CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-395

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

ADDENDUM

NDA NUMBER:	**22-395**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**Oct 13, 2008**
PRODUCT:	**Qutenza™ (Capsaicin Dermal Patch 8%)**
INTENDED CLINICAL POPULATION:	**Patients with neuropathic pain associated with postherpetic neuralgia (PHN)**
SPONSOR:	**NeurogesX Inc.**
DOCUMENTS REVIEWED:	**SD-22, received July 31, 2009**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**L.S. Leshin, DVM, PhD**
PHARM/TOX SUPERVISOR:	**A. Wasserman, PhD**
DIVISION DIRECTOR:	**B. Rappaport, MD**
PROJECT MANAGER:	**T. Clayton**

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability — Approve (no change from the original review)

B. Recommendation for nonclinical studies — None (no change from the original review)

C. Recommendations on labeling — Refer to the original review.
There are no new changes in labelling.

II. Regulatory background

During the initial review of the 6-month transgenic mouse carcinogenicity study a request (IND 63354, May 13, 2009) for a DSI inspection was submitted to examine the extensive discrepancies in the study methodology and results. The inspection group was aware that this study was not essential for approval since the proposed drug would be administered once with possible retreatment at 3 month intervals. Nevertheless, they were able to inspect the contract lab, (b) (4) that conducted the study (Report 7215-150, Oct 6 2006) titled "26-Week Dermal Oncogenicity Study with trans Capsaicin in TgAC Hemizygous Mice." The study was amended and resubmitted (submission July 30, 2009 SD-22 received July 31, 2009) to address the items raised as FDA-483 observations from the FDA surveillance audit of (b) (4) (b) (4) between July 20 and July 22, 2009. The audit is not filed with this application in DARRTS at this time.

III. Summary of nonclinical findings

While a number of critical items were corrected (see Appendix), the underlying problems of the study are still present, making the interpretation of the data problematic. These include a high level of papillomas in the untreated control group (negative controls), upon necropsy not all masses were histologically examined, and there is a trend of increasing papilloma with dose when pooled across sex (there is no obvious mechanistic reason why dose groups should not be pooled). For these reasons, the study is still unacceptable. The carcinogenicity section of the label will indicate that adequate carcinogenic studies have not been conducted. Recall that for the proposed administration frequency, a carcinogenicity study is not essential for approval.

Appendix: Revised Information Provided to Study 7215-150, 26-Week Dermal Oncogenicity Study with trans Capsaicin in TgAC Hemizygous Mice

The major findings of the audit were:

- The conclusions in the final report regarding carcinogenicity of the test article are based on a tabulation of masses (Appendix 6-"Weekly Incidence of Dermal Masses"), which includes masses at sites remote from the treatment sites. Specifically, each page of Appendix 6 states "Note: Numbers indicate the total number of skin masses on the treated site each week". The "treated site" for the animals in this study is defined in the study protocol as an "area approximately 2 cm x 2 cm of dorsal skin at the intrascapular region". However, as identified in the final report Appendix 11, "Individual Anatomic Pathology Data", the number of dermal masses often include masses found at sites other than at the treatment site.
- There was no formal statistical analysis of the tumor data in the final report.
- The final report did not include a description of all circumstances that may have affected the quality or integrity of the data. Specifically, the final report includes data to indicate that 77 of the 350 animals in the study (22%) died prior to completing the 26-week dosing period. There is no discussion in the final report regarding the impact on the study outcomes to having so many study animals die during the study, and no discussion as to the possible cause or relationship of the deaths to the study treatment.
- The final study report did not include the signed and dated reports of each of the individual scientists or other professionals involved in the study.

The Applicant provided the following revisions in the amended final report of July 29, 2009:

- There were numerous administrative changes, including signatures, quality assurance inspections, amendments, etc.
- A statistical report was included, with more detailed statistical methods and analyses.
- Clarification of data entries for statistical analysis was provided.
- A pathology report was included.
- Survival figures were included and a discussion of animal mortality was included.
- The abstract was revised.
- The title and footnote of Appendix 6 was corrected and clarified. The number of papillomas by study week were subdivided into locations in which they were observed.
- A table relating weeks of study to times for various events in the study was included.

Signatures:

Reviewer Signature _____
L. S. Leshin, D.V.M., Ph.D.

Supervisor Signature_____ **Concurrence Yes ___ No**
A. Wasserman, Ph.D.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22395	ORIG-1	NEUROGESX INC	NGX-4010 (CAPSAICIN PATCH 8%)

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/s/

LAWRENCE S LESHIN
10/27/2009

ADAM M WASSERMAN
10/27/2009

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

Supervisory Pharmacologist Memorandum

NDA NUMBER:	**22-395**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**13-OCT-2008**
PRODUCT:	**Qutenza (Capsaicin) patch**
INTENDED CLINICAL POPULATION:	**Neuropathic pain associated with Postherpetic Neuralgia**
SPONSOR:	**NeurogesX**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**L. Steven Leshin, DVM, Ph.D.**
PHARM/TOX SUPERVISOR:	**Adam Wasserman, Ph.D.**
DIVISION DIRECTOR:	**Bob Rappaport, M.D.**
PROJECT MANAGER:	**Tanya Clayton**

EXECUTIVE SUMMARY

I. BACKGROUND

A. Regulatory Summary (Pharmacology/Toxicology)

The present NDA submission submitted by NeurogesX pertains to Qutenza®, a topical patch containing 8% capsaicin (179 mg) in adhesive which is to be used for managing the neuropathic pain of postherpetic neuralgia. The present application proposes up to 4 (20 x 14 cm; 280 cm^2) patches may be applied by a practitioner over painful intact skin areas once every 3 months or greater depending on the return of pain to the site.

Capsaicin, existing as the natural *trans* format, is a significant component of various hot pepper plants and is the main active ingredient in chili peppers used in foods as well as in the defensive weapon "pepper spray" where deployment into mucous membranes of eyes, nose and mouth is profoundly irritating. It is not an approved drug product but is marketed in various forms under a tentative final monograph for external analgesic drug products (proposed for 21 CFR 348.12) published in the Federal Register (February 8, 1983; Volume 48 No. 27). This tentative monograph section (I*rritants that do not produce redness*) applies to topical counterirritants which allow concentrations of capsaicin in capsaicin-related products are acceptable of 0.025 to 0.25%. This in practice has "supported" unapproved topical creams such as Zostrix (0.025%) and Zostrix HP (0.075%) marketed for mild to moderate pain of rheumatoid arthritis or osteoarthritis and myalgia while a recent addition (Zostrix Neuropathy Cream) contains 0.25% capsaicin in an unknown concentration of lidocaine as part of a "*Lidocare*" vehicle which is marketed for the treatment of postherpetic neuropathic pain. The current product proposed for marketing approval represents a markedly higher concentration than all currently marketed and unapproved products.

II. S UMMARY OF NONCLINICAL FINDINGS

A. Brief overview of nonclinical findings

General pharmacology

Capsaicin at high concentrations as used in Qutenza is designed to act as an agonist at epidermal nerve fiber TRPV1 (transient receptor potential vanilloid, type 1) receptors at levels sufficient to cause "defunctionalization" (i.e. calcium-overload-induced local degeneration) of the peripheral ends of these nerve fibers. Capsaicin activates TRPV1 receptors which are non-selective cation channels (though preferring calcium) located on sensory neurons that serve as molecular polymodal integrators of a number of environmental stimuli including heat and protons. Importantly, TRPV1 receptor channels are capable of being functional upregulated or sensitized by protein kinases activated by a number of receptors sensitive to various inflammatory stimuli. Capsaicin-sensitive fibers are generally characterized as unmyelinated C-fibers which represent the peripheral end of bipolar dorsal root ganglion neurons involved in transfer of pain signals to the second order neurons in the spinal cord which provide ascending afferents to the brain. Less common are myelinated Aδ fibers which are capable of responding to capsaicin

stimulation though upregulation or ectopic expression of TRPV1 receptors have been described in certain neuropathic pain states (diabetic neuropathy, thermal injury, etc.)

(b) (4)

ADME

As described by Dr. Leshin, capsaicin contained in the patch appears to have limited penetration into the skin under the conditions of a short (60 min) therapeutic application time; bioavailability in humans appears to be on the order of ~0.9% with in vitro data to indicate that less than 3% of capsaicin is released from the patch over 60 minutes of application to skin. Animal models appear to be reasonable representations of human metabolism, both in the skin where this results in two metabolites while systemic capsaicin is efficiently metabolized by hepatic enzymes into the three different principal metabolites in nonclinical species and human. CYP2C9 and 2C19 appear to be the significant enzymes involved in capsaicin metabolism.

Safety Pharmacology

While safety pharmacology studies which evaluated the effects of capsaicin indicated no significant CNS toxicity with acute patch use in rats, significant clinical observational findings representing effects on the CNS were observed in single- and repeat-dose studies as described in the following section. Higher plasma levels were observed in the latter studies which may explain this discrepancy. Intravenous administration of capsaicin resulted in a dose-dependent and transient increase in cardiorespiratory indices in dogs without evidence of QTc alteration.

General Toxicology

The applicant conducted several general toxicology studies including single-dose capsaicin patch application in rat and dog, and 4-week (once weekly) repeated-dose topical capsaicin studies in rat and minipig using the proposed clinical patch while in mice the Applicant administered a capsaicin solution in diethylene glycol monoethyl ether (DGME; a component of the patch vehicle) daily for 4 weeks. A 2-week study of daily intravenous infusion was also conducted in ketamine/xylazine-sedated dogs

The acute toxicity study in the rat identified dermal irritation characterized by erythema and edema though at the highest patch concentration tested clinical signs including irregular or labored breathing and recumbent posture indicative of transient CNS toxicity were noted. This was not observed in the dog. The Applicant believes this may be due to the different skin characteristics between the species (dog skin is thicker). Dr. Leshin

agrees this may be plausible. If true this may also be an explanation for the observation of systemic exposure in the rodent in contrast to the absence of exposure in the dog.

Repeat dose studies included 2-week (daily) IV study of capsaicin in the dog, and 4-week topical application of the clinical 8% capsaicin patch product with once weekly administration for 3 hr after pre-treatment with a lidocaine local anesthetic (LMX4) in rat and minipig. Satellite groups were allowed recovery periods consisting of 4- and 2-weeks in the rat and minipig, respectively. As the clinical product was used with a uniform density of capsaicin (640 $\mu g/cm^2$) the administered dose was controlled by the size of the patch applied and total capsaicin levels in the 2 dose groups used were 16 and 32 mg patch/animal. Toxicity observed in the rat was primarily local dermal effects observable by gross observation and histopathologic evaluation (hyperkeratosis and acanthosis, inflammation and ulceration) which was observed at all dose levels – unsurprising since the local concentration is constant irrespective of patch size – while the high (systemic) dose was also associated with transient CNS toxicity which was characterized by alterations in posture, breathing, and autonomic signs which returned to normal over the day after patch removal. Microscopic evidence of GI toxicity and immune effects (stomach erosions, GALT hyperplasia of cecum) were observed at termination of the study in the high dose group but despite a request for information this was not provided on low dose group animals. Dr. Leshin surmises these were not collected, though I note there was no evidence of similar findings in the high dose recovery animals, therefore the systemic findings appear to have limited toxicologic significance given the substantial difference in clinical use of the drug product and resulting systemic exposure compared with the repeat-dose toxicology studies. The dermal toxicology study in the minipig utilized the 8% patch with different patch sizes representing 128 mg or 384 mg patch/animal. Patches were applied for 3 hr once weekly for 4 weeks and resulted in similar findings of local toxicity without evidence of systemic toxicity; it is notable that there was little evidence of detectable levels of circulating capsaicin in this model even with this intensive dosing regimen. Intravenous administration of capsaicin in DGME over 15 minutes to anesthetized dogs only resulted in a small increase in ALT at the high dose, 0.3 mg/kg/day, which is not toxicological relevant to human therapy due to the limited severity of the perturbation, lack of other associated findings indicating toxicity and the substantial safety margin over human systemic exposure.

The local effects and safety profile of capsaicin in the Qutenza patch were adequately and appropriately evaluated in multiple animal models including rat, dog, minipig and rabbit. Systemic safety evaluation must be drawn from the rat studies utilizing the capsaicin patch as well as the IV study in dog as no systemic exposure was reliably detected in dermal patch studies in minipig or dog. Nevertheless, the IV study in dog for two weeks is considered sufficient used in combination with the safety data from rat to support this single use clinical product from the systemic safety standpoint as exposures (by C_{max} or AUC) were greatly above the mean - and even maximum - human exposure (see following table).

Systemic Safety Margins from General Toxicology Studies				
	NOAEL or acceptable LOAEL	C_{max} AUC		Human Systemic Safety Margin*
Acute Toxicology				
Rat	16 mg patch; single dose	- 748	ng*h/mL	131X
Dog	256 mg patch; single dose	n.d. n.	d.	N/A
Repeat-Dose Toxicology				
Rat	32 mg patch x 4 weeks	35 357		23X (C_{max}); 63X (AUC)
Minipig	384 mg patch x 4 weeks	n.d. n.	d.	N/A
Dog	0.3 mg/kg IV x 14 days	~ 200 ng/mL	-	133X

*, Human average C_{max} ~ 1.5 ng/mL; AUC ~ 5.7 ng*h/mL
(note: maximum C_{max} in an individual was 4.6 ng/mL)
-, not reported
n.d., not detected
N/A, not applicable

Local toxicity evaluation
Evaluation of the site of application in general toxicity as well as an irritancy study with the 8% capsaicin patch (containing identical or near identical density of capsaicin, respectively) for up to 3 hr revealed mild erythematous and edematous responses with single application. It is notable that concentrations higher than the clinical concentration (640 $\mu g/cm^2$) were not explored in the nonclinical data to determine safety margins for the intended local concentration for clinical use; however, longer durations of exposure were utilized in these studies (3 hr) when compared to the clinical application of 1 hr which provides a durational margin of safety.

Genetic toxicology
Capsaicin was tested in a genetic toxicology battery which included in vitro evaluation in bacteria (Ames assay), murine and human cells (Mouse Lymphoma Assay and Chromosomal Aberration assay in human peripheral blood lymphocytes) as well as an in vivo evaluation in a mouse micronucleus assay. Capsaicin was considered by Applicant and Dr. Leshin to have an equivocal signal in the Mouse Lymphoma Assay. The Applicant argued this was an artifact of the lymphoma cell assay, since structurally similar catecholamines also produce this positive finding in the model. All other studies were negative and valid and therefore I agree with Dr. Leshin that the weight-of-evidence supports a negative conclusion.

Carcinogenicity evaluation
The dermal transgenic Tg.AC evaluation was evaluated by Dr. Leshin as well as members of the Executive Carcinogenicity Assessment Committee who were in agreement that the methodologic flaws and inadequate and conflicting data presentation made interpretation impossible. Upon their recommendation, a DSI inspection has been

requested but due to the sporadic use of the capsaicin patch in clinical use an adequate study is not necessary for approval. I agree with Dr. Leshin that the label will indicate that a valid study has not been conducted.

Reproductive Toxicology

Fertility and Early Embryonic Development

Evaluation of fertility and early embryonic development was conducted in the rat with an intense regimen relative to the single-use patch (3 hr/day for 49 days in males and at least 23 days in females) using the 8% capsaicin patch with systemic dose controlled by patch size: 25, 37.5, 50 cm2 (16, 24, 32 mg/rat) vs. 50 cm^2 control patch. Effects of capsaicin treatment on reproductive parameters were observed in male rats including occasional incidences of small and flaccid testes as well as small epididymides, not observed in control-treated males. Testicular weight was not decreased statistically significantly though cauda epididymal weights were decreased in all male treated groups in a non-dose-dependent manner. Correlating with these changes were decreases in sperm motility (all doses) which were also below the historical control range and a reduction in sperm motility (all doses) and in sperm counts in the vas deferens and cauda epididymis reaching statistical significance in the HD males ($p<0.05$) in the latter organ. Dr. Leshin considers these findings to be not biologically meaningful for the current therapeutic application due to the low transient presence of capsaicin in humans with clinical use. Also, he notes there is significant variability between animals on this parameter, fertility was not affected, and previous toxicology studies with histology of the reproductive organs did not detect abnormal sperm development. While I do not believe there is likely a significant risk due principally to the conditions of use, I believe the data may present a real toxicologic finding. The absence of a statistically significant reduction in fertility in the rat is likely attributable to the large capacity for sperm production in the rodent which renders this model relatively insensitive to perturbation. Nevertheless, Dr. Leshin observes that several of the males with small reproductive organ sizes failed to impregnate their respective females and the fertility index was reduced in treated groups relative to vehicle patch control (72-83% vs. 92%, respectively). Although findings are not statistically significant, there appears to be biologic significance and plausibility to these related findings which may indicate some reduction in male fertility with this admittedly non-representative dosing regimen. Effects on female reproductive parameters appeared to be less pronounced with no effect on estrus cycling and the mild but non-statistically significant reduced fertility parameters mentioned previously. A treatment-related reduction in the number of live embryos (16-18%) compared to control females appeared to be related to a reduced number of corpora lutea (i.e. released eggs) rather than on embryo viability as there was also no increase in non-viable embryos at the time of cesarean section. I believe that although the data appears to reflect a real effect of capsaicin treatment and a No Effect Level was not defined for effects on sperm parameters, gross changes in reproductive organ size or weight were not observed in the general toxicology studies with similar dosing and it is unclear what relevance these findings have for the single-dose treatment regimen as clinically indicated. Although the indication is for the treatment of pain in osteoarthritis and this is generally observed in an older population, some percentage of the female population to use this drug product and certainly most males will be within their reproductive years. Additionally, while even

moderate reductions in sperm quality may have little effect on rodent reproductive capacity, human males are significantly more susceptible to impairment of fertility with reduction in sperm quality due to the lower excess capacity. The nonclinical data will be represented in the approved label.

Embryofetal Developmental Toxicity

Evaluation of embryofetal developmental toxicity was conducted in the rat and rabbit. Administration in the rat model utilized 8% capsaicin patch applied for 3 hr/day during the period of organogenesis with systemic dose controlled by patch size: 25, 37.5, 50 cm^2 (16, 24, 32 mg/rat) vs. a 50 cm^2 control patch. Local anesthesia prior to patch application was produced by topical application of 4% lidocaine (LMX4). Local toxicity consisting of erythema, flaking and scabs were noted in all capsaicin treatment groups. A reduction in ossification was noted in several bones in offspring of the highest systemic dose group but not in the low- or mid-dose group offspring compared with controls and no teratogenicity was observed. Little apparent effect on food consumption or body weight was observed with dosing despite the utilization of an intense regimen relative to the single-use patch.

An embryofetal developmental study in the rabbit was conducted in which the patch was not used but instead during the period of organogenesis a 10% capsaicin liquid was administered as 3, 6.5, or 13 µL/cm2 (60, 130 or 260 mg/rabbit) for a duration of 3 hr/day after local anesthesia was produced by LMX4 (4% lidocaine). Dr. Leshin reports there was no evidence of effects on embryofetal growth, morphogenesis or viability. He is not convinced that an appropriate maximum tolerated dose or maximum feasible dose (MTD or MFD, respectively) was reached in the pregnant rabbit though the Applicant reports that dermal "lesions" which were not further described were observed only in treated groups. He is not requesting repeat of the study to achieve this due to a fairly large margin of exposure (37X) between rabbit in the study and the mean human exposure with single application. I am in agreement with this justification. Dr. Leshin does cite literature which suggests the rabbit may be a relatively insensitive model for capsaicin response but he is not recommending the study be considered invalid. I believe the differences in clinical use of the product compared with the standard dosing methodology of the study does not warrant repeat of the study with a potentially more appropriate species. Furthermore, there is insufficient information for other animal species, other than the primate or dog to suggest an alternative species. Also, evidence of local toxicity due to capsaicin inclusion in the patch was observed when compared with a vehicle patch so some pharmacodynamic response is apparent.

Peri- and Postnatal Developmental Toxicity

A peri- and postnatal development study conducted in the rat using the 8% patch and the intense regimen (3 hr/day from GD7 through post-natal day [PND]20) with the 25, 37.5, or 50 cm^2 patches (16, 24, or 32 mg/rat) did not produce effects on offspring growth, viability or behavior and learning. Toxicokinetic data was not obtained in the study but information is available from the TK bridging study (described below). Levels of capsaicin in milk were obtained on PND14 and while variable (< 2.5 to 770 ng/mL)

appeared to be related to dose and indicate significant exposure can be obtained through this route.

Toxicokinetic data in pregnant rats with use of the 37.5 cm^2 and 50 cm^2 8% capsaicin patch was determined in a separate non-GLP bridging study with dosing from GD7 – GD17. Plasma concentration was maximal at the time of the first sampling (3 hr) equivalent to when the patch was removed and a sharp fall was observed over the next two hours. On GD7, C_{3hr} was ~17 ng/mL for both the 37.5 cm^2 and 50 cm^2 patch while on GD17 C_{3hr} was slightly higher (54 ng/mL) in the 50 cm^2 patch group compared with 37.5 cm^2 group (40 ng/mL) which also demonstrated some plasma accumulation with repeated exposure. Of additional note, significant capsaicin accumulation was observed in milk (92 ng/mL and 338 ng/mL, respectively) which represented a 6-fold higher level than the peak capsaicin concentration in plasma.

Dermal Sensitization

Dr. Leshin notes that a mild dermal sensitization reaction occurred in a study in guinea pigs but that the response should not be considered for regulatory purposes due to the known insensitivity of the guinea pig to capsaicin. I agree with Dr. Leshin this is not a reliable model though I disagree with the reason - the ability of a compound to generate a hypersensitivity reaction is not generally dependent on pharmacologic activity but on molecular weight and structural characteristics; rather, the model has been described as being a poor predictor of human response.

Other Issues Identified:

Cleansing Gel

The Drug Product includes a cleansing gel for the purpose of removing residual capsaicin from the skin after patch removal. I concur with Dr. Leshin that the gel appears to present little risk for human use and the nonclinical studies provided in which the gel was used for both placebo and capsaicin patches supports human use.

Cis-Capsaicin impurity qualification

The Applicant was notified in a 74-day Filing Letter that qualification of the (trans)-capsaicin impurity cis-capsaicin (also know as zucapsaicin) would be necessary to support the proposed drug substance specification levels proposed (NMT (b) (4)) as this exceeds ICH Q3A specifications – though for an oral or systemically available product. They provided on request an evaluation of the impurity in the Ames assay which was negative and provided information that the impurity was contained in genetic toxicity studies of capsaicin as well as being tested in a 2-week IV study in dog. I concur with Dr. Leshin that all data provided supports the safety of the proposed maximal specifications.

III. RECOMMENDATIONS

A. Recommendation on approvability

I concur with Dr. Leshin that this application may be approved pending agreement on labeling.

B. Recommendation for nonclinical studies

I concur no further nonclinical studies are necessary.

C. Recommendations on labeling

The significant changes Dr. Leshin recommends include:

- Section 8.1 Pregnancy
  - Reorganization according to current format utilized for this section. The Applicant is using the format and including additional information recommended in the *Draft* Pregnancy Labeling Rule.
  - Removal of Applicant's margins which were based on mg/m^2 body surface area comparison.
  - Addition of clarifying details to studies

- Section 8.2 Labor and Delivery
  - Adding a statement that effects of the drug product on labor and delivery are unknown.

- Section 11 Description
  - Clarification and removal of promotional statements

- Section 12.1 Mechanism of Action
  - Clarification and language recommendations

- Section 13.1
  - Removal of reference to invalid carcinogenicity study and replacement with a statement that an adequate study has not been conducted.
  - Removal of statement regarding similar mutagenicity findings with catecholamines.
  - Clarification and additional information provided on findings from the Fertility study in rat.

I am in general agreement with Dr. Leshin's recommendations. Final labeling will be negotiated with the Applicant and will be supported by an addendum to the primary review.

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/s/

Adam Wasserman
7/17/2009 03:37:08 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:	**22-395**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**Oct 13, 2008**
PRODUCT:	**Qutenza™ (Capsaicin Dermal Patch 8%)**
INTENDED CLINICAL POPULATION:	**Patients with neuropathic pain associated with postherpetic neuralgia (PHN)**
SPONSOR:	**NeurogesX Inc.**
DOCUMENTS REVIEWED:	**eCTD Module 4** **30-Mar-09, 000-BM (genetic toxicology of impurity)**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**L.S. Leshin, DVM, PhD**
PHARM/TOX SUPERVISOR:	**A. Wasserman, PhD**
DIVISION DIRECTOR:	**B. Rappaport, MD**
PROJECT MANAGER:	**T. Clayton**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability Approve

B. Recommendation for nonclinical studies None

C. Recommendations on labeling

(b) (4)

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(b) (4)

II. Regulatory background

Qutenza would be the first approved capsaicin product. The first approved NDA for capsaicin will be classified as a type 7 NDA, since this highly purified active ingredient would be considered a new molecular entity (NME) and although already marketed, it lacks an approved NDA. It will also be a new chemical entity (NCE) drug since it contains no active ingredient that has been approved by the FDA in any other application submitted under 505(b) of FD&C Act. The Applicant will receive 5 years of marketing exclusivity (5059c)(3)(D)(ii).

Products containing capsaicin 0.025%-0.25% are currently marketed OTC under the Tentative Final External Analgesic monograph of 1983 (FR Vol 48; No. 27; p. 3852; Feb 8, 1983). These products are officially classified as unapproved drugs since this monograph has yet to be finalized after 26 years. The indications for capsaicin creams include the temporary relief of minor aches and pain of muscles and joints associated with arthritis, back pain, and other muscle and joint discomfort.

A pre-IND meeting was held June 26, 2001 at which time the indication was (b) (4) Studies were conducted under IND 63354 which was initiated in Sept 2001. On May 2, 2003 the application was granted orphan product designation (b) (4) The submission is a 505(b)(2) with reference to the scientific literature to support the pharmacology including the mechanism of action and some toxicology safety.

(b) (4)

Capsaicin derived from plants provides the active component of “pepper spray” utilized for personal self-defense and by police for crowd control, and "bear spray or foams" for animal attacks (foams stick to the tissues) that may contain up to 18% capsaicin (bear spray). Some states have limited the amount of capsaicin in these non-drug items, since eye lesions have occurred.

III. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Safety Pharmacology
Safety pharmacology included neurobehavioral assessments in male and female rats and cardiovascular respiratory monitoring in anesthetized (propofol and alfentanil) male dogs following capsaicin administration.

There were no neurobehavioral effects in rats with 32 mg/animal patch applied for 1, 2 or 3 hours resulting in mean plasma concentrations between 10.1 to 37.0 ng/mL from 75 to 360 minutes after patch application. In a local tolerance study examining acute irritation and phototoxicity, it was noted that rats exhibited signs of possible discomfort (increased activity) after capsaicin application, which did not occur but not in groups treated only with anesthetic cream or placebo patches. When the amount of topical anesthesia and the duration of application was increased, the incidence and duration of post capasaicin treatment activity was reduced.

Dogs were administered a 15 minute IV infusion in ascending doses of capsaicin 0.03, 0.1 and 0.3 mg/kg of capsaicin and were monitored for 40 minutes after the start of infusion, with a 15 minute intervals between doses. The high dose resulted in transient increases blood pressure, heart rate, dP/dt_{max}, and femoral blood flow lasting 5 to 10 minutes and returning to baseline. There was no effect on QT_C interval. Respiratory parameters of tidal volume, peak expiratory and inspiratory flow respiratory rate also transiently increased lasting 5 to 10 minutes.

Pharmacokinetics
ADME studies were conducted in rats and pigs following ^{14}C-capsaicin topical patch application. The rat studies yielded different results thought to be attributed to differences in the patches made on location, specifically for each study. The initial rat study indicated that 70% had been absorbed and radioactivity was detectable in plasma from 0.5 to 72 hours (end of study period), while the second rat study indicated radioactivity uptake was substantially less and more consistent with the pig study in which 2% to 6% of ^{14}C-capsaicin was absorbed through the skin, and plasma concentrations were detectable at 1 and 4 hours after application of the patches. (For comparison, human absorption has been estimated at 0.9% of the patch concentration, refer to the Clinical Pharmacology Review).

Capsaicin was highly bound to plasma proteins in human, dog, rabbit and rat, and independent of concentration. In human skin, capsaicin was slowly metabolized to vanìlylamine and vanìlic acid, although the majority is unchanged after 20 hours. Metabolism of capsaicin was much faster in incubations with rat microsomes and enhanced liver enzymes preparations (S9 fraction) compared to human, and both the rat and human were more rapid than dog capsaicin metabolism. Three major metabolites were detected in all three species 16-hydroxy-capsaicin, 17-hydroxy-capsaicin and 16,17-dehydro-capsaicin. Rat microsomes and S9 fraction also formed hydrolytic metabolites vanilylamine and vanillin. The formation of 16-hydroxy-capsaicin and 16,17-dehydro-

capsaicin were primarily formed by the activity of CYP2C9 with some contribution by CYP2C19 to the formation of 16-hydroxy-capsaicin. The CYP enzyme involved in the formation of 17-hydroxy-capsaicin was not identified, but was NADPH-dependent.

General Toxicology
Comment on the general study design: The general procedure for the toxicology studies in which a patch was administered to the animals back for a number of hours (usually 3 hours) each day was to clip the hair, apply an anesthetic cream (b)(4) allowed to stand for 1 hour, wiped away, then the capsaicin patch applied, removed after the appropriate time of exposure, then cleansing cream is used to wipe away residual capsaicin. This is the same procedure used in clinical trials, although the components varied somewhat with the nonclinical studies. For the pivotal toxicology repeated dosing studies, and the reproduction and developmental studies the components were the same as used in the clinical studies. Since the clinical application of the patch was once at 3 month intervals for a 60 to 90 minute period in early studies, the nonclinical application of patches once/day for 3 hours was used to provide an excess exposure over human use.

Single-dose toxicology studies: A single 3 hour duration dose of capsaicin in the form of a patch was applied to the back of rats (16 or 32 mg patch) and dogs (73 and 292 mg patch). The rat, but not the dog exhibited dermal irritation (erythema, edema, fissures). The rat also had detectable dose-related systemic exposure, while almost all plasma samples in the dog were below capsaicin detection limits. A possible reason for the species difference in irritation and systemic exposure is the composition of the skin in which the layers are thicker in the dog than the rat. The rat also exhibited atonia and hypothermia. The only treatment related effect that occured in both species was an increase in urinary potassium 24 hours postdose. There were no other treatment-related effects on clinical hematology, chemistry, organ weight, gross pathology, or histopathology for the the rat or dog. A NOAEL was not attained in the rat due to clinical signs and dermal irritation. The NOAEL in the dog was the high dose 292 mg capsaicin patch.

Repeat-dose toxicology studies: The repeated-dose toxicology studies of topically applied capsaicin were conducted in rats and minipigs with 4-weeks of a once weekly patch application for 3 hours followed by a 4-week recovery period for rats, and a 2-week recovery period in minipigs. The capsaicin patch was the same that was used clinically (capsaicin density 0.64 mg/cm^2).

Rats were treated with patches of 16 mg capsaicin/rat (25 cm^2 patch) or 32 mg capsaicin/rat (50 cm^2 patch) for 3 hours, once a week for 4 weeks. Sores and scabs developed at the site of application. Reversible dermal irritation effects included erythema, edema, atonia, desquamation and fissuring. Animals treated with capsaicin patches showed greater effects than those treated with placebo patches and females tended to have more severe effects. High-dose females also had clinical observation of ataxia and recumbency, irregular/labored respiration, dilated pupils and cold to touch, all reversible (in the days following patch treatment each week. Gross findings included

crusted skin at the treatment site in capsaicin treated females; which was reversible. Histopathology of the treatment site found a slightly greater incidence in capsaicin treated groups of acanthosis/ hyperkeratosis, epidermal inflammation and ulceration. Acanthosis/hyperkeratosis was still noted after the recovery period although the incidence was similar between control and capsaicin groups. Edema at the treatment site was noted in the 2 high-dose females. Slight erosion of the stomach was observed in 3 high dose animals. GALT hyperplasia of the cecum was observed in 6 high dose animals. Maximum plasma concentrations were observed within 2 hours of patch application. AUC increased proportionally to dose on days 1 and 28, except for males on day 1 (supra-proportional). Exposure was significantly greater in females, which could be due to the increased dose on a body weight basis. There was a slow elimination ($t_{1/2}$ = 15.8 to 53.4 hours). A NOAEL dose was not identified as local skin irritation was observed in both dose groups and findings in the stomach and cecum were not assessed in the low dose groups. The Applicant was asked for this histopathology information after the study was initially reviewed for the IND, but never provided this data, apparently those tissues were not collected.

Minipigs were treated with a capsaicin patch (128 mg/minipig, 200 cm^2 patch or 384 mg/minipig 600 cm^2 patch) for 3 hours, once a week for 4 weeks followed by a 2 week recovery period. The high dose patch was locally irritating evidenced by scratching, erythema, and edema. The erythema and edema noted with the high dose was reversed by the end of the study, a week after the last application of capsaicin. There were no treatment related effects on clinical pathology, macroscopic and microscopic pathology findings during the study or following the recovery period. The NOAEL for this study was 384 mg capsaicin/animal. Except for two animals on Day 1, plasma samples were below the limit of detection for capsaicin (LLOQ 5 ng/mL) at all the time points on all sampling days, indicating very little to no systemic exposure to capsaicin for a once weekly 3-hour treatment. Capsaicin metabolites were not evaluated. The NOAEL for this study was 384 mg/minipig, the highest dose.

In dogs, capsaicin dissolved in diethylene glycol monoethyl ether (DGME) and Dulbecco's phosphate buffered saline, was administered via intravenous infusion over a 15 minute period to anesthetised dogs at doses of 0.03, 0.1 or 0.3 mg capsaicin/kg/day daily for 14 days. The control animals had marked vascular irritation at the administration site, and thought to be caused by DGME. There were no deaths, capsaicin-related organ weight changes, macroscopic or microscopic observations. The only capsaicin-related clinical sign was vocalization by dogs receiving the high dose 0.3 mg/kg/day of capsaicin, assumed to occur after awakening from anesthesia. The only capsaicin-related clinical pathology finding was slightly elevated ALT in both males and females at the high dose. The NOAEL for capsaicin was 0.3 mg/kg/day. Capsaicin metabolites were not evaluated in this study.

Genetic Toxicology

Capsaicin was negative in the bacteria reverse mutation assay for mutagenicity and negative in the vivo mouse micronucleus and human peripheral blood lymphocyte chromosomal aberration assays for clastogenicity. Capsaicin showed a (weak) positive response in the in vitro mouse lymphoma assay. There was no follow-up to this positive

result as the Applicant present published evidence that structural similar endogenous compounds (catecholamines) also produced positive results in the mouse lymphoma assay. A number of published studies also indicated that capsaicin produced equivocal findings in a few genotoxic assays. The purity of the plant-isolated capsaicin used in these studies was unknown.

Carcinogenicity
The Applicant was allowed to conduct a single carcinogenicity study in a transgenetic mouse model because the human plasma levels were non-detectable for the majority of samples and they had not yet determined the frequency of application when the discussions concerning initiating the carcinogenicity studies occurred. In retrospect, the study was unnecessary according to the guidance ICH-S1A, since human administration is for a 1 hour duration at 12 week intervals. The Applicant conducted dose ranging studies in FVB mice and received ECAC recommendations for the protocol and doses which the Applicant followed in their 26-week daily dermal application study in transgenic hemizygous FVB/NTac-Tg(v-Ha-ras)TG.ACled mice. There were numerous problems with the analysis and interpretation of this study, which resulted in non-acceptance of the study by the ECAC. The study was also published with the same inappropriate analysis and conclusions. Also in retrospect, but after the initial acceptance of the study by the ECAC, this transgenic model has been determined to be inappropriate for compounds that are skin irritants such as capsaicin. A request for a DSI inspection was submitted to examine the discrepancies in the study results (IND 63354 5/13/09), and is planned to occur in mid-July 2009.

Reproductive and Developmental Toxicology
Fertility: In the fertility and general reproductive toxicology study capsaicin, rats were treated for 3 hours/day with a dermally applied capsaicin patch (identical to the clinical therapeutic patch). Males treated for 49 days developed reduced sperm motility at all dose levels (46-58% vs 85% in controls) and increased non-motile sperm in the vas deferens (25, 37.5 and 50 cm^2 patch sizes, corresponding to 16, 24 and 32 mg/rat, respectively).. Statistically reduced sperm counts were present in the mid dose in the vas deferens and high dose in the cauda epididymis, but these are not likely biologically meaningful since there was large variation associated with mean values and fertility was not affected. Histopathology of testes in previous repeated dose toxicological studies did not find evidence of abnormal sperm development. Females were treated for at least 23 days, a cycling period followed by a mating period and an additional 7 days of gestation. Capsaicin treatments did not affect rat estrous cyclicity or the incidence of pregnancy, even in those mated with males of low sperm counts. The lack of reduced fertility in the males is thought to be attributed to an excess of sperm production in this species, even though motile sperm were reduced by capsaicin treatment. All females appeared normal at necropsy.

Embryofetal development:
Rat: In the rat embryofetal developmental toxicology study, capsaicin patches were also applied daily for 3 hours/day from the beginning of organogenesis, day 7 through day 17, and fetuses were examined after Caesarean-section on day 21, the last of gestation. The low and mid capsaicin doses (25 and 37.5 cm^2 patch sizes, corresponding to 16 and 24

mg/rat, respectively) had no effect on litter reproductive parameters and there was no increase in fetal abnormalities compared to controls or historical background data. Treatment with the high dose patch (50 cm^2 patch, 32 mg/rat) resulted in reduced skeletal ossification in the hind-limb metatarsals, and 1st sternebra. Capsaicin was not a teratogen at the doses studied in the rat.

Rabbit: In the rabbit study, capsaicin was administered as a liquid in a vehicle of diethylene glycol monoethyl ether, applied topically onto the back of the rabbits (at doses of 3, 6.5, or 13 $\mu L/cm^2$ corresponding to 60, 130, or 260 mg/rabbit). There was no effect of any dose on the litter parameters and no fetal abnormalities could be attributed to capsaicin. The developmental NOAEL was the high dose of 13 $\mu L/cm^2$ or 260 mg/rabbit. There were no signs that the maternal maximal tolerated dose or maximal feasible dose was reached. Dermal lesions were reported for all capsaicin treatment groups, but these "lesions" were not described. It is assumed they were breaks in the epidermis due to ulcers or cuts. They could have developed from capsaicin irritation and the resultant scratching or cage rubbing in an attempt to alleviate the irritation, although these behaviors were not mentioned. Although the maximal tolerated dose or maximal feasible dose was not attained in the dose-range or the main study, toxicokinetics indicate that the high dose provided a safety margin of 37X over the mean Cmax values between the first day dosing in the rabbit compared to a single patch application in humans. It is unlikely a repeat of the rabbit study to attain a maximal tolerated dose would provide additional clinically relevant information. A few published studies indicate that the rabbit is relatively insensitive to capsaicin, and this may explain the lack of noted effects, although dermal irritation did occur.

Pre- and post-natal toxicity: There was no major toxicity from capsaicin patch treatments in the pre- and post-natal toxicity study. Capsaicin patches were similar to those in the fertility study described above applied for 3 hours daily from gestation day 7 through postpartum day 20 (lactation day 20). All pups appeared normal at necropsy on Day 28 postpartum. There was no effect on body weight and food consumption of rats from capsaicin treated dams. Capsaicin was detected in milk ranged from < 2.5 ng/mL to 770 ng/mL and tended to be correlated to plasma concentrations of the treated dams. In tests of learning, short-term retention, long-term retention, response inhibition evaluated in a passive avoidance paradigm or water maze, there were no differences between F_1 generation rats from the capsaicin and control treated dams. Sexual development and maturation of F_1 males and females from capsaicin treated dams was similar to F_1 rats from control treated dams. There was no effect on testes or epididymis weights, or the +ratios of these weights to the terminal body weight in F_1 males. Mating and fertility parameters, litter parameters, and fetal abnormalities were similar between rats from capsaicin and control treated dams.

Dermal Irritation

Due to the intense burning sensations from the application of 8% capsaicin in humans, all studies with humans and animals were conducted with a topical anesthetic pretreatment. While this may have greatly attenuated any primary irritation reaction, it is the part of the therapeutic procedure and therefore it would be appropriate to determine the nonclinical primary irritance and hypersensitivity reactions under these conditions.

Acute dermal irritancy was demonstrated in rats with application of an early formulation of the capsaicin patches (124.4 mg capsaicin/200 cm^2; 0.62 mg/cm^2). Upon immediate removal of the patch after a 1 hour application, grade 1 erythema was evident, which resolved after 1 hour.

Dermal Sensitization
An early formulation of capsaicin patches (124.4 mg capsaicin/200 cm^2; 0.62 mg/cm^2) showed a mild dermal sensitization reaction in guinea pigs. This study likely underestimates the sensitization response since publications indicate the guinea pig is relatively insensitive to capsaicin. However, since hypersensitivity reactions are not necessarily dependent on pharmacodynamic action, rather they are responsive to the molecular composition and structure of a compound, it is acceptable.

Phototoxicity
Exposure to simulated solar irradiation (0.5 MED) did not enhance the dermal irritation effect demonstrated without solar irradiation exposure. Application of an early formulation of capsaicin patches (124.4 mg capsaicin/200 cm^2; 0.62 mg/cm^2) on rats for 1 to 3 hours, followed by patch removal, then exposure to simulated solar irradiation did not result in enhanced dermal responses compared to patch treatments without exposure to solar irradiation. There was a 2 hour time interval between patch removal and exposure to simulated solar irradiation, which allowed for recovery of the skin from the erythema produced by the capsaicin patches.

Excipients
The Applicant provided evidence (presented in the Appendix 3of this Review) that the the excipients:
1) exceeded that expected to be systemically absorbed in human patients, by use in the nonclinical toxicological studies in which the frequency, duration, or dose of application was greater than that proposed for clinical use.
2) were used in other approved products, or
3) had an acceptable toxicological profile at the levels applied to the skin and expected to be absorbed systemically.
Therefore, the excipients were not considered to be of toxicological concern.

Cleaning Gel
Evidence was presented that there are no toxicological concerns with the components or use of the Cleansing Gel.

For all in vivo studies, a topical anesthetic, control patch or solution, and cleansing gel were administered to the control groups, confounding the ability to attribute any noted toxicity to a particular component. Toxicity would only be differentiated by the time of its immediate occurance. There were no serious toxicity, except rare occurances of erythema and signs of slight irritation in the control groups, but it could not be attributed to a specific component.

Qutenza (8% capsaicin patch) Safety Margin Table

Parameter or Study	Dose [mg capsaicin patch]	Plasma C_{max}* (ng/mL)	Human Systemic Safety Margin
General Toxicology			
Single-Dose Toxicology			
Rat, No NOAEL Determined	-	-	-
Dog, NOAEL	256	not detectable	-
Repeated-Dose			
Mouse, 1/week for 4 weeks	2.56	43	29X
Rat, 1/week for 4 weeks	32	35.3	23X
Minipig, 1/week for4 weeks	384	not detectable	-
Dog, IV dosing 14 days	0.3 mg/kg	193	130X
Reproductive and Developmental Toxicology			
Rat Embryofetal Development and Postnatal Development			
NOAEL for Teratogenic effects	32	15.7	11X
NOAEL for Non teratogenic effects, reduced fetal skeletal ossification	24	18.4	12X
NOAEL for postnatal development (growth, behavior, sexual maturation)	32	15.7	11X
Rat, Male Fertility			
Sperm motility, No NOAEL Determined	-	-	-
NOAEL for Sperm count (reduced to 79 and 69% of control in mid and high doses)	16	19.3	13X
Rabbit, Embryofetal Development			
NOAEL for Teratogenic and Non-teratogenic effects	260 mg capsaicin solution	54.6	37X

[1] rat plasma data from dose-range study UYQ00004 (not GLP, not QA); the plasma analysis of study 1614-003p was not acceptable, 3 hour daily exposure, daily dosing

[2] rat plasma, male from toxicology study 7215-120, 3 hour patch exposure, once weekly for 4 weeks

[3] rabbit plasma data from dose-range study 1614-002p, 3 hour daily exposure, daily dosing

*C_{max} comparison to the mean human Cmax of 1.48 ng/mL (640 mg capsaicin Quentenza patch, AUC 5.71 ng-h/mL; AUC was not provided or not calculatable for many animal studies.

The C_{max} for animal studies used the first day of dosing toxicokinetic values, since human dose is a single dose, although animals were dosed multiple days.

B. Pharmacologic activity

Trans-capsaicin is the active pharmaceutical ingredient contained in Qutenza. Capsaicin is a pungent molecule contained in chili peppers and is used as a spice in foods consumed worldwide. The capsaicin content of chili peppers ranges from 0.1% to 1% w/w. The synthetic highly purified form used in Qutenza comprises 8% w/w.

Capsaicin is an agonist for the transient receptor potential vanilloid 1 receptor (TRPV1; or vanilloid receptor 1, VR1). TRPV1 is a ligand-gated, non-selective cation channel expressed on small-diameter sensory neurons, those containing nociceptors which specialize in the detection of painful or noxious sensations (thin unmyelinated C-fibers and to a lesser extent thin myelinated Aδ-fibers). TRPV1 responds to noxious stimuli, including capsaicin, heat, and extracellular acidification. In humans, TRPV1 receptors are found in sensory neurons (dorsal root ganglia and trigeminal ganglia), located in pancreas, brain, spinal cord, bladder, kidney, liver, spleen, testis, lung, and gastrointestinal tract. Vanilloids, such as capsaicin, are the only known chemical activators of the TRPV1 receptors.

Activation of TRPV1 at these cutaneous nerve endings by capsaicin results in localized burning sensations, hyperalgesia, allodynia, and erythema. With prolonged exposure to capsaicin, desensitization results in which in spontaneous and evoked painful sensations are reduced and cutaneous nociceptors become less sensitive to a variety of stimuli, including capsaicin or thermal stimuli. This is the rationale for the development of Qutenza for neuropathic pain, to rapidly activate these recptors with a high dose of capsaicin which will desensitize the receptor for a prolonged period of time. Two studies evaluated the pharmacodynamic effects of Qutenza on sensory function and epidermal nerve fiber density in healthy volunteers. Consistent with the known pharmacodynamic effects of capsaicin on TRPV1-expressing nociceptive nerve endings, reduced epidermal nerve fiber density and minor changes in cutaneous nociceptive function (heat detection and sharp sensation) were noted one week after exposure to Qutenza. The epidermal nerve fiber density reduction and sensory changes were fully reversible. Thus desensitization occurs via nerve terminal "pruning" presumably those that have TRPV1 receptors, and that the return of neuropathic pain may be associated with reinnervation.

C. Nonclinical safety issues relevant to clinical use

Local Anesthetic
The topical local anesthetic should be an approved product, but there are unapproved products available which may be used. This is an issue that might be addressed in labeling, but apparently physicians are unaware of that some of the topical anesthetics are unapproved (e.g., the Applicant was unaware that MLX-4, 4% lidocaine cream used in Phase 3 studies was not approved). The Applicant has not addressed differences in the potencies of available topical anesthetics to prevent the burning sensation of capsaicin. This might be addressed in labeling if there is information to determine that different brands and concentrations are more effective. It is noteworthy that the initial

toxicological study with pigs, used a 2% tetracaine topical lotion, which was insufficient to counter signs of pain or distress (vocalization, rubbing and scratching). It was necessary to pretreat the minipigs with Demerol.

Effects on Sensory Neurons
Studies to determine if there is an increase in pain (supersensitivity) after epidermal nerve reinnervation were not conducted in either nonclinical or clinical studies. From a pharmtox perspective, this is more of an academic interest than toxicological concern for clinical treatment. The clinical studies did not address the potential issues of insufficient topical analgesia with patch application, perhaps this is a reason for the low numbers of retreatment patients.

Studies conducted in humans examining epidermal nerve innervation and sensory testing however found no lasting deficits. Deeper layers of the skin were not studied for potential nerve innervation loss. References were provided for potential effects on dorsal root ganglia, but those studies were not well described and not adequate to characterize the response of sensory perikerya, other than to conclude cell death was not apparent.

Reproduction and developmental studies
In the rat fertility studies, sperm motility at all doses and sperm numbers were slightly reduced at the mid and high doses. A safety margin based on plasma toxicokinetic parameters was not available for this reproductive studies, however similar patches were applied to rats in the 4-week, once weekly application repeated dose study. At the low dose, a safety margin of 13X was calculated for reduced sperm counts. There was no NOAEL for sperm motility, since it occurred at all doses. Since rats were administered capsaicin in the fertility study for 49 days, for 3 hours each day, and human therapy will consist of a single application for 1 hour at 3 month intervals, with minimal detectable plasma exposure, the risk to human males is likely very low.

In peri- and post-natal developmental study in rats, capsaicin-treated dams had measureable levels of capsaicin in milk and plasma on day 14 of lactation (rat were weaned at 4 weeks in this study), but there were no effects on viability, growth, learning and memory, sexual development, mating and pregnancy, and their (F_2) fetuses. Although nursing mother would not be expected to comprise a treatment population for this product, human level of capsaicin are very low and transient and very little accumulation in nursing mothers would be expected.

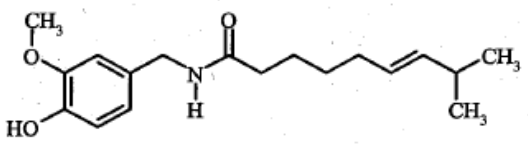
Dermal Irritation and Sensitization
Capsaicin is known dermal irritant which was demonstrated in the nonclinical studies and clinical studies. The nonclinical study to determine if the patch was sensitizing to a second later application was conducted in guinea pig, a common species for this type of study, but is also known to be poorly responsive to capsaicin in comparison to other species. Therefore, although a mild sensitizing response was demonstrated, this response probably underestimated the hypersensitivity reaction that may occur clinically. This is evidenced by the appearance in neuropathic patients of papules, swelling and vesicles and pruritus, not evident in the guinea pig studies. Although multiple applications of the

Qutenza have been applied therapeutically, relatively few patients were treated, and there was minimal information about immediate patient response of the second or third Qutenza application . Furthermore, the dermal hypersensitivity responses have similarity to the primary irritancy response, with perhaps an earlier onset and enhanced severity. A one hour patch application, obscuring the area in which the primary reaction site may develop will not allow monitoring these responses. Fortunately the whole treatment process occurs in a physician's office. The product should be labeled that hypersensitivity reactions may occur, but are not readily apparent due to the treatment process.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	**22-395**
Sequence /date/type of submission:	000/Oct 13, 2008/505(b)(2); 000/March 30, 2009 (impurity genetic tox);
Sponsor and/or agent:	**NeurogesX Inc.** 969C Industrial Park San Carlos, CA 94070-4117
Manufacturer for drug substance:	(b) (4)
Reviewer name:	L.S. Leshin
Division name:	Division of Anesthesia, Analgesia and Rheumatology Products.
HFD	170
Drug:	
	Qutenza™
	(b) (4) (previously identified as (b) (4) in the IND)
Generic name:	**Capsaicin Dermal Patch, NGX-4010**
	Proposed tradename form Oct 14 2003 annual report (b) (4) Patch
Chemical name:	trans-8-methy1-N-vanillyl-6-nonenamide; (E)-8-methyl-n-vanillyl-6-nonenamide; 6-nonenamide, (E)-N-((4-hydroxy-3-methoxy-phenyl)methyl)-8-methyl
CAS registry number:	404-86-4
Molecular formula:	$C_{18}H_{27}NO_3$
Molecular weight:	305.42
Structure:	



Relevant INDs/NDAs/DMFs:

IND 63354 (Capsaicin 8% Patch, NeurogesX Inc., initiated Sept 2001)

(b) (4)

(b) (4)

Drug class:	Non-narcotic analgesic, vaniloid receptor (TRPV1) agonist
Intended clinical population:	Patients with neuropathic pain associated with postherpetic neuralgia (PHN)
Route of administration:	Topical on skin
Clinical formulation:	Topical Dermal Patch consisting of 8% capsaicin in an adhesive mass, 0.64 mg/cm^2, (179 mg capsaicin/280 cm^2 patch, 20 cm x 14 cm) The proposed label indicates up to 4 patches may be applied, covering a skin area of 1120 cm^2.for a total of 716 mg capsaicin.

The patch consists of (b) (4)

The clinical use of this product requires that the treatment site be pretreated with a topical local anesthetic due to the intense burning sensation associated with the capsaicin patch application. There were no studies that examined different types and potencies of topical anesthetics, either clinically or nonclinical studies. These issues are briefly discussed in Appendix 5.

Table 1: Composition of the NGX-4010 Patch

Components of the Patch and Quality Standard (and grade, if applicable)	Intended Functions	Quantitative Composition		
		mg per Unit (280 cm^2)	(b) (4)	
Capsaicin (House Standard)	Active Ingredient	179		
Silicone adhesive (b) (4) (House Standard)				
Silicone adhesive (b) (4) (House Standard)				
Diethylene glycol monoethyl ether (DGME) (b) (4)				
Dimethicone (Silicone oil) (NF)				
Ethylcellulose (b) (4) (NF)				
(b) (4)				
Total	--			
Polyester (b) (4) (b) (4) Film (b) (4)		--	--	--
Polyester film, (b) (4) (House Standard) (b) (4)		--	--	--

(b) (4)

IMPURITIES

Cis-Capsaicin

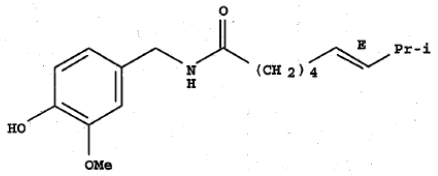
Impurities that can potentially appear in the trans-capsaicin drug substance, include cis-capsaicin (also known as zucapsaicin), (b) (4)

Table 12: API Specifications

Related Substance	Limit w/w %
cis-Capsaicin	(b) (4)
(b) (4)	

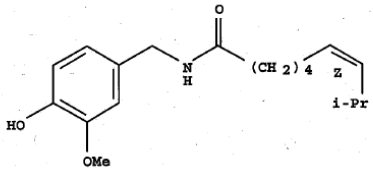
Only cis-capsaicin exceeds the ICH threshold values. The synthetically pure form of capsaicin in NGX-4010 is the trans-isomer, which is identical to the molecule produced by plants. It is separated from cis-capsaicin by (b) (4) the trans isomer is less soluble, leading to an enrichment in this isomer. This process results in a drug substance with a cis-capsaicin level typically not more than (b) (4) Stability studies with the drug substance and drug product demonstrated that cis-capsaicin levels are stable and that trans-capsaicin does not interconvertto the cis form (refer to the CMC Review). The structures of the geometric isomers are presented below.

trans-Capsaicin: CAS Registry 404-86-4 Double bond geometry as showr



Formula: $C_{18}H_{27}NO_3$

cis-Capsaicin: CAS Registry 25775-90-0 Double bond geometry as shown



Formula: $C_{18}H_{27}NO_3$

Following the pre-NDA meeting the Applicant submitted information (SD-183, April 22, 2008) about cis-capsaicin levels in the previously conducted non-clinical studies and a request to discuss the need for additional studies to qualify this impurity. After the NDA was submitted, the toxicological qualification of cis-capsaicin was determined to be incomplete due to inadequate cis-capsaicin concentrations used for the Ames mutagenic assay. That was a study of trans-capsaicin, in which cis-capsaicin was an impurity. They were asked to repeat this study using cis-capsaicin in the 74-day filing letter of Dec 24, 2008. The study was submitted March 30, 2009 to the NDA (000-BM) with negative findings for mutagenic potential (see review in Section 2.6.6.4 Genetic Toxicology). Refer to Appendix 2, for a review of the Applicant's April 22, 2008 submission. The qualification studies for cis-capsaicin include the following:

- *Mutagenicity*, (Ames assay, 5000 µg/plate).

- *Clastogenicity* (in vivo mouse bone marrow chromosomal aberration assay, (b) (4) as an impurity). This is greater than the impurity specification of NMT (b) (4)
- *14-day repeated dose IV administration in the dog*, (b) (4) as an impurity of trans-capsaicin). The high dose of 0.3 mg/kg produced a mean plasma Cmax of 193 ng/mL in males on day 14 (the lowest Cmax for 0.3 mg/kg of day 1 and day 14, males or females), which would be a an amount of cis-capsaicin (b) (4) and (b) (4) The maximal human dose produces a mean Cmax of 1.5 ng/mL. If dermal penetrations was similar to trans-capsaicin, then (b) (4) cis-capsaicin would be expected to produce levels of (b) (4)

Reviewer Comment: *Therefore, in accordance with ICH Q3B (R2) guidance, the impurity cis-capsaicin is qualified (qualification threshold is 0.2%) for the impurity level of NMT (b) (4) of the applied product.*

(b) (4)

(b) (4) (b) (4) (b) (4) . Five capsaicin (b) (4) lots (F0010601, F0010602, F00107001, F00107002 and F00107003) were analyzed for (b) (4) and none was detected. The Applicant proposes to set a specification of NMT (b) (4) for (b) (4) Since (b) (4) is not listed in ICH Q3C solvents table, it was important to determine if there was toxicological concerns with this specification level of (b) (4)

Three isomers, (b) (4) are listed as Class 3 solvents and the Applicant provided the following LD_{50} data to indicate (b) (4) has similar lethal toxicity as these three isomers, perhaps slightly less toxic (references were not provided) and could be considered as a Class 3 solvent.

Table 2: (b) (4) and Isomers LD50 Data

(b) (4)

Reviewer Comment: *Based on LD50 values and similar toxicological profiles for these isomers, it is reasonable to consider (b) (4) a class 3 solvent.*

ICH-Q3C indicated that "Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. However, there are no long-term toxicity or carcinogenicity studies for many of the solvents in Class 3. Available data indicate that they are less toxic in acute or short-term studies and negative in genotoxicity studies."

If (b) (4) was considered as a class 3 solvent, then "amounts of these residual solvents of (b) (4) per day or less (corresponding to (b) (4) under Option 1) would be acceptable without justification."

If the patch contains a capsaicin amount of 179 mg/280 cm^2, then as stated above allowing (b) (4) levels would be (b) (4), and (b) (4) or (b) (4) would be (b) (4). Exposing skin to this amount of (b) (4) for 1-hour patch application at 3 month intervals is unlikely to be toxicologically significant.

Reviewer Comment: *Therefore the specification of NMT (b) (4) for (b) (4) is acceptable from a toxicological perspective.*

EXCIPIENTS

There are no toxicological concerns with the excipients comprising the patch. Refer to the Appendix 3 for the toxicological assessment of these components which is summarized in the table below.

Reviewer comment: *There are no toxicological concerns with the excipients.*

Excipient Components of the Capsaicin Patch

Excipient	mg/Unit (280 cm2)	Formulation (b) (4) (% w/w)	Inactive Ingredient List (max potency and type of formulation))	Toxicological Concern
(b) (4) silicone adhesives (b) (4) (b) (4)	(b) (4)			No Based on use of patch in animal and human studies
diethylene glycol monoethyl ether (DGME) (b) (4) (b) (4)				No Based on Inactive ingredient list and Based on use of patch in animal and human studies
silicone oil (dimethicone) USP/NF				No Based on Inactive ingredient list and Based on use of patch in animal and human studies
ethylcellulose (b) (4) (b) (4)				No Based on Inactive ingredient list and Based on use of patch in animal

				and human studies

CLEANSING GEL

Cleansing Gel is a topically applied semi-solid product in a single use tube container (50 g of topical gel with a nominal overfill of (b) (4) per tube) packaged with the capsaicin dermal patch. It is designed to solubilize residual capsaicin from the skin surface following administration of the capsaicin patch such that it can then be wiped from the skin. The ingredients are listed in the table below. The Applicant states that the cleansing gel does not contain an active ingredient, and has no therapeutic value in treating neuropathic pain. Capsaicin patches and placebo patches (lacking capsaicin) were used in some of the toxicological studies. Since there were no animals without patches and thus without excipients in those studies, any effect of the excipients would have shown up in all groups, and unrelated to capsaicin dose. Except for dermal signs of irritation which could be due to patch removal, there was no indication that the cleasnsing gel produced toxicity. In the NDA 74 day filling letter of Dec 24, 2008, the Applicant was asked to provide a toxicological assessment of the cleansing gel and to justify its safety. In their Jan 29, 2009 response, the Applicant indicated this information was provided in Module 2.4 (Nonclinical Overview), section 2.4.4.7. and Module 2.4 Attachment 2. This material is reviewed in Appendix 4.

Reviewer comment: *There are no toxicological concerns with the cleansing gel.*

Cleansing Gel Components

Inactive Ingredient (Excipient)	Grade	Quantity (% w/w)	50 g tube (g)	Inactive Ingredient List (max potency / type of formulation)	Toxicological Concern
(b) (4) (b) (4)	(b) (4)				No
(b) (4)					No, this formulation lacking (b) (4) is much safer
edetate disodium (EDTA) (b) (4)	USP				No
butylated hydroxyanisole (BHA) (b) (4)	USP-NF				No
sodium hydroxide	USP-NF				-
purified water	USP-NF				-
(b) (4)					

***Reviewer comment**: For all in vivo studies, a topical anesthetic of 4% lidocaine, control patch or solution, and cleansing gel were administered to the control groups, confounding the ability to attribute any noted toxicity to a particular component.*

Studies reviewed within this submission:

Studies submitted to IND 63354 were previously reviewed and included in the NDA review, modified if necessary to include additional information. Previous Reviewers included Dr. Suzanne Thornton, Review 1, November 2, 2001 and Review 4, June 22, 2004; Dr. Violetta Klimek, Review 2, June 27, 2002; and Dr. Timothy J. McGovern, Review 3, June 17, 2003.

Study Number / Location	Title
Pharmacology	
Safety Pharmacology	
2074-001-D6146 m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\2074-001	Cardiovascular and respiratory effects of capsaicin in the anaesthetised dog following intravenous administration
2074-002 m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\2074-002	Effects on General Activity and Behaviour in the Rat Following Application of Capsaicin Dermal Patch
Pharmacokinetics	
Absorption	
7215-105 m4\42-stud-rep\422-pk\4222-absorp\7215-105	Absorption, Distribution, Metabolism, and Elimination of 14C-Capsaicin Following a Dermal Patch Application to Rats
7215-135 m4\42-stud-rep\422-pk\4222-absorp\7215-135	Absorption, Distribution, Metabolism, and Elimination of [14C]Capsaicin Following a Dermal Patch Application to Rats
7215-114 m4\42-stud-rep\422-pk\4222-absorp\7215-114	Absorption, Distribution, Metabolism, and Elimination of 14C-Capsaicin Following a Single Dermal Patch Application to Mini-Swine
Distribution	
7215-143 m4\42-stud-rep\422-pk\4223-distrib\7215-143	The *In Vitro* Protein Binding of *trans*-[14C]Capsaicin in Rat, Rabbit, Dog, and Human Plasma
Metabolism	
7215-125 m4\42-stud-rep\422-pk\4224-metab\7215-125	*In Vitro* Metabolism of [14C]Capsaicin by Rat, Dog, and Human Hepatic Microsomes and S-9 Fraction
XT044001 m4\42-stud-rep\422-pk\4224-metab	*In Vitro* Reaction Phenotyping Study of *trans*-Capsaicin
Tox computer modeling	
nc002-0-rp m4\42-stud-rep\423-tox\4237-other-tox-stud\42375-metab\nc002-0-rp	In silico analysis of capsaicin, capsaicin metabolites and impurities present in synthetic trans-capsaicin
Toxicology	
Single Dose	

Rat	
7215-106 m4\42-stud-rep\423-tox\4231-single-dose-tox	Single-Dose Dermal Toxicity and Toxicokinetic Study with (b) (4) Pain Treatment System (Capsaicin) in Rats
Dog	
7215-107 m4\42-stud-rep\423-tox\4231-single-dose-tox\7215-107	Single Dose Dermal Toxicity and Toxicokinetics Study with (b) (4) Pain Treatment System (Capsaicin) in Dogs
Repeated Dose	
Minipig	
7215-113 m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-113	Subchronic Dermal Toxicity and Toxicokinetics Study with (b) (4) Pain Treatment System (Capsaicin) in Mini-Swine with a 2-Week Recovery Period and an Exploratory Phase
Rat	
7215-120 m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-120	4-Week Dermal Toxicity and Toxicokinetics Study with Capsaicin Patch in Rats with a 2-Week Recovery Period
Dog	
7215-127 m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-127	14-Day Intravenous Infusion Toxicity and Toxicokinetic Study with Capsaicin in Dogs
Genetic Toxicology	
767691 m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\767691	Capsaicin Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA 98 and TA 100 and Escherichia coli WP2uvrA
767707-21007 m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\767707-21007	Capsaicin Mouse Lymphoma Cell Mutation Assay
24641-0-449oecd m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\24641-0-449oecd	Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with Capsaicin
767712 m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\767712	Capsaicin Micronucleus Test in Bone Marrow of CD-1 Mice 0 h + 24 h Oral Dosing and 48 h Sampling
Cis capsaicin Impurity	
8201369 submission 30-mar-09, seq 000, mod type BM NDA022395\0014\M4\42-stud-rep\423-tox\4237-other-tox-stud\42376-imp (b) (4) 8201369-cis-capsaicin-ames-final-report	Bacterial Reverse Mutation Assay with a Confirmatory Assay (response to 74 day filing communication of Dec 24, 2008 and NeurosgesX response of Jan 29, 2009, sequence 2)
Carcinogenicity	
7215-122 m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud	4-Week Dose Range-Finding Dermal Toxicity and Toxicokinetic Study with Capsaicin in FVB/N Mice
7215-150 m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud\7215-150	26-Week Dermal Oncogenicity Study with *trans*-Capsaicin in Tg.AC Hemizygous Mice (FVB/N)
Reproductive Toxicology	
Fertility and Early Embryo Development	

rat	
uyq00003 m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\uyq00003	Percutaneous fertility and general reproduction toxicity study of capsaicin dermal patches in rats
Embryo-Fetal Development	
1614-003p m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-003p	Percutaneous dosage-range-finder developmental toxicity study of capsaicin dermal patches in rats
1614-003 m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-003	Percutaneous developmental toxicity study of capsaicin deermal patches in rats
rabbit	
1614-002p m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-002p	Percutaneous dosage-range-finder developmental toxicity study of capsaicin in rabbits
1614-002 m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-002	Percutaneous developmental toxicity study of capsaicin in rabbits
Pre and Postnatal Development	
uyq00004 m4\43-lit-ref\ UYQ00004-pilot-study.pdf	Percutaneous dosage-range developmental and perinatal/postnatal reproductive toxicity study of capsaicin dermal patches in rats
uyq00005 m4\42-stud-rep\423-tox\4235-repro-dev-tox\42353-pre-postnatal-dev\uyq00005	Percutaneous developmental and perinatal/postnatal reproduction toxicity study of capsaicin dermal patches in rats, including a postnatal behavioral/functional evaluation
Special Dermal Studies	
Dermal Sensitization	
7215-108 m4\42-stud-rep\423-tox\4236-loc-tol\7215-108	Dermal Sensitization Study of (b) (4) Pain Treatment System (Capsaicin) and (b) (4) Placebo Pain Treatment System in Guinea Pigs-Closed Patch Technique
Phototoxicity	
1614-001 m4\42-stud-rep\423-tox\4236-loc-tol\1614-001	Topical phototoxicity screening test of capsaicin patches in rats

Cleansing Gel	
Module 2.4 (Nonclinical Overview), Section 2.4.4.7 and Module 2.4, Attachment 2	Toxicological Assessment of The Cleansing Gel

Studies not reviewed within this submission:

References provided by the Applicant are listed in Appendix 1.

Pharmacokinetics
Analytical Methodology

(b) (4)

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

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

All of the information concerning the pharmacology of capsaicin was obtained from the published literature. The Applicant conducted nonclinical assessments of neurobehavioral, cardiovascular and respiratory safety.

Trans-capsaicin is the active pharmaceutical ingredient contained in Qutenza. Capsaicin is a pungent molecule contained in chili peppers and is used as a spice in foods consumed worldwide. The synthetic highly purified form used in Qutenza comprises 8% w/w to be applied topically on the skin. In comparison, the capsaicin content of chili peppers ranges from 0.1% to 1% w/w consumed orally.

Capsaicin is an agonist for the transient receptor potential vanilloid 1 receptor (TRPV1; or vanilloid receptor 1, VR1). TRPV1 is a ligand-gated, non-selective cation channel expressed on small-diameter sensory neurons, those containing nociceptors which specialize in the detection of painful or noxious sensations (thin unmyelinated C-fibers and to a lesser extent thin myelinated Aδ-fibers). TRPV1 responds to noxious stimuli, including capsaicin, heat, and extracellular acidification. Vanilloids, such as capsaicin, are the only known chemical activators of the TRPV1 receptors.

Activation of TRPV1 at these cutaneous nerve endings by capsaicin results in localized burning sensations, hyperalgesia, allodynia, and erythema. With prolonged exposure to capsaicin, desensitization results in which in spontaneous and evoked painful sensations are reduced and cutaneous nociceptors become less sensitive to a variety of stimuli, including capsaicin or thermal stimuli. This is the rationale for the development of Qutenza for neuropathic pain, to rapidly activate these recptors with a high dose of capsaicin which will desensitize the receptor for a prolonged period of time. Consistent with the known pharmacodynamic effects of capsaicin on TRPV1-expressing nociceptive nerve endings, reduced epidermal nerve fiber density and minor changes in cutaneous nociceptive function (heat detection and sharp sensation) were noted one week after exposure to Qutenza. The epidermal nerve fiber density reduction and sensory changes were fully reversible. Thus desensitization occurs via nerve terminal “pruning” presumably those that have TRPV1 receptors and that the return of neuropathic pain may be associated with reinnervation.

2.6.2.2 Primary pharmacodynamics

The following information was obtained from the Applicants Pharmacology Summary in Section M2.

MECHANISM OF ACTION

Tissue Localization of TRPV1 Receptors:
TRPV1 receptors are expressed on sensory nerve fibers, and throughout the spinal cord and central nervous system (locus ceruleus, preoptic area and medial basal hypothalamus hippocampus, basal ganglia, thalamus,cortex, cerebellum, olfactory bulb, mesencephalon and hindbrain; and on astrocytic foot processes and in pericytes.

Still unclear is whether TRPV1 is also present in a number of non-neural cells as has been reported using a different antibody in immunohistochemical studies. These include mast cells, epidermal keratinocytes, dermal blood vessels, the inner root sheet and the infundibulum of hair follicles, differentiated sebocytes, sweat gland ducts, and the secretory portion of eccrine sweat glands. ,

Ion Channel Effects
Capsaicin activation of the TRPV1 receptors is mediated by an increase in intracellular calcium. Contrasting effects on voltage-activated calcium currents have been reported. One study found that 1 to 25 μM capsaicin increased peak calcium current in guinea pig ventricular myocytes, while others have shown inhibitions in rat trigeminal and hippocampal neurons (with IC_{50} values of 14.5 and 21.2 μM, respectively). Capsaicin also modulates voltage-activated sodium and potassium ion channels. Capsaicin (0.4 to 4 mcM) produced a non-use-dependent reduction of voltage-activated sodium current in isolated rat heart atrial cells and depressed the contractility of isolated electrically driven left rat atria (Milesi et al., 2001; Kopanitsa et al., 1995), and guinea pig smooth muscle cells (IC_{50} = 5.8 μM) (Sim et al., 2001). With respect to voltage-activated potassium channels, capsaicin (10 μM) increased the action potential duration in adult rat ventricular myocytes from 45 ms to 166 ms. This effect was associated with an inhibition of three distinct potassium currents.

The potency of capsaicin for TRPV1 activation varies according to species, expression system and laboratory protocol. The largest comparable data sets for capsaicin potency and efficacy arise from measurements of intracellular calcium responses in cells stably expressing TRPV1. Using fluorescent imaging plate readers under similar (but not identical) conditions of 20 ºC to 25 ºC and pH ~7.4, potency (EC_{50}) values have been obtained for the human, rat and mouse. It is notable that the avain TRPV1 receptor is not sensitive to capsaicin (Jordt, 2002). The rabbit and hamster are relatively insensitive to to capsaicin.

EC_{50} for activation of TRPV1

Species	EC_{50}	Reference
human	74.1 nM	Smart et al., 2000
	12.5 nM	Witte et al., 2002
rat	33.9 nM	Jerman et al., 2000
	13.5 nM	Witte et al., 2002
mouse	3.7 nM	Correll et al., 2004

In all experimental systems, including those using fluorescence plate readers cited above, capsaicin is a full agonist of TRPV1 (i.e., provides maximal activation of TRPV1). Capsaicin potency values are expected to be higher if conducted at physiological temperatures or in acidic conditions (Cortright and Szallasi, 2004), since TRPV1 activation is highlytemperature and pH dependent.

Capsaicin-induced desensitization
Capsaicin induced desensitization of cutaneous nociceptors is mediated by an increase in intracellular calcium, followed by neurochemical and morphological alterations at the peripheral ends of TRPV1-positive, small-diameter, nociceptive afferent neurons (Szallasi and Blumberg, 1999). The onset of an analgesic effect due to repeated topical applications of low-concentration capsaicin cream in humans and superstimulation with excess capsaicin correlates with reduce functionality as measured by quantitative sensory testing (QST) and by loss of immunostaining of the peripheral endings of small-diameter afferent nerves in the epidermis, as measured by the general neuronal marker protein gene product 9.5 (PGP 9.5) (Nolano et al., 1999).

The onset of efficacy and duration of pain relief activity after discontinuation of TRPV1 agonist treatment may be related to the rate at which nociceptive nerve endings lose and then regain functionality (Bjerring, 1990; Nolano, 1999). Although nociceptors can remain functionally inactive for weeks following the topical application of capsaicin, they eventually return to pre-treatment levels of sensitivity. Reductions in nociceptor immunostaining widely interpreted to be degeneration of nociceptor nerve ending also occur, with the same time course as the effect on functionality. The onset of efficacy and duration of pain relief activity after discontinuation of TRPV1 agonist treatment may be related to the rate at which nociceptive nerve endings lose and then regain functionality (Bjerring et al., 1990; Nolano et al., 1999). Although nociceptors can remain functionally inactive for weeks following the topical application of capsaicin, they eventually return to pre-treatment levels of sensitivity. Topical capsaicin treatment only produces alterations of the very tips of peripheral nociceptive nerve fibers, as the vast majority of the neuron and cell body in the dorsal root ganglion remain unaffected (McMahon et al., 1991).

There is evidence that intact nociceptor nerve terminals may also develop abnormal electrophysiological properties due to exposure to abnormal concentrations of neurotrophins such as nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF) family members and pro-inflammatory cytokines. Neutralization of NGF has been reported to reduce pain behaviors in some rodent models of neuropathic pain (Sevcik et al., 2005). Similar results have been obtained both *in vitro* and *in vivo* with GDNF family members (Malin et al., 2006). The long-term desensitization of

nociceptors induced by TRPV1 agonist exposure may not only reduce the ability of nociceptor tips to initiate electrical signals, but it is also known that nociceptor nerve terminals exposed to capsaicin lose the capacity to uptake and retrogradely transport neurotrophic factors such as NGF to the cell body (Miller, 1982; Taylor et al., 1984). Without a constant supply of NGF, many nociceptors lose their ability to maintain a hyperexcitable phenotype (Petruska and Mendell, 2004). Hence, TRPV1 agonist treatments may actually alter the phenotype of nociceptors by depriving them of pro-excitatory influences from their target organs.

DRUG ACTIVITY RELATED TO PROPOSED INDICATION

Pathophysiologieal Activity of Nociceptors:
In chronically painful conditions, partcularly neuropathic pain sytdromes, clinical and nonclinica1 research shows collectively that the most peripheral aspects of damaged sensory nerves often display aberrant "pathophysiological" electrcal hyperactivity (Michaelis, 2002). Spontaneous activity of distal nociceptive fibers following nerve injury has been recorded extensively in nonclinica1 models, and correlated directly with pain behaviors. Transection-of the sciatic nerve in rodents is a long-standing model in which spontaneous or "ectopic" electrcal activity of the resulting neuroma develops, and nerve fibers termnating in the neuroma become extremely sensitive to stimuli (Nall, 1983). Several recent nonclinica1 studies have demonstrated the hyperexcitabilty of intact nociceptors (i.e., those which still innervate their target organ) following mechanical or chemical injures to nerve fibers (Wu, 2001; Wu, 2002; Djouhr, 2006).

In neuropathic pain syndromes, sensory neuron axons are lost due either to cell body or nerve fiber damage following viral, metabolic, traumatic or chemical insults. Immunohistochemical analyses indicate that the density of epidermal nerve fibers in areas of skin reported by patients to be painful is decreased in a wide range of neuropathic pain syndromes; this includes postherpetic neuralgia (Rowbotham, 1996; Oakander, 200n, diabetic neuropathy (Kennedy, 1996), painful HIV-associated neuropathy (Polydetks, 2002), Fabry disease (Scott, 1999), small-fiber neuropathy (Pittenger, 2004) and eryhromelalgia (Davis, 2006). Moreover, data suggest a positive correlation between the extent of epidermal nerve fibers loss and the severity of pain in diabetic neuropathy (Kennedy, 1996) and HIV-associated neuropathy (Po1ydetks, 2002). This observation also extends to nonclinica1 models: in one study with rats following a chronic constriction injury, there was a very significant reduction of PGP 9.5-immunoreactive fibers in the epidermis of the footpad on the same day as maximum pain behavior (Lindenlaub, 2002).

Thus, desensitization of nociceptive nerve fibers may constitute an important therapeutic intervention if nociceptors are hyperactive and is the basis for the development of Qutenza.

Low Dose Capsaicin in Pain Therapy: Several human clinical studies involving populations suffering from pain associated with a variety of conditions (postherpetic neuralgia, painful diabetic neuropathy, post-mastectomy pain syndrome, and others) have suggested that repeated application of low-concentration (up to 0.1% w/w) topical

capsaicin provides beneficial effects (Rains 1995). The onset of a pain relief effect due to repeated topical applications of low-concentration capsaicin cream in humans correlates with loss of function of the peripheral endings of TRPV1-expressing cutaneous sensory nerves, as measured by reduced cutaneous sensitivity to thermal stimuli (as measured by quantitative sensory testing) and loss of immunostaining (using the general neuronal marker protein gene product 9.5 (PGP 9.5) (Nolano 1999). The functionality of the peripheral ends of cutaneous sensory nerve fibers returns after discontinuation of capsaicin treatment and this reinnervation corresponds with return of the ability to detect painful thermal sensations. Importantly, topical low-concentration capsaicin affects only the very ends of peripheral nociceptive nerve fibers, leaving the vast majority of the neuron and the cell body in the dorsal root ganglion unaffected (McMahon 1991).

The duration of pain relief activity after discontinuation of TRPVl agonist treatment may be related to the rate at which nociceptive nerve ending regain fuctionality (Bierrng, 1990; No1ano, 1999). Although nociceptors can remain fuctionally inactive for weeks following the topical application of capsaicin, they eventually return to pre-treatment levels of sensitivity. Changes in nociceptor immunostaining also occur, with the same time course as the effect.

2.6.2.3 Secondary pharmacodynamics

The following information was obtained from the Applicants Pharmacology Summary in Section M2.

Thermoregulation:
TRPV1 localization within the central nervous system may account for the effects of capsaicin exposure on body temperature and body temperature regulation. Intracerebral or subcutaneous administration of capsaicin in rodents induces a fall in body temperature (Fujii, 1986; Ding, 2005) accompanied by heat loss responses such as cutaneous vasodilation (Jansco-Gabor, 1970), which are blocked with a highly selective TRPV1 antagonist (Varga, 2005). Administration of TRPV1 antagonists alone to rats, dogs and monkeys (Gavva et al., 2007) results in hyperthermia. Administration of high dose levels of capsaicin, induces desensitization, animals become insensitive to the hypothermic effect of a subsequent injection (Jansco-Gabor, 1970).

Cardiovascular:
In addition to the skin, TRPV1 receptors are expressed on sensory nerve C-fibers which innervate the heart and vasculature; and may mediate the sensation of burning chest pain associated myocardial ischemia (Vaishnava, 2003; Pan, 2004). Cardiac sensory afferents in rodents consistently respond to regional myocardial ischemia and this response can be blocked by TRPV1 antagonists (Pan, 2004). As stimulation of cardiac output is a physiological response to the detection of cardiac ischemia, bolus systemic exposures to capsaicin would be expected to increase cardiac output. This effect was observed in anesthetized dogs in which a transient tachycardia was induced (cardiac output was not assessed or calculated) during safety studies described in Section 2.6.2.3 of this Review. Subsets of capsaicin-sensitive sensory nerves contain calcitonin gene-related peptide and substance P, which are potent vasodilators and natriuretic/diuretic factors. The effects

produced by loss of capsaicin-sensitive sensory nerves induced by large doses of capsaicin in neonatal rodents revealed that TRPV1-expressing sensory fibers are involved in maintaining the homeostatic blood-pressue regulation altered by sodium loading and activation of various neuron-hormonal systems (Vaishnava, 2003).

