CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-902

PHARMACOLOGY REVIEW

MEMORANDUM
Oct. 27, 2006

TO: File
FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-902

I have read the review by Dr. Jiaqin Yao of the pharmacology/toxicology studies submitted for Veregen ® Ointment, 15% (Kunecatechins) and concur that the marketing application may be approved. The product label is adequate.

_____
Kenneth L. Hastings, Dr.P.H., D.A.B.T.
Associate Director
Office of New Drugs

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Kenneth Hastings
10/27/2006 01:13:19 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-902**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**10/14/2005**
DRUG NAME:	**PolyPhenon® E Ointment, 15%**
INDICATION:	**Treatment of external genital and perianal warts**
SPONSOR:	**MediGene AG, Martinsried, Germany**
DOCUMENTS REVIEWED:	**Vol. 1.1 - 1.52**
REVIEW DIVISION:	**Division of Dermatologic and Dental Products (HFD-540)**
PHARM/TOX REVIEWER:	**Jiaqin Yao**
PHARM/TOX SUPERVISOR:	**Paul Brown**
DIVISION DIRECTOR:	**Susan Walker**
PROJECT MANAGER:	**Millie Wright**

Date of review submission to Division File System (DFS): 6-13-2006

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
The NDA is approvable from a pharmacology/toxicology perspective.

B. Recommendation for nonclinical studies
None

C. Recommendations on labeling

The following wording is recommended for the Carcinogenesis, Mutagenesis and Impairment of Fertility section of the labeling:

The Maximum Recommended Human Dose (MRHD) of [Trade Name] Ointment, 15% was set at three times daily topical administration of 250 mg (in total 750 mg, containing 112.5 mg [USAN Name]) for the animal multiple of human exposure calculations presented in this labeling. Dose multiples were calculated based on the human equivalent dose (HED).

In an oral (gavage) carcinogenicity study, [USAN Name] was administered daily for 26 weeks to p53 transgenic mice at doses up to 500 mg/kg/day (22-fold MRHD). Treatment with [USAN Name] was not associated with an increased incidence of either neoplastic or non-neoplastic lesions in the organs and tissues examined. [Trade Name] Ointment, 15% has not been evaluated in a dermal life-time carcinogenicity study.

[USAN Name] was negative in the Ames test, in vivo rat micronucleus assay, UDS test, and transgenic mouse mutation assay, but positive in the mouse lymphoma tk assay.

Daily vaginal administration of [Trade Name] Ointment, 15% to rats, from Day 4 before mating throughout mating until Day 17 of gestation did not caused adverse effects on mating performance and fertility at doses up to 0.15 mL/rat/day. This dose corresponds to approximately 150 mg/rat/day or 500 mg/kg/day (8-fold MRHD). The vaginal administration is considered a possible unintended route of application in humans since [Trade Name] Ointment, 15% may inadvertently be transferred to the vagina.

The following wording is recommended for the Pregnancy section of the labeling:

Pregnancy Category: C

Embryo-fetal development studies were conducted in rats and rabbits using intravaginal and systemic routes of administration, respectively. Oral administration of [USAN Name] during the period of organogenesis (gestational Days 6 to 15 in rats or 6 to 18 in rabbits) did not caused treatment related effects on embryo-fetal development or

teratogenicity at doses of up to 1,000 mg/kg/day (86-fold MRHD in rats; 173-fold MRHD in rabbits,).

In the presence of maternal toxicity (characterized by marked local irritation at the administration sites and decreased body weight and food consumption) in pregnant female rabbits, subcutaneous administration of 12 or 36 mg/kg/day of [USAN Name] during the period of organogenesis (gestational Days 6 to 19) resulted in corresponding influences on fetal development including reduced fetal body weights and delays in skeletal ossification. No treatment related effects on embryo-fetal development or teratogenicity were noted at 4 mg/kg/day (0.7-fold MRHD). There was no evidence of teratogenic effects at any of the doses evaluated in this study.

A combined fertility/embryo-fetal development study using daily vaginal administration of [Trade Name] Ointment, 15% to rats, from Day 4 before mating throughout mating until Day 17 of gestation did not show treatment-related effects on embryo-fetal development or teratogenicity at doses up to 0.15 mL/rat/day (8-fold MRHD).

A pre- and post-natal development study was conducted in rats using vaginal administration of [Trade Name] Ointment, 15% at doses of 0.05, 0.10, or 0.15 mL/rat/day from Day 6 of gestation through parturition and lactation. The high and intermediate dose levels of 0.15 and 0.10 mL/rat/day resulted in an increased mortality of the F0 dams, associated with indications of parturition complications. The high dose level of 0.15 mL/rat/day also resulted in an increased incidence of stillbirths. No reproductive or developmental toxicities were noted at 0.05 mL/rat/day (2.7-fold MRHD). There were no other treatment-related effects on pre-and post-natal development, growth, reproduction and fertility at any dose tested.

There are no adequate and well-controlled studies in pregnant women. [Trade Name] Ointment, 15% should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Intravaginal application should be avoided.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Polyphenon E 15% Ointment or Polyphenon E drug substance was tested for up to 3 months orally or topically in rats and dogs and for up to 9 months topically in mini-pigs. Gastrointestinal tract, liver, pancreas and lymphoid tissues were primarily affected in rats following oral administration. No apparent systemic toxicity was noted in mini-pigs after topical treatment of Polyphenon E 15% Ointment for 9 months. Polyphenon E Ointment induced minimal to severe local irritation including erythema, edema, and inflammatory reactions when topically applied to rats, rabbits, and mini-pigs. Polyphenon E Ointment caused strong local irritation to vaginal mucosa after vaginal application in female rats and mini-pigs.

Polyphenon E was negative in the Ames test, in vivo rat micronucleus assay, UDS test, transgenic mouse mutation assay, but positive in the mouse lymphoma cell mutation assay.

Oral administration of Polyphenon E at doses up to 500 mg/kg/day for 26 weeks did not increase the incidence of either non-neoplastic or neoplastic lesions in the organs and tissues examined in transgenic heterozygous [redacted] mice.

There were no substantial adverse effects on the reproductive system as determined in a number of fertility and embryo-fetal development studies in rats and rabbits including different routes of administration (oral, subcutaneous, and intravaginal). Polyphenon E was not teratogenic in rats and rabbits. However, subcutaneous application of Polyphenon E drug substance at 12 and 36 mg/kg/day in rabbits caused effects on fetal development such as reduced fetal weights and delayed skeletal ossification. In addition, in the pre- and post-natal development study in rats using vaginal administration of Polyphenon E 15% Ointment, 0.10 and 0.15 mL/rat/day resulted in an increased mortality of the F0 dams, associated with indications of parturition complications. The dose of 0.15 mL/rat/day Polyphenon E 15% Ointment also resulted in an increased incidence of stillbirths. The NOAEL was 4 mg/kg/day Polyphenon E drug substance in the embryo-fetal development study in rabbits by subcutaneous route. The NOAEL for both maternal and developmental toxicity was 0.05 mL/rat/day Polyphenon E 15% Ointment (corresponding to approximately 25 mg/kg/day Polyphenon E drug substance) in the pre- and post-natal development study in rats by vaginal administration.

Polyphenon E Ointment had the potential to induced contact sensitization as shown in the local lymph node assay and was a sensitizer in the guinea-pig.

B. Pharmacologic activity

Polyphenon E drug substance in vitro had anti-oxidative activity, anti-inflammatory activity, the potential to inhibit a variety of enzymes involved in the pathogenesis of cancer and inflammatory diseases, the potential to inhibit HPV associated cervical cancer cell growth, and the immunomodulatory potential. It also reduced the expression of E7 (a major tumor promoter) in human papillomavirus (HPV)-infected cells and activated T cell via predendritic cells by releasing IL-6 in vitro. However, topical application of Polyphenon E Ointment did not cause statistically significant effects on the growth of HPV-6 infected human xenografts in SCID mice and the growth of CRPV-induced skin warts in rabbits. The mode of action of Polyphenon E drug substance involved in the clearance of genital and perianal warts is unknown.

C. Nonclinical safety issues relevant to clinical use

This drug product induced minimal to severe local irritation including erythema, edema, and inflammatory reactions when topically applied to rats, rabbits, and mini-pigs. This drug product also caused strong local irritation to vaginal mucosa after vaginal application in female rats and mini-pigs.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-902
Review number: 1
Sequence number/date/type of submission: 000 / 9-23-2005 / Original submission
000 / 4-25-2006 / BC
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: MediGene AG, Martinsried, Germany

Manufacturer for drug substance: Mitsui Norin Co., Ltd, Shizuoka, Japan

Reviewer name: Jiaqin Yao
Division name: Dermatologic and Dental Product
HFD #: 540
Review completion date: 6-13-2006

Drug:

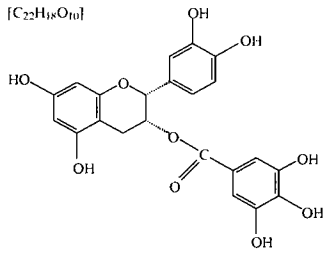
Trade name: PolyPhenon® E Ointment, 15%
Generic name: GTE (green tea extract)
Code name: Tea catechins; Tea polyphenols; Tea tannin; epicatechins; flavan-3-ols;
Chemical name, CAS registry number, Molecular formula/molecular weight: See the next table.

Total catechin content of PolyPhenon E (85 - 95 % w/w)

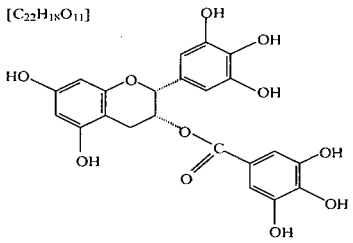
Composition:	Abbr.	Range (%)	Formula	M.W.	CAS #
(-)-Epigallocatechin gallate	EGCg		$C_{22}H_{18}O_{11}$		989-51-5
(-)-Epigallocatechin	EGC		$C_{15}H_{14}O_7$		970-74-1
(-)-Epicatechin gallate	ECg		$C_{22}H_{18}O_{10}$		1257-08-5
(-)-Epicatechin	EC		$C_{15}H_{14}O_6$		490-46-0
(-)-Gallocatechin gallate	GCg		$C_{22}H_{18}O_{10}$		1257-08-5
(+)-Catechin	C		$C_{15}H_{14}O_6$		490-46-0
(-)-Gallocatechin	GC		$C_{15}H_{14}O_7$		3371-27-5
(-)-Catechin gallate	CG		$C_{22}H_{18}O_{10}$		139495-40-2
Total catechin content	-				

Basic PolyPhenon E chemical structures:

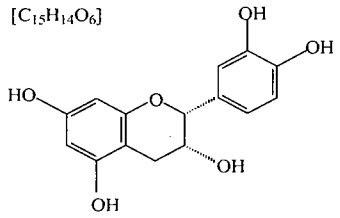
EGCg **ECg**

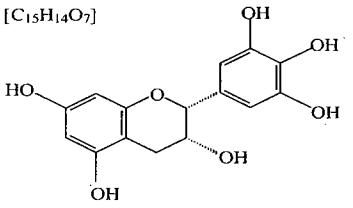


EGC



EC





Relevant INDs/NDAs/DMFs: INDs 56,401, 58367, and 70614, DMF —

Drug class: Immunomodulator

Indication: Treatment of external genital and perianal warts

Clinical formulation:

Ointment Formulation	% (v/v)
Polyphenon E	15.0
Isopropyl Myristate	
White Petroleum	
Oleyl Alcohol	
White Wax (Cera alba)	
Propylene Glycol Monostearate	

Route of administration: Topical

Proposed use: Polyphenon E Ointment, 15% is to be applied topically three times per day to all external genital and perianal warts for up to 16 weeks. The maximal recommended human dose was three times daily topical administration of 250 mg ointment (total 750 mg, containing 112.5 mg or 1.9 mg/kg/day Polyphenon E drug substance, assuming a 60 kg human).

