment related symptoms were observed either on the day of application or throughout the 14-day post-treatment period at any groups of the male and female animals.

13.3. Body weights

(see Tables 3.1.-3.2. and Appendices 3.1.-3.4.)

The mean body weight and body weight gain of the animals corresponded to their species and age throughout the study. No weight loss at any of the males and females was observed.

13.4. Gross pathology

(see Table 4. – 6. and Appendices 4.1.- 4.2., 5.1-5.2 and 6.1- 6.2)

All animals survived until the scheduled autopsy on Day 15. All organs of all male and female rats proved to be free of treatment related gross pathological changes. The absolute weight of the prostates decreased slightly in the high dose male group (-17%), the decrease in the prostate weight related to body weight did not reach the significant level of $p < 0.05$. There was no statistically significant difference among the absolute and relative weights of testes, epididimides, thymus and adrenals of the *Pueraria mirifica*, PE treated males, neither was difference among the uterus', ovaries', thymus' and adrenals' absolute and relative weights of the females compared to the relevant controls.

Pathological changes not considered to be treatment related: Altogether 10 females: #192F, #193F (Group 2F), #196F, #197F, #199F, #200F (Group 4F), and #212F, #213F, #214F, #215F (Group 6F) out of the 15 female rats had uterine horns slightly distended and/or with slight hyperemia. Distended uterine horns (hydrometra) is often observed in normal rats and is a slight physiological disorder connected to the uterine cycle.

14. EVALUATION

No death occurred, no body weight loss was observed. No toxic or other clinical symptoms were apparent. Autopsy revealed no treatment related pathological changes. The slight decrease of the prostate weight of the high dose treated males may be in connection with the phytoestrogenic effect of the test article.

Conclusion:

Single oral LD₅₀ and “no adverse effect dose” are higher than 5,000 mg/kg which corresponds to 2,200 fold of the planned human daily dose of Pueraria mirifica, PE as dietary supplement.

Susan Somfai-Relle

Susan Somfai-Relle, M.D. *May 23, 2006.*
Study Director

References:

1. Certificate of Analysis signed by Youssef Mirhom, issued by Bio-Botanica® Inc., 75 Commerce Drive, Hauppauge, NY 11788 (attached to this report), and Monograph analyzing the structure of Miroestrol, explaining its action as Phytoestrogen (for internal use)
2. E-mail of February 2, 2006: Statement of Prof. Dr. Youssef W. Mirhom, BioBotanica, Inc., forwarded by Mr. Bara
3. US-FDA, Center for Food Safety and Applied Nutrition: Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food; “Redbook II” - DRAFT, Acute oral Toxicity Tests. 1993
4. OECD GUIDELINES FOR TESTING OF CHEMICALS (Guideline No.: 423, adopted: 17th December, 2001, Acute Oral Toxicity - Acute Toxic Class Method).
5. 21 CFR Ch. I (4-1-03 Edition) Subpart B – Food Additive Safety §170.22 Safety factors to be considered

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)**

T a b l e s

Acute oral toxicity study of Pueraria mirifica, PE
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Table 1.

Lethality

Post-treatment observation period (14 days)

Treatment	MALES	FEMALES
	death / number of animals	
Groups 1M / 2F Pueraria mirifica, PE; 5,000 mg/kg, po.	0/5	0/5
Groups 3M / 4F Pueraria mirifica, PE; 2,300 mg/kg, po.	0/5	0/5
Groups 5M / 6F Control, po.	0/5	0/5

**Acute oral toxicity study of Pueraria mirifica, PE
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Table 2.

Clinical Symptoms

During the first four hours after treatment and
the 14 day post-treatment observation period

Treatment	MALES		FEMALES	
	symptom / number of animals			
Groups 1M / 2F Pueraria mirifica, PE; 5,000 mg/kg, po.	0/5		0/5	
Groups 3M / 4F Pueraria mirifica, PE; 2,300 mg/kg, po.	0/5		0/5	
Groups 5M / 6F Control, po. *	0/5		0/5	

* during the 14 day post-treatment observation period only

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Table 3.1.