Studies in a variety of cardiac cells and tissue provide evidence for capsaicin modulation of voltage-activated sodium, calcium, and potassium ion channels. Capsaicin (0.4 to 4 μM) produced a non-use-dependent reduction of voltage-activated sodium current in isolated rat heart atrial cells and depressed the contractility of isolated electrically driven left rat atria (Milesi, 2001). Contrasting effects on voltage-activated calcium currents have been reported. In guinea pig ventricular myocytes, 1 to 25 μM capsaicin increased peak calcium current (Cheng, -2004). With respect to voltage-activated potassium channels, capsaicin (10 μM) increased the action potential duration in adult rat ventricular myocytes from 45 ms to 166 ms. This effect was associated with an inhibition of three distinct potassium currents (and associated IC_{50} values): the transient outward current (I_{TO}; IC_{50} = 6.4 μM), a non-inactivating outward current (I_K; IC_{50} = 11.5 μM), and the inward rectifier current (I_{K1}; IC_{50} = 46.9 μM) (Castle, 1992).

Additional studies have examined the physiologically relevance of the above ionic effects. Recordings from guinea pig papillary muscles demonstrated significant shortening of action potential durations (at 90% repolarization and 1 Hz stimulation) by 9%, 28% and 39% at 10, 30, and 100 μM capsaicin, respectively. No changes in action potential duration were observed with capsaicin (up to 100 μM) at faster stimulation rates (5 Hz) (D'Alonzo, 1995). Similar results were obtained by a different group, using the capsaicin concentration range of 30 to 120 μM) (Li, 2003). In pacemaker cells isolated from sinoatrial nodes of rabbits, capsaicin (10 μM) significantly reduced action potential amplitudes and maximal rates of depolarization. However, the duration of 90% repolarization of the action potential was also prolonged significantly (Cheng, 2003). In the rat, capsaicin (30 μM) also significantly reduced ischemic ventricular tachycardia and ischemic ventricular fibrillation (D'Alonzo, 1995). The dominance of calcium channel during potassium channel inhibition reported in all tissues other than rabbit sinoatrial nodes suggests that exposure of the human heart to high concentrations of capsaicin is likely to result in shortening of cardiac action potentials (and consequently a reduction of QT intervals). However, it is unlikely that such high concentrations are attainable without causing profound cardiovascular effects mediated by massive TRPV1 activation in cardiac sensory nerves.

Non-neural TRPV1 receptors:
TRPV1 is also reported to be expressed in non-neuronal tissues (Gunthorpe and Szallasi, 2008) mast cells, epidermal keratinocytes, dermal blood vessels, the inner root sheet and the infundibulum of hair follicles, differentiated sebocytes, sweat gland ducts, and the secretory portion of eccrine sweat glands (Stander 2004). Using a different anitbody, no evidence for TRPV1 expression in any skin structure other than sensory nerve fibers was found (Lauria, 2006). Also, Lauria (2006) did not find expression of TRPV1 in myelinated fibers, whereas Stander (2004) reported that unmyelinated epidermal nerve fibers were only weakly immunoreactive and dermal myelinated nerve fibers were

strongly stained. Thus the distribution of TRPV1 is controversial due to differences in TRPV1 antibodies used in the immunohistochemical studies.

Inhibition of Nuclear Transcription Factor kappaB (NFκB)
Activation of nuclear transcription factor kappaB (NFκB) is linked with a variety of inflammatory diseases, including cancer, atherosclerosis, myocardial infarction, diabetes, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, Alzheimer's disease, osteoporosis, psoriasis, septic shock, and AIDS. It has been shown that the pathway that activates this transcription factor can be interrupted by chemicals contained in a variety of plants, including capsaicin (Singh et al., 1996; Aggarwal and Shishodia, 2004). A relatively low dose of capsaicin increases anti-inflammatory IL-10 levels and attenuates levels of pro-inflammatory cytokines in rats with experimental septic shock, a condition which displays considerable NFκB expression (Demirbileck et al., 2004). The ability of capsaicin to inhibit NFκB activation may also be related to its anti-psoriatic properties.

Inhibition of Platelet Aggregation:
Capsaicin inhibited the aggregation of rat platelets induced by collagen or thrombin and inhibited ATP release induced by thrombin in the presence of ethylene diamine tetracetic acid (EDTA). The IC_{50} for collagen-induced platelet aggregation was about 85 µg/mL (Wang et al., 1984). *In vivo*, capsaicin dose-dependently prolonged the tail bleeding time in conscious mice and maximal effect was observed at three hours after the oral administration. Although capsaicin was found to suppress platelet aggregation markedly, it did not affect blood coagulation as measured in platelet-poor plasma (Wang et al., 1985).

2.6.2.4 Safety pharmacology

NEUROLOGICAL EFFECTS

Study Title: Effects on General Activity and Behaviour in the Rat Following Application of Capsaicin Dermal Patch

Key Study Findings: There were no behavioral or physiological changes in rats after dermal application of patches containing 32 mg capsaicin for 1, 2, or 3 hours compared to placebo patch control treated rats.

Study no: 2074-002
E-Location: m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\2074-002
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: Sept 9 2003
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
Capsaicin Patch: 640 µg/cm^2, Batch 81101203, Purity not provided (zucapsaicin impurity (b) (4) (lod)
Vehicle Patch: diethylene glycol monoethyl ether, and a pressure sensitive adhesive matrix
ELA-Max (lidocaine 4%)
Cleansing gel (89% polyethylene glycol), Batch 22698

Methods:

Doses: patches (50 cm^2) of 0 or 32 mg capsaicin/rat
Species/strain: Wistar rats
#/sex/group or time point: 6/sex/treatment
Route, form, volume, and infusion rate: dermal patches were applied for 1 to 3 hours to the back of the rats.
Satellite groups used for toxicokinetics or recovery: 3/sex
Age: 9 weeks of age
Weight: males: 317 to 398 g; females: 210 to 251 g

Study design:
Irwin observations for general behavioral, autonomic and motor effects (Irwin, 1968) were performed at the appropriate time-points (75, 135, 195, 270 and 360 minutes) following patch application. The animals were kept for a further 7 days following the day of dosing for general observations. Blood samples were taken from satellite animals pre-dose and at 75, 135, 195, 270 and 360 minutes post-patch application for pharmacokinetic analysis by (b) (4)

Group	Treatment	Dose level (mg/rat)	Length of application (hr)	Number of rats / gender		Observation times (min) post-patch application
				Males	Females	
1	Control Patch	0	3	6	6	75, 135, 195, 270 and 360
2	Capsaicin Dermal Patch	32	1	6	6	75, 135, 195, 270 and 360
3	Capsaicin Dermal Patch	32	2	6	6	135, 195, 270 and 360
4	Capsaicin Dermal Patch	32	3	6	6	195, 270 and 360

* Patch application was taken as the time that the patch was first applied.

Group	Treatment	Dose level (mg/rat)	Animal bleed at time (min)	Number of rats / gender	
				Males	Females
5a	Capsaicin Dermal Patch	32	75, 195, 360	3	3
5b	Capsaicin Dermal Patch	32	Pre-dose, 135, 270	3	3

Note: Patches were removed from all animals at 3 hours post-dose

Patches were applied after clipping the hair of the back, Elizabethan collars were place around the neck, anesthetic cream was applied (40 $\mu L/cm^2$), anesthetic cream removed after 60 min with a dry gauze, collars were removed, patches applied and secured with elastic wrap. After the appropriate duration of exposure, patches were removed and the administration site was wiped with cleansing lotion (3 mL) and dry gauze.

Results

There were no deaths.

Cutaneous application of NGX-4010 at a dose of 32 mg/rat for a duration of 1, 2 and 3 hours produced no behavioral or physiological changes in male and female rats when compared to rats treated with control patches for a duration of 3 hours.

A number of signs were seen in this study that were not considered to be capsaicin-related. These signs were generally mild in severity, occurred in all treatment groups, including the control patch group, and had disappeared in all animals by day 2. Frequently observed signs in both male and female rats were chromodacryorrhoea and red staining of the fur. Increased vocalisation and irritability was also frequently noted for female rats. A slight reddening of the patch application area was seen in two female rats (animal numbers 37 and 38) receiving capsaicin patch for a duration of 2 hours. No further signs were seen during the subsequent 7-day post-dose observation period.

Detectable levels of capsaicin ranging from 10.1 ng/mL to 37 ng/mL were measured in rat plasma at 75, 135, 195 and 270 minutes and were below the limit of quantitation (LOQ=10 ng/mL) at 360 minutes.

Table 1: Treatment Groups in Irwin CNS Safety Pharmacology Study

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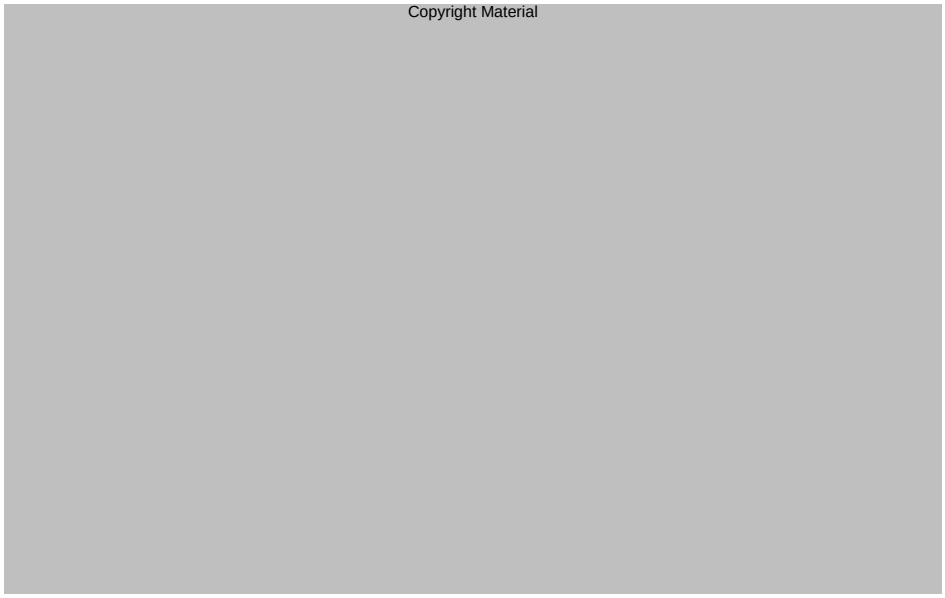


Table 1
Concentration (ng/mL) of Capsaicin in Rat Plasma

Animal	Dose Group	Gender	Timepoint (minute) 0	75	135	195	270	360
49	5a	M		< 10.0		< 10.0		< 10.0
50	5a	M		11.4		13.8		< 10.0
51	5a	M		13.0		13.2		< 10.0
52	5b	M	< 10.0		< 10.0		< 10.0	
53	5b	M	< 10.0		10.1		< 10.0	
54	5b	M	< 10.0		16.7		< 10.0	
55	5a	F		37.0		< 20.0 DS		< 10.0
56	5a	F		35.4		16.8		< 10.0
57	5a	F		11.9		20.3		< 10.0
58	5b	F	< 10.0		35.3		< 10.0	
59	5b	F	< 10.0		20.8		10.5	
60	5b	F	< 10.0		24.5		< 10.0	

DS Diluted sample due to insufficient volume

CARDIOVASCULAR AND PULMONARY EFFECTS

Study Title: Cardiovascular and respiratory effects of capsaicin in the anaesthetised dog following intravenous administration.

This was reviewed by Dr. Suzanne R. Thornton when it was submitted to IND 63354 in 2001.

Key Findings: Capsaicin at 0.3 mg/kg when administered as a timed intravenous infusion produced increases in cardiovascular parameters, mean arterial blood pressure, heart rate and femoral blood flow, and increases in respiratory parameters. There were no detectable changes in QT_{CF} or QT_{CB}. These changes in cardiovascular and respiratory parameters were transient and all values returned to after infusion termination.

Study no: 2074/001-D6146
E-Location: m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol\2074-001
Conducting laboratory and location: (b) (4)
Date of study initiation: 23 July 2001
GLP compliance: No
QA report: No
Drug, lot #, and % purity:
Capsaicin, Lot F0010101, Purity 100%
Vehicle/control: 10% (w/v) hydroxypropyl β-cyclodextrin, Batch 29796-089

Methods:

Doses: 0.03, 0.1, 0.3 mg/kg
Species/strain: Beagle dog/Harlan, UK
#/sex/group or time point: 4/male/timepoint
Route, form, volume, and infusion rate: i.v., 15 min timed infusion 0.10-0.5 mL/kg
Satellite groups used for toxicokinetics or recovery: NA
Age: 10-17 months at dosing
Weight: 19-21.8 kg

Study design:
Dogs were anesthetized, maintained on propofol, and placed on heating blanket. Cannula were placed in the femoral artery and vein. Baseline readings for cardiovascular and respiratory parameters were taken at two 10-min intervals after a 30 min stabilization period, and then at 2, 10, 15, 20, 30, and 40 mins after dose initiation. Dogs wered (as a timed infusion over 15 min) in an ascending order either vehicle, 0.03, 0.1, and 0.3 mg/kg every 25 mins. PK samples were taken 5 mins after dose administration was terminated.

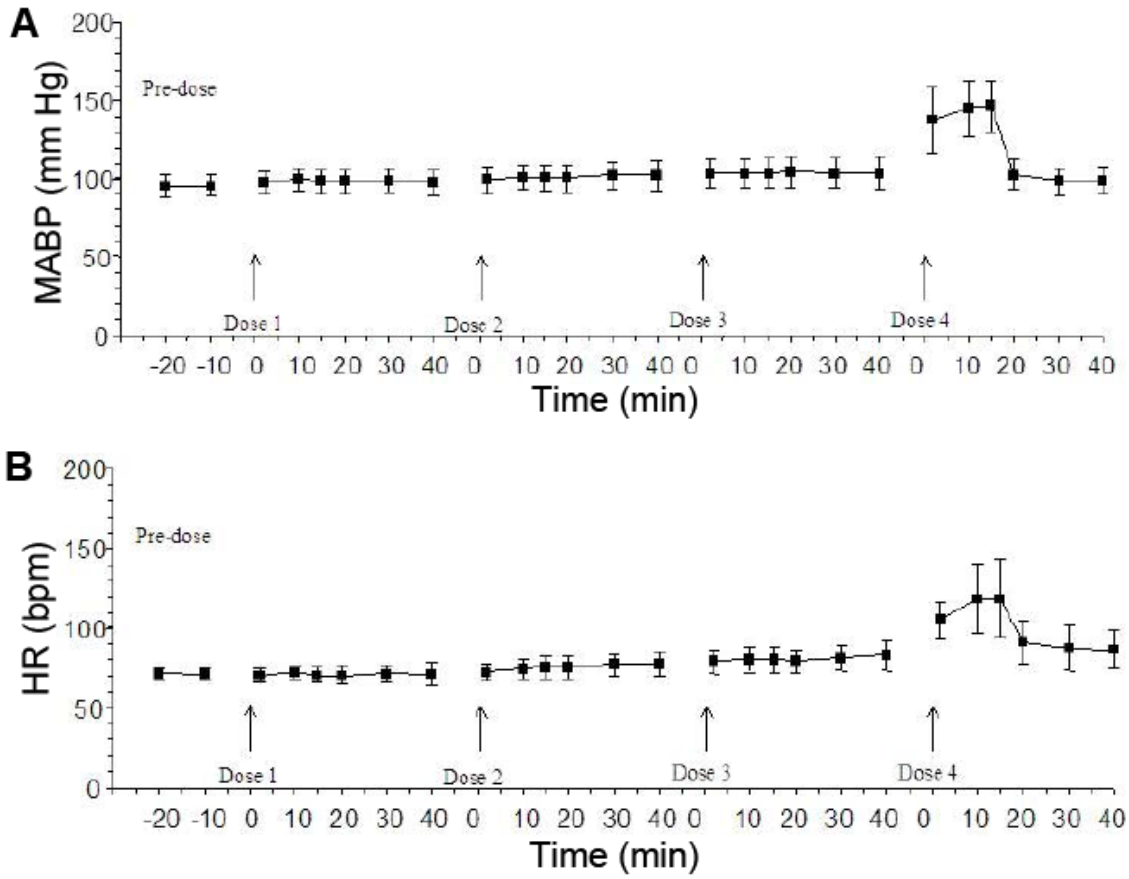
Results

Cardiovascular effects:

At 0.3 mg/kg capsaicin there was an increase in the mean arterial blood pressure (96 to 138 mmHg), increase in heart rate (to 119 bpm), increase in dP/dtmax (to 6679 mmHg/s), and an increase in femoral blood flow (to 174 mL/min) within 2 mins after the start of the administration with a peak at 15 mins. All parameters returned to pre-dose values 5 mins after infusion termination.

Administration of 0.3 mg/kg capsaicin elicited a rise in mean arterial blood pressure from a baseline of 96 ± 7 mm Hg to 138 ± 21 mm Hg within 2 minutes of starting the infusion. This effect peaked at 146 ± 17 mm Hg at the end of infusion (15 min). The hypertensive effect was accompanied by increases in heart rate (from 71 ± 3 bpm at baseline to 119 ± 25 bpm), dP/dtmax (from 4050 ± 91 mm Hg/s at baseline to 6679 ± 1027 mm Hg/s), and femoral blood flow (from 117 ± 27 mL/min at baseline to 174 ± 34 mL/min). These changes were statistically different ($p \leq 0.05$) from the responses observed after administration of the vehicle, 0.03 mg/kg or 0.1 mg/kg capsaicin. However, changes were transient, lasting only about 5 minutes after the end of infusion. The change in heart rate was also associated with decreases in the RR and QT intervals of the ECG. However, the corrected QT intervals (both QTCF and QTCB) were unchanged.

Figure 2: Acute Effects of Intravenous Capsaicin on Mean Arterial Blood Pressure (A) and Heart Rate (B)



Data are presented as mean ± SEM (n = 4) in millimeters of mercury (mm Hg) for arterial blood pressure and beats per min (bpm) for heart rate. Arrows represent initiation of doses. Dose 1 = vehicle only; Dose 2 = 0.03 mg/kg capsaicin; Dose 3 = 0.1 mg/kg capsaicin; Dose 4 = 0.3 mg/kg capsaicin.

Respiratory effects:
The vehicle (hydroxypropyl β-cyclodextrin) produced a decrease in tidal volume (143 to 118 mL), peak inspiratory flow (253 to 198 mL/s), and peak expiratory flow (297 to 246 mL/s). Capsaicin at 0.3 mg/kg produced an increase in peak inspiratory flow (to 418 mL/s), peak expiratory flow (397 mL/s), tidal volume (217 mL), minute volume (2529 mL), and rate of respiration (20 brpm). These changes were transient and lasted 5-10 mins after infusion termination.

Administration of 0.03 mg/kg and 0.1 mg/kg capsaicin did not elicit any changes in respiration in comparison with the vehicle. Administration of the high dose (0.3 mg/kg) of the capsaicin solution elicited increases in peak inspiratory flow (PIF) (to 418 ± 182 mL/s), peak expiratory flow (PEF) (to 397 ± 142 mL/s), tidal volume (TV) (to 217 ± 120 mL), min volume (MV) (to 2529 ± 504 mL) and rate of respiration (to 20 ± 9 brpm). The increase in PIF and PEF following 0.3 mg/kg capsaicin is significantly different ($p \leq 0.05$) from the decrease in these parameters following the vehicle treatment. However, these increases were transient, lasting only about 5 to 10 minutes after the end of infusions. No detectable levels of capsaicin were found 5 minutes after administration of the 0.03 mg/kg capsaicin. Following administration of the intermediate dose (0.1 mg/kg), 2 of the 4 dogs showed detectable levels of capsaicin (approximately 17 and 11 ng/mL). The high dose (0.3 mg/kg) produced plasma levels in all dogs, ranging from 32.2–65.6 ng/mL, with a mean of 47.9 ± 6.4 ng/mL.

Toxicokinetics
No detectable levels of capsaicin were found 5 minutes after administration of the 0.03 mg/kg capsaicin. Following administration 0.1 mg/kg capsaicin, 2 of the 4 dogs had detectable levels of capsaicin (approximately 17 and 11 ng/mL). The high dose level (0.3 mg/kg) produced plasma levels in all dogs, ranging from 32.2 to 65.6 ng/mL, with a mean of 47.9 ± 6.4 ng/mL.

RENAL EFFECTS

No studies were submitted

GASTROINTESTINAL EFFECTS

No studies were submitted.

ABUSE LIABILITY

No studies were submitted.

2.6.2.5 Pharmacodynamic drug interactions

There were no studies submitted for review.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

See the individual study reports in the Safety Pharmacology Section.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The Applicant conducted two single-dose dermal ADME studies in the rat and one single-dose dermal ADME study in the minipigs. *In vitro* metabolism studies were conducted using human, dog and rat liver microsomes treated with ^{14}C-capsaicin and by incubation of ^{14}C-capsaicin with fresh human skin. The human CYP enzymes involved in the metabolism of capsaicin and the plasma protein binding characteristics of capsaicin in rat, rabbit, dog and human plasma were investigated, using ^{14}C-capsaicin. The effect of capsaicin on the expression of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1 and CYP3A4/5 in primary cultures of human hepatocytes was reviewed in the Clinical Pharmacology Review.

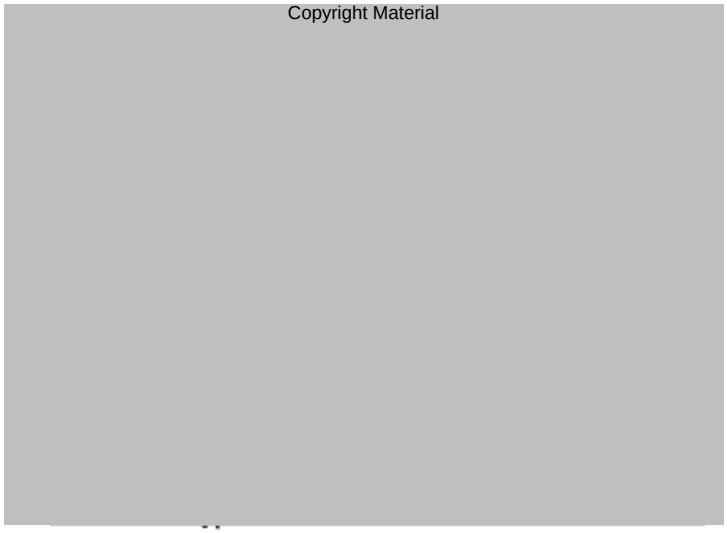
The following is from the Applicant's Pharmacokinetic Summary of Section M2.

Human Absorption: NGX-4010 was designed to have a short application time, in the range of 30 to 90 minutes. This time range was selected to maximize efficacy while minimizig the time a patient experiences discomfort. Based on an in vitro mass-balance study of skin tissue from one subject (Study VAL-07-002-R), during a 90-miute exposure to NGX-4010, no more than 7 µg/cm^2 (approximately 1.1%, or less of capsaicin content in the patch) is predicted to enter the skin. In the same study, the penetration in one subject treated for 1 hour was 0.9% of the applied dose, based on measurements of the amount applied and amounts recovered from surface wipings after removal. Percutaneous absorption of capsaicin from the application of a commercial 0.075% (w/w) gel on healthy human volunteers using a surface recovery technique was approximately 2.28 ± 0.39 µg/cm^2/hour (Fang et al. 1997).

Human Pharmacokinetics: During the clinical development of NGX-4010, blood samples for measurement of capsaicin were collected in patients treated with NGX-4010 and low, transient levels of capsaicin (LLOQ = 0.5 ng/mL) were detected suggesting that systemic exposure in humans is quite low. Of 96 PHN patients receiving NGX-4010 for 60 or 90 minutes in three clinical studies, 31% (30 patients, 19 males and 11 females) displayed quantifiable levels of capsaicin at one or more time points. No metabolites were detected. The highest plasma concentration measured in any PHN patient receiving

a 60-minute treatment was 4.64 ng/mL (Study Cl16: 7215-159) and the highest AUC recorded was 12.7 ng.hr/mL (Pharsight report, August 2008).

Capsaicin Absorption in Other Species: Fang and colleagues have also published an *in vitro* permeation study of a commercial topical 0.075% (w/w) capsaicin cream and hydrophilic ointment comparing different skin types (Fang et al., 1995). The study indicated (Fig 5, below) that human and pig skin had similar flux rates (0.5 to 1 $\mu g/cm^2$/hour), whereas rat, mouse and rabbit skin were more permeable (2 to 6 $\mu g/cm^2$/hour). The flux through skin for both formulations increased in the order of human ≤ pig < rabbit ≤ rat ≤ mouse.



(Fang et al 1995)

ADME Studies in Rats: The absorption of ^{14}C-capsaicin was studied in rats following application of a 50 cm^2 patch over 3 hours with a target dose of 32 mg/rat (Study 7215-105,). After a 3-hour application, approximately 30% of the capsaicin radioactivity was recovered in the patch or readily removed from the surface of the skin, indicating that 70% had been transferred to the skin or other tissues. After 72 hours, approximately 49% of the radioactivity was left on/in the skin of the application site. Tissues with any radioactivity other than skin and blood were liver (approximately 0.1%), lungs (approximately 0.01%) and kidneys (approximately 0.01%). During the test period, the plasma concentration of radioactivity remained relatively constant and elimination of radioactivity in the urine and feces was continuous. To explain these results, it is hypothesized that the rat skin served as a depot for the continuing release of capsaicin and/or capsaicin metabolites after removal of the patch.

A further study in rats was conducted with the application of a 50 cm^2 NGX-4010 patch with ^{14}C-capsaicin with a target dose of 32 mg/animal for 3 hours. The rats were followed for 14 days after the patch application to study the absorption of ^{14}C-capsaicin (Study 7215-135). Limited radioactivity appeared to have been absorbed following application based on radioactivity levels remaining in the patch and the low amounts measured in excreta. In males, a mean of 3.36% of the dose was recovered in urine and 2.42% in feces. In females, a mean of 2.24% of the dose was recovered in urine and

1.35% in feces. The mean mass balance values for males and females were 88.2 and 95.1%, respectively. By 12 hours post-dose and thereafter, radioactivity was not detectable in most tissues, with the exception of skin (at the test site), liver and kidney. The highest mean concentrations were detected in skin (test site), kidney, and liver at 3 hours post-dose. In females, the highest mean concentrations were also detected in skin (test site), with more in the liver than kidney, which was the opposite from that in males.

The differences observed in these two rat studies was thought to be attributed to the differences in the way NGX-4010 patches were prepared. These were not manufactured under GMP conditions but were fabricated by scientists at the study site (b) (4) (b) (4) at the time of the study conduct. Also the two studies were conducted a few years apart. NGX-4010 patches were saved in both studies following 3-hour application. The analysis of the patch from each study revealed that approximately 29% radioactivity was left in the patch in Study 7215-105 as opposed to almost 73% in Study 7215-135. This suggests that the fabrication of the patch was most likely responsible for the differences observed in these two studies.

ADME Studies in Minipigs: The absorption of ^{14}C-capsaicin was studied in minipigs following application of two 50 cm^2 topical patches containing a total target dose of 64 mg/animal for a period of at least 2 hours (Study 7215-114). Skin at the application site contained 1.23% of the administered dose. No radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes and spleen). The patches were not analyzed for radioactivity in this study. Urine and fecal samples collected from 0 to 336 hours (14 days) after 2 hr application of two 50 cm^2 dermal patches in 3 male mini-swine (mean dose of 48.7 mg or 4.73 mg/kg) demonstrated that a mean of 2.8% of the administered radioactive dose was excreted in the urine, primarily in the first 5 days after dosing. In feces, a mean of 0.49% of the administered dose was detected, primarily in the first 8 days. Additional amounts of the dose were detected in the pan/screen rinse (0.59%) and in cage wash (0.12%) and cage wipe (0.23%). Two weeks after patch application, a total of 4.23% of the dose was recovered in excreta, with 2.80% eliminated in urine, 0.49% eliminated in feces and 0.94% in samples of pan/screen rinse, cage wipe and cage wash.

Given the similar permeability of capsaicin in human and pig skin, the unrecovered radioactivity was probably in the patch. Concentrations of radioactivity in all samples of blood were below the limit of quantification (BLQ). Low levels of radioactivity (16 to 38 ng equivalents ^{14}C-capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours post-dose in two out of three animals. The concentrations of unchanged capsaicin in all plasma samples were below detection limits (<5.00 ng/mL).

Plasma Protein Binding
Capsaicin was highly bound to plasma proteins in all four species (human, dog, rabbit and rat) over the concentration range of 50 to 500 ng/mL. Binding was independent of the concentration in all species.

Metabolism
Biotransformation of ^{14}C-capsaicin was rapid in rat liver microsomes and S9 fraction incubations compared to dog and human liver preparations. In humans, the rate of formation of the metabolites was faster than in dog. Metabolism of ^{14}C-capsaicin resulted in the formation of several products all found in the rat, dog and human preparations. Three major metabolites were detected and tentatively identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin and 16,17-dehydro-capsaicin. T hese metabolites are in agreement with published reports. Rat microsomes and S9 fractions also formed vanillylamine and vanillin as hydrolytic metabolites in addition to the major metabolites mentioned above. It was also observed that CYP2C9 is the primary enzyme responsible for converting ^{14}C-capsaicin to 16-hydroxy-capsaicin and 16,17-dehydro-capsaicin. However, CYP2C19 may also contribute to the formation of 16-hydroxy-capsaicin. Although the formation of the third metabolite, 17-hydroxy-capsaicin, was NADPH-dependent, the CYP enzyme(s) responsible for its formation was not identified.

In human skin, biotransformation of capsaicin was slow and capsaicin was metabolized to vanillylamine and vanillic acid, although the majority of the sample radioactivity was associated with unchanged capsaicin.

2.6.4.2 Methods of Analysis

These studies were not reviewed.

(b) (4)

Table 1: Methods of Analysis

Study ID/Design*	Method of Analysis	Detection / Quantification Limit	Impact of Analysis
(b) (4)			

Study ID/Design	Method of Analysis	Detection / Quantification Limit	Impact of Analysis
(b) (4)			

2.6.4.3 Absorption

From the Applicant's Summary in Section M2

Literature Studies with Low-Dose Unpurified Capsaicin
Several *in vitro* flux experiments and human non-invasive surface recovery studies suggest that topically applied capsaicin readily penetrates into skin and even through the skin of some species. An *in vitro* percutaneous penetration study of topically applied 1% (w/w) capsaicin using a flow-through diffusion system on excised human epidermal membranes indicated that percutaneous steady-state penetration of capsaicin was approximately 30 μg/cm^2/hour, depending on the receptor fluid used (Magnusson, 2000). This result was similar to an *in vitro* study that reported a capsaicin flux of approximately 3 μg/cm^2/hour using excised pig skin and a 0.06% capsaicin gel (Fang, 1997). Fang and colleagues also published an *in vitro* permeation study of topical capsaicin comparing different skin types (Fang, 1995). The study indicated that human and pig skin had similar flux rates (0.5–1 μg/cm^2/hour), whereas rat, mouse and rabbit skin were more permeable (2–6 μg/cm^2/hour) to 0.075% (w/w) capsaicin cream. The flux through skin for both formulations (a hydrophilic ointment base and a commercially available 0.075% capsaicin cream) increased in the order of human ≤ pig < rabbit ≤ rat ≤ mouse.

Estimations of capsaicin absorption were determined using a non-invasive surface recovery technique in humans. This study evaluated the uptake and elimination kinetics of capsaicinoids in human stratum corneum following a single topical exposure to 3% solutions containing 55% capsaicin, 35% dihydrocapsaicin and 10% other analogues prepared in three vehicles: mineral oil, propylene glycol, and isopropyl alcohol (Pershing, 2004). Capsaicinoid solutions were evaluated simultaneously in a random application pattern on the volar forearms of 12 subjects using single 150 g doses. Capsaicin and dihydrocapsaicin were recovered from human skin using commercial adhesive discs to harvest stratum corneum from treated sites. Capsaicinoids were extracted from the stratum corneum-adhesive discs and quantified by liquid chromatography/mass spectroscopy (LC/MS). Both capsaicinoids were detected in stratum corneum 1 minute after application with all vehicles and achieved a pseudo-steady state shortly thereafter. Isopropyl alcohol delivered three times more capsaicin (C_{max} 16.1 μg) and dihydrocapsaicin into the human stratum corneum than propylene glycol or mineral oil (C_{max} 6.2and 6.5 μg, respectively) at all time points investigated. The dihydrocapsaicin content was 60% of capsaicin with all vehicles. The estimated $t_{1/2}$ of capsaicin and dihydrocapsaicin in the skin was similar (24 hours) for the three vehicles. Thus, maximal topical capsaicinoid concentrations were achieved quickly in the human stratum corneum and were concentration and vehicle dependent. In contrast, capsaicinoid half-life in skin was vehicle independent.

In vivo percutaneous absorption of capsaicin from ointment bases has also been determined by a pharmacokinetic study in rabbits (Fang, 1996). Using a topically applied capsaicin concentration of 0.25% or 0.35%, the systemic bioavailability was shown to be 34.2% ± 12.6% or 27.3% ± 7.4%, respectively, and the $AUC_{0\text{-}14\ hr}$ was 0.76 ± 0.28 μg hour/mL and 0.85 ± 0.23 μ g hour/mL, respectively. The study was performed by

applying 6 g of ointment base uniformly over a sheet of cotton cloth (6 cm × 10 cm) and placing this piece of cloth onto the shaven skin surface of the rabbit's abdominal region for a 14 hour period by an occlusive dressing technique. Blood samples were taken every hour the first 6 hours and thereafter every other hour up to 14 hours. However, as rabbit skin is much more permeable to capsaicin relative to human skin (Fang, 1995), there are no clear implications of these data for systemic absorption in humans. *In vivo* percutaneous absorption of capsaicin from a 0.075% (w/w) gel on healthy human volunteers using a surface recovery technique was studied by (Fang, 1997). The study was performed by accurately weighing and uniformly spreading 0.2 g of the capsaicin containing gel over a 4 cm^2 sheet of cloth which was then applied on both forearms of the volunteers for 8 hours by occlusive dressing technique. The unabsorbed drug was recovered at intervals after administration. After recovering, the residual base remaining on the skin was withdrawn by a sterile cotton wool swab soaked in methanol. Using this method, flux for capsaicin was determined to be approximately 2.28 ± 0.39 $\mu g/cm^2$/hour.

Studies with the Purified Capsaicin in a Patch Formulation (NGX-4010)
Human skin: More relevant to the degree of capsaicin absorption from NGX-4010 in clinical settings, an *in vitro* mass balance study utilizing NGX-4010 patches, full thickness human skin (abdominal skin from a 53-year-old female) and Franz diffusion cells was conducted by Lohmann Therapie-Systeme (the contract developer and manufacturer of NGX-4010) to measure the amount of capsaicin and diethylene glycol monoethyl ether (DGME) that permeates into and through skin following a 60 minute application. The amount of capsaicin delivered to skin in 60 minutes averaged 5.61 $\mu g/cm^2$. This is about 0.9% of the capsaicin content in NGX-4010, as the average capsaicin content in NGX-4010 is 640 $\mu g/cm^2$, and very low levels of capsaicin were measured in the receptor solution. The exposed patches were analyzed and a total mass balance indicated that 97.1% of the drug was recovered (NeurogesX Report VAL-07-002-R).

Animal Studies: In the *in vivo* animal toxicology studies conducted by NeurogesX with NGX-4010, rats (Study 7215-106 and Study 7215-120) showed extensive systemic exposure to capsaicin while dogs (Study 7215-107) and pigs (Study 7215-113) demonstrated minimal to no systemic exposure to capsaicin.

Rat: NeurogesX studied the absorption, distribution, and elimination of ^{14}C-capsaicin in rats following the application of a 50 cm^2 topical patch with a target dose of 32 mg/animal for 3 hours (Study No. 7215-105). The results of this study reflected the transfer of capsaicin from the patch into the skin and the systemic circulation. Radioactivity was widespread, and was found in various tissues. At 72 hours, the liver had the highest tissue/plasma concentration ratio at 5.93, and the brain had the lowest at 0.888. After the three hour application, 70.2% of capsaicin that had been in the patch was transferred to the skin or other tissues. Over the 72 hour test period, 21.4% of the original amount of ^{14}C -capsaicin in the patch was absorbed into the systemic circulation. Because only radioactivity was measured in these studies, the chemical form of the labeled material is not known.

Subsequent to the previously described rat study, NeurogesX conducted another study in rats with the application of a 50 cm^2 topical patch containing ^{14}C-capsaicin with a target dose of 32 mg/animal for 3 hours (Study 7215-135) in which the rats were followed for 14 days after the patch application to study the absorption, distribution and elimination of ^{14}C-capsaicin. Following application of the ^{14}C-capsaicin patch, limited radioactivity appeared to have been absorbed, based on radioactivity levels remaining in the patch and low amounts measured in excreta. The mean mass balance values for males and females were 88.2% and 95.1%, respectively. Maximum mean concentrations of radioactivity in blood and plasma were highest at 3 hours after patch application. Concentrations of radioactivity in both male and female blood and plasma initially declined after removal of the patch, but then increased slightly at 72 hours post-dose before declining again. In males and females, radioactivity was detected in most of the tissues examined at 3 hours post-dose. By 12 hours post-dose and thereafter, radioactivity was not detectable in most tissues, with the exception of skin (test site), liver and kidney. Metabolic profiles of urine and feces showed that most of the radioactivity was associated with vanillic acid-sulfate and O-demethyl-capsaicin, respectively. Most of the radioactivity in skin (test site) was associated with unchanged drug. Vanillin was the major circulating component in plasma.

The differences observed in the two rat studies above can be attributed to the differences in the way NGX-4010 patches were prepared. These were not manufactured under GMP conditions but were fabricated by scientists at the study site (b) (4) at the time of the study conduct. Also the two studies were conducted a few years apart. NGX-4010 patches were saved in both studies following 3-hour application. The analysis of the patch from each study revealed that approximately 29% radioactivity was left in the patch in study 7215-105 as opposed to almost 73% in study 7215-135. This suggests that the fabrication of the patch was most likely responsible for the differences observed in these two studies. All other experimental conditions were generally similar in both studies.

In a multi-dose rat study (study 7215-120, once weekly patch application) capsaicin exposure occurred in most animals of the receiving capsaicin patches (16 or 32 mg/animal) and increased with increasing dose. A slow elimination was observed ($t_{1/2}$ = 15.8 to 53.4 hours). Maximum plasma concentrations were observed within 2 hours of dosing and Cmax increased proportionally on day 1 but stayed the same or decreased on day 22. AUC increased proportionally on both days except for males on day 1 (supra-proportional). Exposure was significantly greater (2- to 5-fold) in females, likely due to the increased dose on a body weight basis.

Rat Toxicokinetics (Study 7215-120)

Dose level (mg/animal)	Sex	Cmax (ng/ml)	Tmax (hours)	AUC_{0-t} (ng.hr/ml)	AUC_{0-48} (ng.hr/ml)	AUC_{0-72} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	$t_{1/2}$ (hours)
Day 1								
16	M	19.3	2	194	62.8	194	NA	NA
	F	38.6	2	746	381	628	1421	47.6
32	M	41	2	1408	779	1408	2084[a]	25.9[a]
	F	55.4	2	2378	1349	1938	3484	53.4
Day 22								
16	M	32.7	2	127	175	175	NA	NA
	F	127	2	594	664	664	815	26.2
32	M	35.3	2	357	357	399	436[a]	15.8[a]
	F	92.5	2	810	810	1011	NA	NA

a: Values should be approached with caution. Only 2 timepoints used in K_e calculation.

Minipigs: The absorption, distribution and elimination of ^{14}C-capsaicin were also studied in mini-swine following application of 100 cm^2 (two 50 cm^2) topical patches containing a total target dose of 64 mg/animal (Study No. 7215-114). Skin at the application site contained 1.23% of the administered dose. No radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes and spleen). Concentrations of radioactivity in all samples of blood were below the limit of quantification. Low levels of radioactivity (16 to 38 ng equivalents ^{14}C-capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours post-dose in two of the three animals. The concentrations of unchanged capsaicin in all plasma samples were below detection limits (<5.00 ng/mL).

Blood concentrations of radioactively labeled capsaicin after application of two 50 cm^2 dermal patches in 3 male mini-swine were BLQ at all time points from 0.5 to 336 hours after 2 hr patch application. Plasma concentrations of radioactively labeled capsaicin were detected in 2 of 3 animals only from 1 to 4 hours after patch application with a maximum mean level of 0.0185 µg equivalents ^{14}C-capsaicin/g. Plasma concentrations of unchanged capsaicin were BLQ (5 ng/ml) in all animals at all time points up to 336 hours after dose administration. In a multi-dose study in mini-swine (study 7215-113, once weekly patch application), capsaicin plasma levels in all animals at all collections were below the limit of detection (5 ng/ml) for capsaicin except in 2 animals on day 1.

Mouse: In a multi-dose dose range-finding study in mice (study 7215-122, once weekly dosing for 4 weeks, capsaicin in (b) (4) vehicle), exposure to capsaicin occurred in most animals receiving capsaicin and increased in a generally sub-proportional manner with increasing dose. Maximum plasma concentrations were observed between 4 and 12 hours after dosing and measurable values were still present in several animals at 24 hours post-dosing. Calculation of an elimination half-life was only possible for certain groups due to a lack of a distinct elimination phase. Exposures tended to be greater in females, likely due to the administration of dose on a mg/animal basis rather than on a mg/kg basis as females were of lesser body weight values.

Mouse Toxicokinetics (Study 7215-122)

Dose level (mg/animal)	Sex	Cmax (ng/ml)	Tmax (hours)	AUC_{0-t} (ng.hr/ml)	AUC_{0-24} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	$t_{1/2}$ (hours)
0.64	M	9.17	6	NA	NA	NA	NA
	F	24.2	4	150	150	NA	NA
1.28	M	28.4	6	193	193	NA	NA
	F	24.7	6	164	193	NA	NA
2.56	M	43	12	588	588	NA	NA
	F	71.4	4	639	639	736	7.86
5.12	M	51.5	12	909	909	NA	NA
	F	84.8	6	1026	1026	1198	7.48

Study Title: **In Vitro Delivery Kinetics of Capsaicin and Diethylene Glycol Monoethyl Ether from NGX-4010**

Study No. VAL-07-002-R

Key Study Findings: An in vitro mass balance study utilizing NGX-4010 patches, full thickness skin (female abdominal skin) and Franz diffusion cells was conducted to measure the amount of capsaicin and diethylene glycol monoethyl ether that permeated into and through skin following a 60-minute application. The amount of capsaicin delivered to skin in 60 minutes averaged 5.61 micrograms/cm^2. This is about 0.9% of the capsaicin content in NGX-4010, as the average capsaicin content in NGX-4010 is 640 micrograms/cm^2, and very low levels of capsaicin were measured in the receptor solution. The exposed patches were analyzed and a total mass balance indicated that 97.1 % of the drug was recovered.

Study No. VAL-07-002-R
E-Location: m5\53-clin-stud-rep\532-rep-stud-pk-human-biomat\5323-stud-other-human-biomat\val-07-002-r
Conducting laboratory and location: not provided
Date of study initiation: not provided, report dated May 2, 2007
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
Part A

- NGX-401O Patch (Batch No. 81100304); capsaicin content of 619 µg/cm^2 (mean of drug content analysis of 6 patched)
- Low-Concentration Capsaicin Patch (LCCP; Batch No. 81100204); capsaicin content of 2.82 µg/cm^2 (mean of drug content analysis of 6 patches)

Part B

- NGX-4010 Patch (Batch No. 81100304); The capsaicin content was 625 ± 12 µg/cm^2 and DGME (diethylene glycol monoethyl ether) content was 1467 ± 29.5 µg/cm^2 (mean of content analysis of 6 patched)

- Low-Concentration Capsaicin Patch (LCCP; Batch No. 81100204); capsaicin content: 3.15 ± 0.06 µg/cm^2 and DGME content was 1510 ± 29.6 µg/cm^2 (mean of content analysis of 6 patched)
- Full-thickness abdominal skin from a 53-year old female
- Cleansing Gel (Lot No. 22698)
- L.M.X4® (Lot No. 03150A)
- Analytical methods used included:
  - (b) (4), "Capsaicin TDS, Determination of the in vitro release of capsaicin from matrix and TDS"
  - (b) (4) "Capsaicin TDS, Determination of the drug content and the content uniformity"
  - (b) (4), "Determination of capsaicin in TDS, skin samples and receptor medium by HPLC with fluorescence detection"
- Capsaicin was extracted from skin by incubation in 200 µL methanol for 1 hour, followed by intense sonication.

Methods

Part A
The study was performed using heat-separated human epidermis and Franz diffusion cells. The skin (heat-separated epidermis derived from breast skin of a 37-year old female) was mounted in a Franz cell, and round patch samples (1.15 cm^2), slightly smaller than the opening of the cell, were applied to the skin specimen. Slightly larger in diameter than sample patch, another cover patch, comprising a polyester backing and a high-tack silicone adhesive, was applied onto sample patch to ensure completely occlusive conditions.

Part B
In diffusion experiments, patches were applied to the epidermis for intervals of 15, 30, 60 or 90 minutes. All experiments, for each time point studied, were run using skin specimen from the same donor. For 60-minute time points, six replicates were run, while for all other time points three replicates were run. Patches were removed at the end of experiment and capsaicin contents in epidermis specimen, receptor fluid and removed patch were determined by HPLC. Capsaicin delivery kinetics from control, low-concentration capsaicin patches containing 0.04% w/w capsaicin, were also evaluated in an identical fashion.

For analysis, data were normalized to unit area of patch application (i.e., µg/cm^2 of capsaicin delivered per cm^2 of patch). The limit of detection (LoD) of capsaicin was 0.05 ± 0.065 µg/cm^2 of skin, which corresponds to 6.4 ng/mL in receptor solution. Percent recovery refers to percent of capsaicin content originally present in the patch, as collectively found in the removed patch, epidermis and receptor fluid.

Results

Data from Study B are plotted in Figure 2, and show that the amount of capsaicin both in and on the skin (removed by Cleansing Gel and quantified) increases with time, with the

accumulation of capsaicin in the skin being relatively more linear. In Study B (which used full-thickness skin), all capsaicin measurements less than the below the limit of detection(LoD) out to 240 minutes of sampling after patch application. With the use of epidermis only in vitro in Study A, only 0.14% of the total capsaicin measured reached the receptor fluid with a 90-minute patch application. With the 60-rninute application, only 0.06% of the capsaicin reached the receptor fluid. Thus, even in Study A, receptor fluid samples contained -34-fold less capsaicin than did the skin (for the 60-minute patch application).
As summarized in Table 19, the fraction of drug content in the patch that is delivered to the skin ranges from 0.4 to a maximum of 1.1%. Based upon the *240-minute* sampling in Study E, less than 1% of the capsaicin originally contained in the patch was delivered into the skin. Moreover, as shown in Table 18, for all application times, the amount of capsaicin deposited in full-thickness skin is significantly lower than the amount of residual capsaicin removed by the Cleansing Gel.

Differences were noted between NGX-4010 and the low-concentration control patch in these *in vitro* studies. In Study B, NGX-4010 containing 198-times more capsaicin than the low-concentration control patch, and delivered 29-fold more capsaicin into the skin than the low-concentration control, as per the relative skin drug content in the 60-minute patch application with 240-minute sampling. Data from these experiments demonstrate that higher quantities of DGME, relative to capsaicin, are delivered to the skin during NGX-40l0 applications. This is as expected, based on the relatively lower molecular weight of DGME and that this molecule is a skin penetration enhancer and is used in transdermal drug delivery formulations. A plausible explanation of the relatively better performance of the low-concentration patch could the saturation of either skin diffusion pathways or skin reservoir or both, as skin drug contents are only a small fraction of the total capsaicin content originally present in the patch. When assayed, immediately following patch removal after 60-minute patch application, 14-fold more DGME is in the skin than in the receptor fluid. However, if these skin sections are allowed to equilibrate for another 180 minutes with the receptor fluid, the ratio is reversed, and the receptor fluid contains 3-fold more DGME, as seen in case of 240 mintue sampling.

In contrast to capsaicin, only relatively small amounts of DGME could be removed by the Cleansing Gel; this suggests that DGME does not accumulate on the skin surface and/or Cleansing Gel is not effective in removing DGME.

Based upon 240-minute sampling of the 60-minute patch application in vivo, approximately 0.9 % or less of capsaicin originally contained in the patch was delivered into the skin, with no quantifiable levels of capsaicin present in the receptor solution. These data show that NGX-4010 is a cutaneous, not a transdermal patch.

Table 18: Summary of Mass Balance Results for Study B

Test Article	Application Time	Capsaicin Content				Percent Recovery [%]
		Removed Patch [µg/cm²]	Skin [µg/cm²]	Cleansing Gel [µg/cm²]	Total [µg/cm²]	
NGX-4010	15 min	625	2.22	4.09	631	101
	30 min	618	4.43	7.91	630	101
	60 min	590	5.61	11.1	607	97.1
	240 Min	580	5.17	12.8	598	95.6
	90 min	609	7.12	9.28	625	99.7
LCCP	60 min	2.76	0.227	0.134	3.12	99.2
	240-min	3.32	0.181	0.144	3.64	116

Table 19: Summary of Mass Balance Percentage for Study B

Test Article	Application Time	Capsaicin Distribution Between the Compartments (Percent of Recovered Amount)		
		Removed Patch [%]	Skin [%]	Cleansing Gel [%]
NGX-4010	15 min	99.0	0.4	0.6
	30 min	98.1	0.7	1.2
	60 min	97.2	0.9	1.8
	240 min	97.0	0.9	2.1
	90 min	97.4	1.1	1.5
LCCP	60 min	88.5	7.3	4.3
	240 min	91.2	4.9	3.9

Table 20: DGME Mass Balance for 15-Minute Application of NGX-4010

Sample	DGME Content					Percent Recovery [%]
	Removed patch [µg/cm²]	Skin [µg/cm²]	Receptor Fluid [µg/cm²]	Cleansing Gel [µg/cm²]	Total [µg/cm²]	
1	1108	123	<LoD	25.3	1256	85.6
2	1057	138	<LoD	34.4	1229	83.8
3	1034	176	<LoD	30.0	1240	84.5
Mean	**1066**	**146**	**<LoD**	**29.9**	**1242**	**84.7**
RSD	4%	19%	---	15%	1%	1%

Table 21: DGME Mass Balance for 30-Minute Application of NGX-4010

Sample	DGME Content					Percent Recovery [%]
	Removed patch [µg/cm²]	Skin [µg/cm²]	Receptor Fluid [µg/cm²]	Cleansing Gel [µg/cm²]	Total [µg/cm²]	
1	1016	209	<LoD	35.6	1261	85.9
2	861	288	7.53	68.9	1225	83.5
3	982	266	<LoD	66.1	1314	89.6
Mean	**953**	**254**	**2.51**	**56.9**	**1267**	**86.3**
RSD	4%	19%	---	15%	4%	4%

Figure 1: Time Course of DGME Accumulation in Full-Thickness Skin

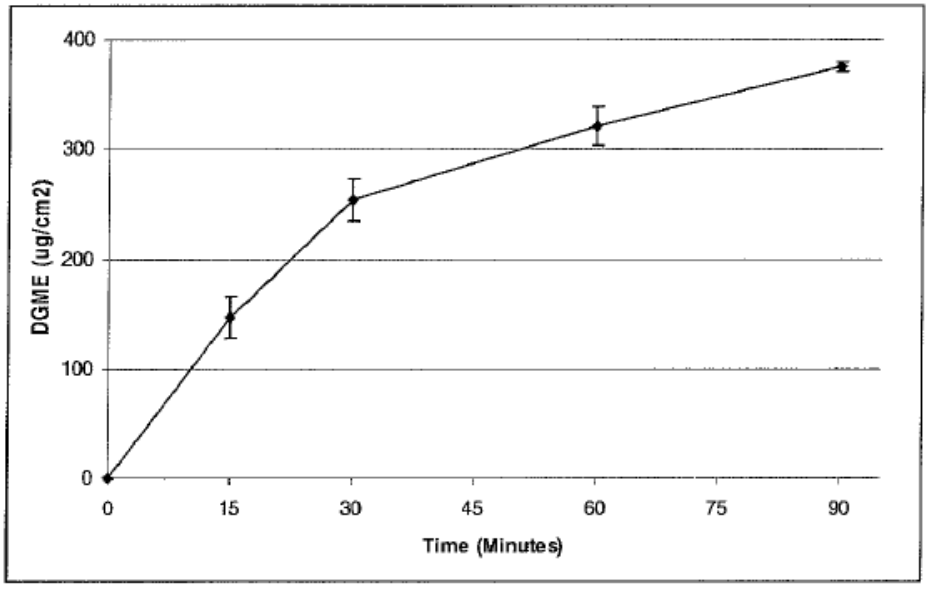
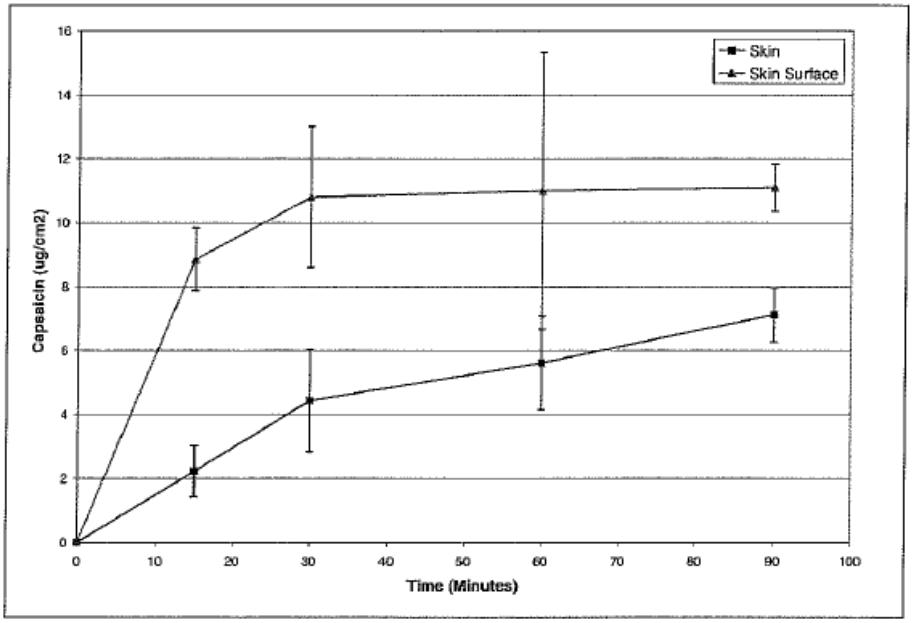


Figure 2: Time Course of Capsaicin Accumulation in Full-Thickness Skin and on the Skin Surface Induced by NGX-4010



Study title: Absorption, distribution, metabolism, and elimination of ^{14}C-capsaicin following a dermal patch application to rats

This study was reviewed by Dr. Suzanne R. Thornton when it was submitted to IND 63354 in 2001.

Key Findings: The skin where the patch was applied maintained approximately half of the dose up to 72 hrs after application. There was no protein-binding or significant retention of capsaicin in other areas of the body.

Study no: 7215-105
E-Location: m4\42-stud-rep\422-pk\4222-absorp\7215-105
Conducting laboratory and location: (b) (4)
Date of study initiation: June 19, 2001
GLP compliance: No
QA report: No
Drug, lot #, radiolabel, and % purity:
[^{14}C]Capsaicin, Lot CSL-99-927-94-14, Purity >98%
Capsaicin, Lot F0010101, Purity 100.3%
Vehicle: transdermal patch

Methods

Dosing: target dose: 0.64 mg/cm^2 actual dose: 0.50 mg/cm^2

*(**Reviewer comment:** These patches were made at the study site and not supplied by the Applicant)*

Capsaicin Concentration	Area Treated or Volume	Average Weight (kg)	Average Dose (mg/kg)
1.19 μCi/mg; 0.50 mg/cm^2	50 cm^2	0.313 – 0.338	74.0 – 79.9

Species/strain: Sprague-Dawley rat Crl:CD(SD)IGS BR
#/sex/group or time point: 15/male/3 per timepoint
Route, form, volume, and infusion rate: dermal patch
Satellite groups used for toxicokinetics or recovery: NA
Age: 10 wks at dosing
Weight: 313-338 g

Study Design:
On the day prior to dosing, the dorsal side was shaved and the skin washed with a mild detergent solution. Approximately 20 mins prior to dosing, 5.7 mL of 2% tetracaine gel was applied to the shaved area and spread with a glass rod. The site was wiped to remove the tetracaine gel just prior to patch application. An Elizabethan collar was placed on each rat and the entire dosing site was wrapped with Elastoplast. The duration of exposure was approximately 3 hrs. The patch was removed and the application site cleaned with 10 mL of PEG 300 "cleansing lotion" and gauze.

Pharmacokinetics/Toxicokinetics:
PK parameters: blood was collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hrs; excreta was collected at 24, 48, and 72 hrs; tissues were collected at 72 hrs.

The target dose was 0.64 mg/cm2 and the dose which was achieved was 0.502 mg/cm^2. The capsaicin patch when administered for 3 hrs resulted in significant amounts of capsaicin remaining in the skin (48% of total radioactivity administered) 72 hrs after administration (see Table 5). There were detectable levels of capsaicin in the blood and plasma 2-3 hrs after patch application There was little systemic exposure (see Table 2, 5). The liver had the highest amount and the brain had the lowest amount. There was also no protein-binding or accumulation of capsaicin in any tissues examined. There was a depot effect in skin with the capsaicin patch as there was a slow release and detection in the blood and plasma over 72 hrs.

Literature indicates that capsaicin is metabolized in liver using a number of different mechanisms: 1) via the liver mixed-function oxidase system which converts capsaicin to a catechol metabolite; 2) metabolism by cytochrome P450 2E1 to a reactive phenoxy radical; and 3) non-oxidative hydrolysis followed by oxidative deamination which produces vanillin (aromatic aldehyde), which in turn either undergoes oxidation to form vanillic acid or reduction to form vanillyl alcohol.

Table 2. Mean Concentrations of Radioactivity in Selected Tissues at 72 Hours After Administration of a Single Dermal Application of ^{14}C-Capsaicin (25.1 mg/animal) to Male Rats (Group 2)

Matrix	µg Equivalents ^{14}C-Capsaicin/g Mean[a]	SD
Adrenal Glands	1.69	0.78
Brain	0.428	0.208
Carcass (residual)	10.0	2.4
Heart	1.44	0.96
Kidneys	1.49	0.22
Liver	2.86	0.71
Lungs	1.07	0.37
Lymph Nodes (mesenteric)	1.67	0.47
Skin (test site)	900	311
Spleen	0.642	0.024
Blood[b]	0.328	0.035
Plasma[b]	0.482	0.126

a Each mean value represents the mean of three animals.
b Values are obtained from Group 1, 72 hour collection.

SD Standard deviation.

Table 5. Mean Normalized Percent of Radioactive Dose Recovered in Each Matrix at Specified Times After Administration of a Single Dermal Application of ^{14}C-Capsaicin (25.1 mg/animal) to Male Rats (Group 2)

Matrix	Collection Interval/Time (Hours)	Percent of Radioactive Dose Mean[a]	SD
Urine	0-24	1.79	0.25
Urine	24-48	1.61	0.21
Urine	48-72	2.72	0.86
Feces	0-24	0.66	0.26
Feces	24-48	1.03	0.35
Feces	48-72	2.03	0.58
Cage Wash	72	1.78	0.36
Cage Wipe	72	2.48	0.88
Subtotal		14.1	2.4
Adrenal Glands	72	0.00	0.00
Brain	72	0.00	0.01
Carcass (residual)	72	7.13	0.65
Heart	72	0.00	0.01
Kidneys	72	0.01	0.00
Liver	72	0.10	0.02
Lungs	72	0.01	0.01
Lymph Nodes (mesenteric)	72	0.00	0.00
Spleen	72	0.00	0.00
Subtotal		7.25	0.67
Dermal Patch	3	20.2	2.0
Skin Wipe	3	9.68	4.52
Subtotal		29.9	3.4
Skin (test site)	72	48.8	6.3
Total		100	0

a Each mean value represents the mean of three animals.

SD Standard deviation.

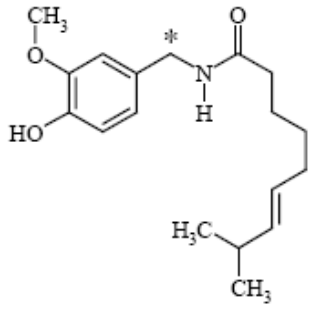
Study title: **Absorption, Distribution, Metabolism, and Elimination of [^{14}C]Capsaicin Following a Dermal Patch Application to Rats**

Key study findings: After patch application to male and female rats for 3 hours, a limited amount of ^{14}C-capsaicin-derived radioactivity appeared to have been absorbed, based on high retention in the dermal patches and low excretion. In both males and females, approximately 4 to 7% of the dose was likely absorbed from the patch. Excretion profiles were similar in both male and female rats. The highest concentrations of radioactivity were detected in skin (test site) through 336 hours postdose. This suggests that the radioactivity left on or in the washed skin may serve as a reservoir for continued absorption after removal of the patch. As proposed in the biotransformation pathway, ^{14}C-capsaicin was converted to several metabolites in rats. The metabolism of ^{14}C-capsaicin involved hydrolysis, demethylation, oxidation, and sulfation. Hydrolysis of the amide bond of capsaicin produced vanillylamine. Further oxidation of vanillylamine generated vanillin and vanillic acid. O-demethylation of capsaicin produced O-demethyl-capsaicin. Vanillic acid-sulfate resulted from sulfation of vanillic acid. In urine (0 to 96 hours postdose), vanillic acid-sulfate was the major metabolite and accounted for 1.26 and 0.67% of the dose in males and females, respectively. In feces (0 to 96 hours postdose) most of the radioactivity excreted was associated with O-demethyl-capsaicin and accounted for 0.88 and 0.45% of the dose in males and females, respectively. In plasma the major circulating metabolite was vanillin.

Study no.: 7215-135
E-location: m4\42-stud-rep\422-pk\4222-absorp\7215-135
Conducting laboratory and location: (b) (4)
(b) (4)
Date of study initiation: Feb 17, 2004
GLP compliance: No
QA report: No
Drug, lot #, and % purity:

Radiolabeled Test Article

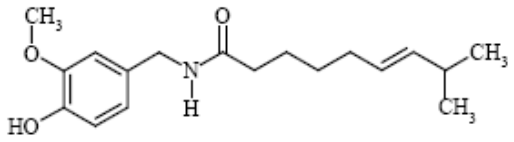
Test article:	[^{14}C]Capsaicin	Structure:
Molecular formula:	$C_{18}H_{27}NO_3$	
Molecular weight:	305.41	
Lot no.:	EPPS-03-028-100-10	
Specific activity:	53.8 mCi/mmol by spectrophotometric assay 0.171 mCi/mg by gravimetric assay	
Radiopurity:	$\geq$ 98% by HPLC	
Storage conditions:	Approximately -20°C, protected from light	



* signifies the position of the radiolabel

Nonradiolabeled Test Article

Test article:	Capsaicin	Structure:
Lot no.:	F0010103	
Chemical purity	101%	
Expiration date:	05 November 2005	
Storage conditions:	Room temperature	



The HPLC analyses performed by (b) (4) showed the radiopurity of [^{14}C]Capsaicin to be 99.4% prior to dosing. The mean specific activity of [^{14}C]Capsaicin in the dermal dose solution was determined by (b) (4) to be 0.902 μCi/mg.

Methods

Doses:
Species/strain: Sprague Dawley rats [Crl:CD(SD)IGS BR]
Number/sex/group or time point (main study): 15/sex/dose or 3/sex/dose
Route, formulation, volume, and infusion rate: 50 cm2 patch size applied for a 3 hour duration to the back of the animal after pretreatment with (b) (4) local anesthetic. The dermal patches were prepared (b) (4) by scientists from (b) (4) The appropriate amounts of radiolabeled and nonradiolabeled capsaicin were formulated with dose vehicle and applied to a strip of plastic backing.
Age: 8 weeks of age
Weight: 190-257 g
Sampling times: Blood (approximately 1 mL) was collected via a jugular vein at predose and at 1.5, 3, 6, 9, 12, 24, 48, 72, 96, 120, 168, 240, and 336 hours postdose from 3 animals/sex/time point.
Unique study design or methodology (if any):

Group	Number of Animals Male	Number of Animals Female	Dose Route	Target Dose Level (cm^2/animal)	Target Capsaicin Dose (mg/rat)	Samples Collected
1	15	15	Dermal	50	32	Blood and Tissues
2	3	3	Dermal	50	32	Urine, Feces, Tissues, and Carcass

Results

Maximum mean concentrations of radioactivity in blood and plasma were highest at 3 hours after patch application. In females, peak mean concentrations of radioactivity in blood and plasma were 163 and 252 ng equivalents ^{14}C-capsaicin/g, respectively. In males, peak mean concentrations of radioactivity in blood and plasma were 107 and 246 ng equivalents ^{14}C-capsaicin/g, respectively. Concentrations of radioactivity in male and female blood and plasma samples initially declined after removal of the patch, but then

increased at 72 hours post-dose. In males and females, blood concentrations increased to 78.9 and 26.1 ng equivalents ^{14}C-capsaicin/g; and in plasma samples, levels increased to 123 and 81.6 ng equivalents ^{14}C-capsaicin/g, respectively, at 72 hours.

In males and females, radioactivity was detected in most of the tissues examined at 3 hours post-dose. By 12 hours post-dose and thereafter, radioactivity was not detectable in most tissues, with the exception of skin (test site), liver and kidney. In males, the highest mean concentrations were detected in skin (test site), kidney, and liver with values of 176000, 1130, and 644 ng equivalents ^{14}C-capsaicin/g, respectively, at 3 hours post-dose. In females, the highest mean concentrations were also detected in skin (test site), liver, and kidney with values of 142000, 1100 and 889 ng equivalents ^{14}C-capsaicin/g, respectively, at 3 hours post-dose.

Most of the radioactivity remained in the dermal patch, dermal wrap, and skin wipe after 3 hours of application and accounted for 80.8 and 90.9% of the dose for males and females, respectively. A limited amounted of radioactivity was therefore absorbed. In males, the absorbed radioactivity resulted in a mean of 3.36% of the dose being excreted in urine, 2.42% in feces, and 1.05% retained in skin (test site) and carcass (residual). In females absorbed radioactivity resulted in a mean of 2.24% of the dose being excreted in urine, 1.35% in feces, and 0.27% retained in skin (test site) and carcass (residual). The radioactivity was excreted through 336 hours postdose. A total mean mass balance for males and females was 88.2 and 95.1%, respectively.

Metabolite Profiles of Capsaicin in Plasma
The profile of capsaicin metabolites in plasma was determined in samples collected from male and female rats at 3 hours postdose. Profiles of male and female plasma samples collected after patch application showed up to five metabolites in addition to unchanged parent drug. Unchanged drug concentrations in male and female plasma at 3 hours postdose were 9.86 and 10.5% of the radioactivity in the samples. At 3 hours postdose, most of the circulating radioactivity was associated with vanillin. The concentrations of vanillin in male and female plasma at 3 hours postdose were 64.1 and 47.3% of the radioactivity in the samples, respectively. Vanillylamine was a minor metabolite in male and female plasma. Vanillic acid was also detected in male and female plasma. Unidentified metabolite M4 was detected in plasma from males and females; unidentified metabolite M6 was detected in plasma from males.

Metabolite Profiles of Capsaicin in Skin (Test Site)
The HPLC profile of male and female skin from the test site showed most of the radioactivity was associated with unchanged drug at 3 hours after application of the patch. The concentrations of parent drug in male and female skin (test site) were 98.9 and 84.8% of the radioactivity in the sample, respectively. Vanillylamine was detected in female skin only, and accounted for 15.2% of the radioactivity in the sample at 3 hours postdose.

Metabolite Profiles of Capsaicin in Urine
The profile of capsaicin metabolites in urine was determined in selected samples collected from male and female rats. The metabolic profile of male and female urine

samples showed two metabolites and low levels of unchanged capsaicin. Unchanged parent drug in urine through 96 hours postdose in male and female rats accounted for 0.04 and 0.03% of the dose, respectively. Vanillic acid-sulfate (M2A) was the major metabolite in male urine and accounted for 1.26% of the dose, while in female the metabolite accounted for 0.67% of the dose. Vanillylamine was the major metabolite in female urine, and accounted for 1.09% of the dose, while in male the metabolite accounted for 1.07% of the dose.

Metabolite Profiles of Capsaicin in Feces

The profile of capsaicin metabolites in feces was determined in selected samples collected from male and female rats. The metabolic profile of male and female feces samples showed six metabolites in addition to unchanged capsaicin through 96 hours postdose. Unchanged parent drug in male and female feces through 96 hours postdose accounted for 0.09 and 0.05% of the dose, respectively. O-demethyl-capsaicin (M6A) was the major metabolite in male and female feces and accounted for 0.88 and 0.45% of the dose, respectively. Vanillylamine, vanillic acid, and vanillin were also detected in male and female feces; none of the metabolites were >0.1% of the dose at any single time point profiled. The unidentified metabolites, M4 and M5, were each <0.1% of the dose through 96 hours postdose.

Distribution and concentration of parent drug and metabolites in pooled plasma collected at 3 hours postdose following a single dermal application of [^{14}C]Capsaici to male and female rats (Group 1, 32 mg/rat)

Peak Identification	Retention Time (Minutes)	Proposed Identification	Male	Female
			Percent of Total Radioactivity in the Sample	
M1	3.0-3.5	Vanillylamine	6.34	9.81
M2	18.1-19.5	Vanillic Acid	3.17	7.46
M3	21.5-22.5	Vanillin	64.1	47.3
M4	23.0-25.0	Unknown	3.70	7.89
M6	30.0-31.5	Unknown	5.99	ND
Parent	33.1-33.5	Capsaicin	9.86	10.5
		Total:	93.1	82.9
			ng Equivalents [^{14}C]Capsaicin/g	
M1	3.0-3.5	Vanillylamine	15.6	24.7
M2	18.1-19.5	Vanillic Acid	7.80	18.8
M3	21.5-22.5	Vanillin	158	119
M4	23.0-25.0	Unknown	9.10	19.9
M6	30.0-31.5	Unknown	14.7	ND
Parent	33.1-33.5	Capsaicin	24.3	26.3
Total ^{14}C Concentration (ng Equivalents/g):			230	209
Pool ^{14}C Concentration (ng Equivalents/g):			246	252

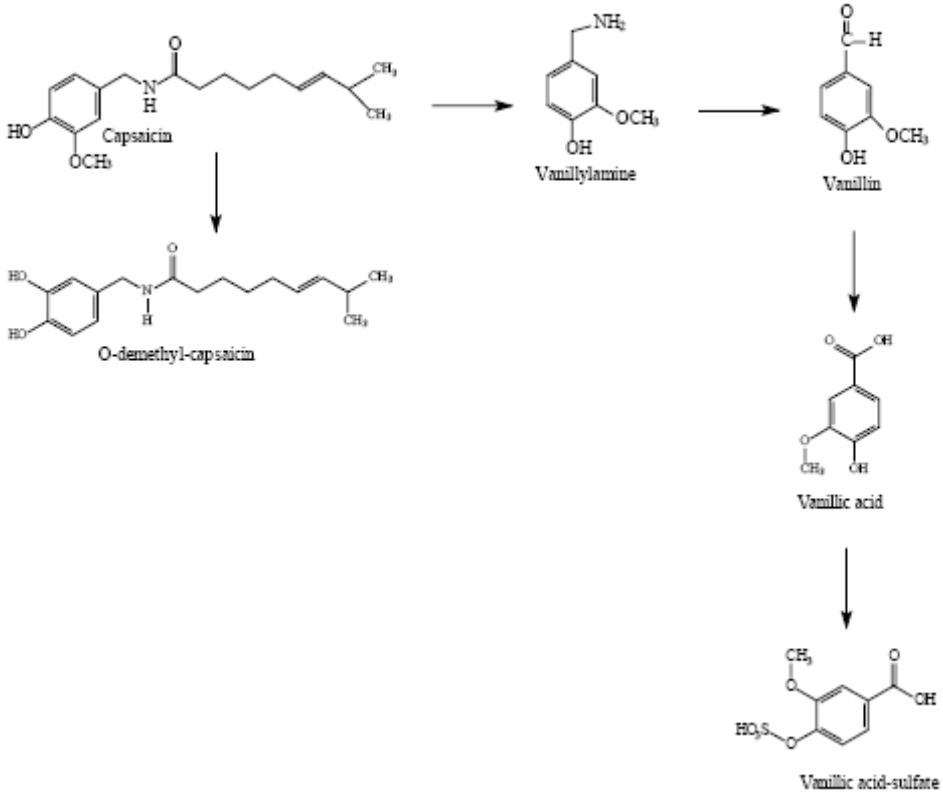
ND Peak not detected; limit of quantitation 2.0 % of run or greater.

Distribution and concentration of parent drug and metabolites in skin (test site) collected at 3 hours postdose following a single dermal application of [^{14}C]Capsaicin to male and female rats (Group 1, 32 mg/rat)

Peak Identification	Retention Time (Minutes)	Proposed Identification	Male	Female
			Percent of Total Radioactivity in the Sample	
M1	3.0-3.5	Vanillylamine	ND	15.2
Parent	33.1-33.5	Capsaicin	98.9	84.8
		Total:	98.9	100
			ng Equivalents [^{14}C]Capsaicin/g	
M1	3.0-3.5	Vanillylamine	ND	21,300
Parent	33.1-33.5	Capsaicin	224,000	119,000
Total ^{14}C Concentration (ng Equivalents/g):			224,000	140,000
Pool ^{14}C Concentration (ng Equivalents/g):			227,000	140,000

ND Peak not detected; limit of quantitation 2.0 % of run or greater.

Proposed biotransformation pathway



Study title: **Absorption, Distribution, Metabolism, and Elimination of [^{14}C]Capsaicin Following a Dermal Patch Application to Miniswine**

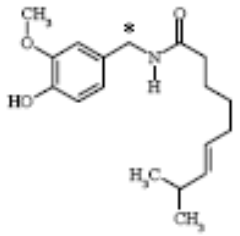
Key study findings: Concentrations of radioactivity in all samples of blood were below the limit of quantitation (BLQ). Low levels of radioactivity (16 to 38 ng equivalents ^{14}C-Capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours postdose in two animals. The concentrations of unchanged Capsaicin in all plasma samples were below detection limits (<5.00 ng/mL). Two weeks after patch application, a total of 4.23% of the dose was recovered in excreta, with 2.80% eliminated in urine, 0.49% in eliminated in feces, and 0.94% in samples of pan/screen rinse, cage wipe, and cage wash. Skin at the application site contained 1.23% of the administered dose. No radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes, and spleen).

Study no.: 7215-114
E-location: m4\42-stud-rep\422-pk\4222-absorp\7215-114

Conducting laboratory and location: (b) (4)
Date of study initiation:
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:

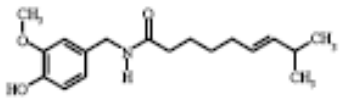
Radiolabeled Test Article

Test article:	^{14}C-Capsaicin	Structure:
Chemical name:	(6-nonenamide, N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-, (6E)-)	
Molecular formula:	$C_{18}H_{27}NO_3$	
Molecular weight:	305.41	
Lot No.:	CSL-99-927-94-14	
Specific activity:	54.0 mCi/mmol UV 51.4 mCi/mmol microgravimetric	
Radiopurity:	≥98%	
Storage conditions:	Approximately -20°C, protected from light	* signifies the position of the radiolabel



Nonradiolabeled Test Article

Test article:	Capsaicin	Structure:
Chemical name:	(6-nonenamide, N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-, (6E)-)	
Molecular formula:	$C_{18}H_{27}NO_3$	
Molecular weight:	305.41	
Lot No.:	F0010101	
Chemical purity	100.3%	
Storage conditions:	Room temperature	



Methods

Group	Number of Male Animals	Target Dose Level	Dose Route	Sample Collection (Hours Postdose)
1	3	100 cm^2	Dermal	Blood: predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and daily through 336 Excreta: Daily through 336 Tissues: 336

Three male Sinclair Mini-swine were received on 23 October 2001 and weighed 7.3, 6.5, and 9.2 kg. The animals were acclimated for approximately 2 weeks prior to dose administration. At dosing, the animals weighed 10.0, 9.2, and 12.0 kg and were approximately 3 months of age.

The dermal patches were prepared (b) (4) by scientists from (b) (4) (b) (4) at a target dose of 64 mg/animal.

The absorption, distribution, and elimination of ^{14}C-Capsaicin were studied in mini-swine following application of two 50-cm2 dermal patches containing a total target dose of 64 mg/animal. On the day prior to dosing, the dorsal side of each animal was shaved and the skin washed with a mild detergent solution. An intramuscular injection of Demerol® (10 mg/kg) was administered approximately 1 hour prior to and approximately 20 to 45 minutes after patch application. Application of the patches was also proceeded by local administration of an anesthetic gel (2% Tetracaine). The patch was applied to each animal for at least two hours. Blood was collected at predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and daily through 336 hours postdose. Samples of excreta were collected at 24-hour intervals through 336 hours postdose. Selected tissues were collected at 336 hours postdose. Samples were analyzed for radioactivity by using liquid scintillation counting. Samples of plasma were also analyzed for unchanged Capsaicin in the Bioanalytical Chemistry department of (b) (4) by using a validated HPLC assay.

Results

Concentrations of radioactivity in all samples of blood were below the limit of quantitation (BLQ). Low levels of radioactivity (16 to 38 ng equivalents ^{14}C-Capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours postdose in two animals. The concentrations of unchanged Capsaicin in all plasma samples were below detection limits (<5.00 ng/mL). Two weeks after patch application, a total of 4.23% of the dose was recovered in excreta, with 2.80% eliminated in urine, 0.49% in eliminated in feces, and 0.94% in samples of pan/screen rinse, cage wipe, and cage wash. Skin at the application site contained 1.23% of the administered dose. No radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes, and spleen).

2.6.4.4 Distribution

Rat
The distribution of ^{14}C-capsaicin was studied in rats following application of a 50 cm2 patch over 3 hours with a target dose of 32 mg/rat (Study 7215-105). After 72 hours, approximately 49% of the radioactivity was left on/in the skin of the application site. Tissues with any radioactivity other than skin and blood were liver (approximately 0.1 %), lungs (approximately 0.01 %) and kidneys (approximately 0.01%). During the test period, the plasma concentration of radioactivity remained relatively constant (the mean plasma concentration at 72 hours was comparable to the mean peak plasma concentration at 3 hours) and elimination of radioactivity in the urine and feces was continuous.

A further study in rats was conducted with the application of a 50 cm^2 NGX-4010 patch containing ^{14}C-capsaicin with a target dose of 32 mg/animal for 3 hours. The rats were followed for 14 days after the patch application to study the distribution of ^{14}C-capsaicin (Study No. 7215-135). In males and females, radioactivity was detected in most of the tissues examined at 3 hours post-dose. By 12 hours post-dose and thereafter, radioactivity was not detectable in most tissues, with the exception of skin (at the test site), liver (not detectable by 336 hours) and kidney (not detectable by 336 hours). The tissue:plasma ratios were <1 for all tissues, where calculable, except skin (test site), liver, kidney and adrenal glands (females only). In males, the highest ratios were in skin (test site), kidney and liver with values of 1730 (12 hours post-dose), 4.38 (3 hours post-dose) and 4.34 (72 hours post-dose), respectively. The highest ratios in females were in skin (test site), liver and kidney with values of 1590 (12 hours post-dose), 7.07 (72 hours postdose), and 3.36 (72 hours post-dose) respectively.

Minipigs
The distribution of ^{14}C-capsaicin was studied in minipigs following application of two 50 cm^2 NGX-4010 patches containing a total target dose of 64 mg/animal for a period of at least 2 hours (Study 7215-114). Two weeks after patch application, radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes and spleen). Concentrations of radioactivity in all samples of blood were below the limits of quantitation. Low levels of radioactivity (16 to 38 ng equivalents 14C-capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours post-dose in two out of three animals. The concentrations of unchanged capsaicin in all plasma samples were below detection limits (<5.00 ng/mL).skin at the application site contained 1.23% ofthe administered dose. No radioactivity was detected in sampled tissues (adrenal glands, brain, heart, kidneys, liver, lungs, lymph nodes and spleen). Concentrations of radioactivity in all samples of blood were below the limits of quantitation. Low levels of radioactivity (16 to 38 ng equivalents ^{14}C-capsaicin/g) were detected only in plasma samples collected at 1 to 4 hours post-dose in two out of three animals. The concentrations of unchanged capsaicin in all plasma samples were below detection limits (<5.00 ng/mL).

Plasma Protein Binding

The plasma protein binding of capsaicin in rat, rabbit, dog and human plasma were evaluated by equilibrium dialysis using ^{14}C-capsaicin at 50, 200, 300 and 500 ng/mL (Study No. 7215-143). Capsaicin was highly bound to plasma proteins in all four species over the concentration range of 50 to 500 ng/mL. The mean percentage of capsaicin bound to plasma proteins ranged from 92.8% to 94.3% in human, 92.0% to 93.2% in dog, 91.1% to 92.4% in rabbit and 90.8% to 91.2% in rats. The binding was independent of the concentration in all species.

