

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-352

PHARMACOLOGY REVIEW(S)



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

Supervisory Pharmacologist Memorandum - Addendum

NDA NUMBER: 22-352
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 6/20/2008
PRODUCT:
 (Proposed) Trade Name:
 Established Name: Colchicine

INDICATION: Familial Mediterranean Fever

INTENDED CLINICAL POPULATION: Adults and children

SPONSOR: Mutual Pharmaceutical Co., Inc.

DOCUMENTS REVIEWED: Primary review of Dr. Leshin; NDA 22-352 as appropriate

REVIEW DIVISION: Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)

PHARM/TOX REVIEWER: L. Steve Leshin, DVM, Ph.D.
PHARM/TOX SUPERVISOR: Adam Wasserman, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Margarita Tossa

ADDENDUM

The purpose of this addendum to the Supervisory Memo is to further clarify the reasoning behind the nonclinical recommendation to require the Applicant to conduct a carcinogenicity evaluation of colchicine as a post-marketing commitment as a basis of approval for NDA 22-352 for Familial Mediterranean Fever (FMF). A Pre-NDA meeting was held with the Applicant on July 31, 2006 to address development questions for their FMF as well as their Treatment of Acute Gout Attack and Prophylaxis of Gouty Flares indications (the latter two have as of December 2008 been submitted independently as NDAs 22-351 and 22-353). The Applicant inquired if the Division agreed that “no additional toxicology studies are required prior to NDA submission”. Our response was the following:

Division Response:

Until the IND package is submitted, we cannot determine if for the existing information is sufficient to assess the safety of colchicine in the proposed indications. Please include copies of all cited literature used to support your drug development program.

If unexpected or additional safety concerns develop during manufacturing or during clinical trials, additional toxicological studies may be required.

With further discussion we informed the Sponsor that “a carcinogenicity evaluation would be desirable but will not be required”. However, the Applicant was “encouraged to summarize the current knowledge concerning the carcinogenic potential of colchicine” in the NDA.

The minutes for a subsequent pre-NDA meeting with the Sponsor (as AR Scientific; an allied company both owned by URL Pharma) for FMF, acute gout flares, and _____ on February 4, 2008 contained the following statement by the Sponsor and Division response:

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Although Mutual is continuing to evaluate the potential for performing such a study, Mutual asks that the Division re-confirm that such a study will not be required prior to the approval of an NDA for treatment and prevention of acute gout flares / _____ of FMF / _____

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FDA RESPONSE:

Carcinogenicity studies will not be required for NDA approval.

From the above interactions it is clear the Division was not requesting carcinogenicity evaluation for these indications as a condition of NDA submission or approval.

Concerns regarding the aneugenic properties of colchicine and the potential for malignancy are described in the primary review of Dr. Leshin as well as my Supervisory

Memo. I continue to recommend the applications for these indications may be submitted, and potentially approved without the evaluation of carcinogenicity for three principal reasons: 1) the long history of use in gout (which is the major indication(s) sought) and the generally older age group which benefits from this treatment as well as 2) the Orphan designations of the other disease indications (FMF, _____) being sought, and finally 3) the drug is currently marketed and unapproved as a single entity (though available as an approved combination product with probenecid as Col-Probenecid® [0.5/500 mg] and other generics); therefore, approval without this information does not expose the public to a greater risk than currently encountered.

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The recommendation to conduct the carcinogenicity evaluation of colchicine as a post-marketing commitment stems from the mechanistic plausibility for the drug to initiate and promote tumor development due to mitotic spindle inhibition and subsequent aneuploidy as well as the observation that FMF (as with _____) strikes a younger population compared to gout and this population theoretically may require the drug for prophylaxis treatment for the remainder of their life. The risk therefore is magnified due to the longer duration of treatment though the severity of this rare disease and associated morbidity and mortality is noted.

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The Phase 4 post-marketing commitment recommendation was discussed with Dr. David Jacobson-Kram, Associate Director for Pharmacology/Toxicology for CDER in an e-mail of November 20, 2008 in which he was supportive of carcinogenicity evaluation, recommending 2 species (one transgenic). Dr. Jacobson-Kram also agreed with the Division's proposed language for the mutagenicity and carcinogenicity sections of the package insert.

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/s/

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12/29/2008 11:30:11 AM
PHARMACOLOGIST



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

Supervisory Pharmacologist Memorandum

NDA NUMBER: 22-352
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 6/20/2008
PRODUCT:
 (Proposed) Trade Name: Not finalized
 Established Name: Colchicine tablets USP, 0.6 mg

INDICATION: **Familial Mediterranean Fever**

INTENDED CLINICAL POPULATION: **Adults and children**

SPONSOR: **Mutual Pharmaceutical Co., Inc.**

DOCUMENTS REVIEWED: **Primary review of Dr. Leshin; NDA 22-352 as appropriate**

REVIEW DIVISION: **Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)**

PHARM/TOX REVIEWER: **L. Steve Leshin, DVM, Ph.D.**
PHARM/TOX SUPERVISOR: **Adam Wasserman, Ph.D.**
DIVISION DIRECTOR: **Bob Rappaport, M.D.**
PROJECT MANAGER: **Margarita Tossa**

EXECUTIVE SUMMARY

I. BACKGROUND

This NDA application, filed by Mutual Pharmaceutical Co, Inc., concerns Colchicine tablets, 0.6 mg, for the treatment of individuals with Familial Mediterranean Fever (FMF) and was previously evaluated under IND 75,040. This disease is rare and the Applicant has received an Orphan designation for their product. Nonclinical studies to support the NDA were largely not conducted by the Applicant, relying almost entirely on the published literature for this 505(b)(2) application. Colchicine has been extensively used for over 100 years in the treatment and prevention of gouty arthritis, and since 1972 for FMF. Although there is no currently approved single colchicine entity there are substantial numbers of marketed (but unapproved) versions available. Additionally, the Agency has approved a combination colchicine-containing product for the gout indication (Colbenemid NDA 12-383, 1961; DESI approval 1972).

A. Regulatory Summary (Pharmacology/Toxicology)

The Applicant has several indications for which they are pursuing approval including acute gout (IND 72,586; NDA 22-351) and _____ . PIND meetings were held with the Applicant on July 31, 2006 for the Gout and FMF indications in which the nonclinical requirements for NDA submission were discussed. At that meeting the Division informed the Applicant that a carcinogenicity evaluation would be desirable but not required and that, for the NDA, the Applicant should summarize the current knowledge concerning the carcinogenic potential of colchicine. Discussion also centered on the need for, and design, of nonclinical cardiovascular safety studies. No other significant nonclinical regulatory agreements were reached.