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology

1. **Anti-oxidative activity of Polyphenon E drug substance in vitro** (PE_PTX_405)
2. **Enzyme inhibitions study in vitro with Polyphenon E drug substance** (PE_PTX_0406)

3. **Inhibition of HPV E7 gene expression by Polyphenon E drug substance in HPV-infected human cell lines in vitro by means of quantitative TaqMan RT-PCR** (PE_PTX_0407)
4. **Inhibition of tumor cell proliferation in vitro by Polyphenon E drug substance** (PE_PTX_0408)
5. **Effects of Polyphenon E drug substance on cytokines in human peripheral blood mononuclear cells in vitro** (PE_PTX_0409)
6. **Modulation of cytokine release of primary keratinocytes in vitro Polyphenon E drug substance** (PE_PTX_0410)
7. **Effects of Polyphenon E drug substance on T cell hybridoma activity** (PE_PTX_0411)
8. **Evaluation of Polyphenon E ointment in the human HPV-6 infected external human xenograft SCID mouse model** (PE_PTX_0311)
9. **Dermal application of Polyphenon E for treatment of CRPV-induced papilloma** (PE_PTX_0306)
10. **Effects of Polyphenon E in the Irwin test in rats** (PE_PTX_0403)
11. **Effects of Polyphenon E on respiration rate and tidal volume in rats** (PE_PTX_0404)

Pharmacokinetics/Toxicokinetics

1. **In vitro per cutaneous absorption of different EGCG formulations in human skin** (PE_PTX_0312)
2. **Pharmacokinetics and tissue distribution studies of ^{3}H-Epigallocatechin Gallate (EGCg) in beagle dogs** (B026-99)
3. **Review and critical evaluation of the literature pertaining to the metabolism of green tea catechins** (11379)
4. **Semiquantitative measurement of total epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epicatechin (EC) in selected minipig plasma samples originating form the "Combined toxicity/kinetics study of Polyphenon E 15% ointment in female minipigs following repeated dermal and intravaginal administration"** (A120461A)

General Toxicology

1. **28-Day toxicity and mutagenicity study of Polyphenon E administered by gavage to male and female [redacted] mice** (G110-01)
2. **Combined toxicity/kinetics study of Polyphenon E 15% ointment in female minipigs following repeated dermal and intravaginal administration** ([redacted]03.PC0206)
3. **13-Week dose-range-finding study for a carcinogenicity study of Polyphenon E 15% Ointment by repeated topical administration to CD rats** (PE_PTX_0219)
4. **9-Month chronic toxicity study of Polyphenon E 15% ointment by dermal administration to minipigs** (PE_PTX_0226)

Genetic Toxicology

1. **Isopropyl myristate - Bacterial reverse mutation test (Plate incorporation and preincubation methods)** ([redacted]03.PC0207)

1. **Polyphenon E crème – pharmacokinetics study in female mini-pig following oral, dermal and vaginal administration** ([redacted]03.PC0005)
2. **Polyphenon E-API – Bacterial reverse mutation test (plate incorporation and preincubation methods)** ([redacted]03.PC0010)
3. **Polyphenon E-API – *In vitro* mammalian cell gene mutation test on L5178Y mouse lymphoma cells $TK^{+/-}$ (microtitre method)** ([redacted]03.PC0013)
4. **Polyphenon E-API – Mammalian erythrocyte micronucleus test in the rat bone marrow** ([redacted]03.PC0015)
5. **Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes using an in vivo procedure with Polyphenon E** ([redacted] 03. PC0130)
6. **Polyphenon E – 7-day pilot study if crème and ointment formulations by vaginal application in the rat** ([redacted] 03.PC0002)
7. **Polyphenon E – Crème and ointment – dose-range finding reproductive toxicity study by intravaginal application in the rat** ([redacted]03.PC0003)
8. **Polyphenon E – 28-day local tolerance study of crème and ointment formulation after vaginal application in the female rat** ([redacted]03.PC0019)
9. **Local tolerance study of 8 different test substances by repeated epicutaneous administration for 7 days** ([redacted]03.PC0131)
10. **Polyphenon E – Local lymph node assay** ([redacted]03.PC0118)

The following studies were reviewed by Dr. Gary Bond in IND 70,614:

1. **Thirteen-week oral (gavage) toxicity study of Epigallocatechin gallate (EGCG) and Polyphenon E (PE) in rats** (l06306 SN1)
2. **Thirteen-week oral (gavage) toxicity study of Epigallocatechin gallate (EGCG) and Polyphenon E (PE) in dogs** (l06306 SN2)
3. **A developmental toxicity study of orally administered Polyphenon E in rats** (1169-2)
4. **A developmental toxicity study of orally administered Polyphenon E in rabbits** (1169-6)

2.6.2 PHARMACOLOGY

1. **Anti-oxidative activity of Polyphenon E drug substance in vitro** (PE_PTX_405)
2. **Enzyme inhibitions study in vitro with Polyphenon E drug substance** (PE_PTX_0406)
3. **Inhibition of HPV E7 gene expression by Polyphenon E drug substance in HPV-infected human cell lines in vitro by means of quantitative TaqMan RT-PCR** (PE_PTX_0407)
4. **Inhibition of tumor cell proliferation in vitro by Polyphenon E drug substance** (PE_PTX_0408)
5. **Effects of Polyphenon E drug substance on cytokines in human peripheral blood mononuclear cells in vitro** (PE_PTX_0409)
6. **Modulation of cytokine release of primary keratinocytes in vitro Polyphenon E drug substance** (PE_PTX_0410)
7. **Effects of Polyphenon E drug substance on T cell hybridoma activity** (PE_PTX_0411)
8. **Evaluation of Polyphenon E ointment in the human HPV-6 infected external human xenograft SCID mouse model** (PE_PTX_0311)
9. **Dermal application of Polyphenon E for treatment of CRPV-induced papilloma** (PE_PTX_0306)

10. Effects of Polyphenon E in the Irwin test in rats (PE_PTX_0403): Five groups of 6 male rats orally received 0, 10, 100, or 1000 mg/kg Polyphenon E drug substance, or 20 mg/kg chlorpromazine (positive control). The animals were evaluated using the Irwin test prior to dosing, 60, 240, and 480 minutes post-dosing. Although a few behavioral and physiological changes were noted in the rats treated with 10 mg/kg Polyphenon E drug substance during the 480 minutes post-dosing period, no significant behavioral and physiological changes were observed in the rats treated with 100 or 1000 mg/kg Polyphenon E drug substance. Characteristic behavioral effects were seen in the rats treated with the positive control.

11. Effects of Polyphenon E on respiration rate and tidal volume in rats (PE_PTX_0404): Five groups of 8 male rats received orally 0, 10, 100, or 1000 mg/kg Polyphenon E, or intravenously 20 mg/kg morphine (positive control). The animals were examined on respiration rate and tidal volume prior to dosing, 60 and 480 minutes post-dosing. No significant effect was observed in Polyphenon E-treated animals. A depression of respiration rate and tidal volume was seen in the rats treated with morphine.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

NA

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

1. In vitro per cutaneous absorption of different EGCG formulations in human skin (PE_PTX_0312): Four different in vitro skin penetration studies with human skin and different Polyphenon E containing formulations to which ^{3}H-labeled EGCg was added

was conducted in 2 different laboratories. Between 0.21% and 8.25% of the applied ^{3}H-labeled EGCg penetrated into skin over a period of up to 48 hours. Only a minor fraction of the applied ^{3}H-labeled EGCg (0.01% to 0.55%) penetrated from the formulation through the skin into the reservoir.

2. Pharmacokinetics and tissue distribution studies of ^{3}H-Epigallocatechin Gallate (EGCg) in beagle dogs (B026-99): Four dogs received intravenously a single dose of ^{3}H-EGCg (25 mg/g), and blood, plasma, urine, and feces samples were collected. The same dogs were treated orally with ^{3}H-EGCg (250 mg/kg) after a wash-out period. Blood, plasma, urine, and feces were again collected. The dogs then received daily doses of 250 mg/g unlabeled EGCg for 26 days, followed by an intravenous dose of ^{3}H-EGCg (about 25 mg/kg). Tissues were collected 1 hr after drug treatment for determination of radioactivity.

After IV administration, the radioactivity was distributed within the first hour after dosing, followed by elimination of the label with a half life of about 6 hr. EGCg or its metabolites were well distributed to several tissues, primarily epithelial, including stomach, mesenteric lymph nodes, kidneys, bladder, colon, esophagus, prostate glands, lungs, small intestine, but radioactivity tends to concentrate in the liver (17.5% of the dose at 1 hr after drug treatment). Approximately 5% of the radioactivity was located in the small intestine and 3% in the gastrointestinal tract contents. Total recovery of radioactivity in the tissues was 46±8% of the dose. The primary route of elimination was in the feces, about 30% of the dose by 24 hr; urinary excretion of radioactivity was about 9% in 48 hr. Total recovery of radioactivity in the excreta after IV EGCg administration was 40±16% of the dose (after 48 hours).

After oral administration, the time to maximum plasma concentration was about 1 hour and the half life for elimination of radioactivity was about 7 hours. Approximately 30% of the radioactivity was excreted in the feces. However, a large fraction of the dose was excreted in feces before 24 hr (approximately 14%), probably due to unabsorbed EGCg. Urinary excretion of radioactivity after oral EGCg administration accounted for approximately 5% of the dose in 72 hr. Total recovery of radioactivity in the excreta after oral EGCg administration was 34±4% of the dose (after 72 hours). Following daily repeat oral dosing with unlabeled EGCg, plasma concentrations of EGCg were accumulated. The estimated oral bioavailability of EGCG based on the total radioactivity and EGCg was 19.6% and 12.7%, respectively.

3. Polyphenon E crème – pharmacokinetics study in female mini-pig following oral, dermal and vaginal administration (MDG03.PC0005, reviewed by Dr. Paul Brown in IND 56401): "Three animals were given 2 mg/kg of the Polyphenon E drug substance by gavage. After 7 days, 2.5 g of a crème containing 10% Polyphenon E was applied to 100 cm^{2} skin on the same three animals three times per day for 5 days. A separate group of three animals was given 20 mg/kg of the Polyphenon E drug substance by gavage. After 7 days, 1 ml of the 10% Polyphenon E crème was administered intravaginally to this second group of three animals three times per day for 5 days. For the oral dosing experiment, blood samples were collected before dosing and again at 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours after dosing. For the dermal and vaginal experiments blood was collected on the first day of dosing before application and 1, 2 and 4 hours following each

application, on the 2nd and 4th day of dosing at 2 hours after the last application, on the 3rd day of dosing prior to application and at 2 hours after the last application and on the fifth day of dosing prior to application, 1, 2, and 4 hours after each application and at 12 and 24 hours after the last application. EGCG concentration was measured as an indicator of exposure to the entire Polyphenon E mixture.

EGCG levels in all samples obtained from animals after the oral dose of [redacted] were below the limit of quantification. The limit of quantification is not specified but appears to be around [redacted] EGCG was quantifiable in the plasma of animals treated with 20 mg/kg at 0.5 hours. The T_{max} appeared to be between 1.5 and 2 hours. No EGCG was detected at 12 or 24 hours after the dose. Half-life was 1.27 hours and clearance was 149,440.6 ml/hr·kg. The average C_{max} was 40.193 ng/ml. EGCG was detected in only a few of the samples taken from animals treated dermally with the Polyphenon E ointment. The highest concentration detected was approximately [redacted] at 8 hours after dosing in one animal. After vaginal administration EGCG was quantifiable at all time points. The T_{max} occurred at between 6 and 8 hours after administration. Half-life was 3.51 hours and clearance was 104,779.9 ml/ hr·kg. The average C_{max} was 1170.267 ng/ml. Relatively little EGCG appears to be absorbed from Polyphenon E applied to the skin of a minipig as a 10% crème formulation. Significant amounts are absorbed, however, after vaginal and oral administration. The half-life of EGCG in the minipig appears to be between 1 and 4 hours. These studies do not measure other components of Polyphenon E."

4. Review and critical evaluation of the literature pertaining to the metabolism of green tea catechins (11379): In this literature review, the sponsor stated that EGCG and ECG were found in plasma after green tea administration to human volunteers. EGCG could be hydrolyzed to EGC and gallic acid, and that gallic acid was further biotransformed to the benzoic and hippuric acid derivatives. Methylation of catechins occurred in vitro and in vivo. The catechins and their 4-Q-methylated metabolites were largely conjugated with glucuronide and sulfate. The sponsor further stated that it is expected that 4-Q-methylation would occur after dermal application (of catechins that are absorbed through the epidermis). However, the extent of methylation may be less than what has been observed after oral ingestion and the degree of conjugation can be expected to be less after dermal application. Obviously, the pharmacokinetic profile can be expected to differ after dermal and oral administrations, with lower plasma concentrations (C_{max}, AUC), a more gradual increase to peak concentrations (longer T_{max}), and a slower decline in plasma levels (longer $t_{½}$) after dermal applications.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

NA

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: Polyphenon E 15% Ointment or Polyphenon E drug substance was tested for up to 3 months orally or topically in rats and dogs and for up to 9 months

topically in mini-pigs. Gastrointestinal tract, liver, pancreas and lymphoid tissues were primarily affected in rats following oral administration. No apparent systemic toxicity was noted in mini-pigs after topical treatment of Polyphenon E 15% Ointment for 9 months. Polyphenon E Ointment induced minimal to severe local irritation including erythema, edema, and inflammatory reactions when topically applied to rats, rabbits, and mini-pigs. Polyphenon E Ointment caused strong local irritation to vaginal mucosa after vaginal application in female rats and mini-pigs.

Genetic toxicology: Treatment with Polyphenon E up to 1,000 mg/kg/day for 28 days did not caused an increased mutation frequency at *cII* gene in [redacted] mice. Polyphenon E was negative in the Ames test, in vivo rat micronucleus assay, and UDS test, but positive in the mouse lymphoma cell mutation assay. Isopropyl myristate was negative in the Ames test, mouse lymphoma cell mutation assay, and in vivo rat micronucleus assay.

Carcinogenicity: Oral administration of Polyphenon E at doses up to 500 mg/kg/day for 26 weeks did not increase the incidence of either non-neoplastic or neoplastic lesions in the organs and tissues examined in transgenic heterozygous [redacted] mice.

Reproductive toxicology: There were no substantial adverse effects on the reproductive system as determined in a number of fertility and embryo-fetal development studies in rats and rabbits including different routes of administration (oral, subcutaneous, and intravaginal). Polyphenon E was not teratogenic in rats and rabbits. However, subcutaneous application of Polyphenon E drug substance at 12 and 36 mg/kg/day in rabbits caused effects on fetal development such as reduced fetal weights and delayed skeletal ossification. In addition, in the pre- and post-natal development study in rats using vaginal administration of Polyphenon E 15% Ointment, 0.10 and 0.15 mL/rat/day resulted in an increased mortality of the F0 dams, associated with indications of parturition complications. The dose of 0.15 mL/rat/day also resulted in an increased incidence of stillbirths. The NOAEL was 4 mg/kg/day Polyphenon E drug substance in the embryo-fetal development study in rabbits using subcutaneous route. The NOAEL for both maternal and developmental toxicity was 0.05 mL/rat/day Polyphenon E 15% Ointment (corresponding to approximately 25 mg/kg/day Polyphenon E drug substance) in the pre- and post-natal development study in rats using vaginal administration.

Special toxicology: Local lymph node assay suggested that Polyphenon E Ointment had the potential to induced contact sensitization and it was a sensitizer in the guinea-pig.