Study title: **The *In Vitro* Protein Binding of *trans*-[14C]Capsaicin in Rat, Rabbit, Dog, and Human Plasma**

Key study findings: *trans*-[^{14}C]Capsaicin was highly bound (90.8 to 94.3%) to proteins in rat, rabbit, dog, and human plasma. In all species tested, there was no evidence of concentration dependence over the target concentration range of 50 to 500 ng/mL.

Study no.: **7215-143**
E-Location: m4\42-stud-rep\422-pk\4223-distrib\7215-143

Conducting laboratory and location: (b) (4)
Date of study initiation: not provided, report date Dec 16, 2004**GLP compliance**: yes
GLP Compliance: yes
QA report: yes

Radiolabeled Test Article

Test article:	*trans*-[^{14}C]Capsaicin	Structure:
Molecular formula:	$C_{18}H_{27}NO_3$	
Molecular weight:	305.41	
Lot no.:	EPPS-03-028-100-10	
Specific activity:	53.8 mCi/mmol by spectrophotometric assay 0.171 mCi/mg by gravimetric assay	
Radiopurity:	≥ 98% by HPLC	
Storage conditions:	Approximately -20°C, protected from light	* signifies the position of the radiolabel

Nonradiolabeled Test Article

Test article:	Capsaicin	Structure:
Lot no.:	F0010103	
Chemical purity:	101%	
Expiration date:	05 November 2005	
Storage conditions:	Ambient	

Drug, lot #, and % purity:

The radiopurities of *trans*-[^{14}C]Capsaicin determined at (b) (4) by HPLC prior to use and at the end of the study were 97.5 and 93.3%, respectively.

The stability of *trans*-[^{14}C]Capsaicin in plasma from each species under the study conditions was determined by profiling the radioactivity in the plasma samples from the time-to-equilibrium experiment. Plasma from the 4-hour time point was analyzed by

HPLC. Following 4 hours of dialysis in plasma, the radiopurity of *trans*-[^{14}C]Capsaicin was 99.0% (rat), 95.6% (rabbit), 99.9% (dog), and 98.6% (human). The results indicate that *trans*-[^{14}C]Capsaicin was stable in plasma from all species through 4 hours, except in rabbit where a slight degradation was observed.

Methods and Results

The protein binding characteristics of *trans*-[^{14}C]Capsaicin in rat, rabbit, dog, and human plasma were evaluated at four concentrations (50, 200, 300, and 500 ng/mL) by equilibrium dialysis. The stability of *trans*-[^{14}C]Capsaicin in plasma from rat, rabbit, dog, and human was evaluated by high-performance liquid chromatographic (HPLC) analysis of samples obtained following incubation of 300 ng/mL of *trans*-[^{14}C]Capsaicin with plasma at 37°C for approximately 4 hours. *trans*-[^{14}C]Capsaicin was stable following incubation with plasma from all species evaluated, except in rabbit where a slight degradation was observed. *trans*-[^{14}C]Capsaicin was highly bound to plasma proteins in all four species over the concentration range of 50 to 500 ng/mL. Over this range, the mean percentage of *trans*-[^{14}C]Capsaicin bound to plasma proteins ranged from 92.8 to 94.3% in human, 92.0 to 93.2% in dog, 91.1 to 92.4% in rabbit, and 90.8 to 91.2% in rat. The binding was independent of the concentration in all species.

2.6.4.5 Metabolism

Existing scientific literature suggests that capsaicin is metabolized to a great extent in the liver following oral administration. Approximately 80% of capsaicin administered orally to rats was found in the urine in the form of unchanged drug, vanillylamine, vanillin, vanillyl alcohol, and vanillic acid. Only 8.7% of the total amount was found as unchanged drug (Geppetti, 1996). Early studies demonstrated that capsaicin is converted to metabolites via hydroxylation of the vanillyl ring moiety, leading to the hypothesis that capsaicin is activated by the liver mixed-function oxidase system to an electrophilic intermediate that is capable of binding to hepatic proteins (Surh, 1995). One-electron oxidation of capsaicin also plays a role in capsaicin metabolism. A recent study showed that liver CYP2E1 activity is responsible for conversion of capsaicin to the reactive phenoxy radical, which in turn can dimerize or bind to CYP2E1, thereby inactivating the enzyme (Surh, 1996). This mechanism may be responsible for the inhibition of further activation and the reported chemoprotective activity of capsaicin against some chemical carcinogens and mutagens. Non-oxidative hydrolysis has also been demonstrated in rat tissues at the acid-amide bond (Surh, 1995). The splitting of the side chain also occurs *in vivo*, which is believed to be the rate-limiting step in overall metabolism of the compound. Oxidative deamination of the resulting vanillylamine produces the aromatic aldehyde, vanillin, which in turn undergoes oxidation to vanillic acid or reduction to vanillyl alcohol for excretion as a free form or as a glucuronic acid conjugate (Surh, 1995). In addition to CYP2E1, capsaicin can also be metabolized by CYP1A2, 3A4, 2B6, 2C8, 2C9, and 2C19 (Reilly, 2002; Reilly, 2006).

Human skin metabolism of capsaicin
In an in vitro metabolism study (Study 274-1060-01) using intact human skin, ^{14}C-capsaicin was incubated (1, 3 and 10μM) for 20 hours with intact fresh human skin

samples. *In vitro* biotransformation of capsaicin was slow: although capsaicin was metabolized to vanillylamine and vanillic acid, the majority of the sample radioactivity was associated with unchanged capsaicin. In the incubation medium and skin, capsaicin, vanillylamine and vanillic acid ranged from 74.0% to 95.6%, 4.37% to 19.8% and 0.11% to 7.97%, respectively, of the radioactivity in each sample at all concentrations (1, 3 and 10 μM).

In Vitro Metabolism, Human, Dog and Rat Liver Microsomes
In an *in vitro* metabolism study (Study No 7215-125) using rat , dog and human liver microsomes, ^{14}C-capsaicin (1 and 10 μM) was incubated for a maximum of 30 minutes with microsomes and S9 fractions prepared from human, dog and rat liver. Biotransformation of ^{14}C-capsaicin was rapid in rat microsomes and S9 fraction incubations compared to dog and human incubations. With human microsomes, the rate of formation of the metabolites was faster than in dog. Metabolism of ^{14}C-capsaicin resulted in the formation of several products. The metabolism of capsaicin was similar in microsomes and S9 fraction from rat, dog and human. In rat, dog and human, three major metabolites were detected and tentatively identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin. These metabolites are in agreement with those reported (Reilly and Yost 2005). Rat microsomes and S9 fractions also formed vanillylamine and vanillin as hydrolytic metabolites in addition to the major metabolites.

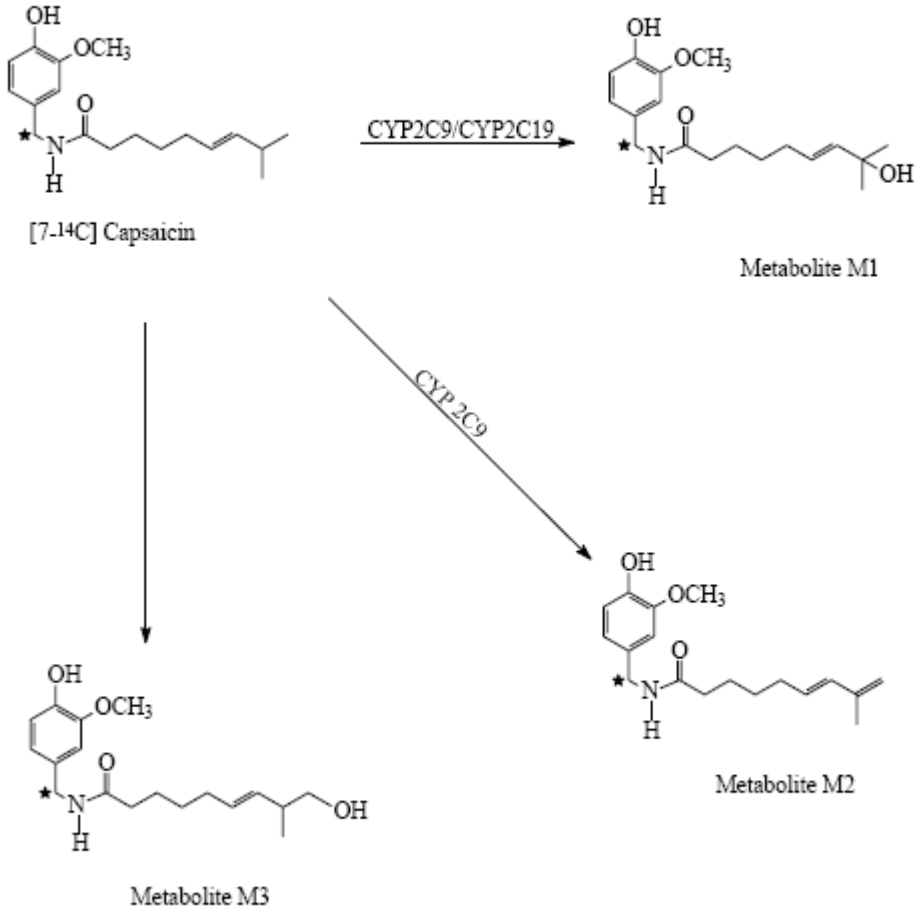
In the study in rats with the application of a 50 cm2 topical patch with ^{14}C-capsaicin with a target dose of 32 mg/animal for 3 hours **(**Study 7215-135**)** where the rats were followed for 14 days after the patch application, metabolic profiles of urine and feces showed most of the radioactivity was associated with vanillic acid-sulfate and O-demethyl-capsaicin, respectively. Most of the radioactivity in skin (test site) was associated with unchanged drug. Vanillin was the major circulating component in plasma.

Indentification of CYP enzymes involved in human liver metabolism of capsacin
In Study XT044001, the human CYP enzymes studied were CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, CYP3A5, CYP4A11. ^{14}C-capsaicin was incubated with a pool of human liver microsomes to assess the proportionality of metabolite formation with respect to incubation time and microsomal protein concentration (to determine initial rate conditions). The results obtained from this experiment were used to design an experiment to determine the Michaelis-Menten kinetic constants, Km and Vmax. Additional experiments were conducted to identify the CYP enzymes involved in the metabolism of ^{14}C-capsaicin. Overall, the results from this study suggested that CYP2C9 is the primary enzyme responsible for converting ^{14}C-capsaicin to 16-hydroxy-capsaicin and 16,17-dehydro-capsaicin. CYP2C19 may also contribute to the formation of 16-hydroxy-capsaicin. Although the formation of the third metabolite, 17-hydroxy-capsaicin, was NADPH-dependent, the CYP enzyme(s) responsible for its formation remained unidentified. It is possible that CYP enzyme(s) other than those evaluated in this study may be responsible for the formation of 17-hydroxy-capsaicin.

Study title: ***In Vitro* Reaction Phenotyping Study of *trans*-Capsaicin**

Key study findings: CYP2C9 is the primary enzyme responsible for converting [^{14}C] *trans*-capsaicin to M1 and M2. CYP2C19 may also contribute to M1 formation. Although the formation of M3 was NADPH-dependent, the CYP enzyme(s) responsible for the formation M3 remain(s) unidentified. Possibly CYP enzyme(s) other than those evaluated are responsible for the formation of M3.

Figure 1: Proposed metabolic pathway of the conversion of [^{14}C] *trans*-capsaicin to metabolites M1, M2 and M3



φ: Indicates position of ^{14}C-label

Study no.: XT044001
E-Location: m4\42-stud-rep\422-pk\4224-metab
Conducting laboratory and location: (b) (4)
Date of study initiation: report date Dec 15, 2004
GLP compliance: yes
QA report: yes

Drug, lot #, and % purity:

Unlabeled *trans*-capsaicin

Lot number:	AD03743
Receival date:	February 25, 2004
Quantity received:	10.0 g
Molecular weight:	305.42 g/mol
Appearance:	White powder
Solvent used for dissolution:	Methanol
Purity:	99.7%
Storage of material:	Room temperature, stored with desiccant

[^{14}C]-labeled *trans*-capsaicin

Lot number:	EPPS-03-028-100-10	EPPS-03-028-100-10
Receival date:	February 25, 2004	May 7, 2004
Quantity received:	1.04 mCi (6.1 mg)	0.5 mCi (2.95 mg)
Molecular weight:	305.41 g/mol	305.41 g/mol
Specific activity:	53.8 mCi/mmol	53.8 mCi/mmol
Appearance:	White solid	White solid
Solvent used for dissolution:	Methanol	Methanol
Purity:	98%	98%
Storage of material:	-80±5°C	-80±5°C
Sample preparation:	The stock solution was prepared in methanol and was stored at –80±5°C, protected from light.	The stock solution was prepared in methanol and was stored at –80±5°C, protected from light.

Methods

A binary gradient HPLC method was provided by the Sponsor for the detection and quantification of [14C] *trans*-capsaicin. One preliminary evaluation was conducted to establish that the HPLC method adequately resolved metabolites from [14C] *trans*-capsaicin. Once the method proved to be adequate, the method was qualified based on criteria defined in this report prior to reaction phenotyping (enzyme mapping). The test article, [14C] t*rans*-capsaicin was incubated with a pool of human liver microsomes to assess the proportionality of metabolite formation with respect to incubation time and microsomal protein concentration (to determine initial rate conditions). The results obtained from this experiment were used to design an experiment to determine the Michaelis-Menten kinetic constants, Km and Vmax. Additional experiments were conducted to identify the CYP enzymes involved in the metabolism of [14C] *trans*-capsaicin. A combination of the following four experimental approaches was taken: (1) analysis of the metabolism of [14C] *trans*-capsaicin by individual recombinant human CYP enzymes (rCYP enzymes); (2) analysis of the correlation between the rate of metabolite formation and the activity of the major CYP enzymes in a bank of 16 samples of human liver microsomes; (3) an evaluation of the effects of specific inhibitory monoclonal and polyclonal antibodies against selected CYP enzymes on the metabolism of [14C] *trans*-capsaicin by human liver microsomes; and (4) an evaluation of the effects of various chemical inhibitors against selected CYP enzymes on the metabolism of [14C] *trans*-capsaicin by human liver microsomes

CYP1A2	7-Ethoxyresorufin *O*-dealkylation
CYP2A6	Coumarin 7-hydroxylation
CYP2B6	*S*-Mephenytoin *N*-demethylation
CYP2C8	Paclitaxel 6α-hydroxylation
CYP2C9	Diclofenac 4′-hydroxylation
CYP2C19	*S*-Mephenytoin 4′-hydroxylation
CYP2D6	Dextromethorphan *O*-demethylation
CYP2E1	Chlorzoxazone 6-hydroxylation
CYP3A4/5	Testosterone 6β-hydroxylation
CYP4A11	Lauric acid 12-hydroxylation
FMO	Benzydamine *N*-oxidation

Direct–acting Inhibitors

CYP enzyme	Inhibitor	Final concentration in incubation (μM)	Solvent used to dissolve inhibitor †
CYP1A2	α-Naphthoflavone	0.1, 1, 10, 50	Methanol (1%)
CYP2A6	Nicotine	100, 500, 1000	Methanol (1%)
CYP2B6	Orphenadrine	100, 500, 1000	Methanol (1%)
CYP2C8	Quercetin	1, 5, 10	Methanol (1%)
CYP2C9	Sulfaphenazole	1, 10, 50	Methanol (1%)
CYP2C19	Modafinil	10, 50, 200	Methanol (1%)
CYP2D6	Quinidine	0.1, 10, 100	Water
CYP2E1	4-Methylpyrazole	1, 10, 100	Water
CYP3A4	Ketoconazole	0.01, 0.1, 0.5, 1, 10	Methanol (1%)
CYP4A11	10-(Imidazolyl)-decanoic acid	0.1, 0.5, 1	Methanol (1%)

† The values in parentheses represent the final concentration of the organic solvent in the incubations. Since [^{14}C] *trans*-capsaicin was added to incubations in 2.5 μL of methanol and inhibitor was added in 2.5 μL of methanol, the final concentration of methanol was 1%.

Metabolism-dependent Inhibitors

CYP enzyme	Inhibitor	Final concentration (μM)	Solvent used to dissolve inhibitor †
CYP1A2	Furafylline	10	Methanol (1%)
CYP2A6	8-Methoxypsoralen	0.5	Methanol (1%)
CYP2C19	Ticlopidine	0.5	Water
CYP3A4	Troleandomycin	50	Methanol (1%)

† The values in parentheses represent the final concentration of the organic solvent in the incubations. Since [^{14}C] *trans*-capsaicin was added to incubations in 2.5 μL of methanol and inhibitor was added in 2.5 μL of methanol, the final concentration of methanol was 1%.

Results

1. The Sponsor.s HPLC method was demonstrated to be suitable for the separation of [^{14}C] *trans*-capsaicin (substrate) and its metabolites when the substrate was incubated with human liver microsomes. The HPLC method was qualified at the Testing Facility.

2. In the presence of NADPH, human liver microsomes converted [^{14}C] *trans*-capsaicin to three primary metabolites, 16-hydroxy-capsaicin (M1), 16,17-dehydro-capsaicin (M2) and 17-hydroxy-capsaicin (M3), whose formation required NADPH and involved no lag in formation.

3. The kinetic constants (Km and Vmax) of M1, M2 and M3 formation were determined to be 1.5 μM and 947 pmol/mg/min, 2.0 μM and 1000 pmol/mg/min and 1.6 μM and 878 pmol/mg/min (respectively) under appropriate initial rate conditions. The *in vitro* intrinsic clearance, Vmax/Km, of metabolites M1, M2 and M3 were 642, 511 and 543 μL/mg protein/min, respectively.

4. [^{14}C] *trans*-Capsaicin (2 and 20 μM), when incubated with recombinant human CYP enzymes (rCYP1A1, rCYP1A2, rCYP2A6, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6, rCYP2E1, rCYP3A4, rCYP3A5 and rCYP4A11), at 25 pmol P450 per incubation, was converted to M1 and M2 predominantly by rCYP2C9 and rCYP2C19. The greatest formation of M3 was observed in incubations with rCYP3A4, but was below the limit of quantification (BLQ).

5. [^{14}C] *trans*-Capsaicin (2 μM) was incubated with a bank of 16 samples of human liver microsomes and formation of each metabolite was correlated with the sample-to-sample variation in various CYP enzyme activities. Formation of M1 moderately correlated with CYP2C9 (diclofenac 4′-hydroxylase) and CYP2C19 (*S*-mephenytoin 4′-hydroxylase) activity with correlation coefficients (r) of 0.680 and 0.504, respectively. Multiple linear regression of M1, CYP2C9 and CYP2C19 activity gave a correlation coefficient of 0.863. Formation of M2 strongly correlated with CYP2C9 activity with a correlation coefficient (r) of 0.856. M3 formation correlated with both CYP2D6 (dextromethorphan *O*-demethylase) and CYP2E1 (chlorozoxazone 6-hydroxylase) activity, with correlation coefficients of 0.698 and 0.704, respectively.

6. Inhibitory monoclonal and polyclonal antibodies against CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) were incubated with a pool of human liver microsomes to examine their effects on the formation of M1, M2 and M3. Polyclonal antibody against CYP2C9 strongly inhibited M1 and M2 formation (greater than 80% inhibition). Purified monoclonal antibodies against CYP2B6 and polyclonal antibodies against CYP2C9 minorly inhibited M3 formation (24 and 19% inhibition, respectively) at 2 μM of [^{14}C] *trans*-capsaicin. However, no inhibition of M3 formation by any antibodies was observed at 20 μM of [^{14}C] *trans*-capsaicin.

7. Various chemical inhibitors against CYP enzymes were incubated with a pool of human liver microsomes to examine their effects on the metabolism of [^{14}C] *trans*-capsaicin (2 μM). Sulfaphenazole, a directacting inhibitor against CYP2C9, strongly inhibited both M1 and M2 formation by as much as 100%. Orphenadrine, an inhibitor of CYP2B6, and ketoconazole, an inhibitor of CYP3A4, completely inhibited M3 formation. No metabolism-dependant inhibition of M1, M2 and M3 by the compounds

evaluated (furafylline, 8-methoxypsoralen, ticlopidine and troleandomycin) was observed.

Study title: ***In Vitro* Metabolism of [14C]Capsaicin by Rat, Dog, and Human Hepatic Microsomes and S-9 Fraction**

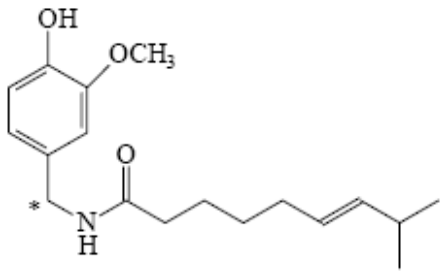
Key study findings:
Biotransformation of [^{14}C]Capsaicin was rapid in rat microsomes and S-9 fraction incubations compared to dog and human incubations. In human, the rate of formation of the metabolites was faster than in dog. Generally, the rate of metabolism in microsomes was faster than the S-9 fraction. For all species, the metabolism of [^{14}C]Capsaicin was less extensive at the 10-µM concentration as compared to the 1-µM concentration, suggesting saturability of metabolism. Metabolism of [^{14}C]Capsaicin resulted in the formation of several products. For rat, dog, and human the number of metabolites detected were 10, 5, and 5, respectively. The metabolism of capsaicin was similar in microsomes and S-9 fraction from rat, dog, and human. In rat, dog, and human, three major metabolites were detected and tentatively identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin. Rat microsomes and S-9 fractions also formed vanillylamine and vanillin as hydrolytic metabolites in addition to the major metabolites mentioned before.

Study no.: **7215-125**
E-Location: m4\42-stud-rep\422-pk\4224-metab\7215-12
Conducting laboratory and location: (b) (4)
Date of study initiation:
GLP compliance: yes
QA report: yes

Radiolabeled Test Article

Test article:	[^{14}C]Capsaicin
Lot no.:	CSL-99-927-94-14
Supplier:	(b) (4)
Specific activity:	54.0 mCi/mmol by UV spectrophotometric assay 51.4 mCi/mmol by microgravimetric assay
Radiopurity:	≥ 98% by HPLC
Storage conditions:	Approximately -20°C, protected from light

Structure:

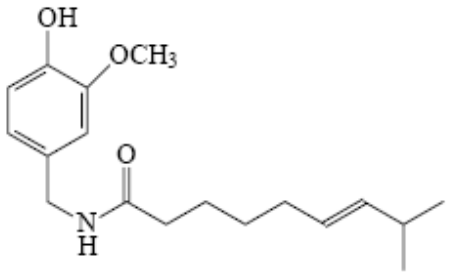


* signifies the position of the radiolabel

Drug, lot #, and % purity:

Nonradiolabeled Test Article

Test article:	Capsaicin	Structure:
Lot no.:	F0010102	
Supplier:	NeurogesX, Inc. (b) (4) (b) (4)	
Expiration date:	25 December 2003	
Storage conditions:	Room temperature	



[^{14}C]Capsaicin dissolved in dimethyl sulfoxide (DMSO) was added to incubation samples. The concentration of DMSO did not exceed approximately 1% of the total volume. A 10 µL addition of the appropriate fortification solution was used to generate final concentrations of 1 and 10 µM in a 1-mL final incubation volume.

The radiopurity of [^{14}C]Capsaicin at the beginning of the study was determined to be 98.3%. The radiopurity of [^{14}C]Capsaicin in fortification solution was determined by HPLC to be 97.0%.

Methods

The metabolism of [^{14}C]Capsaicin was assessed *in vitro* following incubation with hepatic microsomes and S-9 fraction from rat, dog, and human. Biotransformation was determined at 1 and 10 µM [^{14}C]Capsaicin with 1 mg microsomal protein/mL for 0, 5, 10, 20, and 30 minutes. Microsomal and S-9 fraction incubations were performed in 0.1 M phosphate buffer (pH 7.4) containing a NADPH-generating system. Incubations were terminated by the addition of acetonitrile. Identification of metabolites of capsaicin was based on HPLC with radiochemical detection. co-elution with authentic standards and on mass spectral analysis of metabolites in rat, dog, and human microsomes and S-9 fraction. Radioactive peaks in samples were further identified by liquid chromatography/mass spectrometry (LC/MS). Both negative and positive ion electrospray LC/MS were used to analyze metabolites and standards.

Results

In an in vitro metabolism study using rat, dog and human liver microsomes, ^{14}C-capsaicin (1 and 10 µM) was incubated for a maximum of 30 minutes with microsomes and S9 fractions prepared from human, dog and rat liver. Biotransformation of ^{14}C-capsaicin was rapid in rat microsomes and S9 fraction incubations compared to dog and human incubations. With human microsomes, the rate of formation of the metabolites was faster than in dog. Metabolism of ^{14}C-capsaicin resulted in the formation of several products. The metabolism of capsaicin was similar in microsomes and S9 fraction from rat, dog and human. In rat, dog and human, three major metabolites were detected and tentatively identified as 16-hydroxy-capsaicin, 17-hydroxy-capsaicin, and 16,17-dehydro-capsaicin. These metabolites are in agreement with those reported (Reilly and Yost 2005). Rat

microsomes and S9 fractions also formed vanilylamine and vanilin as hydrolytic metabolites in addition to the major metabolites mentioned above.

Human CYP Enzymes Involved in the Metabolism of Capsaicin
A study was designed to evaluate the in vitro metabolism of ^{l4}C-capsaicin with the aim of identifying the human CYP enzymes involved in its metabolism. CYP enzymes studied were CYPIA2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2CI9, CYP2D6, CYP2EI and CYP3A4. ^{14}C-capsaicin was incubated with a pool of human liver microsomes to assess the proportionality of metabolite formation with respect to incubation time and microsomal protein concentration (to determine initial rate conditions). The results obtained from this experiment were used to design an experiment to determine the Michaelis-Menten kinetic constants, Km and Vmax. Additional experiments were conducted to identify the CYP enzymes involved in the metabolism of ^{14}C-capsaicin. A combination of the following four experimental approaches was taken:

- Analysis of the metabolism of 14C-capsaicin by individual recombinant human CYP enzymes (rCYP enzymes)
- Analysis of the correlation between the rate of metabolite formation and the activity of the major CYP enzymes in a bank of 16 samples of human liver microsomes . An evaluation of the effects of specific inhibitory monoclonal and polyclonal antibodies against selected CYP enzymes on the metabolism of 14C-capsaicin by human liver microsomes
- An evaluation of the effects of various chemical inhibitors against selected CYP enzymes on the metabolism of 14C-capsaicin by human liver microsomes.

The results indicate that CYP2C9 is the primary enzyme responsible for converting ^{14}C-capsaicin to 16-hydroxy-capsaicin and 16, 17-dehydrocapsaicin. CYP2C 19 may also contribute to the formation of 16-hydroxycapsaicin. Although the formation of the third metabolite, 17-hydroxycapsaicin, was NADPH-dependent, the CYP enzyme(s) responsible for its formation remained unidentified. It is possible that CYP enzyme(s) other than those evaluated in this study may be responsible for the formation of 17-hydroxy-capsaicin.

Study title: **In silico analysis of capsaicin, capsaicin metabolites and impurities present in synthetic trans-capsaicin**

Key study findings: The only compound identified as carcinogenic risk (of the forestomach) among capsaicin analogs and possible metabolites was methyl nonenoic acid which is a short chain aliphatic carboxylic acid. The "toxophore" identified in the molecule was proprionic acid.

Reviewer Comment: *Since the DEREK analysis software mentioned was not supported with appropriate reference, validation, descriptive information this analysis was not found to be useful for regulatory purposes. Furthermore, it provided a prediction of toxicological risk based on compound structure and known available toxicological findings for similar structure, whereas the Agency prefers an empirical risk determination at this time.*

Study no.: nc002-0-rp
E-Location: m4\42-stud-rep\423-tox\4237-other-tox-stud\42375-metab\nc002-0-rp
Conducting laboratory and location: (b) (4)
Date of study initiation: Report date Aug 13, 2004
GLP compliance: no
QA report: no
Drug, lot #, and % purity:
None, computer modeling study

Methods

This study was to identify any unknown toxicological potential of capsaicin, capsaicin metabolites and its identified impurities present in the Applicant's product.

Methods
This analysis was performed by (b) (4) using (b) (4).

Results

The only compound identified as carcinogenic risk (of the forestomach) among capsaicin analogs and possible metabolites was methyl nonenoic acid which is a short chain aliphatic carboxylic acid. The toxophore identified in the molecule was proprionic acid. This substance is used as a food preservative with ACGIH TLV of 10 ppm. *In silico* analysis of other short chain carboxylic acids up to 8 carbons showed the same risk (except formic acid and acetic acid) of irritation of the forestomach and carcinogenicity of the forestomach because of the presence of the proprionic acid toxophore. In summary, *in silico* analysis of capsaicin, capsaicin metabolites and its impurities present in the *trans*-capsaicin as manufactured for NeurogesX for inclusion in NGX-4010 dermal patches did not identify any previously unknown toxicological potential, including carcinogenicity potential or potential to cause reproductive toxicity.

TABLE 1: Summary of (b) (4) in silico analysis results for naturally occurring capsaicin and its analogs, potential capsaicin metabolites and potential impurities in synthetic capsaicin

Compounds Screened	Results
Naturally Occurring Capsaicinoids	
trans-Capsaicin	Skin sensitization, catechol precursor
Homocapsaicin	Skin sensitization, catechol precursor
Dihydrocapsaicin	Skin sensitization, catechol precursor
Nordihydrocapsaicin	Skin sensitization, catechol precursor
Homodihydrocapsaicin	Skin sensitization, catechol precursor
Metabolites of trans-Capsaicin Identified in NeurogesX Studies and Potential Metabolites Based on Theoretical Structure Analysis	
Vanillylamine	Skin sensitization, catechol precursor
Vanillin	Skin sensitization, catechol precursor
Vanillic Acid	Skin sensitization, catechol precursor
Vanillyl Alcohol	Skin sensitization, catechol precursor
16-Hydroxy capsaicin (ω-1 hydroxylated capsaicin)	Skin sensitization, catechol precursor
16, 17-Dehydro capsaicin (aliphatic dehydrogenated capsaicin)	Skin sensitization, catechol precursor
17-Hydroxy capsaicin (ω hydroxylated capsaicin)	Skin sensitization, catechol precursor
14-Hydroxy capsaicin (Hydroxy capsaicin-3)	Skin sensitization, catechol precursor
Methyl nonenoic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Methyl nonenoyl alcohol	No hazard found
Impurities in Synthetic trans-Capsaicin	
cis-Capsaicin	Skin sensitization, catechol precursor

TABLE 2: Summary of DEREK *in silico* analysis results for catechol moiety-containing compounds, short chain carboxylic acids and positive controls for known gonadotoxicants and developmental toxicants

Compounds Screened	Results
Catechol Moiety-Containing Compounds	
Catechol	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
L-DOPA	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Dopamine	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Epinephrine	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Norepinephire	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Methyldopa	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Isoproterenol	Skin sensitization; Carcinogenicity (forestomach or glandular stomach)
Short Chain Carboxylic Acids	
Formic acid	No hazard identified
Acetic acid	No hazard identified
Proprionic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Butanoic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Pentanoic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Hexanoic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Heptanoic acid	Irritation of the forestomach, carcinogenicity of the forestomach
Octanoic acid	Irritation of the forestomach, carcinogenicity of the forestomach

TABLE 2 (continued)

Compounds Screened	Results
Positive Controls	
Gonadotoxic Drugs	
Cyclophosphamide	Mutagenicity; Carcinogenicity; Skin sensitization
Chlorambucil	Mutagenicity; Carcinogenicity; skin sensitization; Irritation of the forestomach
Developmental Toxicants	
Thalidomide	Teratogenicity
Valproic Acid	Teratogenicity

2.6.4.6 Excretion

From the Applicants Summary in Section M2.

Single-Dose ADME Studies - Rat
Single-dose ADME studies in rats (Study 7215-105 and Study 7215-135) where the elimination of ^{14}C-capsaicin was studied in rats following application of a 50 cm^2 patch over 3 hours with a target dose of 32 mg/rat, the primary route of excretion was urine. In Study 7215-105, approximately 10.4% of the radioactivity excreted in urine (including cage wash and cage wipe), 3.7% excreted in feces, and 7.3% retained in tissues and carcass over 72 hours. In Study 7215-135, over 14 days, with much less radioactivity absorbed, in males 3.36% of the dose was recovered in urine and 2.42% in feces, while in females 2.24% was recovered in urine and 1.35% in feces.

Single-Dose ADME Study - Minipigs
In the single-dose ADME study in minipigs (Study 7215-114) the elimination of ^{14}C-capsaicin was studied following application of two 50 cm^2 topical patches containing a total target dose of 64 mg/animal for a period of at least 2 hours. The primary route of excretion was urine. Two weeks after patch application, a total of 4.23% of the dose was recovered in excreta, with 2.80% eliminated in urine, 0.49% eliminated in feces and 0.94% in samples of pan/screen rinse, cage wipe and cage wash.

2.6.4.7 Pharmacokinetic drug interactions

Refer to the Clinical Pharmacology Review for the review of these studies.
The following is from the Applicants Summary in Section M2:

Cytochrome P450 Inhibition
The abilty of capsaicin to inhibit in vitro the major CYP enzymes in human liver micro somes (CYPIA2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2EI and CYP3A4/5) were investigated (Study XT045002). Capsaicin caused direct inhibition of CYPIA2, CYP2C9 and CYP2C19, with IC_{50} values of 2.1, 2.0 and 3.2 μM, respectively. Capsaicin also appeared to cause direct inhibition of CYP2B6, CYP2D6 and CYP3A4/5 (as measured by both midazolam l'-hydroxylation and nifedipine oxidation), with extrapolated IC_{50} values of 24, 18, 38 and 12 μM, respectively. Capsaicin also showed the potential to cause marked metabolism-dependent inhibition of CYP2B6.

The highest plasma C_{max} concentration of capsaicin observed in any PHN patient following a single 60-minute application of NGX-4010 is approximately 0.015 μM, 4.64 ng/mL (C116 Clinical Study Report, Subject 130-0096 at patch removal). An assessment of the in vivo correlation of the in vitro results for capsaicin was made using the $(I)/K_i$ ratio, where (I) is the plasma C_{max} concentration of the inhibitor and K_i is the inhibition constant. This ratio was calculated assuming the conditions that would provide the most conservative estimate of drug-drug interaction (i.e., competitive inhibition and zero percent protein binding). Thus, despite the CYP enzyme inhibition observed in vitro, it is extremely unlikely that capsaicin wil inhibit in vivo the clearance of concomitantly

administered drugs metabolised by any of the CYP enzymes evaluated because the $(I)/K_i$ ratio is less than 0.02 for each of the enzymes.

Cytochrome P450 Induction
The effect of capsaicin on the expression of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1 and CYP3A4/5 in primary cultures of human hepatocytes was investigated (Study XT043009). Although capsaicin (at 10 μM) caused a statistically significant increase in 7-ethoxyresorufin O-dealkylation (EROD) activity, the increase was only 8.6% of that caused by treatment of hepatocytes with omeprazole, the prototypical inducer for CYP1A2. It is, therefore, unlikely that clinically significant P450 enzyme induction would occur following topical delivery of capsaicin.

2.6.4.8 Other Pharmacokinetic Studies

There were no studies for review.

2.6.4.9 Discussion and Conclusions

Refer to the executive Summary

2.6.4.10 Tables and figures to include comparative TK summary

Human Pharmacokinetics:

Refer to the Clinical Pharmacology Review for additional information.

The highest plasma concentration measured in any PHN patient receiving a 60-minute NGX-4010 treatment was 4.64 ng/mL (Cl16: 7215-159) Subject 130-0096 at patch removal) and the highest AUC was 12.7 ng.hr/mL (Pharsight report, August 2008).

Overall, a total of 173 subjects with either PHN (96 subjects), PDN (33 subjects), or HI-AN (44 subjects) receiving NGX-4010 for 90 or 60 minutes in the above four clincal studies had plasma samples drawn for the determnation of capsaicin levels and its metabolites. Of these, 34 subjects (19 males and 15 females) with a total of44 applications (17 applications were for 90 minutes and 27 applications were for 60 minutes), including retreatments, displayed quantifiable levels of capsaicin at one or more time points. No metabolites were detected. Among the 34 subjects, 4 subjects had the patch applied to the feet and 30 subjects had the patch applied to the trunk. Overall, a total of 73 plasma concentrations of capsaicin were fitted simultaneously from a main dataset including a total of 34 subjects that displayed at least one quantifiable value in a profie. A one-comparent model with first-order absorption provided a better quality of fit than zero-order absorption models. The quality of fit improved markedly when values below the lower limit of quantitation (LLOQ) were set to 'missing' and when a lag time for absorption was used. Setting below LLOQ values to missing was deemed the most conservative approach due to the possible longer elimation half-lives derived when these

values were set to half the LLOQ. The quality of fit of capsaicin concentration profies was fuher improved when an inter-occasion variabilty was added on the apparent volume of distrbution, allowing a different value to be fitted when capsaicin treatments were applied to the painfl area on the same individua1 on different occasions (e.g., a first 90-minute applications versus subsequent 60-minute retreatments). Summary results for the final population PK analysis of the main dataset (n=34) are presented in Table l, where many of the parameters are estimated using Bayesian modeling techniques.

Table 1: Different PK Parameters for NGX-4010 as Determined by the Population PK Analysis

Parameters	Geometric Mean (CV%)
Ka (1/h)	3.40 (18.6%)
Vc/F (L)	147,000 (<1%)
Ke (1/h)	0.285 (272%)
CL/F (L/h)*	34,373 (<1%)
$T_{1/2}$ (h)*	2.16 (72.1%)
AUC (ng.h/mL)*	7.42 (36.4%)
C_{max} (ng/mL)*	1.86 (132%)

Parameters	Geometric Mean (CV%)
T_{max} (h)*	1.31 (94.2%)

Ka: rate constant of absorption, Vc/F: apparent volume of distribution, Ke: rate constant of elimination, CL/F: apparent clearance, $T_{1/2}$: elimination half-life, AUC: area under the curve, C_{max}: maximum plasma concentrations, T_{max}: time to maximum plasma concentrations.
* Bayesian estimates

Toxicokinetic in Dog after IV administration of capsaicin
(Study 7215-127)

Dose of Capsaicin (mg/kg/day, IV)	Day	Cmax (ng/mL)	
		M	F
0.03	**1**	21.4	30.8
	14	21.1	22.2
0.1	**1**	80.7	80.6
	14	60.6	68.6
0.3	**1**	301	311
	14	193	253

Toxicokinetics in Rats, single topical dose (Study 7215-106)

Dose of Capsaicin Patch	Cmax (ng/mL)		$AUC_{0-\tau}$ (ng-h/mL)	
	M	F	M	F
16 mg	38.8	61.8	748	1635
32 mg	106	136	2215	3065

Toxicokinetics in Rats, once weekly repeated dosing (Study 7215-120)

Dose of Capsaicin Patch	Day	Cmax (ng/mL)		$AUC_{0-\tau}$ (ng-h/mL)	
		M	F	M	F
16 mg	**1**	19.3	38.6	194	746
	22	32.7	127	127	594
32 mg	**1**	41.0	55.4	194	1408
	22	35.3	92.5	357	810
day 22 is the 3rd dosing application of this 4 week study (dosed 1 once per week for a 3 hour duration, for 4 weeks)					

Toxicokinetic in Dog after IV administration of capsaicin
(Study 7215-127)

Dose of Capsaicin (mg/kg/day, IV)	Day	Cmax (ng/mL)	
		M	F
0.03	**1**	21.4	30.8
	14	21.1	22.2
0.1	**1**	80.7	80.6
	14	60.6	68.6
0.3	**1**	301	311
	14	193	253

Toxicokinetics in Mouse on Day 1 (Study 7215-122)

Dose of Capsaicin Patch	C_{max} (ng/mL)		AUC_{0-24} (ng-h/mL)		T_{max} (h)	
	M	F	M	F	M	F
0.64 mg (0.16 mg/cm^2)	9.17	24.2	-	150	6	4
1.28 mg (0.32 mg/cm^2)	28.4	24.7	193	193	6	6
2.56 mg (064 mg/cm^2)	43	71.4	588	639	12	4
6.12 (1.28 mg/cm^2)	51.5	84.8	909	1026	12	6
Sampling times were 3, 4, 6, 12 and 24 hours postdose on day 1						

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Refer to the individual studies.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology

Coment on control treatments: A lidocaine or tetracaine-based local anesthetic pretreatment was used was applied to both the control and capsaicin treatment groups. There was no toxicity attributed to the the local anesthetic in these studies assessed by clinical observation, clinical chemistry, hematology and histopathology. Except for the carcinogenicity study, there was also no untreated control group with which to compare the local anesthetic treatment. In fact, for all in vivo studies, a topical anesthetic, control patch or solution, and cleansing gel were administered to the control groups, confounding the ability to attribute any noted toxicity to a particular component. There were no serious toxicity, except rare occurances of erythema and signs of slight irritation in the control groups, but it could not be attributed to a specific component.

SINGLE DOSE

Rat
Capsaicin patches (NGX-4010) were applied topically to male and female rats at 16 mg/rat or 32 mg/rat for a 3 hour duration (Study 7215-106). There were no capsaicin-related deaths. At the site application, erythema, very slight edema, and fissuring occurred within 24 hrs after patch application. An increased incidence of sores/scabs in males treated with 16 mg (1 of 10) or with 32 mg capsaicin (3 of 10) were oberseved. Male rats at 16 and 32 mg/animal exhibited moderate erythema and edema, while female rats at 32 mg/animal exhibited moderate erythema. Clinical signs of recumbency, irregular/labored respiration, and cold to touch were observed in the female rats treated with 32 mg of capsaicin, while only atonia was observed at 16 mg capsaicin. Slight reductions in body weight gain and food consumption occurred at 32 mg/rat. There were no consistent capsaicin-related effects in clinical pathology (except increased urinary potassium on day 2), organ weights, macroscopic or microscopic pathology which were examined on day 2 and at week 5. The increase in Cmax and AUC_{0-t} was roughly dose proportional. Females exhibited higher Cmax and AUC and shorter Tmax than males. Metabolites were not evaluated. The NOAEL was not established due to clinical signs of atonia, hypothermia, and dermal effects of fissuring and sores.

Toxicokinetics in Rats, single topical dose (Study 7215-106)

Dose of Capsaicin Patch	Cmax (ng/mL)		$AUC_{0-\tau}$ (ng-h/mL)	
	M	F	M	F
16 mg	38.8	61.8	748	1635
32 mg	106	136	2215	3065

Dog
Dogs were treated with capsaicin patches (0.73 mg/cm^2) at doses of 73 mg/animal or 292 mg/animal for a 3 hour duration. There were no capsaicin-related effects on mortality, clinical signs, including dermal observations. There were no capsaicin-related effects on clinical hematology or chemistry values. Both males and females had increased urinary potassium at day 2 after treatment. There were no effects on organ weight, and anatomic pathology. There was no systemic exposure in the dogs at the level of detection (10 ng/mL).

REPEAT DOSE

Rat
In rats, once weekly capsaicin patch application (16 or 32 mg/animal; 0.64 mg/cm^2) for a 3 hour duration, resulted in the production of sores/scabs at the dose site. High-dose females demonstrated reversible ataxia and recumbency, irregular/labored respiration, dilated pupils and cold to touch. Reversible dermal irritation effects included erythema, edema, atonia, desquamation and fissuring. Animals treated with capsaicin patches tended to have a higher incidence of effects than those treated with placebo patches, with females having more severe effects than males. Gross findings included crusted skin at the treatment site in the capsaicin treated females; which was reversible. Microscopic findings at the treatment site included a slightly greater incidence in capsaicin treated groups of acanthosis/ hyperkeratosis, epidermal inflammation and ulceration. Slight erosion of the stomach was observed in 3 high dose animals; edema was noted in the 2 high-dose females. GALT hyperplasia of the cecum was observed in 6 high dose animals. Acanthosis/hyperkeratosis was still noted after the recovery period although the incidence was similar between control and capsaicin groups. Systemic exposure to capsaicin occurred in most animals. Cmax and AUC increased proportionally to dose on days 1 and 22 (3rd dose), except for the AUC of males on day 1. A slow highly variable elimination was observed ($t_{1/2}$ = 15.8 to 53.4 hours); maximum plasma concentrations were observed within 2 hours of dosing.. Exposure was significantly greater in females, likely due to the increased dose on a body weight basis. A NOAEL dose was not identified as local skin irritation was observed in both dose groups and findings in the stomach and cecum were not assessed in the low dose groups.

Toxicokinetics in Rats, once weekly repeated dosing (Study 7215-120)

Dose of Capsaicin Patch	**Day**	**Cmax (ng/mL)**		**$AUC_{0-\tau}$ (ng-h/mL)**	
		M	**F**	**M**	**F**
16 mg	**1**	19.3	38.6	194	746
	22	32.7	127	127	594
32 mg	**1**	41.0	55.4	194	1408
	22	35.3	92.5	357	810
day 22 is the 3rd dosing application of this 4 week study (dosed 1 once per week for a 3 hour duration, for 4 weeks)					

Minipig
Male and female Sinclair minipigs were treated with capsaicin patches at either 128 mg/minipig (200 cm^2 patch) or 384 mg/minipig (600 cm^2 patch) for 3 hours, once a week for 4 weeks. This was followed by a 2 week recovery period in some animals. There were no capsaicin-related deaths. and no clinical observations or toxicologically important effects on body weights or clinical pathology findings. The topical application of a capsaicin patch (128 or 384mg/animal, 0.64 mg/cm^2/animal) produced primarily local irritant effects (scratching, erythema, edema) in high-dose mini-pigs. There were no toxicologically important effects on body weight, clinical pathology, and anatomic pathology. Except for two animals on Day 1, plasma samples withdrawn for toxicokinetic analysis were below the limit of detection for capsaicin (LLOQ 5 ng/mL) at all the time points on all sampling days, indicating very little to no systemic exposure to capsaicin after once-a-week patch administration for 3 hours for a period of 4 weeks. The NOAEL for this study is 384 mg/animal or ~22-45 mg/kg based on the initial body weight.

Dog
Capsaicin dissolved in diethylene glycol monoethyl ether and Dulbecco's phosphate buffered saline, was administered via intravenous infusion over a 15 minute period daily for 14 days to anesthetised dogs. The capsaicin doses were 0.03, 0.1 or 0.3 mg/kg/day. The vehicle caused marked vascular irritation at the dose administration sites. There were no deaths, capsaicin-related organ weight changes, macroscopic or microscopic observations. The only capsaicin-related clinical sign was vocalization by dogs receiving 0.3 mg/kg/day of capsaicin. The only capsaicin-related clinical pathology finding was minimally higher ALT for male and female dogs receiving 0.3 mg/kg/day; the difference was statistically significant ($p<0.05$) for females only. On the basis of these results, the NOAEL for capsaicin was 0.1 mg/kg/day. Capsaicin metabolites were not evaluated in this study.

Toxicokinetic in Dog after IV administration of capsaicin
(Study 7215-127)

Dose of Capsaicin (mg/kg/day, IV)	**Day**	**Cmax (ng/mL)**	
		M	**F**
0.03	**1**	21.4	30.8
	14	21.1	22.2
0.1	**1**	80.7	80.6
	14	60.6	68.6
0.3	**1**	301	311
	14	193	253

Mouse
The 4-week study in FVB mice was conducted for the purpose of dose selection for a Tg.AC carcinogenicity assay. Capsaicin was dissolved in [(b) (4)] (b) (4) (DGME), the same vehicle used in developing the patches, and dosed once per week at concentrations

of 0.16, 0.32, 0.64 or 1.28 mg/cm^2/week, at 0.1 mL/animal). Dermal irritation occurred only at the highest dose and included erythema, desquamation, fissuring and eschar formation. A gross sore at the skin treatment site occurred in 1 high dose female. Treatment-related microscopic findings were generally minimal to slight in severity and primarily observed at the site of treatment. They included inflammation of the epidermis and dermis, acanthosis, mast cells in the dermis, increased fibrous connective tissue, erosions and ulcers. Acanthosis was observed in all dose groups and severity increased with increasing dose; severe findings were noted in the high-dose female with the macroscopic sore. Skin erosions and minimal or moderate ulcerationoccurred in a few animals. Exposure to capsaicin increased in a generally sub-proportional manner with increasing dose. Maximum plasma concentrations were observed between 4 and 12 hours after dosing and exposures tended to be greater in females. The MTD was identified as 0.64 mg/cm^2/week, which was equivalent to that proposed for clinical use.

Toxicokinetics in Mouse on Day 1 (Study 7215-122)

Dose of Capsaicin Patch	C_{max} (ng/mL)		AUC_{0-24} (ng-h/mL)		T_{max} (h)	
	M	F	M	F	M	F
0.64 mg (0.16 mg/cm^2)	9.17	24.2	-	150	6	4
1.28 mg (0.32 mg/cm^2)	28.4	24.7	193	193	6	6
2.56 mg (064 mg/cm^2)	43	71.4	588	639	12	4
6.12 (1.28 mg/cm^2)	51.5	84.8	909	1026	12	6
Sampling times were 3, 4, 6, 12 and 24 hours postdose on day 1						

GENETIC TOXICOLOGY

Capsaicin was negative in the bacteria reverse mutation assay for mutagenicity and negative in the vivo mouse micronucleus and human peripheral blood lymphocyte chromosomal aberration assays for clastogenicity. Capsaicin showed a (weak) positive response in the in vitro mouse lymphoma assay. There was no follow-up to this positive result as the Applicant present published evidence that structural similar endogenous compounds (catecholamines) also produced positive results in the mouse lymphoma assay. A number of published studies also indicated that capsaicin produced equivocal findings in a few genotoxic assays. However, the purity of the plant-isolated capsaicin used in these studies was unknown.

An impurity, cis-capsaicin, was also qualified with the submission of a bacterial reverse mutation assay that indicated no potential for mutagenesis.

CARCINOGENICITY

The Applicant was allowed to conduct a single carcinogenicity study in a transgenetic mouse model because the human plasma levels were non-detectable for the majority of samples and they had not yet determined the frequency of application when the discussions concerning initiating the carcinogenicity studies occurred. In retrospect, the study was unnecessary according to the guidance ICH-S1A, since human administration is for a 1 hour duration at 12 week intervals. The Applicant conducted dose ranging studies in FVB mice and received ECAC recommendations for the protocol and doses which the Applicant followed in their 26-week daily dermal application study in transgenic hemizygous FVB/NTac-Tg(v-Ha-ras)TG.ACled mice. There were numerous problems with the analysis and interpretation of this study, which resulted in non-acceptance of the study by the ECAC. The study was also published with the same inappropriate analysis and conclusions. Also in retrospect, but after the initial acceptance of the study by the ECAC, this transgenic model has been determined to be inappropriate for compounds that are skin irritants such as capsaicin. A request for a DSI inspection was submitted to examine the discrepancies in the study results (IND 63354 5/13/09), and is planned to occur in mid-July 2009.

REPRODUCTIVE TOXICOLOGY

Fertility
In the fertility and general reproductive toxicology study capsaicin, rats were treated for 3 hours/day with a dermally applied capsaicin patch (identical to the clinical therapeutic patch). Males treated for 49 days developed reduced sperm motility at all dose levels (46-58% vs 85% in controls) and increased non-motile sperm in the vas deferens (25, 37.5 and 50 cm^2 patch sizes, corresponding to 16, 24 and 32 mg/rat, respectively).. Statistically reduced sperm counts were present in the mid dose in the vas deferens and high dose in the cauda epididymis, but these are not likely biologically meaningful since there was large variation associated with mean values and fertility was not affected. Histopathology of testes in previous repeated dose toxicological studies did not find evidence of abnormal sperm development. Females were treated for at least 23 days, a cycling period followed by a mating period and an additional 7 days of gestation. Capsaicin treatments did not affect rat estrous cyclicity or the incidence of pregnancy, even in those mated with males of low sperm counts. The lack of reduced fertility in the males is thought to be attributed to an excess of sperm production in this species, even though motile sperm were reduced by capsaicin treatment. All females appeared normal at necropsy.

Embryofetal development:
Rat: In the rat embryofetal developmental toxicology study, capsaicin patches were also applied daily for 3 hours/day from the beginning of organogenesis, day 7 through day 17, and fetuses were examined after Caesarean-section on day 21, the last of gestation. The low and mid capsaicin doses (25 and 37.5 cm^2 patch sizes, corresponding to 16 and 24 mg/rat, respectively) had no effect on litter reproductive parameters and there was no increase in fetal abnormalities compared to controls or historical background data. Treatment with the high dose patch (50 cm^2 patch, 32 mg/rat) resulted in reduced skeletal

ossification in the hind-limb metatarsals, and 1st sternebra. Capsaicin was not a teratogen at the doses studied in the rat.

Rabbit: In the rabbit study, capsaicin was administered as a liquid in a vehicle of diethylene glycol monoethyl ether, applied topically onto the back of the rabbits (at doses of 3, 6.5, or 13 $\mu L/cm^2$ corresponding to 60, 130, or 260 mg/rabbit). There was no effect of any dose on the litter parameters and no fetal abnormalities could be attributed to capsaicin. The developmental NOAEL was the high dose of 13 $\mu L/cm^2$ or 260 mg/rabbit. There were no signs that the maternal maximal tolerated dose or maximal feasible dose was reached. Dermal lesions were reported for all capsaicin treatment groups, but these "lesions" were not described. It is assumed they were breaks in the epidermis due to ulcers or cuts. They could have developed from capsaicin irritation and the resultant scratching or cage rubbing in an attempt to alleviate the irritation, although these behaviors were not mentioned. Although the maximal tolerated dose or maximal feasible dose was not attained in the dose-range or the main study, toxicokinetics indicate that the high dose provided a safety margin of 37X over the mean Cmax values between the first day dosing in the rabbit compared to a single patch application in humans. It is unlikely a repeat of the rabbit study to attain a maximal tolerated dose would provide additional clinically relevant information.

Pre- and post-natal toxicity

There was no major toxicity from capsaicin patch treatments in the pre- and post-natal toxicity study. Capsaicin patches were similar to those in the fertility study described above applied for 3 hours daily from gestation day 7 through postpartum day 20 (lactation day 20). All pups appeared normal at necropsy on Day 28 postpartum. There was no effect on body weight and food consumption of rats from capsaicin treated dams. Capsaicin was detected in milk ranged from < 2.5 ng/mL to 770 ng/mL and tended to be correlated to plasma concentrations of the treated dams. In tests of learning, short-term retention, long-term retention, response inhibition evaluated in a passive avoidance paradigm or water maze, there were no differences between F_1 generation rats from the capsaicin and control treated dams. Sexual development and maturation of F_1 males and females from capsaicin treated dams was similar to F_1 rats from control treated dams. There was no effect on testes or epididymis weights, or the ratios of these weights to the terminal body weight in F_1 males. Mating and fertility parameters, litter parameters, and fetal abnormalities were similar between rats from capsaicin and control treated dams.

LOCAL TOLERANCE

Primary Irritation Reaction

Due to the intense burning sensations from the application of 8% capsaicin in humans, all studies with humans and animals were conducted with a topical anesthetic pretreatment. While this may have greatly attenuated any primary irritation reaction, it is the part of the therapeutic procedure and therefore it would be appropriate to determine the nonclinical primary irritance and hypersensitivity reactions under these conditions. The primary irritancy potential of NGX-4010 patches was investigated when topically administered to rats (Study 1614-001). The occurrence of erythema was greatest in the group of rats in which the patches were applied for 1 hour and was least in the groups in

which the patches were applied for 3 hours. Eryhema resolved in all rats 1 hour after patch removaL. No skin responses occurred in rats treated with the anesthetic eream alone or the placebo patch.

Hypersenstivity Reactions
The potential of NGX-40l0 to induce delayed contact hypersensitivity was evaluated in the guinea pig using the closed patch technique (Study 7215-108). Groups were exposed to 9 induction applications of either NGX-4010 or placebo patches. Animals were challenged with NGX~4010 or placebo patches two weeks after the last induction application. Five naive animals served as controls. NGX-4010 was determined to be a weak sensitizer based on a relatively low incidence and mild severity of challenge reactions in the test group to NGX-4010 patch that exceeded the highest respective naive control reaction. The placebo patches did not induce sensitization.

SPECIAL TOXICOLOGY

Phototoxicity
The phototoxicity portion of Study 1614-001 utilzedNGX~4010 patch application times of 1, 2 and 3 hours, an application time for the placebo patch of 3 hours, and a 2 hourtime interval between patch removal and irradiation. 8~MOP (I 00mLl25 cm2) was included as a positive control. No dermal responses indicative of photo toxicity occurred in any of the Confidential Sequence #0009 Page 50 of 116 NDA 2.4 NQnclinical Overview 2.4.4.7 NGX-4010 (NDA 022,395) (groups of rats that received NGX-40 I O. Dermal responses indicative of a phototoxic response, including eryhema grades and scab formation occurred in the group of rats treated with 8~MOP. No mortality occurred and body weights were unaffected during the study.

2.6.6.2 Single-dose toxicity

RAT

Study title: Single-Dose Dermal Toxicity and Toxicokinetic Study with (b) (4) Pain Treatment System (Capsaicin) in Rats

This study was reviewed by Drs. Violetta M. Klimek and Suzanne Thorton when it was submitted to IND 63354.

Key study findings:

- Mild dermal irritation at the application site (dose dependent increase in incidence of sores/scabs and increase in severity of erythema and edema)
- Clinical signs of recumbency, irregular/labored respiration, cold to touch (female rats)
- Slight decrease in male rat body weight during weeks 1 and 2
- Urinalysis: male and female rats exhibited dose-dependent decrease in urine volume at Day 2; dose-dependent increase in chloride and potassium on Day 2;
- Gross pathological changes in the salivary glands in male and female rats, which is likely due to licking and grooming at the site of application, absorbing capsaicin residue orally
- Female rats exhibited higher Cmax and AUC and shorter Tmax
- A NOAEL was determined due to dermal fissures at the site of application, as well as atonia and hypothermia

Study no: 7215-106
E-Location: m4\42-stud-rep\423-tox\4231-single-dose-tox
Conducting laboratory and location: (b) (4)
Date of study initiation: 01 June 2001
GLP compliance: yes
QA report: yes
Drug, lot #, radiolabel, and % purity:
(b) (4) Pain Treatment System (Capsaicin), Lot 8/13009/01.
Control: placebo patch, Lot 8/13010/01
Anaesthetic lotion, 2% Tetracaine gel, Lot GLP-035
Cleansing lotion, PEG 300, lot #: GLP-034
(Note: No purity information was included on the Certificate of Analysis for any products).
Formulation/vehicle: Transdermal patch with clear plastic coating. The System consists of:
the test article - capsaicin at the concentration of 0.729 mg/cm2 ,
the vehicle - monoethyl ether, ethylcellulose, and n-heptane,
the carrier - the physical patches, supplied as 20 cm x 10 cm.

Methods

Approximately 25-30 min prior to dosing, 2.5-5.0 g of 2% tetracaine gel was applied to the site intended for the patch that was followed by application of the capsaicin or placebo dermal patch. The patch was held in place with elastic tape and remained in place for approximately 3-3.5 hrs. The patch was removed and the application site cleaned with 5-10 ml of PEG 300 "cleansing lotion" and gauze.

Dosing:
Species/strain: Rats; Crl:CD□(SD)IGS BR
#/sex/group or time point (main study): 10/male and female/group
Satellite groups used for toxicokinetics or recovery: 9 male and female/3 time points
Age: 46-52 days old
Weight: male: 235-274 g; female:118-202 g
Doses in administered units: target concentration: 0.64 mg/cm^2
actual concentration: 0.73 mg/cm^2

Capsaicin Concentration	*Area Treated or Volume*	*Average Weight (kg)*		*Average Dose (mg/kg)*	
		M	F	M	F
Control: 0.0 mg/cm^2	Control: 50 cm^2	0.255	0.167	0	0
Active: 0.73^ mg/cm^2	Low: 25 cm^2	0.254	0.177	71.9	103.1
	High: 50 cm^2	0.254	0.176	143.7	207.4

Route, form, volume, and infusion rate: dermal patch
Observations and times:
Clinical signs: twice daily (a.m. and p.m.) for mortality and moribundity; once daily, cageside observations) and once a week (detailed observations) for toxicity animals
Body weights: prior to treatment and on the first day of treatment then weekly thereafter for toxicity animals designated for terminal sacrifice
Food consumption: weekly
Ophthalmoscopy: not performed
EKG: not performed
Hematology: 24 hrs and 4 weeks after treatment
Clinical chemistry: 24 hrs and 4 weeks after treatment
Urinalysis: 24 hrs and 4 weeks after treatment
Gross pathology: 24 hrs and 4 weeks after treatment
Organs weighed: 24 hrs and 4 weeks after treatment
Histopathology: 24 hrs and 4 weeks after treatment
Toxicokinetics: pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 72 hrs
Dermal irritation: study days: 2, 8, 15, 22, and 2

Results

Mortality: All animals survived to the end of studies

Clinical signs: Checked daily

(not described in the draft report) Test article related clinical observations include recumbency, irregular/labored respiration in females treated with 32 mg capsaicin (3/9) and cold to touch (2/9 females). Slight increase in the incidence of sores/scabs in males treated with 16 mg/animal (1 out of 10 or 32 mg/animal (3 out of 10) of capsaicin were observed at the dorsal surface of the cervical and the sacral region. In addition, sores/scabs were observed at the dose site of one male and one female treated with 32 mg capsaicin. The hair growth of treated animals was similar to control animals with exception of females treated with 32 mg capsaicin that appeared to show less hair growth on day 21 of the study.

Dermal irritation: Study days 2, 8, 15, 22, 29

All dermal irritation observations appeared within 24 hrs after patch administration and generally remained present throughout the remainder of the study.
Male and female rats in all groups, including the control, exhibited well-defined erythema, very slight edema, and fissuring. Male rats at 16 and 32 mg/animal exhibited moderate erythema and atonia. Female rats at 16 mg/animal exhibited atonia while female rats at 32 mg/animal exhibited moderate erythema.

An increase in the severity of erythema and edema was observed in males treated with 16 (3/10) and 32 mg/animal (3/10) capsaicin. Similar results were observed in control animals (3/10). The increase was well defined to moderate erythema on day 8 and 15. Very slight to slight edema was observed on day 8,15, and 22. In females (32 mg/animal of capsaicin), there was an increase in severity of erythema on day 8 (well defined to moderate). The incidence of fissuring was slightly increased in the same female group on day 8 of the study.

Parameters N=10/group	Males-controls	Males-16 mg	Males-32 mg	Females-controls	Females-16 mg	Females-32 mg
Erythema	3^{C}	1^{A}, 2^{C},1^{D}	1^{C}, 3^{D}	2^{D}	2^{D}	1^{C}, 4^{D}
Edema	5^{A}	5^{A}, 3^{B}	7^{A}, 2^{B}	1^{A}	2^{A}	2^{A}
Atonia	0	2	1	0	1	0
Fissuring	3	3	3	2	2	5

Summary of 1-31 day observation: ^{A} very slight; ^{B} slight; ^{C} moderate; ^{D} well defined

Body weights: measured prior to treatment and first day of treatment

Male rats at 32 mg/animal exhibited a slight, but not statistically significant, decrease in body weight during wk. 2 (6%) and 3 (3%), but returned to control values by wk. 4.

Body weight gains were significantly decreased in male rats at 32 mg/animal during wks. 1-2 and a statistically significant increase during wks. 3-4. There was no treatment-related effects for the female rats.

Male rats treated with 32 mg capsaicin gained statistically significant less body weight then control animals (34% lower) during the first week of the study, then gained it back with a greater rate than controls. At the end of the study, their overall body weight changes were similar to those of the controls. Female rats treated with 32 mg/animal of capsaicin also gained less weight then controls during the first week of the study (40.5% lower, although this was statistically not significant). At the end of the study, the females treated with 32 mg of capsaicin gained less weight by 25.4% then their controls.

Food consumption: measured weekly

A slight, but not statistically significant, decrease in food consumption during wk.1 for male and female rats at 32 mg/animal was observed. This decrease is not considered to be treatment-related.

A slight decrease (statistically not significant) in food consumption of males treated with 32 mg/animal of capsaicin during week1 was observed (9.1% lower the controls).

Ophthalmoscopy: Not performed

Electrocardiography: Not performed

Hematology: 24 hrs and 4 wks after treatment

The determination of the effect of capsaicin on hematology was difficult due to the high variability in the control or treated group values. An increase in N-segs at wk. 5 for male rats was the only dose-dependent change observed. All values, except for the day 2 platelet value for the female rats at 32 mg were within the Historical control value range. There were no treatment-related effects observed in female rats.

The only significant differences for hematology results between the control and treated groups were:
a) mildly lower platelet count at Day 2 for males at the dose of 32mg/animal of capsaicin. This difference was considered incidental, not related to the test article administration as it was absent among females, was small, and was not correlated with any clinical and pathological findings.
b) mildly higher monocyte count (relative to the total white blood cells) at Week 5 for males at dose level of 32mg/animal. This difference was not considered toxicologically relevant because the absolute monocyte counts were within the historical control value range (not described in the draft report).

Parameters	Male – 16 mg		Male – 32 mg		Female – 16 mg		Female-32 mg	
(N=5/group)	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5
Platelets			↓16%				↑52%∴	
Retics		↑24%∴	**↑42%**					↓13%
N-segs	↓71%*	**↑56%**∴	↑17%	**↑67%**∴				

* high variability in placebo patch group values, □high variability in treated group

[Note: Values represent % difference from the control; **Bold** values are dose-dependent findings]

Clinical chemistry: 24 hrs and 4 wks after treatment

The determination of the effect of capsaicin on clinical chemistry was difficult due to the high variability in the control or treated group values. All values were within the Historical control value range. A dose-dependent increase in triglycerides in the male and female rats at wk. 5. The male rats also exhibited a dose-dependent increase in cholesterol at wk. 5 and the female rats exhibited a dose-dependent increase in AST and inorganic phosphate values at wk. 5.

The determination of the effect of capsaicin on clinical chemistry was difficult due to the high variability in the control or treated group values. All values were within the historical control value range. In general there was no other notable differences for clinical chemistry tests results between the control and treatment groups. (see: table below; values are the % difference from the control group)

Parameters	**Male – 16 mg**		**Male – 32 mg**		**Female – 16 mg**		**Female-32 mg**	
(N=5/group)	Day 2	Week 5	Day 2	Week 5	Day 2	Week 5	Day 2	Week 5
A/G Ratio			↑ 23%					
AST				↑ 9%		↑ 9%	↓ 9%	↑ 16%
Alkaline Phos						↑ 9%		↓ 11%
Inorganic Phos			↓ 6%			↑ 8%		↑ 6%
Triglycerides		↑ 72%		↑ 67%	↓ 8%	↑ 27%	↑ 15%	↑ 17%
Cholesterol		↑ 25%	↓ 6%	↑ 27%	↓ 30%			↓ 7%

Urinalysis: 24 hrs and 4 wks after treatment

The urine volume for both male and female rats at 16 and 32 mg/animal were decreased (10-39%) as compared to the placebo control group for the Day 2 collection time. It is unclear if this decrease is treatment-related, but it occurred in only the capsaicin treated groups and there was no historical control data provided when requested from the Sponsor. There were no differences in urine volume for the Week 5 collection. The determination of the effect of capsaicin on urine chemistry was difficult due to the high variability in the control or treated group values and because there were no historical control data provided. For both male and female rats, there were dose-dependent increases in chloride and potassium at Day 2 (statistically not significant due to high individual variability) and dose-dependent decreases in chloride and potassium at Week 5 (statistically significant - males at 16 mg/animal dose). Also for the male rats, there was a dose-dependent increase in excreted chloride at Week 5 and a dose-dependent increase in excreted potassium at Week 5. For the female rats, there was a decrease in excreted potassium at Week 5 for both 16 and 32/mg/animal doses of the capsaicin. The changes in the urine chemistry values on Day 2 may be related to the decrease in urine volume.

Parameters	Male – 16 mg		Male – 32 mg		Female – 16 mg		Female-32 mg	
(N=5/group)	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5
Chloride	**↑10%∴**	**↓17%***	**↑72%∴**	**↓29%***	**↑53%**		**↑79%**	
Potassium	**↑65%∴**	**↓21%***	**↑132%∴**	**↓37%***	**↑59%∴**	**↓44%***	**↑49%**	**↓39%***
Sodium							↑45%	
Excreted Chloride		**↑28%**		**↑56%**				
Excreted Potassium	↓18%	**↑9%∴**	↑15%	**↑36%∴**		**↓48%***		**↓48%***
Excreted Sodium	**↓39%**		**↓37%◆**	↑48%				↓30%

* high variability in placebo patch group values, □high variability in treated group; * $p<0.05$

[Note: Values represent % difference from the control; **Bold** values are dose-dependent findings]

Parameters	Male – 16 mg		Male – 32 mg		Female – 16 mg		Female-32 mg	
(N=5/group)	Day 2	Week 5	Day 2	Week 5	Day 2	Week 5	Day 2	Week 5
Chloride	↑10%	↓17%*	↑72%	↓29%	↑53%		↑79%	
Potasium	↑65%	↓21%*	↑132%	↓37%	↑59%	↓44%	↑49%	↓39%
Sodium							↑45%	
Excreted Chloride		↑28%		↑56%				
Excreted Potassium	↓18%	↑9%	↑15%	↑36%		↓48%		↓48%
Excreted Sodium	↓39%		↓37%	↑48%				↓30%

* statistically significant difference from the control

Organ weights: 24 hrs and 4 wks after treatment (histopathology inventory table)

There was no correlation between organ weight changes and hematology or clinical chemistry values. There was a general dose-dependent trends in changes affecting the male and female rat reproductive organs. In the male rats, there was a dose-dependent increase in the prostate weight at day 2 and wk. 5, an increase in seminal vesicle weight at day 2, a decrease in epididymis and pituitary weights at wk. 5, and an increase in kidney weight at wk. 5. In the female rats, there was a decrease in ovary weight at day 2 and wk. 5, an increase in uterus weight at day 2, and a decrease in uterus weight at wk. 5.

There was no correlation between organ weight changes and hematology or clinical chemistry values. There was no statistically significant organ weight difference (absolute or relative) at either an interim sacrifice (Day 2) or at the terminal sacrifice (Week 5) interval, although prostate was decreased by 35 –38% at Week 5.

[Note: **Bold** values are dose-dependent findings]

Parameters	Male – 16 mg		Male – 32 mg		Female – 16 mg		Female-32 mg	
(N=5/group)	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5	Day 2	Wk. 5
Adrenal				↓11%		↓11%		
Epididymis		**↓9%**		**↓6%**				
Kidney		**↑10%**		**↑7%**		↑9%	↑10%	
Liver				**↑8%**		↑8%		
Lung						↑9%		
Ovary							↓14%	↓7%
Pituitary	**↓12%**		**↓8%**	↑10%			↓11%	↓11%
Prostate	**↑17%**	**↓35%**	**↑13%**	**↓38%**				
Seminal vesicle	↑7%	↑10%						
Spleen				↓17%		↓15%		
Thymus			↓6%	↓10%	↓12%			
Thyroid/parathyroid			↓9%		↑8%			
Uterus					**↑7%**	**↓26%**	**↑28%**	**↓22%**

Gross pathology: 24 hrs and 4 wks after treatment

There were no treatment-related findings and no correlation with organ weights changes. On day 2 (interim euthanasia), the male rats at 32 mg exhibited large mandibular salivary glands (2/5) and 1/5 in each group of the male rats at 16 and 32 mg exhibited diffusely red mandibular salivary glands. Female rats in the control and 16 mg groups at an incidence of 1/5 in each group exhibited diffusely red mandibular salivary glands. There were no macroscopic findings in the male and female rats at the wk. 5 (terminal euthanasia). These changes in the mandibular salivary glands are probably a result oflicking/grooming of the exposure site.

There were no treatment-related findings. On Day 2 (interim euthanasia), the male rats at 32 mg/animal exhibited large mandibular salivary glands (2/5) and 1/5 in each group of the male rats at 16 and 32 mg/animal exhibited diffusely red mandibular salivary glands. Female rats in the control and 16 mg/animal groups at an incidence of 1/5 in each group exhibited diffusely red mandibular salivary glands. There were no macroscopic findings in the male and female rats at the Week 5 (terminal euthanasia). These changes in the mandibular salivary glands are probably a result of licking/grooming of the exposure site.

Parameters	Males			Females		
N = 5/group	0 mg	16 mg	32 mg	0 mg	16 mg	32 mg
Skin (treated) - degeneration	0	0	1	0	0	0
Epididymides –inflammation, chronic	0	0	1	0	0	0
Prostate – inflammation, chronic	0	0	1	0	0	0
Optic nerve, swelling	0	0	1	0	0	0

Histopathology: 24 hrs and 4 wks after treatment (histopathology inventory table)

There were no treatment-related or correlation with changes in organ weightsor gross pathological changes observed. All male and female rats at day 2, including the control, exhibited extramedullary hemaptopoiesis and chronic active inflammation in the liver,

alveolar macrophage infiltration, thyroglossal thyroid cyst, and plasmocytic hyperplasia and hemorrhage in the mandibular salivary glands. Female rats, including the control, also exhibited hepatocellular degeneration/necrosis. All male and female rats at wk. 5, including the control, exhibited alveolar macrophage infiltration in the lung, thyroglossal thyroid cyst, and plasmocytic hyperplasia of the mandibular lymph nodes.

Day 2 (Interim Euthanasia)

1. Prostate – male rats (1/5) at 32 mg exhibited chronic active inflammation and lymphohistiocytic infiltrate
2. Epididymis – male rats (1/5) at 32 mg exhibited chronic active inflammation

Wk. 5 (Terminal Euthanasia)

1. Optic Nerve – 1/5 male rats at 32 mg exhibited myelin swelling
2. Kidney – male rats at 16 mg exhibited cyst (1/5)and increased basophilia tubular epithelium (2/5); female rat at 32 mg (1/5) exhibited lymphocytic infiltrate
3. Liver – female rats at 16 mg (2/5) and 32 mg (2/5) exhibited lymphohistiocytic infiltration
4. Thymus – 2/5 male rats at 16 mg exhibited hemorrhage, 1/5 male rat at 32 mg exhibited lymphoid depletion
5. Pituitary – 1/5 male rats at 32 mg exhibited B-adenoma
6. Pancreas – 1/5 male rats at 32 mg exhibited acinar atrophy
7. Prostate – 1/5 male rats at 16 mg exhibited chronic active inflammation; 1/5 male rats at 32 mg exhibited acute inflammation; all male rats, including the control, exhibited lymphohistiocytic infiltration
8. Epididymis – 1/5 per group male rats at 16 and 32 mg exhibited lymphohistiocytic infiltration

There were no treatment-related gross pathological changes observed other than a slight increase in incidence of skin degeneration (1/5) and optic nerve swelling (1/5) at 32mg/animal in males. All male and female rats at Day 2, including the control, exhibited extramedullary hematopoiesis and chronic active inflammation in the liver, alveolar macrophage infiltration, thyroglossal thyroid cyst, and plasmocytic hyperplasia and hemorrhage in the mandibular salivary glands. Female rats, including the control, also exhibited hepatocellular degeneration/necrosis. Chronic inflammation of epididymides and prostate was not considered drug-related since dosing was only a single administration. All male and female rats at Week 5, including the control, exhibited alveolar macrophage infiltration in the lung, thyroglossal thyroid cyst, and plasmocytic hyperplasia of the mandibular lymph nodes.

Parameters	Males			Females		
N = 5/group	0 mg	16 mg	32 mg	0 mg	16 mg	32 mg
Thymus - lymphoid depletion	0	0	1	0	0	0
Epididymides -inflammation	0	1	1	0	0	0
Liver – lymphohistiocytic infiltrate	0	0	0	0	2	2
- infarct	0	0	1	0	0	0
Pancreas – acinar atrophy	0	0	1	0	0	0
Prostate – inflammation	0	0	1	0	0	0
- lymphohistiocytic infiltrate	2	2	3	0	0	0
Optic nerve – myelin swelling	0	0	1	0	0	0

Toxicokinetics: predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hrs

Detectable plasma levels of capsaicin were present 0.5 h after patch application in both the male and female rats and remained detectable up to 72 hrs. The Cmax and AUC values increased with increasing dose, however, the female rats exhibited a higher Cmax and AUC when compared to the male rats. The Tmax differed between the male and female rats. For the female rats, the Tmax was 3 hrs for both the 16 and 32 mg, while in the male rats, the Tmax was 12 hrs at 16 mg and 8 hrs at 32mg. It is unknown why the Tmax was different for the male rats and the Sponsor offers no explanation.

Table 6: Summary of Toxicokinetic Parameters for Capsaicin in Plasma in Rat Single-Dose Toxicity Study

Sex	Dose Level (mg/animal)	Toxicokinetics		
		C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-t} (ng•hour/mL)
M	16	38.8	12.0	748
F		61.8	3.0	1635
M	32	106	8.0	2215
F		136	3.0	3065

DOG

Study title: Single-dose dermal toxicity and toxicokinetic study with (b) (4) pain treatment system (capsaicin) in dogs.

This study was previously reviewed by Dr. Violetta M. Klimek when it was submitted to IND 63354.

Suzanne R. Thornton reviewed this study in its draft form that was submitted in the original IND package (IND 63,354 – September 21, 2001). The final study report that was requested from the Sponsor at the time of IND submission did not reveal any difference from the draft report. Therefore, Suzanne R. Thornton's conclusions from this study remain as noted (See review of October 30, 2001).

Key study findings:

- Hematology: dose-dependent increase in monocytes in the female dogs
- Clinical chemistry: dose-dependent increase in urea values on Day 2 after treatment in female dogs;
- Urinalysis: dose-dependent increase in potassium on Day 2 in females
- No systemic exposure

Study no: 7215-107
E-Location: m4\42-stud-rep\423-tox\4231-single-dose-tox\7215-107
Conducting laboratory and location: (b) (4)
Date of study initiation: 05 June 2001
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity:
Capsaicin: (b) (4) pain treatment system, Lot 8/13009/01, Purity unknown
Vehicle/Control: Placebo (b) (4) pain treatment system, Lot 8/13010/01
2% Tetracaine gel, Lot GLP-035
Cleansing lotion (PEG 300), Lot GLP-034

No purity information was included on the Certificate of Analysis for any products

Methods

Dose: 0.64 mg/cm^2; (actual dose was 0.73 mg/ cm^2)
Species: Beagle Dog
#/sex/group or time point: 18/male and female
Satellite groups used for toxicokinetics or recovery: NA
Age: 9 months dosing
Weight: male: 7.1-11.4 kg; female: 6.5-9.8 kg

Methods (unique aspects)**:** Approximately 20-30 mins prior to dosing, 0.1 g of 2% tetracaine gel was applied to the pre-clipped area to be treated which was followed by

application of the capsaicin or placebo dermal patch. The patch was wrapped with Vetwrap and secured with surgical tape. The dogs were fitted with Elizabethan collars which remained in place during the treatment, which was approximately 3-3.5 hrs. The patch was removed and the application site cleaned with 5-10 mL of PEG 300 "cleansing lotion" and gauze.

Capsaicin Concentration	*Area Treated or Volume*	*Average Weight (kg)*		*Average Dose (mg/kg)*	
		M	F	M	F
Control: 0.0 mg/cm^2	Control: 400 cm^2	9.1	8.1	0	0
Active: 0.73[A] mg/cm^2	Low: 100 cm^2	8.7	8.1	8.4	9.0
	High: 400 cm^2	8.9	8.1	32.8	36.0

Route, form, volume, and infusion rate: dermal patch
Observations and times:
Clinical signs: Daily
Body weights: prior to treatment, weekly
Food consumption: weekly
Ophthalmoscopy: Not performed.
EKG: Not performed.
Hematology: Study days –6, -2, 2, and 30
Clinical chemistry: Study days –6, -2, 2, 30
Urinalysis: Study days –6, -2, 2, 30
Gross pathology: Day 2 and 30 after treatment
Organs weighed: Day 2 and 30 after treatment (histopathology inventory table)
Histopathology: Day 2 and 30 after treatment (histopathology inventory table)
Toxicokinetics: predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hrs
Dermal irritation: Study days 1 (prior to treatment), 2, 8, 15, 22, 29

Results

Mortality: No deaths occurred.
Clinical signs: No treatment-related effects. All animals, including the control, exhibited red skin inside ears.
Dermal Irritation: No treatment-related effects.
Body weights: No treatment-related effects.
Food consumption: No treatment-related effects.
Ophthalmoscopy: Not performed.
Electrocardiography: Not performed.