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II. MAJOR NONCLINICAL ISSUES IDENTIFIED IN PRIMARY REVIEW

Dr. Leshin has identified a number of nonclinical issues with this NDA application for colchicine which must be weighed in any recommendation for regulatory action. These include the following:

1. Nonclinical studies are almost exclusively old, pre-date GLP regulations instituted to ensure quality and integrity, and in most cases do not use current and preferred evaluation methodologies; they were designed with dose levels to determine the effects of colchicine rather than the safety of colchicine.
2. Nonclinical studies available are almost exclusively of short duration (i.e. ≤ 5 weeks) and do not support chronic dosing;
3. Genetic toxicology studies and knowledge of the mechanism of action indicate colchicine may reasonably be expected to promote or induce neoplasia.
4. Reproductive toxicology studies conducted in nonclinical models indicate a significant risk for embryofetal harm and reduced parental fertility
5. The colchicine product used in the published studies is frequently of unknown quality and comparability when compared with the Applicant's drug product;
6. Nonclinical data provided do not provide full ability to address all standard nonclinical label sections (e.g. carcinogenicity); and,

7. There is a potential drug product photo-degradant impurity which possesses a structural alert for mutagenicity and is not adequately controlled by specifications or qualified through nonclinical studies.

The Agency has undertaken an initiative to bring marketed but unapproved drugs under NDA as well as remove those which remain marketed without regulatory approval. There is demonstrably insufficient nonclinical support provided based on current requirements and standards for a new molecular entity and though there is a regulatory stance that the standard for safety will not be lowered to approve a marketed and unapproved drug, the extensive clinical experience, including human toxicity, obtained with colchicine use over the last 100+ years must be considered when evaluating this problematic nonclinical data package. It is notable that colchicine has been the standard of care for FMF since 1972 and a nearly equivalent colchicine dosage has been in an approved combination product (Colbenemid and generics: colchicine 0.5 mg/probenecid 500 mg) used for gout (initially acute and prophylaxis, now entirely for prophylaxis) since 1961.

Although the purpose of clinical data is not to inform the nonclinical knowledge base, it is important to point out that the mechanism of action of colchicine (as a microtubule inhibitor/mitotic spindle poison) is certainly operative in all eukaryotic organisms. This is the reason that the manifestations of toxicity are so similar across species, including human, as mentioned by Dr. Leshin. This, when combined with the well-understood clinical toxicity of long-term colchicine administration precludes the need to provide modern, GLP-compliant chronic toxicology studies in animals for support of the application.

Regarding the mutagenicity, reproductive toxicity, and potential carcinogenicity of colchicine, the data in the published literature is respectively confounded (genetic toxicology), clear (reproductive toxicology), and absent (carcinogenicity). Colchicine, as a mitotic spindle poison, is not directly genotoxic, but promotes the development of aneuploidy (deviation from the normal 2N complement of chromosomes) in affected cells. This causes instability in all cells without the proper complement of 23 chromosome pairs. The severity of a developmental outcome is temporally dependent on the when and where aneuploidy occurs. Embryonic or germ line aneuploidy "causes developmental abnormalities and reduces organismal fitness in all species where this condition was examined" (Torres et al., 2008) due to imbalances in protein stoichiometry (chromosome gain) or reduced gene dosage or haplo-insufficiency (chromosome loss). Studies cited by the Sponsor and as reviewed by Dr. Leshin indicate colchicine administration in reproductively aged animals induces significant reductions in fertility through direct effects on germ cells as well as hormonal alterations supporting the embryonic environment. Teratogenic effects have been noted in multiple species with maternal exposure to colchicine. The degree and nature of the defects, not surprisingly, are dependent on the developmental stage of embryo exposure. I agree that the level of available information is acceptable for labeling the mutagenesis and reproductive toxicology sections of the package insert. Dr. Leshin recommends a registry for

pregnancy be established for colchicine in this indication. To the extent this appears appropriate with our clinical colleagues I believe this may be warranted and informative.

Colchicine has not been studied in standard rodent carcinogenicity bioassays; however, aneuploidy has for almost 100 years been considered a theoretical risk for tumorigenesis (Boveri, 1914) and this will be indicated in the package insert. Without data for evaluation, however, the published literature is the only source available to gauge the level of concern. Cancerous cells frequently are aneuploid as well as having evidence of gross chromosomal rearrangements (Storchova and Pellman, 2004) and most solid tumors display aneuploid karyotype. A series of elegant studies with genetically modified mice prone to aneuploidy indicates that this condition can paradoxically inhibit cell growth and promote apoptosis as well as provide a staging ground for tumorigenesis (Weaver et al., 2007, Sotillo et al., 2007) and that the balance may depend on the cell types and genetic contexts involved (Pellman, 2007). Weaver and colleagues (2007) demonstrated mice with genetic defects in a spindle motor protein (Centromere-associated Protein-E; *CENP-E*) have significant levels of aneuploidy due to random mis-segregation of chromosomes without direct DNA damage. Such mice have a modest increase in spontaneous spleen and lung tumors in vivo late in life though intriguingly the most common tumors in wild-type background strain are liver tumors which were significantly reduced. To investigate the effects of aneuploidy in the setting of enhanced tumor development, an evaluation of these mice through cross-mating with another group of mice deficient for a tumor suppressor gene (*P19/ARF*) demonstrated that mice progeny with defects in both mitotic spindle protein and tumor suppressor protein had longer tumor-free survival than those with a fully functioning mitotic spindle. These investigators further provided data to suggest that aneuploidy in the setting of chemically induced tumor formation is more of an inhibitor than promoter/initiator of tumorigenesis as mice heterozygous for a defective *CENP-E* protein had reduced tumor burden and incidence compared with wild-type mice after administration of the known mutagenic carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). More recently, Weaver and colleagues (2008) argued based on their 2007 work that the degree of aneuploidy produced may influence the outcome – low levels of aneuploidy promote transformation and tumorigenesis while higher levels encourage reduced cellular growth and apoptosis. This may not be surprising as suppressing the ability of a mutated and transformed cell to divide by mitotic spindle impairment may be expected to inhibit tumor progression. Colchicine, as a whole-chromosome aneuploidy inducer is not mutagenic itself, therefore it may be argued that exposure may be more akin to the effect of *CENP-E*^{+/-} heterozygous mice in the absence of direct application of mutagens as initiating factors. However, as humans are continually exposed to random mutational events as well as direct mutagens, the data from DMBA-exposed mice may be the most appropriate. Evaluation of colchicine in a standard 2-year rat bioassay along with a 6-month transgenic mouse study may give information to address both possibilities, the latter study being considered to be conducted in a genetically “initiated” model, such that transgenic mice have an increased susceptibility for carcinogens. Although Dr. Leshin notes a long-term nonclinical study in which rodents developed respiratory difficulties over time with continual low-dose exposure to colchicine in drinking water, I am not convinced long-term studies (or in the case of a transgenic study, 6 months) – properly conducted with careful dose range-

finding to support doses – cannot be successfully performed. While I agree with Dr. Leshin that there may be some benefit in requesting a registry for malignancy with use of the product should the gout indications be approved at a later date, the incidence of FMF is rare enough in the general population that an epidemiologic evaluation for increased cancer incidence may be exceedingly difficult and further argues for nonclinical evaluation where this may be possible.