Body Weights

MALES

Group / Treatment	Body weights [g]					
	Day of arrival	Day of randomization	Day 1 prior to treatment	Day 2	Day 8	Day 15
Group 1M; Pueraria mirifica, PE; 5,000 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	211	262	246	258	313	361
± S.D.:	2.17	11.0	9.76	11.2	17.5	28.1
Group 3M; Pueraria mirifica, PE; 2300 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	208	258	243	257	313	369
± S.D.:	4.31	8.76	11.6	10.6	12.3	13.8
Group 5M; Control, po.						
Group size:	5	5	5	5	5	5
Mean:	213	259	245	258	309	354
± S.D.:	6.77	8.33	10.6	11.4	19.0	29.1

FEMALES

Group / Treatment	Body weights [g]					
	Day of arrival	Day of randomization	Day 1 prior to treatment	Day 2	Day 8	Day 15
Group 2F; Pueraria mirifica, PE; 5,000 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	160	185	172	179	205	227
± S.D.:	8.04	10.2	11.8	9.32	13.1	18.9
Group 4F; Pueraria mirifica, PE; 2,300 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	162	181	172	179	204	219
± S.D.:	6.90	10.2	7.56	7.85	14.1	13.1
Group 6F; Control, po.						
Group size:	5	5	5	5	5	5
Mean:	160	185	174	180	203	222
± S.D.:	6.73	7.94	7.91	9.55	6.54	9.21

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Table 3.2.

Body Weight Changes

MALES

Group / Treatment	Body weight changes [g]			
	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15	Day 1 through Day 15
Group 1M; Pueraria mirifica, PE; 5,000 mg/kg, po.				
Group size:	5	5	5	5
Mean:	12.0	54.8	47.6	114
± S.D.:	2.21	8.48	13.5	18.4
Group 3M; Pueraria mirifica, PE; 2,300 mg/kg, po.				
Group size:	5	5	5	5
Mean:	14.6	56.1	55.5	126
± S.D.:	5.85	7.40	4.65	5.65
Group 5M; Control, po.				
Group size:	5	5	5	5
Mean:	13.2	50.3	45.6	109.1
± S.D.:	2.07	13.1	13.2	25.1

FEMALES

Group / Treatment	Body weight changes [g]			
	Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15	Day 1 through Day 15
Group 2F; Pueraria mirifica, PE; 5,000 mg/kg, po.				
Group size:	5	5	5	5
Mean:	7.6	26.0	22.0	55.6
± S.D.:	4.53	4.13	6.61	9.71
Group 4F; Pueraria mirifica, PE; 2,300 mg/kg, po.				
Group size:	5	5	5	5
Mean:	6.60	24.8	14.8	46.1
± S.D.:	5.77	8.63	1.47	9.90
Group 6F; Control, po.				
Group size:	5	5	5	5
Mean:	6.04	23.3	19.3	48.6
± S.D.:	2.10	3.85	5.16	7.03

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Table 4.

Gross Pathological Findings

Treatment / Groups	Animal with findings / number of animals			
	external	internal	external	internal
	MALES		FEMALES	
	Group 1M		Group 2F	
<i>Pueraria mirifica</i> , PE; 5,000 mg/kg, po.	0/5	0/5	0/5	2*/5
	Group 3M		Group 4F	
<i>Pueraria mirifica</i> , PE; 2,300 mg/kg, po.	0/5	0/5	0/5	4*/5
	Group 5M		Group 6F	
Control, po.	0/5	0/5	0/5	4*/5

* Uterine horns (slightly) distended and/or (slightly) hyperemic

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Table 5.