2.6.6.2 Single-dose toxicity

No specific single dose toxicity studies were conducted by the sponsor with the drug product.

2.6.6.3 Repeat-dose toxicity

1. 28-Day toxicity and mutagenicity study of Polyphenon E administered by gavage to male and female [redacted] mice (G110-01):

Five groups of 7 male and 7 female [redacted] mice were orally administered vehicle (water), 500, 1000, or 2000 mg/kg/day of Polyphenon E, or 50 mg/kg/day of urethane (positive control) for 28 days, followed by a recovery period of 28 days, to study the toxic effects and potential in vivo mutagenicity of Polyphenon E. All animals in the vehicle, 500 or 1000 mg/kg/day Polyphenon E, and 50 mg/kg/day urethane groups survived until the end of the study and exhibited no significant adverse clinical signs. In contrast, every animal in the 2000 mg/kg/day Polyphenon E dose group died or was euthanized in moribund condition before or on Day 25. Male mice were generally more sensitive, and died by the end of the first week. Both male and female mice in the 2000 mg/kg/day dose group showed severe clinical signs before death including hypoactivity, ruffed fur, hunched posture, low body temperature, and whole body tremors. There were no test article-related effects on body weights, food consumption, hematology, or clinical chemistry in surviving mice, except that increased cholesterol levels (up to 34%) were seen in both males and females in the 1000 mg/kg/day groups. Clinical chemistry data from the 2000 mg/kg/day group were available only from one male euthanized in moribund condition on Day 7. Compared to the vehicle control males, this male mouse has increased aspartate aminotransferase (18816 vs 119 IU/L), alanine aminotransferase (9132 vs 44 IU/L), total bilirubin (1.2 vs 0.2 mg/dL), direct bilirubin, indirect bilirubin, and inorganic phosphate and decreased cholesterol (39 vs 131 mg/dL), triglycerides (39 vs 189 mg/dL), glucose, and BUN/creatinine ratio. In male mice, absolute liver weights and liver-to-body and liver-to-brain weight ratios were significantly lower in the 500 and 1000 mg/kg Polyphenon E groups. Microscopic evaluation of tissues revealed numerous lesions in the animals in the 2000 mg/kg/day dose group that died or were euthanized early. Findings included cardiac myofiber degeneration, hepatic fatty change, hepatic centrolobular necrosis, mesenteric lymph node necrosis, splenic lymphoid depletion, and thymic lymphoid depletion. No treatment-related microscopic changes were observed in other groups.

In the surviving dose groups (500 and 1000 mg/kg/day), no increase in *cII* mutations were observed in the liver, spleen, and lungs compared with the vehicle controls (see the next table). The positive control urethane produces statistically significant increases in mutant frequency (MF) only in lung and spleen in male mice (only up to 2.3 fold of the MF in controls). Likewise, positive DNA samples from previous studies run in parallel with analyses showed strong positive responses, confirming the validity of the assay. Polyphenon E was negative in the [redacted] mouse mutagenesis assay.

Tissue/Organ	Sex	*cII* Mutation Frequency (10^{-5}, mean ± SD)			
		Polyphenon E (mg/kg/day)			Urethane
		0	500	1000	50 mg/kg/day
Liver	M	7.5 ± 2.1	7.0 ± 4.6	6.6 ± 1.9	7.9 ± 2.5
	F	6.0 ± 1.8	7.8 ± 4.4	7.3 ± 1.4	8.1 ± 4.3
Lung	M	5.7 ± 1.7	5.4 ± 3.2	6.2 ± 3.0	12.0 ± 4.3**
	F	5.5 ± 2.0	5.4 ± 2.1	3.6 ± 1.5	9.2 ± 5.0
Spleen	M	4.8 ± 1.8	6.0 ± 3.6	8.4 ± 5.5	11.0 ± 4.0**
	F	4.1 ± 3.6	4.9 ± 1.3	4.8 ± 1.5	6.7 ± 3.0

** Significant different from the control group with Student t-test ($p < 0.01$)

2. Combined toxicity/kinetics study of Polyphenon E 15% ointment in female minipigs following repeated dermal and intravaginal administration (MDG03.PC0206): Four groups of three female minipigs were administered topically with Placebo ointment, Polyphenon E 15% ointment, Placebo ointment without isopropyl myristate (IPM), or Polyphenon E 15% ointment without IPM, three times daily at a dose of 2 g per application (6 g total daily dose) on a 50 cm^2 skin surface area over a period of 28 days. In addition, two groups of 3 female minipigs were administered intravaginally with 0.1 mL or 1.0 mL of Polyphenon E 15% ointment three times daily over a period of nine days. Erythema, necrosis, blister formation, and edema were observed in the animals treated topically with Polyphenon E 15% ointment with or without IPM. Severe local reactions such as redness and swelling of the vulva, sensitivity to pain, and vaginal bleeding were noted in all animals treated intravaginally with Polyphenon E 15% ointment. Moderate changes in some hematology parameters were observed in Polyphenon E-treated animals, maybe due to local inflammatory reactions. None of the minipigs died during the study and no treatment-related effects on body weight, food and water consumption, clinical chemistry, and organ weights were observed. Histopathology did not reveal any findings indicative of systemic toxicity in topically treated animals. However, superficial purulent dermatitis and focal or diffuse necrotic dermatitis and reactive hyperplasia by papillomatous proliferation of the epidermis were observed in all animals treated topically with Polyphenon E 15% ointment with or without IPM. No test article-related histopathological changes were noted in animals treated with Placebo ointments. In animals treated intravaginally, purulent necrotic vaginitis with neutrophilic granulocytes and cell detritus in the vaginal lumen was noted. Reactive purulent inflammatory changes were noted in the kidneys (purulent pyelitis), ureter, urinary bladder, cervix, and uterus.

Topical treatment with Polyphenon E 15% Ointment resulted in EGCg plasma levels in the range of or slightly above the quantitation limit of 5 ng/mL on Days 1 and 7. A peak EGCg plasma level of 16.7 ng/mL was detected for a single animal on Day 7. On Day 28, minimally higher plasma concentrations for EGCg (up to a maximum of 33.0 ng/mL) were found and the mean exposure to EGCg was 285 ng·h/mL. Three times daily vaginal administration of Polyphenon E 15% ointment for a total period of nine days at doses of 0.1 or 1.0 mL per application resulted in substantially higher plasma concentrations and exposures for EGCg than those observed following topical administration. Treatment with the high dose of 1.0 mL/application resulted in mean C_{max} values of 6,066 ng/mL on Day 1 and 1,430 n/mL on Day 9, and AUC values of 34,402 ng·h/mL on Day 1 and 10,729 ng·h/mL on Day 9; while dosing at the low level reached mean C_{max} values of 1,527 ng/mL on Day 1 and 721 ng/mL on Day 9, and AUC values of 10,586 ng·h/mL on Day 1 and 6,576 ng·h/mL on Day 9. Plasma concentrations and AUC values for EGCg did not increase with dose in a linear way and decreased after repeated vaginal application of Polyphenon E 15% ointment.

The sponsor also determined the concentrations of total EGCG, ECG, and EC semiquantitatively in selected minipig plasma samples [**Semiquantitative measurement of total epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epicatechin**

(EC) in selected minipig plasma samples originating from the "Combined toxicity/kinetics study of Polyphenon E 15% ointment in female minipigs following repeated dermal and intravaginal administration" (A120461A, Non-GLP)]. Topical treatment with Polyphenon E 15% Ointment resulted in ECG plasma levels below the quantitation limit and ECG was measurable in most samples from animals treated intravaginally. EC was detected only in a few samples.

3. Thirteen-Week Oral (Gavage) Toxicity Study of Epigallocatechin Gallate (EGCG) and Polyphenon E (PE) in Rats (L06306 SN1, reviewed by Dr. Gary Bond in IND 70,614): "Fisher 344 rats were administered Epigallocatechin Gallate (EGCG) at doses of 0, 45, 150, or 500 mg/kg/day or Polyphenon E (PE) at doses of 0, 90, 300, or 1000 mg/kg/day by oral gavage for 13 weeks. Mortality or early sacrifice occurred in 500 mg/kg EGCG animals, 1000 mg/kg PE animals, and a 300 mg/kg PE female. The most common clinical signs were noisy or labored breathing in high dose EGCG and PE animals, occurring in ~25-50% of the animals. Noisy breathing and red discoloration around the mouth was observed in high dose EGCG and PE animals. Body weight gains during the dosing period for EGCG and PE animals were significantly decreased from 29-41% for HD males and females ($p<0.05$). Food consumption was decreased ~15-25% for HD and ~5-15% for MD animals for both EGCG and PE groups. Decreased absolute and relative weights of the spleen and thymus in the MD or HD EGCG and PE groups were associated with lymphoid depletion of the spleen and necrosis/atrophy of the thymus. WBC and differential counts were sporadically, but significantly increased in HD EGCG and PE female rats ($p<0.05$). Most gross lesions were in spontaneous deaths or moribund sacrifices with dilated and pigmented gastrointestinal tract being most common. Histopathologic changes were induced in a dose-related manner by both EGCG and PE in both sexes. Primary sites of lesions for MD & HD EGCG and PE animals were the gastrointestinal tract (dilation, ulceration, inflammation, necrosis, and hemorrhage), pancreas (apoptotic necrosis), liver (periacinar degeneration/necrosis, karyomegaly/cytomegaly, and bile duct hyperplasia), thymus (necrosis/atrophy), spleen (lymphoid depletion), seminal vesicle (atrophy), and kidney (basophilic tubules, karyomegaly, and tubular necrosis). Based on the observed results, the NOAEL is 45 mg/kg for EGCG and 90 mg/kg for Polyphenon E for 13 week oral administration in rats."

"Toxicokinetics: Blood levels for EGCG increased with increasing dose at 1.5-2.5 hours after dosing on weeks 7 and 13. For EGCG groups, females exhibited increased blood levels compared to males. For PE groups, there were no apparent sex differences in blood levels of EGCG. Levels of EGCG for PE groups were dose proportional. See table."

EGCG Plasma Levels (ng/mL) for Rats Treated Orally with EGCG or Polyphenon E for 13 Weeks						
	EGCG			Polyphenon E		
	45 mg/kg	150 mg/kg	500 mg/kg	90 mg/kg	300 mg/kg	1000 mg/kg
week 7						
males	7 (5/10)[a]	23 (8/10)	141 (10/10)	14 (3/10)	22 (9/10)	167 (9/9)
females	23 (2/10)	44 (8/10)	559 (10/10)	24 (5/10)	36 (8/10)	144 (9/9)
week 13						
males	8 (5/10)	53 (10/10)	129 (10/10)	35 (3/10)	50 (9/10)	359 (10/10)
females	15 (5/10)	54 (10/10)	286 (7/7)	31 (2/10)	50 (9/9)	272 (8/8)

a – number of animals with detectable levels of EGCG per number of animals sampled

4. Thirteen-Week Oral (Gavage) Toxicity Study of Epigallocatechin Gallate (EGCG) and Polyphenon E (PE) in Dogs (L06306 SN2, reviewed by Dr. Gary Bond in IND 70,614): "Beagle dogs were administered Epigallocatechin Gallate (EGCG) at doses of 0, 30, 100, or 300 mg/kg/day or Polyphenon E (PE) at doses of 0, 60, 200, or 600 mg/kg/day by oral capsule for 13 weeks. No mortality was observed. Conjunctivitis and mucus discharge in the eye appeared to occur only in the treated groups and more frequently in the HD groups. Total bilirubin was statistically significantly increased ($p<0.05$) on weeks 4 (514%) and 13 (64%) for HD PE males compared to control values. The toxicological relevance of this finding is unknown as no microscopic changes were observed in the liver of the effected animals. Microscopic observations of pigmented macrophages in the duodenum villus tip occurred in HD EGCG and PE animals. This phagocytic event was not associated with inflammation but was considered to be of toxicological significance. Based on the observed results, the NOAEL is 100 mg/kg for EGCG and 200 mg/kg for Polyphenon E for 13 week oral administration in dogs."

Toxicokinetics: Blood samples were collected at approximately 3 hours after dosing on Weeks 4 and 13. "EGCG was detected in the blood of 2-4 animals/sex/group. Means plasma concentrations were increased in a dose-related, but not dose proportional manner in both sexes with no apparent sex differences. See table."

Mean EGCG Plasma Levels (ng/mL) for Dogs Treated Orally with EGCG or Polyphenon E for 13 Weeks						
	EGCG			Polyphenon E		
	30 mg/kg	40 mg/kg	300 mg/kg	60 mg/kg	200 mg/kg	600 mg/kg
week 4						
males	8 (2/4)[a]	11 (4/4)	26 (4/4)	10 (3/4)	12 (2/4)	93 (4/4)
females	8 (3/4)	10 (4/4)	29 (4/4)	10 (2/4)	16 (4/4)	40 (4/4)
week 13						
males	3 (2/3)	81 (4/4)	87 (4/4)	12 (4/4)	84 (4/4)	331 (4/4)
females	4 (3/4)	53 (4/4)	150 (4/4)	9 (4/4)	172 (4/4)	263 (4/4)

a – number of animals with detectable levels of EGCG per number of animals sampled

5. Study title: 13-Week dose-range-finding study for a carcinogenicity study of Polyphenon E 15% Ointment by repeated topical administration to CD rats

Key study findings: Topical treatment with 50, 200, or 600 µL (corresponding to approximately 50, 200, or 600 mg) of Polyphenon E 15% Ointment in rats once daily for 4 hours over a period of 13 weeks did not cause deaths or test article-related effects on body weights, food and water consumption, hematology, clinical chemistry, organ weights, or microscopic pathology. Restlessness, rough fur, and/or hematomas were

seen in all animals treated with 200 or 600 µL Polyphenon E in a few weeks. Thickened uterus was seen in a few Polyphenon E-treated females (no histopathological examination on uterus was performed). Slight local irritation at the site of administration including very slight erythema and very slight edema was noted in Polyphenon E-treated animals, without test-article related histopathological findings.