The potential presence of photo-degradants bearing a structural alert for mutagenicity (identified as β - and γ -lumicolchicine) has not been adequately addressed at this time. While the Applicant has not observed these degradants in the drug product, they have not developed detection methods which are sensitive enough to preclude such impurities being above 1.5 $\mu\text{g/day}$ total daily intake (TDI), the current standard which the Agency follows for approval of an NDA (McGovern and Jacobson-Kram, 2006) and a level which has been proposed by the EMEA and PhRMA. It is notable from Dr. Craig Bertha's CMC review, that the packaging of the drug product in HDPE bottles or _____ (the latter form for _____) should be sufficiently protective from light such that significant light exposure and resulting photo-degradant development should not occur and the Applicant appears aware of this as they based their choice of packaging on this reasoning. Dr. Leshin notes that the marketed and unapproved colchicine products available currently as well as the approved Colchicine combination products may also contain these degradant impurities and these levels are not known at this time. On balance, and with a drug product that produces whole chromosome loss/gain, this does not appear to be a substantial approval issue. I agree with Dr. Leshin, however, that the Applicant should develop an improved assay to provide for a lower limit of detection as a post-marketing requirement such that the total levels of these photo-degradants will not exceed 1.5 $\mu\text{g/TDI}$. However, I do not believe it necessary to conduct a 28-day repeat-dose toxicology study as the concern is mutagenicity/genotoxicity which is addressed by lowering of the specifications or completion of the genotoxicity assays.

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III. ADVISORY COMMITTEE ISSUES *(if appropriate)*

N/A

IV. RECOMMENDATIONS

A. Recommendation on approvability

I concur with Dr. Leshin's recommendation that the application may be approved pending agreement on labeling.

B. Recommendation for nonclinical studies

There are no additional nonclinical studies considered necessary for taking regulatory action on the current NDA. However, the following nonclinical studies are recommended as post-marketing requirements:

1. The Applicant must evaluate the potential carcinogenicity of colchicine in two rodent species. Studies may consist of a 2-year bioassay in rat and a 6-month

transgenic study in an appropriate mouse model. The Applicant is strongly encouraged to submit protocols for Agency concurrence on study design prior to initiation of studies.

2. The Applicant must improve detection assays to allow reduction of the specifications for the photo-degradant impurities β - and γ -lumicolchicine to ensure a limit of NMT 1.5 μg TDI for the combined degradants. Alternatively, the Applicant may conduct genetic toxicology studies which, if negative, would support the current proposed specifications.

C. Recommendations on labeling

I am in agreement with the proposed changes to the label described in Dr. Leshin's review. The language regarding mutagenicity and carcinogenicity was discussed with Dr. David Jacobson-Kram, Associate Director for Pharmacology/Toxicology. Nonclinical sections of the label remain to be discussed within the review team and negotiated with the Applicant.

REFERENCES

- Boveri T. Zur Frage der Entstehung maligner Tumoren (The Origin of Malignant Tumors)(Jena:Gusav Fischer). 1914.
- European Medicines Evaluation Agency, Committee for Medicinal Products for Human Use (CHMP), Guideline on the limits of genotoxic impurities, CPMP/SWP/5199/02, London, UK, 23 June 2004.
- L. Muller, R.J. Mauthe, C.M. Riley, M.M. Andino, D. De Antonis, C. Beels, J. DeGeorge, A.G.M. De Knaep, D. Ellison, J.A. Fagerland, R. Frank, B. Fritschel, S. Galloway, E. Harpur, C.D.N. Humfrey, A.S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D.K. Ness, M.R. O γ Donovan, M.D. Smith, Vudathala, L. Yotti, A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity. *Regul. Toxicol. Pharmacol.* 44: 198-211, 2006.
- McGovern T, Jacobson-Kram, D. Regulation of genotoxic and carcinogenic impurities in drug substances and products. *Trends in Analytical Chemistry* 25(8): 790-795, 2006.
- Pellman D. Cell biology: aneuploidy and cancer. *Nature* 446(7131): 38-9, 2007.
- Sotillo R, Hernando E, Diaz-Rodriguez E, Teruya-Feldstein J, Cordon-Cardo C, Lowe SW, Benzra R. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. *Cancer Cell* 11(1) 9-23, 2007.
- Storchova Z, and Pellman D. From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 5(1): 45-54, 2004.
- Torres EM, Williams BR, Amon A. Aneuploidy: cells losing their balance. *Genetics* 179(2): 737-46, 2008.
- Weaver BA, Silk AD, Cleveland DW. Low rates of aneuploidy promote tumorigenesis while high rates of aneuploidy cause cell death and tumor suppression. (Comment) *Cell Oncology* 30(5):453.
- Weaver BA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* 11(1): 25-36, 2007.

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/s/

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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: **22-352**

SERIAL NUMBER: **000**

DATE RECEIVED BY CENTER: **June 20, 2008**

PRODUCT: **Colchicine**

INTENDED CLINICAL POPULATION: **Familial Mediterranean Fever**

SPONSOR: **Mutual Pharmaceutical Co., Inc.**

DOCUMENTS REVIEWED: **eCTD Module 4**

REVIEW DIVISION: **Division of Anesthesia, Analgesia, and
Rheumatology Drug Products (HFD-170)**

PHARM/TOX REVIEWER: **L.S. Leshin**

PHARM/TOX SUPERVISOR: **A. Wasserman**

DIVISION DIRECTOR: **B. Rappaport**

PROJECT MANAGER: **M. Tossa**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Approve

B. Recommendation for nonclinical studies:

The photodegradant impurities contain structural alerts for mutagenicity and therefore need specifications set to maintain daily intake at less than 1.5 µg/day. If that is not possible, qualification studies (genetic toxicology and a 28-day repeated dose study) are necessary. These photodegradants are not detected in the clinical product at the current levels of impurity detection. The currently marketed *approved* generics of *Colbenemid*, and the numerous marketed, but *unapproved colchicine only products* have the same potential of containing these impurities, and should all be limited with regards to these impurities. Since these have been on the market for years, this reviewer recommends lowering of specifications or qualification studies could be done postmarketing.