Organ Weights

MALES

Group / Treatment	Testicles mg	Epididymides mg	Prostate mg	Thymus mg	Adrenals mg/
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.					
Group size:	5	5	5	5	5
Mean:	3074	812	700 _a	538	58.0
± S.D.:	373	121	33.6	57.7	4.64
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.					
Group size:	5	5	5	5	5
Mean:	2943	730	852	508	47.2
± S.D.:	201	54.7	122	98.9	5.54
Group 5M: Control, po.					
Group size:	5	5	5	5	5
Mean:	2877	818	846	471	53.4
± S.D.:	208	76.1	67.5	63.0	5.59

FEMALES

Group / Treatment	Thymus mg	Uterus mg	Ovaries mg	Adrenals mg
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.				
Group size:	5	5	5	5
Mean:	545	596	131	64.2
± S.D.:	78.0	286	13.3	9.10
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.				
Group size:	5	5	5	5
Mean:	448	595	156	64.8
± S.D.:	22.1	164	23.1	6.40
Group 6F: Control, po.				
Group size:	5	5	5	5
Mean:	484	716	130	68.8
± S.D.:	49.8	271	15.7	7.30

S.D. = Standard Deviation

"_a" or "A" = Statistically significant difference in comparison with the corresponding control group (p < 0.05 or p < 0.01, respectively)

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Table 6.

Organ Weights Related to Body Weight

MALES

Group / Treatment	Testicles mg/100 g	Epididymides mg/100 g	Prostate mg/100 g	Thymus mg/100 g	Adrenals mg/100 g	* Body weight g
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	852	225	195	149	16.1	361
± S.D.:	76.1	22.4	20.4	15.8	0.68	28.1
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.						
Group size:	5	5	5	5	5	5
Mean:	800	198	231	138	12.8	369
± S.D.:	74.0	20.9	34.9	24.0	1.44	13.8
Group 5M: Control, po.						
Group size:	5	5	5	5	5	5
Mean:	817	233	240	134	15.1	354
± S.D.:	99.3	36.5	22.3	23.0	1.98	29.1

FEMALES

Group / Treatment	Thymus mg/100 g	Uterus mg/100 g	Ovaries mg/100 g	Adrenals mg/100 g	* Body weight g
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.					
Group size:	5	5	5	5	5
Mean:	242	262	57.8	28.3	227
± S.D.:	43.0	126	7.36	3.94	18.9
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.					
Group size:	5	5	5	5	5
Mean:	206	271	71.3	29.6	219
± S.D.:	18.2	68.2	6.56	2.33	13.1
Group 6F: Control, po.					
Group size:	5	5	5	5	5
Mean:	218	325	58.6	31.0	222
± S.D.:	24.8	127.8	6.94	3.30	9.21

* = Body weight at necropsy after exsanguination

S.D. = Standard Deviation

"a" or "A" = Statistically significant difference in comparison with the corresponding control group ($p < 0.05$ or $p < 0.01$, respectively)

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)**

A p p e n d i c e s

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)**

Appendix 1.1.**Individual Data of Lethality**

MALES

Group	Animal code	DAYS OF OBSERVATION PERIOD														
		Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.																
	181	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	182	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	183	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	184	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	185	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.																
	186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	187	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	188	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	189	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 5M: Control, po.																
	201	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	202	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	203	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	204	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Remarks: 0 = No Lethality

* Day 1 = Treatment's day

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 1.2.

Individual Data of Lethality

FEMALES

Group	DAYS OF OBSERVATION PERIOD														
	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.															
191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.															
196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
197	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
198	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 6F: Control, po.															
211	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
212	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
213	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
214	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Remarks: 0 = No Lethality

* Day 1 = Treatment's day

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 2.1.

Individual Clinical Symptoms

during the first four hours after treatment

MALES

Animal code	After treatment min	Observations
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.		
181	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
182	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
183	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
184	0 – 240	Symptom-free. 151 min (30 min after feeding time) starts to eat
185	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.		
186	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
187	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
188	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
189	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
190	0 – 240	Symptom-free. 121 min (feeding time) starts to eat

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 2.2.