Study no.: PE_PTX_0219
Volume #23, and page #: NA
Conducting laboratory and location: [redacted]

Date of study initiation: 8-28-2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E 15% ointment, 678, [redacted]

Methods

Doses: 0, 50, 200, and 600 µL Polyphenon E 15% Ointment/25 cm^2 surface area (approximately 50, 200, and 600 mg ointment)

Group	Treatment	Number of animals, Main study (Satellite)	
		Male	Female
1	Sham control	10 (6)	10 (6)
2	600 µL Placebo	10 (6)	10 (6)
3	50 µL Polyphenon E	10 (6)	10 (6)
4	200 µL Polyphenon E	10 (6)	10 (6)
5	600 µL Polyphenon E	10 (6)	10 (6)

Species/strain: [redacted] CD rats
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Topical, 15% ointment as clinical formulation.
Satellite groups used for toxicokinetics or recovery: 6/sex/group for Groups 2 - 5.
Age: 6 - 7 weeks
Weight (nonrodents only): Male, 150 - 159 g; female, 150 - 159 g
Unique study design or methodology (if any): Test articles were applied to an area of approximately 25 cm^2 on the back, shaved skin without any dressing for 4 hours per day, 91 consecutive days. Residuals of the test substance were removed at the end of the 4-hour restraining period each day. The sponsor stated that the low-dose (50 µL/animal/day) and the high-dose (600 µL/animal/day) were the minimum and maximum feasible dose volume that could be administered daily by topical application. A 7-day rat topical study (PE_PTX_0218) showed that 600 µL/rat/day was tolerated.

Observation times and results

Mortality: Observed twice daily. No deaths occurred.

Clinical signs: Observed twice daily. The sponsor stated that restlessness and/or hematomas seen in all animals treated with 200 or 600 µL Polyphenon E in a few weeks were caused by the collar used for restraining the animals. Rough fur was also seen in those animals.

Skin responses: The application sites were assessed twice daily. No erythema or edema was noted in the sham control or placebo animals. In males treated with 50 µL Polyphenon E, very slight erythema and very slight edema were noted in 1 or 2 of 10 males for a few days; well-defined erythema were further seen in 3 males on Days 86-91 and even a moderate to severe erythema was observed in 1 male for one day (Day 85). However, females treated with 50 µL Polyphenon E had no erythema or edema. In the group treated with 200 µL Polyphenon E, only very slight erythema and very slight edema were noted in some, but not all males or females during a few weeks. In the group treated with 600 µL Polyphenon E, only very slight erythema and very slight edema were noted in all males and in 3 of 10 females during Weeks 2 to 4 and only very slight erythema in up to 4 males during Weeks 7 to 11.

Body weights: Recorded weekly. Compared to the sham controls, the body weights of the males treated with placebo was lower. However, no apparent test article-related effects on body weights was seen.

Food and drinking water consumption: Recorded weekly. No apparent treatment-related effects were noted.

Ophthalmological and auditory examination: Carried out prior to the initiation of the study and at the end of Week 13. No apparent treatment-related effects were noted.

Hematology: Blood samples were collected from the first 5 animals/sex/group on Day 92. Thromboplastin Time was slightly, but statistically significantly decreased in males treated with 200 or 600 µL Polyphenon E and Polyphenon E-treated females. No other apparent treatment-related effects were noted.

Clinical chemistry: Blood samples were collected from the second 5 animals/sex/group on Day 92. No apparent treatment-related effects were noted.

Urinalysis: NA

Gross pathology: Thickened uterus was seen in a few Polyphenon E-treated females.

Organ weights (specify organs weighed if not in histopath table): No apparent treatment-related effects were noted.

Histopathology: Only the following organs from animals in the sham control, placebo, and high-dose groups were examined microscopically: epididymis, heart, kindney and ureter, liver, lungs with bronchi/bronchioles, lymph node (cervical and mesenteric), pancreas, skin (treated and untreated), spleen, testicle, thymus, thyroid and parathyroids.

No treatment-related microscopic findings were noted. Minimal to mild focal hyperplasia of the epidermis was seen in a few animals in all groups examined.

Toxicokinetics: Blood samples were collected from 3 rats/sex/group in Groups 2-5 pre-dose and at 1, 2, 4, 8, and 12 hr post-dose on Day 3 and Week 12. There was no indication for an accumulation of EGCg in the plasma with repeated topical administration of Polyphenon E in rats for 12 weeks. There was roughly a dose-dependent increase for the plasma levels of EGCg. No apparent sex difference was seen.

Test Day	Dose (μL/animal/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-24h} (h)
Day 3	50	M	89.9	4	11.3	592
		F	192.2	1	9.0	952
	200	M	67.6	4	21.2	688
		F	114.4	1	17.5	682
	600	M	430.0	8	7.2	3064
		F	463.0	8	25.3	3448
Week 12	50	M	NC			
		F	49.2	2	11.5	250
	200	M	227.0	1	4.9	1060
		F	246.4	1	9.2	1111
	600	M	345.0	2	(120.0)*	3476
		F	360.3	4	2.9	2482

NC: Most samples were below the lower limit of quantification (5 ng/mL)
*: Outlier, value not used for further calculations.

6. Study title: 9-Month chronic toxicity study of Polyphenon E 15% ointment by dermal administration to minipigs

Key study findings: There were no deaths and no findings indicative of systemic toxicity. There were no apparent treatment-related effects on food consumption, electrocardiogram, heart rate, blood pressure, ophthalmology, hematology, clinical chemistry, urinalysis, bone marrow, organ weights, and gross pathology. Only the males in the high-dose group had 20% lower body weights than those in the control group at the end of treatment. Very slight to well-defined erythema and very slight edema formation were seen at the application site in some animals treated with the placebo ointment on several days during the treatment period. Very slight to severe erythema and very slight to moderate edema formation were seen in the low-dose group. The incidence and severity of erythema and edema in the middle- and high-dose group were increased compared to the low-dose group. Moderate to severe erythema and moderate to severe edema were noted in the middle-dose group animals. Severe erythema and moderate to severe edema were seen in all high-dose group animals. The incidence and severity of erythema and edema were most pronounced during Weeks 2 to 6 of the study. Subsequently, the severity of skin reactions decreased over time despite the continued treatment with Polyphenon E 15% Ointment. In addition, transient eschar formation surrounding the administration site and red spots either at the administration site or at

other areas of the body of the animals, partly with eschar formation, were also noted. The incidence of these findings was increased in the Polyphenon E-treated groups. The skin reactions had subsided within the first three weeks of the recovery period. No treatment-related microscopic findings were seen in other organs than the skin. Minimal to moderate hyperplasia of the epidermis (psoriasiform reaction pattern) and subcutaneous inflammatory reaction were seen at the Polyphenon E-treated sites and slightly more pronounced in the high-dose group. Minimal superficial purulent dermatitis was also noted in 1 or 2 animals of all Polyphenon E-treated groups, except low-dose females. Minimal to moderate inflammatory reactions with lympho-histiocytic infiltration and minimal mixed cell infitration in the subepithelium were noted in some animals from all groups. In addition, mild epidermal hyperplasia and inflammatory reactions were also seen at untreated, but shaved skin in several animals of all treatment groups including the placebo control, particularly those sections covered with Curafix® fixative bandage. At the end of the 6-week recovery period, minimal to mild hyperplasia and subcutaneous inflammatory reaction were still seen at the Polyphenon E-treated sites. The systemic NOEL for Polyphenon E 15% Ointment in the 9-month minipig chronic toxicity study was above 2.0 g/50 cm^2/application (3 applications/day). However, No local NOAEL was established in this study.

Study no.: PE_PTX_0226
Volume #15, and page #: NA
Conducting laboratory and location: [redacted]

Date of study initiation: 8-28-2002
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E 15% ointment, 000.00402, [redacted] for EGCg

Methods

Doses: 0, 0.25, 1.0, and 2.0 g Ointment/50 cm^2 skin surface area
Species/strain: [redacted] minipigs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: Topical, 15% ointment as clinical formulation.
Satellite groups used for toxicokinetics or recovery: None.
Age: 14 - 19 weeks
Weight (nonrodents only): Male, 5.3 - 11.8 kg; female, 5.2 - 12.6 kg
Unique study design or methodology (if any): An area of approximately 50 cm^2 on the left site of each animal's spine was treated three times daily at 7 am, 11 am, and 3 pm for a total exposure period of 12 hours followed by a 12 hour exposure-free period. The right side of each animal's spine remained untreated but was shaved as control. The administered area was covered with a porous gauze dressing. After 39 weeks of treatment, four male and four female animals per group were sacrificed, whereas the remaining two male and two female animals per group were observed during and sacrificed after a 6-week recovery period.

Observation times and results

Mortality: Observed three times daily. No death occurred.

Clinical signs: Observed before and after dosing and the application site was assessed once daily. There were no apparent treatment-related effects except skin responses.

Skin responses: Very slight to well-defined erythema and very slight edema formation were seen at the application site in some animals treated with the placebo ointment on several days during the treatment period. Very slight to severe erythema and very slight to moderate edema formation were seen in the low-dose group. The incidence and severity of erythema and edema in the middle-dose group were increased compared to the low-dose group. Moderate to severe erythema and moderate to severe edema were noted in the middle-dose group animals. Severe erythema and moderate to severe edema were seen in all high-dose group animals. The incidence and severity of erythema and edema were most pronounced during Weeks 2 to 6 of the study. Subsequently, the severity of skin reactions decreased over time despite the continued treatment with Polyphenon E 15% Ointment. In addition, transient eschar formation surrounding the administration site and red spots either at the administration site or at other areas of the body of the animals, partly with eschar formation, were also noted. The incidence of these findings was increased in the Polyphenon E-treated groups. The skin reactions had subsided within the first three weeks of the recovery period.

Body weights: Recorded weekly. The body weights of males in the high dose group were lower (20%) than those of males in the control group at the end of treatment.

Food and drinking water consumption: Recorded daily. No apparent treatment-related effects were noted.

Ophthalmological and auditory examination: Carried out prior to the initiation of the study and at the end of Weeks 13, 26, 39, and 45. No apparent treatment-related effects were noted.

EKG and blood pressure: Carried out before and 4 hours after the first administration on Day 1, Weeks 13, 26, 39, and 45. No apparent treatment-related effects were noted.

Hematology: Blood samples were collected prior to the first administration and at the end of Weeks 13, 26, 39, and 45. No apparent treatment-related effects were noted.

Clinical chemistry: Blood samples were collected prior to the first administration and at the end of Weeks 13, 26, 39, and 45. No apparent treatment-related effects were noted.

Urinalysis: Urine samples were collected over a period of 3 hours prior to the first administration and at the end of Weeks 13, 26, 39, and 45. No apparent treatment-related effects were noted.

Gross pathology: No apparent treatment-related effects were noted.

Organ weights (specify organs weighed if not in histopath table): No apparent treatment-related effects were noted.

Histopathology: No treatment-related microscopic findings were seen in other organs than the skin. Minimal to moderate, regular hyperplasia of the epidermis (psoriasiform reaction pattern) and subcutaneous inflammatory reaction were seen at the Polyphenon E-treated sites and slightly more pronounced in the high-dose group. Minimal superficial purulent dermatitis was also noted in 1 or 2 animals of all Polyphenon E-treated groups, except low-dose females. Minimal to moderate inflammatory reactions with lympho-histiocytic infiltration and minimal mixed cell infitration in the subepithelium were noted in some animals from all groups. In addition, mild epidermal hyperplasia and inflammatory reactions were also seen at untreated, but shaved skin in several animals of all treatment groups including the placebo control, particularly those sections covered with Curafix® fixative bandage. At the end of the 6-week recovery period, minimal to mild hyperplasia and subcutaneous inflammatory reaction were still seen at the Polyphenon E-treated sites.

Toxicokinetics: Blood samples were collected from 4 minipigs/sex/group in Groups 1- 4 pre-dose and at 1, 2, 6, and 10 hr post-dose on Days 3, 85, 176, and 267. EGCg was detected in some plasma samples in each group (LLOQ 5 ng/mL). A maximum plasma EGCg concentration of 217 ng/mL with an $AUC_{0\text{-}24h}$ of 403 ng·h/mL was reached in a high-dose male on Day 85. There was no clear dose-response relationship for the plasma levels of EGCg (See the next table). There was no indication for an accumulation of EGCg in the plasma with repeated topical administration of Polyphenon E in minipigs for 9 months.