C. Recommendations on labeling: refer to the Table below

Reviewer's Comment on Labeling:

The Sponsor provided summaries and published literature to support labeling. There are no toxicological data that provide nonclinical NOAEL values as a guideline for colchicine use. Most nonclinical toxicological findings were close to or within the dose range of clinical therapeutic use, there is a very low threshold between symptomatic adverse effects and lethal doses for animals and humans. Recommendations by the Maternal Health Team are indicated in red.

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

 § 552(b)(4) Draft Labeling

 § 552(b)(5) Deliberative Process

II. Summary of nonclinical findings

A. Regulatory Background

This NDA is being submitted by Mutual Pharmaceutical Co., Inc under Section 505(b)(2) of the Federal Food, Drug and Cosmetic Act relying on publicly available information, supplemented by nonclinical pharmacology studies performed by the Applicant. They developed colchicine tablets USP, 0.6 mg for the treatment of children and adults with Familial Mediterranean Fever (FMF). An Orphan Drug Designation was granted for this indication (ODD 07-2458, Sept 25 2007). Mutual requested and obtained priority review status for this NDA since treatment of FMF is an unmet medical need with serious morbidity and mortality associated with inadequate treatment.

Colchicine has been in use in the United States as a single active ingredient since prior to 1938 (and likely before 1908). Despite its extensive use primarily in the treatment and prevention of acute attacks of gout, colchicine as a single use product has not been approved by the FDA. All previous approved products were a combination of colchicine and probenecid (ColBenemid, NDA 12-383, combination of colchicine 0.5 mg and probenecid 500 mg, up to four tablets (i.e. 2.0 mg colchicine) daily for the management of chronic gout; approved in 1961 followed by DESI approval in 1972 Fed Reg 37, no. 146, p. 15189; July 28, 1972). This application, if approved, would represent the first approved colchicine-only product.

This NDA is the outcome of meetings with the Applicant concerning colchicine use for gout (IND 72,586), FMF (PIND 75,040), and _____ . A PIND meeting was held in July 31 2006 which included a discussion of the nonclinical program requirements for colchicine's development for all the above indications. An IND to conduct clinical studies for FMF was never submitted or initiated since it was agreed the Applicant could submit published clinical studies for support of the FMF indication (refer to the Clinical Review of Dr. Hull).

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Nonclinical information for all three indications was submitted to IND 72,586 to support the gout clinical program. This included a literature review of colchicine toxicology and Sponsor-conducted cardiovascular safety studies and genetic toxicology studies. Upon review, the Division determined that there was sufficient nonclinical pharmacology and toxicology information to enable labeling of nonclinical sections for which clinical information is lacking. The Division agreed that carcinogenicity studies would not be necessary for the NDA. Drug interaction studies involving cytochrome P450 (CYP) metabolism, inhibition, and induction are reviewed in the Clinical Pharmacology Review.

B. Brief overview of nonclinical findings

With a long clinical history for the use of colchicine, the Applicant submitted published nonclinical literature to support the NDA. In addition they conducted genetic toxicology and cardiovascular safety toxicology studies. The genetic toxicology studies

were conducted to address potential issues that might arise concerning a conformational isomer impurity that exists in equilibrium with colchicine. The cardiovascular safety studies were conducted because the Applicant determined there was insufficient information available to adequately assess cardiovascular safety. Both types of studies were initiated by the Applicant before review and discussion of the development program.

General toxicology

Historical information provided by the Applicant and obtained from published animal studies and human use indicates similar toxicities with increasing doses. However, the published nonclinical literature contains inadequate long-term toxicology studies. Almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely collected such as clinical pathology and histopathology findings. In general, the acute toxic signs in animals (rats, dogs, rabbits, cats) with short-term colchicine administration are gastrointestinal tract-related and include emesis, distended intestines, diarrhea (bloody in more severe cases), lack of appetite and lethargy. With increasing doses these signs become more severe, and there is a loss of body tone, abnormal gait and hindlimb paralysis and wasting atrophy, ascites and eventually death. For comparison, in humans, at sufficient doses, colchicine can produce gastrointestinal disorders, profound muscle weakness, respiratory insufficiency, and peripheral neuropathy.

In the Applicant's cardiovascular safety dose-range finding study in rabbits, administration of a single daily colchicine dose of 1.5 mg/kg (divided into three smaller intravenous doses administered 15 min apart) resulted in no clinical signs or mortality, but 3.0 mg/kg total dose (also administered as three 1 mg/kg doses) resulted in deaths by the following day. In the cardiovascular safety dose-range finding study in dogs, deaths occurred at an oral dose of 0.9 mg/kg, but not 0.45 mg/kg. For comparison, in humans, fatalities have resulted from colchicine doses between 0.5 to 0.8 mg/kg (30 to 48 mg for a 60 kg individual) with 100% fatality at doses greater than 0.8 mg/kg.

Cardiovascular Toxicology

In the cardiovascular safety studies, colchicine at 10 μ M (4 μ g/mL) produced no significant inhibition of hERG (K^+ repolarization) current. In anesthetized rabbits administration of 9 mg/kg colchicine intravenously as three 3 mg/kg doses 30 min apart, resulted in a dose-related decreased heart rate (12, 18 and 22%), decreased body temperature (2, 3, and 4%) and increased QTc (7, 9 and 13%), with each 3 mg/kg dose administered, respectively. However, this dose exceeds the lethal dose as noted in the above and the significance of these changes is unclear. In the conscious telemetered dog, oral doses of colchicine (0.1, 0.3 and 0.5 mg/kg) administered at 1 week intervals resulted in no significant cardiovascular or ECG findings, including QTc. Plasma colchicine concentrations were 7.6 to 49.2 ng/mL at the 1 hour postdose timepoint (highest of the 1 and 4 hour timepoints of sample collection). **For comparison, the Applicant's pharmacokinetic studies with healthy adult humans found lower mean colchicine**

concentrations, 3.6 and 3.1 ng/mL after 10 and 14 days, respectively, of 0.6 mg b.i.d. doses. The Applicant's study of ECG in human volunteers receiving colchicine also did not indicate an effect on QTc.

Genetic Toxicology

The Applicant conducted assays to determine the mutagenic and clastogenic potential of purified colchicine which contains a conformational isomer impurity that exists in equilibrium with colchicine at approximately a 1% concentration and cannot be eliminated. The bacterial reverse mutation (Ames) assay was negative at doses up to 5000 µg/plate, with or without the presence of metabolic enzymes. A chromosomal aberration assay with human white blood cells (enriched lymphocytes) was negative at doses up to those that disrupted mitosis. At the highest concentrations, 860 and 2000 ng/mL, an increased proportion of cells exhibited mitotic disruption in the form of elevated mitotic index (mitotic arrest) and centromeric disruption (dissociated chromatids). These are not considered clastogenic effects.