Individual Clinical Symptoms

Post-treatment observation period (14 days*)

MALES

Group	Animal code	DAYS OF OBSERVATION PERIOD														
		Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.																
	181	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	182	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	183	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	184	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	185	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.																
	186	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	187	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	188	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	189	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	190	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
Group 5M: Control, po.																
	201	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	202	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	203	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	204	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	205	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF

Remarks: SF = Symptom Free

Day 1 = Treatment's day, symptoms of males observed during the first 4 hours of the treatment's day are shown in App. 2.1 on the previous page

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 2.3.

Individual Clinical Symptoms

during the first four hours after treatment

FEMALES

Animal code	After treatment min	Observations
Group 2F: <i>Pueraria mirifica</i>, PE; 5,000 mg/kg, po.		
191	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
192	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
193	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
194	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
195	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
Group 4F: <i>Pueraria mirifica</i>, PE; 2,300 mg/kg, po.		
196	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
197	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
198	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
199	0 – 240	Symptom-free. 121 min (feeding time) starts to eat
200	0 – 240	Symptom-free. 121 min (feeding time) starts to eat

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 2.4.

Individual Clinical Symptoms

Post-treatment observation period (14 days*)

FEMALES

Group	Animal code	DAYS OF OBSERVATION PERIOD														
		Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.																
	191	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	192	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	193	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	194	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	195	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.																
	196	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	197	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	198	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	199	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	200	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
Group 6F: Control, po.																
	211	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	212	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	213	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	214	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
	215	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF

Remarks: SF = Symptom Free

* Day 1 = Treatment's day, symptoms of females observed during the first 4 hours of the treatment's day are shown in App. 2.3 on the previous page

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 3.1.

Individual Body Weights

MALES

Group	Animal code	Body weights [g]					
		Day of arrival	Day of randomization	Day 1, prior to treatment	Day 2	Day 8	Day 15
Group 1M: <i>Pueraria mirifica</i>, PE; 5,000 mg/kg, po.							
	181	210.5	279.5	258.5	273.0	342.5	398.9
	182	211.2	263.1	251.7	262.3	312.1	372.5
	183	213.1	260.8	245.0	256.2	307.3	359.2
	184	210.6	257.8	243.5	257.6	306.7	349.7
	185	207.1	249.5	232.4	241.9	296.3	322.8
	Group size:	5	5	5	5	5	5
	Mean:	211	262	246	258	313	361
	± S.D.:	2.17	11.0	9.76	11.2	17.5	28.1
Group 3M: <i>Pueraria mirifica</i>, PE; 2,300 mg/kg, po.							
	186	210.2	269.4	259.8	270.4	334.5	390.6
	187	212.2	261.5	239.1	263.1	311.2	362.1
	188	209.5	258.8	242.4	257.2	305.7	360.2
	189	201.9	255.6	244.0	252.9	311.1	374.2
	190	204.4	245.4	227.5	242.3	303.8	356.8
	Group size:	5	5	5	5	5	5
	Mean:	208	258	243	257	313	369
	± S.D.:	4.31	8.76	11.6	10.6	12.3	13.8
Group 5M: Control, po.							
	201	212.0	268.4	259.9	272.2	336.3	387.4
	202	216.8	264.2	250.5	266.2	318.8	371.7
	203	216.3	259.9	243.0	258.0	288.8	313.2
	204	219.2	251.8	240.4	251.4	296.3	338.3
	205	202.1	248.5	231.9	243.7	303.0	360.7
	Group size:	5	5	5	5	5	5
	Mean:	213	259	245	258	309	354
	± S.D.:	6.77	8.33	10.6	11.4	19.0	29.1

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 3.2.