Dose (g/50 cm^2)	C_{max} for EGCg (ng/mL)			
	Day 3	Day 85	Day 176	Day 267
0.25	18.8 (1/8)*	10.4 (3/8)	7.8 (1/8)	5.2 (2/8)
1.0	6.5 (2/8)	9.9 (5/8)	10.7 (3/8)	6.2 (2/8)
2.0	12.8 (5/8)	41.0 (6/8)	6.9 (7/8)	7.6 (7/8)

* Number of animals with detectable levels of EGCg per number of animals sampled

Histopathology inventory (optional)

Study	PE_PTX_0219	PE_PTX_0226		
Species	Rats	Minipigs		
Adrenals	*	X*		
Aorta		X		
Bone Marrow smear		X		
Bone (femur)		X		
Brain	*	X*		
Cecum		X		
Cervix		X		
Colon		X		

Duodenum		X		
Epididymis	X	X		
Esophagus		X		
Eye		X		
Fallopian tube				
Gall bladder		X		
Gross lesions		X		
Harderian gland		X		
Heart	*	X*		
Ileum		X		
Injection site				
Jejunum		X		
Kidneys	X*	X*		
Lachrymal gland				
Larynx		X		
Liver	X*	X*		
Lungs	X*	X*		
Lymph nodes, cervical	X*	X*		
Lymph nodes mandibular				
Lymph nodes, mesenteric	X*	X*		
Mammary Gland		X		
Nasal cavity				
Optic nerves		X		
Ovaries	*	X*		
Pancreas	X	X		
Parathyroid	X*	X*		
Peripheral nerve				
Pharynx		X		
Pituitary	*	X*		
Prostate		X		
Rectum		X		
Salivary gland		X		
Sciatic nerve		X		
Seminal vesicles				
Skeletal muscle		X		
Skin	X	X		
Spinal cord		X		
Spleen	X*	X*		
Sternum				
Stomach		X		
Testes	X*	X*		
Thymus	X*	X*		
Thyroid	X*	X*		
Tongue		X		
Trachea				
Urinary bladder		X		
Uterus	*	X		
Vagina		X		
Zymbal gland				

Drug, lot #, radiolabel, and % purity: Isopropyl myristate, CB11630002, —

Methods:
Strains/species/cell line: L5178Y TK +/- mouse lymphoma cell line.

Doses used in definitive study: See the next table

Basis of dose selection: Cytotoxicity up to 5,000 µg/mL

Negative controls: DMSO

Positive controls: Methyl methanesulfonate and cyclophosphamide

Incubation and sampling times: Three-hour exposures were used both with and without activation and 24 hour exposure time without activation. After 48 hr expression, cells were diluted and plated for mutant frequency in selective medium containing 4 µg/mL 5-triflurothymidine (TFT) in 96-well microtitre plates. Cells were also diluted and plated for viability in non-selective medium. Plates were scored after 10-13 days incubation.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The vehicle controls had acceptable mutant frequency values, which were within the normal range for the L5178Y cell line at the TK locus. The positive controls, both in the absence and presence of metabolic activation, induced marked increases in the mutant frequency, indicating the satisfactory performance of the test and of the activity of the metabolizing system (see the next table).

Study outcome: Isopropyl myristate did not induce a statistically significant or dose-related increase in the mutant frequency, at any dose level, with or without metabolic activation for 4-hour treatment and without metabolic activation for 24-hour treatment (see the next table). Isopropyl myristate was considered to be non-mutagenic to L5178Y cells under the conditions of the test.

Treatment (µg/mL)	4-hour -S9		Treatment (µg/mL)	4-hour +S9	
	Relative total growth (%)	Mutation frequency (X 10^{-6})		Relative total growth (%)	Mutation frequency (X 10^{-6})
0	100	219	0	100	215
87	105	207	87	110	190
155	72	183	155	107	192
276	73	252	276	93	245
492	97	188	492	103	198
878	83	218	878	116	206
1568	82	200	1568	133	163
2800	79	174	2800	118	186
5000	36	186	5000	118	195
4 (MMS)	87	321	2.5 (CP)	61	1440
7.5 (MMS)	61	1020	5 (CP)	7	2795

Treatment (μg/mL)	24-hour -S9		Treatment (μg/mL)	24-hour -S9	
	Relative total growth (%)	Mutation frequency (X 10^{-6})		Relative total growth (%)	Mutation frequency (X 10^{-6})
0	100	193	0	100	167
1.7	82	210	1.7	90	147
5.4	74	209	5.4	66	186
17	71	202	17	76	154
52	95	178	52	59	180
164	62	196	164	10	211
512	20	243	512	1	240
1600	23	268	1600	20	164
5000	29	212	5000	0	295
4 (MMS)	21	2518	4 (MMS)	26	2346
7.5 (MMS)	5	4121	7.5 (MMS)	5	3478

Positive controls: MMS, methylmethanesulfonate; CP, cyclophosphamide

3. Study title: Isopropyl myristate - Mammalian erythrocyte micronucleus test in the rat

Key findings: Isopropyl myristate at a single intraperitoneal dose up to 8500 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes (PCE) in rat bone marrow, either 24 or 48 hours after treatment.

Study no: [redacted]03.PC0209
Volume # 23 **and page** # NA
Conducting laboratory and location: [redacted]
Date of study initiation: 2-25-2002
GLP compliance: Yes
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Isopropyl myristate, CB11630002,[redacted]

Methods:
Strains/species/cell line: Sprague-Dawley rats, 7/sex/dose group

Doses used in definitive study: 0 and 8500 mg/kg

Basis of dose selection: A range-finding experiment showed that administration of a single intraperitoneal dose at 1063, 2125, 1250, or 8500 mg/kg isopropyl myristate in rats (3/sex/dose) did not cause mortality, clinical signs, or reduction in the PCE/NCE ratio.

Negative controls: Vehicle, 0.9% NaCl/saline

Positive controls: A single dose of 60 mg/kg cyclophosphamide (CPA) for 24 hours.

Incubation and sampling times: Rats were treated via a single intraperitoneal injection. Bone marrow sampling took place at 24 and 48 hours after treatment. The number of PCEs containing micronuclei were counted in a total of at least 2000 PCEs per animals.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The positive control induced a statistically significant increase in the frequency of micronucleated PCE; the negative and positive controls were consistent with the historical control data.

Study outcome: No mortality and no clinical signs were noted at the single dose level of 8500 mg/kg (the maximum feasible dose). Treatment with isopropyl myristate caused slight reductions in the PCE/NCE ratio. There were no statistically significant increases in micronucleated PCE in the isopropyl myristate-treated groups, compared to the vehicle control.

Treatment (μg/kg)	Kill time (hr)	Sex	PCE/NCE	Frequency of micronucleated PCE per 1000 cells	
				Per sex	Per dose group
Saline	24	Male	0.62	1.59	1.45
		Female	0.76	1.30	
	48	Male	0.49	1.49	1.34
		Female	0.49	1.20	
Isopropyl myristate 8500 mg/kg	24	Male	0.43	1.10	1.10
		Female	0.58	1.09	
	48	Male	0.63	1.10	0.85
		Female	0.47	0.60	
CPA 60 mg/kg	24	Male	0.22	18.68	21.90
		Female	0.26	25.11	

4. 28-Day toxicity and mutagenicity study of Polyphenon E administered by gavage to male and female [redacted] mice (G110-01, See Repeat-dose Toxicity): Compared with the vehicle control groups, no increase in *cII* mutations were observed in the liver, spleen, and lungs of mice treated with (500 and 1000 mg/kg/day Polyphenone E, Polyphenon E was negative in the [redacted] mouse mutagenesis assay.

The following studies were reviewed by Dr. Paul Brown in IND 56401.

1. Polyphenon E-API – Bacterial reverse mutation test (plate incorporation and preincubation methods) ([redacted] 03.PC0010)
2. Polyphenon E-API – *In vitro* mammalian cell gene mutation test on L5178Y mouse lymphoma cells $TK^{+/-}$ (microtitre method) ([redacted] '03.PC0013)
3. Polyphenon E-API – Mammalian erythrocyte micronucleus test in the rat bone marrow ([redacted] 03.PC0015)
4. Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes using an *in vivo* procedure with Polyphenon E ([redacted] G03. PC0130)

"Polyphenon E appeared to cause a slight increase in mutation rate in the Ames assay in salmonella strain TA102 in the absence of metabolic activation. However, since this did not exceed a 2 fold increase over control it was not considered a positive result. A positive genotoxic effect was observed in the mouse L5178Y $TK^{+/-}$ lymphoma cell assay both in the presence and absence of metabolic activation. No increase in

micronucleated polychromatic erythrocytes was noted in the rat after doses of Polyphenon E of up to 170 mg/kg. Polyphenon E at oral doses of up to 2000 mg/kg did not appear to produce unscheduled DNA synthesis in rat livers." Oral administration with Polyphenone E up to 1000 mg/kg/day for 28 days was not associated an increased mutation frequency in t mouse mutagenesis assay.

"Green tea has been shown to decrease the genotoxicity of some mutagens in bacterial systems (Jain et al., Mutat. Res. 210:1, 1989). Hydrosylates of green tea, however, are mutagenic in the Ames assay but this mutagenicity seems to be due largely to the presence of kaempferol, quercitin and myricetin (Uyeta et al., Mutat. Res. 88:233, 1981). EGCG has been shown to decrease the spontaneous mutation rate in NIG 1125 *Bacillus subtilis* carrying a mutation in DNA-polymerase III, but failed to lower the frequency of chemically or ultraviolet radiation induced reverse mutations in *S. typhimurium* or *E. coli* (IARC Monograph Vol. 51, 1991). Green tea extract containing 15-18% EGCG was not mutagenic in the Ames assay with or without metabolic activation at concentrations up to 5000 µg/plate (Yamane et al., Cancer 77:1662, 1996)."

2.6.6.5 Carcinogenicity

Study title: A twenty-six week oral (gavage) carcinogenicity study in transgenic heterozygous mice with Polyphenon-E

Key study findings: Oral administration of Polyphenon E at doses up to 500 mg/kg/day for 26 weeks appeared to be tolerated in the transgenic heterozygous mouse. No notable treated-related toxicity was observed except a slight decrease in body weights, food consumption, and absolute thyroid weights in females treated with 500 mg/kg/day Polyphenon E. Oral administration of Polyphenon E did not increase the incidence of either non-neoplastic or neoplastic lesions in the organs and tissues examined. The positive control, p-Cresidine, produced urinary bladder hyperplasia and carcinomas, which are consistent with the known carcinogenic effects of p-Cresidine. In addition, there was a dose-related increase in plasma EGCg concentrations in Polyphenon E-treated mice.

Adequacy of the carcinogenicity study and appropriateness of the test model: The use of the p53 transgenic mouse model appears appropriate for Polyphenon-E since it was positive for genotoxicity in the mouse lymphoma cell assay. Although the intended route of exposure in humans is topical, the use of the oral route is appropriate since it reached high enough systemic exposure in this study. As recommended previously, histopathological evaluation was conducted on all tissues in all groups.

Evaluation of tumor findings: Oral administration of Polyphenon E up to 500 mg/kg/day did not increase the incidence of non-neoplastic or neoplastic lesions in the organs and tissues examined. The 2 tumors noted in Polyphenon E-treated males (prostatic carcinoma in 1/25 in Group 3 and gastric sarcoma in 1/25 in Group 4) were not considered to be related to the administration of Polyphenon E.

Study no.: N01-CN-05134
Volume # 26, and page # NA
Conducting laboratory and location:

Date of study initiation: 7-29-02
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E, PE-000920, EGCg (Total Catechin

CAC concurrence: The Executive CAC Committee concurred with the study protocol on 6-11-2002 (IND Review 3).

Methods

Doses:

Group	Treatment	No. of mice		Dose (mg/kg/day)	Dosage conc. (mg/mL)	Dose volume (ml/kg)
		Male	Female			
1	Vehicle (water)	25	25	0	0	10
2	Polyphenon-E	25	25	125	12.5	10
3	Polyphenon-E	25	25	250	25	10
4	Polyphenon-E	25	25	500	50	10
5	p-Cresidine	25	25	400	40	10

Basis of dose selection (MTD, MFD, AUC etc.): MTD from a 28 day range-finding study

Species/strain: Transgenic heterozygous mouse

Number/sex/group (main study): 25/sex/group

Route, formulation, volume: Oral (gavage), Polyphenon E in water, 10 mL/kg

Frequency of dosing: Once daily

Satellite groups used for toxicokinetics or special groups: None

Age: 7-8 weeks

Animal housing: Suspended steel cages

Restriction paradigm for dietary restriction studies: Not applicable

Drug stability/homogeneity: Drug stability was analyzed by measuring EGCg levels in samples of the dose solutions collected on Days 0, 3, and 10 after preparation. Homogeneity was assessed by testing samples obtained from the top, middle, and bottom of the 12.5 and 50 mg/mL dose groups.

Dual controls employed: Water and p-Cresidine

Interim sacrifices: None

Deviations from original study protocol: No deviations affected the integrity or validity of this study.

Observation times

Mortality: Twice daily

Clinical signs: A detailed examination for clinical signs was performed once per week.

Body weights: Weekly

Food consumption: Weekly

Hematology: Blood was collected for hematology from the first 10 animals per sex in each group at the end of the study.

Clinical chemistry: Blood was collected for clinical chemistry from the second 10 animals per sex in each group at the end of the study.

Gross pathology: All animals were subjected to a complete gross necropsy examination.

Organ weighed: Adrenals, brain, heart, kidneys, liver, spleen, testes, thyroid/parathyroid, and tissue masses

Histopathology: All tissues in the list below from all animals were examined microscopically. Tissue list: adrenals, aorta, bone marrow, brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, fallopian tubes, femur, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs with bronchi, ovaries, mammary gland, mesenteric lymph node, pancreas, pituitary, prostate, salivary gland, seminal vesicles, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, stomach, sternum, testes, thymus thyroids/parathyroids, trachea, urinary bladder, uterus, and vagina.

Toxicokinetics: Blood was collected for drug level analysis from the last 5 animals per sex from Groups 1 - 4 at approximately 15 minutes post-dose (estimated T_{max}) at the end of the study.

Results

Mortality: One vehicle-treated male died on Day 88 (with thymic malignant lymohoma); one male on Group 4 died on Day 178 (with gastric sarcoma); three p-Cresidine-treated females died on Days 22, 84, and 122.

Clinical signs: No notable clinical abnormalities were observed in water- or Polyphenon E-treated animals that survived to study termination. Post-dose decreased activity and orange staining of the cage paper were seen in p-Cresidine-treated animals.