Published studies reported colchicine was positive in mutagenic (*in vitro* mouse lymphoma thymidine kinase [TK] assay) and clastogenic (*in vitro* mammalian cell micronucleus assay *in vivo* in mice, hamsters, and rats) assays. In retrospect, these were probably false positives, a result of the cellular proliferation essential for these assays. The study of Honma et al 2001 determined that the mutagenic effects in the mouse lymphoma TK assay was due to loss of a functional *tk* allele generated by the loss of the entire chromosome 11 where the *tk* gene resides. There were no mutants with structural changes such as deletions or translocations involving chromosome 11. The mutations described in these assay arose through mitotic nondisjunction without structural DNA changes. In the published clastogenicity assays, cells with micronuclei were counted, but the chromosomes were not examined for signs of clastogenicity, rather only micronuclei were counted. The study of Jie and Jia (2001) examined the micronuclei and found they were composed mainly of whole chromosomes. These findings are consistent with colchicine's well characterized induction of aneuploidy. The conformational isomer did not alter the genotoxic results compared to literature reported effects. In retrospect, all colchicine preparations probably contained this isomer. Micronuclei can arise from acentric fragments induced by substances causing chromosomal breakage (clastogens) as well as from whole lagging chromosomes induced by those causing aneuploidy.

Reproduction and Developmental Toxicology

Colchicine disruption of microtubule formation results in reproductive and developmental toxicity by cells involved in meiosis and mitosis. The effects are species and dose dependent, with the timing of exposure also critical for the effects on embryonic development. In general, published nonclinical studies indicated adverse effects on sperm development and fertility, early embryonic development and implantation, organogenesis (teratology), and late-stage embryonic development. In males, colchicine interfered with seminiferous tubule fluid secretion, testosterone production/release from rat Leydig cells, and disrupted microtubules in Sertoli cells in the epididymides, resulting

in abnormal sperm production. In females, colchicine administration can result in eggs with Y chromosomes, and interfere with sperm penetration, the second meiotic division, and normal cleavage, and produce triploid and mosaic embryos. Published nonclinical studies demonstrated that colchicine is embryo-lethal early in development, is associated with the production of skeletal abnormalities during organogenesis, and causes slow embryofetal development. Published studies indicate that colchicine-mediated microtubular disruption inhibits the secretion of various hormones. It also can reduce milk yield and alter milk composition (reduced fat content).

C. Pharmacologic activity

Colchicine binds to the intracellular protein tubulin, preventing its alpha and beta forms from polymerizing into microtubules. This disruption of the microtubular network results in impaired protein assembly in the Golgi apparatus, decreased endocytosis and exocytosis, altered cell shape, depressed cellular motility and arrest of mitosis. Of particular importance to FMF, colchicine also interferes with the formation of the inflammasome, a newly appreciated and identified cellular structure involved in the production of inflammatory-related cytokines.

D. Nonclinical safety issues relevant to clinical use

Findings of nonclinical toxicology closely match those known historically from clinical colchicine use. Published clinical studies submitted in support of this NDA, lacked for the most part, the toxicities of colchicine that are observed with higher doses and overdosing. These toxicities have been described in abundance in review articles and medical databases. Colchicine has a very narrow therapeutic window, with human deaths reported at doses not much greater than therapeutic doses. Comparing human lethality with the limited nonclinical data at nonlethal doses, indicates that human deaths have been reported at doses lower than those that affect rodents, implying that NOAEL determinations may not provide a useful margin of safety. A direct NOAEL comparison could not be conducted since for the most part, nonclinical studies were not conducted to identify a NOAEL, but to identify toxicities.

Genetic toxicology studies indicate that colchicine treatment results in aneuploid cells through mitotic or meiotic non-disjunction, but colchicine is not considered mutagenic or clastogenic although results from these assays often result in positive results (a false positive finding, from different mechanism leading to a similar result). The significance of aneuploidy toward carcinogenic potential in comparison with a pure clastogenic mechanism cannot be quantitatively assessed. However, most tumors consist of aneuploid cells and both mechanism can result in tumors (Weaver et al 2007, *Cancer Cell* 11:25-36; Torres et al 2008, *Genetics* 179:737-746).

Carcinogenicity studies have not been requested due to the long history of clinical experience, although specific documentation of any relationship between colchicine use and carcinogenicity is lacking. They are still possible options if clinical findings from expanded safety surveillance result in signals for further study. From the few repeated

dose studies reported in the literature (possibly the same studies conducted for the original approval of colchicine/probenecid combination in 1961, original reviews were not found), there was a low threshold between adverse effects and the lethal dose in studies of less than 1 month duration in rats. With low doses of colchicine administered in drinking water of spontaneously hypertensive and nonhypertensive rats, respiratory difficulties developed within 4 to 13 months (Cicogna et al 1997). While there may be greater susceptibility in this strain, overall studies do not provide much confidence that a 2 year oral study in rats or mice would be productive. A study was conducted with dermally applied colchicine twice weekly in mice for 6 months, and this could be conducted with transgenic mice, but those mice would not be an appropriate model for an orally ingested drug since they have a high spontaneous background rate of internal tumor formation that may confound and mask colchicine induced effects. The reviewer recommends that the Applicant maintains a record of malignancies in patients on colchicine therapy for eventual comparison with population cancer rates.

GLP studies of reproductive and developmental toxicology and carcinogenicity have not been conducted. For reproductive and developmental toxicology, there is sufficient information from published literature to convey the risk in the label. Furthermore there are recent clinical epidemiology studies of pregnancies in colchicine treated women with FMF that have not found detrimental effects that could be attributed to colchicine, but there were limited number of pregnancies studied. The reviewer recommends that the Applicant maintains a record of males and female reproductive problems, pregnancies, and their outcomes for eventual comparison with population data to determine if adverse events occur at a greater rate than the general population.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

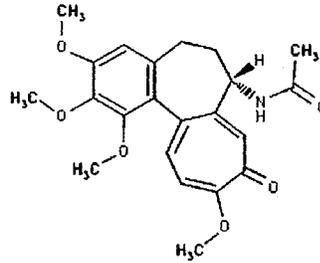
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-352
Review number: 1
Sequence /date/type of submission: 000/June 20, 2008/505(b)(2)
Information to sponsor: No
Sponsor and/or agent: Mutual Pharmaceutical Co., Inc.,
 Philadelphia, PA
Manufacturer for drug substance: for DMF

b(4)

Reviewer name: L.S. Leshin
Division name: Division of Anesthesia, Analgesia and
 Rheumatology Products.
HFD 170
Review completion date: Nov 18, 2008