Individual Body Weights

FEMALES

Group	Animal code	Body weights [g]					
		Day of arrival	Day of randomization	Day 1, prior to treatment	Day 2	Day 8	Day 15
Group 2F: <i>Pueraria mirifica</i>, PE; 5,000 mg/kg, po.							
	191	166.6	199.8	188.2	193.0	225.4	256.9
	192	170.6	192.2	180.1	184.4	211.7	233.1
	193	155.7	180.3	161.9	176.6	199.0	223.9
	194	154.3	178.2	166.4	171.0	196.4	210.9
	195	152.8	176.1	162.1	171.7	194.2	211.8
	Group size:	5	5	5	5	5	5
	Mean:	160	185	172	179	205	227
	± S.D.:	8.04	10.2	11.8	9.32	13.1	18.9
Group 4F: <i>Pueraria mirifica</i>, PE; 2,300 mg/kg, po.							
	196	167.0	192.7	177.5	189.0	227.9	240.6
	197	170.9	188.2	181.7	184.9	204.1	220.5
	198	153.8	178.7	167.3	169.6	194.7	209.6
	199	160.1	177.8	172.8	174.6	198.6	212.7
	200	158.1	166.4	162.9	177.0	193.6	209.5
	Group size:	5	5	5	5	5	5
	Mean:	162	181	172	179	204	219
	± S.D.:	6.90	10.2	7.56	7.85	14.1	13.1
Group 6F: Control, po.							
	211	170.7	195.7	182.3	191.3	212.3	233.6
	212	161.4	188.9	179.9	187.3	207.4	229.0
	213	158.2	186.7	174.9	178.8	199.9	211.5
	214	154.4	178.5	167.5	172.3	197.3	222.3
	215	154.5	176.2	163.8	168.9	198.2	215.2
	Group size:	5	5	5	5	5	5
	Mean:	160	185	174	180	203	222
	± S.D.:	6.73	7.94	7.91	9.55	6.54	9.21

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 3.3.

Individual Body Weight Changes

MALES

Group	Animal code	Body weight changes [g]				
		Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15	Day 1 through Day 15	
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.						
	181	14.5	69.5	56.4	140.4	
	182	10.6	49.8	60.4	120.8	
	183	11.2	51.1	51.9	114.2	
	184	14.1	49.1	43.0	106.2	
	185	9.50	54.4	26.5	90.4	
	Group size:	5	5	5	5	5
	Mean:	12.0	54.8	47.6	114	
	± S.D.:	2.21	8.48	13.5	18.4	
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.						
	186	10.6	64.1	56.1	130.8	
	187	24.0	48.1	50.9	123.0	
	188	14.8	48.5	54.5	117.8	
	189	8.90	58.2	63.1	130.2	
	190	14.8	61.5	53.0	129.3	
	Group size:	5	5	5	5	5
	Mean:	14.6	56.1	55.5	126	
	± S.D.:	5.85	7.40	4.65	5.65	
Group 5M: Control, po.						
	201	12.3	64.1	51.1	127.5	
	202	15.7	52.6	52.9	121.2	
	203	15.0	30.8	24.4	70.2	
	204	11.0	44.9	42.0	97.9	
	205	11.8	59.3	57.7	128.8	
	Group size:	5	5	5	5	5
	Mean:	13.2	50.3	45.6	109.1	
	± S.D.:	2.07	13.1	13.2	25.1	

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 3.4.