Body weights: A slight, but statistically significant decrease in body weights was observed in females treated with 500 mg/kg/day Polyphenon E; mean body weights in these animals were approximately 5% below controls at the end of study. A statistically significant decrease in body weights was noted in both males and females treated with p-Cresidine.

Food consumption: A slight, but statistically decrease in food consumption was observed in females treated with 500 mg/kg/day Polyphenon E. A statistically significant decrease in food consumption in both males and females treated with p-Cresidine.

Hematology: Only the mean platelet count showed a minimal but statistically significant increase (119%) in the high dose (500 mg/kg/day) males at study termination. Hematological abnormalities in the p-Cresidine-treated animals were consistent with those observed on previous studies.

Clinical chemistry: Several statistically significant changes were noted in the Polyphenon E-treated animals, but not apparently dose-related. A statistically significant increase in total bilirubin, calcium, and cholesterol was observed in both males and females treated with p-Cresidine.

Gross pathology: The male in Group 1 died on Day 66 had a thymic mass and dark red lungs. The male in Group 4 died on Day 178 had an adhesion in the abdominal cavity. Animals in Group 5 had pitted/nodular kidneys, discolored renal papilla, dilated renal pelvis, and thickened/nodular urinary bladder.

Organ weighed: No apparent treatment-related changes were observed in Polyphenon E-treated animals, except a decrease in absolute thyroid weights in the females of Groups 4. However, a variety of statistically significant organ weight changes were observed in the p-Cresidine-treated animals.

Histopathology:

Non-neoplastic: Oral administration of Polyphenon E did not increase the incidence of non-neoplastic lesions in the organs and tissues examined. Treatment with p-Cresidine induced transitional cell hyperplasia in urinary bladder (92% - 100% of the mice). In addition, treatment with p-Cresidine was associated with an increased incidence/severity of a variety of non-neoplastic lesions affecting liver (hepatocellular hypertrophy and hepatocellular degeneration/apoptosis), kidney (chronic nephropathy, papillary necrosis, hydronephrosis, and transitional cell hyperplasia), spleen (pigment accumulation within macrophages and extramedullary hematopoiesis), and urinary bladder (squamous metaplasia and chronic inflammation), which were consistent with those reported previously.

Neoplastic: Oral administration of Polyphenon E did not increase the incidence of neoplastic lesions in the organs and tissues examined. Treatment with p-Cresidine caused urinary bladder transitional cell carcinoma (44% - 64% of the mice, see the next table). The 2 tumors noted in Polyphenon E-treated males (1/25 in Group 3 and 1/25 in Group 4) were not considered to be related to the administration of Polyphenon E.

Treatment (mg/kg/day)	Male (lesions/animals tested)	Female (lesions/animals tested)
Vehicle (0)	Thymic malignant lymohoma (1/25)	Osteosarcoma (1/25) Uterine stromal polyp (1/25)
Polyphenon-E (125)	None	None
Polyphenon-E (250)	Prostatic carcinoma (1/25)	None
Polyphenon-E (500)	Gastric sarcoma (1/25)	None
p-Cresidine (400)	Urinary bladder transitional cell carcinoma (16/25)	Urinary bladder transitional cell carcinoma (16/25) Urinary bladder squamous cell papilloma (2/25)

		Abdominal cavity mass (urinary bladder) leiomyosarcoma (1/25)

Toxicokinetics: Due to system failures, the sample analysis for EGCg was delayed and used the pooled samples per sex in each group from the small volume of plasma remaining after the initial processing of the samples collected from the mice. As shown in the following table, there was a dose-related increase in plasma EGCg concentrations in Polyphenon E-treated mice and no EGCg was detected in the vehicle control plasma samples.

Group	Polyphenon E (mg/kg/day)	Male		Female	
		EGCg (ng/mL)	No. of samples analyzed	EGCg (ng/mL)	No. of samples analyzed
1	0	ND	4	ND	2
2	125	14.45	3	22.29	2
3	250	45.87	3	53.40	2
4	500	378.34	3	204.20	1

ND: Not detected (The lower limit of quantification was 5.7 ng/mL)

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

1. Polyphenon E – 7-day pilot study if crème and ointment formulations by vaginal application in the rat (03.PC0002, reviewed by Dr. Paul Brown in IND 56401): The maximum feasible volume of crème or material that could be administered intravaginally to 8 week old Sprague-Dawley rats was less than 0.2 ml per rat.

2. Polyphenon E – Crème and ointment – dose-range finding reproductive toxicity study by intravaginal application in the rat (03.PC0003, reviewed by Dr. Paul Brown in IND 56401): Administration of the Polyphenon E crème or ointment formulation at volumes of up to 0.15 ml/rat by the intravaginal route did not appear to cause any adverse effects on reproductive ability or embryo-fetal development in this preliminary study. The report concluded that the dose of 0.15 ml/rat/day would be acceptable as the high dose in a definitive fertility/embryofetal toxicity study.

3. Study title: Polyphenon E – Creme and ointment – combined mating performance and embryo toxicity study by intravaginal application in the female rat

Key study findings: There were no treatment-related deaths, abortions, or effects on clinical signs, food consumption, lesions upon necropsy, ovary weights, mating performance, fertility, late resorptions, dead fetuses, fetal weigh, fetal sex ratio, and abnormalities of the soft tissues and/or skeleton. There was a slight, but not statistically significant, dose-dependent reduction in body weight gain during gestation in animals treated with Polyphenon E cream. Treatment with Polyphenon E might cause disrupted estrus cycles in a few females. The incidence of early resorptions was slightly, but not statistically significantly higher in the Polyphenon E Cream high dose group than in the saline or placebo groups.

Study no.: [redacted]03.PC0104
Volume #29, and page #1
Conducting laboratory and location: [redacted]
Date of study initiation: 10-2-2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E Cream 10%, 000.35401, [redacted] Polyphenon E ointment 15%, LB-020, [redacted]

Methods

Doses: Saline 0.15 mL/rat/day, 0.05 or 0.15 mL/rat/day Placebo cream, Polyphenon E cream, Placebo ointment, or Polyphenon E ointment (9 groups)

Species/strain: SD rats - [redacted]

Number/sex/group: 25 femals/group

Route, formulation, volume, and infusion rate: Intravaginal, once daily, [redacted]

Satellite groups used for toxicokinetics: No

Study design: Nine groups of 25 female rats were treated from Day 4 before mating, through mating until Day 17 of gestation.

Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, estrus cycle, ovary weights, and macroscopic examination on ovaries, uterus, and vagina on moms; body weight, external examination, skeletal examination, and sex of fetuses; hysterectomies (number of corpora lutea, number and distribution of live and dead resorptions, and number and distribution of early and late resorptions).

Results

Mortality: There were no treatment-related deaths or abortions.

Clinical signs: No treatment-related clinical signs were noted

Body weight: There was a slight, but not statistically significant, dose-dependent reduction in body weight gain during gestation in animals treated with Polyphenon E cream.

Food consumption: There were no treatment-related effects.

Estrus Cycle: Three to seven females treated with Polyphenon E and three treated with high dose of Placebo ointment became acyclic during the treatment period.

Toxicokinetics: Blood samples were taken on the 4th day of dosing for EGCg kinetic measurements. The maximum EGCg plasma concentrations were generally found 30 or 60 minutes post treatment and up to more than 10-fold difference between rats in the same group were noted. The mean maximum EGCg plasma concentration was 485 and 891 ng/mL in animals treated with the low dose and high dose of Polyphenon E ointment, and 161 and 647 ng/mL in animals treated with the low dose and high dose of

Polyphenon E cream, respectively. The mean AUC value for EGCg were 553 and 1,450 ng·h/mL in animals treated with the low dose and high dose of Polyphenon E ointment, and 381 and 506 ng·h/mL in animals treated with the low dose and high dose of Polyphenon E cream, respectively.

Necropsy: there were no treatment-related lesions upon necropsy. The ovary weights were similar in all groups.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): The incidence of early resorptions was slightly, but not statistically significantly higher in the Polyphenon E Cream high dose group than in the saline or placebo groups. There were no treatment-related effects on mating performance, fertility, late resorptions, dead fetuses, fetal weigh, fetal sex ratio, abnormalities of the soft tissues and/or skeleton,

Embryofetal development

4. A range-finding developmental toxicity study of orally administered Polyphenon E in rats (1169-1): Six groups of 6-7 sperm-positive female rats were administered Polyphenon E (PE) orally at 0 (water vehicle), 125, 250, 500, 750, or 1000 mg/kg daily on Days 6-15 of gestation. There were no treatment-related effects, except a complete resorption of fetuses in 2 out of 6 dams treated with 1000 mg/kg. The dose of 1000 mg/kg/day was chosen as the highest dose for the definitive study.

5. A developmental toxicity study of orally administered Polyphenon E in rats (1169-2, reviewed by Dr. Gary Bond in IND ——): "Sprague Dawley female rats were administered Polyphenon E (PE) orally at 0 (water vehicle), 250, 500 or 1000 mg/kg by gavage daily for days 6-15 of gestation. Minimal clinical signs were observed (2/27 HD females with increased salivation and redness around nose fur). Body weight gains during the dosing period were -14, -7, & -10% for LD, MD, & HD groups compared to controls, respectively. No effects on fertility or embryo-fetal development were observed at any dose. Polyphenon E was not teratogenic in rats."

6. A range-finding developmental toxicity study of orally administered Polyphenon E in rabbits (1169-3): Six groups of 6-8 sperm-positive female rabbits were administered Polyphenon E (PE) orally at 0 (water vehicle), 62.5, 125, 250, 500, or 1000 mg/kg daily on Days 6-18 of gestation. There were no treatment-related effects and the sponsor concluded that 1000 mg/kg/day was chosen as the highest dose for the definitive study.

7. A developmental toxicity study of orally administered Polyphenon E in rabbits (1169-6, reviewed by Dr. Gary Bond in IND ———): "Female ——— White rabbits were administered Polyphenon E (PE) orally at 0 (water vehicle), 100, 300 or 1000 mg/kg by gavage daily for days 6-18 of gestation. Increased mortality of 0, 6, 5, & 7 rabbits in the control, LD, MD, & HD, respectively was reported to be due to gavage trauma. One animal for each PE treated group was sacrificed as moribund with clinical signs of discoloration around the mouth, labored breathing, salivation, dyspnea, or hypoactivity. Changes in body weight gain from days 6-18 of dosing were -31, +10, and -

84% for LD, MD, & HD, respectively compared to control. Food consumption was decreased 19% for HD females, but not the other treatment groups. No effects on fertility or embryo-fetal development were observed at any dose. Polyphenon E was not teratogenic in rabbits."

8. Polyphenon E-API / Isopropyl myristate - Dose range-finding study by subcutaneous route in the pregnant rabbits [redacted] 03.PC0210): Five groups of 6 mated female rabbits were administered subcutaneously saline, 37.5 or 150 mg/kg/day Polyphenon E-API (PE-API), or 85 or 340 mg/kg/day Isopropyl myristate (IPM) on Days 6-19 of gestation. Treatment with PE-API at the high dose (150 mg/kg/day) caused marked local irritation at the injection sites (erythema, induration and/or edema) resulting in severe subcutaneous lesions of dark appearance indicative of necrosis and preventing completion of the planned treatment period. Dosing was therefore discontinued after six daily injections. One rabbit in this group was subsequently sacrificed after aborting on Day 22 of gestation. Body weight loss, reduced food consumption and embryonic resorptions (mainly early resorptions with few live fetuses) were noted in this group. PE-API at 37.5 mg/kg/day also caused local irritation and slightly reduced body-weight gain, although much less severe than the high dose group. There were no significant macroscopic lesions indicative of local irritation in the groups given IPM. Slight increases in early and/or late resorption incidences were observed in the low dose PE-API and high dose IPM groups. The mean number of corpora lutea and consequent number of uterine implantation sites were incidentally lower in the high dose PE-API group than in the other treated and control groups. Mean live litter sizes reflected the incidental differences in the number of implantation sites, but were not clearly influenced by the relatively high resorption rates in the low dose PE-API and high dose IPM groups. The fetal weights were not adversely influenced by treatment in any group. Apart from the local irritation findings at the administration sites, there were no treatment-related lesions found at necropsy examination of the dams injected with either PE-API or IPM. Two fetuses from separate litters in the high dose PE-API group had an umbilical hernia (in one case associated with hyperflexion of a limb). Another fetus from one of the same litters had a short tail. Based on the results from this dose range finding study, the sponsor chose 4, 12, and 36 mg/kg of PE-API as well as 850 mg/kg of IPM for the main study.

Blood samples were collected from all rabbits treated with PE-API prior to dosing and 30 and 90 minutes post-dosing on the first day of treatment. The maximum EGCg plasma concentrations were generally found at the last blood sampling time point (90 minutes). The mean maximum EGCg plasma concentration was 5492 and 76171 ng/mL with a mean $AUC_{0\text{-}1.5h}$ value of 5497 and 6773 ng·h/mL in animals treated with 37.5 and 150 mg/kg/day PE-API, respectively.

9. Study title: Polyphenon E-API / Isopropyl myristate - Study on embryo-fetal development by the subcutaneous route in the pregnant rabbits (Segment II)

Key study findings: The high-dose PE-API (36 mg/kg/day) caused severe local irritations at the injection sites, marked reductions in maternal body weight and food consumption during the whole treatment period, reduced fetal weight, and retarded

(From Day 26) or aborted on the day of scheduled necropsy. In addition, one rabbit at the 12 mg/kg/day PE-API group aborted on the day of scheduled necropsy.

Clinical signs (dams): Treatment with the high-dose PE-API (36 mg/kg/day) caused severe signs of local irritation (erythema, induration and / or edema as well as dark subcutaneous lesions indicative of necrosis) at the injection sites, becoming more severe with each administration. Similar, but much less severe, local reactions were noted in the mid-dose PE-API group. Isolated local reactions were seen in the low-dose PE-API and IPM groups. Three of the aborting rabbits given IPM had shown lame limbs and an unsteady gait for a period of about one week prior to abortion or sacrifice.