Drug:
 Generic name: **Colchicine**
 Code name: 4/TP5011
 Chemical name: (S)-N-[5,6,7,9-Tetrahydro-1,2,3,10,
 tetramethoxy 9-oxobenzo(a)heptalen-7-yl]
 acetamide;
 and
 acetamide, N-[5,6,7,9-tetrahydro-1,2,3,10-
 tetramethoxy 9-oxobenzo[a]heptalen-7-yl],
 (S)-
 CAS registry number: 64-86-8
 Molecular Formula: C₂₂H₂₅NO₆
 Molecular Weight: 399.43
 Structure:



Relevant INDs/NDAs/DMFs:

NDA 12,383 (Colbenemid, Colchicine + Probenecid; Merck; approved 1961 and DESI 1972; indicated for the treatment of gout)

PIND 75040 (colchicine; for the treatment of Familial Mediterranean Fever)
 IND 72586 (colchicine; received Feb 12, 2007; for gout)

b(4)

Drug class:

Spindle poison (mitotic inhibitor, blocks tubulin polymerization to form into microtubules)

Intended clinical population:

Adults and children greater than 4 years of age with Familial Mediterranean Fever (FMF), specifically for the _____

b(4)

Route of administration:

Oral

Clinical formulation:

Colchicine Tablets USP, 0.6 mg

The recommended dosage of colchicine:

- Adults and adolescents older than 16 years of age is 1.8 mg daily. Colchicine should be increased as needed to control disease and as tolerated in increments of 0.3 mg/day to a maximum recommended daily dose of 2.4 mg. If intolerable side effects develop, the dose should be decreased in increments of 0.3 mg/day.
- Pediatric patients 4 years of age and older is based on age (doses may be given once or twice daily) as follows:
 - Children 4 – 6 years: 0.3 to _____ daily
 - Children > 6 – 12 years: _____ daily
 - Adolescents > 12 – 16 years: _____ daily

b(4)

The drug product is a purple film-coated, capsule-shaped tablet _____ debossed 'AR 374' on one side and scored on the other side. The tablet may be split, if necessary, to achieve lower doses.

The components and quantitative composition of the drug product are listed in Table 2.3.P:1.

Reviewer's Comment: The excipients are acceptable and do not require additional toxicological testing.

**Table 2.3.P:1
Composition of Colchicine Tablets USP, 0.6 mg**

Ingredient	mg/Tablet	% w/w	Function
Colchicine active ingredient	0.60 ¹		Active ingredient
Lactose monohydrate, NF			
Pregelatinized starch, NF			
Microcrystalline cellulose, NF			
Sodium starch glycolate, NF			
Magnesium stearate, NF			
Caruba Wax, NF			
Total			

b(4)

¹ Registered trademark of _____ contains FD&C Blue #2 _____ FD&C Red #40 _____ hypromellose, polydextrose, polyethylene glycol, titanium dioxide, and triacetin. The quantitative composition is provided in Module 3.

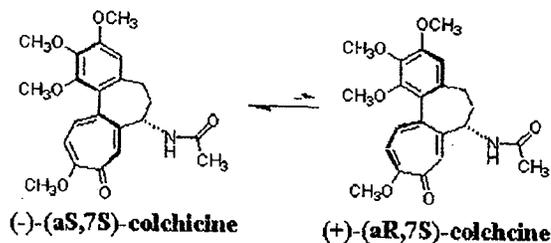
Impurities/Degradants

Colchicine, is extracted and purified from the seeds of *Gloriosa superba* Linn, of the Liliaceae family. Three cGMP batches of drug product (batches BB 374 0215, BB 374 0217, BB 374 0218) were manufactured by Mutual in November 2006. All three batches were manufactured using colchicine, USP drug substance lot COL 0906004 from _____

b(4)

Isomers: Natural colchicine exists in a predominantly [(-)*aS*, 7*S*] form with aromatic ring moieties (rings A and C) arranged in a counterclockwise helicity, and as the conformational isomer [(+)*aR*, 7*S*] form with a clockwise helicity (Impurity B in the British Pharmacopoeia). The conformational isomer _____ and an _____ . Thus it is present in the drug substance at a level of approximately _____. The conformational isomer is present in the 3 registration batches at a level of _____ with a specification limit set at _____

b(4)



Reviewer comment: We previously agreed at the preNDA meeting that qualification studies of the conformational isomer would not be necessary. Due to its existence in equilibrium with colchicine, it would not be possible to perform qualification studies using either the impurity alone or to use drug substance spiked with the impurity. The Sponsor demonstrated that the conformational isomer attains complete equilibrium in about _____

_____ The Sponsor noted that the isomer lacks the tubulin-binding properties of colchicine based on structural spatial analysis of molecular interaction between colchicine and tubulin binding sites (described in the following paragraph), but this has not been demonstrated empirically.

b(4)

The conformational analysis colchicine forms and circular dichroic evaluation of the interaction of these compounds with tubulin (Brossi, 1990), demonstrated that the colchicine binding to tubulin requires a specific stereochemical arrangement of the A and C rings. The αS isomer of colchicine binds to the C-domain on the β-subunit of tubulin, with the appropriate torsion angle between rings A and C (about 53 degrees) required for binding. Because the αR conformer has the opposite helicity as that of tubulin, this colchicine isomer is expected to have only a low affinity for tubulin binding, and would not be expected to have appreciable pharmacologic or toxicologic activity (Hastie, 1991).

_____ that exceeded recommended specifications during the IND phase for a Gout indication (conducted by the same Applicant) was _____ (also known as _____ This has since been reduced and is currently below the detection limits in the clinical batches.

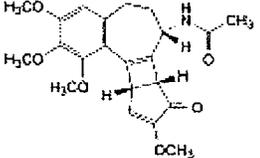
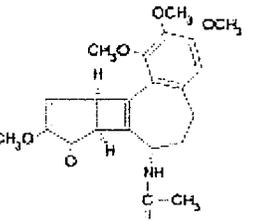
b(4)

Gamma- and Beta-Lumicolchicine: There are two potential photodegradants impurities which contain a structural alert for mutagenicity (see table below), however they were not identified in the clinical product.

Reviewer' Comment: The photodegradant impurities contain structural alerts for mutagenicity and therefore need specifications set to maintain daily intake at less than 1.5 µg/day. If that is not possible, qualification studies (genetic toxicology and a 28-day repeated dose study) are necessary. These photodegradants are not detected in the clinical product at the current levels of impurity detection, due to package protection from light. The currently marketed approved generics of Colbenemid, and the numerous marketed, but unapproved colchicine only products have the same potential of containing these impurities, and should all be limited with regards to these impurities. Since these

have been on the market for years, this reviewer recommends lowering of specifications or qualification studies could be done postmarketing.