Hematology: There were no treatment-related effects in the male dogs. The determination of the effect of capsaicin on hematology was difficult due to the high variability in the control or treated group values. All values were within Historical control values. In the female dogs, there was an 11% and 25% increase from the mean baseline values in monocytes detected via antibodies at 64 mg and 256 mg/animal, respectively. Clinical chemistry: The determination of the effect of capsaicin on clinical chemistry was difficult due to the high variability in the control or treated group values. All values were within the Historical control value range. There were no treatment-related effects in the male

dogs, however, there was an 8% increase from mean baseline values in the total cholesterol at 64 mg. Female dogs exhibited a dose-dependent increase in urea 24 hrs after treatment and an increase in AST values 30 days after treatment. All values, except for the AST, were within historical control values.

Parameters	**Female – 64 mg**		**Female-256 mg**	
(N=3-6/group)	Day 2	Day 30	Day 2	Day 30
AST		**↑145%***	↓22%	**↑76%***
Glucose	↑8%			
Urea	**↑31%**		**↑27%**	

* high variability

[Note: Values represent % change from mean baseline values; **Bold** values are dose-dependent findings.]

Urinalysis: The determination of the effect of capsaicin on urine chemistry was difficult due to the high variability in the control or treated group values and because there were no Historical control data provided. For the male and female dogs, there was a dose-dependent increase in chloride at day 30. For the male dogs, there was also a dose-dependent increase in potassium at days 2 and a dose-dependent decrease at day 30. Female dogs exhibited a dose-dependent increase in excreted potassium at day 2.

Parameters	**Male – 64 mg**		**Male – 256 mg**		**Female – 64 mg**		**Female-256 mg**	
(N=3-6/group)	Day 2	Day 30	Day 2	Day 30	Day 2	Day 30	Day 2	Day 30
Chloride	**↑20%**	**↑89%**		**↑79%**	**↑34%**	**↑69%**		**↑37%**
Potassium	**↑21%**	**↓46%**	**↑10%**	**↓14%**		**↑11%**		**↑23%**
Excreted Chloride					↑132%		↑32%	
Excreted Potassium				↑248%	**↑150%**		**↑4%**	

[Note: Values represent % change from mean baseline values; **Bold** values are dose-dependent findings.]

Organ weights:

There was no correlation between organ weight changes and hematology or clinical chemistry values. There were no treatment-related changes observed for the male or female dogs. The decrease in brain weights for the male dogs at 64 mg is a result of them weighing less than the control group.

Parameters	Male – 64 mg		Male – 256 mg		Female – 64 mg		Female-256 mg	
(N=3-6/group)	Day 2	Day 30	Day 2	Day 30	Day 2	Day 30	Day 2	Day 30
Adrenal		↓6%		↓6%				↑15%
Brain	↓6%	↓9%		↓9%				
Epididymis	↓6%		↑7%	↓9%				
Heart							↓9%	
Kidney	↓10%	↑12%	↓8%			↓7%		↓4%
Liver/gall bladder					↑9%		↑6%	
Lung	↓7%	↓6%		↓10%		↓8%		↓7%
Ovary							↑37%	↓27%
Pituitary					↑17%	↑11%	↑19%	↑5%
Prostate	**↓29%**	↑24%	**↓9%**	↓18%				
Seminal vesicle								
Spleen			↑26%			↑12%		
Testes	↓14%							

Gross pathology:
There were no treatment-related findings and no correlation with organ weights changes. At the day 2 (interim euthanasia), the male dogs at 256 mg, 1/3 exhibited aplasia kidney. One female dog in the 256 mg group exhibited an incidence of 1/3 exhibited unequally sized ovaries, distended uterus, and thickened mammary tissue. The female dog was not cycling as she had a normal luteal phase documented in the clinical observations. At the day 30 (terminal euthanasia), a male dog (1/3) at 64 mg exhibited a dark area in the cecum. One female dog at 256 mg at an incidence of 1/3 exhibited unequally sized ovaries, and thickened mammary tissue. All female dogs, including the control group, exhibited thickened wall of the uterus and vagina, which may indicate that they were cycling (i.e, coming into heat).

Histopathology:
There were no treatment-related findings and no correlation with organ weights gross pathological findings.
Day 2 (Interim Euthanasia)
Male Dogs:
1. Epididymis – 1/3 at 256 mg exhibited focal lymphocytic infiltration
2. Mandibular salivary glands– 1/3 at 64 and 256 mg exhibited subacute/chronic inflammation.
3. Pituitary – 1/3 at 64 and 256 mg exhibited craniopharyngeal pituitary cysts
4. Prostate – 1/3 chronic inflammation
Female Dogs:
1. Pituitary – 1/3 at 64 mg exhibited craniopharyngeal pituitary cycts and 1/3 at 256 mg exhibited a cyst
2. Thyroid – 1/3 at 64 mg exhibited a diffuse C-cell hyperplasia
Day 30 (Terminal Euthanasia)
Male Dogs:
1. Pituitary – 1/3 at 64 and 256 mg exhibited craniopharyngeal pituitary cysts
2. Mandibular salivary glands– 1/3 at 64 and 256 mg exhibited follicular lymphoid aggregates
Female Dogs:
1. Pituitary – 2/3 at 64 mg and 1/3 at 256 mg exhibited craniopharyngeal pituitary cysts

2. Skin – 1/3 at 256 mg exhibited focal acanthosis with hyperkeratosis
Toxicokinetics: There was no systemic exposure in the dogs at the level of detection (10 ng/mL).

Histopathology Inventory

Study	7215-106	7215-107	Study	7215-106	7215-107
Species	Rat	Dog	Species	Rat	Dog
Adrenals	X*	X*	Nasal cavity		
Aorta			Optic nerves	X	
Bone Marrow smear (femur)	X	X	Ovaries	X*	X*
Bone (femur)			Pancreas	X	X
Brain	X*	X*	Parathyroid	X*	X
Cecum	X	X	Peripheral nerve		
Cervix			Pharynx		
Colon	X	X	Pituitary	X*	X*
Duodenum	X	X	Prostate	X*	X*
Epididymis	X*	X*	Rectum	X	X
Esophagus	X	X	Salivary gland, mandibular	X*	X
Eye	X	X	Sciatic nerve		X
Fallopian tube			Seminal vesicles	X*	
Gall bladder		X*	Skeletal muscle (thigh)		X
Gross lesions	X	X	Skin	X	X
Harderian gland	X		Spinal cord	X	X
Heart	X*	X*	Spleen	X*	X*
Ileum	X	X	Sternum w/bone marrow	X	X
Injection site			Stomach	X	X
Jejunum	X	X	Testes	X*	X*
Kidneys	X*	X*	Thymus	X*	X
Lachrymal gland			Thyroid	X*	X
Larynx			Tongue	X	X
Liver	X*	X*	Trachea	X	X
Lungs w/bronchi	X*	X*	Urinary bladder	X	X
Lymph nodes, cervical			Uterus	X*	X
Lymph nodes mandibular	X		Vagina	X	X
Lymph nodes, mesenteric	X	X	Zymbal gland		
Mammary Gland	X	X			

X, histopathology performed
*, organ weight obtained

2.6.6.3 Repeat-dose toxicity

MINIPIG

Study title: Subchronic dermal toxicity and toxicokinetics study with (b) (4) Pain Treatment System (Capsaicin) in mini-swine with a 2-week recovery period and an exploratory phase

This study was previously reviewed by Dr. Timothy J. McGovern when it was submitted to IND 63354.

Key study findings:

- Topical application of a capsaicin patch produced primarily local irritant effects (scratching, erythema, edema) in high-dose mini-pigs.
- No definitive systemic effects were observed and there was no significant systemic exposure to capsaicin.
- The NOAEL for this study is 384 mg/animal (~ 22-45 mg/kg based on initial body weights)

Study no: 7215-113
E-location: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-113
Conducting laboratory and location: (b) (4)
Date of study initiation: September 2001
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity:
(b) (4) Pain Treatment System (capsaicin), Lot 81114301, Purity not provided; 0.64 mg/cm^2 patch concentration
Vehicle/Control: (b) (4) Pain Treatment System (placebo), Lot No. 81113301. This is a 200 cm patch containing monoethyl ether, ethyl cellulose, and n-heptane.
Anesthetic lotion containing 2% tetracaine
Cleansing lotion, 89% PEG
Demerol™ (IM, 50 or 100 mg/mL concentration)
Methods

Dose: 0, 128 mg (200 cm^2 patch), 384 mg (600 cm^2 patch) capsaicin/animal
The high dose was determined by being that area of back that could be treated without the animals could not access, the patches could be maintained for the 3 hour duration.
Species/strain: Sinclair mini-swine
#/sex/group or time point (main study): 3/sex/dose
Route, form, volume, and infusion rate: dermal topical patches were applied for a 3 hour duration, once a week for 4 weeks
Satellite groups used for toxicokinetics or recovery:; 3/sex/group; control: 2/sex/group
Age: 2-5 months old
Weight: 8.5-17.5 kg

Study design:

Group	No. of Animals Male	Female	Dose Level (mg/animal)	Dose Concentration (mg/cm^2)	Area of Patch (cm^2)
1 (Control)[a,d]	5	5	0	0	600
2 (Low)[b]	6	6	128	0.64	200
3 (High)[b]	6	6	384	0.64	600
Exploratory Phase					
4 (High)[c]	1	1	384	0.64	600
5 (High)[d]	1	1	384	0.64	600

a Animals designated for recovery sacrifice (two animals/sex/group) underwent 2 weeks of recovery following 5 weeks of dose administration.
b Animals designated for recovery sacrifice (three animals/sex/group) underwent 2 weeks of recovery following 5 weeks of dose administration.
c Animals designated for the exploratory phase were treated once on Day 7 (minimum of 3 hours) to investigate the feasibility of using Demerol™ (single dose of 2 mg/kg) as an analgesic during dosing.
d Originally Group 1 had 6 animals/sex. However, after the first dosing, one animals/sex was transferred for the exploratory phase and assigned to Group 5. These animals were subsequently treated once on Day 8 (minimum of 3 hours) to further investigate the use of Demerol™ (10 mg/kg) as an analgesic during dosing.

At least 24 hrs before dosing and weekly thereafter, the skin in the dorsal thoracic area was shaved. Sixty to 85 minutes prior to dosing, an anesthetic lotion was applied to the treatment site. Animals in groups 1-3 were exposed on days 1, 15, 22, 29 and 36 for a 3 hr exposure period. On day 1, dosing was suspended due to apparent pain behaviors displayed (scratching/rubbing at the dose site and vocalization) by animals in Group 3. Patches were removed early and 4 Group 3 animals were not dosed. An exploratory phase was added (Groups 4 and 5) to determine the feasibility of using an IM injection of Demerol as an analgesic. On Day 7, at least 30 minutes prior to dosing, the anesthetic lotion and Demerol (2 mg/kg) were applied to Group 4 animals. Since pain behaviors were still present, higher Demerol doses (10 mg/kg) were administered on Day 8 (Group 5). The 2 Group 5 animals tolerated the patches for the 3-hr interval. For the remaining doses after Day 8, 60 to 85 minutes before dosing, anesthetic lotion (0.03 g/cm2; 18 g for Groups 1 and 3, 6 grams for Group 2) was applied to the treatment site. Demerol (10 mg/kg, IM) was also given 60 to 85 minutes before dosing and a second dose was given ~ 1 hr after patch application. Patches were held in place with multi-layered sheet of gauze material, and the trunk of the animal was encased with a Spandage Instant Stretch Bandage (or equivalent). After treatment, bandage and patch were removed and the dose site was wiped with 0.06 g/cm2 (36 g for Groups 1 and 3; 12 g for Group 2 of cleansing lotion, then dried with Kim towels.

Results

Mortality: checked twice daily

There was one unscheduled death, a low dose female sacrificed in a moribund condition on day 14, with signs of hypoactivity and weight loss in the days prior to sacrifice, and histopathology indicated moderately severe chronic-active bronchopneumonia and slight

subacute inflammation in the cerebellum. This was not considered to be capsaicin related.

Clinical signs: checked twice daily

Except for scratching and rubbing of the dose site there were no capsaicin-related clinical observations. Scratching and rubbing of the dose site occurred in 3 high dose females on day 15, and in 1 low dose male and 1 high dose male on day 30.

Body weights: measured weekly

There was no effect of capsaicin on body weight.

Hematology: blood samples collected on days 5, 19, 42 and 58

There was no effect of capsaicin on hematology.

Clinical chemistry: blood samples collected on days 5, 19, 42 and 58

There was no effect of capsaicin on clinical chemistry.

Gross pathology: main study animal were anesthetized and necropsied on day 42, recovery animals were processed on day 58 after a 2 week recovery period

There were no effects of capsaicin on macroscopic observations.

Organ weights (specify organs weighed if not in histopath table):

There were no effects of capsaicin on organ weights.

Histopathology: Adequate Battery: yes
Peer review: no

There were no effects of capsaicin on histopathology. Lesions observed in several organs at terminal sacrifice, particularly the lungs, kidneys, and intestines, were present in control animals and capsaicin-treated animals. The lesions were considered to be caused by an infectious agent. A malignant melanoma arising from untreated skin (mammary gland of one LD male was not considered capsaicin-related, as they are known to occur as congenital lesions in several breeds of miniature swine.

Toxicokinetics: blood samples collected from the anterior vena cava on day 1, at 0.5, 2, 4, 7, 12, 24, 48, 72, and 96 hours postapplication. On days 15 and 36 samples were collected at 0.5, 2, 4, 7, and 24 hours, then once daily on the following 6 days (corresponding to hours 48, 72, 96, 120, 144, and 168 postapplication). Recovery animals had 1 sampel collected on day 58.

Except for two animals on Day 1, plasma samples were below the limit of detection for capsaicin (5 ng/mL) at all the time points on all the days. ,

RAT

Study title: 4-week dermal toxicity and toxicokinetics study with capsaicin patch in rats with a 2-week recovery period

This study was previously reviewed by Dr. Timothy J. McGovern when it was submitted to IND 63354.

Key study findings: Rats were treated with NGX-4010 at 16 mg/rat (25 cm^2 patch) or 32 mg/rat (50 cm^2 patch) for 3 hours, once a week for 4 weeks.

- A NOAEL dose was not identified as local skin irritation was observed in both dose groups and findings in the stomach and cecum were not assessed in the low dose groups.
- Once weekly capsaicin patch application resulted in the production of sores/scabs at the dose site. High-dose (32 mg) females demonstrated reversible ataxia and recumbency, irregular/labored respiration, dilated pupils and cold to touch.
- Reversible dermal irritation effects included erythema, edema, atonia, desquamation and fissuring. Animals treated with capsaicin patches showed greater effects than those treated with placebo patches; females tended to have more severe effects.
- Gross findings included crusted skin at the treatment site in capsaicin treated females; this finding was reversible.
- Microscopic findings at the treatment site included a slightly greater incidence in capsaicin treated groups of acanthosis/hyperkeratosis, epidermal inflammation and ulceration. Slight erosion of the stomach was observed in 3 high dose animals; edema was noted in the 2 highdose females. GALT hyperplasia of the cecum was observed in 6 high dose animals. Acanthosis/hyperkeratosis was still noted after the recovery period although the incidence was similar between control and capsaicin groups.
- Exposure to capsaicin occurred in most animals; a slow elimination was observed (t1/2 = 15.8 to 53.4 hours); maximum plasma concentrations were observed within 2 hours of dosing. AUC increased proportionally on both days except for males on day 1 (supraproportional). Exposure was significantly greater in females, likely due to the increased dose on a body weight basis.

Toxicokinetics in Rats, once weekly repeated dosing (Study 7215-120)

Dose of Capsaicin Patch	Day	Cmax (ng/mL)		$AUC_{0-\tau}$ (ng-h/mL)	
		M	F	M	F
16 mg	1	19.3	38.6	194	746
	22	32.7	127	127	594
32 mg	1	41.0	55.4	194	1408

	22	35.3	92.5	357	810
day 22 is the 3rd dosing application of this 4 week study (dosed 1 once per week for a 3 hour duration, for 4 weeks)					

Study no: 7215-120
E-location: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-120
Conducting laboratory and location: (b) (4)
Date of study initiation: February 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity:
Capsaicin patch, Lot 81114301, Purity was not provided.
Patch (10 cm x 20 cm, 200 cm^2) contains 0.64 mg/cm^2 capsaicin in a matrix of silicone adhesive, diethylene glycol monoethyl ether, and ethyl cellulose.
Vehicle/Control: Patch (200 cm^2), (b) (4) Pain Treatment System (placebo), Lot 81113301.

Methods:

Doses: placebo patch (50 cm^2), 16 mg patch (25 cm^2, 5 x 5 cm), 32 mg patch (50 cm^2, 6.25 x 8 cm)
The patches were cut to the appropriate size using a template at the study location. Since the flux rate from the patch was maximized, the only means to deliver a higher cutaneous dose to the animals was to increase the treated surface area and/or increase the duration of treatment. The highest dose level selected was defined as the portion of the animal's back that could not be groomed with the mouth if the animal was unrestrained. In addition, a dose (area) that was 1/2 of the maximal dose level was selected to investigate a dose-response relationship. The dose concentration (mg/cm^2) is equivalent to that proposed for clinical use.
Species/strain: Crl:CD (SD) IGS BR rats
#/sex/group or time point (main study): 10/sex/group
Route, form, volume, and infusion rate: patches were applied to the back for a 3 hour duration, once a week, for 4 weeks
Satellite groups used for toxicokinetics or recovery: Recovery: 5/sex/group
TK: 9/sex/group
Age: 69-75 days old
Weight: males: 325-432 g; females: 211-266 g
Study design:

	# of animals					
Group	Male	Female	Dose (mg/animal)	Dose (mg/kg)*	Dose Conc. (mg/cm^2)	Area of patch (cm^2)
1 (control)[a]	15	15	0	0	0	50
2 (low)[a]	15	15	16	45-64	0.64	25
3 (high)[a]	15	15	32	90-128	0.64	50
Exploratory						
4 (low)[c]	9	9	16	45-64	0.64	25
5 (high)[d]	9	9	32	90-128	0.64	50

a animals designated for recovery sacrifice (2/sex/group) underwent 2 weeks of recovery following 5 weeks of dose administration

* Reviewer's calculations using approximated body weights of 350 mg (males) and 250 mg (females)

Site of application was a patch size area (25 cm^2 or 50 cm^2) of dorsal skin at the intrascapular region for the once weekly dermal application to intact skin for a 3 hr exposure period. At least once prior to day 1 and ~ 1-4 hrs prior to patch application (days 1, 8, 15 and 22), an area of dorsal skin larger but including the application site was clipped free of hair. On dosing days, an anesthetic lotion was applied to the site and was removed prior to patch application. Patches were held in place by elastic tape over the entire patch area. At the end of the ~ 3 hr patch application, the dressing was removed, patches were discarded or collected and the sites were wiped with cleansing lotion to remove any remaining test or control article.

Results

Mortality: One male from the TK group died on day 7 and a HD female died on day 8 after anesthetic exposure but before patch application. These deaths were not considered to be treatment related.

Clinical signs: checked twice daily

Sores/scabs at the dose site were the predominant findings. High-dose females demonstrated significant ataxia and recumbency as well as irregular/labored respiration. Females may have been more sensitive due to their smaller size compared to males. Additional findings in females included dilated pupils and cold to touch. These findings were reversible during the recovery period. Clinical signs in rats following dosing with Capsaicin patch (days 1-29).

Observation		Males			Females		
		C	LD	HD	C	LD	HD
	n=15	15	15	15	15	15	15
Ataxic		0	0	0	1	0	9
Hypoactive		0	1	2	2	0	1
Recumbent	Lateral	0	0	0	7	1	10
	Sternal	0	0	0	0	0	2
Sensitive to touch		0	0	1	0	0	0
Clear, oral discharge		0	0	0	0	0	1
Dilated pupils		0	0	0	1	0	6
Irregular respiration		0	0	0	5	0	9
Labored respiration		0	0	0	0	0	3
Red skin, dose site		1	0	0	0	2	0
Sore/scab – dose site		9	11	13	13	15	14
Cold to touch	Limbs	0	0	0	0	0	4
	Tail	0	0	0	0	0	2
	Entire body	0	0	0	0	0	5
Recovery phase	n=5						
Sore/scab	dose site	1	0	1	1	0	0
	sacral	2	3	3	2	4	2

Dermal irritation: scored on days 2, 9, 16, 23 and 29 and days 36 and 43 (recovery)

Dermal irritation correlated with the above noted clinical signs. Irritation effects included very slight to moderate/severe erythema, very slight to slight edema, atonia, desquamation and fissuring. Animals treated with capsaicin patches showed greater effects that those treated with placebo patches and females tended to have more severe effects. No irritation was noted during the recovery period.

Dermal irritation in rats following dosing with Capsaicin patch (days 1-29).

Observation	Males			Females		
	C	LD	HD	C	LD	HD
n =	15	15	15	15	15	15
Erythema						
None	15	15	15	15	15	15
Very slight	4	15	13	14	15	14
Well-defined	0	2	2	0	6	7
Moderate to severe	0	1	0	0	0	0
Edema						
None	15	15	15	15	15	15
Very slight	2	8	3	4	11	10
Slight	0	2	0	0	0	2
Atonia	15	14	15	15	12	15
Desquamation	0	5	5	2	10	7
Fissuring	0	3	2	3	8	4

Body weights:

No significant treatment-related effects were observed.

Food consumption: days 1-7, 8-14, 15-21, 22-27, 29-35 and 36-41.

No significant treatment-related effects were observed.

Ophthalmoscopy: once pre-dose and during week 4.

No significant drug-related effects were observed.

EKG: not assessed

Hematology: day 29 and 43 (recovery)

No significant drug-related effects were observed.

Clinical chemistry: day 29 and 43 (recovery)

No significant drug-related effects were observed.

Urinalysis: day 29 and 43 (recovery)

No significant drug-related effects were observed.

Gross pathology: at necropsy on day 29 and 2 weeks later (recovery)

The only drug-related finding was an increased incidence of crusted skin at the treatment site in capsaicin treated females. This observation was no longer apparent at the end of the recovery period.

Gross observations in rats following repeat dosing with Capsaicin patch.

Observation	Males			Females		
	C	LD	HD	C	LD	HD
n=	10	10	10	10	10	10
Skin, treated area - crusted	3	4	3	3	8	8
Recovery n=	5	5	5	5	5	5
Skin, treated area - crusted	1	0	1	0	0	0

Organ weights: at necropsy; see Histopathology Inventory table

No significant treatment-related effects were observed.

Histopathology: at necropsy, tissues from animals in the control and high-dose groups were examined as well as animals that died or were sacrificed at an unscheduled interval; see Histopathology Inventory table for tissues that were assessed.

Microscopic findings were noted at the treatment site with a slightly greater incidence in capsaicin treated groups. Findings included acanthosis/hyperkeratosis, epidermal inflammation and ulceration. Acanthosis/hyperkeratosis was still noted after the recovery period although the incidence was similar between control and capsaicin groups. Slight erosion of the stomach was observed in 3 high dose animals; edema was noted in the 2

females. GALT hyperplasia of the cecum was observed in 6 high dose animals. Stomach and cecum findings were not evaluated in the low-dose group.

Microscopic observations in rats following repeat dosing with Capsaicin patch.

Observation	Males			Females		
	C	LD	HD	C	LD	HD
n=	10	0	10	10	0	10
Stomach, glandular						
Erosion	0		1	0		2
Edema	0		0	0		2
Cecum						
Hyperplasia (GALT)	1		3	0		3
Skin, treated n=	10	4	10	10	8	10
Acanthosis/hyperkeratosis	10	4	10	8	8	9
Suppurative epidermal inflam	0	0	2	2	6	2
Ulceration	0	0	1	2	3	3
Recovery n=	5	0	5	5	0	4
Skin, treated area						
Acanthosis/hyperkeratosis	5		5	1		1

Toxicokinetics: TK animals were divided into 3 groups of 3/sex/treatment group. One group was bled pre-dose and ~ 7 hours and 2 and 5 days following patch application. The second group was bled ~ 0.5 and 12 hours and 3 and 6 days after patch application. The third group was bled ~ 2 and 24 hours and 4 and 7 days after patch application. Post-dose collection intervals began at the time when patches were applied to each animal. Analysis by HPLC method with lower limit of detection of 10 ng/ml.

Exposure to capsaicin occurred in most animals of the TK groups receiving capsaicin patches and increased with the increase in dose. A slow elimination was observed (t1/2 = 15.8 to 53.4 hours). Maximum plasma concentrations were observed within 2 hours of dosing and Cmax increased proportionally on Day 1 but stayed the same or decreased on day 22. AUC increased proportionally on both days except for males on day 1 (supraproportional). Exposure was significantly greater (2 to 5-fold) in females, likely due to the increased dose on a body weight basis.

Group	Dose level (mg/animal)	Sex	Cmax (ng/ml)	Tmax (hours)	AUC_{0-t} (ng.hr/ml)	AUC_{0-48} (ng.hr/ml)	AUC_{0-72} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	$t_{1/2}$ (hours)
Day 1									
Group 4	16	M	19.3	2	194	62.8	194	NA	NA
		F	38.6	2	746	381	628	1421	47.6
Group 5	32	M	41	2	1408	779	1408	2084[a]	25.9[a]
		F	55.4	2	2378	1349	1938	3484	53.4
Day 22									
Group 4	16	M	32.7	2	127	175	175	NA	NA
		F	127	2	594	664	664	815	26.2
Group 5	32	M	35.3	2	357	357	399	436[a]	15.8[a]
		F	92.5	2	810	810	1011	NA	NA

a: Values should be approached with caution. Only 2 timepoints used in K_e calculation.

DOG

Study Title: 14-Day Intravenous Infusion Toxicityand Toxicokinetic Study with Capsaicin in Dogs

Key study findings: Capsaicin dissolved in [redacted] (diethylene glycol monoethyl ether, DGME) and Dulbecco's Phosphate Buffered Saline, was administered via intravenous infusion over a 15-minute period to anesthetized dogs at capsaicin dose levels of 0.03, 0.1 or 0.3 mg/kg/day each day for 14 days. The vehicle itself caused marked vascular irritation at the dose administration sites. There were no deaths, no capsaicin-related organ weight changes, and no macroscopic or microscopic observations. The only capsaicin-related clinical signs was vocalization during dosing (under anesthesia) by the dogs receiving 0.3 mg/kg/day capsaicin. An elevation in ALT occurred in male and female at the high dose, 0.3 mg/kg/day capsaicin. On the basis of these results, the NOAEL for capsaicin was 0.1 mg/kg/day, corresponding to a Cmax of 81 mg/mL on the first dosing day and 64.5 ng/mL on day 14. Capsaicin metabolites were not evaluated in this study

Toxicokinetic in Dog after IV administration of capsaicin
(Study 7215-127)

Dose of Capsaicin (mg/kg/day, IV)	Day	Cmax (ng/mL)	
		M	F
0.03	**1**	21.4	30.8
	14	21.1	22.2
0.1	**1**	80.7	80.6
	14	60.6	68.6
0.3	**1**	301	311
	14	193	253

Data from this study have been published (Chanda S, Mould A, Esmail A, Bley K. Toxicity studies with pure *trans*-capsaicin delivered to dogs via intravenous administration. Regul Toxicol Pharmacol 2005;43:66–75).

Study No.: 7215-127
E-Location: m4\42-stud-rep\423-tox\4232-repeat-dose-tox\7215-127
Conducting laboratory and location: [redacted]
Date of study initiation: July 7, 2003
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
Capsaicin, Lot F0010103, purity 98.7% (impurity: zucapsaicin 0.73%)

Vehicle: (b) (4) (Diethylene glycol monoethyl ether, 99%) and Dulbecco's phosphate bufered saline (2:1 v/v final dilution)

Toxicokinetic in Dog (IV administration of capsaicin) Study 7215-127

Dose of Capsaicin (mg/kg/day, IV)		0.03		0.1		0.3	
	Day	M	F	M	F	M	F
Cmax (ng/mL)	1	21.4	30.8	80.7	80.6	301	311
	14	21.1	22.2	60.6	68.6	193	253

Methods

Doses: 0, 0.03, 0.1, 0.3 mg capsaicin/kg/day
Analysis of the concentration for capsaicin was within +10% of the target concentration, except for the 0.06 mg/mL (0.03 mg/kg/day) group on Day 8 when the mean value was 111.2%.

Species/strain: beagle dogs

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: intravenous infusion over 15 minutes for all doses, 0.5 mL/kg, administered via the cephalic veins (or saphenous vein if necessary).

Satellite groups used for toxicokinetics or recovery: used the same animals as the main study

Age: 8-10 months

Weight: males: 8.0 to 9.7 kg; females: 6.7 to 8.9 kg

Unique study design or methodology (if any):

Group	No. of Dogs Male	No. of Dogs Female	Capsaicin Dose Level[b] (mg/kg/day)	Capsaicin Dose Concentration (mg/mL)
1 (Control)[a]	3	3	0	0
2 (Low)	3	3	0.03	0.06
3 (Mid)	3	3	0.1	0.2
4 (High)	3	3	0.3	0.6

a Group 1 received the vehicle only.
b Doses were administered at a volume of 0.5 mL/kg over a period of approximately 15 minutes/day for 14 days.

Prior to dosing, dogs were lightly anesthetized with with a half-dose (0.055 mL/kg) of a ketamine HCl/xylazine cocktail administered IM (the cocktail is made by mixing 7 mL of 100 mg/mL ketamine HCI with 3 mL of 100 mg/mL xylazine). Anesthetic doses were adjusted as necessary. The dogs were suspended in slings during dosing.

***Reviewer Comment*:** There were a few important protocol deviations not mentioned in the text of the study report. On day 12, dose administration to a high dose male (#H04622) was ended early due to signs of distress. This animal was not dosed the following day, on day 13. On day 13, the dosing catheter was placed in the right jugular vein for a control female (#H04635) and a low dose female (#H04633). Also on day 13, a high dose female (#H04628) ended early due to signs of distress. These were unlikely to alter the study results or conclusion, but there were only 3 animals per sex per group, so the potential for strongly influencing the interpretation of the results exists.

Results

Mortality: checked twice daily

There were no deaths.

Clinical signs: checked twice daily

All dogs administered the high dose, 0.3 mg/kg/day,) vocalized during dosing despite anesthesia, but not on all days. In general, this occurred more frequently later in the study and more in male dogs than in female dogs. The anesthetic was increased to attempt to counter an apparent tolerance. However, toward the end of the study, there were 3 cases in which infusions were terminated early due to animal distress, but these distress observations were not described.

Clear nasal discharge and excessive salivation occurred in all groups, but tended toward a greater indence in the 0.1 or 0.3 mg/kg/day dose groups compared to controls. Slight tremors (head, limbs, and/or body) also occurred in all groups during the study. These mainly occurred during the dosing period, but some occurred before or afterwards. One male and one female in the high dose group urinated during the infusions. This occurred on multiple occasions in both animals.

Occasional incidences of swollen and/or limited use of the limbs were noted in some animals, and these were considered to be caused by the dosing procedure. As the study progressed, the condition of the dosing veins deteriorated (as indicated by the pathology findings for the dosing sites), so that, in addition to using the cephalic veins, the saphenous, and occasionally jugular veins, were used for dosing.

NUMBER OF ANIMALS AFFECTED

CATEGORY / KEYWORD / QUALIFIER	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	DOSE:	0	0.03	0.1	0.3	0	0.03	0.1	0.3
	NUMBER:	3	3	3	3	3	3	3	3
*** TOP OF LIST ***									
APPEARANCE									
LIMITED USE									
LIMB-FRONT-LEFT		0	0	0	0	0	1	0	0
LIMB-HIND-LEFT		0	0	1	0	0	0	0	0
SWOLLEN									
LIMB-FRONT-LEFT		0	0	0	1	0	1	0	0
LIMB-HIND-LEFT		0	1	1	0	1	0	0	0
LIMB-HIND-RIGHT		0	1	0	1	0	0	0	0
TREMORS									
HEAD-ENTIRE		2	0	3	3	1	0	0	3
LIMB-FRONT-LEFT		0	0	0	1	0	0	1	1
LIMB-FRONT-RIGHT		0	0	0	0	0	0	1	0
LIMB-HIND-LEFT		0	0	0	3	0	0	0	2
LIMB-HIND-RIGHT		0	0	0	1	0	0	1	0
LIMBS-ALL		1	0	0	1	0	1	2	0
LIMBS-HIND		0	0	0	1	0	0	0	0
BODY-ENTIRE		2	0	2	2	2	0	0	2
BEHAVIOR									
VOCALIZATION DURING DOSING		0	0	0	3	0	0	0	3
DISCHARGE									
NASAL									
CLEAR		1	2	2	3	2	2	1	3
VOMITUS									
YELLOW IN COLOR		0	0	1	0	1	0	1	0

NUMBER OF ANIMALS AFFECTED

CATEGORY / KEYWORD / QUALIFIER	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	DOSE:	0	0.03	0.1	0.3	0	0.03	0.1	0.3
	NUMBER:	3	3	3	3	3	3	3	3
DISCHARGE									
VOMITUS									
CONTAINING FOOD		0	0	1	0	2	0	0	1
FOAMY		0	0	0	0	1	0	0	0
EXCESSIVE SALIVATION		1	2	3	2	0	0	1	3
URINE DISCOVERED WHEN DOG REMOVED FROM SLING									
FOLLOWING DOSE		0	0	0	1	0	0	0	0
URINATION DURING DOSING		0	0	0	0	0	0	0	1
APPEARS TO BE CYCLING		0	0	0	0	1	0	0	2
DISCHARGE UNKNOWN SOURCE									
FOUND IN PAN									
RED IN COLOR		0	0	0	1	0	0	1	0
EXCRETION									
FEW FECES		0	0	0	0	0	1	0	0
MUCOID FECES		0	0	0	0	0	0	0	1
NON-FORMED FECES		3	3	1	2	3	3	3	2
EYES									
CLEAR DISCHARGE									
EYE-LEFT		0	0	0	1	0	0	1	1
EYE-RIGHT		0	0	0	1	1	0	0	1
EYES		0	1	1	3	1	1	1	2
CHERRY EYE									
EYE-LEFT		0	0	0	0	0	0	0	1
CLOUDY DISCHARGE									
EYE-LEFT		0	0	0	0	0	0	0	1

NUMBER OF ANIMALS AFFECTED

CATEGORY / KEYWORD / QUALIFIER	SEX:	MALE				FEMALE			
	GROUP:	1	2	3	4	1	2	3	4
	DOSE:	0	0.03	0.1	0.3	0	0.03	0.1	0.3
	NUMBER:	3	3	3	3	3	3	3	3
SKIN & PELAGE									
ALOPECIA									
DORSAL		0	1	0	0	0	0	0	0
BROKEN SKIN									
PAW-HIND-RIGHT		0	0	0	0	0	0	1	0
RED SKIN									
LIMB-FRONT-LEFT		0	0	0	0	0	1	0	0
LIMB-HIND-LEFT		0	1	1	1	0	0	1	0
PAW-FRONT-LEFT		0	0	0	0	0	0	0	1
INSIDE EARS		0	0	0	0	0	0	0	1

Body weights: obtained weekly

There was a general trend for a slow reduction in body weight, but this was unlikely to be toxicological important for the 2 weeks of the study. The body weights of males at the high dose, 0.3 mg/kg/day were approximately 7% lower than the controls (8.5 vs 9.1 kg). Over the duration of the study, males in this group lost approximately 0.4 kg, whereas the controls gained 0.1 kg. Female controls lost 0.2 kg and the high dose females lost 0.4 kg.

Food consumption: obtained weekly

By the end of the study, the food consumption of dogs administered 0.3 mg/kg/day was approximately 11% and 12% lower, for males and female respectively, than the controls,.

Hematology: Samples were obtained from the jugular vein twice prior to initiation of treatment and at termination, day 15.

A few animals, including controls, had notably high neutrophil counts that were likely secondary to inflammatory lesions at injection sites.

Clinical chemistry: Samples were obtained from the jugular vein twice prior to initiation of treatment and at termination, day 15.

An elevation in ALT occurred in male and female at the high dose, 0.3 mg/kg/day capsaicin, statistically significant for females.

Serum ALT concentrations

		Males				**Females**			
Dose (mg/kg/day)		**0**	**0.03**	**0.1**	**0.3**	**0**	**0.03**	**0.1**	**0.3**
ALT (IU/L)	**Day -1**	30	32	26	29	26	27	29	27
	Day 15	41	**75**	39	**55**	32	34	40	**52**

Urinalysis: Samples were taken twice prior to initiation of treatment and at termination. Urinalysis was excluded from the second prestudy interval.

There were no capsaicin-related effects.

Gross pathology: Animals were sacrificed and necropsied on Day 15.

There were no capsaicin-related macroscopic observations. Thrombosis was noted at the intravenous injection sites in all dose groups and therefore due to administration of the vehicle, (b) (4) , Other lesions observed with the thrombosis included inflammation, fibrosis, edema, and hemorrhage.

Organ weights (specify organs weighed if not in histopath table):

Differences from the control weight occurred for prostate and female adrenal weights, however there were no correlating macroscopic or microscopic findings.

	Males				**Females**			
Dose (mg/kg/day)	**0**	**0.03**	**0.1**	**0.3**	**0**	**0.03**	**0.1**	**0.3**
Prostate (g) **(% BW)**	8.95 0.099	8.99 0.100	6.99 0.077	**5.39** **0.010**				
Adrenal (g) **(% BW)**					0.85 0.011	0.84 0.011	**1.15** **0.016**	1.06 **0.014**

Histopathology: Adequate Battery: yes
Peer review: no

There were no capsaicin-related histopathological observations other than the vein lesions noted in the macroscopic observations.

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		SEX:	-MALE-				-FEMALE-			
		GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
		NUMBER:	3	3	3	3	3	3	3	3
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:		3	3	3	3	3	3	3	3
	NOT REMARKABLE:		2	3	3	2	3	3	2	2
--CYST			1	0	0	0	0	0	0	0
--VACUOLATION			0	0	0	1	0	0	1	1

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		3	3	3	3	3	3	3	3
LT CEPHALIC VEIN (IS0)	NUMBER EXAMINED:	3	3	3	3	3	3	3	3
	NOT REMARKABLE:	0	0	0	0	1	1	0	0
--DEMODEX SP.		0	1	0	0	0	0	0	0
--EDEMA		0	0	0	1	0	1	0	0
--FIBROSIS		0	0	0	1	1	0	1	0
--HEMORRHAGE		1	1	2	1	0	1	0	1
--INFILTRATE, LYMPHOHISTIOCYTIC		0	1	1	0	2	0	1	0
--INFILTRATE, LYMPHOHISTIOCYTIC, HAIR FOLLICLES		1	1	0	0	0	0	0	0
--INFLAMMATION, ACUTE		0	0	1	0	0	1	0	0
--INFLAMMATION, CHRONIC-ACTIVE		0	1	0	1	0	1	1	0
--THROMBUS		2	2	1	3	2	1	3	2
RT CEPHALIC VEIN (IS1)	NUMBER EXAMINED:	3	3	3	3	3	3	3	3
	NOT REMARKABLE:	0	1	0	0	0	1	0	0
--ACUTE INFLAMMATORY EXUDATE		0	0	0	0	0	1	0	0
--EDEMA		0	0	0	1	1	0	0	1
--FIBROSIS		1	1	0	1	1	1	1	0
--HEMORRHAGE		2	0	0	1	1	0	0	3
--INFILTRATE, LYMPHOHISTIOCYTIC		1	1	0	0	2	0	3	1
--INFILTRATE, LYMPHOHISTIOCYTIC, HAIR FOLLICLES		0	1	1	0	0	0	0	0
--INFLAMMATION, ACUTE		0	0	1	0	0	0	0	0
--HEMORRHAGE		2	2	0	2	2	3	1	3
--INFILTRATE, LYMPHOHISTIOCYTIC		1	0	1	0	2	1	2	0
--INFILTRATE, LYMPHOHISTIOCYTIC, HAIR FOLLICLES		0	1	0	1	0	0	0	0
--INFLAMMATION, ACUTE		0	1	0	0	0	0	1	0
--INFLAMMATION, CHRONIC-ACTIVE		1	0	1	1	1	2	1	1
--THROMBUS		2	2	2	3	2	3	3	1
	NOT REMARKABLE:	0	1	0	0	0	1	0	0
--INFLAMMATION, CHRONIC-ACTIVE		0	0	0	1	0	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	0	0	1	0	0	0	0
--THROMBUS		1	1	2	2	3	1	3	3
LEFT SAPHEN VEIN (IS2)	NUMBER EXAMINED:	3	3	3	3	3	3	3	3
	NOT REMARKABLE:	1	0	0	0	0	0	0	1
--ACUTE INFLAMMATORY EXUDATE		0	0	0	0	0	1	0	0
--DEGENERATION, MYOFIBER		0	0	1	0	0	0	0	0
--DEMODEX SP.		0	0	0	1	0	0	0	0
--EDEMA		1	0	2	2	0	0	0	0
--FIBROSIS		0	0	0	1	2	1	0	0
--HEMORRHAGE		2	1	1	2	2	2	1	1
--INFILTRATE, LYMPHOHISTIOCYTIC, HAIR FOLLICLES		0	0	0	1	2	2	1	1
--INFLAMMATION, ACUTE		0	0	0	0	0	0	1	0
--INFLAMMATION, CHRONIC-ACTIVE		2	0	2	0	1	0	1	0
--NECROSIS		0	1	0	0	0	0	0	0
--THROMBUS		1	3	1	3	3	3	2	0
RT SAPHEN VEIN (IS3)	NUMBER EXAMINED:	3	3	3	3	3	3	3	3
	NOT REMARKABLE:	0	0	1	0	0	0	0	0
--ACUTE INFLAMMATORY EXUDATE		0	0	0	0	0	0	1	0
--EDEMA		0	1	0	1	1	0	1	0
--FIBROSIS		0	1	0	0	1	0	0	0

Toxicokinetics: Samples were taken predose, approximately 15 (end of infusion) and 30 minutes, and 1, 3, 6, 12, and 24 hours after the start of infusion on days 1 and 14. Plasma and toxicokinetic analysis was analyzed for capsaicin by (b) (4). Metabolites were not determined. Most levels in plasma were below the limit of quantitation by 0.5 hours after the start of infusion. Thus, toxicokinetic analysis was limited to the examination of the maximum observed concentration (C_{max}), which occurred immediately following end of

infusion (0.25 hours). C_{max} was determined by visual inspection of the plasma concentration-time profiles.

After intravenous administration, capsaicin was rapidly eliminated and measurable values were only obtained immediately after the end of infusion (0.25 hours) in the 0.03 and 0.1 mg/kg/day dose groups. In the 0.3 mg/kg/day dose group, measurable values were obtained out to 0.5 hours in all dogs on Day 1, and on day day 14, only one dog still had a measurable value at this timepoint. The lower limit of quantification was 10.0 ng/mL.

Mean C_{max} values on Day 1 were generally higher than on Day 14. The increases in mean C_{max} for males and females were roughly proportional to the increase in the dose.

Summary of Plasma Concentrations Immediately Post-Infusion for Capsaicin in Dog Plasma (C_{max})

Dose Group	Dose Level (mg/kg/day)	Gender		Day 1 C_{max} (ng/mL)	Day 14 C_{max} (ng/mL)
2	0.03	M	Mean	21.4	21.1
			SD	3.5	2.0
			N	3	3
		F	Mean	30.8	22.2
			SD	9.4	7.3
			N	3	3
3	0.1	M	Mean	80.7	60.6
			SD	17.7	32.5
			N	3	3
		F	Mean	80.6	68.6
			SD	3.5	13.4
			N	3	3
4	0.3	M	Mean	301	193
			SD	90	110
			N	3	3
		F	Mean	311	253
			SD	91	49
			N	3	3

MOUSE

Study title: <u>Four-week dose range-finding dermal toxicity and toxicokinetic study with Capsaicin in FVB/N mice</u>

This study was reviewed by Dr. Timothy J McGovern in June 2003 when it was submitted to IND 63354

Key study findings:

- Dermal sores were observed in several male animals with an increase in number relative to capsaicin dose (capsaicin dissolved in (b) (4) dosing once per week at concentrations of 0.16, 0.32, 0.64 or 1.28 mg/cm /week); 3 HD females exhibited sores.
- Dermal irritation was noted only at the highest dose and included erythema, which became well defined and persisted until terminal sacrifice in one of the

females. Desquamation was also observed in high dose mice while fissuring and eschar formation were noted in one female.

- A gross sore at the skin treatment site occurred in 1 high dose female.
- Treatment-related microscopic findings were generally minimal to slight in severity and primarily observed at the site of treatment. They included inflammation of the epidermis and dermis, acanthosis, mast cells in the dermis, increased fibrous connective tissue, erosions and ulcers.
- Acanthosis was observed in all dose groups and severity increased with increasing dose; severe findings were noted in one high-dose female that was correlated with a gross sore.
- Skin erosions were observed in males at doses of 1.28 mg/animal/wk or greater; the finding was noted in a control female as well as some treated animals. Minimal ulceration was observed in one male and one female while moderate ulceration was noted in one high-dose female.
- Exposure to capsaicin occurred in most animals of the TK groups and increased in a generally sub-proportional manner with increasing dose. Maximum plasma concentrations were observed between 4 and 12 hours after dosing. Exposures tended to be greater in females.
- The MTD was identified as 1.28 mg patch (0.64 mg/cm^2) per week; this concentration is equivalent to that currently proposed for clinical use.

Toxicokinetics in Mouse on Day 1 (Study 7215-122)

Dose of Capsaicin Patch	C_{max} (ng/mL)		AUC_{0-24} (ng-h/mL)		T_{max} (h)	
	M	F	M	F	M	F
0.64 mg (0.16 mg/cm^2)	9.17	24.2	-	150	6	4
1.28 mg (0.32 mg/cm^2)	28.4	24.7	193	193	6	6
2.56 mg (064 mg/cm^2)	43	71.4	588	639	12	4
6.12 (1.28 mg/cm^2)	51.5	84.8	909	1026	12	6
Sampling times were 3, 4, 6, 12 and 24 hours postdose on day 1						

Study no: 7215-122
E-Location: m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud
Conducting laboratory and location: (b) (4)
Date of study initiation: January 2003
GLP compliance: Yes
QA report: No; the protocol states that study specific audits will not be conducted but departmental audits are routinely conducted
Drug, lot #, radiolabel, and % purity:
Synthetic capsaicin, F0010102, Purity 99.1%
vehicle: (b) (4) (diethylene glycol monoethyl ether, 99%

Methods

Vehicle or test article was applied to the dorsal skin on day 1 and every 7 days thereafter for at least 4 weeks (dosing based on most recent body weight).
The vehicle or test article will be applied using a microliter pipette. After application, the dose area was covered with a Johnson & Johnson 2 x 2 piece of gauze, secured with 3M Micropore Surgical Tape, wrapped with a Co-Flex Cohesive Flexible Bandage and an Elizabethan-type collar was placed on each mouse for ~ 3 hours. An anesthetic cream (ELA-Max) was applied (~ 0.1 ml/animal) to the treatment site ~ 30 minutes before the application of test article or vehicle. After 30 minutes, any residual ELA-Max was wiped off before the vehicle or test article application. Approximately 3 hours after application of vehicle or test article, the dose area was cleaned with a cleansing lotion (~0.2 ml/animal)

Dosing:
Doses: 0, 0.64, 1.28, 2.56, 5.12 mg/animal/week see table below
Species/strain: Mice/FVB/NTac
#/sex/group or time point (main study): 6
Satellite groups used for toxicokinetics or recovery: 15/sex for TK
Age: 8 weeks
Weight: males: 20.6-27.6 g; females: 18.1-23.3 g

	# of animals				
Group	Male	Female	Dose* (mg/animal/week)	Dose** (mg/kg)	Dose/surface area/wk (mg/cm^2/week)
1 (b) (4) - 100%	6	6	0	0	0
2 low	6	6	0.64	21	0.16
3 mid-low	6	6	1.28	43	0.32
4 mid-high	6	6	2.56	85	0.64
5 high	6	6	5.12	171	1.28
6 Transcutol – 100%	3	3	0	0	0
7 low	15	15	0.64	21	0.16
8 mid-low	15	15	1.28	43	0.32
9 mid-high	15	15	2.56	85	0.64
10 high	15	15	5.12	171	1.28

* Based on an application area of ~ 4 cm^2 on the back of a mouse
**Reviewer's calculations using approximated body weights of 350 (males) and 250 mg (females)

Route, form, volume, and infusion rate: topical, solution, 0.1 ml/animal

Observations and times:
Clinical signs: 2 times daily
Body weights: once pre-dose, day 1 and weekly
Food consumption: weekly
Ophthalmoscopy: not assessed
EKG: not assessed
Hematology: 2 days prior to scheduled sacrifice

Clinical chemistry: at scheduled sacrifice
Urinalysis: not assessed
Gross pathology: at necropsy on day 30
Organs weighed: at necropsy; see Histopathology Inventory table
Histopathology: at necropsy, all tissues processed and examined microscopically; see Histopathology Inventory table for tissues that were assessed
Toxicokinetics: Group 6 (b) (4) sample taken on day 1 of week 1 at ~ 3 hours postdose.
Samples taken from Groups 7-10; 3/sex/dose on day 1 of Week 1 at ~ 3, 4, 6, 12 and 24 hours post-dose
Other: dermal irritation: treatment sites scored daily (prior to application of dose formulation on treatment days) and on day of necropsy. Scoring was done according to the following modified Draize scale:

Erythema	Edema	Atonia	Desquamation	Fissuring	Eschar
0 none	0 none	No	No	No	No
1 very slight	1 very slight	Yes	Yes	Yes	Yes
2 well-defined	2 slight				
3 moderate to severe	3 moderate				
4 severe to slight eschar formation	4 severe				

Results:

Mortality: There were no early deaths or moribund sacrifices.

Clinical signs: The study report indicates that test article treated mice often exhibited a short period of clinical signs that included hyperactivity, salivation, running around cage, and/or rapid respiration. The findings were not tabulated due to their short duration. Dermal sores were also observed in several male animals with an increase in number relative to capsaicin dose. In addition, 3 HD females exhibited sores. The timing of findings in males occurred on days 16-17 in the LD, day 9 in the LMD, days 9-15 in the UMD and days 9-15 and 18-20 at the HD. In females findings in the LMD occurred on days 9-20 and 17-20 and 21-30 at the HD.

Clinical signs in mice following dosing with Capsaicin (days 1-30).

Observation	Males					Females				
n=6	C	LD	LMD	UMD	HD	C	LD	LMD	UMD	HD
Skin & Pelage										
Sore, right flank	0	0	1	0	0	0	0	1	0	0
Sore, left flank	0	1	0	1	2	0	0	0	0	0
Sore, left & right flank	0	0	0	0	2	0	0	0	0	0
Sores, several areas on dorsal area posterior to dose site	0	0	0	0	1	0	0	0	0	0
Sore, right shoulder	0	0	0	0	1	0	0	0	0	1
Sores, posterior to dosing site and on right shoulder	0	0	0	0	0	0	0	0	0	1
Severe sore, dorsal area	0	0	0	0	0	0	0	0	0	1

Dermal irritation was noted only at the highest dose. Very slight erythema was observed in one male (day 27) and 2 females (day 18 in one, day 16 onward in the other). The erythema became well defined and persisted until terminal sacrifice in one of the females.

Desquamation was also observed in high dose males (days 11-14; dyas 26-28 in one) and females (days 17-22 and 24-30) while fissuring (days 21-28) and eschar formation (day 23-28) were only noted in one female.

Dermal irritation in mice following dosing with Capsaicin (days 1-30).

Observation	Males					Females				
	C	LD	LMD	UMD	HD	C	LD	LMD	UMD	HD
Erythema &Eschar formation										
None	6	6	6	6	6	6	6	6	6	6
Very slight	0	0	0	0	0	0	0	0	0	2
Well-defined	0	0	0	0	1	0	0	0	0	1
Edema										
None	6	6	6	6	6	6	6	6	6	6
Atonia										
No	6	6	6	6	6	6	6	6	6	6
Desquamation										
No	6	6	6	6	6	6	6	6	6	6
Yes	0	0	0	0	3	0	0	0	0	2
Fissuring										
No	6	6	6	6	6	6	6	6	6	6
Yes	0	0	0	0	0	0	0	0	0	1
Eschar										
No	6	6	6	6	6	6	6	6	6	6
Yes	0	0	0	0	0	0	0	0	0	1

Body weights: Body weight gain was comparable among groups after 4 weeks dosing. Between weeks 4 and 5, most groups including controls lost weight ranging from 0.1 to 0.7 g.

Food consumption: Food consumption was comparable among all groups.

Hematology: No treatment related effects were observed.

Clinical chemistry: No treatment related effects were observed other than a reduction in triglyceride levels (25-38%) at the two highest doses in males and females. The decrease was not statistically significant.

Organ weights: No treatment related effects were observed.

Gross pathology: The only treatment related gross observation was a sore at the skin treatment site in 1 of 6 high dose females.

Histopathology: Microscopic findings are summarized in the table below. The treatmentrelated findings were primarily observed at the site of treatment and included inflammation of the epidermis and dermis, acanthosis, mast cells in the dermis, increased fibrous connective tissue, erosions and ulcers. Inflammation was generally of a minimal to slight severity. Acanthosis was observed in all dose groups and severity increased with increasing dose; severe findings were noted in one high-dose female that was correlated with a gross sore. Skin erosions were observed in males at doses of 1.28 mg/animal/wk or greater; the finding was noted in a control female as well as some treated animals.

Minimal ulceration was observed in one male and one female while moderate ulceration was noted in one high-dose female. Single findings of skin erosion at a non-treatment site and liver necrosis were observed in one high-dose female.

Microscopic observations in mice following dosing with Capsaicin (days 1-30).

Observation	Males					Females				
n=6	C	LD	LMD	UMD	HD	C	LD	LMD	UMD	HD
Skin, treated n=	6	6	6	6	6	6	6	6	5	6
Inflammation, acute, focal, epidermis	2	1	5	0	2	1	0	1	1	4
Minimal	2	0	0	0	2	1	0	1	1	3
Slight	0	1	5	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	0	0	1
Acanthosis	5	6	6	6	6	6	6	6	5	6
Minimal	5	5	4	1	0	3	4	2	3	2
Slight	0	1	2	2	4	3	2	4	3	3
Moderate	0	0	0	3	2	0	0	0	0	1
Severe	0	0	0	0	0	0	0	0	0	1
Inflammatory cell infiltrate, dermis	2	3	5	4	6	3	4	1	3	4
Minimal	2	3	2	2	1	2	2	1	1	2
Slight	0	0	3	2	5	1	2	0	2	2
Increased mast cells, dermis	6	3	5	6	6	6	6	6	4	6
Minimal	6	3	5	6	6	5	6	6	4	6
Slight	0	0	0	0	0	1	0	0	0	0
Increased fibrous connective tissue, dermis	0	4	5	6	6	3	5	2	4	3
Minimal	0	4	5	5	3	3	5	2	4	2
Slight	0	0	0	1	3	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	1
Erosion	0	0	5	2	1	1	0	2	0	1
Minimal	0	0	3	2	1	1	0	2	0	1
Slight	0	0	2	0	0	0	0	0	0	0
Ulcer	0	0	1	0	0	0	0	0	1	1
Minimal	0	0	1	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	0	0	0	1
Skin, untreated n=	6	6	6	6	6	6	6	6	5	6
Erosion - minimal	0	0	0	0	0	0	0	0	0	1
Liver n=	6	6	6	6	6	6	6	6	6	6
Necrosis, focal, acute	0	0	0	0	0	0	0	0	0	1
Kidney n=	6	6	6	6	6	6	6	6	6	6
Cystic tubules, cortex	0	0	0	0	1	0	0	0	0	0
Pelvis dilatation	0	0	0	0	0	0	0	0	0	1

Toxicokinetics: Exposure to capsaicin occurred in most animals of the TK groups receiving capsaicin and increased in a generally sub-proportional manner with increasing dose. Maximum plasma concentrations were observed between 4 and 12 hours after dosing and measurable values were still present in several animals at 24 hours post-dosing. Calculation of an elimination half-life was only possible for certain groups due to a lack of a distinct elimination phase. Exposures tended to be greater in females, likely due to the administration of dose on a mg/animal basis rather than on a mg/kg basis as females were of lesser body weight values.

Group	Dose level (mg/animal)	Sex	Cmax (ng/ml)	Tmax (hours)	AUC_{0-t} (ng.hr/ml)	AUC_{0-24} (ng.hr/ml)	AUC_{0-inf} (ng.hr/ml)	$t_{1/2}$ (hours)
Group 7	0.64	M	9.17	6	NA	NA	NA	NA
		F	24.2	4	150	150	NA	NA
Group 8	1.28	M	28.4	6	193	193	NA	NA
		F	24.7	6	164	193	NA	NA
Group 9	2.56	M	43	12	588	588	NA	NA
		F	71.4	4	639	639	736	7.86
Group 10	6.12	M	51.5	12	909	909	NA	NA
		F	84.8	6	1026	1026	1198	7.48

a: Values should be approached with caution. Only 2 timepoints used in K_e calculation.

Histopathology inventory (optional)

Study	7215-113	7215-120	7215-122	7215-127
Species	pig	rat	mouse	dog
Adrenals	*X	*X	*X	*X
Aorta		X		
Bone Marrow smear				
Bone (femur)	X	X	X	X
Brain	*X	*X	*X	*X
Cecum	X	X	X	X
Cervix		X		
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	*X	*X	*X	*X
Esophagus	X	X	X	X
Eye	X	X	X	X
Fallopian tube				
Gall bladder	X		X	X
Gross lesions	X	X	X	X
Harderian gland		X	X	
Heart	*X	*X	*X	*X
Ileum	X	X	X	X
Injection site				X
Jejunum	X	X	X	X
Kidneys	*X	*X	*X	*X
Lachrymal gland			X	
Larynx				
Liver	*X	*X	*X	*X
Lungs	*X	*X	X	*X

Lymph nodes, cervical				
Lymph nodes mandibular		X		
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	
Nasal cavity				
Optic nerves		X		
Ovaries	*X	*X	X	*X
Pancreas	X	X	X	X
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary	*X	*X	*X	*X
Prostate	*X	*X	*X	*X
Rectum	X	X	X	X
Salivary gland	X	*X	X	*X
Sciatic nerve	X	X		X
Seminal vesicles		*X	X	
Skeletal muscle	X	X		X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	*X	*X	*X	*X
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	*X	*X	*X	*X
Thymus	X	*X	X	*X
Thyroid	X	*X	X	*X
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X	*X	X	*X
Vagina	X	X	X	X
Zymbal gland				

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

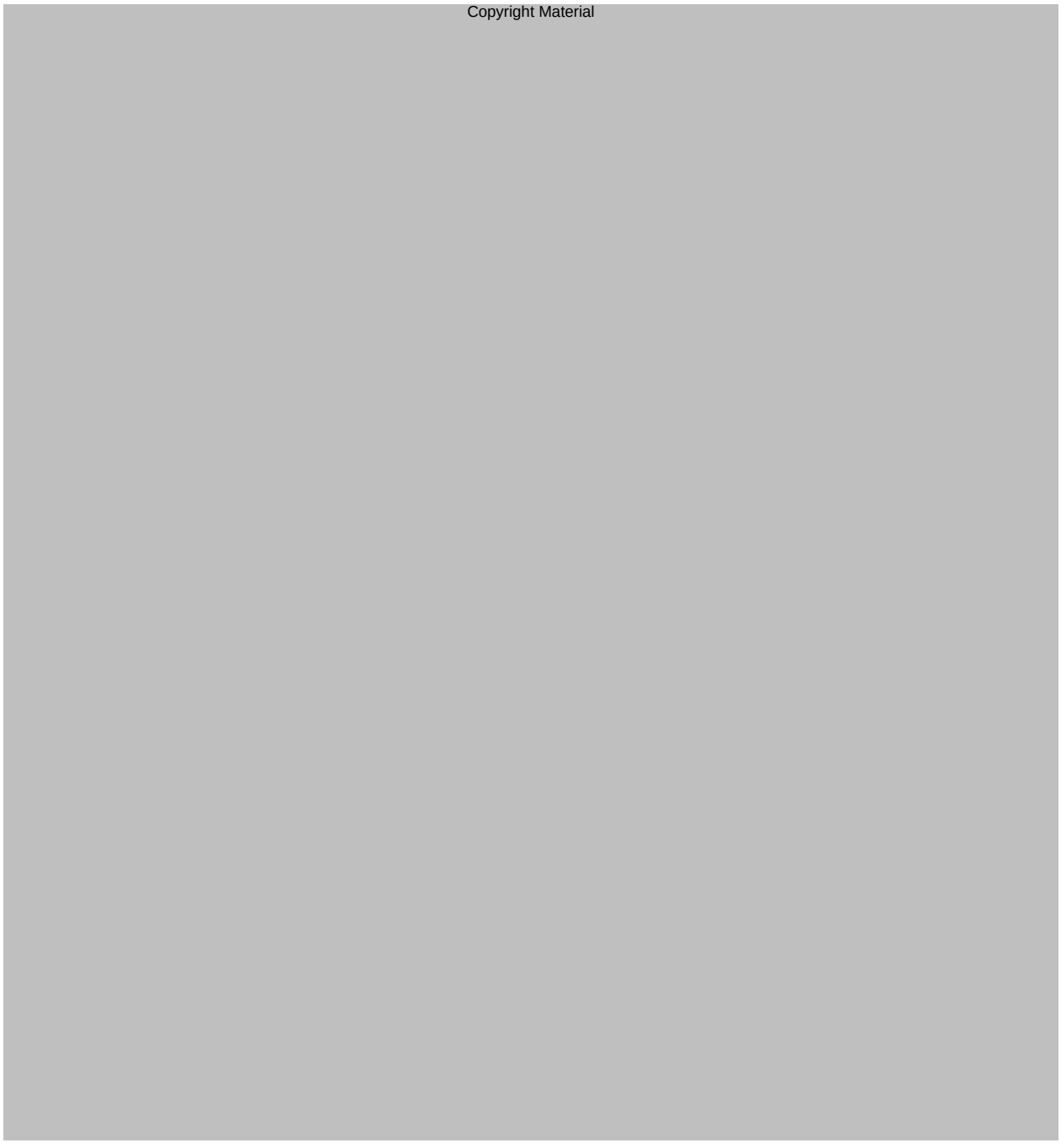
Summary of Genotoxicity Studies conducted by NeurogesX

Assay/study #	Cell or Species	Findings
Reverse Bacteria Mutagenic Assay 767691	S.typhimurium E. coli	Negative
Chromosomal Aberrations 24641-0-449oecd	Cultured Human Peripheral Blood Lymphocytes	Negative
Micronucleus Test in Bone Marrow Cells 767712	CD-1 Mice	Negative
Mouse Lymphoma 767707-21007	Mouse Lymphoma Cells	Positive in the presence and absence of metabolic enzymes (S9)with 4 hour exposure, Small colonies predominated, indicative of clastogenicity; Negative in 24 hour exposure assay without metabolic enzymes (S9)

The trans-capsaicin developed by the Sponsor for clinical use and used in all the drug development nonclinical studies is synthesized and highly purified. Except for the mouse lymphoma assay, all tests were negative for genotoxicity. The Applicant submitted information indicating that naturally occurring catechol compounds such as L-DOPA, dopamine, and epinephrine, gave positive responses in the mouse lymphoma assay.

In a review of the literature by the Applicant, various tests were performed with natural capsaicin extracts and were not conducted under GLP. Eight bacterial point mutation tests (including Ames assay) were performed between 1981 and 1995 using various strains of *S. typhimurium* and various forms of S9 activation. Four tests resulted in positive findings and four were negative. Two tests in Chinese hamster V79 cells were conducted; one was positive and one was negative. The in vivo micronucleus test was conducted in mice and it was positive. (refer to the Table from Chanda et al 2007). However, much of the published toxicology of capsaicin relates to extracts of capsaicin which are typically a mixture of capsaicin and other capsaicinoids (including norhydrocapsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin). A review of safety assessments including genetic toxicology assays of capsaicin extracts can be found (Johnson 2007). The actual percentage of capsaicin and other capsaicinoids will vary depending on the peppers used and method of extraction. The US Pharmacopoeia, 2008, defines capsaicin as a product which contains ≥ 55% capsaicin and the combination of capsaicin and dihydrocapsaicin to be ≥ 75%; total capsaicinoid content may be as little as 90%. Additionally, extracts may be expected to contain chemical entities other than vanilloid compounds. As a result of these various known and unknown impurities, results obtained from these studies may differ from results obtained with pure capsaicin as used in NGX-4010. It is likely that many of the published capsaicin equivocal genotoxicity studies are due to variations in the compounds present in the extracts.

From Chanda et al (2007)



Study title: **Capsaicin Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA 98 and TA 100 and Escherichia coli WP2uvrA**

This study was previously reviewed by Dr. Timothy J. McGovern when it was submitted to IND 63354.

Key findings: Under the conditions tested, capsaicin was negative in the Ames bacterial reverse mutation assay in concurrence with the sponsor's conclusion: Capsaicin was not mutagenic to *Salmonella typhimurium* or *Escherichia coli* when tested in dimethylsulphoxide at concentrations extending into the toxic range.

Study no.: 767691
E-Location: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\767691
Conducting laboratory and location: (b) (4)
Date of study initiation: Sept 6, 2001
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin, Batch No. F0010101, Purity 100.3%
Vehicle: dimethyl sulfoxide

Methods

Strains/species/cell line:
Salmonella typhimurium TA 1535, TA 1537, TA 98 and TA 100
Eschirichia coli WP2uvrA
Doses used in definitive study: With S9: 17, 50, 167, 500, 1667, 5000 µg/plate
Without S9: 1.7, 5, 17, 50, 167, 500 µg/plate.
Basis of dose selection:
Basis of dose selection: A toxicity test using strain TA 100 only was conducted with and without metabolic activation. Range finding studies: In the toxicity test using strain TA 100 only with and without metabolic activation at capsaicin doses of 17, 50, 167, 500, 1667 and 5000 µg/plate, toxicity (reduction in the number of revertant colonies by 45-47%) was observed at 5000 µg/plate in both activation conditions. Precipitation occurred at 1667 µg/plate and above in the absence of S9 mix and at 5000 µg/plate in the presence of S9 mix. In the absence of S9 mix, excessive precipitation at 5000 µg/plate obscured the background lawn of microcolonies making accurate assessment of toxicity difficult. The lawn was assumed to be normal.

Test agent stability: expiry date of Oct 9 2002; precipitation of test article was observed

Metabolic activation system: S9 fraction (10%) from Aroclor induced male Fischer 344 rats

Negative controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls:

With activation: 2-aminoanthracene (20 µg/plate) with E. coli; 2 µg/plate with TA 1535 and 1537; 0.5 µg/plate with TA 98 and 100

Without activation: N-ethyl-N-nitro-N-nitrosoguanidine (2 µg/plate) with E. coli; Sodium azide (1 µg/plate) with TA 1535 and TA 100; 2-nitrofluorene (1 µg/plate) with TA 98; 9-amoniacridine (80 µg/plate) with TA 1537

Comments: The controls are acceptable

Incubation and sampling times:

Exposure conditions: Direct Plate method: 2 ml soft agar was dispensed into sterile tubes; 0.5 ml S9 mix or 0.05 M phosphate buffer, pH 7.4, were added, followed by 0.1 ml bacteria. Solvent or test solution was added last. Tube contents were then mixed and poured onto minimal medium plates. When soft agar had set, plates were inverted and incubated.

Pre-Incubation method: S9 mix or 0.05 M phosphate buffer, pH 7.4, were added to sterile tubes followed by 0.1 ml bacteria and finally solvent or test solution (0.1 ml per tube). Tubes were then placed in a shaking incubator at 37C for 20 minutes. Soft agar was then added to the tubes and then the cooled contents were added to agar plates. When soft agar had set, plates were inverted and incubated.

Incubation and sampling times: Two independent mutation tests were conducted; the first used the Direct Plate method and the second used the Pre-incubation method. For both methods, the incubation was for 2-3 days at 37C. Doses used in definitive study: With S9: 17, 50, 167, 500, 1667, 5000 µg/plate in first and second mutation assays; toxicity in the second assay was more pronounced and the test was repeated at doses of 5, 17, 50, 167, 500, 1667 µg/plate. Without S9, the doses were 1.7, 5, 17, 50, 167, 500 µg/plate.

Analysis:

No. of replicates: triplicate

Counting method: Cardinal Colony Counter set at maximum sensitivity (colonies of 0.1 mm or more in diameter were counted)

Criteria for positive results: A doubling of the mean concurrent vehicle control values at some concentration of the test item for strains TA 1535, TA 1537, and TA 98 and for E. coli; a 1.5 fold increase over control value for TA 100; a dose-related response and a reproducible effect in independent tests.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Study validity: The study is considered valid in that appropriate bacterial strains were used, dose selection was adequate based upon observations of cytotoxicity or precipitation, assay methodology was appropriate, vehicles and positive controls were

appropriate, criteria for a positive response were adequate, and vehicle controls and positive controls responded as expected.

Study outcome:
In the first mutation assay, precipitation was observed at the concentration of 5000 µg/plate with all strains in the presence of S9. In the absence of S9, precipitation was noted at concentrations of 1667 and 5000 µg/plate. Under both conditions, there was no significant increase in mean number of revertant colonies compared to control and the positive controls produced expected results.
The second mutation assay resulted in less than 4 non-toxic level for several strains due to thinning of the background lawn and precipitation. In a repeat of the second mutation assay with S9, precipitation was observed at 1667 µg/plate and thinning of the background lawn was observed at this concentration for all S. typhimurium strains; cytotoxicity was also observed at 500 µg/plate for TA 1537. Without S9, cytotoxicity was observed at 167 µg/plate or greater for strains TA 1535 and TA 100 and at 500 µg/plate for the other 3 strains. Under both conditions, there was no significant increase in mean number of revertant colonies compared to control and the positive controls produced expected results.

First Mutation Assay – Direct Plate

Metabolic Activation	Test Article	Concentration mcg/plate	Revertant Colonies (Mean ± SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2*uvr*A
Without Activation	DMSO	100 mcL/plate	11 ± 5	7 ± 3	14 ± 8	92 ± 5	7 ± 3
	Capsaicin	17	8 ± 3	7 ± 1	12 ± 1	90 ± 8	9 ± 3
		50	9 ± 2	7 ± 4	9 ± 2	103 ± 14	9 ± 1
		167	7 ± 3	4 ± 1	11 ± 4	84 ± 5	8 ± 2
		500	13 ± 2	4 ± 2	10 ± 2	91 ± 16	9 ± 1
		1667[a]	10 ± 4	4 ± 3	10 ± 2	60 ± 0	1 ± 1
		5000[a]	7 ± 2	0 ± 1	2 ± 1	46 ± 3	0 ± 0
	NaN_3	1	192 ± 36	-	-	566 ± 26	-
	9-Aminoacridine	80	-	1726 ± 601	-	-	-
	2-Nitrofluorene	1	-		582 ± 158	-	-
	MMS	200	-	-	-	-	-
	ENNG	2	-	-	-	-	245 ± 2
With Activation	DMSO	100 mcL/plate	9 ± 1	7 ± 3	13 ± 6	108 ± 16	9 ± 3
	Capsaicin	17	11 ± 4	5 ± 4	16 ± 1	100 ± 5	8 ± 4
		50	9 ± 7	5 ± 2	22 ± 0	106 ± 6	8 ± 3
		167	11 ± 0	5 ± 3	19 ± 2	92 ± 12	7 ± 3
		500	10 ± 2	6 ± 4	22 ± 7	118 ± 14	8 ± 2
		1667	9 ± 4	5 ± 1	17 ± 5	97 ± 22	5 ± 2
		5000[a]	8 ± 3	2 ± 1	9 ± 3	63 ± 13	0 ± 0
	2-Aminoanthracene	2	508 ± 32	164 ± 50	-	-	-
		0.5	-	-	997 ± 254	1809 ± 290	-
		20	-	-	-	-	574 ± 35

Second Mutation Assay – Pre-Incubation Method

Metabolic Activation	Test Article	Concentration mcg/plate	Revertant Colonies (Mean ± SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2*uvrA*
Without Activation	DMSO	100 mcL/plate	10 ± 4	8 ± 3	11 ± 6	78 ± 2	12 ± 4
	Capsaicin	17	6 ± 1	4 ± 2	10 ± 4	85 ± 8	8 ± 1
		50	8 ± 4	3 ± 1	15 ± 3	88 ± 17	10 ± 4
		167	8 ± 3 (STL)	5 ± 2	14 ± 6	76 ± 3	15 ± 1 (STL)
		500	5 ± 3 (STL)	2 ± 2 (TL)	2 ± 3 (TL)	65 ± 5 (TL)	8 ± 3 (TL)
		1667*	7 ± 3 (TL)	0 ± 0 (NL)	# (TL)	47 ± 11 (VTL)	5 ± 3 (TL)
		5000*	4 ± 2 (TL)	0 ± 0 (NL)	# (TL)	58 ± 5 (VTL)	# (TL)
	NaN_3	1	216 ± 27	-	-	652 ± 29	-
	9-Aminoacridine	80	-	1901 ± 395	-	-	-
	2-Nitrofluorene	1	-		350 ± 21	-	-
	MMS	200	-	-	-	-	-
	ENNG	2	-	-	-	-	209 ± 12
With Activation	DMSO	100 mcL/plate	11 ± 4	9 ± 3	18 ± 7	97 ± 14	14 ± 3
	Capsaicin	17	5 ± 1	8 ± 2	21 ± 7	95 ± 11	17 ± 6
		50	4 ± 4	9 ± 4	21 ± 3	102 ± 10	15 ± 1
		167	7 ± 2	5 ± 1	18 ± 2	111 ± 8	13 ± 8
		500	5 ± 3	7 ± 2 (TL)	24 ± 3	105 ± 8	12 ± 2
		1667	8 ± 7 (STL)	3 ± 2 (TL)	14 ± 4	97 ± 2	12 ± 3
		5000*	3 ± 2 (STL)	3 ± 2 (TL)	12 ± 2 (STL)	90 ± 19 (STL)	8 ± 3 (STL)
	2-Aminoanthracene	2	400 ± 6	172 ± 54	-	-	-
		0.5	-	-	732 ± 97	1451 ± 26	-
		20	-	-	-		719 ± 14

Study title: **Cis Capsaicin Impurity: Bacterial Reverse Mutation Assay with a Confirmatory Assay**

This study was requested in the 74-day filing letter, since the cis-capsaicin levels were not previously qualified in a mutagenicity assay.

Key findings: *cis-Cap*saicin was negative in the Bacterial Reverse Mutation Assay for mutagenic potential.

cis-Capsaicin was evaluated in the dose range-finding assay in tester strains TA100 and WP2*uvr*A. Ten doses of test article, from 6.67 to 5000 µg/plate, were evaluated with and without S9. Inhibited growth (characterized by a reduced background lawn and/or a decrease in revertant frequency) was observed in tester strain TA100 at doses ≥3300 µg/plate with and without S9. In addition, the test article was found to precipitate from solution in the aqueous top agar at doses of 3330 and/or 5000 µg/plate with S9. Based upon the results of the dose range-finding assay, *cis*-capsaicin was evaluated in the initial mutagenicity assay, in all five tester strains, at doses of 313, 625, 1250, 2500 and 5000 µg/plate with and without S9. All doses of the test article, as well as the concurrent positive and vehicle controls, were evaluated in triplicate plates. Inhibited background growth again was observed in all four *Salmonella* tester strains at the highest two or three doses evaluated with and without S9. Significant reductions in revertant counts also were observed in tester strain WP2uvrA at the highest two dose levels with and without S9. In addition, the test article was found to be incompletely soluble in the aqueous top agar at doses ≥2500 µg/plate with and without S9. Revertant frequencies for all doses of *cis*-capsaicin, in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures. However, toxicity was too great in tester strains TA98, TA100 and TA1537, and thus these strains had fewer than three acceptable dose levels.

cis-Capsaicin was re-evaluated in the confirmatory mutagenicity assay, at doses of 78.1, 156, 313, 625, and 1250 µg/plate with S9 and without S9. Inhibited background growth again was observed in all four *Salmonella* tester strains at doses ≥1250 µg/plate with and without S9. In addition, the test article again was found to be incompletely soluble in the aqueous top agar at a dose of 2500 µg/plate with and without S9. Revertant frequencies for all doses of *cis*-capsaicin, in all tester strains with and without S9, approximated or were less than vehicle control values.

cis-Capsaicin subsequently was re-tested in tester strains TA98, TA100 and TA1537, under identical conditions, and similar results were observed. Inhibited growth again was observed in all three tester strains at doses ≥1250 µg/plate with and without S9, and the test article again was found to be incompletely soluble in the aqueous top agar at a dose of 2500 µg/plate with and without S9. Revertant frequencies for all doses of *cis*capsaicin,

in all tester strains with and without S9, approximated or were less than control values. All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met.

These results indicate *cis-cap*saicin was negative in the Bacterial Reverse Mutation Assay with a Confirmatory Assay under the conditions, and according to the criteria, of the test protocol.

Study no.: 8201369
E-Location: Submission 30-Mar-09, Seq 000, Mod Type BM; NDA022395\0014\M4\42-stud-rep\423-tox\4237-other-tox-stud\42376-imp\ (b) (4) 8201369-cis-capsaicin-ames-final-report
Conducting laboratory and location: (b) (4)
Date of study initiation: Jan 2, 2009
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
cis-capsaicin, Lot F001B090R174, purity 96.3% (made by (b) (4) (b) (4)
Vehicle: DMSO

Analysis of the concentration of cis-capsaicin determined it to be within 3% of the theoretical concentration. Stability test up to 8 days at room temperature or refrigerated (>0 to 10°C) indicated it to be stable during this time.

Methods

Tester Strain Genotypes

Tester Strain	*his/trp* Mutation	Additional Mutations		Plasmid
		Repair	LPS	
TA98	*his*D3052	*uvr*B	*rfa*	pKM101
TA100	*his*G46	*uvr*B	*rfa*	pKM101
TA1535	*his*G46	*uvr*B	*rfa*	–
TA1537	*his*C3076	*uvr*B	*rfa*	–
WP2*uvr*A	*trp*	*uvr*A	–	–

Strains/species/cell line:

Doses used in definitive study: 313, 625, 1250, 2500, and 5000 µg/plate with and without S9; with a confirmatory mutagenicity assay at doses of 78.1, 156, 313, 625, 1250, and 2500 µg/plate with and without S9.

Basis of dose selection: The dose range-finding assay was performed using 10 doses of cis-capsaicin, and vehicle control, in tester strains TA100 and WP2*uvr*A. cis-Capsacin was evaluated at doses of 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate with and without S9 (one plate per dose; the highest dose level represents the limit dose for this assay).
Inhibited growth (characterized by a reduced background lawn and/or a decrease in revertant frequency) was observed in tester strain TA100 at doses ≥3300 µg/plate with S9. In addition, the test article was found to precipitate from solution in the aqueous top agar at doses of 3330 and/or 5000 µg/plate with S9.

Negative controls:
dimethylsulfoxide (DMSO)
Positive controls:

Tester Strain(s)	S9	Positive Control	Dose (μg/plate)
TA98	–	2-nitrofluorene	1.0
TA100, TA1535	–	sodium azide	2.0
TA1537	–	ICR-191	2.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0
TA98	+	benzo[a]pyrene	2.5
TA100, TA1535, TA1537	+	2-aminoanthracene	2.5
WP2*uvr*A	+	2-aminoanthracene	25.0

Incubation and sampling times:
Based upon the results of the dose range-finding assay, the cis-capsaicin was evaluated in the initial mutagenicity assay at doses of 313, 625, 1250, 2500, and 5000 μg/plate with and without S9. All doses of cis-capsaicin as well as the concurrent vehicle and positive controls were evaluated in triplicate plates. The test article was re-evaluated in a confirmatory mutagenicity assay at doses of 78.1, 156, 313, 625, 1250, and 2500 μg/plate with and without S9. A re-test subsequently was performed, in tester strains TA98, TA100, and TA1537, under identical conditions. All doses of the test article, as well as the concurrent positive and vehicle controls, were evaluated in triplicate plates. Cultures were incubated for 52 ± 4 hours at 37°C. Revertant colonies were counted by automated colony counter or by hand.

Results

Study validity:
All tester strains were checked, at a minimum, for the following phenotypic characteristics.
Histidine/Tryptophan Auxotrophy (his-/trp- Mutation)
rfa Cell Wall Mutation
pKM101 Plasmid
uvrA and uvrB Mutation
The condition of the bacterial background lawn was evaluated macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that dose level. Lawns were scored as normal (N), reduced (R), obscured by precipitate (O), macroscopic precipitate present (P), absent (A), or enhanced (E); contaminated plates (C) were also noted.