Individual Body Weight Changes

FEMALES

Group	Animal code	Body weight changes [g]				
		Day 1 through Day 2	Day 2 through Day 8	Day 8 through Day 15	Day 1 through Day 15	
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.						
	191	4.80	32.4	31.5	68.7	
	192	4.30	27.3	21.4	53.0	
	193	14.7	22.4	24.9	62.0	
	194	4.60	25.4	14.5	44.5	
	195	9.60	22.5	17.6	49.7	
	Group size:	5	5	5	5	
	Mean:	7.6	26.0	22.0	55.6	
	± S.D.:	4.53	4.13	6.61	9.71	
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.						
	196	11.5	38.9	12.7	63.1	
	197	3.20	19.2	16.4	38.8	
	198	2.30	25.1	14.9	42.3	
	199	1.80	24.0	14.1	39.9	
	200	14.1	16.6	15.9	46.6	
	Group size:	5	5	5	5	
	Mean:	6.6	24.8	14.8	46.1	
	± S.D.:	5.77	8.63	1.47	9.90	
Group 6F: Control, po.						
	211	9.0	21.0	21.3	51.3	
	212	7.40	20.1	21.6	49.1	
	213	3.90	21.1	11.6	36.6	
	214	4.80	25.0	25.0	54.8	
	215	5.10	29.3	17.0	51.4	
	Group size:	5	5	5	5	
	Mean:	6.04	23.3	19.3	48.6	
	± S.D.:	2.10	3.85	5.16	7.03	

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 4.1.

Gross Pathological Findings

MALES

Group	Animal code	Day 15	
		external	internal
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.			
	181	No Finding	No Finding
	182	No Finding	No Finding
	183	No Finding	No Finding
	184	No Finding	No Finding
	185	No Finding	No Finding
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.			
	186	No Finding	No Finding
	187	No Finding	No Finding
	188	No Finding	No Finding
	189	No Finding	No Finding
	190	No Finding	No Finding
Group 5M: Control, po.			
	201	No Finding	No Finding
	202	No Finding	No Finding
	203	No Finding	No Finding
	204	No Finding	No Finding
	205	No Finding	No Finding

„No Finding” stands here for:

external: Animal of average development. Skin, fur, visible mucous membranes are intact.

internal: Organs are without pathological changes.

Gastric mucosa: intact.

Acute oral toxicity study of *Pueraria mirifica*, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 4.2.

Gross Pathological Findings

FEMALES

Group	Day 15	
	external	internal
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.		
191	No Finding	No Finding
192	No Finding	The uterine horns are slightly distended, diameter of approx. 3 mm, Gastric mucosa: intact. The rest of the organs: intact.
193	No Finding	The uterine horns are slightly distended, diam. approx. 3 mm, slightly hyperemic. Gastric mucosa: intact. The rest of the organs: intact.
194	No Finding	No Finding
195	No Finding	No Finding
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.		
196	No Finding	The uterine horns are slightly hyperemic. Gastric mucosa: intact. The rest of the organs: intact.
197	No Finding	The uterine horns are slightly distended, diam. approx. 2 mm. Gastric mucosa: intact. The rest of the organs: intact.
198	No Finding	No Finding
199	No Finding	The uterine horns are slightly distended, diam. approx. 4 mm. Gastric mucosa: intact. The rest of the organs: intact.
200	No Finding	The uterine horns are slightly hyperemic. Gastric mucosa: intact. The rest of the organs: intact.
Group 6F: Control, po.		
211	No Finding	No Finding
212	No Finding	The uterine horns are slightly distended, diam. approx. 3 mm. Gastric mucosa: intact. The rest of the organs: intact.
213	No Finding	The uterine horns are slightly distended, diam. approx. 4 mm. Gastric mucosa: intact. The rest of the organs: intact.
214	No Finding	The uterine horns are slightly distended, diam. approx. 3 mm, hyperemic. Gastric mucosa: intact. The rest of the organs: intact.
215	No Finding	The uterine horns are slightly distended, diam. approx. 4 mm, slightly hyperemic. Gastric mucosa: intact. The rest of the organs: intact.

„No Finding” stands here for:

external: Animal of average development. Skin, fur, visible mucous membranes are intact.

internal: Organs are without pathological changes. Gastric mucosa: intact.

**Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)**

Appendix 5.1.