Structural Alerts for Mutagenicity

<p>β-Lumi-colchicina</p>	<p>N-[(7S,7bR,10aS)-1,2,3,9-tetramethoxy-8-oxo-5,6,7,7b,8,10a-hexahydrobenzo[α]cyclopenta[3,4]cyclobuta[1,2-c]cyclohepten-7-yl]acetamide</p>		<p>Photo-degradation product of colchicinas</p>
<p>γ-Lumi-colchicina</p>	<p>N-[(7S,7bR,10aS)-1,2,3,9-tetramethoxy-8-oxo-5,6,7,7b,8,10a-hexahydrobenzo[α]cyclopenta[3,4]cyclobuta[1,2-c]cyclohepten-7-yl]acetamide</p>		<p>Photo-degradation product of colchicinas</p>

Regulatory Background

This NDA is being submitted by Mutual Pharmaceutical Co., Inc under Section 505(b)(2) of the Federal Food, Drug and Cosmetic Act relying on publicly available information, supplemented by nonclinical pharmacology studies performed by the Applicant.. They developed colchicine tablets USP, 0.6 mg for the treatment of children and adults with Familial Mediterranean Fever (FMF). An Orphan Drug Designation was granted for this indication (ODD 07-2458, Sept 25 2007). Mutual requested and obtained Priority review status for this NDA since treatment of FMF is an unmet medical need that is clinically significant given the serious morbidity and mortality associated with lack of, or suboptimal, treatment.

Colchicine has been in use in the United States as a single active ingredient since prior to 1938 (and likely before 1908). Despite its extensive use primarily in the treatment and prevention of acute attacks of gout, colchicine as a single use product has not been approved by the FDA. All previous approved products were a combination of colchicine and probenecid (ColBenemid, NDA 12-383, combination of colchicine 0.5 mg and probenecid 500 mg, up to four tablets (i.e. 2.0 mg colchicine) daily for the management of chronic gout; approved in 1961 followed by DESI approval in 1972 Fed Reg 37, no. 146, p. 15189; July 28, 1972). This application, if approved, would represent the first approved colchicine-only product.

This NDA is the outcome of meetings with the Applicant concerning colchicine use for gout (IND 72,586), FMF (PIND 75,040), and _____ . A PIND meeting was held in July 31 2006 which included a discussion of the nonclinical program requirements for colchicine’s development for all the above indications. An IND to conduct clinical studies for FMF was never submitted or initiated since it was agreed the

b(4)

Applicant could submit published clinical studies for support of the FMF indication (refer to the Clinical Review of Dr. Hull).

Nonclinical information for all three indications was submitted to IND 72,586 to support the gout clinical program. This included a literature review of colchicine toxicology and Sponsor-conducted cardiovascular safety studies and genetic toxicology studies. Upon review, the Division determined that there was sufficient nonclinical pharmacology and toxicology information to enable labeling of nonclinical sections for which clinical information is lacking. The Division agreed that carcinogenicity studies would not be necessary. Drug interaction studies involving cytochrome P450 (CYP) metabolism, inhibition, and induction are reviewed in the Clinical Pharmacology Review.

Injectable Colchicine: On Feb 6, 2008, a news release by the FDA indicated that the Agency would take enforcement action against companies marketing injectable colchicine (this route of administration has never been approved for any colchicine-containing product) after receiving 50 reports of adverse events, including 23 deaths, associated with its use. Injectable colchicine has been manufactured or compounded independently and used to treat gout.

Data reliance for (b)(2) applications:

Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-352 are owned by Mutual Pharmaceutical Co., Inc. or are data for which Mutual Pharmaceutical Co., Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-352 that Mutual Pharmaceutical Co., Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Mutual Pharmaceutical Co., Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-352.

Studies reviewed within this submission:

Report Number / Location	Title
Safety Pharmacology	
Cardiovascular	
0406LU21.001 Module 4.2.1.3 Also in IND 72586, SD-44, April 30, 2008	Dose-Range-Finding (Pyramid) Intravenous Toxicity Study of Colchicine in Rabbits
1235LU21.001 Module 4.2.1.3 Also in IND 72586, SD-44, April 30, 2008	Effects of Colchicine on Electrocardiogram, Heart Rate and QTc in Anesthetized Rabbits
0433DU21.001 Module 4.2.1.3 Also in IND 72586, SD-44, April	Dose-Range-Finding (Pyramid) Oral Toxicity Study in Dogs with Colchicine

30, 2008	
1259DU21.001 Module 4.2.1.3 Also in IND 72586, SD-44, April 30, 2008	Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs
1273HU21.001 Module 4.2.1.3 Also in IND 72586, SD-44, April 30, 2008	Effects of Colchicine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Genetic Toxicology	
In vitro	
MPC-004-07-0002 Module 4.2.3.3.1	Colchicine: Bacterial Mutation Test
MPC-004-07-0003 Module 4.2.3.3.1	Colchicine: Chromosome Aberration Test

Studies not reviewed within this submission:

Full text publications considered relevant to nonclinical safety and pharmacology were submitted and listed in the Appendix. Of the 169 publications, 49 publications pertaining to the safety of colchicine were incorporated into the Applicant's review of the literature.

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

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Colchicine binds to the intracellular protein tubulin, preventing its alpha and beta forms polymerizing into microtubules. This disruption of the microtubular network throughout the cell results in impaired protein assembly in the Golgi apparatus, decreased endocytosis and exocytosis, altered cell shape, depressed cellular motility and arrest of mitosis usually in metaphase. Since its dissociation is slow, its action is essentially irreversible. The conformational structure of colchicine is required for tubulin binding and its three stereoisomers do not bind to tubulin.

Traditionally, it has been thought that the anti-inflammatory response of colchicine was due to its prevention of activation, prevention of secretory functions, and migration of neutrophils *via* a mechanism that involves preventing β -tubulin polymerization into microtubules and the disruption of cytoskeletal functions. Recently colchicine was found to interfere with the formation of the inflammasome, a newly identified cellular structure involved in the production of inflammatory-related cytokines.

2.6.2.2 Primary pharmacodynamics

MECHANISM OF ACTION

Colchicine binds to the β subunits of tubulin $\alpha\beta$ heterodimers, at the interface with α subunits to the helical portion of tubulin which prevents tubulin polymerization into microtubules. High affinity binding requires that colchicinoids exhibit the proper stereochemical arrangement of the A and C rings. The affinity of colchicine for the colchicine site on tubulin is directly related to the effectiveness in inhibition of microtubule polymerization. The dissociation of colchicine from tubulin is slow and therefore its action is considered essentially irreversible. Bound colchicine alters the lateral contacts between tubulin subunits preventing the straightening of the curved tubulin shape. With a low concentrations of colchicine the number of missing lateral contacts is small and the microtubule mass is preserved, although some of the normal cellular functions might be disrupted. At high colchicine concentrations, the proportion of missing lateral contact increases, the ends destabilize, and the microtubule mass disassembles and disappears from the cell. This disruption of the microtubular network impairs a variety of cellular processes that include protein assembly in the Golgi apparatus, formation of the inflammasome complex (discussed in the next section), endocytosis and exocytosis, cell shape, cellular motility, and mitotic and meiotic cellular division.