The following criteria were used to determine a valid assay:

- *Tester Strain Integrity*
  Histidine/Tryptophan Auxotrophy (his-/trp- Mutation)

Salmonella tester strains must exhibit the presence of the *his*- mutation, as demonstrated by the ability to grow on minimal bottom agar plates supplemented with histidine/biotin (but not tryptophan/biotin). Tester strain WP2*uvr*A must exhibit the presence of the *trp*mutation, as demonstrated by the ability to grow on bottom agar plates supplemented with tryptophan/biotin (but not histidine/biotin).

rfa Cell Wall Mutation

Salmonella tester strains must exhibit the presence of the *rfa* mutation, as demonstrated by sensitivity to crystal violet. Tester strain WP2*uvr*A must exhibit resistance to crystal violet.

pKM101 Plasmid

Tester strains TA98 and TA100 must exhibit the presence of the pKM101 plasmid by demonstrating resistance to ampicillin. In contrast, tester strains TA1535, TA1537, and WP2*uvr*A must be sensitive to ampicillin.

uvrA and uvrB Mutation

All tester strains must exhibit the presence of the *uvr*A or *uvr*B mutations, as demonstrated by their sensitivity to ultraviolet light.

- *Characteristic Number of Spontaneous Revertants*

  The average revertants/plate of the vehicle control cultures must be within the following acceptance limits which are based upon historical data and published reports

TA98	8 - 60
TA100	60 - 240
TA1535	4 - 45
TA1537	2 - 25
WP2*uvr*A	5 - 40

- *Tester Strain Culture Density*

  Each culture must reach a target OD650(1:4) = 0.4 to 0.6, which has been demonstrated to be representative of cultures in the late log stage with ≥0.5 x 109 bacteria/mL.

- *Positive Control Values*

  The positive controls must induce a ≥3-fold increase in revertants/plate as compared to the concurrent vehicle controls. A 3-fold increase in the absence of S9 indicates that the tester strain can identify a mutagen, while a 3-fold increase in the presence of S9 will be considered to have demonstrated the integrity of the S9 mix as well as the ability of the tester strain to detect a mutagen.

- *Number of Dose Levels*

  A minimum of three non-toxic doses is required to evaluate assay data.

- *Maximum Dose Level*

  The highest dose evaluated was the limit dose of 5000 µg/plate, or produced significant cytotoxicity.

Criteria for a Positive Response

A test article is considered to have produced a positive response if it induces a dosedependent increase in revertant frequency that is ≥2.0-fold vehicle control values for tester strains TA98, TA100, and WP2*uvr*A, or ≥3.0-fold vehicle control values for tester strains TA1535 and TA1537. In addition, any response should be reproducible.

Criteria for a Negative Response

A test article is considered to have produced a negative response if no dose-dependent, ≥2.0-fold or ≥3.0-fold increases are observed in tester strains TA98, TA100, and WP2*uvr*A, or TA1535 and TA1537, respectively.

Criteria for an Equivocal Response

Even after repeated trials, a test article may produce results that are neither clearly positive nor clearly negative (e.g., responses that do not meet the dose-dependency or fold increase requirements but are reproducible). In those rare instances, the test article may be considered to have produced an equivocal response. Other criteria also may be used in reaching a conclusion about the study results (e.g., comparison to historical control values, biological significance, etc.). In such cases, the Study Director will use sound scientific judgment and clearly report and describe any such considerations.

Study outcome:

All positive and vehicle control values were within acceptable ranges, and all criteria for a valid study were met. Background growth was inhibited in all four *Salmonella* tester strains at the highest two or three doses evaluated with and without S9. Significant reductions in revertant counts were observed in tester strain WP2uvrA at the highest two dose levels with and without S9. In addition, cis-capsaicin was found to be incompletely soluble in the aqueous top agar at doses ≥2500 µg/plate with and without S9.

Revertant frequencies for all doses of *cis*-capsaicin, in all tester strains with and without S9, approximated or were less than those observed in the concurrent vehicle control cultures. However, toxicity was too great in tester strains TA98, TA100 and TA1537, and thus these strains had fewer than three acceptable dose levels. *cis*-Capsaicin was re-evaluated in the confirmatory mutagenicity assay, at doses of 78.1, 156, 313, 625, and 1250 µg/plate with S9 and without S9. Inhibited background growth again was observed in all four *Salmonella* tester strains at doses ≥1250 µg/plate with and without S9. In addition, cis-capsaicin was again incompletely soluble in the aqueous top agar at a dose of 2500 µg/plate with and without S9. Revertant frequencies for all doses of *cis*-capsaicin, in all tester strains with and without S9, approximated or were less than vehicle control values.

Table 5
Confirmatory Mutagenicity Assay Results with S9

Assay No.: 8201369-409OECD
Trial No.: 8201369C1
Plating Method: Plate incorporation assay
Study No.: 8201369
Date Plated: 1/29/2009
Date Counted: 2/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	***Cis*-Capsaicin**	2500	*16.7*	*2.3*	*0.9*	18 P M R, 18 P M R, 14 P M R
		1250	*13.0*	*3.5*	*0.7*	11 R, 17 R, 11 R
		625	*17.3*	*4.0*	*0.9*	13 N, 21 N, 18 N
		313	*20.3*	*9.1*	*1.1*	19 N, 30 N, 12 N
		156	*11.0*	*5.2*	*0.6*	8 N, 8 N, 17 N
		78.1	*20.3*	*5.9*	*1.1*	27 N, 16 N, 18 N
	Dimethyl Sulfoxide		*18.7*	*2.9*		17 N, 17 N, 22 N
TA100	***Cis*-Capsaicin**	2500	*34.0*	*2.0*	*0.4*	34 P M R, 32 P M R, 36 P M R
		1250	*50.0*	*4.6*	*0.5*	55 R, 46 R, 49 R
		625	*80.7*	*13.3*	*0.9*	73 N, 73 N, 96 N
		313	*90.3*	*9.0*	*1.0*	95 N, 80 N, 96 N
		156	*74.0*	*9.5*	*0.8*	63 N, 80 N, 79 N
		78.1	*72.3*	*7.4*	*0.8*	75 N, 78 N, 64 N
	Dimethyl Sulfoxide		*94.7*	*6.7*		99 N, 87 N, 98 N
TA1535	***Cis*-Capsaicin**	2500	*2.7*	*0.6*	*0.3*	3 P M R, 3 P M R, 2 P M R
		1250	*7.3*	*4.0*	*0.8*	5 R, 5 R, 12 R
		625	*10.0*	*5.2*	*1.2*	16 N, 7 N, 7 N
		313	*11.3*	*3.5*	*1.3*	11 N, 15 N, 8 N
		156	*7.7*	*2.1*	*0.9*	10 N, 6 N, 7 N
		78.1	*11.0*	*4.0*	*1.3*	15 N, 7 N, 11 N
	Dimethyl Sulfoxide		*8.7*	*2.9*		12 N, 7 N, 7 N
TA1537	***Cis*-Capsaicin**	2500	*1.3*	*0.6*	*0.3*	2 P M R, 1 P M R, 1 P M R
		1250	*2.7*	*1.5*	*0.5*	3 M R, 4 M R, 1 M R
		625	*4.3*	*0.6*	*0.8*	5 N, 4 N, 4 N
		313	*6.0*	*1.7*	*1.1*	5 N, 8 N, 5 N
		156	*7.7*	*3.2*	*1.4*	9 M N, 4 N, 10 N
		78.1	*5.0*	*3.6*	*0.9*	2 N M, 9 M N, 4 N M
	Dimethyl Sulfoxide		*5.3*	*4.5*		10 N, 5 N, 1 M N

Key to Plate Postfix Codes

P	Precipitation of test article observed
M	Plate counted manually
R	Reduced background bacterial lawn
N	Normal background bacterial lawn

Assay No.: 8201369-409OECD
Trial No.: 8201369C1
Plating Method: Plate incorporation assay

Study No.: 8201369
Date Plated: 1/29/2009
Date Counted: 2/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvr*A	***Cis*-Capsaicin**	2500	*11.7*	*3.5*	*0.9*	12 P M N, 8 P M N, 15 P M N
		1250	*16.3*	*8.1*	*1.2*	15 N, 9 N, 25 N
		625	*16.0*	*1.0*	*1.2*	15 N, 17 N, 16 N
		313	*15.7*	*2.5*	*1.2*	18 N, 16 N, 13 N
		156	*12.7*	*2.1*	*1.0*	11 N, 12 N, 15 N
		78.1	*17.0*	*13.2*	*1.3*	12 N, 32 N, 7 N
	Dimethyl Sulfoxide		*13.3*	*4.6*		16 N, 8 N, 16 N
TA98	**BP**	2.5	*468.7*	*7.5*	*25.1*	460 N, 473 N, 473 N
TA100	**2AA**	2.5	*1469.7*	*74.5*	*15.5*	1385 N, 1499 N, 1525 N
TA1535	**2AA**	2.5	*189.3*	*17.4*	*21.8*	209 N, 176 N, 183 N
TA1537	**2AA**	2.5	*90.0*	*13.0*	*16.9*	75 N, 98 N, 97 N
WP2*uvr*A	**2AA**	25.0	*395.7*	*29.3*	*29.7*	391 N, 369 N, 427 N

Key to Positive Controls		Key to Plate Postfix Codes	
BP	Benzo{a}pyrene	P	Precipitation of test article observed
2AA	2-amino anthracene	M	Plate counted manually
		R	Reduced background bacterial lawn
		N	Normal background bacterial lawn

Table 6
Confirmatory Mutagenicity Assay Results without S9

Assay No.: 8201369-409OECD
Trial No.: 8201369C1
Plating Method: Plate incorporation assay

Study No. 8201369
Date Plated: 1/29/2009
Date Counted: 2/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	***Cis*-Capsaicin**	2500	*5.3*	*3.8*	*0.3*	8 P M R, 1 P M R, 7 P M R
		1250	*10.0*	*3.0*	*0.6*	7 R, 10 M R, 13 R
		625	*9.3*	*3.8*	*0.6*	5 N, 11 N, 12 N
		313	*12.0*	*1.0*	*0.7*	11 N, 13 N, 12 N
		156	*8.7*	*1.2*	*0.5*	8 N, 8 N, 10 N
		78.1	*10.7*	*3.2*	*0.6*	7 N, 13 N, 12 N
	Dimethyl Sulfoxide		*16.7*	*1.5*		17 N, 18 N, 15 N
TA100	***Cis*-Capsaicin**	2500	*14.7*	*5.7*	*0.2*	21 P M R, 10 P M R, 13 P M R
		1250	*36.0*	*11.1*	*0.4*	24 M R, 38 M R, 46 M R
		625	*79.3*	*12.3*	*1.0*	93 N, 76 N, 69 N
		313	*83.7*	*7.5*	*1.0*	76 N, 84 N, 91 N
		156	*76.7*	*11.5*	*1.0*	70 N, 90 N, 70 N
		78.1	*81.3*	*12.9*	*1.0*	92 N, 67 N, 85 N
	Dimethyl Sulfoxide		*80.7*	*17.2*		84 N, 96 N, 62 N
TA1535	***Cis*-Capsaicin**	2500	*4.0*	*1.7*	*0.3*	3 P M R, 6 P M R, 3 P M R
		1250	*4.3*	*1.2*	*0.3*	5 M R, 3 M R, 5 M R
		625	*8.0*	*2.0*	*0.6*	8 N, 10 N, 6 N
		313	*10.7*	*4.6*	*0.8*	8 N, 16 N, 8 N
		156	*7.7*	*0.6*	*0.6*	8 N, 7 N, 8 N
		78.1	*10.7*	*4.5*	*0.8*	6 N, 11 N, 15 N
	Dimethyl Sulfoxide		*13.7*	*6.7*		21 N, 8 N, 12 N
TA1537	***Cis*-Capsaicin**	2500	*0.7*	*0.6*	*0.1*	1 P M R, 1 P M R, 0 P M R
		1250	*1.7*	*0.6*	*0.3*	2 M R, 1 M R, 2 M R
		625	*4.3*	*3.1*	*0.9*	5 N, 1 N, 7 N
		313	*5.3*	*1.2*	*1.1*	6 N, 4 N, 6 N
		156	*5.3*	*0.6*	*1.1*	6 N, 5 N, 5 N
		78.1	*4.0*	*1.7*	*0.8*	2 N, 5 N, 5 N
	Dimethyl Sulfoxide		*5.0*	*1.7*		4 N, 4 N, 7 N

Key to Plate Postfix Codes

P	Precipitation of test article observed
M	Plate counted manually
R	Reduced background bacterial lawn
N	Normal background bacterial lawn

Table 6 (cont.): Confirmatory Mutagenicity Assay Results without S9

Assay No.: 8201369-409OECD
Trial No.: 8201369C1
Plating Method: Plate incorporation assay

Study No.: 8201369
Date Plated: 1/29/2009
Date Counted: 2/1/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
WP2*uvr*A	***Cis*-Capsaicin**	2500	*6.0*	*1.7*	*0.4*	5 P M N, 5 P M N, 8 P M N
		1250	*10.7*	*3.2*	*0.6*	12 N, 13 N, 7 N
		625	*13.7*	*2.1*	*0.8*	12 N, 13 N, 16 N
		313	*18.3*	*5.5*	*1.1*	13 N, 24 N, 18 N
		156	*15.0*	*3.0*	*0.9*	12 N, 18 N, 15 N
		78.1	*18.3*	*3.2*	*1.1*	17 N, 16 N, 22 N
	Dimethyl Sulfoxide		*17.0*	*4.0*		21 N, 17 N, 13 N
TA98	**2NF**	1.0	*329.7*	*60.3*	*19.8*	260 N, 364 N, 365 N
TA100	**SA**	2.0	*1039.0*	*65.0*	*12.9*	991 N, 1013 N, 1113 N
TA1535	**SA**	2.0	*773.3*	*51.7*	*56.6*	745 N, 742 N, 833 N
TA1537	**ICR**	2.0	*340.7*	*77.0*	*68.1*	264 N, 340 N, 418 N
WP2*uvr*A	**4NQO**	1.0	*253.3*	*12.3*	*14.9*	250 N, 267 N, 243 N

Key to Positive Controls		Key to Plate Postfix Codes	
2NF	2-nitrofluorene	P	Precipitation of test article observed
SA	Sodium Azide	M	Plate counted manually
ICR	ICR-191	R	Reduced background bacterial lawn
4NQO	4-nitro-quinoline oxide	N	Normal background bacterial lawn

Table 7
Re-test Mutagenicity Assay Results with S9

Assay No.: 8201369-409OECD
Trial No.: 8201369-D1
Plating Method: Plate incorporation assay

Study No.: 8201369
Date Plated: 2/4/2009
Date Counted: 2/6/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	***Cis*-Capsaicin**	2500	*13.7*	*1.2*	*0.7*	13 P M R, 15 P M R, 13 P M R
		1250	*13.3*	*2.9*	*0.7*	15 R, 10 R, 15 R
		625	*13.0*	*3.6*	*0.7*	12 N, 17 N, 10 N
		313	*18.7*	*3.1*	*1.0*	22 N, 16 N, 18 N
		156	*17.7*	*0.6*	*0.9*	18 N, 17 N, 18 N
		78.1	*13.0*	*5.6*	*0.7*	8 N, 19 N, 12 N
	Dimethyl Sulfoxide		*19.3*	*4.0*		17 N, 17 N, 24 N
TA100	***Cis*-Capsaicin**	2500	*10.3*	*8.6*	*0.1*	1 P M R, 12 P M R, 18 P M R
		1250	*24.7*	*16.2*	*0.3*	34 R, 34 R, 6 M R
		625	*64.3*	*10.1*	*0.7*	58 M B N, 59 N, 76 N
		313	*81.7*	*12.6*	*0.9*	95 N, 70 N, 80 N
		156	*80.3*	*13.8*	*0.9*	96 N, 70 N, 75 N
		78.1	*87.3*	*8.5*	*1.0*	96 N, 87 N, 79 N
	Dimethyl Sulfoxide		*88.7*	*11.2*		76 N, 97 N, 93 N
TA1537	***Cis*-Capsaicin**	2500	*0.3*	*0.6*	*0.0*	0 P M R, 0 P M R, 1 P M R
		1250	*2.0*	*1.7*	*0.2*	3 R M, 3 R M, 0 R M
		625	*4.7*	*1.2*	*0.5*	4 N, 6 N, 4 N
		313	*11.0*	*5.0*	*1.2*	11 N, 16 N, 6 N
		156	*7.7*	*2.5*	*0.9*	5 N, 8 N, 10 N
		78.1	*5.0*	*1.0*	*0.6*	6 N, 5 N, 4 N
	Dimethyl Sulfoxide		*9.0*	*1.7*		7 N, 10 N, 10 N
TA98	**BP**	2.5	*413.7*	*44.1*	*21.4*	400 N, 378 N, 463 N
TA100	**2AA**	2.5	*819.3*	*256.7*	*9.2*	1096 N, 589 N, 773 N
TA1537	**2AA**	2.5	*56.7*	*20.4*	*6.3*	39 N, 52 N, 79 N

Key to Positive Controls	
BP	Benzo{a}pyrene
2AA	2-amino anthracene

Key to Plate Postfix Codes	
P	Precipitation of test article observed
M	Plate counted manually
R	Reduced background bacterial lawn
B	Bubbles or Split in agar
N	Normal background bacterial lawn

Table 8
Re-test Mutagenicity Assay Results without S9

Assay No.: 8201369-409OECD
Trial No.: 8201369-D1
Plating Method: Plate incorporation assay

Study No.: 8201369
Date Plated: 2/4/2009
Date Counted: 2/6/2009

Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle	Individual revertant colony counts
TA98	***Cis*-Capsaicin**	2500	*4.3*	*2.1*	*0.4*	2 P M R, 5 P M R, 6 P M R
		1250	*10.7*	*2.1*	*1.1*	13 R, 9 R, 10 R
		625	*13.3*	*5.5*	*1.3*	16 N, 17 N, 7 N
		313	*11.3*	*3.5*	*1.1*	11 N, 15 N, 8 N
		156	*14.7*	*3.2*	*1.5*	17 N, 11 N, 16 N
		78.1	*13.7*	*3.1*	*1.4*	17 N, 11 N, 13 N
	Dimethyl Sulfoxide		*10.0*	*7.0*		5 N, 18 N, 7 N
TA100	***Cis*-Capsaicin**	2500	*30.3*	*5.0*	*0.5*	35 P M R, 25 P M R, 31 P M R
		1250	*45.3*	*16.0*	*0.7*	30 R, 62 R, 44 R
		625	*57.7*	*5.1*	*0.9*	52 N, 59 N, 62 N
		313	*78.3*	*9.3*	*1.2*	72 N, 74 N, 89 N
		156	*71.7*	*7.1*	*1.1*	73 N, 64 N, 78 N
		78.1	*71.0*	*9.8*	*1.1*	68 N, 82 N, 63 N
	Dimethyl Sulfoxide		*67.0*	*9.5*		56 N, 73 N, 72 N
TA1537	***Cis*-Capsaicin**	2500	*0.0*	*0.0*	*0.0*	0 P M R, 0 P M R, 0 P M R
		1250	*0.7*	*0.6*	*0.1*	0 R M, 1 R M, 1 R M
		625	*6.3*	*2.1*	*0.9*	8 N, 4 N, 7 N
		313	*4.3*	*3.8*	*0.6*	6 N, 0 N, 7 N
		156	*5.0*	*1.0*	*0.7*	6 N, 4 N, 5 N
		78.1	*2.3*	*2.3*	*0.3*	1 N, 1 N, 5 N
	Dimethyl Sulfoxide		*7.0*	*1.0*		8 N, 7 N, 6 N
TA98	**2NF**	1.0	*326.3*	*57.5*	*32.6*	391 N, 281 N, 307 N
TA100	**SA**	2.0	*1137.0*	*57.0*	*17.0*	1075 N, 1149 N, 1187 N
TA1537	**ICR**	2.0	*490.0*	*75.0*	*70.0*	547 N, 518 N, 405 N

Key to Positive Controls	
2NF	2-nitrofluorene
SA	Sodium Azide
ICR	ICR-191

Key to Plate Postfix Codes	
P	Precipitation of test article observed
M	Plate counted manually
R	Reduced background bacterial lawn
N	Normal background bacterial lawn

Study title: **Capsaicin Mouse Lymphoma Cell Mutation Assay**

This study was reviewed June 17, 2003 after submission to IND 63354 by Dr. Timothy J. McGovern, Ph.D.

Key findings:

- Capsaicin tested positively in the in vitro mouse lymphoma assay following 4 hours exposure in the presence and absence of S9 under the conditions tested.
- Small colonies predominated indicating that the alterations were associated with large chromosomal deletions.
- Negative findings were observed following 24 hour exposure without S9.

Study no.: 767707-21007
E-Location: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\767707-21007
Conducting laboratory and location: (b) (4)
Date of study initiation: Sept 6, 2001
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin, Batch No. F0010101, Purity 100.3%
Vehicle: DMSO

Methods

The microwell method (Cole *et al* (1983) was used in this study.

Strains/species/cell line: tk+tk- -3.7.2.C mouse lymphoma L5178Y cell line
Doses used in definitive study:
Assay 1 (-S9, 4 hr): 2.5, 15, 27.5, 40, 52.5, 65, 77.5, 90 µg/ml
Assay 2 (+S9, 4 hr): 3, 6, 9, 12, 15, 18, 21, 24 µg/ml
Assay 3 (-S9, 24 hr): 4, 12, 20, 28, 36, 44, 52, 60 µg/ml
Assay 4 (+S9, 4 hr): 3, 6, 9, 12, 15, 18, 21, 24 µg/ml
Basis of dose selection:
Initial toxicity tests in absence and presence of SP Range finding studies: A single culture was prepared for each capsaicin treatment (0.5-5000 µg/ml) using 4 hr exposure period. A second experiment was performed in the absence of S9 with 24 hr exposure to the test item. In the absence of S9, RSG was reduced to 32.9% at 50 µg/ml. Complete cell death occurred at 150 µg/ml. In the presence of S9, toxicity was greater with an RSG value of 31% at 5 µg/ml and 18.1% at 15 µg/ml; complete cell death occurred at 50 µg/ml or greater. At 24 hr exposure, RSG was 9.8% at 50 µg/ml and complete cell death occurred at 150 µg/ml. Precipitation of the test item occurred at concentrations of 150 µg/ml and above.
Test agent stability: expiry date of Oct 9 2002; freely soluble in DMSO
Metabolic activation system: S9 fraction (10%) from Aroclor induced male Fischer 344 rats

Negative controls:

Vehicle: DMSO
Negative controls: DMSO

Positive controls:

In the absence of S9 (4 hr exposure), 250 µg/ml ethyl methanesulphonate (EMS dissolved in DMSO), a large colony inducer, and 10 µg/ml methyl methanesulphonate (MMS dissolved in water), which usually induces greater numbers of small colonies. For the 24 hr exposure period, concentrations were
reduced to 150 µg/ml EMS and 5 µg/ml MMS. In the presence of S9, 3-methylcholanthrene (3-MC dissolved in DMSO), a large and small colony inducer, was used at a concentration of 2.5 µg/ml.

Comments: The controls are acceptable

Incubation and sampling times:

Incubation and sampling times: 4 and 24 hr incubations

Study design: 4 hr treatments were conducted with and without metabolic activation; 24 hour exposure was conducted in the absence of S9. Samples of cell culture were dispensed to sterile tubes. If appropriate, S9 mix was added followed by 0.1 ml of test solution in DMSO. The final reaction mixture contained 10 ml of cells at a population density of ca 3 x 10^5 cells/ml on Day 1. Samples of cells taken at end of treatment period were used to estimate the survival of the treated cultures, relative to the vehicle controls. Cell densities were assessed, cultures diluted into cloning medium as appropriate, and two 96-well dishes seeded with an estimated 1.6 cells per well (in 200 µl). The numbers of empty wells after 9 days, plus the post-treatment cell densities, were used to calculate the Day 0 Relative Survival (RS) for each cell culture.

Cell counts were determined following exposure and provided a measure of suspension growth and RSG. In addition to all vehicle and positive control cultures, the cultures from the 4 highest concentrations of test item giving at least 10% RSG were selected for assessment. The selected cultures were then assessed for expression of genetic damage by performing two parallel cloning assays: the cloning efficiency assay and the mutant selection assay. For the cloning efficiency assay, each culture was diluted into cloning medium to give an estimated 8 cells/ml. Two 96 well dishes were filled with 200 µl cell culture per well giving an estimated 1.6 cells per well. For the mutant selection assay, TFT stock solution was added to cloning medium to give a final concentration of 3 µg/ml. Cell cultures were added to this medium to give an estimated 1 x 10^4 cells/ml. Two 96 well dishes were filled with 200 µl cell culture per well giving an estimated 2000 cells per well. All dishes were incubated at 37 C until the colonies were fully developed (9 days for cloning efficiency assay, 12 days for mutant selection assay).

Analysis:

No. of replicates: duplicate, vehicle control an quadruplicate
Counting method: dissecting microscope
Criteria for positive results: An experiment was considered to be positive if one or more concentrations were statistically significant and there was a significant linear trend. Absolute increases in numbers of mutant colonies provide supporting evidence of a

positive response. A test may also be considered positive in the absence of a linear trend due to mitigating evidence: presence of a similar level of toxicity at all concentrations, presence of a steep toxicity curve. A test item is positive if 2 positive experiments out of 2 were recorded within the same activation condition.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

The study is considered valid in that appropriate bacterial strains were used, dose selection was adequate based upon observations of cytotoxicity or precipitation, assay methodology was appropriate, vehicles and positive controls were appropriate, criteria for a positive response were adequate, and vehicle controls and positive controls responded as expected.

Study outcome:

The results of the mutation assays are summarized in the tables below. In assay 1, the 2 highest concentrations were deemed too toxic. The highest assessed concentration of 65 µg/ml gave a log mutant fraction that was significantly greater than the vehicle control group and the test for linear trend of mutant fraction was significant. The mutant fraction at 65 µg/ml was above the vehicle control range of 43-131 and was also higher than both "no effect" maximum values, having an induced mutant fraction of 88 mutants per million and a fold increase of 1.8.

In assay 2, the highest concentration was deemed to be too toxic and 21 µg/ml produced an RTG < 10% and was excluded. Concentrations of 12 and 18 µg/ml gave log mutant fractions that were significantly higher than the vehicle control and the test for linear trend was significant.

In assay 3, the two highest concentrations tested were too toxic; the next two highest gave RTGs that were below 10% and were excluded. No significant evidence of mutagenic activity was observed although the test item cultures that were assessed were increased over the vehicle control culture.

Assay 4 also demonstrated similar results to assay 2 as a significant increase in log mutant fraction at concentrations of 12, 15, and 18 µg/ml and the test for linear trend was significant.

Colony sizing for the positive assays tended either to give ratios of small to large colonies similar to vehicle controls, or to give higher numbers of small type colonies. Thus, the positive results observed in this assay may be associated with large chromosomal deletions as opposed to small chromosomal deletions and point mutations associated with a predominance of large cultures.

The solvent control and positive control mutant fractions were within the expected ranges for these parameters.

Based upon the results of this assay, capsaicin tested positively in the in vitro mouse lymphoma assay following 4 hours exposure in the presence and absence of S9 under the conditions tested.

Small colonies predominated indicating that the alterations were associated with large chromosomal deletions. Negative findings were observed following 24 hour exposure without S9. The study report concludes that capsaicin is weakly mutagenic in the presence of S9 mix with limited evidence of very weak activity in the absence of S9.

Assay 1: Absence of S9, 4 hr exposure

Chemical	Concentration (µg/ml)	Day 0	Day 2		
		Relative survival %	Relative total growth %	Mutant fraction (x 10^{-6})	Ratio of small to large colonies
DMSO	100 µl added	100	100	110	1.31
EMS	250	83	62	568*	0.6
MMS	10	89	35	1362*	1.8
Capsaicin	2.5	98	NPS	NPS	-
	15	87	NPS	NPS	-
	27.5	79	58	150	1.42
	40	79	58	151	1.54
	52.5	58	40	147	1.77
	65	28	15	198*	2.06
	77.5	11	NPT	NPT	-
	90	NPT	NPT	NPT	-

Assay 2: Presence of S9, 4 hr exposure

Chemical	Concentration (µg/ml)	Day 0	Day 2		
		Relative survival %	Relative total growth %	Mutant fraction (x 10^{-6})	Ratio of small to large colonies
DMSO	100 µl added	100	100	111	1.07
3-MC	2.5	77	56	886*	1.13
Capsaicin	3	74	NPS	NPS	-
	6	78	NPS	NPS	-
	9	60	52	138	1.26
	12	42	31	224*	1.06
	15	31	21	160	1.6
	18	20	12	301*	1.28
	21	13	8	(208)#	(1.05)
	24	5	NPT	NPT	-

Assay 3: Absence of S9, 24 hr exposure

Chemical	Concentration (µg/ml)	Day 0	Day 2		
		Relative survival %	Relative total growth %	Mutant fraction (x 10^{-6})	Ratio of small to large colonies
DMSO	100 µl added	100	100	92	1.08
EMS	150	51	27	2088*	0.48
MMS	5	50	31	2142*	1.66
Capsaicin	4	67	NPS	NPS	-
	12	58	38	129	1.28
	20	36	28	135	1.49
	28	20	15	135	2.13
	36	14	8	(254)#	(1.72)
	44	14	7	(167)#	(2.42)
	52	9	NPT	NPT	-
	60	NPT	NPT	NPT	-

Assay 4: Presence of S9, 4 hr exposure

Chemical	Concentration (µg/ml)	Day 0	Day 2		
		Relative survival %	Relative total growth %	Mutant fraction (x 10^{-6})	Ratio of small to large colonies
DMSO	100 µl added	100	100	110	1.40
3-MC	2.5	108	79	734*	1.75
Capsaicin	3	96	NPS	NPS	-
	6	79	70	105	0.84
	9	48	46	90	1.31
	12	46	33	171*	4.1
	15	28	23	232*	2.29
	18	17	14	215*	3.36
	21	8	9	(259)#	(2.07)
	24	5	NPT	NPT	-

*: significant difference in log mutant fraction compared with vehicle control ($P < 0.05$)
Test for linear trend of mutant fraction with concentration of Capsaicin = significant (P=0.001-0.003 for assays 1, 2 and 4; P=0.022 for assay 3)
#: Treatment giving unacceptably low RTG (<10%). Mutant data included for information only; not included in statistical analysis.
NPS = not plated – surplus
NPT = not plated- toxic

a) Metabolic Activation	Test Article	Concentration or Dose Level (mcg/mL)	Day 0 Relative Survival (%)	Relative Total Growth (%)	Mutant Fraction (× 10^{-6})	Ratio of Small to Large Colonies	Statistical Comparison
Without Activation (4 hr)	Vehicle	(100 mcL added)	100	100	110	1.31	-
	Capsaicin	2.5	98	NPS	NPS	-	-
		15	87	NPS	NPS	-	-
		27.5	79	58	150	1.42	-
		40	79	58	151	1.54	-
		52.5	58	40	147	1.77	-
		65	28	15	198	2.06	a
		77.5	11	NPT	NPT	-	-
		90	NPT	NPT	NPT	-	-
	EMS	250	83	62	568	0.60	a
	MMS	10	89	35	1362	1.80	a

a $p < 0.05$
Test for dose-related trend = significant (p = 0.003)

b) Metabolic Activation	Test Article	Concentration or Dose Level (mcg/mL)	Day 0 Relative Survival (%)	Relative Total Growth (%)	Mutant Fraction (× 10^{-6})	Ratio of Small to Large Colonies	Statistical Comparison
With Activation (4 hr)	Vehicle	(100 mcL added)	100	100	111	1.07	-
	Capsaicin	3	74	NPS	NPS	-	-
		6	78	NPS	NPS	-	-
		9	60	52	138	1.26	-
		12	42	31	224	1.06	*
		15	31	21	160	1.60	-
		18	20	12	301	1.28	*
		21	13	8	208	1.05	u
		24	5	NPT	NPT	-	-
	3 MC	2.5	77	56	886	1.13	*

* $p < 0.05$
Test for dose-related trend = significant ($p < 0.001$)
u = treatment giving unacceptably low RTG (< 10%). Mutation data included for information only. Data excluded from statistical analysis
NPS = not plated – surplus, NPT = not plated – toxic

c) Metabolic Activation	Test Article	Concentration or Dose Level (mcg/mL)	Day 0 Relative Survival (%)	Relative Total Growth (%)	Mutant Fraction ($\times 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
Without	Vehicle	(200 mcL added)	100	100	92	1.08	-
Activation	Capsaicin	4	67	NPS	NPS	-	-
(24 hr)		12	58	38	129	1.28	-
		20	36	28	135	1.49	-
		28	20	15	135	2.13	-
		36	14	8	254	1.72	u
		44	14	7	167	2.42	u
		52	9	NPT	NPT	-	-
		60	NPT	NPT	NPT	-	-
	EMS	150	51	27	2088	0.48	a
	MMS	5	50	31	2142	1.66	a

[a] $p < 0.05$
Test for dose-related trend = significant ($p = 0.022$)
u = treatment giving unacceptably low RTG (< 10%). Mutation data included for information only. Data excluded from statistical analysis
NPS = not plated – surplus, NPT = not plated – toxic

d) Metabolic Activation	Test Article	Concentration or Dose Level (mcg/mL)	Day 0 Relative Survival (%)	Relative Total Growth (%)	Mutant Fraction ($\times 10^{-6}$)	Ratio of Small to Large Colonies	Statistical Comparison
With Activation	Vehicle	(100 mcL added)	100	100	110	1.40	-
(4 hr)	Capsaicin	3	96	NPS	NPS	-	-
		6	79	70	105	0.84	-
		9	48	46	90	1.31	-
		12	46	33	171	4.10	a
		15	28	23	232	2.29	a
		18	17	14	215	3.36	a
		21	8	9	259	2.07	u
		24	5	NPT	NPT	-	-
	3 MC	2.5	108	79	734	1.75	a

[a] $p < 0.05$
Test for dose-related trend = significant ($p < 0.001$)
u = treatment giving unacceptably low RTG (< 10%). Mutation data included for information only. Data excluded from statistical analysis.
NPS = not plated – surplus, NPT = not plated – toxic

Study title: **Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes with Capsaicin**

This study was previously reviewed by Dr. Timothy J. McGovern when it was submitted to IND 63354.

Key findings: Capsaicin tested negatively in the assay with cultured human peripheral blood lymphocytes in

Study no.: 24641-0-449OECD
E-Location: m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro\24641-0-449oecd
Conducting laboratory and location: (b) (4)
Date of study initiation: Nov 27, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin, Batch/Lot No. F0010102, Purity 99.1%
Vehicle: DMSO

Methods

Strains/species/cell line: Human peripheral blood lymphocytes from healthy, adult donors

Doses used in definitive study:
Initial without activation: 118, 168, 240, 343, 490, 700, 1000 µg/ml
Initial with activation: 168, 240, 343, 490, 700, 1000 µg/ml
Confirmatory without activation: 24.6, 49.2, 98.4, 123, 154, 192, 240, 320 µg/ml
Confirmatory with activation: 25, 98.4, 123, 154, 192 µg/ml

Basis of dose selection:
An initial chromosomal aberration assay was performed with duplicate cultures at each test article concentration, the negative and vehicle controls and for each of 3 concentrations of the positive control articles; ~ 22 hr harvests were conducted.
Range finding studies: None

Test agent stability: expiry date of December 25, 2003; freely soluble in DMSO at concentrations less than or equal to 310 µg/ml
Metabolic activation system: S9 fraction (1.5%) from Aroclor induced rats and an energyproducing system (NADP plus isocitric acid)

Negative controls:
Vehicle: DMSO
Negative controls: DMSO

Positive controls: mitomycin C (0.75, 1 and 1.5 µg/ml, ~ 3 hr treatment; 0.2, 0.3 and 0.4 µg/ml, ~ 22 hr treatment) for the nonactivation series and cyclophosphamide (20, 25 and 40 µ⌠g/ml) for the activation series. Both positive controls were dissolved in sterile, deionized water.

Comments: The controls are acceptable.

Incubation and sampling times:

Exposure conditions:
Incubation and sampling times: In the initial assay, the incubation time was 3 hours with and without metabolic activation. In the confirmatory assay, the incubation time was 22 hours without metabolic activation and 3 hours with metabolic activation.
Harvest time ~ 22 hours from the initiation of treatment.

Analysis: Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number of 46 +/- 2 were analyzed. 100 cells, if possible, from each duplicate culture from at least 3 concentrations of the test article, the negative vehicle and vehicle controls, and one dose from the positive control cultures were analyzed for the different types of chromosomal aberrations. At least 25 cells were analyzed from those cultures that had greater than 25% of cells with one or more aberrations. Mitotic index was evaluated from the negative control, vehicle control and a range of test article concentrations by analyzing the number of mitotic cells in at least 1000 cells per culture and the ratio expressed as a percentage of mitotic cells. Percent polyploidy and endoreduplication were also analyzed by evaluating at least 100 metaphases per culture, if available.

No. of replicates: duplicate
Counting method: light microscopy

Criteria for positive results: A test article was considered positive for inducing chromosomal aberrations if a significant increase ($p < 0.01$) in the number of cells with chromosomal aberrations was observed at one or more concentrations. The linear trend test evaluated the dose responsiveness. If a significant increase was seen at one or more concentrations, a dose-response should be observed.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

The study is considered valid in that appropriate cells were used, dose selection was adequate based upon observations of cytotoxicity or precipitation, assay methodology was appropriate, vehicles and positive controls were appropriate, criteria for a positive response were adequate, and vehicle controls and positive controls responded as expected.

Study outcome:

Without metabolic activation, precipitation was observed at concentrations of 240 µg/ml or greater. Hemolysis was observed at 343 ⌈g/ml or greater; only dead cells were present

on slides at these concentrations. Reductions of 0%, 39% and 65% were observed in the mitotic indices of the cultures tested with 118, 168 and 240 µg/ml as compared with vehicle controls.
No significant increases in chromosomal aberrations, polyploidy or endoreduplication were observed at concentrations up to 240 µg/ml.
With metabolic activation, precipitation was observed at concentrations of 240 µg/ml or greater. Hemolysis was observed at 343 µg/ml or greater; only dead cells were present on slides at these concentrations. Reductions of 0% and 56% were observed in the mitotic indices of the cultures tested with 168 and 240 µµg/ml as compared with vehicle controls. No significant increases in chromosomal aberrations, polyploidy or endoreduplication were observed at concentrations up to 240 µg/ml.

Confirmatory assay:
Without metabolic activation, precipitation was observed at concentrations of 192 µg/ml or
greater. Hemolysis was observed at 320 µg/ml and slight hemolysis was observed at 240 µg/ml. Only dead cells were present on slides at 154 µg/ml or greater. Reductions of 0%, 30%, 92% and 73% were observed in the mitotic indices of the cultures tested with 24.6, 49.2, 98.4 and 123 µg/ml as compared with vehicle controls. Due to toxicity, only 51 and 38 metaphases were available for analysis in the duplicate cultures treated with 123 µg/ml. No significant increases in chromosomal aberrations, polyploidy or endoreduplication were observed at concentrations up to 123 µg/ml.
With metabolic activation, precipitation was observed at concentrations of 192 µg/ml or greater. Hemolysis was observed at 320 µg/ml; only dead cells were present on slides at this concentration. Reductions of 0%, 0% and 100% were observed in the mitotic indices of the cultures tested with 154, 192 and 240 µg/ml as compared with vehicle controls. No significant increases in chromosomal aberrations, polyploidy or endoreduplication were observed at concentrations up to 192 µg/ml.

Based upon the results of this assay, capsaicin tested negatively in the in vitro chromosome aberrations assay using human peripheral blood lymphocytes under the conditions tested. This conclusion is in concurrence with that of the sponsor.

Trial #2 (Confirmatory; 22 hr continuous treatment –S9 and 3 hr pulse treatment and 19 hr recovery +S9)							
Cytotoxic Effects: Dose related decreases in mitotic indices							
Genotoxic Effects: None							
Metabolic Activation	Test Article	Concentration (mcg/mL)	Cytotoxicity (% of control)[a]	Aberrant Cells Mean –g	Aberrant Cells Mean +g	% Polyploid	% Endo-reduplicate
Without Activation	Vehicle DMSO	10 mcL/mL	100	0.0	1.0	0.0	0.0
	Negative RPMI 1640		-	0.0	0.0	0.0	0.3
	Capsaicin	24.6	100	0.5	4.0	0.0	0.3
		49.2	70	0.5	2.5	0.0	0.0
		123[b]	27	0.0	1.1	0.0	0.0
	Mitomycin	0.300[c]	-	29.0[d]	41.0	0.0	0.0
With Activation	Vehicle DMSO	10 mcL/mL	100	0.5	1.5	0.0	0.0
	Negative RPMI 1640		-	0.0	0.0	0.0	0.0
	Capsaicin	98.4	-	0.0	0.5	0.0	0.3
		123	-	0.0	0.0	0.3	1.3
		154	100	0.0	0.5	0.0	0.5
		192	100	1.5	2.0	0.0	1.5
	Cyclophosphamide	25[c]	-	29.0[d]	42.0	0.0	0.0

[a] Based on mitotic indices.
[b] Only 51 cells available for analysis from the A culture and 38 cells from the B culture.
[c] Only 50 cells analyzed/culture.
[d] Fisher's Exact Test: * - $p \leq 0.01$

Study title: **Capsaicin Micronucleus Test in Bone Marrow of CD-1 Mice 0 h + 24 h Oral Dosing and 48 h Sampling**

This study was previously reviewed by Dr. Timothy J. McGovern when it was submitted to IND 63354.

Key findings: Capsaicin was negative in the in vivo mouse micronucleus assay under the conditions tested.

Study no.: 767712 (in listed as Study no: 21123)
E-Location: m4\42-stud-rep\423-tox\4233-genotox\42332-in-vivo\767712
Conducting laboratory and location: (b) (4)
Date of study initiation: Sept 6, 2001
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin, Batch No. F0010101, Purity 100.3%
Vehicle: 0.5% carboxymethylcellulose

Methods
Strains/species/cell line: CD-1 mice
Doses used in definitive study: First micronucleus study: 0, 200, 400, 800 mg/kg;
Second study: 200 mg/kg (females only.)
Basis of dose selection:

Dose Range finding studies: Three toxicity tests were conducted.

In the first, 5 groups of 1 male and 1 female were dosed orally at 0 and 24 hr with Capsaicin (50, 125, 350, 800 and 2000 mg/kg). The HD female died with labored breathing, subdued behavior, prostration, unable to stand, unable to move limbs, muscle tenses, cold to touch, hunched appearance and piloerection.

In the second test, 3 groups of 3 males and 3 females were dosed orally at 0 and 24 hr with Capsaicin (800, 1200 and 1600 mg/kg). One male and one female from the LD group died, 3 males and 2 females from the MD died and 3 males and 1 female from the HD died. Similar clinical signs as above were observed. The MTD was considered to be 800 mg/kg.

In the third test was performed with one group of 5 females dosed orally at 0 and 24 hr with 400 mg/kg. This study was conducted due to high mortality observed in the first micronucleus assay. Four animals died with similar clinical signs as above. Therefore, the MTD in females was considered to be in the region of 200 mg/kg.

Test agent stability: expiry date of Oct 9 2002; precipitation of test article was observed
Metabolic activation system: NA

Negative controls:
Vehicle: 0.5% carboxymethylcellulose
Negative controls: NA
Positive controls:
cyclophosphamide, 50 mg/kg

Comments: The controls are acceptable

Incubation and sampling times:
Exposure conditions:
Incubation and sampling times: sampling time at 48 hrs after initial dosing
Study design: Animals used for the micronucleus tests were dosed at 0 and 24 hr. The first test included 5/sex in the control group, 5 males in the LD and MD groups, and 10/sex in the HD group. The positive control group included 5 males. The second test included 5 control females and 10 test drug treated females. Mice were killed by cervical dislocation, one femur from each was dissected out and the marrow flushed and flushed into a centrifuge tube containing 3 ml of a 1:1 mixture of fetal calf serum and 0.8% trisodium citrate in Sorensen's buffer, pH 6.8. The cells were pelleted and resuspended and then a smear was made; two slides were made for each animal. The better of the two slides was assessed. Slides from males dosed in the first micronucleus test and females dosed in the second test were randomised together. Slides from females in the first test (vehicle control and high dose) were retained but not assessed.

Two thousand PCEs per animal were scored for micronuclei and the frequency of micronucleated cells (MN-PCE) determined. The numbers of MN-NCE in mature red blood cells were also recorded. The PCE/NCE, a measure of any induced systemic toxicity was determined by counting a minimum total of 100 erythrocytes (PCE+NCE) per marrow preparation.

Analysis:

No. of replicates: 1
Counting method: Leitz Dialux 20 binocular microscopes were used.
Criteria for positive results:
An increase in the number of micronucleated PCE obtained for one or more of the test item treated groups greater than 10% over the expected historical control range for a group of animals. The increase should be biologically relevant and statistically significant relevant to concurrent and historical control frequencies for MN-PCE and /or MN-NCE induction.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The study is considered valid in that dose selection was adequate based upon observations in the toxicity tests, assay methodology was appropriate, vehicles and positive controls were appropriate, criteria for a positive response were adequate, and vehicle controls and positive controls responded as expected.

Study outcome:
In the first micronucleus test, 3 males and 7 females died in the HD group. Clinical signs included subdued behavior, hunched appearance, piloerection, and prostration.
In the second test, 2 of 5 females died. Capsaicin did not induce bone marrow micronuclei in any test groups. There was no indication of of bone marrow toxicity. The positive controls produced expected results as the mean MN-PCE frequency was 1.57% versus 0.07-0.11% in the vehicle control group; bone marrow toxicity was also noted in this group.

Thus, under the conditions tested, capsaicin was negative in the in vivo mouse micronucleus assay in concurrence with the sponsor's conclusion.

Test Article	Dose (mg/kg/day)	No. of Animals Scored	No. of Micronucleated Normochromatic Erythrocytes (MN-NCE)	No. of Polychromatic Erythrocytes Analyzed	Micronucleated Polychromatic Erythrocytes (% MN-PCE)	PCE/NCE (Mean ± SD)
0.5% carboxy methylcellulose	10 mL/ kg/day	4M[a]	7	8014	0.07	0.89 ± 0.08
0.5% carboxy methylcellulose	10 mL/ kg/day	5F	9	10006	0.11	0.92 ± 0.12
capsaicin	200	5M	10	10015	0.04	0.84 ± 0.12
capsaicin	400	5M	8	10007	0.10	0.85 ± 0.13
capsaicin	800	5M	13	10022	0.09	0.89 ± 0.20
capsaicin	200	5F	6	10018	0.05	0.85 ± 0.10
cyclophosphamide	50	5M	51	10014	1.57	0.67 ± 0.09

[a] One male died immediately after dosing – considered to be due to dosing error

2.6.6.5 Carcinogenicity

The Applicant was allowed to conduct a single carcinogenicity study in a transgenetic mouse model because the human plasma levels were non-detectable for the majority of samples and they had not yet determined the frequency of application when the discussions concerning initiating the carcinogenicity studies occurred. In retrospect, the study was unnecessary according to the guidance ICH-S1A, since human administration is for a 1 hour duration at 12 week intervals. The Applicant conducted dose ranging studies in FVB mice and received Executive Carcinogenic Assessment Committee (ECAC) recommendations for the protocol and doses which the Applicant followed in their 26-week daily dermal application study in transgenic hemizygous FVB/NTac-Tg(v-Ha-ras)TG.ACled mice. There were numerous problems with the analysis and interpretation of this study, which resulted in non-acceptance of the study by the ECAC. The study was also published with the same inappropriate analysis and conclusions. Also in retrospect, but after the initial acceptance of the study by the ECAC, this transgenic model was probably inappropriate for compounds that are skin irritants such as capsaicin. A request for a DSI inspection was submitted to examine the discrepancies in the study results (IND 63354 5/13/09), and is planned to occur in mid-July 2009.

Appendix 6 contains a summary table the studies that contributed to decisions about the protocol and doses to be eventually used in the carcinogenicity study, and the ECAC meeting minutes in which these studies were discussed.

The carcinogenicity study was published before the submission to IND 63354 as "Chanda S, Erexson G, Frost D, Babbar S, Burlew JA, Bley K. 26-Week dermal oncogenicity study evaluating pure trans-capsaicin in Tg.AC hemizygous mice (FBV/N). Int J Toxicol. 2007 Mar-Apr; 26(2):123-33." The presentation of results in that publication are different from Study Report, but similar to the summary tables in the NDA. The data is the same, just presented differently, and the Applicant's conclusions are the same.

BACKGROUND:
The initial 4-week dose-range finding study in the mouse (Study 7215-122) was reviewed in the Repeated Dose Section, 2.6.6.3 of this Review. In response to ECAC concerns about the use of (b) (4) (diethylene glycol monoethyl ether) additional studies were conducted and these are reviewed below.

From Dr.Suzanne R. Thornton-Jones Review of June 2004:
The Sponsor previously submitted a special protocol for a Tg.AC hemizygous transgenic mouse which was presented to the Executive CAC on 12 June 2003. The original proposal was to administer the positive control, TPA, and the *trans*-capsaicin in the drug product vehicle (b) (4) (diethylene glycol monoethyl ether) at concentrations of 0.32, 0.64, and 1.28 mg/cm^2/week for 3 hours for 26 weeks. The Executive CAC recommendations at that time were: 1) they did not concur with the Sponsor's proposal to administer TPA in (b) (4) due to the lack of information regarding the (b) (4)®, proposed dosing regimen, and proposed dose; 2) TPA should be administered in (b) (4) with the same frequency (once per week), duration of

exposure (3 hours), and wiped with the cleansing agent as proposed for capsaicin; 3) addition of an untreated control group; 4) recommended concentrations of 0.16, 0.32, and 0.64 mg/cm^2; 5) consider conducting a traditional dermal carcinogenicity study with weekly or less frequent dosing; and 6) submit all revised protocols and supporting dose range-finding data to be reviewed by the Executive CAC.

Study title: **Pilot study to validate the use of tetradecanoyl phorbol-13-acetate (TPA) dissolved in (b) (4) as a positive control in Tg.AC hemizygous mouse (FVB/N).**

This study was reviewed by Dr Suzanne R. Thornton-Jones in June 2004 when it was submitted to IND 63543.

Key study findings

- TPA in (b) (4) at a concentration of 6.25 µg/application given once or twice a week produced papilloma masses at an incidence of 50-67%, but not at the expected 100% frequency

TPA at a dose of 6.25 µg/application dissolved in the (b) (4)® vehicle administered topically to FVB/N mice either once or twice a week for 26 weeks produced papilloma masses [at a frequency of 50-67%], but not at the 100% frequency generally observed when TPA is dissolved in methanol, DMSO, or other suitable solvent. Due to the lack of 100% frequency of papilloma masses with TPA in (b) (4)® the Sponsor believes that it is not a 'suitable vehicle control article' that can be used in the Tg.AC hemizygous mouse model with capsaicin, and I concur.

Study no.: 7215-131
Location: Not submitted to the NDA, submittted to IND 63354 SD-79, May 28 2004
Conducting laboratory and location: (b) (4)
Date of study initiation: 29 July 2003
GLP compliance: Yes
QA report: No
Drug, lot #, and % purity:
TPA (tetradecanoyl phorbol-13-acetate), Lots: 121K1740, 093K0741, 042K1555, 102K1528; Purity 99%
(b) (4)® (diethylene glycol monoethyl ether), Lot 07212TA, Purity 98.9%

Methods:

Doses: 6.25 µg/application TPA in (b) (4)®
Species/strain: FVB/NTac-Tg(v-Ha-ras)TG.ACLED hemizygous mouse
Number/sex/group or time point (main study): 6/sex/group
Route, formulation, volume, and infusion rate: dermal application of 200 µL for 3 hrs
Age: 5-6 weeks at start of treatment
Weight: males 22.1-27.3 g; females 18.3-22.6 g

Unique study design or methodology: Mice were dosed either once or twice a week with TPA in (b) (4)® at a 2 x 2 cm (4 cm^2) site at the intrascapular dorsal region for 26 weeks. The concentration of TPA used in the study is higher than the standard concentration of 2.5 µg/200 µL application. The Sponsor based the higher dose on a two published papers:

- Spaulding et al. (1993) indicated that a total concentration of 25-30 µg of TPA administered at 3-10 sites may be sufficient to induce an average of 11-15 papillomas in Tg.AC mice.
- Thompson et al. (1998) administered 6.25 µg of TPA/application twice a week for 26 weeks. No control group was used in the study.

Observation times and results:

Observations	Times	Results
Mortality	daily	One female mouse in the once a week group was humanely euthanized during SW19 with non treatment-related findings. All other animals survived to scheduled euthanasia.
Clinical signs	daily – included dermal mass mapping and Draize irritation scoring	Unremarkable clinical observations. **Masses were observed in male mice at incidences of 1/6 and 4/6 and female mice at 3/6 and 4/6 for the once a week and twice a week groups, respectively.**
Body weights	pre-dose, first day of dosing, weekly	Unremarkable for male mice. Female mice dosed twice a week had a decrease in body weight gain vs. the once a week group (7.6 g vs. 10.2 g).
Food consumption	weekly	Unremarkable.
Opthalmoscopy	not performed	---.
EKG	not performed	---
Hematology	not performed	---
Clinical chemistry	not performed	---
Urinalysis	not performed	---
Gross pathology	not performed	Unremarkable.
Organ weights	not performed	---
Histopathology	not performed Adequate Battery: NA Peer review: NA	---
Toxicokinetics	not performed	---

[Note: SW=study week]

Study title: **4-week dose range-finding dermal toxicity and toxicokinetic study with *trans*-capsaicin in FVB/N mice.**

Key study findings:
The Sponsor conducted a 4-week study in FVB/N mice treated with methanol as the vehicle control or *trans*-capsaicin dissolved in methanol administered for a 3 hour exposure every day for 4 weeks. Findings from the study included slight erythema and atonia at doses 0.64 mg/animal (0.16 mg/cm^2) with corresponding histopathological changes of acanthosis, epidermal serocellular crust, hyperkeratosis, and chronic (active) inflammation. Transient decreases in body weight gains were observed in male mice during week 1 (20-34%) at doses 0.64 mg/animal (0.16 mg/cm^2) with corresponding decreases in food consumption. Body weight gains were also decreased in female mice during week 2 (25-45%) at doses ≥0.32 mg/animal (0.08 mg/cm^2). Adrenal weights were decreased in female mice at doses >0.32 mg/animal (0.08 mg/cm^2). The MTD for this study was 1.28 mg/animal (0.32 mg/cm^2) for male and female mice.

- Dermal irritation: very slight erythema and atonia at doses ≥0.64 mg/animal (0.16 mg/m^2); well-defined erythema and very slight edema at a dose of 1.28 mg/animal (0.32 mg/m^2)
- Body weight gains: decrease during SW1 for male mice at doses ≥0.64 mg/animal (0.16 mg/m^2) and female mice during SW2 at doses ≥0.32 mg/animal (0.08 mg/m^2)
- Food consumption: decreased in male mice during SW1 at doses ≥0.32 mg/animal (0.08 mg/m^2)
- Hematology: decrease in WBC and lymphocytes in all treated male mice; increase in platelets in female mice at a dose of 1.28 mg/animal (0.32 mg/m^2)
- Clinical chemistry: decrease in glucose in male and female mice at most doses, increases in AST, ALT
- Organ weights: decreases in adrenal weight in female mice at doses ≥0.32 mg/animal (0.08 mg/m^2)
- Histopathology: acanthosis, epidermal serocellular crust, hyperkeratosis, and chronic (active) inflammation
- MTD = 1.28 mg/animal (0.32 mg/m^2) male and female mice

Study no.: 7215-137
Location: Not submitted to the NDA, submittted to IND 63354 SD-79, May 28 2004
Conducting laboratory and location: (b) (4)
Date of study initiation: 24 February 2004
GLP compliance: Yes
QA report: No, unaudited draft report submitted
Drug, lot #, and % purity:
trans-capsaicin, Lot F0010102, Purity 100.2%
L-MX4 (4% lidocaine), Lot 03150A, no purity information provided
cleansing agent, Lot 22698, no purity information provided

Methods:
Doses: 0.16, 0.32, 0.64, 1.28 mg/animal/day (0.04, 0.08, 0.16, 0.32 mg/cm^2/day)
Species/strain: FVB/NTac-Tg(v-Ha-ras)TG.ACLED hemizygous mouse

Number/sex/group or time point (main study): 6/sex/group
Route, formulation, volume, and infusion rate: dermal application of 50 µL for 3 hrs
Satellite groups used for toxicokinetics or recovery: 15/sex/capsaicin groups
3/sex/group for control group
Age: 7 weeks at start of treatment
Weight: males 16.4-27.1 g; females 15.6-24.2 g
Unique study design or methodology: Mice were treated with the control (90% methanol), or *trans*-capsaicin dissolved in methanol, applied daily to a 2 x 2 cm (4 cm^2) site at the intrascapular dorsal region for 4 weeks. The site of administration was anesthetized with LMX-4 cream for 30 mins, then the residual LMX-4 wiped away and then the vehicle or *trans*-capsaicin were administered for 3 hrs. At the end of treatment, the area was washed with the 200 µL of the'cleansing lotion'.

Observation times and results:

Observations	Times	Results
Mortality	daily	One male at a dose of 0.32 mg/animal was humanely euthanized on SD 18. Clinical observations included thin appearance, hypoactivity, recumbency, squinted eyes, cold to touch, irregular/labored breathing, rough hair and yellow hair coat in the perineal area. Cause of death was undetermined but histopathological findings included lymphoid necrosis (slight-minimal) in the mesenteric lymph node, spleen, and thymus, and hemorrhage and alveolar macrophage infiltration in the lung. All other animals survived to scheduled euthanasia.
Clinical signs[1]	daily - included dermal mass mapping and Draize irritation scoring	Male mice at doses of 0.16-0.32 mg/animal exhibited rough hair coat and yellow hair coat in the perineal area. There were no other clinical observations. Dermal irritation findings included very slight erythema and atonia at doses ≥0.64 mg/animal, while at a dose of 1.28 mg/animal there was well-defined erythema and very slight edema. Eschar was observed in male mice at doses of 0.16-0.32 mg/animal.
Body weights	pre-dose, first day of dosing, weekly	Unremarkable for body weight in male and female mice. Body weight gains were decreased for male mice at doses ≥0.64 mg/animal during SW1 (20-34%), and in female mice at doses ≥0.32 mg/animal during SW2 (25-45%).
Food consumption	weekly	Decreased in male mice at doses ≥0.32 mg/animal during SW1 (11-17%). Unremarkable for female mice.

Opthalmoscopy	not performed	---
EKG	not performed	---
Hematology	SW 4	Male mice at all doses exhibited decreases in WBC (12-35%) and lymphocytes (12-38%). Female mice exhibited increases in platelets (16%) at a dose of 1.28 mg/animal.
Clinical chemistry[1]	SW 4	Male mice at all doses exhibited a decrease in glucose (10-11%). Also in male mice there was an increase in AST (12-21%) at doses of 0.32-0.64 mg/animal, and ALT (39-68%) at doses of 0.16-0.32 mg/animal. Female mice exhibited increases in AST (38-57%) and ALT (23-67%) at doses of 0.16, 0.64-1.28 mg/animal, and a dose-related increase in inorganic phosphate (12-17%) at doses ≥0.64 mg/animal. Female mice also exhibited a decrease in urea nitrogen (15-26%) at doses of 0.16-0.64 mg/animal and a decrease in glucose at doses of 0.32 and 1.28 mg/animal.
Urinalysis	not performed	---
Gross pathology	SW 4	Unremarkable for male mice. Female mice (2/6) at a dose of 1.28 mg/animal exhibited distended and fluid-filled lumen of the uterus.
Organ weights	SW 4	Unremarkable for male mice. Female mice at doses ≥0.32 mg/animal exhibited a decrease in adrenal weight (13-25%) [statistically significant at a dose of 1.28 mg/animal].
Histopathology[1]	SW 4 Adequate Battery: yes Peer review: unknown	Skin findings in male and female mice consisted of acanthosis, epidermal serocellular crust, hyperkeratosis, and chronic (active) inflammation. Male mice (5/6) at a dose of 1.28 mg/animal exhibited subacute inflammation in the liver. Female mice at doses ≥0.32 mg/animal exhibited dilated uterus and/or endometrial edema.
Toxicokinetics	SD 1	A complete TK profile was not performed. Measurable values were only observed at a dose of 1.28 mg/animal. Plasma concentrations were generally between 10-20 ng/mL, but there were a few animals that exhibited values greater than 20 ng/mL.

[Note: SD=study day; SW=study week; [1] See Appendix for further details]

Appendix A. Study results for Study 7215-137, 4-week dose range-finding study in FVB/N mice

Clinical Observations – Dermal Irritation Scores

Dose (mg/animal)	0.16	0.32	0.64		1.28	
	M	M	M	F	M	F
Erythema & eschar formation						
Very slight erythema (barely perceptible)			1/6	1/6	6/6	5/6
Well-defined erythema					1/6	1/6
Edema formation						
Very slight edema (barely perceptible)					5/6	2/6
Atonia - Yes			1/6		2/6	
Eschar - Yes	2/6	1/5				

[M=male, F=female; Values represent number of animals/total number examined.]

Clinical chemistry:

Dose (mg/animal)	0.16		0.32		0.64		1.28	
	M	F	M	F	M	F	M	F
ALT	↑68%	↑67%	↑39%			↑29%		↑23%
AST		↑57%	↑21%		↑12%	↑49%		↑38%
Glucose	↓11%		↓10%	↓11%	↓10%		↓11%	↓11%
Inorganic phosphate						↑12%		↑17%
Urea nitrogen		↓15%		↓26%		↓15%		

Values represent percent difference from control; M=male, F=female

Histopathology:

Dose (mg/animal)	0.16		0.32		0.64		1.28	
Skin, treated	M	F	M	F	M	F	M	F
Acanthosis		1/6	5/5	5/6	6/6	5/6	6/6	6/6
Slight		1/1	3/5	1/5	5/6	3/5	3/6	3/6
Minimal			2/5	4/5	1/6	1/5	2/6	
Moderate						1/5	1/6	3/6
Crust, serocellullar, epidermal		1/6	1/5		3/6	2/6	3/6	3/6
Slight		1/1			2/3		1/3	
Minimal			1/1		1/3	2/2	1/3	2/3
Moderate							1/3	1/3
Hyperkeratosis		2/6	5/5	5/6	6/6	5/6	6/6	6/6
Slight		1/2		1/5	2/6	1/5	3/6	4/6
Minimal		1/2	5/5	4/5	4/6	4/5	3/6	2/6
Inflammation, chronic-active	1/6	2/6	5/5	5/6	6/6	5/6	6/6	6/6
Slight	1/1		2/5	1/5	3/6	2/5	2/6	3/6
Minimal		2/2	3/5	4/5	1/6	1/5	1/6	
Moderate					2/6	2/5	3/6	3/6

Values represent number of animals/total number examined.

Study title: **26-Week Dermal Oncogenicity Study with *trans*-Capsaicin in Tg.AC Hemizygous Mice (FVB/N)**

Study no.: 7215-150
E-location: M4\4234-carcigen\42342-smt-stud\7215-150
Conducting laboratory and location: (b) (4)
Date of study initiation: March 17, 2005
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
Synthetic trans-capsaicin, Lot AD3743, purity 99.7%
Vehicle control: Diethylene glycol monoethyl ether (DGME, also known as (b) (4)®), Purity 99.6 and 99.7%
Positive control: TPA, tetradecanoyl phorbol-13-acetate (phorbol 12 myristate 13-acetate, purity 100%
Anesthetic cream: L-M-X4™ (lidocaine, 4%), Purity not provided

CAC concurrence: The ECAC made recommendations at the July 6, 2004 meeting (refer to Appendix 6), and the Applicant appears to have complied with the recommendations.

Key study findings:
Histopathological analysis (refer to the table below) of dermal masses obtained at necropsy from the site of capsaicin application revealed that, of the confirmed masses, most were benign squamous cell papillomas. The incidence of papillomas in the capsaicin groups was greater than in the vehicle control group. The anesthetic control group and untreated control groups had similar low incidence of papillomas as the vehicle control group. The positive control group was clearly positive, with most animals having squamous cell papillomas in the treatment area.

Male and female Model TGAC-T (hemizygous), FVB/NTac-Tg(v-Ha-ras)TG.ACled mice were assigned to 7 groups, 25 mice/sex/group. Treatment groups 2, 3, and 4 received trans-capsaicin (0.64, 1.28, or 2.56 mg/mouse; 0.16, 0.32, or 0.64 mg/cm^2, respectively) in diethylene glycol monoethyl ether (DGME) once weekly by topical application to the dorsal skin (4 cm^2) for 26 weeks. Capsaicin or vehicle was applied for a 3 hour period then wiped away with a cleansing gel. Due to the intense burning sensation, a topical local anesthetic was applied prior to the capsaicin and removed after 30-45 minutes prior to the capsaicin application. There were 4 control groups, a DGME vehicle control, an anesthetic control, a positive control tetradecanoylphorbol-13-acetate (TPA in DGME, 6.25 µg/mouse, 1.56 µg/cm^2, treated twice weekly), and an untreated control group.

- There were no substantial differences in weight gain and food consumption between groups during the study.
- Clinical signs of malocclusion, swollen gums, rough haircoat, and random masses occurred in all groups except the untreated group.

- Dermal irritation at the site of application was assessed weekly but 7 days after the previous dose (just before the next dose was applied). Erythema and desquamation were still noted for males at a low frequency (<7/group). The only female with signs was desquamation in the high capsaicin group. The positive control and untreated groups lacked any signs of dermal irritation. There was no indication of dermal atonia, edema, eschar or fissuring in any group.
- Gross and histopathological analysis of dermal masses at the site of application at necropsy revealed that of the confirmed masses by gross pathology, most all were benign squamous cell papillomas with one incidence of a malignant fibrosarcoma. The incidence of these was much greater for the capsaicin and positive control groups than in the vehicle, lidocaine, or untreated control groups.
- Non-neoplastic microscopic findings in the site of application included acanthosis, hyperkeratosis/parakeratosis (primarily females), epidermal crusts, subepidermal fibrosis, epidermal ulcerations/erosions, and chronic-active inflammation.

In the summary table that follows, there are discrepancies between the Sponsor's and the Reviewer Analysis in the number of animals with papilloma for the Treated skin and for the Non-Treated skin. There were a multitude of instances in the individual pathology tables where masses were not indicated as confirmed and/or lacked histopathological information, and instances in which histopathology was not linked to a specific mass when more than one mass was identified. There were also cases in which the treatment site listed masses of the neck, ear or abdomen.

In the table top rows are the group identification numbers, treatments, number of animals initially, mortalities and survivors to the end of the study. The number of animals with at least one mass or papilloma at necropsy (combined unscheduled early death or euthanasia and scheduled at study termination) are presented for the Treated Skin or Non-Treated Skin. Non-Treated Skin means all skin other than the site of application (treatment site).

Summary of Results for Treated and Non-Treated Skin

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Mortalities (unscheduled)	1	4	5	9	3	3	2	5	3	7	19	17	1	3
N at end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
TREATED SKIN														
Sponsor Summary [condensed by Reviewer from Chanda et al 2007: Tables 4, 5, 6, same as Module 2 Summary Tables],														
Copyright Material														
Reviewer Analysis from Individual Pathology Data Tables														
Masses	0	0	2	2	7	3	4	3	0	1	21	20	0	1

Papillomas	0	0	1	1	4	2	1	2	0	0	21[a]	17[a]	0	1
Papillomas Combined Sexes	**0**		**2**		**6**		**3**		**0**		**38[a]**		**1**	
* These incidences are the same as the statistical data set supplied by the Sponsor														
NON-TREATED SKIN														
Sponsor Summary [condensed by Reviewer from Chanda et al 2007: Tables 4, 5, 6, same as Module 2 Summary Tables],														

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Reviewer Analysis from Individual Pathology Data Tables														
Masses	7	11	7	6	21	6	11	17	11	10	24	20	16	10
Papillomas*	3	2	1	2	6	3	3	8	4	4	10[b]	10[b]	6	5
Papillomas Combined Sexes	**5**		**3**		**9**		**11**		**7**		**20[b]**		**11**	

Notes: Numbers represent the incidences of animals with at least one mass or papilloma.

[a] includes animals with papillomas listed in the "treatment area", but were their sites were actually in other skin areas

[b] there were 13 males and 8 females with masses that lacked adequate histopathology information; per protocol non-treated skin did not require histopathology

Adequacy of the carcinogenicity study and appropriateness of the test model:

- In summary, this Reviewer does not consider this study acceptable due to deficiencies in the individual study data tables, lack of appropriate summary tables and lack of appropriate statistical analysis, as indicated below. However, there is sufficient information despite these deficiencies to judge capsaicin's carcinogenic potential in this study.
- *Study Design and Appropriateness:*
  - Although the Tg.AC mouse model was determined as an adequate substitute for a 2-year (lifetime) bioassay at the time of the carcinogenicity study discussions (refer to Nov 9, 2004 EOP2 Meeting Minutes, discussion of question 7 and Oct 26, 2005 EOP2 Meeting Minutes, discussion of question 2) for this drug development program, it has since been questioned whether this model is an appropriate model for known skin irritants. However, it was decided at that time to be acceptable and was not revisited since then.
  - The appropriate control groups were included in the study upon ECAC previous recommendations.
  - *Positive Control*: The positive control treatment, 1.25 µg TPA, was applied twice as frequently (twice weekly) and at a volume twice as large (0.2 mL) as the test substances. Although the recommended doses in this animal model are 1.25 to 2.5 µg TPA, three times weekly (Tennet et al, 2001), the results of this study indicated the animal model was responsive at this twice weekly dosing frequency.
  - *Once weekly dosing frequency*: The once weekly dosing frequency of a test substance has not been validated as an appropriate dosing frequency for this animal model, and it should be dosed at the same frequency as the

positive control, or the positive control dosed at the same frequency as the test substances.

- *Once weekly assessment of tumors*: The basis for the with the Tg.AC mouse model was developed with the multiple applications of test compounds each week (usually 3-5 days/week). With less frequent applications such as once-weekly as used in this study, a once weekly assessment may not be appropriate. The use of this model is highly dependent on the time course of papilloma growth, persistence, and regression. The once weekly frequency of assessment has not been validated for a once weekly test substance application.
- A change in dosing strategy occurred half way into the study, that spread the dosing over a 2 day period. This eliminated the occurrence of deaths in one of the groups. While this change allowed more time for dosing and observation, and should not have impacted the study, there was too little explanation concerning this change. Perhaps anesthetic was left on too long or not applied due to being rushed to treat so many animals in a multistep process, all in one day. It was not explained. It was not mentioned why it took until half way through the study to alter the treatments to extend over a 2 day period.

- *Statistical Analysis:*
  - The statistical basis for the determination of neoplastic potential of capsaicin was not presented. In general the methodology and presentation of results was unclear and misleading (tables did not reflect what they purported to indicate).
  - Requests by the reviewing Statistician for a proper data set for this type of study, resulted in submission of only the presence or absence of masses, lacking the number of masses observed.
- *Results and Presentation:*
  - No pathology report was submitted, although summary and individual data tables were provided, but with insufficient explanation.
  - The study report presentation was not an accurate reflection of the data in the individual animal study tables and the individual study tables, specifically Appendix 6 and 11, appeared to be incomplete and inconsistent with the stated methodology. The Quality Assurance Statement that "This report has been reviewed by the Quality Assurance Unit of (b) (4). and accurately reflects the raw data." is not true. For example, in Appendix 6: Weekly incidence of Dermal Masses the data is misleading. The note at bottom of each page, "Note: Numbers indicate the total number of skin masses on the treated site each week", is not accurate, it is a table of all dermal masses (the distinction is essential for the proper analysis of this type of study). This was discovered when summarizing the incidence of masses at the treatment sites as indicated in the Appendix 6 (a summary table was not provided in the study) and noting the control animals had very high incidences of "treatment site" masses. However, the individual pathology tables indicated the vast majority of these were not "treatment site" locations. In addition, the week headings are also misleading in that week 1 is actually

day 1, week 2 are the results from assessing week 1 application of treatments (applied on day 1), etc and week 28 is actually 1or 2 days after week 27. Apparently necropsies were conducted over a few days and this was not mentioned. It is presented as if the animals were dosed for another week, which was not the case.

- There are deficiencies in the Appendix 11 pathology tables for individual animals in which masses were not confirmed, histology was not conducted, or results were not reported, for a specific mass. This was common when more than one mass was identified, and the result was not linked to a specific mass. It is unclear if the result was from only a representative mass, or all masses had this result.
- Table presentations lacked necessary explanation such as Table 3: Summary of Dermal Observations in which conflicting information was presented such as rows indicating "Atonia, no, dose site," and a row indicating "Atonia not applicable, dose site," both with N=25, but there are only 25 in the dose group. The table appears to be created by "dumping" raw data lines into a table, without any effort to convey the study results in a meaningful and interpretable manner.

Evaluation of tumor findings:

- Conclusions from this study are based on histopathological findings since the study lacked the presentation of data that would allow standard Tg.AC analysis procedures.
- Statistical evaluation of the tumor data was not conducted by the Applicant as far as this reviewer could determine. The mass and papilloma incidences used in the statistical analysis are consistent with the number presented in the Applicants Summary Tables of the application Module 2 and the tables of Chanda et al 2007, but these numbers conflict with the individual animal pathology tables of Appendix 11 due to inadequate and missing information in this table. Conclusions from a draft of the Statistical Review by Dr. Steve Thomson are as follows:
  - There was no difference in the survival patterns between the vehicle control and capsaicin treatment groups (Groups 1-4).
  - In males the pairwise comparison for the number of animals with papilloma in treated skin in the mid dose group is statistically significantly higher than the no treatment group ($p=0.0082$) and barely statistically significantly higher in the vehicle treatment group ($p=0.0491$).
  - In females there is a clear trend for papillomas with dose ($p=0.0002$), while the test of differences between vehicle and the high dose group and the medium dose group were also statistically significant ($p=0.0004$ and $p=0.0353$, respectively).
  - The corresponding pairwise tests with the no-treatment group were not quite statistically significant at the usual 0.05 level (all $p \geq 0.0776$). This is clearly due to the large number of papillomas in the no treatment group.
  - Particularly with the relatively small sample sizes in this study (25 animals) it seems that the evidence for a trend over several treatment

- groups is much stronger than the evidence in a single treatment group like the untreated group. However there is not consistency across genders.

Incidence of animals with Squamous Cell Papillomas
(from the Draft Statistical Review, animal incidences values from the Applicants provided data)

Veh	Low	Mid	Hi	Lido	TPA	None	Trend	VvsH	VvsM	VvsL	VvsN	LvsN	MvsN	HvsN
TREATED SKIN														
Males														
1	2	6	3	1	21	0	.1615	.2890	.0491	.5000	.5000	.2449	.0082	.1092
Females														
0	1	2	2	0	17	1	.1280	.2220	.2449	.4634	.4898	.7225	.5000	.4511
SKIN/SUBQ, OTHER														
Males														
1	0	4	1	3	8	6	.3663	.7449	.1743	.5000	.9509	.9889	.6099	.9453
Females														
2	2	4	8	3	8	5	.0061	.0301	.3326	.6388	.7742	.7353	.4439	.2045
ANY SKIN														
Males														
9	4	14	6	10	36	9	.7969	.8870	.2836	.9018	.3812	.7519	.0989	.7275
Females														
2	7	10	16	8	29	9	.0002	.0004	.0353	.0780	.9573	.5124	.5400	.0776

- The data were not presented as the table information stated and implied (refering to mass weekly counts of Appendix 6). What was originally thought to be a lack of determination of the non-site of application tumors during the study, and the high number of tumors in the untreated control group, was actually a table of all dermal tumors in contrast to a note at the bottom of each page indicating masses at the site of treatment.
- There are deficiencies in the pathology tables for individual animals in which masses were not confirmed and histological results were not indicated for a specific mass when more than one mass was identified.

Methods
Doses: 0, 0.16, 0.32. 0.64 mg/cm^2; (0, 0.64, 1.28, 2.56 mg/mouse)
Basis of dose selection (MTD, MFD, AUC etc.): MTD; Excessive toxicity erosions, ulcer and fissuring at the dose of 1.28 mg/cm^2, once weekly administration.

- Dose levels for *trans*-Capsaicin were selected based on data from a 1-month range-finding study in FVB/N mice ([(b) (4)]: 4-Week Dose Range-Finding Dermal Toxicity and Toxicokinetic Study with Capsaicin in FVB/N mice).
  (***Reviewer Comment***: *This was acceptable according to the June 12, 2003 ECAC meeting minutes presented in Appendix 6.*
- The vehicle for the positive control was selected on the basis of a pilot study validating TPA dissolved in DGME as a positive control in Tg.AC hemizygous [[(b) (4)]: Pilot Study to Validate the use of Tetradecanoyl Phorbol-13-Acetate (TPA) Dissolved in [(b) (4)]® as a Positive Control in Tg.AC Hemizygous Mice (FVB/N)] and in consultation with the sponsor and FDA.

*(**Reviewer Comment**: This was acceptable according to the July 6 2004 ECAC meeting minutes).*

Species/strain: Tg.AC transgenic mouse; TGAC-T (hemizygous), FVB/NTac-Tg(v-Ha-ras)TG.ACled

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

Route, formulation, volume:

- Capsaicin or vehicle was administered dermally once weekly for 26 weeks at a fixed dose volume of 0.1 mL/animal/week to a 4 cm^2 area (2 cm x 2 cm) of the intrascapular region of dorsal skin (positive control was 0.2 mL). After 3 hours, the test article was removed from the skin by use of cleansing gel (approximately 0.2 mL/animal on gauze). A larger area was clipped free of hair as needed to allow dosing and observation of the site. The Untreated Group 7 also had the intrascapular region clipped.
- An anesthetic cream (LMX4™) was applied (approximately 0.1 mL/animal, just enough to cover slightly beyond the application area) to the treatment site for 30 to 45 minutes before the application of the test or vehicle control article. After 30 to 45 minutes, any residual LMX4™ was wiped off with dry gauze before the test or vehicle control article application. Approximately 3 hours after application of the test or vehicle control article, the application area was cleaned with the cleansing gel. For Group 5, the LMX4™ only treatment, the anesthetic cream was wiped off after 30-45 minutes with dry gauze (no cleansing gel) and no further treatment was applied.

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Group	Identification	Dose Volume mL	Dose Level (mg/mouse/week)[a]	Dose Concentration (mg/mL)	Dose/Surface Area/Week (mg/cm^2/week)[b]	Number of Mice	
						Male	Female
1 - Vehicle Control	DGME	0.1	0	0	0	25	25
2 - Low Dose	*trans*-Capsaicin in DGME	0.1	0.64	6.4	0.16	25	25
3 – Mid Dose	*trans*-Capsaicin in DGME	0.1	1.28	12.8	0.32	25	25
4 - High Dose	*trans*-Capsaicin in DGME	0.1	2.56	25.6	0.64	25	25
5 - Anesthetic Control	Lidocaine only (L·M·X4™)[c]	0.1	0	0	0	25	25
6 - Positive Control	TPA[d]	0.2	12.5	31.25	3.125	25	25
7 - Untreated Control	Untreated	0	0	0	0	25	25

a Based on an application area of approximately 4 cm^2 on the back of a mouse.
b Mice in Groups 1-4 were dosed once a week for approximately 3 hours.
c Lidocaine (L·M·X4™) were dosed once a week by topical administration for 30 to 45 minutes.
d TPA were dissolved in (b) (4) and dosed twice/week by topical administration for approximately 3 hours. The dose unit for Dose Level was µg/mouse/week, for Dose/Surface Area/Week was µg/cm^2/week, and for Dose Concentration was mg/mL/dose.

Frequency of dosing: once weekly (positive control dosed twice weekly)

Satellite groups used for toxicokinetics or special groups: none

Age: 8 (males) and 10 (females) weeks old

Animal housing: housed individually in stainless-steel cages
Restriction paradigm for dietary restriction studies: No
Drug stability/homogeneity:

Information on synthesis methods, stability, purity, composition, or other characteristics of the test, vehicle/control, and positive control articles, anesthetic cream and cleansing gel were not provided in the study report, but are on file with the Applicant.
Capsaicin Formulation: Dose analysis of capsaicin prepared in weeks 1, 4, 12, 13 and 26 indicated doses ranged from 93.9 to 108.3% of target concentrations, within the acceptable target range of ±10% of theoretical.
Positive Control Formulations: Dose analyses for TPA concentrations indicated doses ranged from (88.4 to 106.6%). It was prepared on the day of use (twice weekly) until stability was established. Stability analyses of the TPA at 20 and 40 µg/mL indicated it was stable up to 14 days under room temperature and refrigerated conditions. Beginning at week 4, the positive control formulation was prepared at least weekly. There was a low recovery of TPA from the first mix during week 1 (26.8% of target) and week 13 (88.4% of target), but adjustments were made to mixing procedure to correct these lower than expected recoveries to within ±10% of theoretical.

Dual controls employed:

- Negative Control groups included vehicle control, anesthetic cream control, and untreated (area hair clipped) control; Cleansing gel treatment was incorporated into all the groups except the anesthetic cream group and the untreated control group.
- Positive Control, following anesthetic cream treatment as described above for the other groups, TPA in DGME (Group 6) received twice weekly applications in 0.2 mL which was wiped away with cleansing gel after 3 hours.