Body weight (dams): Treatment with the high-dose PE-API (36 mg/kg/day) caused a marked reduction in maternal body weight gain during the whole treatment period. A slight reduction in body weight gain was caused by the treatment with mid-dose PE-API. Treatment with IPM caused a marked (less than by the high-dose PE-API), transient reduction in body weight gain at the start of treatment period.

Food consumption (dams): Marked reductions in food consumption were noted in the high-dose PE-API and IPM groups.

Toxicokinetics: Blood samples were collected from 6 rabbits in the PE-API-treated groups prior to dosing and 30 and 90 minutes post-dosing on the first and last day of treatment (gestation Days 6 or 19). The maximum EGCg plasma concentrations were generally found at either 30 or 90 minutes post-dosing. The mean maximum EGCg plasma concentration was 1379, 2897, and 5152 ng/mL with a mean $AUC_{0\text{-}1.5h}$ value of 1391, 3396, and 5641 ng·h/mL on the first day and the mean maximum EGCg plasma concentration was 1561, 3798, and 3752 ng/mL with a mean $AUC_{0\text{-}1.5h}$ value of 1648, 4653, and 4307 ng·h/mL on the last day of treatment in animals treated with 4, 12, and 36 mg/kg/day PE-API, respectively. There was no indication of an accumulation of EGCg in the plasma between Days 6 and 19 of gestation.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): The IPM-treated rabbit sacrificed on Day 20 of gestation was found to have undergone total litter loss. The number of corpora lutea, pre-implantation loss, the number of uterine implantations, and the sex ratio of fetuses were comparable in all treatment groups. Resorption indices and post-implantation loss were not obviously influenced by treatment with PE-API, except that one dam in the high-dose PE-API group had 10 early resorptions and no live fetuses.

Offspring (malformations, variations, etc.): Mean fetal weight was slightly reduced in the IPM and high-dose PE-API groups. There were total three malformed fetuses (from two litters) in each of the control and high-dose PE-API groups, four (from three litters) in the IPM group, seven (from four litters) in the low-dose PE-API group, and five (from four litters) in the mid-dose PE-API group. In addition, one aborted fetus from a dam in the mid-dose PE-API group had a domed head (see the next table).

	Control	IPM 850 mg/kg/day	Polyphenon® E drug substance 4 mg/kg/day	Polyphenon® E drug substance 12 mg/kg/day	Polyphenon® E drug substance 36 mg/kg/day
Total *	3 (2)	4 (3)	7 (4)	6 (5)	3 (2)
Hydrocephaly / domed head	1	1	0	1 [a]	1
Vertebral defects	2 (2)	3 (2)	2 (2)	2 (2)	1
Umbilical hernia	0	0	1	0	1
Malpositioned kidney	0	0	3 (2)	3 (2)	0
Thoracic vessel defect	0	1	0	1	0
Open eye	1	0	0	1	0
Limb / paw defect	2 (2)	0	1	1	0

* Each fetus may have more than one defect
a - Aborted fetus

The types of malformations and their distribution over the treatment groups did not suggest a teratogenic potential of either test article. However, the types and incidences of skeletal anomalies and variations indicated a generalized retardation of ossification (in the thoracic vertebrae, paws, sternum, and pubis) in the high-dose PE-API group. There was a similar, but less marked delay in ossification in the mid-dose PE-API and IPM groups. There was no clear retard of ossification in the low-dose PE-API group.

Prenatal and postnatal development

10. Study title: Polyphenon E 15% Ointmnet - Pre- and post-natal development study by vaginal application in the rat (Segment III)

Key study findings: Four groups of 25 mated females were treated intravaginally with 0.15 mL/rat/day Placebo Ointment, 0.05, 0.10, or 0.15 mL/rat/day Polyphenon E 15% Ointment from Day 6 of gestation until the end of lactation (weaning). Four high-dose and three mid-dose dams died or were sacrificed as the result of presumptive parturition complications. Another high-dose dam was sacrificed on Day 14 of lactation following the death of both liveborn pups. There were no treatment-related effects on clinical signs, body weight, and food consumption. The incidence of stillborn pups was increased in the high-dose group (23 stillborn pups from six dams) compared to the control group (5 stillborn pups from three dams). The mean live litter size and the live birth index were consequently reduced in the high-dose group. There were no other treatment-related effects on pre-and post-natal development under the conditions of this study. The NOEL for both maternal and developmental toxicity was the low-dose level of Polyphenon E 15% Ointment (0.05 mL/rat/day).

Study no.: PE_PTX_0402
Volume #37, and page #1
Conducting laboratory and location: [redacted]
Date of study initiation: 10-29-2004
GLP compliance: Yes

QA reports: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E 15% Ointment, 39075-1,

Methods

Doses: 0.15 mL/rat/day Placebo Ointment, 0.05, 0.10, or 0.15 mL/rat/day Polyphenon E 15% Ointment

Species/strain: SD rats

Number/sex/group: 25 mated females

Route, formulation, volume, and infusion rate: Intravaginal, 15% ointment, 0.05, 0.10, or 0.15 mL/rat/day

Satellite groups used for toxicokinetics: None

Study design: Four groups of 25 mated females were treated intravaginally with 0.15 mL/rat/day Placebo Ointment, 0.05, 0.10, or 0.15 mL/rat/day Polyphenon E 15% Ointment from Day 6 of gestation until the end of lactation (weaning). The F1 generation (25/sex/group) was selected from the Fl offspring and was maintained, untreated, and mated on the basis of one male and one female from the same group.

Parameters and endpoints evaluated: In the F0 (maternal) generation: viability, clinical signs, body weight, feed consumption, pregnancy and parturition, duration of gestation, maternal behavior, litter size, pup viability and pup body weights, gross pathology at necropsy, implantation sites. In the F1 generation: viability, external abnormalities, physical development (the days of pinna unfolding, incisor eruption or eye opening), behavioral (water maze and open field tests) and functional tests (surface righting, gripping, pupillary, and auditory reflex), sexual maturation, mating and fertility parameters (pre-coital interval, pregnancy, number of corpora lutea counts, pre-and post-implantation loss, and live litter sizes).

Results

F_0 in-life: Four high-dose and three mid-dose dams died or were sacrificed for ethical reasons as the result of presumptive parturition complications. Another high-dose dam was sacrificed on Day 14 of lactation following the death of both liveborn pups. There were no treatment-related effects on clinical signs, body weight, and food consumption. Twenty-two (22) dams in each of the low- and mid-dose groups, 20 in the high-dose group, and 23 in the control group successfully completed delivery. Excluding the dam that died immediately after giving birth, there were 23 stillborn pups from six high-dose dams, 9 stillborn pups from seven mid-dose dams, 7 stillborn pups from five high-dose dams, and 5 stillborn pups from three control dams. The mean live litter size on Day 1 post-partum and the live birth index were consequently reduced in the high-dose group. The pup viability and lactation indices were similar in all groups. The proportion of male pups was normal in all treated groups.

F_0 necropsy: There were no treatment-related lesions.

F_1 physical development: There were no unscheduled deaths amongst the F1 rats derived from dams treated with Polyphenon E Ointment. One male from the mid-dose group had a missing tip of the tail and one female from the control group had dental abnormalities. There were no treatment-related effects on clinical signs, body-weight gains, the days of

pinna unfolding, incisor eruption or eye opening, surface righting, gripping, pupillary, and auditory reflex, and the age of vaginal opening or balano-preputial separation. No treatment-related lesions were found at necropsy examination.

F_1 behavioral evaluation: The water maze and open field tests did not reveal any behavioral defects.

F_1 reproduction: All mating and fertility parameters in the F1 generation were unaffected by maternal treatment. The mean pre-coital interval was comparable in all groups. Two females in each of the low- and high-dose groups, and three in the mid-dose group failed to become pregnant. There were no treatment-related effects on the number of corpora lutea counts, pre-and post-implantation loss, and live litter sizes.

2.6.6.7 Local tolerance

1. Polyphenon E – 28-day local tolerance study of crème and ointment formulation after vaginal application in the female rat ([redacted] 03.PC0019, reviewed by Dr. Paul Brown in IND 56401): The intravaginal administration of the Polyphenon E 10% crème and 15% ointment appeared to produce some damage in the vagina. This included ulceration and erosion of the vaginal mucosa with accompanying inflammation. This effect appeared to be due to the active ingredient since essentially no such changes were observed in the placebo treated animals. Most of the effects appeared to be reversible since little evidence of the damage was noted in animals that had been allowed to recover for 4 weeks after the administration of 4 weeks of treatment.

2. Polyphenon E - Cutaneous irritation study of different formulations in the rabbit after repeated applications for 28 days ([redacted] 03.PC0017): This study was terminated prematurely on Day 6 due to severe local toxicity, although it was planned initially as a 28-day dermal irritation study. In this study, 1 g of Polyphenon E 15% Ointment or 1 g of Polyphenon E 10% Cream was applied three times daily to the skin of rabbits (surface area 25 cm^2), resulting in a total daily dose of 3 g. Erythema and edema were seen in all treated sites (including placebos). After 6 days of treatment, the daily irritation index reached the upper limit (4.7 to 5) of the irritant classification range for the cream formulations (Placebo and Polyphenon E) and irritation was slightly less pronounced with ointment than with cream. Histopathological examination revealed that the epidermis (acanthosis, hypertrophy/hyperplasia of the sebaceous glands, hyperkeratosis) and the superficial dermis (vascular hemorrhage, edema, congestion, mixed cell infiltration) were affected. The more severe changes were seen in the test article-treated sites than in corresponding placebo-treated sites and a lower severity of irritation was seen in treated sites with ointment than with cream.

3. Local tolerance study of 8 different test substances by repeated epicutaneous administration for 7 days [redacted] 03.PC0131, reviewed by Dr. Paul Brown in IND 56,401): The Polyphenon E ointment was clearly very irritating when applied to rabbits (0.5 g) in this study. Polyphenon E moistened with water was not irritating. Much of the irritation caused by the Polyphenon E ointment may be due to the excipients although the

placebo ointment was not as irritating as the Polyphenon E ointment suggesting that at least some of the irritation is due to Polyphenon E.

2.6.6.8 Special toxicology studies

1. Polyphenon E – Local lymph node assay (03.PC0118, reviewed by Dr. Paul Brown in IND 56401): All three vehicles (Placebo ointment, Placebo cream, and acetone/olive oil) tested in this study produced a greater than 3 fold increase in labeling compared to untreated animals. Compared to the vehicle controls, a large additional increase (>6) in labeling was observed when Polyphenon E (Polyphenon E 10% cream, Polyphenon E 15% ointment, and Polyphenon E-API 20% or 40% in acetone/olive oil) was included in any of the three vehicles. Polyphenon E may have the potential to induce contact sensitization although alternative mechanisms for the increase in labeling index can not be ruled out.

2. Study title: Polyphenon E - Sensitising potential in the guinea-pig: Magnusson & Kligman test (G.P.M.T.)

Key study findings: Polyphenon E 15% Ointment was a sensitizer in the animal model.

Study no.: 03.PC0133
Volume # 40, and page # NA
Conducting laboratory and location:
Date of study initiation: 11-15-2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Polyphenon E-API, PE-010612, Total catechin content Polyphenon E 15% Ointment, LB-020, label claim.
Formulation/vehicle: Polyphenon E-API in water; Polyphenon E 15% Ointment 5%,

Methods and Results:

When guinea-pigs were treated twice with Polyphenon E-API dissolved in water and subsequently challenged at a distant site topically with Polyphenon E-API in water, no skin reaction/sensitization was observed (See the next table). However, when the animals during the induction phase were treated twice with Polyphenon E 15% Ointment and subsequently challenged with Polyphenon E 15% Ointment at a distant site, a positive reaction was observed. In addition, after these sensitized animals were challenged a second time with either Polyphenon E-API or placebo ointment, both groups showed a positive response, although less pronounced in the placebo ointment group. Microscopical changes indicative of a response to a combined irritant and hypersensitivity challenge were seen at the treatment site in nearly all animals in both the test article and the placebo groups.

Induction [a] (Days 0 and 8)	First challenge [b] (Day 21)	Results (incidence of sensitized animals)	Second challenge [b] (4 weeks after first challenge)	Results (incidence of sensitized animals)
Water for injection	Polyphenon® E drug substance [redacted]	0 / 10	-	-
Polyphenon® E drug substance [redacted]	Polyphenon® E drug substance [redacted]	0 / 10	-	-
Sterile Codex liquid paraffin	Polyphenon® E Ointment, 15% [redacted]	0 / 10	-	-
Polyphenon® E Ointment, 15% [redacted]	Polyphenon® E Ointment, 15% [redacted]	19 / 20 (1 / 20 equivocal)	Polyphenon® E drug substance [redacted]	10 / 10
			Placebo ointment as supplied (MNIC)	9 / 10

a - Intradermal injection with and without Freund's complete adjuvant on Day 0, followed by topical occlusive application on Day 8.
b - Topical occlusive application for 24 hours.
MNIC: Maximum non irritant concentration, as determined in a preliminary experiment.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Polyphenon E drug substance in vitro had anti-oxidative activity, anti-inflammatory activity, the potential to inhibit a variety of enzymes involved in the pathogenesis of cancer and inflammatory diseases, the potential to inhibit HPV associated cervical cancer cell growth, and the immunomodulatory potential. It also reduced the expression of E7 (a major tumor promoter) in human papillomavirus (HPV)-infected cells and activated T cell via predendritic cells by releasing IL-6 in vitro. However, topical application of Polyphenon E Ointment did not cause statistically significant effects on the growth of HPV-6 infected human xenografts in SCID mice and the growth of CRPV-induced skin warts in rabbits. The mode of action of Polyphenon E drug substance involved in the clearance of genital and perianal warts is unknown.