Individual Organ Weights

MALES

Group / Animal code	Testicles mg	Epididymides mg	Prostate mg	Thymus mg	Adrenals mg/
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.					
181	3529	948	686	525	63
182	3188	936	691	587	63
183	3190	740	667	511	56
184	2521	690	756	603	54
185	2943	746	702	462	54
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.					
186	2655	646	761	652	50
187	2815	750	831	420	39
188	3072	797	983	456	44
189	3132	725	973	444	51
190	3042	731	713	570	52
Group 5M: Control, po.					
201	2990	721	913	474	59
202	2878	761	760	464	49
203	3111	833	789	527	57
204	2554	904	892	369	46
205	2853	872	877	519	56

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 5.2.

Individual Organ Weights

FEMALES

Group / Animal code	Thymus mg	Uterus mg	Ovaries mg	Adrenals mg
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.				
191	591	443	134	68
192	427	797	137	74
193	526	997	116	54
194	634	387	119	70
195	549	354	148	55
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.				
196	431	728	189	73
197	446	653	170	62
198	467	397	133	70
199	474	753	151	61
200	423	445	139	58
Group 6F: Control, po.				
211	526	300	118	68
212	479	941	150	77
213	536	925	112	74
214	412	592	130	67
215	468	821	141	58

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 6.1.

Individual Organ Weights Related to Body Weight

MALES

Group / Animal code	Testicles mg/100 g	Epididymides mg/100 g	Prostate mg/100 g	Thymus mg/100 g	Adrenals mg/100 g	* Body weight g
Group 1M: Pueraria mirifica, PE; 5,000 mg/kg, po.						
181	885	238	172	132	16	398.9
182	856	251	186	158	17	372.5
183	888	206	186	142	16	359.2
184	721	197	216	172	15	349.7
185	912	231	217	143	17	322.8
Group 3M: Pueraria mirifica, PE; 2,300 mg/kg, po.						
186	680	165	195	167	13	390.6
187	777	207	229	116	11	362.1
188	853	221	273	127	12	360.2
189	837	194	260	119	14	374.2
190	853	205	200	160	15	356.8
Group 5M: Control, po.						
201	772	186	236	122	15	387.4
202	774	205	204	125	13	371.7
203	993	266	252	168	18	313.2
204	755	267	264	109	14	338.3
205	791	242	243	144	16	360.7

* = Body weight at necropsy

Acute oral toxicity study of Pueraria mirifica, PE
with 14-day post-treatment observation period in the rat (Limit test)

Appendix 6.2.

Individual Organ Weights Related to Body Weight

FEMALES

Group / Animal code	Thymus mg/100 g	Uterus mg/100 g	Ovaries mg/100 g	Adrenals mg/100 g	* Body weight g
Group 2F: Pueraria mirifica, PE; 5,000 mg/kg, po.					
191	230	172	52.2	26.5	256.9
192	183	342	58.8	31.7	233.1
193	235	445	51.8	24.1	223.9
194	301	183	56.4	33.2	210.9
195	259	167	69.9	26.0	211.8
Group 4F: Pueraria mirifica, PE; 2,300 mg/kg, po.					
196	179	303	78.6	30.3	240.6
197	202	296	77.1	28.1	220.5
198	223	189	63.5	33.4	209.6
199	223	354	71.0	28.7	212.7
200	202	212	66.3	27.7	209.5
Group 6F: Control, po.					
211	225	128	50.5	29.1	233.6
212	209	411	65.5	33.6	229.0
213	253	437	53.0	35.0	211.5
214	185	266	58.5	30.1	222.3
215	217	382	65.5	27.0	215.2

* = Body weight at necropsy

Botanical Extracts
Tinctures
Nutraceuticals
Cosmetic Intermediates
Phytochemicals
Spray Drying
Product Development



BIO-BOTANICA® INC

Code: 4523
Lot #: 031615
Analysis Date: 02/02/06

Pueraria mirifica, PE

Certificate of Analysis

Product Description:

MANUFACTURER: Bio-Botanica, Inc.
PLANT: *Pueraria mirifica* Family: Fabaceae
PART USED: Root
ACTIVE CONSTITUENTS: Miroestrol and Isoflavonoids
APPEARANCE: Free-flowing Powder
COLOR: Light Beige
AROMA: Characteristic, Aromatic
TASTE: Aromatic, Characteristic, Slightly Sweet
METHOD OF ASSAY: HPLC
METHOD OF MANUFACTURING: Extraction by *Bio-Chelation[®]
SOLVENT FOR EXTRACTION: Aqueous Alcohol
EXCIPIENT: Maltodextrin, Capsul, Colloidal Silicon Dioxide
ADDITIVE: None
SOLUBILITY: Miscible with Water
RECOMMENDED STORAGE: Store in a cool dry place, away from excessive heat, light or freezing temperatures.
SHELF LIFE: 12 Months – from the date of analysis

Specification:

MOISTURE: ≤7.0% (2 hrs@ 105°C)
FOREIGN ORGANIC MATTER: ≤0.02 %
BULK DENSITY: 0.25-0.60 g/CC
ASH: ≤10.00 % (2 hrs@ 600°C)
MESH SIZE: ≥95.00% through #40 mesh
ASSAY: Each 100 g contains:
≥ 20 mg Miroestrol and ≥ 20 mg Isoflavonoids
(Including: Daidzin, 3-11 mg; Puerarin, 12-30 mg; Genistin, 0.5-2 mg; Daidzein, 1.1-3.6 mg; Genistein, 0.2-2 mg)

Analysis:

MOISTURE: 2.29%
FOREIGN ORGANIC MATTER: N/A
BULK DENSITY: 0.46
ASH: 6.00%
MESH SIZE: 95.60%
ASSAY: Each 100 g contains: Miroestrol, 34.14 mg; Daidzin, 11.71 mg; Puerarin, 23.72 mg; Genistin, 1.27 mg; Daidzein, 1.67 mg; Genistein, 1.39 mg.

Microbial:

TOTAL PLATE COUNT: ≤ 5000 C.F.U. @72hrs@ 37°C
YEAST & MOLD COUNT: ≤ 500 C.F.U./gm
STAPHYLOCOCCUS AUREUS: Negative
SALMONELLA: Negative
E. COLI: Negative

Analysis:

TOTAL PLATE COUNT: 600
YEAST&MOLD COUNT: 60
STAPHYLOCOCCUS AUREUS: Neg.
SALMONELLA: Neg.
E. COLI: Neg.

*Bio-Chelation[®] is a registered trademark of Bio-Botanica, Inc.. Bio-Chelation[®] is a proprietary cold extraction process.

Written by:

Quality Control:

Approved by Vice President of Research & Development





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HUNGARY

Budapest, 25th June 2004
No.: 337/48/2004
Our ref.: Éva Perjés/KK
Annex:
Subject:

STATEMENT OF GLP COMPLIANCE

Date of inspection: 13-14 April, 2004

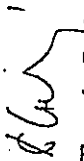
Assessment of conformity with GLP Principles has been performed according to Council Directives 87/18/EEC and 88/320/EEC and Commission Directives 1999/11/EC and 1999/12/EC.

According to the criteria specified in paragraph 5 of Article 3 of the Joint Decree No 9/2001. (III.30.) EüM-FVM of the Minister of Health and the Minister of Agriculture and Regional Development on the application and verification of good laboratory practice the Director-General of the National Institute of Pharmacy certifies that the test facility

Pharmaceutical Control and Development Laboratory Co. Ltd. (PCDL)
H-1149 Budapest, Mexikói út 9., Hungary

is able to carry out *toxicity studies and safety pharmacology testing* in compliance with the Principles of Good Laboratory Practice as established by the OECD and the European Community.




Prof. Dr. Tamás L. Paál
Director-General