Interim sacrifices: No
Deviations from original study protocol: At week 12, the dosing schedule was changed to dose the groups over a 2-day period, rather than all groups on one day. Groups 2, 4, and the first weekly dose for group 6 on Tuesday and groups 1, 3, and 5 on Wednesday; Group 6 received a second dose on Friday). The Applicant notes that "once this change in dosing procedure was implemented, the result was a notable decrease in the number of unscheduled deaths for the remainder of the study." *(Reviewer comment: this should not have impacted the study; obviously something was wrong with either the dosing treatments or observations. Perhaps anesthetic was left on too long or not applied due to being rushed to treat so many animals in a multistep process, all in one day. It was not explained. It was not mentioned why it took until half way through the study to alter the treatments to extend over a 2 day period).*

Analysis: The following statistical methods were used to analyze the body weight, body weight change, and food consumption data.

- Levene's test (Levene, 1960; Draper and Hunter, 1969) was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, rank transformation was used to stabilize the variance. Comparison tests took variance heterogeneity into consideration.

- One-way analysis of variance [ANOVA (Winer, 1971a)] was used to analyze body weight and food consumption data.
- If the ANOVA was significant ($p \leq 0.05$), Dunnett's t-test (Dunnett, 1955, 1964) was used for control versus treated group comparisons. For data that exhibited heterogeneous variances after transformations, Dunnett's t-test for unequal variances with Welch's degrees of freedom (Welch, 1947) was employed.

For each sex, the group comparisons listed below were evaluated at the 5%, two-tailed probability level. Data collected on or after the first day of treatment were analyzed statistically.

(1) Group 1 with 2-7
(2) Group 2 with 3-7
(3) Group 3 with 4-7
(4) Group 4 with 5-7
(5) Group 5 with 6 and 7
(6) Group 6 with 7

Results

Mortality: Animals were checked twice daily

High mortality occurred in the TPA positive control group with 19 of 25 males and 17 of 25 females either dead or moribund prior to terminal sacrifice. Among mice given the vehicle control or test article, early deaths and moribund sacrifices ranged from 1 of 25 (vehicle control males) to 9 of 25 (0.64 mg/animal/week females). The cause of early death or moribundity could not determined from pathology for many animals. In the positive control animals, early death and moribundity was often considered related to large numbers of skin papillomas. As indicated in the table below, hematopoietic system alterations (erythrocytic leukemia and lymphosarcoma) and odontogenic (tooth) neoplasms were also associated with early death or moribundity, which are known to be common spontaneous neoplasms in Tg.AC mice (Mahler et al., 1998).

A change in dosing procedure to dose the animals over a two day period halfway through the study resulted in a "notable decrease in the number of unscheduled deaths for the remainder of the study." This was particularly the case with deaths in females of the 0.64 mg dose of capsaicin.

Mortality incidence among the dose groups

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose (mg/mouse)	**0**		**0.64**		**1.28**		**2.56**		**0**		**6.25 µg/application; 12.5 µg/week**		**0**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 $\mu g/cm^2$/ twice weekly; 3.25 $\mu g/cm^2$/ week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25

Mortalities (unscheduled)	**1**	**4**	**5**	**9**	**3**	**3**	**2**	**5**	**3**	**7**	**19**	**17**	**1**	**3**
Survivors to end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
Probable Cause of Early Death (from Microscopic Analysis, Applicant Table 12)														
Undetermined		**4**	**3**	**7**	**2**	**3**	**1**	**4**	**1**	**5**	**18**	**13**	**1**	**1**
Neoplasia, total	**1**		**2**	**2**	**1**		**1**	**1**	**2**	**3**	**1**	**5**		**2**
Fibrosarcoma							1							
Fibroma											1			
Lymphosarcoma			1						1					
Ameloblastoma	1		1	1				1		1				
Odontoma														1
Erythrocytic leukemia				1	1				1	2		4		1
Yolk sac carcinoma												1		

Clinical signs: Animals were checked twice daily (a.m. and p.m.). Weekly detailed observations were also obtained. Information (i.e., time of onset, location, appearance, progression) on each grossly visible or palpable mass on the treatment site was recorded. *(Reviewer comment: These weekly detailed observations occurred prior to each weekly dose administration, and therefore events that may have occurred (such as papilloma onset and disappearance during the 7 day period after each dose administration would not be observed).*

The most common adverse clinical signs were malocclusion, swollen gums, rough haircoat, and random masses although these occurred with approximately equal incidence in the dose groups with the exception of the untreated group.

Selected Dermal Clinical Signs*

(Reviewer Modified From Applicant Table 2: Summary of Clinical Signs)

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 µg/cm^2/ twice weekly; 3.25 µg/cm^2/ week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Mass	7	11	**6**	**7**	**13**	**7**	**10**	**14**	8	7	24	20	12	8
combined sexes	18		**13**		**20**		**24**		15		44		20	
all digits					1									
dorsal abdomen (back)									1					
mouth													1	
Rough haircoat	4	3	13	6	13	4	4	4	7	5	7	4	8	3

* number of animals with observed sign

Dermal/Local Irritation: Observations were "done daily on all animals and groups during Week 1 prior to dosing, at least weekly thereafter, and on the day of scheduled sacrifice; the application site was scored/graded using a modified Draize technique."
From protocol amendment 3, number 1: "beginning week 12, irritation scoring will be done prior to dosing on Tuesdays (groups 2, 4, and 6) and Wednesdays (Group 1, 3, and 5). Group 7 weekly irritation scoring will be done on Tuesdays."

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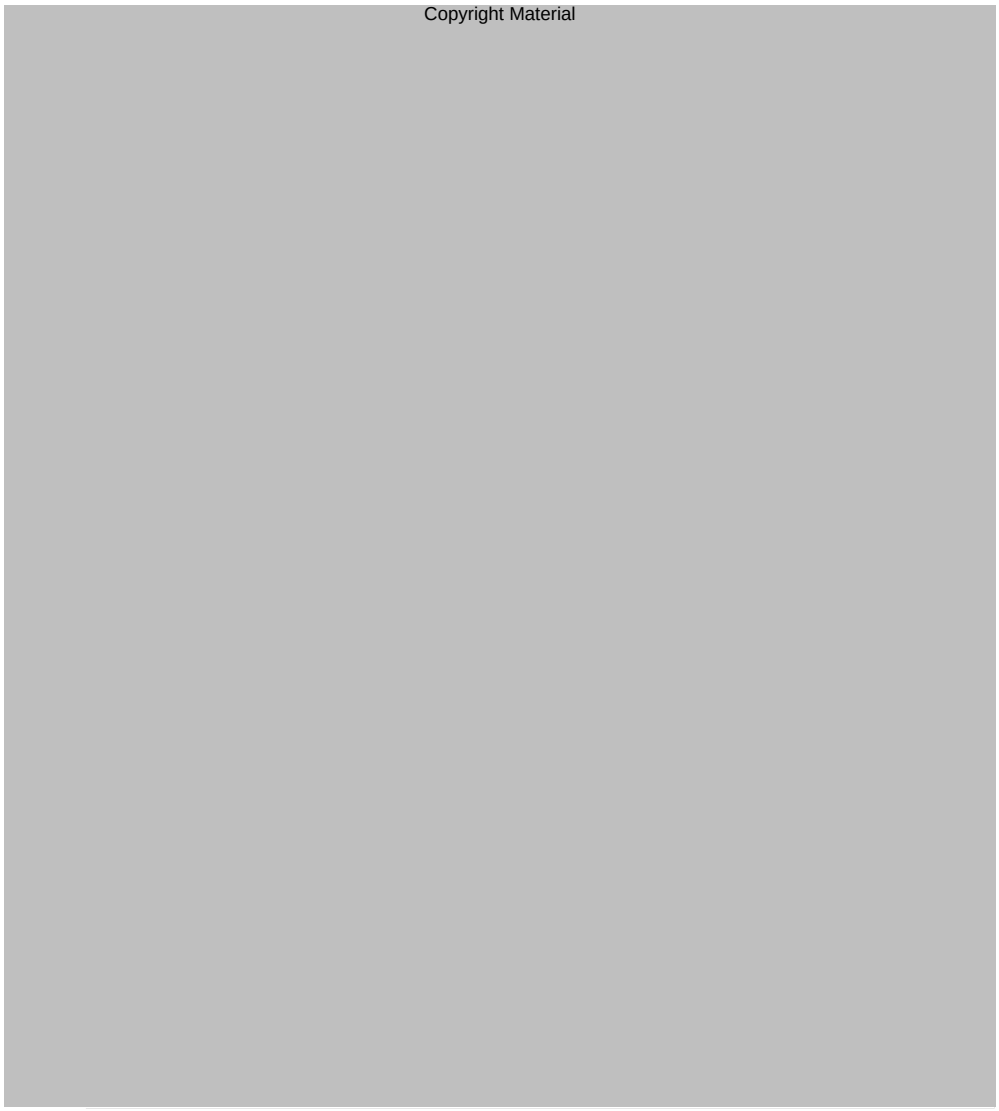


Draize, J. H., *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics,* pp. 46-49, The Association of Food and Drug Officials of the United States, Austin, TX (1959).

Except for erythema in 3 lidocaine treated control males, the control groups did not exhibit signs of dermal irritation when assessed a week after application. In fact only one

female mouse had signs dermal irritation which was desquamation. Signs of erythema were present in a few males following capsaicin treatment a week earlier.

Total body incidence of sores and scabs (not further defined) were increased in mid and high capsaicin treated groups. Note that the untreated control also had substantial high incidence than any of the other control groups.

Reviewer Comment: *Dermal Irritation scoring was conducted 7 days after the dose was applied, thus it is not very informative in terms of drug induced irritation.*

Dermal Observations (Draize Assessment)

(Reviewer Modified From Applicant Table 3: Summary of Dermal Observations)

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 µg/cm^2/ twice weekly; 3.25 µg/cm^2/ week**		**0**	
Gender	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
From Applicant Table 3: Summary of Dermal Observations														
Only at Dose Site														
Atonia[1]														
Desquamation	2	0	2	0	7	0	4	1	0	0	0	0	0	0
Edema[1]														
Erythema														
Very slight	0	0	1	0	3	0	4	0	2	0	0	0	0	0
Slight[2]	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Well-defined	1	0	0	0	1	0	2	0	0	0	0	0	0	0
Eschar	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fissuring[1]														
From Applicant Table 2: Summary of Clinical Signs[3]														
Sore/Scab, total, all locations	19	6	**25**	**4**	**40**	**12**	**48**	**30**	14	8	3	1	23	17

[1] Unknown incidence, data table indicates both "No" and "Not Applicable," but lack of a "yes" may indicate that no animals exhibited these signs

[2] The category "slight erythema" is not defined according the Irritation Scoring Scale and is therefore assumed to be "well-defined," since it is not "very slight."

[3] more than one location are counted as separate incidences

Body weights: weighed once prior to treatment, prior to dosing on the first day of dosing, and weekly thereafter; body weight change weeks presented in the data tables denote the measure at the end of each study week. The Applicant noted that a body weight recorded on day 1 is considered a week 1 body weight, a body weight recorded on day 8 is considered a week 2 body weight. Body weight change and food consumption data presented are from the end of each study week.

All group mean body weights increased from the beginning to end of the study(weeks 1-26). There were no dose-related decreases in mean body weight or overall mean body weight change. The occasional significant changes in body weight and body weight gain compared to controls were sporadic.

Body Weight (g) and Body Weight Change (g)
(Modified from Applicant Table 4* Mean Body Weight and Table 5* Mean Body Weight Change)

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 µg/cm^2/ twice weekly; 3.25 µg/cm^2/ week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Day 1	27.4	22.2	27.4	21.4	27.0	21.0	26.8	21.3	27.0	21.4	27.2	21.5	28.0	21.9
N	24	21	20	16	22	22	23	20	22	18	6	8	24	22
Week 28, Day of necropsy	32.1	27.8	31.8	28.4	29.0	25.7	31.9	27.1	31.9	25.2	33.0	28.1	32.6	26.0
Gain	4.7	5.6	4.4	7.0	2.0	4.7	5.1	5.8	4.9	3.8	5.8	6.6	4.6	4.1

* Reviewer calculation (day to necropsy at the end of week 26).
In the Applicant's table, a body weight recorded on day 1 was considered a week 1 body weight, a body weight recorded on day 8 was considered a week 2 body weight. Body weight change and food consumption data presented are from the end of each study week thus the table indicating body weight gain for table 5 is actually data from day 8 to the end of week 25. Week 27 is the last day of the study; necropsy on the next day was identified as week 28.

Food consumption: measured weekly; food consumption weeks presented in the data tables denote the measure at the end of each study week

There were no consistent effects of treatments on food consumption.

Gross pathology:
A separate pathology report was not included, although summary individual tables were present.
A necropsy was done on animals that died or were sacrificed at an unscheduled interval. All positive control animals having 15 or more dermal masses (clumped multiple dermal masses) or 15 or more individual dermal masses per animal were euthanized. After 26 weeks of treatment, all surviving animals were necropsied.

For the Applicant tables skin and masses were defined as indicated in the following table:

Treated Skin	Dose site
Untreated Skin	Untreated skin from the dorsal sacral region

Skin/subQ, other	Findings in the skin observed at sites other than the standard skin sections (treated and untreated)
mass	a mass observed at necropsy, not mentioned previously in clinical observations
confirmed mass	a mass noted during the last clinical observations, confirmed at necropsy

Histopathology: Peer review: No

Skin containing masses and areas of skin clear of masses were processed for histological analysis as indicated in the table below. Untreated skin was obtained from skin overlying the sacral vertebrates. Macroscopic lesions at non-skin locations were also processed for histological analysis, except for those in group 6, the positive control.

	Microscopic Examination of Skin			
	Treated skin		**Untreated skin**	
	Region without mass	**Mass**	**Region without mass**	**Mass**
All animals from				
group 1 (vehicle) group 4 (high dose) group 6 (positive control)	**yes**	**yes**	**yes**	**yes**
Scheduled sacrifice animals groups 2 (low dose) group 3,(mid dose) group 5 (lidocaine control) group 7 (untreated controls)	**no**	**yes**	**no**	**yes**
Unscheduled death animals groups 2 (low dose) group 3,(mid dose) group 5 (lidocaine control) group 7 (untreated controls)	**no**	**yes**	**no**	**yes**

Reviewer comment: *A separate pathology report was not included, although summary individual tables were present. There was minimal and inadequate explanation for some of the results present in the pathology table. For example, when histopathology was conducted on a representative mass, but multiple masses were present from different locations, the individual histopathological result was always not identified with a specific location.*

DERMAL NEOPLASTIC LESIONS

Weekly Mass Counts

The numbers of animals with masses at the treatment site as indicated in a note at the bottom of each page of the weekly counts of Appendix 6 were actually counts of all dermal masses observed, not just those of the treatment site. These were summarized in a table of the incidences of dermal masses at the application site by the Reviewer before realizing it represented all dermal masses as indicated below..

Incidences of All Dermal Masses

Group	1		2		3		4		5		6		7	
Group Type	Vehicle control		Capsaicin low		Capsaicin mid		Capsaicin high		Lidocaine control		Positive control		Untreated control	
Dose/ application area (mg/cm^2)	0		0.16		0.32		0.64		0		1.56 $\mu g/cm^{2}$-/ 2X/weekly		0	
Gender	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
DURING STUDY (from Appendix 6 tables, this table includes all dermal masses observed)														
Animal incidence	6	11	6	6	13	7	10	13	8	7	24	19	12	9
Combined sexes	17		12		19		23		15		43		21	
AT NECROPSY (from Appendix 11 tables, individual animal pathology listings)														
Animal incidence	5	9	5	6	13	6	9	12	7	7	24	19	11	8
Combined sexes	14		11		19		21		14		43		19	
Total number of masses	7	9	8	6	23	7	14	19	10	9	331	181	16	11
Total confirmed masses	3	3	9	5	16	6	12	15	6	6	233	169	9	10

Histopathological analysis of dermal masses at necropsy revealed that many of the masses noted in the table above were not confirmed as masses and many were not neoplastic.

Histopathological analysis (refer to the table below) of dermal masses obtained at necropsy from the site of capsaicin application revealed that, **of the confirmed masses,** most were benign squamous cell papillomas with one incidence of a malignant fibrosarcoma (low dose capsaicin, male). The incidence of papillomas in the capsaicin groups was greater than in the vehicle control group. (***Reviewer comment:*** *refer to the Statistical Review, forthcoming*). The anesthetic control group and untreated control groups had similar low incidence of papillomas as the vehicle control group. The positive control group was clearly positive, with most animals having squamous cell papillomas in the treatment area.

For non-treated skin masses, there was a increase in papillomas in the control group, but this might be expected since the non-treated area encompasses the entire body surface area of the mouse except for the 4 cm^2 treatment area. Also there was a large number of non-treated skin masses in the positive control group which is possibly related to the high dose of TPA, 6.25 µg/mouse twice weekly, compared to the recommended 1.25 to 2.5 µg/mouse applied 3 times/week (Tennet et al, 2001).

Summary of Treated Skin Incidences of Dermal Masses and Papillomas at Necropsy

Group	1		2		3		4		5		6		7	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Mortalities (unscheduled)	1	4	5	9	3	3	2	5	3	7	19	17	1	3
N at end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
TREATED SKIN														
Sponsor Summary [condensed by Reviewer from Chanda et al 2007: Tables 4, 5, 6 or Module 2 Summary Tables],														
Copyright Material														
Reviewer Analysis from Individual Pathology Data Tables														
MASSES DURING ENTIRE STUDY														
Animal Incidence	2	0	3	2	6	2	4	3	0	1	21	17	0	1
Total Masses	2	0	3	2	7	3	5	5	0	1	152	64	0	1
Notes: The above 2 rows include masses at necropsy and weekly mass observations not present at necropsy														
MASSES AT NECROPSY														
Animal Incidence	0	0	3[b]	2	6	2	3	3	0	1	21[c]	17[c]	0	1
Total Masses	**0**	**0**	**2**	**2**	**7**	**3**	**4**	**3**	**0**	**1**	**152**	**63**	**0**	**1**
Confirmed Masses	0	0	2	1	6	3	2	3	0	0	101	56	0	1
Unknown[a]	0	0	0	0	1	1	1	0	0	1	-[d]	-[d]	0	0
Total Papilloma	**0**	**0**	**1**	**1**	**4**	**2**	**1**	**2**	**0**	**0**	**21[d]**	**15[d]**	**0**	**1**
Combined Sexes	**0**		**2**		**6**		**3**		**0**		**36[d]**		**1**	
Other neoplasia	0	0	0	0	0	0	0	0	0	0	-[d]	-[d]	0	0
Non-Neoplastic Masses	0	0	1	1	2	0	1	1	0	0	-[d]	-[d]	0	0

Notes:
Masses at necropsy indicated in table at the site of "dorsal thorax" were considered to be the site of treatment. However if one also considers "dorsal abdomen" as site of treatment, the number tend to match closer to the above rows from the Sponsor Summary.

[a] Unknown indicates the information is unclear or missing that a mass was confirmed and/or histopathology was conducted. It could be that only a representative mass was processed and examined as stated in the methods.

[b] 1 mass found at necropsy

[c] includes animals with papillomas listed in the "treatment area", but were the papilloma sites were actually in other skin areas

[d] only a representative mass was histologically analyzed

Summary of Non-Treated Skin Incidences of Dermal Masses and Papillomas at Necropsy

Group	1		2		3		4		5		6		7	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Mortalities (unscheduled)	1	4	5	9	3	3	2	5	3	7	19	17	1	3
N at end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
NON-TREATED SKIN														
Sponsor Summary [condensed by Reviewer from Chanda et al 2007: Tables 4, 5, 6 or Module 2 Summary Tables],														
Copyright Material														
Reviewer Analysis from Individual Pathology Data Tables														
MASSES DURING ENTIRE STUDY														
Animal Incidence	5	11	5	6	12	6	8	13	7	7	24	20	12	9
Total Masses	8	12	7	6	21	6	11	18	11	10	192	143	18	10
Notes: The above 2 rows include masses at necropsy and weekly mass observations not present at necropsy.														
MASSES AT NECROPSY														
Animal Incidence	5	10	5	6	12	6	8	13	7	7	24	20	12	9
Total Masses	**7**	**11**	**7**	**6**	**21**	**6**	**11**	**17**	**11**	**10**	**192**	**143**	**16**	**10**
Confirmed Masses	3	3	7	4	10	3	10	12	6	6	132	113	9	9
Unknown[a]	4	8	1	1	11	3	0	4	3	4	-[b]	-[b]	7	1
Total Papilloma	**3**	**2**	**1**	**2**	**6**	**3**	**3**	**8**	**3**	**4**	**10**[b]	**10**[b]	**6**	**5**
Combined Sexes	**5**		**3**		**9**		**11**		**7**		**20**[b]		**11**	
Other neoplasia														
fibrosarcoma			1				1							
fibroma											1			
ameloblastoma			1											
Non-neoplastic masses	0	1	3	3	4	0	6	4	1	1	-[b]	-[b]	2	3

Notes:
Masses at necropsy indicated in table at the site of "untreated skin" and "skin/subQ, other" were considered to non-treatment skin sites incorporated into this table. There were a large number of masses without confirmation of mass, histopathology findings, or unclear which site histopathology referred, and these were enter as "unknown."
[a] Unknown indicates the information is unclear or missing that a mass was confirmed and/or histopathology was conducted. It could be that only a representative mass was processed and examined as stated in the methods.
[b] there were 13 males and 8 females with masses that lacked adequate histopathology information; per protocol non-treated skin did not require histopathology

For the treated skin, most masses were benign squamous cell papillomas, there was one incidence of a malignant fibrosarcoma (Group 2, male). The incidence of these papillomas present at necropsy was greater than the vehicle control (***Reviewer comment:*** *refer to the Statistical Review, forthcoming*). The vehicle control, anesthetic control, and untreated control had similar incidences of papillomas. The positive control group was clearly positive.

For non-treated skin masses, there was a increase in papillomas in the control group, but this might be expected since the non-treated area encompasses the entire body surface area of the mouse except for the 4 cm^2 treatment area.

The further delineation of the types and incidences of dermal neoplasia was provided in the Tables, below. Note that comparisons of squamous cell papilloma incidences are slightly different from the Applicants summary tables. Since there was minimal explanation for any of the submitted tables and data, the reason for the discrepancies is unknown.

Incidences and Type of Dermal Neoplasia*

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Treated skin														
B-Papilloma, Squamous Cell	**1**	**0**	**2**	**1**	**6**	**2**	**3**	**2**	**1**	**0**	**21**	**17**	**0**	**1**
M-Fibrosarcoma			**1**											
Non-Treated Skin (combined 'Untreated Skin' and 'Skin/SubQ, other' categories)														
B-Papilloma, Squamous Cell	**1**	**2**		**2**	**4**	**4**	**1**	**8**	**3**	**4**	**8**	**8**	**6**	**5**
B-osteoma	**1**													
B-Acanthoma					**1**									
B-Fibroma											**1**			
M-Fibrosarcoma					**1**		**1**							
B - benign, M - malignant														

* at necropsy, Reviewer Modified From Applicants Tables 9 Macroscopic Observations and Table 12 Microscopic Observations; these numbers are subject to discrepancies that appeared in the Sponsor's individual pathology tables

Further description and tables of the dermal non-neoplastic lesions are presented in the next section

Reviewer comments: *There was no analysis of dermal masses at the treatment site typical of studies with Tg.AC mice. The Applicant presented a summary of the data which was quite different from the Study Contract Organization. The Reviewer's analysis is fairly close to the Applicant's with differences probably due to differences in the assignment of histopathology to specific locations based on incompleteness of the pathology information. However, the interpretation of the study by the Reviewer (positive for carcinogenic potential) is opposite that of the Sponsor (negative).*

The study report presentation was not an accurate reflect presented correctly in the report. For Appendix 6: Weekly incidence of Dermal Masses, the set up of the table in misleading. The note at bottom of each page "Note: Numbers indicate the total number of skin masses on the treated site each week", is not accurate. This was determined when this Reviewer summarizing the incidence of masses at the treatment sites as indicated in the Appendix 6 (a summary was not provided by the Sponsor) and noting the control animals had very high incidences of "treatment site" masses. Checking with Appendix 11

Individual Anatomic Pathology Data, the weekly mass counts were actually a table of the occurrence of all skin masses, not just at the treatment site. In addition, the week headings are also misleading in that week 1 is actually day 1, week 2 are the results from assessing week 1 (day 1) application of treatments, etc and week 28 is actually 1 day after week 27. Apparently necropsies were conducted over 2 days, but the presentation would indicated incorrectly as if the animals developed or carried masses during another 7 day interval. In general the methodology and presentation of results was unclear and misleading (tables did not reflect what they purported to indicate).

DERMAL NON-NEOPLASTIC LESIONS

Non-neoplastic lesions included acanthosis (thickening of the epidermis), hyperkeratosis/parakeratosis (primarily females), epidermal crusts, subepidermal fibrosis, epidermal ulcerations/erosions, and chronic active inflammation. There was no evidence of a dose response in incidence or severity. Acanthosis (12/25), crust (serocellular) formation (11/25) and subepidermal fibrosis (9/25) were more frequent in the high-dose males, and ulcer/erosion and chronic-active inflammation were more frequent in the mid- and high-dose males (3/25, 4/25; 1/25, 3/25, respectively). In the females, hyperkeratosis/parakeratosis (14/25) was most frequent in the low-dose animals, acanthosis had a higher incidence in the low- and mid-dose groups (10/25, 12/25, respectively), and epidermal crust formation (6/25) was more frequent in high-dose animals.

Similar lesions were also present in the treated skin collected from control animals, suggesting that the control article and/or treatment procedure, such as weekly shaving of the treatment site, contributed to lesion incidence and severity. A higher incidence of acanthosis and hyperkeratosis/parakeratosis occurred in nontreated skin sites of the untreated controls (group 7), sites in which the hair was not shaved.

Microscopic Observations*

Group	1		2		3		4		5		6		7	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose (mg/mouse)	**0**		**0.64**		**1.28**		**2.56**		**0**		**6.25 µg/application; 12.5 µg/week**		**0**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 $\mu g/cm^2$/ twice weekly; 3.25 $\mu g/cm^2$/ week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Treated Skin														
hyperplasia, squamous cell			1		1									
epidermal polyp							1							
acanthosis	1	2	5	10	6	12	12	4	4	3	1	2		
inflammation, subacute														
hyperkeratosis/ parakeratosis	2		4	14	5	3	1	2	7	1	2	2		
fibrosis, sub-epidermal		2	2	2	2	1	9					1		
ulcer/erosion					3		4							
crust, serocellular, epidermal		2	2		9	2	11	6	3	3	2		1	
inflammation, subacute	1						1							
inflammation, chronic active					1		3	1			1			
mast cell increased							4							
Untreated Skin														
hyperkeratosis/ parakeratosis	1		1	6								1		
crust, serocellular, epidermal						1	1							
Skin/SubQ, other														
N examined	4	5	6	6	11	5	7	13	7	5	11	12		
unremarkable	1	2		2	1				1		1	2		
polyp, epidermal		1												
keratin cyst										1				
fibrosis, sub-epiderma											1	1	1	
acanthosis			2	2	3	1	4	5				2	3	5
hyperkeratosis/	1		2	2	3	2	2	1	1			2	1	5

parakeratosis														
inflammation, chronic active	1		4				3	2					3	
ulcer/erosion	1		2				3	1	1		1		2	
crust, serocellular, epidermal			1		1		2	5	3	1	1		2	
Head **N examined** **N unremarkable**	3	4 2	4 1	6	6 2	5 1	3	2	2	3	3	4		
fibrosis, sub-epidermal		1				1						1		
polyp, epidermal							2					1		
fibroma											1			
abscess						1								
hyperkeratosis					1									
inflammation, chronic-active					1									
acanthosis					1									
ulcer/erosion					1									

* Reviewer Modified From Applicant Table 12; these numbers are subject to discrepancies that appeared in the Sponsor's individual pathology tables

NON-DERMAL NEOPLASTIC LESIONS

There were no obvious differences in findings between animal sacrificed early and those sacrificed at the end of the study. The most frequently observed gross lesions were masses in the nonglandular and glandular regions of the stomach, oral cavity (head), and salivary gland. Squamous papilloma was the most frequent microscopic diagnosis for the masses in the stomach, skin (other – skin other than treatment site, often involving the anterior one half of the body), and treated skin. Oral cavity masses consisted primarily of odontogenic tumors, such as ameloblastoma and odontoma and the salivary gland masses consisted of one adenoma and two carcinomas.

Non Dermal Masses and Histopathological Determination at Necropsy*

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose (mg/mouse)	**0**		**0.64**		**1.28**		**2.56**		**0**		**6.25 µg/application; 12.5 µg/week**		**0**	
Dose/ application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 $\mu g/cm^2$/ twice weekly; 3.25 $\mu g/cm^2$/ week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Head														
mass	21	10	21	50	20	30	20	10	20	30	30	21	20	10
confirmed mass														
Histopath														
N examined	3	42	41	6	62	51	3	2	2	3	3	4		
N-unremarkable														
M-Osteosarcoma				2										
M-Fibrosarcoma						1								
M-Ameloblastoma	1		2	3	2	1	1	1	2	1		1	2	
M-Odontoma			1	1		1		1		2				3
B-Papilloma, Squamous Cell	1										2	2		
B-Osteoma	1	1												
Liver														
C-hematopoietic neoplasm				1					2	2		3		1
Lung,														
mass							1							
B-adenoma, bronchiolar alveolar							1							
C-hematopoietic neoplasm												1		
Spleen														
C-hematopoietic neoplasm			1	1	2				2	2		4		1
Stomach, glandular,														
mass	2	2		2	3	2	5	3	2	1			2	
Stomach, nonglandular														
mass	8	4	3	4	6	7	2	8	6	5	5	4	5	5
B-papilloma, squamous cell	6		2	4	4	4	2	6	6	4	5	2	3	3
Salivary Gland, mandibular,														
mass		1		1	1				1					

B-Adenoma		1												
M carcinoma				1					1					
Pancreas, mass			1											
Eye, confirmed mass										1				
Ovary, mass										1		1		1
M-Malignant Granulosa/Theca Cell tumor										1				
M-Yolk Sac Carcinoma												1		
B-Cystadenoma										1				
B- Teratoma														1
Vagina, confirmed mass				1										
Zymbal's gland, mass								1						
B-adenoma								1						
Abdominal cavity, mass									1				1	
M-Lymphosarcoma					1				1					
M-Leukemia, erythrocytic				12	1				2	2		4		1

* Reviewer Modified From Applicants Tables 9 Macroscopic Observations and Table 12 Microscopic Observations; these numbers are subject to discrepancies that appeared in the Sponsor's individual pathology tables
B – benign, M - malignant

NON-DERMAL, NON-NEOPLASTIC LESIONS

Macroscopic and microscopic pathology findings, including those involving the spleen, liver, and lung were consistent with changes commonly associated with the Tg.AC mouse (Mahler et al.,1998) or were considered to be incidental and unrelated to test article administration. Gross observations occasionally noted in males consisted of distended urinary bladder and paraphimosis/abrasion of the penis, which were usually also confirmed microscopically.

Toxicokinetics: Not conducted

The tables from Chanda et al (2007) summarized the study data more clearly and in a way that was lacking in the Study Report and the Applicants Module 2 summary. Therefore the tables from that study are attached on the following pages. Their summarized analysis was divided into scheduled and unscheduled deaths. The Reviewer then combined selected relevant data across both of these groups for presentation in the beginning of this ECAC presentation. The data from which those values are obtained are presented in the next two tables which further summarized the Chanda data.

Reviewer Summary (from mass and papilloma totals of the next table)

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose / application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 $\mu g/cm^2$ / 2X/week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Mortalities (unscheduled)	1	4	5	9	3	3	2	5	3	7	19	17	1	3
N at end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
TREATED SKIN														
Total Masses	1	0	3	1	7	2	6	6	1	0	23	19	0	1
Total Papilloma	1		2	1	6	2	3	2	1	0	21	19	0	1
NON-TREATED SKIN														
Total Masses	4	4	3	4	9	4	4	11	4	6	11	15	6	9
Total Papillomas	1	2	0	2	4	4	1	8	3	3	8	8	6	5

For treated and non-treated skin regions, only benign squamous cell papillomas were indicated in the tables of Chanda et al 2007

Reviewer Summary (Totals of the unscheduled and final phase results From Chanda et al 2007: Tables 4, 5, 6 on next pages)

Group	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
Group Type	**Vehicle control**		**Capsaicin low**		**Capsaicin mid**		**Capsaicin high**		**Lidocaine control**		**Positive control**		**Untreated control**	
Dose / application area (mg/cm^2)	**0**		**0.16**		**0.32**		**0.64**		**0**		**1.56 µg/cm^2 / 2X/week**		**0**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
N	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Mortalities (unscheduled)	1	4	5	9	3	3	2	5	3	7	19	17	1	3
N at end of study	24	21	20	16	22	22	23	20	22	18	6	8	24	22
TREATED SKIN														
Masses														
unscheduled	0	0	0	0	0	0	0	0	0	0	19	11	0	0
final phase	1	0	3	1	7	2	6	6	1	0	4	6	0	1
Total Masses	**1**	**0**	**3**	**1**	**7**	**2**	**6**	**6**	**1**	**0**	**23**	**19**	**0**	**1**
Papillomas														
unscheduled	0	0	0	0	0	0	0	0	0	0	18	11	0	0
final phase	1	0	2	1	6	2	3	2	1	0	3	6	0	1
Total	**1**	**0**	**2**	**1**	**6**	**2**	**3**	**2**	**1**	**0**	**21**	**19**	**0**	**1**
NON-TREATED SKIN														
Masses														
unscheduled	1	0	0	0	0	0	1	1	0	0	7	8	0	0
final phase	3	4	3	4	9	4	3	10	4	6	4	7	6	9
Total Masses	**4**	**4**	**3**	**4**	**9**	**4**	**4**	**11**	**4**	**6**	**11**	**15**	**6**	**9**
Papillomas														
unscheduled	0	0	0	0	0	0	0	1	0	0	7	4	0	0
final phase	1	2	0	2	4	4	1	7	3	3	1	4	6	5
Total Papillomas	**1**	**2**	**0**	**2**	**4**	**4**	**1**	**8**	**3**	**3**	**8**	**8**	**6**	**5**

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: **Percutaneous fertility and general reproduction toxicity study of capsaicin dermal patches in rats**

Key study findings: The NOAEL for maternal and paternal toxicity of capsaicin dermal patch is <25 cm^2 (16 mg/rat). The reproductive NOAEL is also <25 cm^2 (16 mg/rat).

- Fertility was reduced in all three capsaicin dose groups compared to controls although the reductions were not dosage dependent (76, 72, and 83% for doses of 25, 37.5, and 50 cm^2, or 16, 24, and 32 mg/rat, respectively).
- Males had increased incidences of small and/or flaccid testes and/or epididymides, the reduced weight of the caudal epididymides, and reduced sperm motility and density in all capsaicin-treated groups.
- The numbers of pregnancies were reduced and the average numbers of corpora lutea, implantations and viable embryos were slightly reduced in all capsaicin-treated groups.

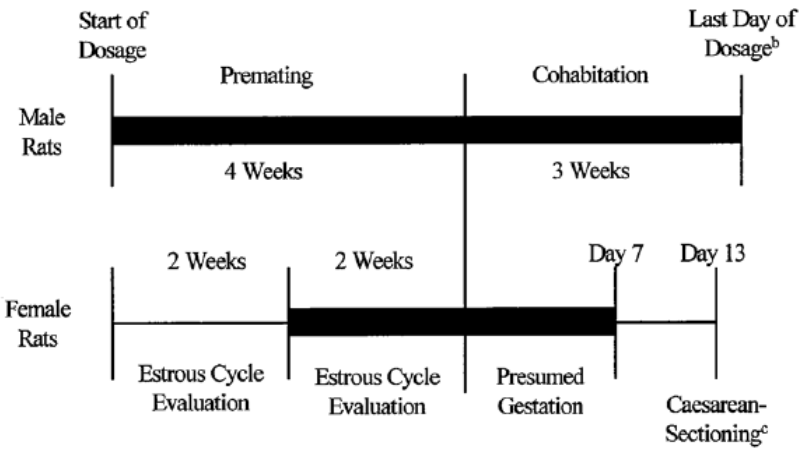
Study no.: UYQ00003
E-location: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42351-fert-embryo-dev\uyq00003
Conducting laboratory and location (b) (4)
Date of study initiation: Aug 9, 2005
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin Dermal Patch (8% w/w, *trans*-capsaicin), Lot 7006753 (81101203), Purity ?, (impurities <lod)
Vehicle Patch Opaque patch, Lot 81100104
Anesthetic Cream, (Lidocaine 4%/L-M-X-4™)
Cleansing Gel, (89% polyethylene glycol)

Methods

Doses: 0, 25, 37.5 50 cm^2 patch sizes
Doses were selected based on the results of a dose-range developmental and perinatal/postnatal reproductive toxicity study of capsaicin dermal patches in rats (Protocol UYQ00004, located in the literature references section). Patches of vehicle 50 cm^2, or capsaicin 37.5 or 50 cm^2 were applied once daily on GD 7 through postpartum day 20 (n=8/dose group). Three fetuses from one litter in the 50 cm^2 test article-treated group had domed heads with extreme dilation of the lateral and third ventricles of the brain.

Reviewer comment: *The maximal tolerated dose or maximal feasible dose was not attained in the dose-range study.*

Species/strain: Rat/Crl:CD(SD)
62 days of age, weight at study assignment males: 331 – 388 g, females: 224 – 272 g.
Number/sex/group: 25/sex/group
Route, formulation, volume, and infusion rate: Patches were applied to the back of the rats once/day for a 3 hour duration.
Satellite groups used for toxicokinetics: There were no toxicokinetic groups.
Study design:
Male rats were treated from beginning 28 days before cohabitation, through cohabitation (maximum of 14 days) and continuing through the day before sacrifice which occurred after completion of the cohabitation period. Female rats were treated from 15 days before cohabitation, through cohabitation (maximum of 19 days) and continuing through day 7 of presumed gestation (DG 7) at the same time each day. Estrous cycling was evaluated by examination of vaginal cytology for 14 days before cohabitation, and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed *in situ* during the cohabitation period.



Males: After completion of the cohabitation period, all surviving male rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Daily patch application continued until the day before sacrifice. To assess the potential toxicity of the test article on the male reproductive system, reproductive organs were weighed and retained for possible histopathological evaluation and sperm evaluations were conducted.
Females: All female rats were sacrificed on GD 13 and a gross necropsy was performed.

Results

Mortality:

There was one death which occurred in a male rat (#1376) in the 50 cm^2 capsaicin group found dead on during the 31st patch application. Necropsy revealed enlarged kidneys, a tan discolored area on the left kidney, marked dilation of and a yellow granular material in the pelvis of both kidneys, moderate dilation of the ureters, distended urinary bladder filled with red fluid, tan gelatinous substance and numerous calculi, thickened bladder walls and small seminal vesicles and prostate. Adverse clinical observations included ungroomed coat on study days (SDs) 8 to 31, urine-stained abdominal fur on SDs 19 to 31, chromodacryorrhea and cloudy urine on DS 30, chromorhinorrhea, dehydration and hunched posture on SDs 30 and 31, and cold to touch on SD 31. This rat generally gained weight until SD 29 and then lost 23% of the SD 29 body weight by SD 31.

Clinical signs: observed 3-times daily, before dosage administration, at the time of the 3-hour post-exposure cleansing, and once during the postdose period (female rats). Before each daily patch application the site of application was observed for signs of skin irritation and evaluated by Draize scoring.

Males: Grade 1 erythema occurred more frequently in male rats in the 25, 37.5 and 50 cm^2 capsaicin groups compared to the vehicle group, but at similar frequency among the capsaicin-treated groups. Grade 2 erythema in male rats occurred only capsaicin groups with greatest incidences in the 37.5 cm^2 capsaicin group. In female rats, Grades 1 and 2 erythema occurred in similar incidences in all groups with no differences between them. Grade 3 erythema occurred in female rats, twice in the 25 cm^2 and once in the 37.5 cm^2 capsaicin-treated groups, on one or two days during the precohabitation period. Grade 1 flaking occurred increased numbers of female rats in the 25, 37.5 and 50 cm^2 capsaicin - treated groups during the precohabitation and gestation periods with no differences between these groups. Grade 2 flaking occurred at increased incidences for male and female rats in the 37.5 and 50 cm^2 test article-treated groups. Scabs at the application site occurred in increased numbers of male rats in the 25, 37.5 and 50 cm^2 capsaicin-treated groups, but were not dose-dependent. There was one rat in each of the 25 and 37.5 cm2 capsaicin-treated groups with ulcerations at the application site. Ungroomed coat occurred in increased numbers of male and female rats in the 37.5 and 50 cm^2 capsaicin-treated groups, but was significantly increased only in male rats.

Other clinical observations included urine-stained abdominal fur, chromodacryorrhea, chromorhinorrhea, dehydration, hunched posture, cold to touch, cloudy urine, missing, broken and/or misaligned incisors, localized alopecia on the limbs, sparse hair coat, scabs on the paw, lacrimation and swollen snout. These were not considered related to the capsaicin treatment because: 1) the incidences were not dosage dependent; 2) the observations occurred in only one or two rats per group; and/or 3) they occurred in the male rat that was found dead.

TABLE B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)	I 50 (VEHICLE)	II 25	III 37.5	IV 50
FOUND DEAD	0	0	0	1a
MAXIMUM POSSIBLE INCIDENCE	1286/ 25	1287/ 25	1288/ 25	1270/ 25
CLINICAL OBSERVATIONS:				
UNGROOMED COAT	1/ 1	4/ 2	22/ 11**	37/ 10a**
SOFT OR LIQUID FECES	34/ 16	8/ 5**	21/ 6**	3/ 3**
URINE-STAINED ABDOMINAL FUR	6/ 2	0/ 0	1/ 1	15/ 2a
CHROMODACRYORRHEA	0/ 0	43/ 2	20/ 1	1/ 1a
CHROMORHINORRHEA	1/ 1	5/ 1	3/ 1	2/ 1a
DEHYDRATION	0/ 0	0/ 0	0/ 0	2/ 1a
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	2/ 1a
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1a
CLOUDY URINE	0/ 0	0/ 0	0/ 0	1/ 1a
INCISOR(S): TOTAL	19/ 2	37/ 1	25/ 2	0/ 0
MISSING/BROKEN	6/ 1	0/ 0	14/ 2	0/ 0
MISALIGNED	13/ 1	37/ 1	12/ 1	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	40/ 2	0/ 0	14/ 1	0/ 0
SPARSE HAIR COAT	0/ 0	0/ 0	1/ 1	0/ 0
LEFT FOREPAW: SCAB	0/ 0	6/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 1376 was found dead on day 31 of study.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE B1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)	I 50 (VEHICLE)	II 25	III 37.5	IV 50
FOUND DEAD	0	0	0	1a
MAXIMUM POSSIBLE INCIDENCE	1286/ 25	1287/ 25	1288/ 25	1270/ 25
CLINICAL OBSERVATIONS (Cont.):				
LACRIMATION	0/ 0	6/ 1	0/ 0	0/ 0
SWOLLEN SNOUT	0/ 0	2/ 1	0/ 0	0/ 0
SKIN REACTION OBSERVATIONS:				
ERYTHEMA: GRADE 1	12/ 7	469/ 25**	426/ 24**	317/ 23a**
GRADE 2	0/ 0	21/ 5	75/ 11**	18/ 3
FLAKING: GRADE 1	295/ 25	658/ 25	680/ 25	697/ 25a
GRADE 2	0/ 0	13/ 4	68/ 10**	34/ 7**
BACK: SCAB(S)	4/ 1	162/ 17**	196/ 16**	208/ 12**
BACK: ULCERATION	0/ 0	11/ 1	14/ 1	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Rat 1376 was found dead on day 31 of study.
** Significantly different from the vehicle control group value (p≤0.01).

Females: Grades 1 and 2 erythema occurred in similar numbers of female rats in the four dose groups. Grade 3 erythema occurred in 2 rats in the 25 cm^2 and 1 rat in the 37.5 cm^2 capsaicin-treated groups, on one or two days during the precohabitation period. Grade 1 flaking occurred in increased numbers of female rats in the 25, 37.5 and 50 cm^2 capsaicin-treated groups during the precohabitation and gestation periods, but was not dose-dependent; the incidences were generally comparable among the three groups. Grade 2 flaking occurred in increased numbers of female rats in the 37.5 and 50 cm^2 capsaicin-treated groups during the precohabitation and gestation periods. Scabbing at the application site occurred slightly increased frequency in the capsaicin-treated groups during the precohabitation period, but the numbers of rats affected were similar among all four dosage groups during the gestation period. Ungroomed coat occurred in slightly increased numbers of female rats in the 37.5 and 50 cm^2 test article-treated groups during the gestation period.

Other clinical observations included missing and/or broken incisors, localized alopecia on the limbs, spare hair coat, ptosis, urine-stained abdominal fur and soft or liquid feces. These were considered unrelated to the capsaicin dermal patch because: 1) the incidences were not dosage dependent; 2) the observations occurred in only one rat in a group; and/or 3) the observations occurred only in the vehicle control group.

TABLE C1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - FEMALE RATS

GROUP PATCH SIZE (CM2~)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
MORTALITY	0	0	0	0
PRECOHABITATION [DAY 1 OF STUDY TO THE DAY OF COHABITATION (DAY 15 OF STUDY)]:				
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
CLINICAL OBSERVATIONS:				
UNGROOMED COAT	0/ 0	7/ 1	36/ 4	23/ 2
INCISOR(S): MISSING/BROKEN	0/ 0	0/ 0	0/ 0	13/ 1
LOCALIZED ALOPECIA: LIMB(S)	1/ 1	0/ 0	0/ 0	0/ 0
SKIN REACTION OBSERVATIONS:				
FLAKING: GRADE 1	2/ 1	111/ 25**	89/ 25**	72/ 22**
GRADE 2	0/ 0	6/ 3	12/ 5**	27/ 8**
ERYTHEMA: GRADE 1	51/ 13	94/ 19	95/ 18	121/ 20
GRADE 2	19/ 6	17/ 5	14/ 5	21/ 5
GRADE 3	0/ 0	3/ 2	2/ 1	0/ 0
BACK: SCAB(S)	0/ 0	29/ 5	25/ 5	8/ 2

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE C1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - FEMALE RATS

GROUP PATCH SIZE (CM2~)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
MORTALITY	0	0	0	0
PRESUMED GESTATION:				
MAXIMUM POSSIBLE INCIDENCE	350/ 25	350/ 25	350/ 25	350/ 25
CLINICAL OBSERVATIONS:				
UNGROOMED COAT	10/ 1	4/ 1	32/ 6	53/ 4
LOCALIZED ALOPECIA: LIMB(S)	18/ 2	6/ 1	13/ 3	14/ 1
SPARSE HAIR COAT	0/ 0	3/ 1	1/ 1	3/ 1
PTOSIS	0/ 0	0/ 0	3/ 1	0/ 0
URINE-STAINED ABDOMINAL FUR	4/ 1	0/ 0	0/ 0	0/ 0
SOFT OR LIQUID FECES	1/ 1	0/ 0	0/ 0	0/ 0
SKIN REACTION OBSERVATIONS:				
FLAKING: GRADE 1	92/ 17	251/ 25**	231/ 24**	208/ 23**
GRADE 2	10/ 4	17/ 3	41/ 7	84/ 12**
ERYTHEMA: GRADE 1	77/ 17	102/ 18	118/ 21	116/ 21
GRADE 2	83/ 11	54/ 10	30/ 9	47/ 8
BACK: SCAB(S)	110/ 16	85/ 14	95/ 9	61/ 10

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body weight: Body weights for male and female rats were recorded daily during the dose and postdose (female rats only) periods and at sacrifice.

Males: Body weights and body weight gains in the male rats were unaffected by treatment.
Females: Body weight gains were significantly reduced ($p \leq 0.01$) in all three capsaicin-groups for the entire precohabitation period, study days (SD) 1 to 15, SD1 is the first day of patch application). During this period, body weight gains were significantly reduced on SDs 1 to 8 and 8 to 15 and average body weights were significantly reduced on SD 15 in the 25 cm^2 capsaicin-treated group. Body weight gain was significantly increased in the 25 cm^2 capsaicin-treated group during the gestation dosage period (GDs 0 to 8 of gestation). However, body weight gains for the postdose period (GDs 8 to 13) and the entire gestation period (GDs 0 to 13) and average body weights were similar among the four dose groups.

TABLE C3 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - FEMALE RATS

GROUP PATCH SIZE (CM2~)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+8.4 ± 6.1	+1.8 ± 6.1**	+4.6 ± 9.3	+5.4 ± 9.1
DAYS 8 - 15b	MEAN±S.D.	+10.0 ± 6.1	+4.6 ± 3.6**	+7.4 ± 12.2	+6.4 ± 5.8
DAYS 1 - 15b	MEAN±S.D.	+18.4 ± 7.1	+6.4 ± 6.2**	+12.0 ± 6.9**	+11.8 ± 8.2**

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Last value recorded before cohabitation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE C2 (PAGE 1): BODY WEIGHTS - PRECOHABITATION - SUMMARY - FEMALE RATS

GROUP			I	II	III	IV
PATCH SIZE (CM2~)a			50 (VEHICLE)	25	37.5	50
RATS TESTED		N	25	25	25	25
BODY WEIGHT (G)						
DAY 1		MEAN±S.D.	242.3 ± 13.8	242.8 ± 13.9	244.0 ± 14.2	242.5 ± 11.7
DAY 8		MEAN±S.D.	250.7 ± 16.0	244.6 ± 13.4	248.6 ± 15.0	247.9 ± 11.5
DAY 15b		MEAN±S.D.	260.7 ± 15.6	249.2 ± 12.9**	256.0 ± 15.4	254.3 ± 11.1

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.
b. Last value recorded before cohabitation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE C5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - FEMALE RATS

GROUP			I	II	III	IV
PATCH SIZE (CM2~)a			50 (VEHICLE)	25	37.5	50
RATS TESTED		N	25	25	25	25
PREGNANT		N	23	19	18	20
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 8		MEAN±S.D.	+23.3 ± 5.9	+29.1 ± 9.7*	+20.7 ± 7.3	+23.2 ± 9.4
DAYS 8 - 10		MEAN±S.D.	+10.2 ± 4.2	+10.0 ± 3.6	+11.4 ± 4.8	+12.0 ± 6.8
DAYS 10 - 13		MEAN±S.D.	+17.4 ± 5.1	+14.2 ± 7.4	+18.0 ± 7.2	+16.6 ± 7.8
DAYS 8 - 13		MEAN±S.D.	+27.6 ± 5.9	+24.2 ± 7.9	+29.4 ± 8.2	+28.6 ± 10.4
DAYS 0 - 13		MEAN±S.D.	+50.9 ± 8.2	+53.3 ± 16.0	+50.1 ± 11.3	+51.7 ± 10.9

DAYS = DAYS OF GESTATION
a. Dosage occurred on day 1 of study through day 7 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Food consumption: Feed consumption values for male rats were recorded weekly during the dosage period, except during cohabitation. Feed consumption values for female rats were recorded weekly to cohabitation and on GDs 0, 8, 10 and 13.

Males: In the male rats absolute and relative feed consumption (g/kg/day) values were reduced on study days 1 to 8 in the 37.5 (relative only) and 50 cm^2 test article-treated groups. After these initial reductions in feed consumption, absolute and relative feed consumption values were increased or significantly increased in the 25 and 50 cm^2 test article-treated groups on study days 8 to 15 and 35 to 42.

Females: Relative feed consumption values were significantly increased in the 37.5 and 50 cm^2 test article-treated groups for the entire precohabitation period (SDs 1 to 15). Absolute feed consumption values for the precohabitation period were similar among the four dose groups. During the gestation dosage period (GDs 0 to 8), absolute and relative feed consumption values were significantly increased in the 25 cm^2 test article-treated group and the relative feed consumption value was significantly increased in the 50 cm^2 test article-treated group. Relative feed consumption values for the entire gestation period (GDs 0 to 13) were significantly increased in these two dose groups.

Toxicokinetics: not conducted

Necropsy: All surviving male rats were sacrificed after completion of the cohabitation period and a gross necropsy was performed. Reproductive organs (right testis, left testis, left epididymis (whole and caudal), right epididymis, seminal vesicles (with and without fluid) and prostate) were weighed and retained for possible histopathological evaluation and sperm evaluations were conducted. All female rats were sacrificed on GD 13 and a gross necropsy was performed. The uterus of each rat was excised and examined for pregnancy, number and distribution of corpora lutea, implantation sites, and viable and nonviable embryos. A viable embryo was defined as oval or crescent shaped, pink, firm and enclosed in an amniotic sac filled with clear fluid. A nonviable embryo was defined as amorphous, small, pale pink to tan or deep red to black, soft and enclosed in an amniotic sac filled with clear, cloudy or opaque fluid. Placentae were examined for size, color and shape.

Males: Small and/or flaccid testes occurred in 0, 2, 3 and 3 male rats in the 50 (vehicle), 25, 37.5 and 50 cm^2 capsaicin-treated groups, respectively, and small epididymides occurred in single male rats in the 37.5 and 50 cm^2 test article-treated groups. All of the male rats with small and/or flaccid testes and/or epididymides mated the cohort female; of these, two rats in each of the 25 and 50 cm^2 test article-treated groups (#1329, #1386) had bilateral small testes and did not impregnate the female, and one in the 37.5 cm^2 test article-treated group (#1374) had small left caudal and whole epididymis and small and flaccid left testis and did not impregnate the female. Other male rats with small testes and/or epididymides impregnated the cohort female rat.

Terminal body weights were generally comparable among the four dosage groups and did not significantly differ. The weights of the left caudal epididymis and the ratios of this organ weight to the terminal body weight were reduced or significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 25, 37.5 and 50 cm^2 test article-treated groups. The weights of the epididymides, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organ weights to the terminal body weights were unaffected by application of the capsaicin dermal patch at sizes as large as 50 cm^2.

TABLE B7 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS EXAMINED a	N	25	25	25	25
FOUND DEAD	N	0	0	0	1b
APPEARED NORMAL	N	22	23	22	20
EPIDIDYMIDES: RIGHT OR LEFT, SMALL	N	0	0	1	1
TESTES: RIGHT AND/OR LEFT, SMALL AND/OR FLACCID	N	0	2	3	3
CAUDA EPIDIDYMIS: LEFT, SMALL	N	0	0	1	0

TABLE B7 (PAGE 2): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS EXAMINED a	N	25	25	25	25
FOUND DEAD	N	0	0	0	1b
APPEARED NORMAL	N	22	23	22	20
SEMINAL VESICLES: BILATERAL, SMALL	N	0	0	0	1b
PROSTATE: SMALL	N	0	0	0	1b
EXTERNAL ILIAC LYMPH NODES: LARGE	N	1	0	0	0
DEEP INGUINAL LYMPH NODES: LARGE	N	1	0	0	0

a. Refer to the individual clinical observations table (Table B15) for external observations confirmed at necropsy.
b. Rat 1376 was found dead on day 31 of study.

TABLE B8 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	25	24a
TERMINAL BODY WEIGHT	MEAN±S.D.	467.0 ± 35.9	468.0 ± 34.9	470.2 ± 49.0	465.8 ± 40.9
EPIDIDYMIS LEFT	MEAN±S.D.	0.7298 ±0.0776	0.6670 ±0.0985	0.6828 ±0.1044	0.6829 ±0.0878
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.3213 ±0.0501	0.2786 ±0.0492**	0.2852 ±0.0628*	0.2814 ±0.0458*
TESTIS LEFT	MEAN±S.D.	1.7438 ±0.1166	1.5589 ±0.3624	1.6072 ±0.3469	1.6037 ±0.3023
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	1.9034 ±0.2673	1.8830 ±0.3088 [24]b	1.9348 ±0.3528 [24]b	1.8321 ±0.1808 [23]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.8671 ±0.1470	0.8460 ±0.1811	0.8582 ±0.1684	0.8140 ±0.1396
EPIDIDYMIS RIGHT	MEAN±S.D.	0.7710 ±0.0917	0.7160 ±0.1186	0.6959 ±0.0851	0.6848 ±0.1703
TESTIS RIGHT	MEAN±S.D.	1.7390 ±0.1367	1.5731 ±0.3432	1.6371 ±0.3038	1.5403 ±0.3640
PROSTATE	MEAN±S.D.	0.8693 ±0.2838	0.7366 ±0.2509	0.8147 ±0.2034	0.7532 ±0.2794

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 1376, which was found dead on day 31 of study.
b. Excludes values for rats that had organs damaged (weight affected) or values that were not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE B9 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	25	24a
TERMINAL BODY WEIGHT	MEAN±S.D.	467.0 ± 35.9	468.0 ± 34.9	470.2 ± 49.0	465.8 ± 40.9
EPIDIDYMIS LEFT	MEAN±S.D.	0.156 ± 0.018	0.143 ± 0.022	0.147 ± 0.029	0.147 ± 0.017
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.068 ± 0.012	0.059 ± 0.011*	0.061 ± 0.018	0.059 ± 0.010*
TESTIS LEFT	MEAN±S.D.	0.376 ± 0.041	0.334 ± 0.083	0.344 ± 0.079	0.344 ± 0.067
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.409 ± 0.059	0.404 ± 0.064 [24]b	0.414 ± 0.079 [24]b	0.394 ± 0.045 [23]b
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.185 ± 0.032	0.181 ± 0.041	0.183 ± 0.040	0.175 ± 0.031
EPIDIDYMIS RIGHT	MEAN±S.D.	0.165 ± 0.020	0.153 ± 0.025	0.148 ± 0.025	0.148 ± 0.038
TESTIS RIGHT	MEAN±S.D.	0.374 ± 0.044	0.337 ± 0.079	0.351 ± 0.070	0.330 ± 0.076
PROSTATE	MEAN±S.D.	0.187 ± 0.061	0.158 ± 0.054	0.175 ± 0.049	0.160 ± 0.054

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 1376, which was found dead on day 31 of study.
b. Excludes values for rats that had organs damaged (weight affected) or values that were not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Females: All female rats appeared normal at necropsy.

Sperm Parameters: Sperm concentrations and motility were evaluated using computer-assisted sperm analysis (CASA). Motility was evaluated by the Hamilton Thorne IVOS, using a sample collected from the left vas deferens. A homogenate was prepared for evaluation by the Hamilton Thorne IVOS to determine sperm concentration (sperm per gram of tissue weight).

Sperm motility was reduced in all three capsaicin-treated groups. There was a significant reduction in the number and percent of motile sperm and significant increase in the number of nonmotile sperm. The total sperm count from the vas deferens sample was significantly reduced in the 37.5 cm^2 capsaicin treated group. Values for the number and percent motile sperm were below the historical control ranges in all three dosage groups (with the exception of number motile in the 50 cm^2 test article-treated group).

Cauda epididymal sperm count and density were reduced in the 25, 37.5 and 50 cm^2 capsaicin-treated groups; the reduction was significant ($p \leq 0.05$) for sperm count in the 50 cm^2 test article-treated group. Values ranged from 68.7% to 86.4% of the control group value.

[*Historical controls:* In male control group rats from studies conducted at the testing facility from February 1998 to October 2005, the average number of motile sperm in 102

studies was 337.2 (range 209.4 to 565.8) and the average percent motile sperm in 103 studies was 86.2% (62.5% to 96.4%)].

TABLE B10 (PAGE 1): SPERM MOTILITY, COUNT AND DENSITY - SUMMARY - MALE RATS

GROUP		I			II			III			IV		
PATCH SIZE (CM^2)		50 (VEHICLE)			25			37.5			50		
RATS TESTED	N	25			25			25			24a		
VAS DEFERENS SPERM MOTILITY													
NUMBER MOTILE	MEAN±S.D.	349.2	±	158.9	201.4	±	140.2**	147.9	±	127.8**	225.8	±	192.1*
MOTILE PERCENT	MEAN±S.D.	85.1	±	21.0	57.8	±	27.6**	46.6	±	33.0**	52.4	±	27.2**
STATIC COUNT (NONMOTILE)	MEAN±S.D.	51.6	±	66.2	139.0	±	122.4**	150.8	±	92.6**	172.7	±	117.1**
TOTAL COUNT b	MEAN±S.D.	400.8	±	136.0	340.3	±	165.1	298.7	±	101.4*	398.5	±	193.1
CAUDA EPIDIDYMAL SPERM COUNT													
SPERM COUNT c	MEAN±S.D.	131.8	±	70.7	101.7	±	137.2	105.1	±	69.0	90.5	±	65.8*
SPERM DENSITY d	MEAN±S.D.	1193.68	±	618.69	1013.15	±	1338.41	1031.27	±	574.58	923.13	±	615.35

a. Excludes values for rat 1376, which was found dead on day 31 of study.
b. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
c. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
d. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^6 mL), multiplying by 2 (dilution factor) and multiplying by 10^6 to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table B16 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
* Significantly different from the vehicle control group value (p<0.05).
** Significantly different from the vehicle control group value (p<0.01).

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

The number of estrous stages per 14 days was similar among the four dose groups before the start of administration and during the precohabitation period.

Capsaicin patches resulted in a generalized reduction in fertility compared to the control group. The fertility index (number of pregnancies per number of rats that mated) and the number of pregnancies per number of rats in cohabitation were reduced in the capsaicin-treated groups. Although these reductions were neither statistically significant nor dose dependent, the values were below the ranges observed historically at the testing facility in the 37.5 cm^2 test article-treated group. All other mating and fertility parameters (numbers of days in cohabitation, rats that mated and rats with confirmed mating dates during the first or second week of cohabitation) were unaffected by capsaicin treatment.

[*Historical controls:* In control group male rats from studies conducted at the testing facility from January, 2000 to December, 2005, the average fertility index in 122 studies was 94.5% (range 80.0% to 100%) and the average number of pregnancies per number of rats in cohabitation in 116 studies was 93.3% (range 78.3% to 100%). In female rats from studies conducted at the testing facility from September, 1992 to March, 2005, the average fertility index in 190 studies was 94.1% (range 75.9% to 100%) and the average number of pregnancies per number of rats in cohabitation in 185 studies was 93.4% (range 73.3% to 100%)]

Caesarean-sectioning and litter observations were based on 23 (92.0%), 19 (76.0%), 18 (72.0%) and 20 (80.0%) pregnant rats in the vehicle and capsaicin 25, 37.5 and 50 cm^2,

respectively. The litter averages for corpora lutea, implantations, and viable embryos were reduced in the capsaicin-treated groups ranging from 81.6% to 90.2% of the control group values, but not statistically different. There were 0, 5, 3 and 3 litters in the four respective groups with nine or fewer implantations. No other Caesarean-sectioning or litter parameters were affected by application of the capsaicin dermal patch at sizes as large as 50 cm^2. The litter averages for nonviable embryos and the percentage of nonviable embryos per litter were similar among the four dose groups. No dam had a litter consisting of only nonviable embryos. All placentae appeared normal.

TABLE C10 (PAGE 1): MATING AND FERTILITY, ESTROUS CYCLING AND DAYS IN COHABITATION - SUMMARY - FEMALE RATS

GROUP PATCH SIZE (CM2~)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
ESTROUS CYCLING OBSERVATIONS					
PRETREATMENT ESTROUS CYCLING					
RATS EVALUATED	N	25	25	25	25
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	3.4 ± 0.6	3.6 ± 0.5	3.6 ± 0.7	3.8 ± 0.4
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	0	0	0	0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0
TREATMENT ESTROUS CYCLING					
RATS EVALUATED	N	25	25	25	25
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.9 ± 0.8	2.9 ± 0.6	2.8 ± 0.8	2.8 ± 0.8
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	2	0	0	1
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0

a. Dosage occurred on day 1 of study through day 7 of presumed gestation.

TABLE B6 (PAGE 1): MATING AND FERTILITY - SUMMARY - MALE RATS

GROUP PATCH SIZE (CM2~)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	2.4 ± 2.0	2.4 ± 1.7	3.2 ± 2.4	3.2 ± 3.4
RATS THAT MATED b	N(%)	25(100.0)	25(100.0)	25(100.0)	24(96.0)
FERTILITY INDEX c,d	N/N (%)	23/ 25 (92.0)	19/ 25 (76.0)	18/ 25 (72.0)	20/ 24 (83.3)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	24
MATED WITH FEMALE e					
DAYS 1-7	N(%)	24(96.0)	25(100.0)	24(96.0)	23(95.8)
DAYS 8-14	N(%)	1(4.0)	0(0.0)	1(4.0)	1(4.2)
RATS PREGNANT/RATS IN COHABITATION d	N/N (%)	23/ 25 (92.0)	19/ 25 (76.0)	18/ 25 (72.0)	20/ 25 (80.0)

a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Includes only one mating for each male rat.
c. Number of pregnancies/number of rats that mated.
d. Includes only one pregnancy for each rat that impregnated more than one female rat.
e. Restricted to rats with a confirmed mating date.

TABLE C12 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - FEMALE RATS

GROUP PATCH SIZE (CM2~)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	23(92.0)	19(76.0)	18(72.0)	20(80.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	23	19	18	20
CORPORA LUTEA	MEAN±S.D.	16.4 ± 2.3	14.5 ± 5.6	14.8 ± 4.5	14.1 ± 4.2
IMPLANTATIONS	MEAN±S.D.	16.0 ± 2.1	13.6 ± 5.8	13.8 ± 5.0	13.2 ± 5.0
VIABLE EMBRYOS	N	339	233	221	239
	MEAN±S.D.	14.7 ± 2.4	12.3 ± 5.8	12.3 ± 5.1	12.0 ± 4.6
NONVIABLE EMBRYOS	N	29	25	27	26
	MEAN±S.D.	1.3 ± 1.6	1.3 ± 1.7	1.5 ± 2.2	1.3 ± 1.3
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	13(56.5)	8(42.1)	9(50.0)	12(60.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	23(100.0)	19(100.0)	18(100.0)	20(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	19(100.0)	18(100.0)	20(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	7.9 ± 9.8	9.9 ± 15.8	10.3 ± 15.8	8.8 ± 8.7

a. Dosage occurred on day 1 of study through day 7 of gestation.

Embryofetal development

Study title: Percutaneous dosage-range-finder developmental toxicity study of capsaicin dermal patches in rats

Key study findings: Since, toxicokinetics was not obtained in the main study, # 1614-003, this study was examined to determine if similar results were obtained possibly allowing the capsaicin concentrations to be extrapolated to the main study. However, this was not the case. The toxicokinetic study of capsaicin in pregnant rats was not adequately conducted, yielding in accurate data.

Reviewer comment: The 50 cm^2 dose is not a MTD, and the reported plasma concentrations are not representative of the actually capsaicin plasma concentration. Almost all samples were <LLOQ (<10 ng/mL) indicated as "diluted sample due to insufficient volume." The study should be repeated with appropriate plasma volumes collected and assayed to determine capsaicin concentration.

Study no.: **1614-003p**
E-Location: m4\
Conducting laboratory and location: (b) (4)

Date of study initiation: Feb 10, 2004
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin patch, Batch 7006753, 177.9 mg/280 cm^2; total impurities <lod
Vehicle Patch Opaque patch, 20 x 10 cm
Lidocaine 4% ((L.M.X.4™), Lot 03150A
Cleansing Gel (89% polyethylene glycol)

Methods

Doses: Vehicle patch: 50 cm^2; capsaicin patches: 25, 37.5 or 50 cm^2, (corresponding to 16, 24, and 32 mg capsaicin/animal, respectively)
Species/strain: pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats
62 days of age at arrival, 219-246 g at study assignment
Number/sex/group: 8/group
Route, formulation, volume, and infusion rate: capsaicin and vehicle patches were applied to the backs of the rats once daily for 3 hours from gestation days (GDs) 7 to 17.
Satellite groups used for toxicokinetics: 9/group
Study design: After clipping the hair along the back of the animal, the application site (approximately 25, 37.5 or 50 cm^2) was demarcated using an indelible marker with one mark at each corner of the site on the animal's back. A "map" of the clipped area was made for all rats with areas of apparent hair tufts, skin defects or natural differences in skin colorization, before administration. On the days of dosing, the rats were fitted with

Elizabethan collars, and the anesthetic cream (LMX4™) was topically applied to the previously clipped application site at an approximate volume of 32 µL/cm^2. Just enough cream was applied to cover the patch application area only (plus 0.5 cm beyond the boundaries), and any excess was removed. At least 60 minutes later, the anesthetic cream was wiped off with dry gauze. Tape was overlaid on the patch. After three hours of application exposure, the patches were removed from the backs of the rats, and the application sites were gently wiped with the cleansing gel (89% polyethylene glycol, approximately 3 mL) and dry gauze.

Results

Mortality (dams): (checked twice daily)

There were no test article related necropsy observations.

Clinical signs (dams): clinical observations made daily, also signs of skin irritation were noted at time of post-dose cleaning, and body and feed weights were recorded. After the 3 hour dose period, skin sites were observed for signs of skin irritation and graded once daily until precluded by hair growth.

Grade 2 flaking and grade 2 erythema occurred only in the treated groups; however the incidences of these skin observations were not necessarily dose dependent. Grade 1 erythema and scabs on the back occurred in increased numbers of rats in the all capsaicin -treated groups, as compared to the vehicle control group. Ptosis was also observed in one or two rats in each of the capsaicin groups. Urine-stained abdominal fur and ungroomed coat occurred only in the capsaicin-treated groups and were considered related to an aversion to normal grooming behavior due to possible capsaicin residue. The Applicant did not considered these to be a direct effect of the capsaicin patch.
*(**Reviewer comment:** Not sure why since it is likely due to capsaicin on the animal's haicoat, possibly making a distinction concerning absorption through skin versus grooming by licking and taste aversion).*

All other clinical observations were considered unrelated to the Capsaicin Dermal Patch because: 1) the incidences were not dose-dependent; and/or 2) the observations occurred in only one or two rats per dosage group. These observations included chromodacryorrhea; chromorhinorrhea; red perivaginal substance; cold to touch; soft or liquid feces; misaligned, missing or broken incisors; swollen neck (related to wearing Elizabethan collars); localized alopecia (limbs); lacrimation; periorbital swelling and grade 1 flaking.
*(**Reviewer comment:** Although perhaps few in number, chromodacryorrhea; chromorhinorrhea, cold to touch, soft or liquid feces, lacrimation; periorbital swelling and grade 1 flaking these were noted in other studies in only capsaicin-treated animals and are likely related to capsaicin treatment here).*

Persistent adverse clinical observations were confirmed at necropsy. No additional gross lesions were identified.

Body weight (dams): Body weights were recorded weekly during the acclimation period, on GD 0 and daily during the dose and post-dose periods.

Body weight loss occurred in the 50 cm^2 dosage group on GDs 7 to 10; however during all other tabulated intervals, body weight gains were generally increased in the treated groups as compared to the vehicle control group. Body weight gains and absolute and relative feed consumption values were increased in the treated groups for the entire dosing period (calculated as GDs 7 to 18), the entire gestation period after the initiation of dosage (GDs 7 to 21), and the gestation period (GDs 0 to 21).

Food consumption (dams): Feed consumption values were recorded on GDs 0, 7, 10, 12, 15, 18 and 21.

Absolute and relative feed consumption values were increased in the treated groups for the entire dosing period, the entire gestation period after the initiation of dosage, and the entire gestation period.

Necropsy: All female rats assigned to the main study were sacrificed by carbon dioxide asphyxiation on GD 21, a Caesarean-sectioning examination was conducted and a gross necropsy was performed. Fetuses were weighed and examined for gross external alterations and sex. Rats that died were examined for the cause of death on the day the observation was made, and pregnancy status and uterine contents were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color and shape.

There were no effects of capsaicin on necropsy observations.

Toxicokinetics: Tail vein blood samples were collected at approximately 2, 3, 4, 6 and 24 hours post-application on GDs 7 and 17 from rats assigned for use in toxicokinetic sample collection. All surviving female rats assigned for use in toxicokinetic sample collection were sacrificed on GD 17 or 18 after the last blood sample collection, examined for pregnancy status and the carcasses were discarded without further evaluation. Plasma was analyzed by (b) (4) (b) (4)

Two rats in the 50 cm^2 satellite dosage group were found dead on GDs 8 and 10, at the time of postdose observations. Observations in these rats are described below. The cause of death for these rats was not readily apparent but it is likely the deaths were related to restraint because they occurred while the rats were wrapped with the elastic wrapping material; all other rats in the satellite dosage groups survived to scheduled sacrifice.

*(**Reviewer comment**: do they mean they were wrapped too tightly?)*

Rat 6659 was found dead on GD 8, 3 hours and 4 minutes after the patch was applied. There were no adverse clinical observations before death. This rat lost weight on GD 8. All tissues appeared normal at necropsy. The litter consisted of 17 embryos.

Rat 6660 was found dead on GD 10, 3 hours and 4 minutes after the patch was applied. This rat had grade 1 erythema and urine-stained abdominal fur on GDs 9 and 10. Body weights were unremarkable. All tissues appeared normal at necropsy. The litter consisted of 15 embryos.

Adverse clinical observations in the 25, 37.5 and 50 cm^2 dosage groups were similar to those observed in the main study group. Body weights and feed consumption values in the rats assigned for use in toxicokinetic sample collection were comparable to the main study groups. Three rats in the 25 cm^2 dosage group were not pregnant; all other rats assigned for use in toxicokinetic sample collection were pregnant. Results of the plasma concentration analyses are presented in the table below.

Plasma levels were generally undetectable for up to 24 hours after patch application on GD 7 in the three treated groups, with the exception of one sample in the 25 cm^2 dose group at 6 hours post-application. After application on GD 17, plasma levels of capsaicin were detected in the 37.5 and 50 cm^2 dosage groups at 2 hours post-application; the average values were dose-dependent as levels were present in only one sample in the 37.5 cm^2 dose group while capsaicin was detectable in all three samples in the 50 cm^2 group. Capsaicin was detectable in the plasma sample of just one rat at 3 hours post-dosage in the 37.5 cm^2 dose group.

***Reviewer Comment**: Examination of the individual sample values (Tables 1 and 2 below) indicated that the reported plasma concentrations may not be representative of the actually capsaicin plasma concentration. Almost all samples were <LLOQ (<10 ng/mL) indicated as "diluted sample due to insufficient volume." The study should be repeated with appropriate plasma volumes collected and assayed to determine capsaicin concentration.*

Concentration of Capsaicin in Rat Plasma

	Group II 25 cm^2	Group III 37.5 cm^2	Group IV 50 cm^2
Day 7 of Gestation			
N	3	3	3
Predosage (ng/mL)	<LLOQ	<LLOQ	<LLOQ
2 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ
3 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ
4 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ
6 hours (ng/mL)	97.6 [1]	<LLOQ	<LLOQ
24 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ
Day 17 of Gestation			
N	3	3	3
Predosage (ng/mL)	<LLOQ	<LLOQ	<LLOQ [2]
2 hours (ng/mL)	<LLOQ	73.2 [1]	44.8 ± 18.5
3 hours (ng/mL)	<LLOQ	59.8 [1]	<LLOQ [2]
4 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ [2]
6 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ
24 hours (ng/mL)	<LLOQ	<LLOQ	<LLOQ [2]

LLOQ = Lower limit of quantitation
[ ] = Number of values averaged if less than three.

Table 1
Concentration (ng/mL) of Capsaicin in Rat Plasma: Day 7

Animal	Dose Group	Gender	Timepoint: Day 7 Hour 0	Day 7 Hour 2	Day 7 Hour 3	Day 7 Hour 4	Day 7 Hour 6	Day 7 Hour 24
(b) (4)								

Table 2
Concentration (ng/mL) of Capsaicin in Rat Plasma: Day 17

Animal	Dose Group	Gender	Day 17 Hour 0	Day 17 Hour 2	Day 17 Hour 3	Day 17 Hour 4	Day 17 Hour 6	Day 17 Hour 24

(b) (4)

Terminal and microscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

All Caesarean-sectioning or litter parameters were similar between treatment groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses or late resorptions. There were no fetal gross alterations.

Offspring (malformations, variations, etc.):

There were no fetal gross alterations.

Study title: **Percutaneous developmental toxicity study of capsaicin dermal patches in rats**

Key study findings: The maternal NOAEL for the capsaicin dermal patch was <25 cm^2 (16 mg/animal), and the developmental NOAEL was 37.5 cm^2 (24 mg/animal).

Caesarean-sectioning observations were based on 24 (96.0%), 24 (96.0%), 25 (100%) and 23 (92.0%) pregnant rats with one or more live fetuses in respectively vehicle, 25, 37.5, 50 cm^2 casaicin groups. There were no Caesarean-sectioning or litter parameters affected by application of the capsaicin dermal patch. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percentage of resorbed conceptuses, and percent live male fetuses were similar among the four treatment groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

Study no.: 1614-003
E-Location: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-003
Conducting laboratory and location: (b) (4)
Date of study initiation: March 23, 2004
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin dermal patch, Batch 81101203; Purity not stated; total impurities <lod (limit of detection, and below specifications)
Vehicle Patch Opaque patch, Batch 81113301
Lidocaine 4% (LMX4™), Lot 03175A
Cleansing Gel (89% polyethylene glycol), Lot 22698

Methods

Doses: vehicle patch size: 50 cm^2; capsaicin (8% w/w) patch sizes: 25, 37.5 or 50 cm^2 (corresponding to 16, 24, and 32 mg capsaicin/animal, respectively)
Species/strain: pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats;
62 days of age at arrival, 219-246 g at start or study
Number/sex/group: 25/group
Route, formulation, volume, and infusion rate: capsaicin and vehicle patches were applied to the backs of the rats once daily for 3 hours from gestation days (GDs) 7 to 17.
Satellite groups used for toxicokinetics: There were no toxicokinetic groups.
Study design: The study design is illustrated below.

After clipping the hair along the back of the animal, the application site (approximately 25, 37.5 or 50 cm^2) was demarcated using an indelible marker with one mark at each corner of the site on the animal's back. A "map" of the clipped area was made for all rats with areas of apparent hair tufts, skin defects or natural differences in skin colorization, before administration. On the days of dosing, the rats were fitted with Elizabethan collars, and the anesthetic cream (LMX4™) was topically applied to the previously clipped application site at an approximate volume of 32 $\mu L/cm^2$. Just enough cream was applied to cover the patch application area only (plus 0.5 cm beyond the boundaries), and any excess was removed. At least 60 minutes later, the anesthetic cream was wiped off with dry gauze. Tape was overlaid on the patch. After three hours of application exposure, the patches were removed from the backs of the rats, and the application sites were gently wiped with the cleansing gel (89% polyethylene glycol, approximately 3 mL) and dry gauze.

Results

Mortality (dams): checked twice daily

There were no deaths.

Clinical signs (dams): checked daily at predosing, 3 hour post-exposure cleansing, and during postdosing period. Before each dose and after patch removal, the application site skin was graded for signs of irritation according to Draize scale.

Grade 1 erythema occurred in significantly increased ($p \leq 0.01$) numbers of rats in the capsaicin-treated groups compared to the control group, but the erythema incidence was not dose-dependent. Lacrimation occurred in significantly increased ($p \leq 0.05$ or $p \leq 0.01$) numbers of rats in the 37.5 and 50 cm^2 capsaicin-treated groups. A significantly increased ($p \leq 0.01$) number of rats in the 50 cm^2 test article-treated group were cold to touch. Soft or liquid feces and a perivaginal substance (red, brown or black) occurred in slightly increased numbers, but nonsignificant, of rats in the 50 cm^2 capsaicin-treated group. Observations of urine-stained abdominal fur and ungroomed coat occurred in increased or significantly increased ($p \leq 0.01$) numbers of rats in the capsaicin groups and were considered possibly related to an aversion to normal grooming behavior due to capsaicin residue.

Persistent adverse clinical observations were confirmed at necropsy. No additional gross lesions were identified.

Other observations included chromodacryorrhea, chromorhinorrhea, areas of red fur, ptosis, yellow fore- and/or hindpaws, light brown feces, localized alopecia (limbs), yellow perinasal substance, red substance in the cage pan, abrasion on the back, excess salivation, grade 1 flaking and scabs or red skin at the application site. The numbers of rats with yellow fore- and/or hindpaws was significantly increased ($p \leq 0.01$) in the 25 and 37.5 cm^2 test article-treated groups, a non-dosage dependent observation considered unrelated to the test article. These findings were considered unrelated to the capsaicin dermal patch because: 1) the incidences were not dosage dependent; and/or 2) the observations occurred in only one or two rats per group.