The plasma pharmacokinetic properties of Polyphenon E 15% Ointment were studied following application to the rat and the mini-pig by oral, topical, or intravaginal routes. Catechins were absorbed and systemically available only minimally in minipigs and patients following dermal application. No significant accumulation of plasma EGCg concentrations was observed following repeated topical or intravaginal applications of Polyphenon E Ointment in mini-pigs, rabbits, or rats. It was noted that EGCg and its metabolites tended to concentrated in the liver after intravenous administration and in the gastrointestinal tract after oral administration in dogs.

No significant behavioral and physiological changes were observed in the rats treated orally with 100 or 1000 mg/kg Polyphenon E drug substance during a 480 minutes post-dosing period, although a few behavioral and physiological changes were noted in the rats treated with 10 mg/kg Polyphenon E drug substance. No significant effect on respiration rate and tidal volume was observed in rats treated orally with up to 1000 mg/kg Polyphenon E.

Polyphenon E 15% Ointment or Polyphenon E drug substance was tested for up to 3 months orally or topically in rats and dogs and for up to 9 months topically in mini-pigs. Gastrointestinal tract, liver, pancreas and lymphoid tissues were primarily affected in rats following oral administration. The NOAEL was 90 mg/kg/day for Polyphenon E in the 13-week oral toxicity study in rats. In the 13-week oral toxicity study in dogs, the NOAEL was 200 mg/kg/day for Polyphenon E. Topical treatment with 50, 200, or 600 µL (corresponding to approximately 50, 200, or 600 mg) of Polyphenon E 15% Ointment in rats once daily for 4 hours over a period of 13 weeks did not cause deaths or test article-related effects on body weights, food and water consumption, hematology, clinical chemistry, organ weights, or microscopic pathology. Restlessness, rough fur, and/or hematomas were seen in all animals treated with 200 or 600 µL Polyphenon E in a few weeks. Thickened uterus was seen in a few Polyphenon E-treated females (no histopathological examination on uterus was performed). Slight local irritation at the site of administration including very slight erythema and very slight edema was noted in Polyphenon E-treated animals, without test-article related histopathological findings.

No apparent systemic toxicity was noted in mini-pigs after topical treatment of Polyphenon E 15% Ointment for 9 months. There were no apparent treatment-related effects on food consumption, electrocardiogram, heart rate, blood pressure, ophthalmology, hematology, clinical chemistry, urinalysis, bone marrow, organ weights, and gross pathology. Only the males in the high-dose group had 20% lower body weights than those in the control group at the end of treatment. Very slight to well-defined erythema and very slight edema formation were seen at the application site in some animals treated with the placebo ointment on several days during the treatment period. Very slight to severe erythema and very slight to moderate edema formation were seen in the low-dose group. The incidence and severity of erythema and edema in the middle- and high-dose group were increased compared to the low-dose group. Moderate to severe erythema and moderate to severe edema were noted in the middle-dose group animals. Severe erythema and moderate to severe edema were seen in all high-dose group animals. The incidence and severity of erythema and edema were most pronounced during Weeks 2 to 6 of the study. Subsequently, the severity of skin reactions decreased over time despite the continued treatment with Polyphenon E 15% Ointment. In addition, transient eschar formation surrounding the administration site and red spots either at the administration site or at other areas of the body of the animals, partly with eschar formation, were also noted. The incidence of these findings was increased in the Polyphenon E-treated groups. The skin reactions had subsided within the first three weeks of the recovery period. No treatment-related microscopic findings were seen in other organs than the skin. Minimal to moderate hyperplasia of the epidermis (psoriasiform reaction pattern) and subcutaneous inflammatory reaction were seen at the

in the Ames assay with or without metabolic activation at concentrations up to 5000 µg/plate.

Oral administration of Polyphenon E at doses up to 500 mg/kg/day for 26 weeks appeared to be tolerated in the [redacted] mouse. No notable treated-related toxicity was observed except a slight decrease in body weights, food consumption, and absolute thyroid weights in females treated with 500 mg/kg/day Polyphenon E. Oral administration of Polyphenon E did not increase the incidence of either non-neoplastic or neoplastic lesions in the organs and tissues examined. The 2 tumors noted in Polyphenon E-treated males (prostatic carcinoma in 1 out of 25 males treated with 250 mg/kg/day and gastric sarcoma in 1 out of 25 males treated with 500 mg/kg/day) were not considered to be related to the administration of Polyphenon E. The positive control, p-Cresidine, produced urinary bladder hyperplasia and carcinomas, which are consistent with the known carcinogenic effects of p-Cresidine. In addition, there was a dose-related increase in plasma EGCg concentrations in Polyphenon E-treated mice.

Anti-tumor effects of green tea extracts have been shown in vitro. Several published studies demonstrated a preventive or growth inhibitory effect of topically applied green tea catechin extracts on carcinogen- or UV-induced skin cancers in murine models. In addition, it has been reported that application of green tea polyphenons/extracts before or after UV irritation in humans reduced the phototoxicity and inflammation in skin. Topical application of Polyphenon E 15% Ointment in humans resulted in low blood levels of EGCg which were either below or just above the detection limit of 5 ng/mL. For this indication, a 2-year carcinogenicity study with Polyphenon E is not currently recommended.

There were no substantial adverse effects on the reproductive system as determined in a number of fertility and embryo-fetal development studies in rats and rabbits including different routes of administration (oral, subcutaneous, and intravaginal). Polyphenon E was not teratogenic in rats and rabbits. However, subcutaneous application of Polyphenon E drug substance at 12 and 36 mg/kg/day in rabbits caused the effects on fetal development such as reduced fetal weights and delayed skeletal ossification. These doses resulted in a high systemic exposure (the mean maximum EGCg plasma concentrations > 2800 with mean $AUC_{0\text{-}1.5h}$ values > 3300 ng·h/mL) and maternotoxicity. In addition, in the pre- and post-natal development study in rats using vaginal administration of Polyphenon E 15% Ointment, 0.10 and 0.15 mL/rat/day resulted in an increased mortality of the F0 dams, associated with indications of parturition complications. The dose of 0.15 mL/rat/day also resulted in an increased incidence of stillbirths. The NOAEL was 4 mg/kg/day Polyphenon E drug substance in the embryo-fetal development study in rabbits using subcutaneous route. The NOAEL for both maternal and developmental toxicity was 0.05 mL/rat/day Polyphenon E 15% Ointment (corresponding to approximately 25 mg/kg/day Polyphenon E drug substance) in the pre- and post-natal development study in rats using vaginal administration. Because of the low systemic toxicity following dermal application in patients, such adverse effects, as observed in rabbits and rats, are unlikely in humans.

Polyphenon E Ointment induced minimal to severe local irritation including erythema, edema, and inflammatory reactions when topically applied to rats, rabbits, and mini-pigs. Polyphenon E Ointment caused strong local irritation to vaginal mucosa after vaginal application in female rats and mini-pigs. Local lymph node assay suggested that Polyphenon E Ointment had the potential to induced contact sensitization and it was a sensitizer in the guinea-pig.

Isopropyl myristate, an excipient in this drug product, is not present in any previously-approved drug product at a concentration of 35%. However, it is used in a wide variety of cosmetic products (up to 50%) and may be applied to all areas of the skin. The sponsor submitted a report, "Safety assessment of myristyl myristate and isopropyl myristate" and this review concluded that isopropyl myristate was safe as a cosmetic ingredient.

"Isopropyl Myristate was tested for acute oral toxicity in both rats and mice. In rats, the acute oral LD50 was estimated to be greater than 16.0 ml/kg; in mice, the LD50 was calculated to be 49.7 ml/kg. There were no signs of acute dermal toxicity when Isopropyl Myristate was applied undiluted and in product formulations. A guinea pig immersion study with Isopropyl Myristate at 0.5 percent produced only mild skin irritation. Subchronic dermal toxicity studies with product formulations containing 16-47 percent Isopropyl Myristate applied for four weeks showed no systemic toxicity. In primary skin irritation studies with rabbits, undiluted Isopropyl Myristate produced no more than mild irritation in 24 hours; it produced moderate to severe irritation when applied for three consecutive days. Subchronic skin irritation studies for 28 days with mice and 14 days with rabbits showed moderate irritation. Acute inhalation of aerosols of product formulations containing up to 20 percent Isopropyl Myristate produced no systemic toxicity. Subchronic 13-week inhalation studies in guinea pigs and monkeys on a product containing 16-20 percent Isopropyl Myristate showed only local lung effects. Acute parenteral studies with intracutaneous, subcutaneous, and intraperitoneal injection into rabbits, mice, or rats produced no systemic toxicity and high LD50 values. Intramuscular injection of 25 percent Isopropyl Myristate in peanut oil for 12 weeks produced only minor local damage in rats, dogs, and monkeys. Isopropyl Myristate was minimally irritating to the rabbit eye, and was not a skin sensitizer in studies with guinea pigs. Isopropyl Myristate was not carcinogenic on the skin of mice, but a mixture of Isopropyl Myristate and isopropyl alcohol significantly accelerated the carcinogenic activity of ——— on the skin.

Human primary skin irritation studies showed no reactions to Isopropyl Myristate alone and up to mild irritation from product formulations containing 15-58 percent Isopropyl Myristate. Repeated application of undiluted Isopropyl Myristate for 21 days produced only slight irritation. Isopropyl Myristate was not a human skin sensitizer when in petrolatum or in product formulations at 15-58 percent, although one woman who had suffered from a vulvar dermatitis caused by a feminine hygiene spray was found to be sensitized to this ingredient. A product containing 43 percent Isopropyl Myristate produced no phototoxicity and no photo-contact allergenicity in human studies."

In addition, isopropyl myristate was negative in the Ames test, mouse lymphoma TK assay, and in vivo rat micronucleus assay. It was not teratogenic in the rabbit embryo-fetal development toxicity study (Segment II). Isopropyl myristate in the drug product was also tested in the 9-month topical mini-pig study and several reproductive toxicology studies (Segment I and III). From a Pharmacology/Toxicology perspective, isopropyl myristate is reasonably safe for clinical use as an excipient in this drug product.

Conclusions: The NDA is approvable from a pharmacology/toxicology perspective.

Unresolved toxicology issues (if any): None

Recommendations: No further recommendations.

Suggested labeling:

The sponsor suggested the following wording for the Carcinogenesis, Mutagenesis and Impairment of Fertility section of the labeling:

3 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

6 § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Withheld Track Number: Pharm/Tox-_____

APPENDIX/ATTACHMENTS

1. Executive CAC
Date of Meeting: March 28, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Karen Davis Bruno, Ph.D., DMEP, Alternate Member
Paul Brown, Ph.D., HFD-540, Supervisor
Jiaqin Yao, Ph.D., HFD-540, Presenting reviewer

Author of Minutes: Jiaqin Yao

The following information reflects a brief summary of the Committee discussion and its recommendations.

IND/NDA: IND 56,401 and NDA 21-902
Drug Name: Polyphenon E Ointment, 15%
Sponsor: MediGene AG, Martinsried, Germany

Background:
Polyphenon E is a mixture of polyphenolic compounds isolated from green tea. Polyphenon E was negative in the Ames assay, the in vivo rat micronucleus assay, the UDS assay, and the ——— transgenic mouse mutagenesis assay. Polyphenon E was positive in the mouse lymphoma cell mutation assay. The Executive CAC Committee concurred with the study protocol on 6-11-2002. Histopathological evaluation was conducted on all tissues in all groups.

P53 Mouse Carcinogenicity Study:
The sponsor submitted a 26-week carcinogenicity study in which transgenic heterozygous ——— mice were orally administered 0, 125, 250, or 500 mg/kg/day Polyphenon E once daily for 26 weeks. The 2 tumors noted in Polyphenon E-treated males (prostatic carcinoma in 1/25 in Group 3 and gastric sarcoma in 1/25 in Group 4) were not considered to be related to the administration of Polyphenon E. Oral administration of Polyphenon E up to 500 mg/kg/day for 26 weeks in the transgenic mice did not increase the incidence of non-neoplastic or neoplastic lesions in the organs and tissues examined.

Executive CAC Recommendations and Conclusions:
The Committee agreed that the study was adequate and the Committee found that the study was negative for drug related neoplasms.

David Jacobson-Kram, Ph.D
Chair, Executive CAC

cc:\
Division File, DDP, HFD-540
PBrown/Supervisor, HFD-540
JYao/Reviewer, HFD-540
MWright/CSO/PM, HFD-540
EAbraham/PM, HFD-560
ASeifried, OND IO

2. Dose Calculation/Comparison in the Labeling

Species	Study	Route	Test Article	Dose			
				mL/rat/day	mg/kg/day	mg/m^2/day	Fold MRHD
Human		Topical	Ointment		12.5	462.5	
			Poly. E		1.875	69.4	
Mouse	Carcinogenicity	Oral	Poly. E		500	1500	22
Rat *	Fertility/Embryo-fetal	Vaginal	Ointment	0.15	500	3700	8
	Pre- and Post-natal	Vaginal	Ointment	0.05	167	1233	2.7
	Embryo-fetal	Oral	Poly. E		1000	6000	86
Rabbit	Embryo-fetal	Oral	Poly. E		1000	12000	173
	Embryo-fetal	S.C.	Poly. E		4	48	0.7

* The Km is 7.4 for a 300 g rat.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Jiaqin Yao
6/13/2006 04:18:50 PM
PHARMACOLOGIST

Paul Brown
6/14/2006 07:34:26 PM
PHARMACOLOGIST