Reviewer comment: *Although perhaps few in number, chromodacryorrhea; chromorhinorrhea, ptosis, salivation, lacrimation; grade 1 flaking, scabs and red skin these were noted in other studies in only capsaicin-treated animals and are likely related to capsaicin treatment.*

PROTOCOL 1614-003: PERCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF CAPSAICIN DERMAL PATCHES IN RATS

TABLE 1 (PAGE 1): SKIN REACTIONS AND CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
MORTALITY	0	0	0	0
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
CLINICAL OBSERVATIONS:				
CHROMODACRYORRHEA	138/ 25	140/ 25	139/ 25	141/ 25
CHROMORHINORRHEA	104/ 24	171/ 25	167/ 25	193/ 25
UNGROOMED COAT	22/ 5	146/ 23**	197/ 24**	233/ 25**
URINE-STAINED ABDOMINAL FUR	15/ 5	85/ 14	152/ 20**	221/ 25**
SOFT OR LIQUID FECES	4/ 3	2/ 2	3/ 2	8/ 8
PERIVAGINAL SUBSTANCE b	0/ 0	3/ 3	2/ 2	7/ 6
LACRIMATION	0/ 0	0/ 0	4/ 3*	5/ 5**
RED FUR c	6/ 2	24/ 5	17/ 4	18/ 4
COLD TO TOUCH	0/ 0	1/ 1	0/ 0	4/ 4**
PTOSIS	1/ 1	2/ 2	0/ 0	2/ 2
BOTH FOREPAWS AND/OR HINDPAWS: YELLOW	0/ 0	9/ 7**	7/ 5**	1/ 1
LIGHT BROWN FECES	1/ 1	1/ 1	2/ 2	1/ 1
LOCALIZED ALOPECIA: LIMB(S)	5/ 2	0/ 0	0/ 0	2/ 1

STATISTICAL ANALYSES OF SKIN REACTION AND CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP ON DAYS 7 THROUGH 21 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 through 17 of presumed gestation.
b. Red, brown or black.
c. Located on the nose, mouth, neck, lower midline, left and right inguinal area, both forepaws, both hindpaws and/or both hindlimbs.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE 1 (PAGE 2): SKIN REACTIONS AND CLINICAL AND NECROPSY OBSERVATIONS - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
MORTALITY	0	0	0	0
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25
CLINICAL OBSERVATIONS:				
YELLOW PERINASAL SUBSTANCE	0/ 0	0/ 0	0/ 0	1/ 1
RED SUBSTANCE IN CAGE PAN	0/ 0	0/ 0	0/ 0	1/ 1
BACK: ABRASION	0/ 0	1/ 1	4/ 1	0/ 0
EXCESS SALIVATION	0/ 0	0/ 0	1/ 1	0/ 0
SKIN REACTION OBSERVATIONS:				
FLAKING: GRADE 1	103/ 24	223/ 25	250/ 25	239/ 25
ERYTHEMA: GRADE 1	32/ 12	174/ 24**	203/ 25**	151/ 22**
SCAB(S)	27/ 10	36/ 15	35/ 13	34/ 13
RED SKIN	0/ 0	0/ 0	0/ 0	1/ 1

PERSISTENT ADVERSE CLINICAL OBSERVATIONS WERE CONFIRMED AT NECROPSY, NO ADDITIONAL GROSS LESIONS WERE IDENTIFIED

STATISTICAL ANALYSES OF SKIN REACTION AND CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP ON DAYS 7 THROUGH 21 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 through 17 of presumed gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body weight (dams): recorded daily during the dose and postdose periods

Body weights were similar among the four dose groups. Body weight gains tended to be increased, though not significantly, throughout the study in the capsaicin-treated groups compared to the vehicle control group. Body weight gains for the entire dosage period (calculated as GDs 7 to 18) were 117%, 108% and 108% of the vehicle control group value in the three capsaicin-treated groups.

TABLE 3 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	25	23
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+39.7 ± 11.7	+38.1 ± 9.5	+37.8 ± 9.5	+38.3 ± 8.6
DAYS 7 - 10	MEAN±S.D.	+2.0 ± 7.3	+7.9 ± 8.8	+4.9 ± 7.3	+3.5 ± 6.7
DAYS 10 - 12	MEAN±S.D.	+9.1 ± 6.8	+8.5 ± 4.8	+9.6 ± 5.8	+10.9 ± 5.5
DAYS 12 - 15	MEAN±S.D.	+11.2 ± 8.4	+14.5 ± 7.9	+13.6 ± 5.3	+16.0 ± 7.7
DAYS 15 - 18	MEAN±S.D.	+30.1 ± 8.2	+30.2 ± 5.1	+28.4 ± 4.4	+26.5 ± 10.1
DAYS 7 - 18	MEAN±S.D.	+52.4 ± 12.7	+61.1 ± 13.7	+56.5 ± 8.7	+56.9 ± 16.5
DAYS 18 - 21	MEAN±S.D.	+52.8 ± 11.3	+53.1 ± 6.8	+56.7 ± 8.1	+56.0 ± 6.4
DAYS 7 - 21	MEAN±S.D.	+105.1 ± 19.9	+114.2 ± 15.8	+113.2 ± 13.3	+112.9 ± 19.6
DAYS 0 - 21	MEAN±S.D.	+144.8 ± 27.4	+152.4 ± 16.9	+151.0 ± 15.4	+151.2 ± 23.3

DAYS = DAYS OF GESTATION
a. Dosage occurred on days 7 through 17 of gestation.

Food consumption (dams): Feed consumption values were recorded on GDs 0, 7, 10 12, 15, 18 and 21.

Absolute and relative feed consumption values were increased or significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in all three treated groups on GDs 7 to 10 and for the entire dosage period (calculated as GDs 7 to 18). Absolute and relative feed consumption values were also significantly increased ($p \leq 0.01$) on GDs 10 to 12 in the 37.5 and 50 cm^2 capsaicin-treated groups. Absolute and relative feed consumption values were significantly increased ($p \leq 0.01$) in the 50 cm^2 test article-treated group for the entire gestation period after the initiation of dosage (GDs 7 to 21) and the entire gestation period (GDs 0 to 21).

TABLE 4 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	25	23
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 0 - 7	MEAN±S.D.	23.2 ± 2.2	23.5 ± 1.8	23.4 ± 2.1	24.1 ± 1.9
DAYS 7 - 10	MEAN±S.D.	21.9 ± 3.6	24.0 ± 2.2*	23.8 ± 2.5*	24.6 ± 2.4**
DAYS 10 - 12	MEAN±S.D.	22.1 ± 3.6	23.8 ± 2.1	24.8 ± 2.8**	25.3 ± 3.0**
DAYS 12 - 15	MEAN±S.D.	24.1 ± 3.0	25.5 ± 2.9 [23]b	25.1 ± 2.8 [23]b	25.9 ± 4.4 [20]b
DAYS 15 - 18	MEAN±S.D.	27.0 ± 3.4	28.3 ± 3.4 [20]b	27.8 ± 2.4 [24]b	29.8 ± 4.7 [22]b
DAYS 7 - 18	MEAN±S.D.	23.9 ± 2.8	25.7 ± 2.2* [20]b	25.4 ± 1.9 [24]b	26.6 ± 2.6** [22]b
DAYS 18 - 21	MEAN±S.D.	28.8 ± 4.2	29.3 ± 2.7 [23]b	29.3 ± 3.3 [24]b	31.1 ± 2.7
DAYS 7 - 21	MEAN±S.D.	25.0 ± 2.8	26.3 ± 2.1 [23]b	26.3 ± 1.8 [24]b	27.6 ± 2.4**
DAYS 0 - 21	MEAN±S.D.	24.4 ± 2.4	25.4 ± 1.8 [23]b	25.3 ± 1.7 [24]b	26.3 ± 1.8**

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation.
b. Excludes values that were associated with spillage or wet/soiled feed.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

Necropsy: All female rats were sacrificed by carbon dioxide asphyxiation on GD 21. Caesarean sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently nonpregnant rats were examined to confirm the absence of implantation sites.

There were no adverse necropsy observations.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Non-responding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis

indicated that the fetus was a late resorption. Placentae were examined for size, color and shape. Each fetus was subsequently weighed and examined for sex and gross lesions. Live fetuses were sacrificed by an intraperitoneal injection of sodium pentobarbital. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations, using a variation of the microdissection technique of Staples. The remaining fetuses (approximately one-half of the fetuses in each litter) were eviscerated, cleared and stained with alizarin red S and examined for skeletal abnormalities.

Caesarean-sectioning observations were based on 24 (96.0%), 24 (96.0%), 25 (100%) and 23 (92.0%) pregnant rats with one or more live fetuses in respectively vehicle, 25, 37.5, 50 cm^2 casaicin groups. There were no Caesarean-sectioning or litter parameters affected by application of the capsaicin dermal patch. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percentage of resorbed conceptuses, and percent live male fetuses were similar among the four treatment groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	24(96.0)	25(100.0)	23(92.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	24	25	23
CORPORA LUTEA	MEAN±S.D.	16.1 ± 2.7	16.4 ± 2.3	17.7 ± 2.8	17.0 ± 3.1
IMPLANTATIONS	MEAN±S.D.	15.1 ± 2.4	14.4 ± 1.6	15.6 ± 2.2	15.3 ± 2.5
LITTER SIZES	MEAN±S.D.	14.8 ± 2.4	13.7 ± 1.6	15.0 ± 2.2	14.8 ± 2.2
LIVE FETUSES	N	355	329	374	340
	MEAN±S.D.	14.8 ± 2.4	13.7 ± 1.6	15.0 ± 2.2	14.8 ± 2.2
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.3 ± 0.6	0.7 ± 0.9	0.6 ± 0.8	0.5 ± 0.8
EARLY RESORPTIONS	N	7	16	15	11
	MEAN±S.D.	0.3 ± 0.6	0.7 ± 0.9	0.6 ± 0.8	0.5 ± 0.8
LATE RESORPTIONS	N	0	1	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	6(25.0)	11(45.8)	11(44.0)	8(34.8)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	24(100.0)	25(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	24(100.0)	25(100.0)	23(100.0)

a. Dosage occurred on days 7 through 17 of gestation.

TABLE 7 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	24	25	23
IMPLANTATIONS	MEAN±S.D.	15.1 ± 2.4	14.4 ± 1.6	15.6 ± 2.2	15.3 ± 2.5
LIVE FETUSES	N	355	329	374	340
	MEAN±S.D.	14.8 ± 2.4	13.7 ± 1.6	15.0 ± 2.2	14.8 ± 2.2
LIVE MALE FETUSES	N	189	162	203	170
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	52.8 ± 11.3	49.4 ± 13.6	53.7 ± 14.8	50.3 ± 13.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	4.79 ± 0.40	5.00 ± 0.32	4.83 ± 0.26	4.86 ± 0.36
MALE FETUSES	MEAN±S.D.	4.92 ± 0.40	5.13 ± 0.32	4.96 ± 0.29	4.97 ± 0.34
FEMALE FETUSES	MEAN±S.D.	4.64 ± 0.42	4.87 ± 0.30	4.69 ± 0.30	4.74 ± 0.37
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.8 ± 3.4	4.8 ± 6.0	3.8 ± 4.7	2.8 ± 4.5

a. Dosage occurred on days 7 through 17 of gestation.

Offspring (malformations, variations, etc.): Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Fetal evaluations were based on 355, 329, 374 and 340 live, GD 21 Caesarean-delivered fetuses in 24, 24, 25 and 23 litters in the vehicle, 25, 37.5 and 50 cm^2 groups, respectively. Each of these fetuses was examined for gross external alterations. Of these respective fetuses, 170, 159, 182 and 164 fetuses were examined for soft tissue alterations, and 185, 170, 192 and 176 fetuses were examined for skeletal alterations and fetal ossification site averages.

Delays in skeletal ossification, evident as significant reductions ($p \leq 0.01$) in the average number of ossified hindlimb and forelimb phalanges and metatarsals and a significant increase ($p \leq 0.01$) in the litter incidence of incompletely ossified 1st sternebra, occurred in the 50 cm^2 test article-treated group. No other gross external, soft tissue or skeletal fetal alterations (malformations or variations) were related to capsaicin treatment.

Litters with fetuses with alterations numbered 12 (50.0%), 4 (16.7%), 13 (52.0%) and 12 (52.2%), respectively. The numbers of fetuses with any alteration observed were 21 (5.9%), 6 (1.8%), 16 (4.3%) and 19 (5.6%) and the percentages of fetuses with any alteration per litter were 5.8, 1.9, 4.3 and 5.6 in these same respective groups. The percentages of *litters with* fetuses with alterations and *fetuses with* any alteration were significantly reduced ($p \leq 0.01$) in the 25 cm^2 test article-treated group. The Applicant interpreted this as a non-dosage dependent observation that was considered unrelated to the test article.

Reviewer comment: *On the contrary, this indicates a NOAEL value, with a dose >25 cm^2 resulting in a capsaicin related alteration.*

TABLE 8 (PAGE 1): PETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
LITTERS EVALUATED	N	24	24	25	23
FETUSES EVALUATED	N	355	329	374	340
LIVE	N	355	329	374	340
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	12(50.0)	4(16.7)**	13(52.0)	12(52.2)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	21(5.9)	6(1.8)**	16(4.3)	19(5.6)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	5.8 ± 7.4	1.9 ± 4.6	4.3 ± 4.8	5.6 ± 6.7

a. Dosage occurred on days 7 through 17 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE 9 (PAGE 1): PETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
LITTERS EVALUATED	N	24	24	25	23
FETUSES EVALUATED	N	355	329	374	340
LIVE	N	355	329	374	340
EYES: LEFT, BULGE DEPRESSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	2(0.6)b	0(0.0)	0(0.0)
EYES: RIGHT, LID OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)c
EARS: BILATERAL, LOW-SET					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.3)b	0(0.0)	0(0.0)
JAW: AGNATHIA					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.3)b	0(0.0)	0(0.0)
ORAL OPENING: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.3)b	0(0.0)	0(0.0)
BODY: TRUNK SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)c
FORELIMBS AND HINDLIMBS: BILATERAL, SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)c
FORELIMBS AND HINDLIMBS: BILATERAL, DIGITS FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)c

a. Dosage occurred on days 7 through 17 of gestation.
b. Fetus 9232-15 had other gross external alterations.
c. Fetus 9299-5 had other gross external alterations.

TABLE 10 (PAGE 1): PETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
LITTERS EVALUATED	N	24	24	25	23
FETUSES EVALUATED	N	170	159	182	164
LIVE	N	170	159	182	164
EYES: MICROPHTHALMIA					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	2(1.2)b,c	0(0.0)	0(0.0)
TONGUE: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	2(1.2)b,c	0(0.0)	0(0.0)
SITUS INVERSUS					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.6)c	0(0.0)	0(0.0)
NASAL SEPTUM: INCOMPLETE					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
NASOPHARYNX: NOT PATENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)
PRIMORDIAL TEETH: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.6)b	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 17 of gestation.
b. Fetus 9232-15 had other soft tissue alterations.
c. Fetus 9232-17 had other soft tissue alterations.

Sternum**:** An incompletely ossified 1st sternebra occurred in 6, 0, 8 and 12 fetuses from 6, 0, 7 and 9 litters in the four respective groups. The fetal incidence of incompletely ossified 1st sternebra was significantly increased ($p \leq 0.05$) in the 50 cm^2 capsaicin-treated group. The fetal and litter incidences of this observation were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 25 cm^2 test article-treated group, reflecting the increased incidence in the vehicle control group and considered unrelated to the capsaicin-treated. The fetal and litter incidences of an incompletely ossified 1st sternebra in the 50 vehicle, 37.5 and 50 cm^2 groups were all outside the ranges observed historically at the Testing Facility. One fetus in the vehicle control group (9222-3) also had alterations of the cervical vertebrae, as previously described, and one fetus (9299-5) in the 50 cm^2 capsaicin-treated group also had incompletely or not ossified pubes. No additional alterations occurred in these fetuses. Asymmetric sternal centra occurred in one fetus in each of the 25 and 37.5 cm^2 capsaicin-treated groups. No additional alterations occurred in these fetuses.

TABLE 11 (PAGE 1): PETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table)

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
LITTERS EVALUATED	N	24	24	25	23
FETUSES EVALUATED	N	185	170	192	176
LIVE	N	185	170	192	176
SKULL: SQUAMOSAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(8.3)	0(0.0)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	2(1.1)b,f	0(0.0)	0(0.0)	0(0.0)
SKULL: ZYGOMATIC, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(8.3)	0(0.0)	1(4.0)	0(0.0)
PETAL INCIDENCE	N(%)	2(1.1)b,f	0(0.0)	1(0.5)i	0(0.0)
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	3(12.5)	2(8.3)	4(16.0)	3(13.0)
PETAL INCIDENCE	N(%)	7(3.8)e,h	3(1.8)	4(2.1)	4(2.3)j,k
CERVICAL VERTEBRAE: 6TH CERVICAL ARCH HAD THE APPEARANCE OF THE 7TH					
LITTER INCIDENCE	N(%)	5(20.8)	0(0.0)	1(4.0)	3(13.0)
PETAL INCIDENCE	N(%)	6(3.2)d,e,g	0(0.0)**	1(0.5)**	3(1.7)j,k
CERVICAL VERTEBRAE: 5TH CERVICAL ARCH HAD THE APPEARANCE OF THE 7TH					
LITTER INCIDENCE	N(%)	2(8.3)	0(0.0)	0(0.0)	0(0.0)
PETAL INCIDENCE	N(%)	3(1.6)d,g	0(0.0)**	0(0.0)**	0(0.0)**
THORACIC VERTEBRAE: 12 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)k
THORACIC VERTEBRAE: CENTRUM, BIPID					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
RIBS: 12 PRESENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)k
RIBS: SHORT					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	1(0.5)h	0(0.0)	0(0.0)	1(0.6)

TABLE 11 (PAGE 2): PETAL SKELETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
LITTERS EVALUATED	N	24	24	25	23
FETUSES EVALUATED	N	185	170	192	176
LIVE	N	185	170	192	176
RIBS: WAVY					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	1(4.0)	1(4.3)
PETAL INCIDENCE	N(%)	1(0.5)c	0(0.0)	1(0.5)i	1(0.6)
RIBS: INCOMPLETELY OSSIFIED (HYPOPLASTIC)					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	1(4.0)	0(0.0)
PETAL INCIDENCE	N(%)	1(0.5)c	0(0.0)	1(0.5)i	0(0.0)
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	6(25.0)	0(0.0)**	7(28.0)	9(39.1)
PETAL INCIDENCE	N(%)	6(3.2)g	0(0.0)*	8(4.2)	12(6.8)*l
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	1(4.0)	0(0.0)
PETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	1(0.5)	0(0.0)
PELVIS: PUBES, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)l
PELVIS: PUBES, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
PETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)l

a. Dosage occurred on days 7 through 17 of gestation.
b. Petus 9206-7 had other skeletal alterations.
c. Petus 9206-9 had other skeletal alterations.
d. Petus 9208-5 had other skeletal alterations.
e. Petus 9217-1 had other skeletal alterations.
f. Petus 9221-9 had other skeletal alterations.
g. Petus 9222-3 had other skeletal alterations.
h. Petus 9224-8 had other skeletal alterations.
i. Petus 9270-13 had other skeletal alterations.
j. Petus 9289-1 had other skeletal alterations.
k. Petus 9293-7 had other skeletal alterations.
l. Petus 9299-5 had other skeletal alterations.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Fetal Ossification

The average number of ossified hindlimb and forelimb phalanges and metatarsals were significantly reduced ($p \leq 0.01$) in the 50 cm^2 test article-treated group. The values for ossified fore- and hindlimb phalanges were within the ranges observed historically at the testing facility, however the values for ossified metatarsals were outside the historical range. There were no other statistically significant or biologically important differences among the four groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers and xiphoid), forelimbs (carpals and metacarpals) or hindlimbs (tarsals).

TABLE 12 (PAGE 1): FETAL OSSIPICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
LITTERS EXAMINED	N	24	24	25	23
FETUSES EXAMINED	N	185	170	192	176
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.02	0.99 ± 0.04	0.98 ± 0.06	0.99 ± 0.04
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.13 ± 0.18	13.08 ± 0.15	13.03 ± 0.07	13.06 ± 0.09
LUMBAR	MEAN±S.D.	5.87 ± 0.18	5.92 ± 0.16	5.96 ± 0.08	5.93 ± 0.10
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	6.94 ± 0.54	7.08 ± 0.79	6.99 ± 0.58	6.75 ± 0.58
RIBS (PAIRS)	MEAN±S.D.	13.09 ± 0.13	13.05 ± 0.11	13.03 ± 0.06	13.04 ± 0.08
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.99 ± 0.03	4.00 ± 0.00	3.98 ± 0.09	3.98 ± 0.04
XIPHOID	MEAN±S.D.	1.00 ± 0.02	1.00 ± 0.00	1.00 ± 0.02	0.97 ± 0.08
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.02
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	8.44 ± 0.56	8.48 ± 0.53	8.25 ± 0.69	7.71 ± 0.75**
HINDLIMB b					
TARSALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
METATARSALS	MEAN±S.D.	4.85 ± 0.22	4.89 ± 0.15	4.80 ± 0.23	4.50 ± 0.36**
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	6.90 ± 1.24	6.92 ± 1.12	6.50 ± 1.03	5.47 ± 0.69**

a. Dosage occurred on days 7 through 17 of gestation.
b. Calculated as average per limb.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Study title: **Percutaneous dose-range-finder developmental toxicity study of capsaicin in rabbits**

Key study findings: Since toxicokinetics were not obtained in the main study, #1614-003, this study was reviewed to determine if similar results were obtained to possibly allow the capsaicin concentrations to be extrapolated to the main study. There was sufficient similarity in the findings that the toxicokinetics of this dose-range study were able to be extrapolated to the main study to determine safety margins based on Cmax. AUC was not calculated in this study.

Reviewer Comment: *The Sponsor selected for the main study a 13 uL/cm^2 dose as the dose that would "produce minimal maternal toxicity and little or no developmental toxicity" which is an inappropriate maximal tolerated or maximal feasible dose.*

Rabbit Dose and Toxicokinetics

Dose (µL/cm^2)		**3**	**10**	**30**
mg/cm^2		0.3	1.0	3.0
mg/rabbit		60	200	600
mg/kg (~3.5 kg/rabbit)		17	57	171
HED (based on body surface area)		5.7	19	57
Cmax (ng/mL)	**GD 7**	18.2	13.5	54.6
	GD 19	148	350	966
t_{max} (h)	**GD 7**	6	3	3
	GD 19	5 min	30 min	6
AUC		not calculated		

Study no.: 1614-002p
E-Location: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-002p
Conducting laboratory and location (b) (4)
Analytical Report: (b) (4)
Date of study initiation:
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Trans-capsaicin, Lot F0010102, Purity 100.02% (related compounds, impurities, total 0.62%)
Vehicle: Diethylene glycol monoethyl ether, 99%
Topical anesthetic: Lidocaine 4%, LMX4, Lot 03150A
Cleansing Gel (89% polyethylene glycol), Lot 22698

The results of concentration analyses indicated no capsaicin contamination of the vehicle and that the 10% (w/v) capsaicin formulation was 98.4% to 99.3% of the targeted concentration.

Methods

Doses: 30 (Vehicle), 3, 10 and 30 µL/cm^2 to a10 cm x 20 cm area on the back

Amount of Capsaicin in Dose

Dose (µL/cm^2)	30	3	10	30
mg/cm^2	0	0.3	1.0	3.0
mg/rabbit	0	60	200	600

The results of concentration analyses indicated no casaicin contamination of the vehicle and that the 10% (w/v) capsaicin formulation was 98.4% to 99.3% of the targeted concentration.

Species/strain: New Zealand White [Hra:(NZW)SPF] rabbits

Number/sex/group: 5 pregnant rabbits/dose

Route, formulation, volume, and infusion rate:

Satellite groups used for toxicokinetics: 3 pregnant rabbits/dose

Study design:

Parameters and endpoints evaluated:

Rabbits were habituated to Elizabethan collars on 3 days before the start of the study. The site of application was clipped of hair was clipped. The area of application was approximately 10 cm x 20 cm and marked with indelible ink at the corners. The rabbits were fitted with Elizabethan collars, topical lidocaine cream was applied to the application site for at least 1 hour (40 uL/cm^2), then wiped off with dry gauze, after which vehicle or capsaicin was applied for a 3 hour duration, then the area wiped with cleansing lotion (approximately 10 mL) and a dry gauze. The vehicle, diethylene glycol monoethyl ether (DGME, 99%) or capsaicin in DGME (10% w/v capsaicin) was applied topically once daily for a 3-hour on GDs 7 through19.

Parameters and endpoints evaluated:

All surviving female rabbits assigned to the main study were sacrificed on GD 29 and a Caesarean-sectioning examination was conducted, and a gross necropsy was performed. Fetuses were weighed and examined for gross external alterations and sex. Rabbits that died were examined for the cause of death on the day the observation was made, and pregnancy status and uterine contents were recorded.

Results

Mortality (dams):

Two rabbits in the 30 µL/cm^2 dose group (1 in the main study found dead on GD 19 before administration of the final dose, and 1 in the toxicokinetic group was found dead on GD 16 before administration of that days' dose) were found dead.

Clinical signs noted for this rabbit included erythema grade 1 (GDs 12, 13, 17 and 18), erythema grade 2 (GDs 14 and 16) and wrinkling (GDs 16 to 18). This rabbit lost weight after GD 15 and feed consumption values were reduced after GD 14. At necropsy, there were numerous red areas on the mucosal surface of the stomach (ranging in size from

pinpoint to 0.6 cm x 3.4 cm); all other tissues appeared normal. The litter consisted of 13 fetuses that appeared normal at gross external examination.

The toxicokinetic rabbit had grade 1 erythema on GDs 11 to 13, grade 2 erythema on GDs 14 and 15, and wrinkling and vocalization on GD15. This rabbit generally lost weight and feed consumption values were reduced after GD 7. All tissues appeared normal at necropsy. This rabbit was pregnant.
***Reviewer comment*:** *The Applicant attributed the two deaths to capsaicin since they both occurred in the high dose group. I concur with this assessment.*

Clinical signs (dams):

Increased incidences of skin reactions occurred in all treated groups. The severity increased with dose. Grade 1 and 2 erythema occurred in all rabbits in the treated groups, while grade 3 erythema occurred in one or two rabbits in the 10 and 30 µL/cm^2 dosage groups. Flaking occurred in all rabbits doses, with grade 1 flaking occurred in 2 to 4 rabbits, grade 2 flaking occurred in 4 or 5 rabbits and grade 3 flaking occurred in 1 or 2 rabbits in all treated groups. Wrinkling occurred in 4 or 5 rabbits and skin thickening occurred in 1 to 3 does in the capsaicin treated groups. Vocalization occurred in one or two rabbits of the capsaicin groups, and occurred only on one day for each affected rabbit.

Body weight (dams):

Mean body weight gains were reduced in the 10 and 30 µL/cm^2 groups for the entire dosing period (calculated from GDs 7 to 20), and in the 30 µL/cm^2 group for the entire gestation period after the initiation of dosage (GDs 7 to 29) and the entire gestation period (GDs 0 to 29).

Maternal Body Weight Gains (mean kg)

Dose (µL/cm^2) (mg/rabbit)	**30 vehicle 0**	**3 60**	**10 200**	**30 600**
N	**5**	**5** (4 pregnant)	**5**	**5** (1 died on GD 19)
Interval				
GDs 0-7	0.09	0.12	0.08	0.14 (n=5)
GDs 7-20 (Dosing period)	0.27	0.28	0.18 (-33%)*	0.12 (-56%)*
GDs 7-29	0.58	0.39 (-33%)*	0.43 (-26%)*	0.24 (-59%)*
GDs 0-29	0.68	0.51 (-25%)*	0.51 (-25%)*	0.38 (-44%)*

* compared to control (0 dose)

Food consumption (dams):

Absolute and relative feed consumption values were reduced in the 30 µL/cm^2 dosage group for the entire dosage period (calculated as GDs 7 to 20).

Necropsy

There were no capsaicin-related necropsy observations. Findings included par-ovarian cysts (n=3), clear, fluid-filled cysts on the surface of the kidneys (n=1), black areas on all lung lobes (n=3), right horn of the uterus was adhered to the rectum and ended in a blind sac (n=1, 30 µL/cm^2 dose group, the pregnancy was unilateral).

Toxicokinetics: Blood samples were collected at 0, 5, 15, 30 min, 1, and 6 hours in relation to dose application. Pharmacokinetic analysis was not conducted.

Following topical administration on GD 7, plasma concentrations of capsaicin were first detected at 6 hours, 15 minutes and 5 minutes in the 3, 10 and 30 µL/cm^2 dose groups, respectively. The highest concentrations of capsaicin occurred in the 3 µL/cm^2 dosage group at 6 hours postdose and at 3 hours postdose in the 10 and 30 µL/cm^2 groups. Capsaicin was detected predose on GD 19. On GD 19, application of capsaicin, resulted in variable increases in plasma concentrations, but increased in a dosage-dependent manner at all timepoints.

Concentration of Capsaicin in Rabbit Plasma

	Group II 3 mcL/cm^2	Group III 10 mcL/cm^2	Group IV 30 mcL/cm^2
Day 7 of Gestation			
N	3	3	3
Predosage (ng/mL)	<LLOQ	<LLOQ	<LLOQ
5 minutes (ng/mL)	<LLOQ	<LLOQ	14.4 [1]
15 minutes (ng/mL)	<LLOQ	5.97 [1]	25.45 ± 23.73
30 minutes (ng/mL)	<LLOQ	10.63 ± 3.219	25.95 ± 26.30
1 hour (ng/mL)	<LLOQ	12.13 ± 6.490	33.10 ± 33.37
3 hours (ng/mL)	<LLOQ	13.52 ± 9.098	54.65 ± 69.17
6 hours (ng/mL)	18.2 [1]	12.34 ± 2.130	49.50 ± 33.26
Day 19 of Gestation			
N	3	3	2
Predosage (ng/mL)	57.53 ± 13.48	190.8 ± 175.5	286.0 ± 35.36
5 minutes (ng/mL)	148.2 ± 137.5	198.4 ± 138.2	402.5 ± 84.14
15 minutes (ng/mL)	106.0 ± 7.994	280.3 ± 151.0	821.5 ± 365.6
30 minutes (ng/mL)	141.7 ± 23.44	350.3 ± 177.5	734.5 ± 23.33
1 hour (ng/mL)	143.0 ± 22.72	333.3 ± 140.7	809.0 ± 123.0
3 hours (ng/mL)	84.23 ± 24.29	237.0 ± 112.8	779.0 ± 106.1
6 hours (ng/mL)	77.27 ± 24.22	178.3 ± 100.0	966.5 ± 415.1

LLOQ = Lower limit of quantitation
[ ] = Number of values averaged

Although there is substantial variation in the group III data on day 19, individual animals data indicate 2 of three samples were generally similar.

Table 1
Concentration (ng/mL) of Capsaicin in Rabbit Plasma

Animal	Dose Group	Gender	Timepoint: Day 7 Hour 0 Minute 0	Day 7 Hour 0 Minute 5	Day 7 Hour 0 Minute 15	Day 7 Hour 0 Minute 30	Day 7 Hour 1 Minute 0	Day 7 Hour 3 Minute 0	Day 7 Hour 6 Minute 0
(b) (4)									

Final Report

Table 1 (Continued)
Concentration (ng/mL) of Capsaicin in Rabbit Plasma

Animal	Dose Group	Gender	Timepoint						
			Day 19 Hour 0 Minute 0	Day 19 Hour 0 Minute 5	Day 19 Hour 0 Minute 15	Day 19 Hour 0 Minute 30	Day 19 Hour 1 Minute 0	Day 19 Hour 3 Minute 0	Day 19 Hour 6 Minute 0

(b) (4)

Terminal and necroscopic evaluations:

Pregnancy occurred in 5 (100%), 4 (80.0%), 5 (100%) and 5 (100%) of the does in the 0, 3, 10, and 30 µL/cm^2 dose groups, respectively. Caesarean-sectioning data were based on 4 pregnant does for the 30 µL/cm^2 dose group due to the previously described early death of one rabbit.

No litter parameters were affected by capsaicin treatment. Only one litter in the 30 µL/cm^2 dosage group had any early resorptions (three early resorptions in one litter). The averages for corpora lutea, implantations per doe, average fetal body weights, percent resorbed conceptuses, percent live male fetuses per litter were similar among the four dose groups. There was no litter consisting of only resorbed conceptuses, and there were no dead fetuses or late resorptions. There were no fetal gross alterations.

Offspring (malformations, variations, etc.):

There were no capsaicin-related gross alterations in the fetuses.

Study title: **Percutaneous developmental toxicity study of capsaicin in rabbits**

Key study findings:
The maternal NOAEL of capsaicin is <3 µcL/cm^2 (corresponding to <60 mg/rabbit) due to erythema (grades 1 and 2), flaking (grades 2 and 3), wrinkling and lesions at the application site with all capsaicin doses, although the effects were not dose-dependent. There was no effect of capsaicin treatment on body weight or weight gain. There were no adverse effects on embryo-fetal development, therefore the developmental NOAEL was >13 µL/cm^2 (corresponding to >260 mg/rabbit).

A few published studies indicate that the rabbit is relatively insensitive to capsaicin, and this may explain the lack of noted effects, although dermal irritation did occur. (Glinsukon et al., (1980) Acute toxicity of capsaicin in several animal species. *Toxicon* 18: 215–220; Szallasi and Blumberg (1993) [3H]Resiniferatoxin binding by the vanilloid receptor: Species-related differences, effects of temperature and sulfhydryl reagents. *Naunyn-Schmiedeberg's Arch Pharmacol* 347**:**84–91).

Study no.: **1614-002**
E-Location: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1614-002
Conducting laboratory and location: (b) (4)
Date of study initiation: March 26, 2004
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin, Lot F0010102, Purity 100.02% (related compounds: zucapsaicin 0.59%, total impurities 0.67%).
Vehicle, diethylene glycol monoethyl ether (DGME, 99%)
Anesthetic Cream (Lidocaine, 4% LMX4™)
Cleansing Gel (89% polyethylene glycol)

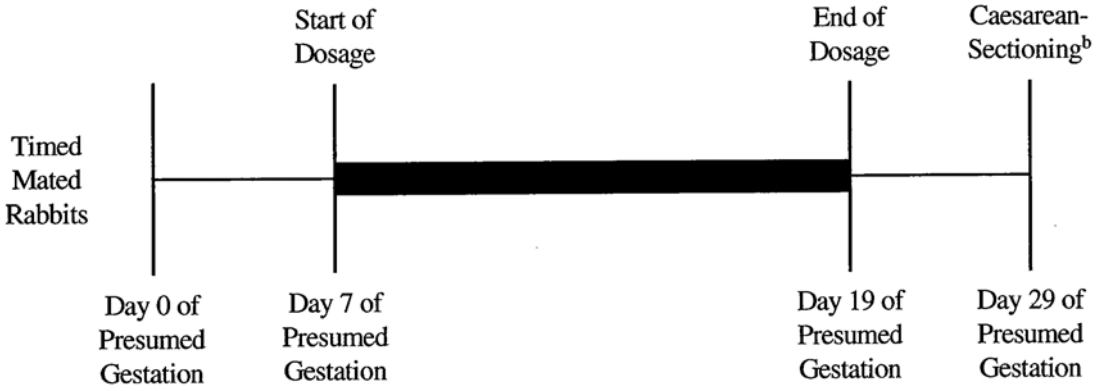
Methods
Doses: 3, 6.5, 13 µL/cm^2 of 10% w/v capsaicin = 60, 130, 260 mg total capsaicin applied to a 10 cm x 20 cm area (200 cm^2)

Amount of Capsaicin Applied

µL/cm^2	13	3	6.5	13
total mL	2.6	0.6	1.3	2.6
mg/cm^2	0	0.3	0.65	1.3
mg/rabbit	0	60	130	260

Species/strain: New Zealand White [Hra:(NZW)SPF] rabbits,
(approximately 6 months of age)
Number/sex/group: 20 pregnant females/dose

Route, formulation, volume, and infusion rate: Capsaicin or the vehicle (diethylene glycol monoethyl ether, 99%) was applied topically to a 10 x 20 cm^2 area on the back of each rabbit once daily for a 3 hour duration from gestation days (GDs) 7 through 19.
Satellite groups used for toxicokinetics: not conducted
Study design: An illustration of the study design is presented below.



Rabbits were mated for 4 consecutive days before being shipped to the study lab. Capsaicin or the vehicle, diethylene glycol monoethyl ether, 99%, was administered to the backs of the rabbits habituated to Elizabethan collars once daily for three hours on gestation days (GD) 7 through 19 at doses of 13 (vehicle), 3, 6.5 and 13 µL/cm^2 at approximately the same time each day. Rabbits were habituated to Elizabethan collars on 3 days before the start of the study. The site of application was clipped of hair was clipped. The area of application was approximately 10 cm x 20 cm and marked with indelible ink at the corners. The rabbits were fitted with Elizabethan collars, topical lidocaine cream was applied to the application site for at least 1 hour (40 µL/cm^2), then wiped off with dry gauze, after which vehicle or capsaicin was applied for a 3 hour duration, then the area wiped with cleansing lotion (approximately 10 mL) and a dry gauze and Elizabethan collars removed.

Results

Mortality (dams): checked twice daily

There were 6 mortalities, 4 due to sacrifice in moribund condition (3 in the vehicle control and 1 in the 13 µL/cm^2 dose groups). There was 1 rabbit found dead in the 3 µL/cm^2 group, and one rabbits in the vehicle control group aborted and was sacrificed. The moribund condition of the doe in the high dosage group (13 µL/cm^2) was attributed to an apparent back injury, and there was no obvious cause of death for the doe in the 3

µL/cm^2 dosage group, although this rabbit had lesions of the stomach mucosa. All other unscheduled deaths occurred in the vehicle control group.

Mortalities

Dose	**3 µL/cm^2**	**13 vehicle**	**3**	**6.5**	**13**
	mg/rabbit	**0**	**60**	**130**	**260**
	N	20	20	20	20
Mortalities	**total**	4	1	0	1
	sacrifice moribund	3 #6401 #6409 #6413			1 #6480 back injury
	found dead		1 #6423 unknown cause, but had stomach lesions		
	sacrifice due to abortion	1 #6406			

Vehicle control
Rabbit 6401 sacrificed in moribund condition on GD 18, before administration of the 12th dose. This rabbit had erythema grade 1 on GD 13, scant feces on DGs 16 to 18, and decreased motor activity, enophthalmos, cold to touch, pale mucous membranes, emaciation and soft or liquid feces on GD 18. This rabbit lost weight and feed consumption values were reduced after GD 11. All tissues examined appeared normal at necropsy. The litter consisted of nine embryos that appeared normal at gross external examination.
Rabbit 6406 aborted and was sacrificed on GD 20. This rabbit had erythema grade 1 on GD 13, scant feces on DGs 16 to 18 and no feces in the cage pan on GD 19. This rabbit generally lost weight and feed consumption values were reduced after GD 11. The stomach contained a trichobezoar; all other tissues examined appeared normal at necropsy. The litter consisted of four fetuses and eight late resorptions. All fetuses appeared normal at gross external and soft tissue evaluations.
Rabbit 6409 was sacrificed due to moribund condition on GD 20. This rabbit had flaking grade 1 on GDs 15 to 20, scant feces on GDs 15 to 18, no feces in cage pan on GDs 18 to 20 and emaciation, hunched posture and dehydration on GD 20. This rabbit lost weight after GD 9 and feed consumption values were reduced after GD 7. The stomach contained a trichobezoar; all other tissues examined appeared normal at necropsy. The litter consisted of 12 fetuses. All fetuses appeared normal at gross external and soft tissue evaluations.
Rabbit 6413 was sacrificed on GD 11 before administration of the 5th daily dosage as the result of a back injury. This rabbit had no adverse clinical observations before injury. Decreased motor activity, spastic paralysis, lost proprioceptive positioning and hypotension of the hindlimbs occurred on GD 11. This rabbit generally lost weight and

feed consumption values were reduced after GD 7. Necropsy revealed an intervertebral disk fracture at the level of the L2-L3 vertebrae, mottled red and dark red lung lobes and a pink, foamy material in the trachea. All other tissues examined appeared normal. The litter consisted of six embryos.

3 µL/cm^2 dosage group

Rabbit 6423 was found dead on GD 21. This rabbit had erythema grade 1 on GDs 11 to 12, erythema grade 2 on GDs 13 to 20, flaking grade 1 on GD 16, flaking grade 2 on GDs 17 to 20, wrinkling on GDs 16 to 20, scant feces on GDs 16 to 18 and no feces in cage pan on GDs 19 and 20. This rabbit generally lost body weight and feed consumption values were reduced after GD 8. Necropsy revealed black areas on the mucosal surface of the stomach (ranging in size from pinpoint to 0.1 cm x 0.4 cm). All other tissues examined appeared normal. The litter consisted of seven late resorptions and four early resorptions.

13 µL/cm^2 dosage group

Rabbit 6480 was sacrificed on GD 11 before administration of the 5th daily dosage because of a suspected back injury. This rabbit had erythema grade 1 on GDs 8 to 11, and decreased motor activity, spastic paralysis and impaired proprioceptive positioning on GD 11. This rabbit lost weight on GD 11 and feed consumption values were slightly reduced on GDs 7 to 8 and 9 to 10. All tissues examined appeared normal at necropsy; no fractures in either the spinal column or the hindlimbs were detected. The litter consisted of seven embryos.

Clinical signs (dams): checked twice daily; Before the first daily dosage application, and at 24-hour intervals each day thereafter, each skin site was observed for signs of skin irritation and graded using the endpoints; Skin sites were also observed for signs of skin irritation and graded once daily during the postdose period until precluded by hair growth.

Erythema (grades 1 and 2), flaking (grades 2 and 3), wrinkling and lesions at the application site occurred at statistically significant incidences ($p \leq 0.05$ or $p \leq 0.01$) in the 3, 6.5 and 13 uL/cm2 dosage groups. However, these observations tended to occur at similar incidences in the three treated groups with no dosage-dependent differences among the dosage groups. Additionally the numbers of affected vehicle control group does with grade 1 erythema and grade 2 flaking were similar to the numbers of affected does in the treated groups (16 in the vehicle group compared to 19 or 20 in the treated groups). All other skin reactions and all clinical observations were considered unrelated to capsaicin.

All other skin reactions and all clinical observations were considered unrelated to capsaicin because: 1) the incidences were not dosage dependent; 2) the observations occurred in only one or two rabbits in a group; and/or 3) the observations were related to an injury. These clinical observations included discolored application site, decreased motor activity, spastic paralysis, impaired or lost proprioceptive positioning, localized alopecia on the limbs, soft or liquid feces, vocalization, scant feces, no feces in the cage pan, ungroomed coat, emaciation, hypotension of the hindlimbs, enophthalmos, cold to touch, pale mucous membranes, hunched posture, dehydration, grade 3 erythema, grades 1 and 2 edema and grade 1 flaking.

TABLE 1 (PAGE 1): SKIN REACTIONS AND CLINICAL OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MCL/CM2~)a	I 13 (VEHICLE)	II 3	III 6.5	IV 13
MAXIMUM POSSIBLE INCIDENCE	413/ 20	452/ 20	460/ 20	442/ 20
CLINICAL OBSERVATIONS:				
APPLICATION SITE: RED, BROWN, OR PURPLE	24/ 4	23/ 5	11/ 2	40/ 7
DECREASED MOTOR ACTIVITY	2/ 2c,e	0/ 0	0/ 0	1/ 1f
SPASTIC PARALYSIS	1/ 1e	0/ 0	0/ 0	1/ 1f
IMPAIRED PROPRIOCEPTIVE POSITIONING	0/ 0	0/ 0	0/ 0	1/ 1f
LOCALIZED ALOPECIA: LIMB(S)	3/ 1	0/ 0	0/ 0	1/ 1
SOFT OR LIQUID FECES	1/ 1c	2/ 2	2/ 2	0/ 0
VOCALIZATION	0/ 0	1/ 1	3/ 2	0/ 0
SCANT FECES	10/ 3c,d,g	7/ 2b	0/ 0	0/ 0
CLINICAL OBSERVATIONS:				
NO FECES IN CAGE PAN	4/ 2d,g	2/ 1b	0/ 0	0/ 0
UNGROOMED COAT	0/ 0	1/ 1	0/ 0	0/ 0
EMACIATION	2/ 2c,d	0/ 0	0/ 0	0/ 0
LOST PROPRIOCEPTIVE POSITIONING	1/ 1e	0/ 0	0/ 0	0/ 0
HINDLIMBS HYPOTENSION	1/ 1e	0/ 0	0/ 0	0/ 0
ENOPHTHALMOS	1/ 1c	0/ 0	0/ 0	0/ 0
COLD TO TOUCH	1/ 1c	0/ 0	0/ 0	0/ 0
PALE MUCOUS MEMBRANES	1/ 1c	0/ 0	0/ 0	0/ 0
HUNCHED POSTURE	1/ 1d	0/ 0	0/ 0	0/ 0
DEHYDRATION	1/ 1d	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dosage occurred on days 7 through 19 of presumed gestation.
b. Doe 6423 was found dead on day 21 of gestation.
c. Doe 6401 was moribund sacrificed on day 18 of gestation.
d. Doe 6409 was moribund sacrificed on day 20 of gestation.
e. Doe 6413 was moribund sacrificed on day 11 of gestation.
f. Doe 6480 was moribund sacrificed on day 11 of gestation.
g. Doe 6406 aborted and was sacrificed on day 20 of gestation.

TABLE 1 (PAGE 3): SKIN REACTIONS AND CLINICAL OBSERVATIONS - SUMMARY

DOSAGE GROUP	I	II	III	IV
DOSAGE (MCL/CM2~)a	13 (VEHICLE)	3	6.5	13
MAXIMUM POSSIBLE INCIDENCE	413/ 20	452/ 20	460/ 20	442/ 20
MORTALITY	3	1	0	1
FOUND DEAD	0	1b	0	0
MORIBUND SACRIFICED	3c,d,e	0	0	1f
ABORTED AND SACRIFICED	1g	0	0	0
SKIN REACTION OBSERVATIONS:				
ERYTHEMA: GRADE 1	37/ 16c,g	123/ 20b**	78/ 19**	80/ 20f**
GRADE 2	13/ 4	179/ 20b**	250/ 20**	213/ 19**
GRADE 3	0/ 0	0/ 0	0/ 0	5/ 2
EDEMA: GRADE 1	1/ 1	10/ 2	14/ 2	15/ 3
GRADE 2	0/ 0	0/ 0	0/ 0	1/ 1
FLAKING: GRADE 1	86/ 15d	30/ 15b	34/ 18	32/ 17
GRADE 2	102/ 16	236/ 20b**	258/ 20**	245/ 19**
GRADE 3	0/ 0	23/ 6**	31/ 7**	23/ 7**
WRINKLING	0/ 0	127/ 15b**	177/ 20**	210/ 19**
LESION	9/ 1	39/ 9**	6/ 5*	35/ 6**

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 7 THROUGH 29 OF PRESUMED GESTATION.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dosage occurred on days 7 through 19 of presumed gestation.
b. Doe 6423 was found dead on day 21 of gestation.
c. Doe 6401 was moribund sacrificed on day 18 of gestation.
d. Doe 6409 was moribund sacrificed on day 20 of gestation.
e. Doe 6413 was moribund sacrificed on day 11 of gestation.
f. Doe 6480 was moribund sacrificed on day 11 of gestation.
g. Doe 6406 aborted and was sacrificed on day 20 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Body weight (dams): Body weights were recorded on GD 0, the day of arrival at the testing facility, and daily during the dosage and postdosage period. Feed consumption values were recorded daily throughout the study.

Body weights and body weight gains were not altered by capsaicin treatment compared to the control group. Values were generally similar among the four groups throughout the dosing and the postdosing periods.

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~)a		13 (VEHICLE)	3	6.5	13
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	19	20	19	19
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.09 ± 0.08	+0.13 ± 0.10	+0.11 ± 0.11	+0.09 ± 0.08
DAYS 7 - 10	MEAN±S.D.	+0.00 ± 0.06	+0.01 ± 0.08	+0.01 ± 0.07	+0.00 ± 0.05
DAYS 10 - 13	MEAN±S.D.	-0.01 ± 0.08 [18]b	+0.00 ± 0.11	+0.05 ± 0.03*	+0.05 ± 0.06 [18]b
DAYS 13 - 16	MEAN±S.D.	+0.02 ± 0.14 [18]b	+0.06 ± 0.08	+0.06 ± 0.03	+0.04 ± 0.07 [18]b
DAYS 16 - 20	MEAN±S.D.	+0.07 ± 0.09 [16]b	+0.04 ± 0.12	+0.08 ± 0.04	+0.07 ± 0.03 [18]b
DAYS 7 - 20	MEAN±S.D.	+0.15 ± 0.21 [16]b	+0.12 ± 0.25	+0.19 ± 0.07	+0.17 ± 0.08 [18]b
DAYS 20 - 24	MEAN±S.D.	+0.14 ± 0.06 [15]b	+0.12 ± 0.05 [19]b	+0.12 ± 0.04	+0.12 ± 0.05 [18]b
DAYS 24 - 29	MEAN±S.D.	+0.09 ± 0.07 [15]b	+0.13 ± 0.12 [19]b	+0.10 ± 0.07	+0.12 ± 0.07 [18]b
DAYS 20 - 29	MEAN±S.D.	+0.23 ± 0.08 [15]b	+0.25 ± 0.13 [19]b	+0.22 ± 0.09	+0.23 ± 0.11 [18]b
DAYS 7 - 29	MEAN±S.D.	+0.43 ± 0.14 [15]b	+0.42 ± 0.17 [19]b	+0.41 ± 0.10	+0.40 ± 0.14 [18]b
DAYS 0 - 29	MEAN±S.D.	+0.52 ± 0.15 [15]b	+0.56 ± 0.19 [19]b	+0.52 ± 0.14	+0.49 ± 0.18 [18]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values for does that were found dead, moribund sacrificed or aborted.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Food consumption (dams): recorded daily throughout the study.
Absolute and relative feed consumption values were unaffected by dosages of capsaicin as high as 13 mcL/cm^2. The statistically significant increases ($p \leq 0.01$) in absolute and relative feed consumption values in the 6.5 mcL/cm2 dosage group on DGs 10 to 13 and 13 to 16 were considered unrelated to the test article because the increases were not dosage dependent.

Necropsy: All surviving rabbits were sacrificed on GD 29 by intravenous administration of Beuthanasia®-D Special euthanasia solution and Caesarean-sectioned. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were preserved in neutral buffered 10% formalin for possible future evaluation; all other tissues were discarded. Uteri of apparently nonpregnant does were examined to confirm the absence of implantation sites. Fetuses were weighed and examined for gross external, soft tissue and skeletal alterations and sex.

There were no effect of capsaicin treatment on necropsy observations.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): The number and distribution of corpora lutea were recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, early and late resorptions and live and dead fetuses. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Nonresponding term fetuses were considered to be dead (there were no dead fetuses).

Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

There was no effect of capsaicin treatment on Caesarean-sectioning findings or litter parameters. Pregnancy occurred in 19 or 20 does in each dosage group. As a result of the unscheduled deaths described earlier, Caesarean-sectioning observations were based on 15, 19, 19 and 18 pregnant does with one or more live fetuses in the vehicle, 3, 6.5, and 13 $\mu L/cm^2$ dose groups, respectively. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were similar among all treatment groups No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal.

TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (MCL/CM2-)a		I 13 (VEHICLE)	II 3	III 6.5	IV 13
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	19(95.0)	20(100.0)	19(95.0)	19(95.0)
FOUND DEAD	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
MORIBUND SACRIFICED	N(%)	3(15.8)	0(0.0)	0(0.0)	1(5.3)
ABORTED AND SACRIFICED	N(%)	1(5.3)	0(0.0)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	15	19	19	18
CORPORA LUTEA	MEAN±S.D.	10.2 ± 2.1	10.9 ± 2.2	10.1 ± 1.9	10.9 ± 2.7
IMPLANTATIONS	MEAN±S.D.	9.1 ± 1.6	9.6 ± 2.1	8.0 ± 2.1	8.9 ± 2.3
LITTER SIZES	MEAN±S.D.	8.9 ± 1.6	9.2 ± 2.0	7.7 ± 2.2	8.8 ± 2.4
LIVE FETUSES	N	133	175	147	159
	MEAN±S.D.	8.9 ± 1.6	9.2 ± 2.0	7.7 ± 2.2	8.8 ± 2.4
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.3 ± 0.4	0.4 ± 0.5	0.3 ± 0.6	0.1 ± 0.5
EARLY RESORPTIONS	N	3	5	5	0
	MEAN±S.D.	0.2 ± 0.4	0.3 ± 0.4	0.3 ± 0.6	0.0 ± 0.0
LATE RESORPTIONS	N	1	2	1	2
	MEAN±S.D.	0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.2	0.1 ± 0.5
DOES WITH ANY RESORPTIONS	N(%)	4(26.7)	7(36.8)	5(26.3)	1(5.6)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	15(100.0)	19(100.0)	19(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	15(100.0)	19(100.0)	19(100.0)	18(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (MCL/CM2~)a		I 13 (VEHICLE)	II 3	III 6.5	IV 13
LITTERS WITH ONE OR MORE LIVE FETUSES	N	15	19	19	18
IMPLANTATIONS	MEAN±S.D.	9.1 ± 1.6	9.6 ± 2.1	8.0 ± 2.1	8.9 ± 2.3
LIVE FETUSES	N	133	175	147	159
	MEAN±S.D.	8.9 ± 1.6	9.2 ± 2.0	7.7 ± 2.2	8.8 ± 2.4
LIVE MALE FETUSES	N	60	79	70	73
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	44.7 ± 13.8	44.6 ± 13.8	45.6 ± 19.6	45.1 ± 18.7
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	42.76 ± 6.31	43.06 ± 5.96	44.63 ± 5.63	44.13 ± 4.14
MALE FETUSES	MEAN±S.D.	43.71 ± 6.77	42.95 ± 6.51	44.64 ± 5.85	45.00 ± 3.91
FEMALE FETUSES	MEAN±S.D.	42.21 ± 6.18	42.91 ± 5.75	44.70 ± 5.79	43.37 ± 4.85
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.8 ± 4.7	3.7 ± 5.0	4.4 ± 8.4	1.4 ± 5.9

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 3 (PAGE 1): UTERINE CONTENTS AND LITTER DATA FOR INDIVIDUAL RABBITS THAT DIED OR ABORTED

DOSAGE GROUP DOSAGE (MCL/CM2~)c	RABBIT NUMBER	DAY OF DEATH	CORPORA LUTEA R	L	T	IMPLANTATIONS R	L	T	EMBRYOS/FETUSES a R	L	A	T	RESORPTIONS b R	L	A	T
I 13 (VEHICLE)	6401	MORIBUND SACRIFICED ON DAY 19 OF GESTATION	6	3	9	6	3	9	6	3	0	9	0	0	0	0
	6406	ABORTED AND SACRIFICED ON DAY 20 OF GESTATION	9	4	13	8	4	12	3	1	0	4	4	2	2	8
	6409	MORIBUND SACRIFICED ON DAY 20 OF GESTATION	5	8	13	5	7	12	5	7	0	12	0	0	0	0
	6413	MORIBUND SACRIFICED ON DAY 11 OF GESTATION	5	7	12	2	4	6	2	4	0	6	0	0	0	0
II 3	6423	FOUND DEAD ON DAY 21 OF GESTATION	5	6	11	5	6	11	0	0	0	0	5 [3ER]	6 [1ER]	0	11
III 6.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV 13	6480	MORIBUND SACRIFICED ON DAY 11 OF GESTATION	4	3	7	4	3	7	4	3	0	7	0	0	0	0

R = RIGHT L = LEFT T = TOTAL A = ABORTED ER = EARLY RESORPTION
a. Refer to the individual fetal alterations table (Table 22) for fetal gross, soft tissue, and skeletal observations.
b. Late resorptions, unless noted otherwise.
c. Dosage occurred on days 7 through 19 of gestation.

Offspring (malformations, variations, etc.): The fetuses were weighed, examined for gross external alterations. Live fetuses were sacrificed by an intraperitoneal injection of Beuthanasia®-D Special. All fetuses were examined internally to identify sex. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and the frontal bones, and the brain was examined *in situ*. All fetuses were examined for skeletal alterations after staining with alizarin red S. Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Fetal evaluations were based on 133, 175, 147 and 159 live, GD 29 Caesarean-delivered fetuses in 15, 19, 19 and 18 litters in the vehicle, 3, 6.5 and 13 $\mu L/cm^2$ dosage groups, respectively. There were no gross external, soft tissue or skeletal fetal alterations (malformations or variations) attributable to capsaicin treatment. There were no

capsaicin-related fetal abnormalities. *Litters with* fetuses with alterations numbered 10 (66.7%), 12 (63.2%), 12 (63.2%) and 7 (38.9%) respectively. The numbers of *fetuses with* alterations observed were 13 (9.8%), 23 (13.1%), 19 (12.9%) and 14 (8.8%), and the percentages of fetuses with any alteration per litter were 10.0, 13.2, 13.1 and 8.6 in these same respective dose groups.

TABLE 10 (PAGE 1): FETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~)a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	175	147	159
LIVE	N	133	175	147	159
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	10(66.7)	12(63.2)	12(63.2)	7(38.9)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	13(9.8)	23(13.1)	19(12.9)	14(8.8)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	10.0 ± 9.2	13.2 ± 12.2	13.1 ± 12.0	8.6 ± 15.0

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 11 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~)a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	175	147	159
LIVE	N	133	175	147	159
LEFT OR RIGHT FOREPAW: FLEXED					
LITTER INCIDENCE	N(%)	2(13.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.5)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on days 7 through 19 gestation.

TABLE 12 (PAGE 1): FETAL SOFT TISSUE ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~)a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	175	147	159
LIVE	N	133	175	147	159

NO FETAL ALTERATIONS WERE IDENTIFIED AT SOFT TISSUE EXAMINATION

a. Dosage occurred on days 7 through 19 of gestation.

Sternum

Fused sternal centra occurred in 1, 2, 7 and 8 fetuses from 1, 2, 5 and 3 litters in the four respective dosage groups. One fetus (6457-2) in the 6.5 µL/cm^2 dosage group also had asymmetric sternal centra. The fetal incidences of this alteration were significantly increased ($p \leq 0.01$) in the 6.5 and 13 µL/cm2 dosage groups. These increases were considered unrelated to the test article because: 1) the values were within or slightly increased over the ranges observed historically at the testing facility (range of 0 to 4.8%; values of 4.8% and 5.0% in the respective groups); and 2) the litter incidence, the more important parameter, was not significantly increased.

*Fetal Ossification Site*s

There were no statistically significant or biologically important differences among the four dosage groups in the average numbers of ossification sites per fetus for the hyoid, vertebrae (cervical, thoracic, lumbar, sacral and caudal), ribs, sternum (manubrium, sternal centers and xiphoid), forelimbs (carpals and metacarpals) or hindlimbs (tarsals,metatarsals and phalanges). The statistically significant reduction ($p \leq 0.05$) in the numbers of ossified forelimb phalanges was considered unrelated to the test article because it was not dosage dependent and was within the ranges observed historically at the testing facility.

TABLE 13 (PAGE 1): FETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~)a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	174b	147	159
LIVE	N	133	174	147	159
SKULL: NASAL(S), IRREGULAR OSSIFICATION (INCLUDES NASAL-FRONTAL, SUTURE IRREGULAR; MIDLINE SUTURE DISPLACED; INTER/INTRA NASAL					
LITTER INCIDENCE	N(%)	4(26.7)	8(42.1)	4(21.0)	4(22.2)
FETAL INCIDENCE	N(%)	4(3.0)	12(6.9)	5(3.4)	5(3.1)
SKULL: NASAL - FRONTAL, SUTURE IRREGULAR					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	1(0.6)
SKULL: NASALS, MIDLINE SUTURE DISPLACED					
LITTER INCIDENCE	N(%)	4(26.7)	5(26.3)	4(21.0)	3(16.7)
FETAL INCIDENCE	N(%)	4(3.0)	8(4.6)	5(3.4)f	3(1.9)j
SKULL: NASAL, CONTAINED AN INTRANASAL					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	1(5.3)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	1(0.7)h	1(0.6)
SKULL: NASALS, CONTAINED AN INTERNASAL					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.1)	0(0.0)	0(0.0)
SKULL: OTHER ALTERATIONS:					
SKULL: PARIETAL, CONTAINED A HOLE					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)d	0(0.0)	0(0.0)
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	2(13.3)	3(15.8)	5(26.3)	1(5.6)
FETAL INCIDENCE	N(%)	3(2.2)	4(2.3)	7(4.8)f,g	1(0.6)

TABLE 13 (PAGE 2): PETAL SKELETAL ALTERATIONS - SUMMARY
(See footnotes on the last page of this table.)

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~) a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	174	147	159
LIVE	N	133	174	147	159
THORACIC VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)e	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRA, FUSED					
LITTER INCIDENCE	N(%)	1(6.7)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.8)c	1(0.6)e	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	1(6.7)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.8)c	0(0.0)	0(0.0)	0(0.0)
LUMBAR VERTEBRAE: ARCH, OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)d	0(0.0)	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)	0(0.0)
RIBS: FUSED					
LITTER INCIDENCE	N(%)	1(6.7)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.8)c	1(0.6)e	0(0.0)	0(0.0)
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)	0(0.0)	1(5.6)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	1(0.6)

TABLE 13 (PAGE 3): PETAL SKELETAL ALTERATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2~) a		13 (VEHICLE)	3	6.5	13
LITTERS EVALUATED	N	15	19	19	18
FETUSES EVALUATED	N	133	174b	147	159
LIVE	N	133	174	147	159
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	1(6.7)	2(10.5)	5(26.3)	3(16.7)
FETAL INCIDENCE	N(%)	1(0.8)	2(1.1)	7(4.8)**g,i	8(5.0)**j
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(13.3)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.5)c	0(0.0)	1(0.7)	0(0.0)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)i	0(0.0)
SCAPULAE: ALA, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(6.7)	1(5.3)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.8)	2(1.1)	0(0.0)	0(0.0)
SCAPULAE: ALA, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)g	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes fetus 6437-8, which was inadvertently not examined for skeletal alterations.
c. Fetus 6416-8 had other skeletal alterations.
d. Fetus 6427-6 had other skeletal alterations.
e. Fetus 6437-1 had other skeletal alterations.
f. Fetus 6441-1 had other skeletal alterations.
g. Fetus 6447-3 had other skeletal alterations.
h. Fetus 6454-7 had other skeletal alterations.
i. Fetus 6457-2 had other skeletal alterations.
j. Fetus 6465-7 had other skeletal alterations.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE 14 (PAGE 1): FETAL OSSIFICATION SITES - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MCL/CM2-)a		13 (VEHICLE)	3	6.5	13
LITTERS EXAMINED	N	15	19	19	18
FETUSES EXAMINED	N	133	174b	147	159
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.75 ± 0.24	12.68 ± 0.32	12.70 ± 0.26	12.73 ± 0.29
LUMBAR	MEAN±S.D.	6.25 ± 0.23	6.31 ± 0.30	6.29 ± 0.26	6.26 ± 0.29
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	17.16 ± 0.40	17.02 ± 0.19	16.93 ± 0.34	16.95 ± 0.36
RIBS (PAIRS)	MEAN±S.D.	12.71 ± 0.25	12.59 ± 0.30	12.60 ± 0.29	12.68 ± 0.29
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	3.96 ± 0.09	3.97 ± 0.07	3.98 ± 0.11	4.04 ± 0.15
XIPHOID	MEAN±S.D.	0.96 ± 0.09	0.98 ± 0.05	1.00 ± 0.00	0.96 ± 0.12
FORELIMB c					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.97 ± 0.10	4.99 ± 0.03	4.99 ± 0.04	5.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.93 ± 0.20	13.93 ± 0.16	13.77 ± 0.35*	14.00 ± 0.00
HINDLIMB c					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	11.99 ± 0.02	12.00 ± 0.00	11.99 ± 0.04	12.00 ± 0.00

a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes fetus 6437-8, which was inadvertently not examined for skeletal alterations.
c. Calculated as average per limb.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

Pre and Postnatal Development

Study title: **Percutaneous dosage-range developmental and perinatal/postnatal reproduction toxicity study of capsaicin dermal patches in rats**

This non-GLP, non-QA study was used to provide plasma and milk capsaicin levels in pregnant and lactating rats.

Key study findings:

Study no.: UYQ00004

E-Location: m4\43-lit-ref\ UYQ00004-pilot-study.pdf
Conducting laboratory and location: (b) (4)

Date of study initiation: April 12, 2005
GLP compliance: no
QA reports: no
Drug, lot #, and % purity:
Capsaicin Dermal Patch (8% w/w, *trans*-capsaicin), Lot 81101203, Purity not provided; (impurities <lod); 177.9 mg/280 cm^2
Vehicle Patch, Lot 81100104
Anesthetic Cream, (Lidocaine 4%/L-M-X-4^{TM})
Cleansing Gel, (89% polyethylene glycol)

Methods
Doses: Vehicle patch: 50 cm^2; capsaicin patches: 37.5 or 50 cm^2, (corresponding to 24 and 32 mg capsaicin/animal, respectively)
Species/strain: pregnant Crl:(CD) SD
Number/sex/group: 8/group
Route, formulation, volume, and infusion rate: capsaicin and vehicle patches were applied to the backs of the rats once daily for 3 hours from gestation days (GDs) 7 to postpartum day 20 (Lactation Day 20).
Satellite groups used for toxicokinetics: 6/group
Study design: The vehicle or capsaicin dermal patch was applied to the backs of the rats for a 3 hour duration once daily from gestation days 7 through 24 of presumed gestation (rats that did not deliver a litter) or day 20 postpartum (=Lactation Day (LD) 20; rats that delivered a litter) at approximately the same time each day.
Parameters Examined: Viabilities, clinical observations, signs of skin irritation and body and feed weights were recorded. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, maternal behavior, litter sizes and pup viability at birth. Viabilities, clinical observations and body weights of the pups were recorded. After completion of the 21-day postpartum period, female rats and all pups were sacrificed by carbon dioxide asphyxiation and a gross necropsy was performed.

Blood samples were collected on GDs 7 and 17 from rats assigned for use in toxicokinetic sample collection. Milk samples were collected from all rats assigned to the main study on LD 14 after patch application..

Results

Mortalities:
There were no capsaicin-related deaths. One dam in the 50 cm^2 capsacin-treated group was sacrificed because of moribund condition that was attributed to an injury related to the daily wrapping.

Clinical Observations: The only clinical observation considered related tocapsaicin administration was ungroomed coat which occurred in slightly increased numbers of rats in the 37.5 and 50 cm^2 capsaicin-treated groups. Grade 1 erythema and flaking occurred in slightly increased numbers of rats in the capsaicin-treated groups, while grade 2 erythema and flaking occurred in increased numbers of rats in these groups. Scabbing at the application site also occurred. Additionally, residue occurred only in the capsaicin-treated groups.

Toxicokinetics: Plasma samples were collected from the tail vein on GD 7 and 17 at 3, 4, 5 and 6 hours after patch application. Milk samples (at least 100 µL/dam when possible) were collected on LD 14. Milk samples were obtained at least 5 min after dams were injected IV with 1 unit of oxytocin. These were analyzed for capsaicin and its three metabolites (M1, M2, and M3). The lower limit of quantification (LLOQ) for capsaicin in plasma and milk was 0.5 ng/mL. Concentrations are expressed as mean ± sd.

Adverse clinical and skin reaction observations for rats assigned for use in toxicokinetic sample collection in the 37.5 and 50 cm^2 capsacin -treated groups were similar to those observed in the main study groups. Body weights and feed consumption values in these rats were comparable to the main study groups. All rats assigned for use in toxicokinetic sample collection were pregnant.

F_0 Plasma
On GD 7 (first day of dosing), peak plasma levels of capsaicin occurred at 3 hours after patch application in both capsaicin-treated groups. Plasma levels in the two groups were not proportional to dose. Mean peak plasma levels were 18.4 ± 7.4 ng/mL and 15.8 ± 8.9 ng/mL in the 37.5 and 50 cm^2 capsaicin-treated groups, respectively. M1 metabolite was also detected in plasma samples of some rats (one rat in 37.5 cm^2 and two rats in 50 cm^2 groups, but at different timepoints on GD 7. No M2 or M3 metabolites were detected. On GD 17 (last day of dosing), peak plasma levels were seen again at 3 hours after patch application in both test article-treated groups. The increases in plasma levels on GD 17 were proportional or greater than proportional to the increase in dose. Mean peak plasma levels were 40.2 ± 6.5 ng/mL and 54.4 ± 9.8 ng/mL in the 37.5 and 50 cm^2 capsaicin-treated groups, respectively. Peak plasma levels of M1 were also detected at 3 hours after patch application in both the groups. Mean peak plasma levels of M1 were 1.4 ± 0.7 ng/mL and 3.4 ± 1.3 ng/mL in the 37.5 and 50 cm^2 capsaicin-treated groups, respectively. No M2 or M3 were detected.

Mean Concentration of Capsaicin in Rat Plasma

	Group II 37.5 cm^2	Group III 50 cm^2
Day 7 of Gestation		
N	3	3
3 hours (ng/mL)	18.4 ± 7.4	15.7 ± 8.9
4 hours (ng/mL)	0.4 ± 0.3	2.0 ± 0.4
5 hours (ng/mL)	0*	0.3 ± 0.4
6 hours (ng/mL)	2.0 ± 3.0	1.8 ± 1.8
Day 17 of Gestation		
N	3	3
3 hours (ng/mL)	40.2 ± 6.5	54.4 ± 9.8
4 hours (ng/mL)	7.2 ± 3.6	11.1 ± 13.2
5 hours (ng/mL)	1.9 ± 0.3	4.6 ± 3.4
6 hours (ng/mL)	7.9 ± 8.2	38.9 ± 62.5

* = all values below LLOQ (lower limit of quantitation)

Table 18
Concentration (ng/mL) of Capsaicin in Rat Plasma

			Timepoint							
Animal Number	Dose Group	Gender	Day 7 Hour 3	Day 7 Hour 4	Day 7 Hour 5	Day 7 Hour 6	Day 17 Hour 3	Day 17 Hour 4	Day 17 Hour 5	Day 17 Hour 6

(b) (4)

Table 19
Concentration (ng/mL) of M1 in Rat Plasma

Animal Number	Dose Group	Gender	Timepoint: Day 7 Hour 3	Day 7 Hour 4	Day 7 Hour 5	Day 7 Hour 6	Day 17 Hour 3	Day 17 Hour 4	Day 17 Hour 5	Day 17 Hour 6
(b) (4)										

Milk:
In rat milk samples, vehicle, 37.5 and 50 cm^2 capsaicin-treated groups had mean capsaicin concentrations of 11.6 ± 9.2 (n = 6) ng/mL, 91.7 ± 58.7 (n = 5) ng/mL, and 337.7 ±264.8 (n = 3) ng/mL, respectively. The contamination in the vehicle control group may be attributed to ingestion of capsaicin by the vehicle control group rats through fur clippings in the air after trimming of the application sites. The concentration of capsaicin in rat milk in the treated groups increased with increasing dose and the increase was greater than proportional to the increase in dose. No detectable levels of any metabolites were seen in the vehicle patch group. In 37.5 cm^2, four out of six rats had detectable levels of M1 (mean = 1.3 ± 2.0 ng/mL), two out of six rats had detectable levels of M2 (mean = 0.5 ± 0.8 ng/mL), and one out of six rats had a detectable level of M3 (mean 0.2 ± 0.4 ng/mL). In the 50 cm^2 group, six out of seven rats had detectable levels of M1 (mean 1.2 ± 0.8 ng/mL). No M2 or M3 were detected in the 50 cm^2 group.

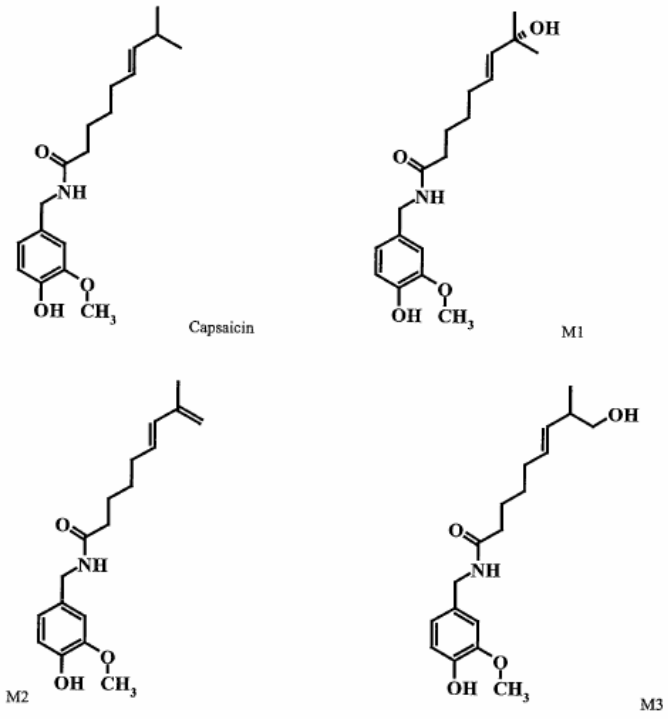
Reviewer comment: *The Applicant suggested that the contamination in the vehicle control group may be attributed to ingestion of capsaicin by the vehicle control group rats through fur clippings in the air after trimming of the application sites. This may be seems unlikely in view of the amount detected in control milk samples, a contamination from a treated milk sample or an assay standard seems more likely. Nevertheless, even if a vehicle "baseline" was subtracted from all samples, there was still substantial capsaicin in the milk from capsaicin-treated animals.*

Concentration of Capsaicin in Rat Milk

	Group I 50 (Vehicle) cm^2	Group II 37.5 cm^2	Group III 50 cm^2
N	7	6	7
Concentration	11.6 ± 9.2 [6]a	91.7 ± 58.7 [5]a	337.7 ± 264.8 [3]a

a. Excludes values for samples with insufficient volume for analysis.

Structures of capsaicin and metabolites M1, M2, M3P:



Study title: **Percutaneous developmental and perinatal/postnatal reproduction toxicity study of capsaicin dermal patches in rats, including a postnatal behavioral/functional evaluation**

Key study findings: There was no major toxicity from capsaicin patch treatments in the pre- and post-natal toxicity study. Capsaicin patches were similar to those in the fertility study described previously and applied for 3 hours daily from gestation day 7 through postpartum day 20 (lactation day 20). F_1 pups appeared normal at necropsy on Day 28 postpartum. There was no effect of capsaicin-treated dams on body weight and food consumption of F_1 males and females. Capsaicin was detected in F_0 dam milk, ranging ranged from < 2.5 ng/mL to 770 ng/mL. There were no differences in F_1 rats from different maternal capsaicin treatments in tests of learning, short-term retention, long-term retention, response inhibition evaluated in passive avoidance and water maze paradigm at various ages. Sexual development and maturation of F_1 males and females from capsaicin treated dams were similar to F_1 rats from control treated dams. There was no effect on testes or epididymis weights, or the ratios of these weights to the terminal body weight in F_1 males, a finding in capsaicin-treated F_0 males during fertility studies. Mating and fertility parameters, litter parameters, and fetal abnormalities were similar between rats from capsaicin and control treated dams.

Based on the results of this study, the NOAEL for maternal toxicity was <25 cm^2 (16 mg/rat) due to skin erythema and lesions. The reproductive NOAEL and NOEL for viability, growth and development and reproduction in the F_1 generation of treated dams is >50 cm^2 (32 mg/rat).

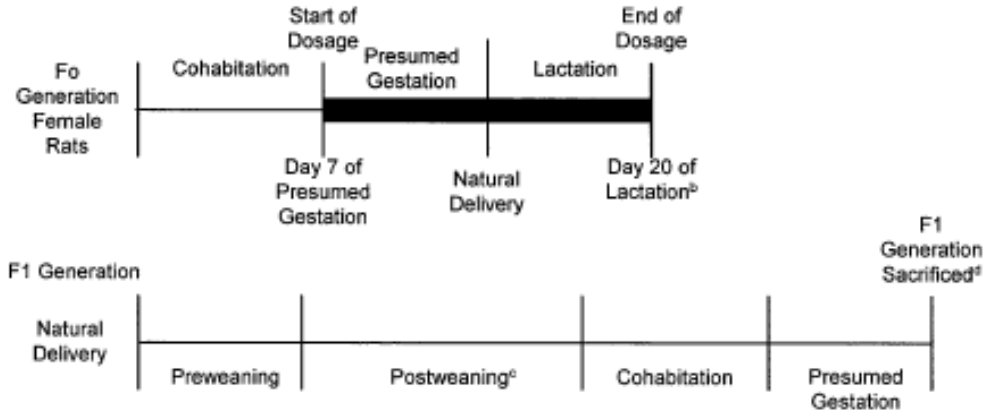
Study no.: UYQ00005
E-Location: m4\42-stud-rep\423-tox\4235-repro-dev-tox\42353-pre-postnatal-dev\uyq00005
Conducting laboratory and location: (b) (4)
Date of study initiation: Oct 2, 2005
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Capsaicin Dermal Patch (8% w/w, *trans*-capsaicin), Lot 81101203, Purity not provided (impurities <0.1% (lod); 177.9/280 cm^2
Vehicle Patch Opaque patch, Lot 81100104
Anesthetic Cream, (Lidocaine 4%/L-M-X-4^{TM})
Cleansing Gel, (89% polyethylene glycol)

Methods
Doses: Patch size: 50 (Vehicle), 25, 37.5 or 50 cm^2 (corresponding to 0, 16, 24, and 32 mg/animal, respectively)

Doses were selected based on the results of a dose-range developmental and perinatal/postnatal reproductive toxicity study of Capsaicin Dermal Patches in rats (Protocol UYQ00004, see the study review prior to the current study)

Species/strain: pregnant Crl:CD(SD) female rats

Number/sex/group: 25/group
Route, formulation, volume, and infusion rate: The vehicle patch or a capsaicin dermal patch was applied daily for 3 hours duration to the backs of the rats from gestation day (GD) 7 through day 20 of lactation (LD 20) or GD 24 (rats that did not deliver a litter).
Satellite groups used for toxicokinetics: not conducted
Study design:



After clipping the hair along the back of the animal, the application site (approximately 25, 37.5 or 50 cm^2) was demarcated using an indelible marker with one mark at each corner of the site on the animal's back. A "map" of the clipped area was made for all rats with areas of apparent hair tufts, skin defects or natural differences in skin colorization, before administration. On the days of dosing, the rats were fitted with Elizabethan collars, and the anesthetic cream (LMX4™) was topically applied to the previously clipped application site at an approximate volume of 32 $\mu L/cm^2$. Just enough cream was applied to cover the patch application area only (plus 0.5 cm beyond the boundaries), and any excess was removed. At least 60 minutes later, the anesthetic cream was wiped off with dry gauze. Tape was overlaid on the patch. After three hours of application exposure, the patches were removed from the backs of the rats, and the application sites were gently wiped twice with the cleansing gel (89% polyethylene glycol, approximately 3 mL) and dry gauze.

The Applicant did not cull litters during the lactation period because they stated random selection of pups for culling could have resulted in potential biases in pup viabilities and body weight gains during this period.

On day 21 postpartum, 25 male and 25 female pups per group were randomly selected for for continued evaluation. At least one male pup and one female pup per litter, when possible, were selected. After weaning on day 28 postpartum, F1 generation rats were individually housed.

F_1 generation pups were not directly given the test article or vehicle, but may have been possibly exposed to the test article or vehicle during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period

F_0 Generation Rats

Dosage Group	Dosage Administration			Number of Rats
	Test Article	Dosage (cm^2 patch)	Duration of Application	
I	Vehicle Patch	50	3 hours	25
II	Capsaicin Dermal Patch	25	3 hours	25
III	Capsaicin Dermal Patch	37.5	3 hours	25
IV	Capsaicin Dermal Patch	50	3 hours	25

F_1 Generation Rats

Dosage Group	Maternal Dosage (cm^2 patch)	Number of Rats Per Sex
I	50 (Vehicle Patch)	25
II	25	25
III	37.5	25
IV	50	25

Parameters examined: For F_0 generation rats, viabilities, clinical observations, signs of skin irritation and body and feed weights were recorded. Rats were evaluated for clinical signs during parturition, duration of gestation, maternal behavior, litter sizes and pup viability at birth. Before the first daily application, and at 24-hour intervals each day thereafter, each skin site was observed for signs of skin irritation and graded using the endpoints described by Draize. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter sizes (all pups delivered) and pup viability at birth. Because pups begin to consume maternal feed on or about LD 14, feed consumption values were not tabulated after LD 14. Viabilities, clinical observations and body weights of the pups were recorded. Maternal behavior was evaluated on LDs 1, 4, 7, 14, 21 and 28. After completion of the 28-day postpartum period, female rats and all pups not selected for continued evaluation were sacrificed by carbon dioxide asphyxiation and a gross necropsy was performed.

On LD 14, milk samples (at least 100 µL per dam) were collected from eight rats per dosage group after patch application. Dams were selected for milk sample collection based on delivery dates. Each dam was removed from the nesting box and individually housed for approximately four hours. The dams were injected intravenously with one unit of oxytocin at least five minutes before milk samples were collected.

Female F_1 rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum. Beginning at 24 ± 1 day postpartum, one male rat and one female rat

from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Beginning at approximately 70 days postpartum, one male rat and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory.

At approximately 90 days of age, the F1 generation rats within each dosage group were randomly assigned (with the exclusion of sibling matings) to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 21 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. Female rats not mated within the first 14 days of cohabitation were assigned alternate male rats from the same dosage group that had mated.

Results

F_0 Generation

Mortality:
There were no deaths. One F_0 generation female rat (#3721) in the vehicle control group was sacrificed on lactation day (LD) day 5 due to respiratory distress that was presumed to be related to the wrapping procedure.

Adverse clinical observations before sacrifice included grade 1 erythema on gestation days (GDs) 12 to 21 and LDs 1 to 5, grade 1 flaking on GDs 16 to 21 and LDs 1 to 5 and prostrate, dehydration, brown perioral substance and dyspnea on LD 5. This rat generally gained weight throughout gestation, and lactation body weights were unremarkable. Necropsy revealed pale, overinflated lobes of the lungs; all other tissues appeared normal. This dam delivered 17 pups. One pup was found dead on LD 5 with no milk in the stomach; all other pups appeared normal at necropsy following sacrifice.

Clinical Observations:
In the 37.5 and 50 cm^2 groups during the gestation period, there were increased numbers of F_0 generation rats with ungroomed coat and urine-stained abdominal fur. During the lactation period, the numbers of rats with grade 2 skin flaking were significantly increased in all three capsaicin-treated groups. There were no rats in the vehicle control group with grade 2 flaking.

TABLE B1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
UNSCHEDULED SACRIFICE	1b	0	0	0
PRESUMED GESTATION:				
MAXIMUM POSSIBLE INCIDENCE	382/ 25	384/ 25	378/ 25	390/ 25
CLINICAL OBSERVATIONS:				
UNGROOMED COAT	0/ 0	0/ 0	41/ 5	129/ 14**
URINE-STAINED ABDOMINAL FUR	4/ 2	3/ 2	20/ 7**	51/ 8**
RED OR PINK PERIVAGINAL SUBSTANCE	0/ 0	0/ 0	2/ 2	4/ 4
INCISOR(S): TOTAL	0/ 0	0/ 0	0/ 0	19/ 2
MISALIGNED	0/ 0	0/ 0	0/ 0	11/ 1
MISSING/BROKEN	0/ 0	0/ 0	0/ 0	8/ 1
BACK: SCAB	0/ 0	0/ 0	0/ 0	6/ 1
BACK: ABRASION	0/ 0	0/ 0	0/ 0	1/ 1
NECK: SWOLLEN	0/ 0	0/ 0	0/ 0	1/ 1
EXCESS SALIVATION - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1
LEFT FOREPAW (THIRD DIGIT): SWOLLEN	0/ 0	0/ 0	3/ 1	0/ 0
LOCALIZED ALOPECIA: LIMB(S)	3/ 1	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Dam 3721 was sacrificed on day 5 of lactation due to respiratory distress.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Grade 1 erythema and flaking and scabbing at the application site occurred in all four groups with similar incidences during the gestation and lactation periods. The numbers of rats with scabbing at the application site was significantly reduced in the 37.5 cm^2 capsaicin-treated group during the lactation period.

TABLE B1 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
UNSCHEDULED SACRIFICE	1b	0	0	0
PRESUMED GESTATION: (CONT.)				
MAXIMUM POSSIBLE INCIDENCE	382/ 25	384/ 25	378/ 25	390/ 25
SKIN REACTION OBSERVATIONS:				
ERYTHEMA: GRADE 1	243/ 25b	248/ 24	243/ 24	236/ 24
FLAKING: GRADE 1	135/ 23b	258/ 25	237/ 25	263/ 25
SCAB(S)	15/ 4	8/ 2	8/ 2	1/ 1

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Dam 3721 was sacrificed on day 5 of lactation due to respiratory distress.

Other clinical observations included dental problems (misaligned, missing and/or broken incisors), scab or abrasion on the back, swollen neck, excess salivation (slight or moderate), swollen forepaw digit, localized alopecia on the limbs, soft or liquid feces, dehydration, mass on the lower midline or in the axilla, scab or ulceration in the axilla, gasping, prostrate, brown perioral substance, and dyspnea. The numbers of rats with pink or red perivaginal substance during the gestation period were slightly increased in the 37.5 and 50 cm^2 capsaicin-treated groups but these observations generally occurred at the time of parturition (GDs 20, 21 or 22) and were not considered a capsaicin related. The

number of rats with a red perivaginal substance was significantly increased ($p \leq 0.01$) in the 37.5 cm^2 capsaicin-treated group during the lactation period, a non dosage-dependent event considered unrelated to the test article; all observations occurred on LDs 1 or 2 after delivery of a litter. These clinical observations and skin reactions during the gestation and lactation periods were considered unrelated to the test article because: 1) the incidences were not dosage dependent; 2) the observations occurred in only one rat in a group; and/or 3) the observations occurred only in the vehicle control group.

TABLE B1 (PAGE 3): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
UNSCHEDULED SACRIFICE	1b	0	0	0
LACTATION:				
MAXIMUM POSSIBLE INCIDENCE	649/ 24	700/ 25	700/ 25	645/ 24
CLINICAL OBSERVATIONS:				
UNGROOMED COAT	109/ 8	88/ 11	100/ 13	217/ 14
SOFT OR LIQUID FECES	51/ 14	47/ 19	28/ 11	23/ 14
URINE-STAINED ABDOMINAL FUR	178/ 15	103/ 14	106/ 16	79/ 9
LOCALIZED ALOPECIA: LIMB(S)	43/ 4	41/ 3	26/ 1	56/ 4
DEHYDRATION	5/ 3b	6/ 1	16/ 2	4/ 2
LEFT AXILLA OR LOWER MIDLINE: MASS	0/ 0	0/ 0	7/ 1	1/ 1
EXCESS SALIVATION - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1
RED PERIVAGINAL SUBSTANCE	0/ 0	0/ 0	3/ 3**	0/ 0
LEFT FOREPAW (THIRD DIGIT): SWOLLEN	0/ 0	0/ 0	28/ 1	0/ 0
LEFT AXILLA: ULCERATION	0/ 0	0/ 0	7/ 1	0/ 0
RIGHT OR LEFT AXILLA: SCAB	0/ 0	0/ 0	8/ 1	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Dam 3721 was sacrificed on day 5 of lactation due to respiratory distress.
** Significantly different from the vehicle control group value (p≤0.01).

TABLE B1 (PAGE 4): CLINICAL OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

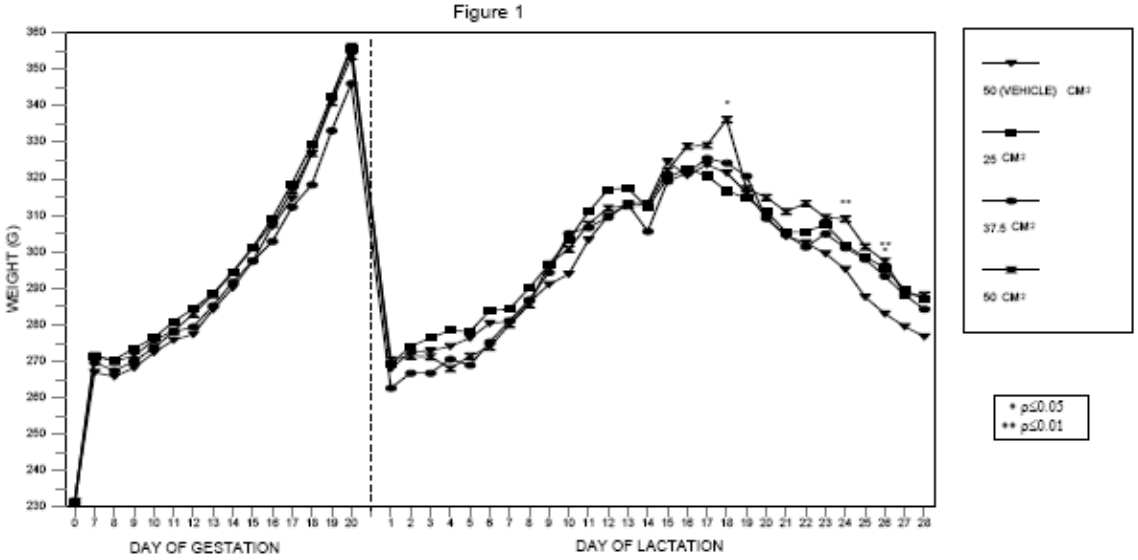
DOSAGE GROUP PATCH SIZE (CM^2)a	I 50 (VEHICLE)	II 25	III 37.5	IV 50
UNSCHEDULED SACRIFICE	1b	0	0	0
LACTATION:				
MAXIMUM POSSIBLE INCIDENCE	649/ 24	700/ 25	700/ 25	645/ 24
CLINICAL OBSERVATIONS: (CONT.)				
GASPING	0/ 0	0/ 0	1/ 1	0/ 0
PROSTRATE	1/ 1b	0/ 0	0/ 0	0/ 0
BROWN PERIORAL SUBSTANCE	1/ 1b	0/ 0	0/ 0	0/ 0
EXCESS SALIVATION - SLIGHT	1/ 1	0/ 0	0/ 0	0/ 0
DYSPNEA	1/ 1b	0/ 0	0/ 0	0/ 0
SKIN REACTION OBSERVATIONS:				
ERYTHEMA: GRADE 1	564/ 24b	457/ 25	505/ 25	519/ 23
FLAKING: GRADE 1	505/ 24b	554/ 25	418/ 25	470/ 24
SCAB(S)	380/ 23	195/ 16**	236/ 21	312/ 20
FLAKING: GRADE 2	0/ 0	122/ 20**	254/ 23**	154/ 20**

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Dam 3721 was sacrificed on day 5 of lactation due to respiratory distress.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

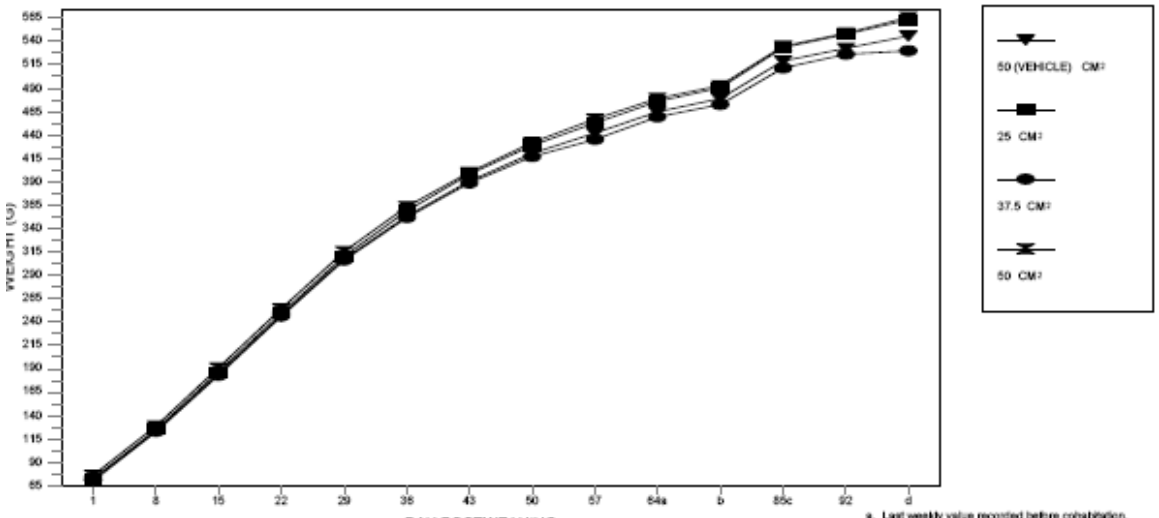
Body Weight

Body weights and body weight gains during the gestation period were unaffected by capsaicin treatment. Body weights during the gestation period were similar among the four groups. An initial, statistically significant ($p \leq 0.05$) body weight loss occurred in the 50 cm^2 capsaicin-treated group on LDs 1 to 4 and body weight gains were significantly reduced in the 37.5 cm^2 capsaicin-treated group on GDs 10 to 14. However, despite these transient changes, body weight gains were significantly increased in the 25, 37.5 and 50 cm^2 capsaicin-treated groups for the entire lactation period (LDs 1 to 28). Lactation body weights were significantly increased in the 50 cm^2 capsaicin-treated group on LDs 18 and 24 and in the 25, 37.5 and 50 cm^2 capsaicin-treated groups on LD 26.

MATERNAL BODY WEIGHTS
F0 GENERATION FEMALE RATS
Figure 1



BODY WEIGHTS
F1 GENERATION MALE RATS
Figure 2



a. Last weekly value recorded before cohabitation.
b. Precohabitation body weight.
c. First weekly value recorded after cohabitation.
d. Terminal body weight.

BODY WEIGHTS
F1 GENERATION FEMALE RATS
Figure 3

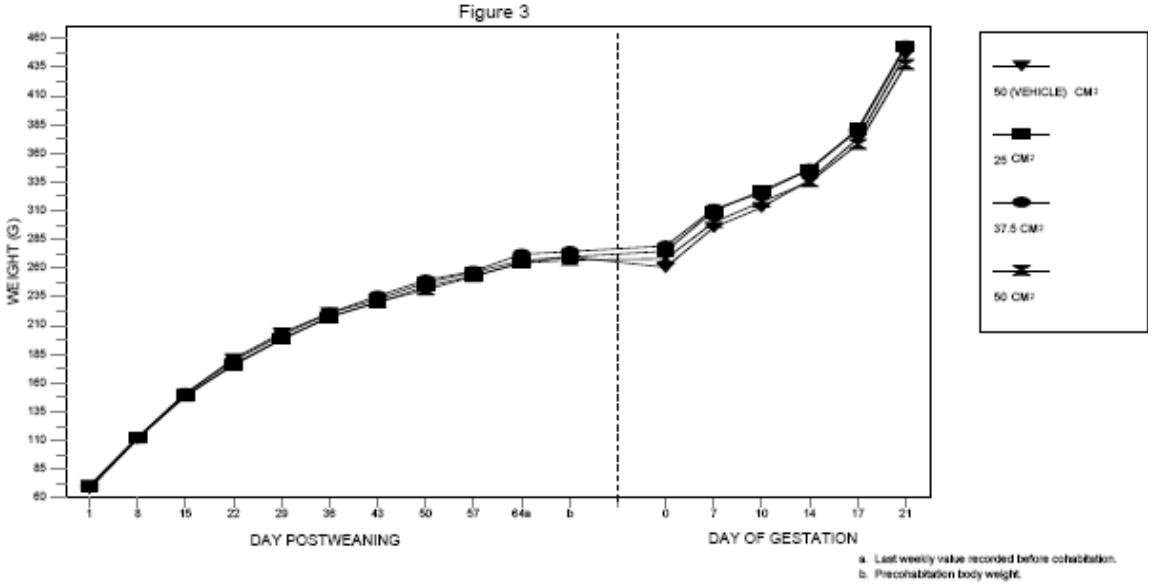


TABLE B4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	24
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+35.2 ± 7.6	+40.4 ± 7.4*	+38.4 ± 7.0	+40.2 ± 6.6*
DAYS 7 - 10	MEAN±S.D.	+5.4 ± 6.8	+4.9 ± 6.0	+4.0 ± 6.3	+4.1 ± 6.0
DAYS 10 - 12	MEAN±S.D.	+5.2 ± 7.2	+8.0 ± 6.0	+5.7 ± 8.0	+7.2 ± 3.4
DAYS 12 - 15	MEAN±S.D.	+20.0 ± 5.6	+16.8 ± 6.0	+18.0 ± 5.2	+18.2 ± 4.4
DAYS 15 - 18	MEAN±S.D.	+29.6 ± 6.5	+28.2 ± 6.9	+20.8 ± 14.4	+25.9 ± 8.6
DAYS 18 - 20	MEAN±S.D.	+29.2 ± 11.5	+26.5 ± 5.0	+27.9 ± 17.5	+26.5 ± 8.5
DAYS 7 - 20	MEAN±S.D.	+89.4 ± 20.0	+84.4 ± 12.8	+76.5 ± 19.8	+81.9 ± 13.4
DAYS 0 - 20	MEAN±S.D	+124.6 ± 19.6	+124.8 ± 17.4	+114.9 ± 20.3	+122.2 ± 16.2

DAYS = DAYS OF GESTATION
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
* Significantly different from the vehicle control group value ($p \leq 0.05$).

TABLE B6 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP PATCH SIZE (CM^2)a		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	25	25	24
DELIVERED A LITTER	N	24	25	25	24
INCLUDED IN ANALYSES	N	24	25	25	23b
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 1 - 4	MEAN±S.D.	+6.1 ± 16.1	+9.3 ± 9.2	+7.9 ± 11.6	-1.3 ± 7.4*
DAYS 4 - 7	MEAN±S.D.	+6.1 ± 15.2 [23]c	+5.8 ± 15.0	+10.5 ± 13.0	+12.0 ± 9.3
DAYS 7 - 10	MEAN±S.D.	+13.0 ± 17.1 [23]c	+19.0 ± 11.8	+24.0 ± 10.8	+20.6 ± 15.3
DAYS 10 - 14	MEAN±S.D.	+19.3 ± 22.9 [23]c	+8.7 ± 19.8	+0.6 ± 20.7**	+12.2 ± 17.1
DAYS 14 - 21	MEAN±S.D.	-9.0 ± 15.6 [23]c	-6.6 ± 13.9	-1.0 ± 14.0	-1.8 ± 14.5
DAYS 21 - 28	MEAN±S.D.	-27.5 ± 8.3 [23]c	-18.5 ± 10.5	-20.4 ± 13.6	-23.0 ± 12.8
DAYS 1 - 28	MEAN±S.D.	+8.0 ± 11.8 [23]c	+17.8 ± 12.4*	+21.6 ± 19.4**	+18.7 ± 13.5*

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dam 3783, which was sacrificed on day 1 of lactation due to no surviving pups.
c. Excludes values for dam 3721, which was sacrificed on day 5 of lactation due to respiratory distress.
* Significantly different from the vehicle control group value (p≤0.05).
** Significantly different from the vehicle control group value (p≤0.01).

Feed Consumption:
Absolute and relative feed consumption values during the gestation period were significantly increased in the 25, 37.5 and 50 cm^2 capsaicin-treated groups for the entire gestation dosage period (GDs 7 to 20) and the entire gestation period (GDs 0 to 20). Absolute and relative feed consumption values during the lactation period were significantly increased for the entire tabulated lactation period (LDs 1 to 14) in the 25, 37.5 and 50 cm^2 capsaicin-treated groups. Within this period, feed consumption values in the capsaicin-treated groups were increased or significantly increased at all tabulated intervals.

Deliver and F_1 viability:
Pregnancy occurred in 24, 25, 25 and 24 of the 25 mated female rats in the vehicle, 25, 37.5 and 50 cm^2 capsaicin -treated groups, respectively. All pregnant dams delivered litters. Values for the numbers of dams delivering litters, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, lactation index, surviving pups per litter, percent male pups per number of pups sexed per litter, live litter size at weighing and pup weight per litter were similar among the four groups.

A significant increase in the duration of gestation and a significant reduction in the numbers of pups found dead or missing on LD 1 in the 25 cm^2 capsaicin-treated group. There was a significantly increased numbers of pups were found dead or missing and presumed cannibalized in the 37.5 and 50 cm^2 capsaicin groups on LDs 2 to 4, resulting in a significant reduction in the viability index for the 50 cm^2 capsaicin-treated group.

Reviewer comment: The Applicant suggests the reduced number of pups in the 25 cm^2 capsaicin-treated group was not considered to be capsaicin related because they were not

dose dependent. However coupled with the next finding of significantly increased numbers of pups were found dead or missing and presumed cannibalized in the 37.5 and 50 cm^2 capsaicin groups on LDs 2 to 4, suggests that there is a dose-relationship and pup loss is capsaicin related. The Applicant also suggests these apparent increases in pup mortality was not toxicologically important because the viability index was within the ranges observed historically at the testing facility and the average numbers of surviving pups per litter and live litter sizes at weighing were similar among the four groups. A more appropriate explanation would be that this findings are due to capsaicin, somehow influencing aversion responses and maternal bonding, important for the rat, but this pardigm is not particularly relevant for human birth and maternal responses.

TABLE B11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	25(100.0)	25(100.0)	24(96.0)
DELIVERED A LITTER	N(%)	24(100.0)	25(100.0)	25(100.0)	24(100.0)
DURATION OF GESTATION b	MEAN±S.D.	23.0 ± 0.2	23.2 ± 0.4*	23.0 ± 0.3	23.1 ± 0.3
IMPLANTATION SITES	N	347	366	375	356
PER DELIVERED LITTER	MEAN±S.D.	14.4 ± 2.0	14.6 ± 1.6	15.0 ± 1.9	15.5 ± 1.7 [23]c
DAMS WITH STILLBORN PUPS	N(%)	3(12.5)	1(4.0)	3(12.0)	2(8.3)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX d	%	100.0	100.0	100.0	100.0
	N/N	24/ 24	25/ 25	25/ 25	24/ 24
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
DAMS WITH ALL PUPS DYING DAYS 5-28 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
c. Excludes value for rat 3777, which was not recorded.
d. Number of rats with live offspring/number of pregnant rats.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

TABLE B12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	25	25	24
PUPS DELIVERED (TOTAL)	N	337	354	363	346
	MEAN±S.D.	14.0 ± 1.9	14.2 ± 1.8	14.5 ± 2.2	14.4 ± 1.7
LIVEBORN	MEAN±S.D.	13.9 ± 1.9	14.1 ± 1.8	14.4 ± 2.2	14.1 ± 2.4
	N(%)	334(99.1)	352(99.4)	360(99.2)	338(97.7)
STILLBORN	MEAN±S.D.	0.1 ± 0.3	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.4
	N(%)	3(0.9)	2(0.6)	3(0.8)	3(0.9)
UNKNOWN	N	0	0	0	5
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	4/334(1.2)	0/352(0.0)*	1/360(0.3)	7/338(2.1)
DAYS 2- 4	N/N(%)	1/330(0.3)	2/352(0.6)	9/359(2.5)**	8/330(2.4)**e
DAYS 5- 7	N/N(%)	3/312(1.0)b	3/350(0.8)	3/350(0.8)	6/322(1.9)
DAYS 8-14	N/N(%)	4/309(1.3)	6/347(1.7)	2/347(0.6)	0/316(0.0)
DAYS 15-21	N/N(%)	1/305(0.3)	0/341(0.0)	0/345(0.0)	0/316(0.0)
DAYS 22-28	N/N(%)	1/304(0.3)	0/341(0.0)	0/345(0.0)	0/316(0.0)
VIABILITY INDEX c	%	98.5	99.4	97.2	95.5**e
	N/N	329/334	350/352	350/360	321/337
LACTATION INDEX d	%	97.1	97.4	98.6	98.1
	N/N	303/312b	341/350	345/350	316/322

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes pups of litter 3721 that were sacrificed on day 5 postpartum due to sacrifice of dam.
c. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
d. Number of live pups on day 28 postpartum/number of live pups on day 4 postpartum.
e. Excludes values for one pup in litter 3786 that was sacrificed after escaping from the nesting box.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Lactation Period:
Body weight gains were significantly increased in the 25, 37.5 and 50 cm^2 capsaicin-treated groups for the entire lactation period (LDs 1 to 28). Lactation body weights were significantly increased in the 50 cm^2 test article-treated group on LDs 18 and 24 and in the 25, 37.5 and 50 cm^2 capsaicin -treated groups on LD 26. Absolute and relative feed consumption values during the lactation period were significantly increased for the entire tabulated lactation period (LDs 1 to 14) in all three capsaicin-treated groups.

Milk capsaicin concentrations:
On LD 14, milk samples (at least 100 µL per dam) were collected from eight rats per dosage group after patch application. Dams were selected for milk sample collection based on delivery dates. Each dam was removed from the nesting box and individually housed for approximately four hours. The dams were injected intravenously with one unit of oxytocin at least five minutes before milk samples were collected.

Milk capsaicin levels were observed in all three capsaicin-treated groups. There was a considerable variability in the levels of capsaicin in milk. With the exception of metabolite M1 in one rat in the 37.5 cm^2 capsaicin-treated group, none of the metabolites were detected in any of the milk samples in any test article-treated groups. No capsaicin or any of the metabolites were detected in the vehicle-treated group.

Concentration of Capsaicin and its Metabolites (M1, M2 and M3) in Rat Milk

Dosage Group	Test Article	Dosage (cm^2 patch)	No. of rats	Capsaicin Levels (ng/mL)	M1 Levels (ng/mL)	M2 Levels (ng/mL)	M3 Level (ng/mL)
I	Vehicle Patch	50	8	< 2.50	< 2.50	< 2.50	< 2.50
II	Capsaicin Dermal Patch	25	8	6.21 - 181	< 2.50	< 2.50	< 2.50
III	Capsaicin Dermal Patch	37.5	8	4.95 - 770	(3.21)[a]	< 2.50	< 2.50
IV	Capsaicin Dermal Patch	50	8	23.7 - 369	< 2.50	< 2.50	< 2.50

a. Value seen in only one of eight rats.

F_0 necropsy:
There was no effect of capsaicin treatment on necropsy observations since the findings identified occurred in only one animal and were not dose-dependent. Necropsy observations included pale, overinflated lungs in the vehicle control rat that was sacrificed due to respiratory distress, as previously described; yellow areas and adhesions of the liver in one rat in the 25 cm^2 capsaicin-treated group; tissue in the stomach of one dam in the 50 cm^2 capsaicin -treated group that was sacrificed on LD 1 due to no surviving pups; and urinary tract lesions (i.e., moderate dilation of the pelvis of both kidneys, adhesion of the right kidney to the abdominal wall and liver, thickened walls of the urinary bladder, slight dilation of the ureters and calculi in the bladder and ureters) in one dam in the 50 cm^2 capsaicin -treated group.

TABLE B2 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
PATCH SIZE (CM^2)a		50 (VEHICLE)	25	37.5	50
RATS EXAMINED b	N	25	25	25	25
UNSCHEDULED SACRIFICE	N	1c	0	0	0
APPEARED NORMAL	N	24	24	25	23
LUNGS:					
ALL LOBES, PALE; OVERINFLATED	N	1c	0	0	0
LIVER:					
YELLOW AREAS; CAUDATE LOBE ADHERED TO THE RIGHT LATERAL LOBE	N	0	1	0	0
STOMACH:					
TISSUES PRESENT	N	0	0	0	1
KIDNEYS:					
BILATERAL, PELVIS, MODERATE DILATION; RIGHT, ADHERED TO ABDOMINAL WALL AND RIGHT AND CAUDATE LOBE OF LIVER	N	0	0	0	1
URINARY BLADDER:					
THICK WALLS; CONTAINED ONE CALCULUS	N	0	0	0	1
URETER:					
BILATERAL, SLIGHT DILATION; CONTAINED ONE CALCULUS	N	0	0	0	1

a. Dosage occurred on days 7 of presumed gestation through day 20 of lactation or day 24 of presumed gestation (rats that did not deliver a litter).
b. Refer to the individual clinical observations table (Table B15) for external observations confirmed at necropsy.
c. Dam 3721 was sacrificed on day 5 of lactation due to respiratory distress.

F_1 Generation

F_1 Mortality:

There were no capsaicin-related deaths in the F_1 generation rats. One male rat (#12046) from the 25 cm^2 maternal dose group was sacrificed on day 89 (week 13) postweaning due to injuries to the snout and teeth. Adverse clinical observations before sacrifice included bent tail, ulceration of the palate, chromodacryorrhea, chromorhinorrhea, red substance in the cage pan, decreased motor activity, swollen left ear, swollen face, missing, broken and/or misaligned incisor(s), ungroomed coat, soft or liquid feces and scant feces during week 13 postweaning. Necropsy did not reveal any additional gross lesions.

F_1 Clinical and Necropsy Observations

All clinical observations in the F_1 generation male and female rats were considered unrelated to maternal application of the Capsaicin Dermal Patch because: 1) the incidences were not dose dependent; 2) the observation occurred in only one or two rats; and/or 3) the observation was common in this species and strain. These clinical observations included ulceration on the neck or palate, scabbing on the neck, ear, axilla, back or limb, dental problems (misaligned, missing and/or broken incisors), swollen ears, chromodacryorrhea, red perioral substance, bent tail, sparse hair coat, abrasion on the neck, ungroomed coat, soft or liquid feces, scant feces, swollen face, chromorhinorrhea, dehydration, localized alopecia of the limbs, torn ear and missing forepaw digit.

F_1 pups appeared normal at necropsy on day 28 postpartum. These clinical observations included dehydration, cold to touch, no milk band present, pale or purple body or limb, not nesting, decreased motor activity, black tip of tail, scab on the lower midline, labored breathing, swollen limb, abrasion on the back, bradypnea, not nursing, ungroomed coat,

missing tip of tail and absent digit. No milk in the stomach occurred in 6, 4, 4 and 3 pups that were found dead and necropsied in the four respective groups. The incidence was significantly reduced in the 50 cm^2 maternal treatment group as described earlier in the *Deliver and F_1 viability* subsection, and considered not toxicological relevant for humans.

F_1 Body weight:
Body weights and body weight gains of the F_1 generation male and female rats during the postweaning, precohabitation and gestation periods were similar among groups. Occasional occurrences of statistically significant reductions in body weight gain on GDs 10 to 14 in the 25 and 50 cm^2 maternal dosage groups were not considered to be toxicologically important because they were not dose dependent and did not persist.

F_1 Food consumption:
Absolute and relative feed consumption values during the postweaning, precohabitation and gestation periods were similar among groups. Occasional occurrences of statistically significant reductions in absolute and relative feed consumption values on postweaning days 8 to 15 for the F_1 generation female rats in the 37.5 and 50 cm^2 maternal dose groups were not considered to be toxicologically important because they did not persist.

F_1 Necropsy:
All necropsy observations in the F_1 generation male and female rats were considered unrelated to maternal application of the Capsaicin Dermal Patch because: 1) the incidences were not dosage dependent; and 2) the observation occurred in only one or two rats. These necropsy observations included small and flaccid testis, large testis, small epididymis, free floating mass on the liver and ovarian cyst. Male rats with lesions of the testes and/or epididymides (12004 in the vehicle control group and 12027 and 12038 in the 25 cm^2 maternal dosage group) still impregnated the cohort female rat. The female rat with the ovarian cyst (12135 in the 25 cm^2 maternal dose group) had a normal litter.

F_1 behavioral evaluation:
Beginning at 24 ± 1 day postpartum, one male rat and one female rat from each litter, where possible, were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Beginning at approximately 70 days postpartum, one male rat and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory.

There were no effects from maternal capsaicin treatment in the measures for learning, short-term retention, long-term retention, or response inhibition in the F_1 generation male and female rats, evaluated by performance in a passive avoidance or the watermaze paradigm. The number of trials to criterion during session 1 were significantly increased for F_1 male rats in the 37.5 and 50 cm^2 dose groups, but was not considered toxicologically important since these values were within the historical control range for the testing facility.

TABLE C19 (PAGE 1): PASSIVE AVOIDANCE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL PATCH SIZE (CM^2)		50 (VEHICLE)	25	37.5	50
MALE RATS:					
SESSION 1a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	4.0 ± 1.7	3.5 ± 0.7	4.0 ± 1.0	4.0 ± 0.9
LATENCY TRIAL 1b	MEAN±S.D.	11.1 ± 12.5	8.8 ± 6.9	7.8 ± 8.0	11.0 ± 7.1
LATENCY TRIAL 2b	MEAN±S.D.	45.6 ± 20.4	50.6 ± 18.7	41.4 ± 22.4	38.7 ± 20.2
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	3.4 ± 0.9	3.2 ± 0.8	3.6 ± 1.2	3.4 ± 0.6
LATENCY TRIAL 1b	MEAN±S.D.	15.8 ± 17.4	16.7 ± 18.1	11.6 ± 13.8	11.0 ± 13.0
FEMALE RATS:					
SESSION 1a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	3.6 ± 0.9	3.9 ± 1.2	3.7 ± 0.7	3.7 ± 0.9
LATENCY TRIAL 1b	MEAN±S.D.	12.1 ± 16.1	13.7 ± 13.8	11.8 ± 12.4	10.6 ± 9.5
LATENCY TRIAL 2b	MEAN±S.D.	41.3 ± 21.8	40.3 ± 20.7	44.0 ± 18.4	46.9 ± 19.6
FAILED TO LEARN c	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SESSION 2a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	3.3 ± 0.6	3.1 ± 0.4	3.3 ± 0.9	3.3 ± 0.6
LATENCY TRIAL 1b	MEAN±S.D.	10.0 ± 8.5	12.6 ± 15.7	16.7 ± 18.8	13.0 ± 16.4

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from group averages and statistical analyses.

TABLE C20 (PAGE 1): WATERMAZE PERFORMANCE - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL PATCH SIZE (CM^2)		50 (VEHICLE)	25	37.5	50
MALE RATS:					
SESSION 1a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	8.2 ± 2.2	9.4 ± 2.8	10.2 ± 2.9*	10.5 ± 2.8*
ERRORS PER TRIAL	MEAN±S.D.	0.40 ± 0.20	0.36 ± 0.17	0.42 ± 0.17	0.46 ± 0.23
LATENCY TRIAL 2b	MEAN±S.D.	14.5 ± 12.5	14.1 ± 12.0	14.7 ± 6.9	16.2 ± 15.3
FAILED TO LEARN c	N(%)	0(0.0)	2(8.0)	1(4.0)	1(4.3)
SESSION 2a	N	23	23	24	22
TRIALS TO CRITERION	MEAN±S.D.	5.8 ± 1.6	7.1 ± 2.9	6.2 ± 1.9	6.7 ± 2.3
ERRORS PER TRIAL	MEAN±S.D.	0.09 ± 0.19	0.16 ± 0.19	0.11 ± 0.15	0.08 ± 0.10
LATENCY TRIAL 1b	MEAN±S.D.	9.3 ± 10.3	11.2 ± 8.7	8.4 ± 6.2	7.1 ± 4.7
FEMALE RATS:					
SESSION 1a	N	23	25	25	23
TRIALS TO CRITERION	MEAN±S.D.	8.5 ± 2.2	9.2 ± 2.5	9.6 ± 3.2	10.0 ± 2.4
ERRORS PER TRIAL	MEAN±S.D.	0.44 ± 0.32	0.42 ± 0.17	0.36 ± 0.16	0.49 ± 0.27
LATENCY TRIAL 2b	MEAN±S.D.	17.2 ± 13.2	13.2 ± 9.3	12.2 ± 8.2	16.8 ± 8.4
FAILED TO LEARN c	N(%)	0(0.0)	2(8.0)	2(8.0)	0(0.0)
SESSION 2a	N	23	23	23	23
TRIALS TO CRITERION	MEAN±S.D.	6.0 ± 1.4	7.5 ± 2.6	7.0 ± 2.4	7.1 ± 2.8
ERRORS PER TRIAL	MEAN±S.D.	0.08 ± 0.12	0.18 ± 0.17	0.14 ± 0.13	0.11 ± 0.12
LATENCY TRIAL 1b	MEAN±S.D.	8.4 ± 3.3	12.5 ± 12.0	10.1 ± 6.0	8.0 ± 3.9

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. The latency was recorded in seconds.
c. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

F_1 Sexual Development, Mating and Pregnancy

Female F_1 rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum. At approximately 90 days of age, the F_1 rats within each dose group were randomly assigned (with the exclusion of sibling matings) to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 21 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory

plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. Female rats not mated within the first 14 days of cohabitation were assigned alternate male rats from the same dosage group that had mated.

Sexual maturation in F_1 rats was not affected by maternal capsaicin treatment. The average day on which preputial separation or vaginal patency occurred was similar among the four dose groups. Testes and epididymides weights or the ratios of these weights to the terminal body weight were not affected by the maternal capsaicin dose.

TABLE C18 (PAGE 1): SEXUAL MATURATION - SUMMARY - F1 GENERATION RATS

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
MALE RATS	N	25	25	25	25
PREPUTIAL SEPARATION a	MEAN±S.D.	46.7 ± 3.4	46.3 ± 3.7	46.5 ± 2.5	46.2 ± 2.7
FEMALE RATS	N	25	25	25	25
VAGINAL PATENCY b	MEAN±S.D.	32.7 ± 1.3	32.7 ± 1.0	32.9 ± 1.4	32.8 ± 1.2

a. Average day postpartum that the prepuce was observed to be separated.
b. Average day postpartum that the vagina was observed to be patent.

There were no effect of maternal capsaicin treatment on the mating and fertility parameters evaluated in the F_1 generation male and female rats. The number of days in cohabitation, number of rats that mated, fertility index (number of pregnancies per number of rats that mated), number of rats with confirmed mating dates during the first and second weeks of cohabitation, and number of pregnancies per number of rats in cohabitation were similar among the four dose groups.

Pregnancies from F_1 matings occurred in 20 (80.0%), 23 (92.0%), 22 (88.0%) and 25 (100%) rats in the four respective groups. One rat in the 50 cm^2 maternal dosage group without a confirmed mating date delivered a litter; therefore, Caesarean-sectioning observations in this group were based on 24, rather than 25 pregnant rats. Caesarean-sectioning or litter parameters were not affected by maternal capsaicin treatments. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were similar among the four dose groups. There were no litters of consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal. There were no capsaicin-related fetal alterations. Only one fetus (#12172-15) from the 37.5 cm^2 maternal treatment group had gross external alterations consisting of exencephaly, open eyelids, a short snout, a cleft palate, a fleshy protrusion at the oral opening and a protruding tongue.

TABLE C21 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	3.4 ± 3.5	3.0 ± 2.7	4.8 ± 4.3	3.2 ± 2.7 [24]
RATS THAT MATED b	N(%)	23(92.0)	24(96.0)	22(88.0)	24(96.0)
FERTILITY INDEX c,d	N/N (%)	20/ 23 (87.0)	22/ 24 (91.7)	20/ 22 (90.9)	24/ 24 (100.0)
RATS WITH CONFIRMED MATING DATES	N	23	24	22	23
MATED WITH FEMALE e					
DAYS 1-7	N(%)	23(100.0)	24(100.0)	21(95.4)	23(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION d	N/N (%)	20/ 25 (80.0)	22/ 25 (88.0)	20/ 25 (80.0)	24/ 25 (96.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Includes only one mating for each male rat.
c. Number of pregnancies/number of rats that mated.
d. Includes only one pregnancy for each rat that impregnated more than one female rat.
e. Restricted to rats with a confirmed mating date.

TABLE C22 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a	MEAN±S.D.	3.8 ± 4.8	3.1 ± 2.9	5.2 ± 5.3	3.3 ± 3.3 [24]
RATS THAT MATED	N(%)	24(96.0)	25(100.0)	24(96.0)	25(100.0)
FERTILITY INDEX b	N/N (%)	20/ 24 (83.3)	23/ 25 (92.0)	22/ 24 (91.7)	25/ 25 (100.0)
RATS WITH CONFIRMED MATING DATES	N	24	25	24	24
MATED BY FIRST MALE c					
DAYS 1-7	N(%)	23(95.8)	24(96.0)	21(87.5)	22(91.7)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.2)	1(4.2)
MATED BY SECOND MALE c					
DAYS 15-21	N(%)	1(4.2)	1(4.0)	2(8.3)	1(4.2)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	20/ 25 (80.0)	23/ 25 (92.0)	22/ 25 (88.0)	25/ 25 (100.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Restricted to rats with a confirmed mating date and rats that did not mate.
b. Number of pregnancies/number of rats that mated.
c. Restricted to rats with a confirmed mating date.

TABLE C23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	20(80.0)	23(92.0)	22(88.0)	25(100.0)
DELIVERED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	20	23	22	24
CORPORA LUTEA	MEAN±S.D.	16.5 ± 2.0	16.2 ± 2.2	16.3 ± 2.2	15.9 ± 3.0
IMPLANTATIONS	MEAN±S.D.	16.0 ± 2.0	15.1 ± 2.6	15.9 ± 2.0	14.7 ± 2.9
LITTER SIZES	MEAN±S.D.	15.4 ± 2.0	14.4 ± 2.4	14.8 ± 3.3	14.2 ± 2.8
LIVE FETUSES	N	307	331	325	340
	MEAN±S.D.	15.4 ± 2.0	14.4 ± 2.4	14.8 ± 3.3	14.2 ± 2.8
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.6 ± 0.7	0.7 ± 1.0	1.1 ± 1.8	0.5 ± 0.8
EARLY RESORPTIONS	N	12	17	22	13
	MEAN±S.D.	0.6 ± 0.6	0.7 ± 1.0	1.0 ± 1.8	0.5 ± 0.8
LATE RESORPTIONS	N	1	0	2	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	11(55.0)	12(52.2)	12(54.5)	9(37.5)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	20(100.0)	23(100.0)	22(100.0)	24(100.0)
PLACENTAE APPEARED NORMAL	N(%)	20(100.0)	23(100.0)	22(100.0)	24(100.0)

TABLE C24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
LITTERS WITH ONE OR MORE LIVE FETUSES	N	20	23	22	24
IMPLANTATIONS	MEAN±S.D.	16.0 ± 2.0	15.1 ± 2.6	15.9 ± 2.0	14.7 ± 2.9
LIVE FETUSES	N	307	331	325	340
	MEAN±S.D.	15.4 ± 2.0	14.4 ± 2.4	14.8 ± 3.3	14.2 ± 2.8
LIVE MALE FETUSES	N	179	164	176	183
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	59.0 ± 15.0	50.0 ± 12.9	53.6 ± 15.2	53.7 ± 15.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.38 ± 0.30	5.32 ± 0.28	5.24 ± 0.36	5.32 ± 0.27
MALE FETUSES	MEAN±S.D.	5.52 ± 0.36	5.44 ± 0.31	5.37 ± 0.38	5.44 ± 0.33
FEMALE FETUSES	MEAN±S.D.	5.22 ± 0.29	5.20 ± 0.26	5.07 ± 0.37	5.15 ± 0.28
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.1 ± 4.4	4.5 ± 6.0	8.0 ± 15.5	3.5 ± 5.2

TABLE C25 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - F2 GENERATION LITTERS/FETUSES

MATERNAL DOSAGE GROUP MATERNAL PATCH SIZE (CM^2)		I 50 (VEHICLE)	II 25	III 37.5	IV 50
LITTERS EVALUATED	N	20	23	22	24
FETUSES EVALUATED	N	307	331	325	340
LIVE	N	307	331	325	340
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)
EYE: LIDS OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)
SNOUT: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)
ORAL OPENING: FLESHY PROTRUSION					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)
TONGUE: PROTRUDES					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.3)a	0(0.0)

a. Fetus 12172-15 had other gross external alterations.

2.6.6.7 Local tolerance

DERMAL IRRITATION

Study title: **Topical Phototoxicity Screening Test of Capsaicin Patches in Rats**

This study contains both local irritancy and phototoxicity studies. The local irritancy study is presented here and the phototoxicity study is presented under the Special Toxicology subheading 2.6.6.8

Key study findings: Application of an 8% w/w capsaicin dermal patch to the back of Crl:CD® (SD)IGS BR VAF/Plus® rats elicited cutaneous erythema that was observed immediately after patch removal. With increasing duration of patch exposure (0, 1, 2, 3 hours), the severity of erythema was reduced. No skin responses occurred in rats treated with the anesthetic cream alone or the placebo patch.

Study no.: 1614-001
E-location: m4\42-stud-rep\423-tox\4236-loc-tol\1614-001
Conducting laboratory and location: (b) (4)
Date of study initiation: April 8, 2003
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
8% w/w Capsaicin Patch (20 cm x 10 cm), Lot 81107802, Purity not provided; concentration density 124.4 mg/200 cm^2
Placebo patch, Lot 81113301, 200 cm^2
Cleansing Gel (89% polyethylene glycol), Batch 710
Ela-Max® (lidocaine 4%, topical anesthetic cream; (b) (4)
(b) (4)
8-methoxypsoralen (8-MOP), Lot 97H0359 (b) (4)

Methods
Doses: 0 (no patch) or 25 cm^2 patch (=15.55 mg capsaicin)
Species/strain: female Rats, Crl:CD® (SD)IGS BR VAF/Plus®
Number/sex/group or time point (main study): 3 females/group
Route, formulation, volume, and infusion rate: a single patch was applied once to the back of rats for 0 (no patch), 1 , 2 or 3 hours in duration
Age: 7 weeks at arrival
Weight: at study assignment 158-181 g

Study design or methodology:

Dosage Group	Number of Rats Per Group	Treatment Regimen Descriptor			Duration of Patch Contact with the Skin (Hours)	Assigned Rat Numbers
		Pretreatment[a]	Pretreatment Anesthetic Cream Administration Volume Rate (mcL/cm^2)	Test Article, Control Article or Comparator Article Administration		
I	3	Anesthetic Cream	25	None	NA	8831 - 8833
II	3	Anesthetic Cream	25	Control Article (Vehicle) Patch	3	8834 - 8836
III	3	Anesthetic Cream	25	8% w/w capsaicin Patch	1	8837 - 8839
IV	3	Anesthetic Cream	25	8% w/w capsaicin Patch	2	8840 - 8842
V	3	Anesthetic Cream	25	8% w/w capsaicin Patch	3	8843 - 8845
VI	3	Anesthetic Cream	38	8% w/w capsaicin Patch	3	8828 - 8830

NA - Not applicable.

a. Anesthetic cream was in contact with the skin of rats assigned to Groups I through V for approximately 30 minutes; anesthetic cream was in contact with the skin of rats assigned to Group VI for approximately 60 minutes.

Primary Irritancy test

All female rats were acclimated to Elizabethan collars. Hair was clipped from the backs of the rats at least 24 hours before treatment. Rats were selected for inclusion in the study based on availability of appropriate skin sites for UVR exposure for the phototoxicity study. The rats were fitted with Elizabethan collars, and the anesthetic cream was topically applied (25 or 38 µL/cm^2) to the area to which the patches were to be applied. The administration site was demarcated with an indelible marker at each corner of the site. Approximately 30 minutes later (Groups I through V) or 60 minutes later (Group VI), the anesthetic cream was wiped off with a dry gauze and control (vehicle) or test article patches (5 cm x 5 cm) were applied to rats in Groups II through VI. The patches were secured using an elastic wrapping material overlay. No patches were applied to rats in Group I. After the patches had been in contact with the skin for 1, 2 or 3 hours, the patches were removed, and the administration skin sites were gently wiped with the cleansing lotion (approximately 3 mL) and dry gauze.

Parameters Monitored:

The rats were observed for viability at least twice daily and were individually examined for clinical signs before treatments. Observations for clinical signs and treatment effects were made immediately after removal of the patches (Groups II through V) or at a comparable time (Group I). Observations included, but were not limited to, changes in skin, eyes, mucus membranes, respiratory and central nervous system and somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation and ocular discharge, diarrhea, lethargy, sleep and coma. Rats were also examined immediately (within 15 minutes), 1 hour ± 10 minutes, 2 hours ± 10 minutes, 4 hours ± 10 minutes and 6 hours ± 15 minutes after patch removal (Groups II through VI) or a comparable time (Group I), and on study day 1 (SD 1) for signs of skin

irritation and general behavioral patterns. Rats were examined one, two and three days after application/dosage administration (SDs 2 through 4, respectively) for general appearance, including general behavioral patterns and signs of skin irritation. Body

GRADING VALUES FOR SKIN REACTIONS

ERYTHEMA
- Erythema Grade 1 = Barely perceptible light redness
- Erythema Grade 2 = Distinct redness
- Erythema Grade 3 = "Beet red" color

EDEMA
- Edema Grade 1 = Mild, raised <1 mm
- Edema Grade 2 = Moderate, raised 1 mm to 2 mm
- Edema Grade 3 = Marked, raised >2 mm

FLAKING
- Flaking Grade 1 = Barely perceptible scales[a]
- Flaking Grade 2 = Distinct scales
- Flaking Grade 3 = Pronounced flaking with denuded[b] sites

ULCERATION
- 1 = Yes

SCAB
- 1 = Yes

RESIDUE
- 1 = Yes (Residue of test article present)

weights were recorded weekly, including on the day of treatment and prior to sacrifice

Results

Erythema grade 1 (barely perceptible redness) was observed in all groups of rats in which capsaicin patches were applied (Groups III-VI) immediately after patch removal. The occurrence of erythema was greatest in the group of rats in which the patches were applied for 1 hour (3/3) and was least in the groups in which the patches were applied for 3 hours (Groups V and VI). This erythema resolved in all rats 1 hour after patch removal (Groups III-VI). No skin responses occurred in the group of rats administered the anesthetic cream alone (Group I) or the placebo patch (Group II).

For up to 45 minutes after patch application in Groups III-V, some rats in the groups exhibited signs of possible discomfort (increased activity), as compared with the rats administered only the anesthetic cream (Group I) or the placebo patch (Group II). Because of this activity, the volume of anesthetic cream administered was increased from 25 $\mu L/cm^2$ to 38 $\mu L/cm^2$ and the duration of administration was increased to approximately 60 minutes (Group VI). This increased anesthetic cream and administration time reduced both the duration and incidence of the activity of rats to approximately 5 minutes in 1 of 3 rats.

Dosage Group	Number of Rats Per Group	Treatment Regimen Descriptor		Duration of Patch Contact with the Skin (Hours)	Skin Responses[a]
		Anesthetic Cream Administration Volume and Time	Test Article, Control Article or Comparator Article Administration		
I	3	30 min, 25 mcL/cm^2	None	NA	0
II	3	30 min, 25 mcL/cm^2	Control Article (Vehicle) Patch	3	0
III	3	30 min, 25 mcL/cm^2	8% w/w capsaicin Patch	1	3
IV	3	30 min, 25 mcL/cm^2	8% w/w capsaicin Patch	2	2
V	3	30 min, 25 mcL/cm^2	8% w/w capsaicin Patch	3	1
VI	3	60 min, 38 mcL/cm^2	8% w/w capsaicin Patch	3	2

a. Number of rats observed with a response of erythema grade 1 for any of the observation time points.

DERMAL SENSITIZATION

Study title: Dermal sensitization study of (b) (4) pain treatment system (capsaicin) and (b) (4) placebo pain treatment system in guinea pigs-closed patch technique.

This study was reviewed previously as an IND submission by Drs. Violetta M. Klimek and Suzanne R. Thornton.

Key study findings: The potential of NGX-4010 to induce delayed contact hypersensitivity was evaluated in the guinea pig using the closed patch technique. Based on the relatively low incidence and mild severity of challenge reactions to the capsaicin patch (b) (4) Pain Treatment System), capsaicin in this patch formulation is considered to be a mild dermal sensitizer in guinea pigs. The placebo patch produced no sensitization reaction. The patch itself is a mild irritant since it caused erythema with increasing severity with increasing number of treatment days leading to hemorrhaging and desquamation in some animals toward the later part of the induction period.

Reviewer Comment: *The reactions were examined 24 and 48 hours after application, for which very faint erythema was usually present during the induction phase, although by the 7 treatment, an increase in severity was noted (faint erythema to moderate erythema) in some animals. The 6 hour patch application with occlusion in guinea pigs contrasts with the treatment of rats up to 3 hours without occlusion in which erythema resolved by 1 hour. The optimal time points for observing a maximal hypersensitivity reaction were not determined here and were likely missed. It has since been learned that the guinea pig vanilloid receptors are relatively insensitive to capsaicin (Szallasi 1994 Gen Pharmac 25:223-243). Therefore, the findings presented here that capsaicin is a weak sensitizer to a later capsaicin exposure likely underestimates its sensitizing capabilities in humans. However, a hypersensitivity reaction does not require pharmacodynamic sensitivity, being an immunologic response, it is more dependent on molecular size and structure.*

Study no: 7215-108
E-Location: m4\42-stud-rep\423-tox\4236-loc-tol\7215-108
Conducting laboratory and location: (b) (4)
Date of study initiation: 15 June 2001
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity:
Capsaicin patch ((b) (4) pain treatment system), Batch 8/13009/01, Purity not specified (capsaicin density 0.729 mg/cm^2)
Placebo patch ((b) (4) pain treatment system, pacebo)
[Note: lot number does not match Certificate of Analysis included in the Appendix of the report]
Cleansing lotion (PEG 300), Lot 36

Methods

Doses: 4.91 cm^2/animal, (0.73 mg capsaicin/cm^2 patch; 8.8 mg/kg applied topically)
Species/strain: Crl:HA(BR) guinea pigs, males
Number/sex/group or time point (main study): 10/group, and 5 naïve control animals
Route, form, volume, and infusion rate: a 2.5 cm2 dermal patch was applied to the back for 6 hrs/application on days 1, 4, 6, 8, 11, 13, 15, 18, and 20 at the same location, then challenged 2 and 4 weeks later with patch applications at a different location.
Age: not stated, indicated as young adult
Weight: 380-461 g
Study design:

Group	Number of Animals	Descriptions
1 (Test article 1)	10	(b) (4) Pain Treatment System (capsaicin)
2 (Test article 2)	10	Placebo Pain Treatment System
3 (Naïve Control)	5	Pain Treatment System (capsaicin) and (b) (4) Placebo Treatment System (at challenge only)

Induction Phase: The study consisted of a 9 treatment induction phase over 3 weeks, 6 hrs/day, with either capsaicin patch (Group 1; (b) (4) Pain Treatment System) or placebo patch (Group2; (b) (4) Placebo Pain Treatment System). The induction applications occurred on days 1, 4, 6, 8, 11, 13, 15, 18, and 20. Each application was made on the same site during the induction phase. The naive control animals (Group 3) were not treated during this phase.

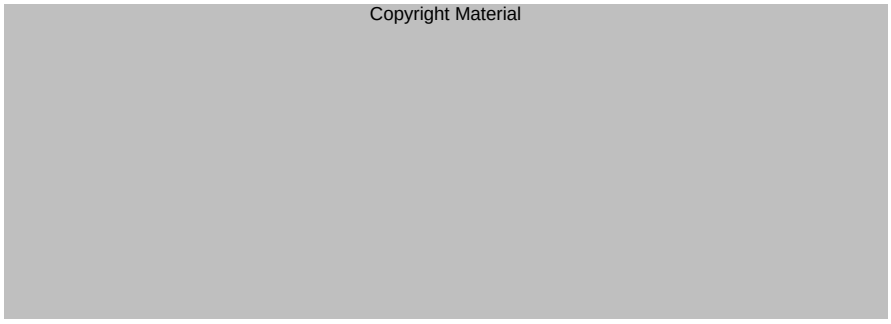
Animal in Groups 1 and 2 were clipped free of hair, 25-mm diameter sized circular patches were applied by placing one 25-mm section under a Hilltop Chamber patch and placing the patch on the induction site along the dorsal anterior left quadrant. The patches were covered with dental dam and secured with Elastoplast™ tape. The dressing remained in place for approximately 6 hours, then removed, and the induction sites wiped with the cleansing lotion.

Challenge Phase: All animals were challenged 2 wks and 4 wks after the ninth induction. The animals were shaved and depilated with Neet® depilatory one and two days, respectively, prior to the challenge application to reduce mechanical irritation upon patch removal. A capsaicin or placebo patch was applied to the depilated area on dorsal anterior right quadrant in the same manner as during the induction phase of the study. Group 1 was challenged with a capsaicin patch and Group 2 with a placebo patch. The Group 3 naive control animals were treated with both capsaicin and placebo patches on the right and left dorsal anterior quadrants, respectively.

A second challenge application was conducted 2 weeks after the initial challenge application, with patches applied to new naive sites on each animal in the test groups (Groups 1 and 2) and on five new naive control animals in the same manner as the initial challenge application. The initial naive control animals were not treated during the second challenge phase.

Scoring: Animals were scored for dermal reactions according to the Buehler sensitization scale presented below.

BUEHLER SENSITIZATION SCORING SCALE



Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

Results

There were no deaths. Mean body weights were not affected by capsaicin treatment. There were four animals in the (b) (4) pain treatment capsaicin group with notable clinical observations, hypothermia (E25762, E25768), few feces and thin appearance (E25764), and left eye closed (E25767).

Reviewer Comment: *The Applicant claims these were not a result of treatment. These findings occur in other toxicological studies and are now considered due to capsaicin treatment. Hypothermia is a reaction to the pharmacodynamic effect on vanilloid receptors, whether this is mediated at the cutaneous nerve endings in addition to CNS action via systemic capsaicin is unknown. Eye closing could reflect an acute pain phenomena via contact with aerosolized capsaicin known to occur with patch removal and handling. Weight loss could also be a centrally mediated reaction or an aversion response to the irritant effects of ingested capsaicin from aerosolization or grooming behavior.*

Dermal Findings:

> In the induction period, for the first treatment with a capsaicin patch, dermal scores ranged from 9 animals were either 0.5 or 1 at 24 hours, but by 48 hours only 2 animals had score of 0.5, the rest were 0. By the 9th treatment, 3 weeks later, the 24 hour scores were either 1 or 2 in 7 animals, and were still 1 or 2 in 5 animals at 48 hour after treatment. Subcutaneous hemorrhaging, pinpoint areas of subcutaneous hemorrhaging, desquamation, and/or denuded areas were also observed within the application sites of all Group 1 animals, with the hemorrhaging occurring more frequently late during the induction phase. The

first treatment with placebo patches resulted in scores of 0.5 in 8 animals and score of 1 in 1 animal, and by the 9th treatment a score 0.5 occurred in only 3 animals. For placebo patches, only denuded areas were observed, there were no hemorrhaging or desquamation sites.

For the challenge periods, with one exception, there were no new reactions that developed at 48 hours if not present at 24 hour after challenge. The exception occurred during the second challenge in which no reaction was observed at 24 hrs, but a very faint erythema was observed at 48 hours after challenge. Reactions from placebo patches did not exceed those from naive controls reactions. There were 3 animals in the first challenge and 2 animals in the second challenge with scores that exceeded those of the placebo patch group.

Incidences of Dermal Reactions

Score	Induction		First Challenge		Second Challenge	
	1st	9th				
Group1, Capsaicin Patch, n=10						
0	1	1	3		5	
0.5	6	2	4		3	
1.0	3	3	3		1	
2.0		4			1	
3.0						
Group2, Placebo Patch, n=10						
0	1	7	7		6	
0.5	8	3	3		4	
1.0	1					
2.0						
3.0						
Group 3, Naive Control, n=5						
	No treatments		**capsaicin**	**placebo**	**capsaicin**	**placebo**
0			2	3	4	3
0.5			3	2	1	2
1.0						
2.0						
3.0						

	Induction Phase (Dose Number)																		Initial Challenge Phase		Second Challenge Phase	
	1		2		3		4		5		6		7		8		9					
Animal	Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour	
Number	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48

(b) (4)

	Induction Phase (Dose Number)																		Initial Challenge Phase		Second Challenge Phase	
	1		2		3		4		5		6		7		8		9					
Animal	Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour		Hour	
Number	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48

(b) (4)

2.6.6.8 Special toxicology studies

PHOTOTOXICITY

Study title: **Topical Phototoxicity Screening Test of Capsaicin Patches in Rats**

Dermal irritation was also determined in this study and is presented in the Local Tolerance Section (#2.6.6.7) of this review.

Key study findings: Application of a dermal patch containing 8% w/w capsaicin to the back of Crl:CD® (SD)IGS BR VAF/Plus® rats elicited cutaneous erythema observed immediately after patch removal. In an additional study with a similar design followed after patch removal by exposure to solar-simulated ultraviolet (UV) radiation (0.5 MED), there was the dermal response and severity was not enhanced.

Study no.: **1614-001**
E-location: m4\42-stud-rep\423-tox\4236-loc-tol\1614-001
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation:
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
8% w/w Capsaicin Patch (20 cm x 10 cm), Lot 81107802, Purity not provided; concentration density 124.4 mg/200 cm^2
Placebo patch, Lot 81113301, 200 cm^2
Cleansing Gel, Batch 710
Ela-Max (lidocaine 4%, topical anesthetic cream; (b) (4) (b) (4)
8-methoxypsoralen (8-MOP), Lot 97H0359 (b) (4)

Methods
Doses: 0 (no patch) or 25 cm^2 patch (=15.55 mg capsaicin)
Species/strain: female Rats, Crl:CD® (SD)IGS BR VAF/Plus®
Number/sex/group or time point (main study): 5 females/group
Route, formulation, volume, and infusion rate: a single patch was applied once to the back of rats for 0 (no patch), 1 , 2 or 3 hours in duration
Age: 7 weeks at arrival
Weight: at study assignment 155-188 g

Study design or methodology:

Dosage Group	Number of Rats Per Group	Treatment Regimen Descriptor		Duration of Patch Contact with the Skin (Hours)	Assigned Rat Numbers
		Pretreatment	Test Article, Control Article or Comparator Article Administration		
1	5	None	None	NA	8846 - 8850
2	5	None	8-MOP (0.1%)	NA	8851 - 8855
3	5	Anesthetic Cream	Control Article (Vehicle) Patch	3	8856 - 8860
4	5	Anesthetic Cream	8% w/w capsaicin Patch	1	8861 - 8865
5	5	Anesthetic Cream	8% w/w capsaicin Patch	2	8866 - 8870
6	5	Anesthetic Cream	8% w/w capsaicin Patch	3	8871 - 8875

NA - Not applicable.

Phototoxicity Screening

Rats were acclimated to Elizabethan collars and hair clipped from the back of the rats a few days before the study initiation. Rats were selected for inclusion in the study based on availability of appropriate skin sites for UVR exposure, and one spot was tattooed on each rat as close as possible to the midline in the center of the back. At least 24 hours before UVR exposure, hair was again removed from the backs of the rats and a "map" of the area was made for any rat with areas of apparent hair tufts, skin defects or natural differences in skin colorization. Rats were fitted with Elizabethan collars, and the anesthetic cream was topically applied (38 µL/cm^2) to the area to which the patch was applied to rats in Groups 3 through 6. The administration site was demarcated at each corner of the site. Approximately 60 minutes later, the anesthetic cream was wiped off with a dry gauze and control article (vehicle) or capsaicin patches (5 cm x 5 cm, approximately 25 cm^2) were applied to rats in Groups 3 through 6. The patches were positioned so that the site of UVR exposure demarcated by the tattoo was approximately in the center of the patch site. The patches were secured using an elastic wrapping material overlay. No patches were applied to rats in Groups 1 and 2. The positive control, 8-MOP, was topically administered (100 µL/25 cm^2) to the rats in Group 2. After the patches had been in contact with the skin for a duration of 3, 1, 2 and 3 hours (Groups 3, 4, 5 and 6, respectively), the patches were removed, and the administration skin sites of rats assigned to Groups 3 through 6 were gently wiped with the cleansing lotion (approximately 3 mL) and dry gauze.

The rats were then exposed to UVR two hours after removal of the patches as determined from the primary irritancy test in Groups 1, and 3 through 6, and one hour after 8-MOP administration in Group 2. All rats were anesthetized [ketamine (100 mg/mL), 40 to 60 mg/kg, and xylazine (20 mg/mL), 5 to 8 mg/kg; IM], positioned on plastic trays covered with an aluminum foil mask containing a 1.3 cm diameter hole that served as the site of cutaneous UVR exposure. The rats were positioned approximately one meter from the UVR source. An instrumental UVR exposure dose equivalent to approximately 0.5 minimal erythema dose (MED, eliciting a barely perceptible response in skin) was administered. The source of irradiation, a 6.5 kw long-arc xenon water-cooled lamp, was used to simulate mid-latitude summer sunlight. One filter (Schott WG 320, doped glass,

1 mm thick) was used to attenuate mid-range ultraviolet radiation (UVB). A Solar Light detector was used to monitor the incident UV irradiation, and an instrumental dose of about 0.5 MED was delivered in an exposure period of approximately one-half hour.

Reviewer comment: *It was not clear why there was a two hour interval for the capsaicin treatments and only 1 hour interval for the 8-MOP treatment until UV irradiation. Perhaps it was to allow for erythema to resolve before irradiating, which state occurred by 1 hour after patch removal. An intensity measurement of the irradiation was not provided to validate that the animals were receiving and appropriate 0.5 MED dose of UV irradiation.*

Parameters monitored:
The rats were observed for viability at least twice daily. Rats were individually examined for clinical signs before and immediately after patch removal (Groups 3 through 6) or a comparable time (Groups 1 and 2). Observations included but were not limited to, changes in the skin, eyes, mucus membranes, respiratory and central nervous system, activity, and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation and ocular discharge, diarrhea, lethargy, sleep and coma. Adverse clinical signs observed during and immediately after UVR exposure were recorded for individual rats when observed. Rats were individually examined 4 hours after dosage/UVR exposure on DS 1, as well as one, two and three days later for general appearance, including general behavioral patterns and signs of skin irritation. Body weights were recorded weekly including on the day of dosage administration and prior to sacrifice.

GRADING VALUES FOR SKIN REACTIONS

ERYTHEMA
- Erythema Grade 1 = Barely perceptible light redness
- Erythema Grade 2 = Distinct redness
- Erythema Grade 3 = "Beet red" color

EDEMA
- Edema Grade 1 = Mild, raised <1 mm
- Edema Grade 2 = Moderate, raised 1 mm to 2 mm
- Edema Grade 3 = Marked, raised >2 mm

FLAKING
- Flaking Grade 1 = Barely perceptible scales[a]
- Flaking Grade 2 = Distinct scales
- Flaking Grade 3 = Pronounced flaking with denuded[b] sites

ULCERATION
- 1 = Yes

SCAB
- 1 = Yes

RESIDUE
- 1 = Yes (Residue of test article present)

Results

There were no responses due to UV irradiation in groups that received no vehicle or capsaicin patches (Group 1), the vehicle patch (Group 3), or the 8% w/w capsaicin dermal patch administered for 1, 2 or 3 hours (Groups 4, 5 and 6, respectively). Cutaneous responses indicative of a phototoxic response, including erythema grades 1 and 2, edema grades 1 and 2 and scab occurred in the rats administered the positive control 8-MOP (Group 2).

Skin responses to UV irradiation

Dosage Group	Number of Rats Per Group	Treatment Regimen Descriptor		Duration of Patch Contact with the Skin (Hours)	Skin Responses[a]
		Anesthetic Cream Administration Volume and Time	Test Article, Control Article or Comparator Article Administration		
1	5	None	None	NA	0
2	5	None	8-MOP (0.1%)	NA	33
3	5	60 minutes, 38 mcL/cm^2	Control Article (Vehicle) Patch	3	0
4	5	60 minutes, 38 mcL/cm^2	8% w/w capsaicin Patch	1	1
5	5	60 minutes, 38 mcL/cm^2	8% w/w capsaicin Patch	2	0
6	5	60 minutes, 38 mcL/cm^2	8% w/w capsaicin Patch	3	0

a. Number of skin reactions (erythema, edema, flaking, scab) that occurred in the administration skin site for any of the observation time points.

2.6.6.9 Discussion and Conclusions

Refer to the Executive Summary

2.6.6.10 Tables and Figures

These are incorporated in the reviews of each study.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Refer to tables within each reviewed study.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions	Refer to the Executive Summary
Unresolved toxicology issues (if any)	Refer to the Executive Summary
Recommendations	Refer to the Executive Summary
Suggested labeling	Refer to the Executive Summary

Signatures (optional):

Reviewer Signature _____
L. S. Leshin, D.V.M., Ph.D.

Supervisor Signature_____ **Concurrence Yes ___ No**
A. Wasserman, Ph.D.

APPENDIX/ATTACHMENTS

APPENDIX 1: References provided by the Applicant

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APPENDIX 6: Carcinogenicity ECAC Meetings

The meeting minutes of June 12, 2003 and July 6, 2004 for the protocol and dose determination of the carcinogenicity study are included in the pages that follow.

List of Carcinogenicity Dose Finding and Preliminary Studies

ECAC Meeting	Topic	Study number / Location	Study
June 12, 2003 s	**May 1, 2003 SN-031 draft protocol review** 26-week dermal oncogenicity study with capsaicin in Tg.AC hemizygous mice (FVB/N)	**7215-122** m4\42-stud-rep\423-tox\4234-carcigen\42342-smt-stud **reviewed June 17, 2003**	4-Week Dose Range-Finding Dermal Toxicity and Toxicokinetic Study with Capsaicin in FVB/N Mice
-	**July 10, 2003 SN-034-GC** Sponsor's reply to recommendations and email of clarifications prior to final submission of response; **no response communicated**		
July 6, 2004	**Revised draft protocol SN-60 SX, of May 28 2004**	**7215-131** IND 63354, SN-060, Vol. 1, pp 1-14 **not submitted to the NDA reviewed June 22, 2004**	Pilot study to validate the use of tetradecanoyl phorbol-13-acetate (TPA) dissolved in (b) (4)® as a positive control in Tg.AC hemizygous mice (FVB/N)
		7215-137 IND 63354, SN-060, Vol. 2, pp 2-1 (unaudited draft report) **not submitted to the NDA reviewed June 22, 2004**	4-week dose range-finding dermal toxicity and toxicokinetics study with *trans*-capsaicin in FVB/N mice

June 12, 2003,

Executive CAC
Date of Meeting: June 12, 2003

Committee: Abby Jacobs, Ph.D., HFD-540, Acting Chair
Ken Hastings, Ph.D., HFD-590, Alternate Member
David Jacobson-Kram, Ph.D., HFD-024, Alternate Member
Tim McGovern, Ph.D., HFD-170, Team Leader and Presenting Reviewer

Author of Draft: Tim McGovern, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 63,354
Drug Name: Capsaicin Dermal Patch
Sponsor: NeurogesX, Inc.

Capsaicin is a pungent ingredient in chili peppers and is a highly selective agonist for the vanilloid receptor 1 (VR1), a ligand-gated, non-selective, cation channel, preferentially expressed in small diameter, primary afferent neurons, especially nociceptive sensory nerves. Based on the agonistic action of capsaicin at the VR1 receptor, with resultant inactivation of cutaneous nociceptors, the sponsor is developing a high concentration dermal patch using pure (≥ 98%) synthetic capsaicin for the treatment of neuropathic pain. The maximum expected clinical use of Dermal Capsaicin Patch is treatment of areas up to 1000 cm^2 at a capsaicin concentration of 0.64 mg/cm^2 (up to 640 mg capsaicin). The sponsor anticipates that the patch will be used once every few months for a duration of one hour although this use has not yet been finalized.

In tests performed by the sponsor with purified synthetic capsaicin, results were negative in an Ames bacterial reverse mutation assay, an in vitro chromosome aberration assay in cultured human peripheral lymphocytes, and an in vivo mouse micronucleus assay. Capsaicin was mutagenic in a mouse lymphoma L51178Y cells in the presence and absence of S9. The sponsor submitted information indicating that naturally occurring catechol compounds such as L-DOPA, dopamine, epinephrine, etc, have also shown positive responses in mouse lymphoma assays. In a review of the literature by the sponsor, various tests were performed with natural capsaicin extracts and were not conducted under GLP. Eight bacterial point mutation tests (including Ames assay) were

performed between 1981 and 1995 using various strains of *S. typhimurium* and various forms of S9 activation. Four tests resulted in positive findings and four were negative. Two tests in Chinese hamster V79 cells were conducted; one was positive and one was negative. The in vivo micronucleus test was conducted in mice and it was positive.

(b) (4)

[redacted]

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the sponsor's proposed study design for a Tg.AC study, due to excessive toxicity at the highest proposed dose as well as a lack of information regarding the proposed test article vehicle and the proposed dosing regimen and vehicle for the positive control.

2. The Committee recommended a high capsaicin concentration of 0.64 mg/cm^2/week (~ 85 mg/kg/dose). The recommended mid- and low-concentrations are 0.32 and 0.16 mg/cm^2/week, respectively.

3. The Committee recommended that the positive control (TPA) be administered in the same vehicle ((b) (4) and with the same frequency (once per week) and duration of exposure (3 hours) as that proposed for capsaicin. Also, the TPA treatment site should be cleansed in a manner similar to that proposed for capsaicin treatment. This evaluation of the positive control is essential because of concerns about validity of the assay at short exposure times.

4. The Committee recommended that an untreated control group be added to the protocol, due to a lack of sufficient information with the use of Transcutol as a vehicle in this model.

5. The Committee recommended that the sponsor submit a modified protocol for concurrence. Data to support the validity of TPA as a positive control under the conditions listed in recommendation # 3 should be submitted in order to obtain concurrence with the modified protocol.

6. Alternatively, the sponsor may consider conducting a traditional dermal carcinogenicity study with weekly or less frequent dosing. Should the sponsor chose to follow this option, the protocol should be submitted to the executive CAC for concurrence, together with supporting dose-ranging data.

Abby Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-170
McGovern/Team leader, HFD-170
Malandro/CSO/PM, HFD-170
/ASeifried, HFD-024

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/s/

Abby Jacobs
6/13/03 02:55:44 PM

July 6, 2004

Executive CAC
Date of Meeting: July 06, 2004

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Karen Davis-Bruno, Ph.D., HFD-510, Alternate Member
R. Daniel Mellon, Ph.D., Team Leader
Suzanne Thornton-Jones, Ph.D., Presenting Reviewer

Author of Draft: Suzanne Thornton-Jones

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-year carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 63,354 (N060SX)
Drug Name: capsaicin patch
Sponsor: NeurogesX, Inc.
Sponsor contact name: Karen J. Harder
Sponsor/Applicant telephone and fax number: 650-508-2116; 650-649-1798

Background:
Capsaicin is a vanilloid receptor agonist being developed for treatment of neuropathic pain. In mutagenicity studies with purified synthetic capsaicin, the compound tested negative in the *in vitro* Ames bacterial reversion assay, *in vitro* chromosomal aberration assay in cultured human peripheral lymphocytes, and in an *in vivo* mouse micronucleus assay. In contrast, capsaicin treatment resulted in a positive finding in the *in vitro* mouse lymphoma L5178Y ($TK^{\pm}$) assay ± S9. Similar catechol moieties (L-DOPA, dopamine, epinephrine) have been reported to test positive in the mouse lymphoma assay. Metabolism information is scarce but it appears to be metabolized in the liver via mixed function oxidase systems, CYP 2E1, and/or non-oxidative hydrolysis. Protein binding is unknown. The sponsor is proposing a Tg.AC transgenic mouse bioassay where capsaicin will be administered topically for 26-weeks.

Dose Selection:
The Sponsor previously submitted a special protocol for a Tg.AC hemizygous transgenic mouse, which was presented to the Executive CAC on 12 June 2003. (b) (4)

(b) (4)

Relevant recommendations conveyed to the Sponsor by the Executive CAC at that time were: 1) they did not concur with the Sponsor's proposal to administer TPA in (b) (4) due to the lack of information regarding the (b) (4) proposed dosing regimen, and proposed doses; 2) TPA should be administered in (b) (4) with the same frequency (once per week), duration of exposure (3 hours), and wiped with the cleansing agent as proposed for capsaicin; 3) addition of an untreated control group; and 4) recommended doses of 0.16, 0.32, and 0.64 mg/cm^2.

Following the Executive CAC recommendations, the Sponsor conducted a pilot study with TPA dissolved in (b) (4) in FVB/N mice. TPA in (b) (4) was administered either once or twice a week to a 2 x 2 cm (4cm^2) site for 26 weeks at a concentration of 6.25 µg/application. TPA in (b) (4) produced papilloma masses at a frequency of 50-67%. The Sponsor did not consider (b) (4) to be an acceptable vehicle for TPA for the Tg.AC mouse assay because of not achieving 100% papilloma mass frequency, and decided to use methanol as the vehicle. The Sponsor conducted a 4-week dose range-finding study in FVB/N mice with TPA and capsaicin dissolved in methanol where mice were dermally treated with 90% methanol (vehicle control) or *trans*-capsaicin dissolved in methanol daily for 3 hrs. Findings from the study included slight erythema and atonia at doses 0.64 mg/animal (0.16 mg/cm^2) with corresponding histopathological changes of acanthosis, epidermal serocellular crust, hyperkeratosis, and chronic (active) inflammation. Transient decreases in body weight gains were observed in male mice during SW1 (20-34%) at doses 0.64 mg/animal (0.16 mg/cm^2) with corresponding decreases in food consumption. Body weight gains were also decreased in female mice during SW2 (25-45%) at doses $\geq$0.32 mg/animal (0.08 mg/cm^2). Adrenal weights were decreased in female mice at doses >0.32 mg/animal (0.08 mg/cm^2). The MTD for this study was 1.28 mg/animal (0.32 mg/cm^2) for male and female mice. Based on the results of the study the sponsor proposed doses of (b) (4)

(b) (4) respectively) in male and female mice with TPA and capsaicin being administered in methanol.

Executive CAC Recommendations and Conclusions:

- The Committee concurred with the use of the Tg.AC transgenic mouse assay.
- The Committee found the tumor incidence with TPA in (b) (4) acceptable.
- The Committee recommended (b) (4) as the vehicle for the positive control (TPA) and capsaicin administration, not methanol.
- The Committee recommended including the following control groups: an untreated control group (or submit Historical Control Data), a lidocaine alone group, and a lidocaine plus Transcutol® group.
- The Committee did not concur with the proposed doses and again recommended doses of 0, 0, 0, 0.16, 0.32, and 0.64 mg/cm^2 based on the previously conducted dose range-finding study where TPA and capsaicin were administered in (b) (4).
- The Committee advises that hematological evaluations are not necessary in this type of study.

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

cc:\
/Division File, HFD-170
/Dan Mellon, HFD-170
/Suzanne Thornton-Jones, HFD-170
/Lisa Malandro, HFD-170
/Adele Seifried, HFD-024

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/s/

David Jacobson-Kram
7/8/04 02:58:52 PM

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/s/

Lawrence Leshin
7/17/2009 11:50:34 AM
PHARMACOLOGIST

Adam Wasserman
7/17/2009 12:44:45 PM
PHARMACOLOGIST
I concur with Dr. Leshin that this application may be approved. See the Supervisory Memo for further details.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: **22-395** Applicant: **NeurogesX Inc.** Stamp Date: **Oct 13, 2008**

Drug Name: **Qutenza™ (proposed) capsaicin patch 8%** NDA Type: **505(b)(2)**

On **initial** overview of the NDA application for RTF:

	Content Parameter	Yes	No	Comment
1	On its face, is the pharmacology/toxicology section of the NDA organized (in accord with 21 CFR 314 and current guidelines for format and content) in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section of the NDA indexed and paginated in a manner allowing substantive review to begin?	X		
3	On its face, is the pharmacology/ toxicology section of the NDA legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted in this NDA (carcinogenicity, mutagenicity*, teratogenicity*, effects on fertility, juvenile studies, acute and repeat dose adult animal studies*, animal ADME studies, safety pharmacology, etc)?			administratively change from b1 to b2 b2 will be for nonclinical pharmacology based on published literature
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	On its face, does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted a rationale to justify the alternative route?	X		
7	Has the sponsor submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
8	Has the sponsor submitted all special studies/data requested by the Division during pre-submission discussions with the sponsor?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	If there are any impurity – etc. issues, have these been addressed? (New toxicity studies may not be needed.)	X		no response was sent to Applicant after the follow-up March 6 2008 PreNDA meeting request for impurity information and Applicants request for a meeting was denied (see Additional Comments below) Cleansing gel: toxicological assessment requested, contains (b) (4) considered a novel excipient cis-capsaicin impurity qualification (see June 2008 follow-up to PreNDA meeting)
11	Has the sponsor addressed any abuse potential issues in the submission?			not applicable
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?			not applicable
13	From a pharmacology/toxicology perspective, is the NDA fileable? If ``no`` please state below why it is not.	X		can administratively change from b1 to b2, rather than deny filing and have them resubmit

Any Additional Comments:

In response to nonclinical comments in the preNDA meeting minutes, the Sponsor submitted a request for further information pertaining the qualification of cis-capsaicin which had a specification of NMT (b) (4). They provided information that although specific studies were not conducted to address the toxicology of cis-capsaicin, this impurity was present in the trans-capsaicin drug substance used in the nonclinical studies. They provided amounts and safety margins and additional information. The amount of the cis-capsaicin in the genetic toxicology studies does not provide sufficient support to qualify the safety of this impurity for mutagenicity. The in vivo chromosomal aberration assay of mouse bone marrow cells provided sufficient support for clastogenicity safety. The 14-day IV study in the dog provided sufficient support for qualification in general toxicology studies.

Review of the previous and this new information indicated that cis-capsaicin lacks support for mutagenicity safety, thus it is not yet fully qualified. The Sponsor should conduct a GLP genetic toxicology study of cis-capsaicin to determine its mutagenic potential.

Comments to send to the Sponsor:

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

The following information is requested:

The impurity, cis-capsaicin, in your product Capsaicin Patch 8% (w/w), is incompletely qualified at this time. A point mutation assay with the isolated impurity tested up to the limit dose for the assay is required. If a positive test result is obtained, a second alternative assay should be conducted such as the in vitro mouse lymphoma assay. Provide an estimated date for this study's submission.

The impurities were not included in the certificates of analysis submitted with the original nonclinical studies. Include the report(s) for the analysis of cis-capsaicin in drug substance lots referenced in Table 2 and Table 4 of the April 23, 2008 submission.

Provide a toxicological assessment of the cleansing gel and its components and justification for its safety.

Reviewing Pharmacologist: L. Steven Leshin Date

Team Leader/Supervisor: Adam Wasserman Date

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/s/

Lawrence Leshin
12/18/2008 03:48:53 PM
PHARMACOLOGIST

Adam Wasserman
12/18/2008 10:37:54 PM
PHARMACOLOGIST
Concur. Comments should be conveyed to Applicant in the 74-day letter at the latest.