DRUG ACTIVITY RELATED TO PROPOSED INDICATION

Colchicine effectiveness in ameliorating symptoms in FMF occurs by blocking the processes of inflammation and amyloidosis. The anti-inflammatory response of

colchicine is partly attributed to the prevention of activation, degranulation, and migration of neutrophils *via* a mechanism that involved β -tubulin binding and the disruption of cytoskeletal functions. Recent studies suggest that colchicine disrupts the formation of an inflammasome (or pyroprotosome) complex of proteins through disruption of cytoskeletal function. The inflammasome is primarily present in neutrophils and monocytes and is involved internal cellular surveillance. When activated by the interaction with pyrin protein, this complex activates caspase 1 which then cleaves portions of the inactive cytokines pro-IL-1 β and pro-IL-18 to produce the active proinflammatory cytokines IL-1 β and IL-18.

Pyrin protein (encoded by the MEFV gene, MEditerranean FeVer) is mutated as a result of the genetic defect in FMF subjects. There are two hypothesis as to the mechanism of pyrin's effect. In the sequestration hypothesis, pyrin competitively binds an accessory protein ASC through its pyrin domain sequence and pro-caspase-1 through another domain B30.2 and prevents them from being incorporated into the inflammasome. In the pyrin inflammasome hypothesis, pyrin forms its own inflammasome through binding ASC and another unidentified adaptor protein resulting in caspase-1 activation. With human pyrin mutations it is not clear if caspase activation is constitutively turned-on, but colchicine appears to prevent the inflammsome complex from either forming or functioning, resulting in a reduction of proinflammatory cytokines. (See reviews by Simon and van der Meer 2007: *Am J Physiol Reg Integ Comp Physiol* 292:R86-R98; and McDermott and Tschopp 2007 *Trends in Molecular Med* 13: 381-388).

Other functions of pyrin and the inflammasome that may be involved are also under active investigation. These include pyrin's role in the NF-kappa B cell-signaling cascade, and involvement of the inflammasome in the suppression of genes involved in chemotaxis (*e.g.*, eNOS3) and fiber deposition.

Anti-inflammatory Activity of Colchicine Metabolites

The anti-inflammatory activity of two primary metabolites of colchicine, 2-demethylcolchicine (2-DMC) and 3-demethylcolchicine (3-DMC), were tested *in vivo* using the rat carrageenin-induced footpad edema model (100 μ g injected of each compound/foot). 2-DMC did not inhibit edema but 3-DMC was as effective as colchicine. Specifically, at 3 and 5 hours after injection, 3-DMC inhibited edema by 39 and 47% and colchicine inhibited edema by 44% and 53%.

Reviewers Comment: As indicated in the partially reproduced Table from Sugio et al 1987 below, 2-DMC, while not inhibiting edema, had substantial (50%) binding to tubulin.

Colchicine analogs (100 µg/foot)	Carrageenin edema ^b (% of inhibition)		Tubulin binding in vitro (%) ^c
	3 hr	5 hr	
Colchicine (1)	44**	53**	90
1-Demethylcolchicine (2)	30**	46**	26
2-Demethylcolchicine (3)	-6	-11	50
3-Demethylcolchicine (4)	39**	47**	68

Inhibition of Amyloidosis

In various mouse models, amyloidosis is ameliorated by colchicine. Colchicine blocks amyloid enhancing factor (AEF)-induction of amyloid A protein by blocking the production of amyloid enhancing factor, and in the later, inflammatory stage, blocks amyloid fibril deposition.

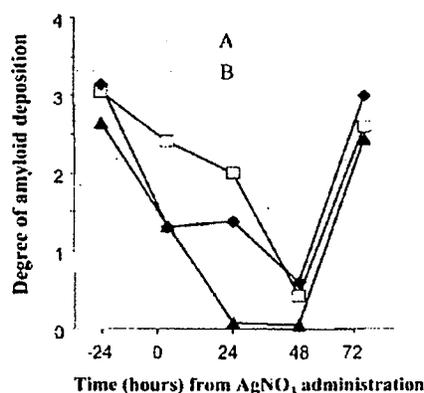
Brandwein et al (1985) demonstrated that colchicine administration inhibited amyloidosis induced in CBA/J mice during chronic inflammation caused by repeated injections of silver nitrate. Colchicine (10 µg, ~0.4 mg/kg, i.p.) was administered daily for 3 days then co administered for the next 25 days with silver nitrate (0.2%, 0.5 mL; s.c). Amyloid A (AA) protein levels (which correlate with amyloid deposition) was then measured in the spleen. Silver nitrate treatment induced deposition of 667 ± 68 ng of AA/mg of pooled splenic tissue in control mice. Treatment with colchicine lowered AA levels to 12 ± 1 ng/mg of spleen ($P < 0.001$). Serum AA levels declined similarly in both silver nitrate and control treatment groups suggesting that serum AA decline was not the primary event inhibiting amyloid deposition. When preformed amyloid enhancing factor was administered in a model of accelerated amyloid deposition, colchicine only partially prevented the amyloid deposition (control: 974 ± 46 ng AA/mg vs. colchicine 578 ± 91 ng AA/mg spleen). The authors concluded that colchicine inhibited amyloidosis in the predisposition phase, possibly by blocking the formation of amyloid-enhancing factor.

Using the silver nitrate induced chronic inflammatory condition in mice, Shtrasburg et al (2001) found that colchicine prevented amyloid deposition after inflammation has been established. Male Swiss mice were injected with exogenous amyloid enhancing factor (1 µg, i.v.) either prior to or together with silver nitrate (2%, 0.5 mL, s.c.). Silver nitrate was injected daily for 3 days concomitantly with AEF as the "standard protocol" and in another study of prolonged induction, silver nitrate was administered either 24 hours or 7 days after administration of amyloid enhancing factor. Based on previous experience, a single colchicine dose of 30 µg per mouse (estimated to be approximately ~1 mg/kg) was administered intravenously with or after the AgNO₃ injection. Six days after the first silver nitrate injection the spleens were examined for amyloid deposition. With the standard induction protocol, colchicine exhibited a time-dependent inhibitory effect on amyloidogenesis with the maximal effect at an interval of 48 hours between the first silver nitrate injection and colchicine treatment. When colchicine was administered

either before or 72 hours after initiation, there was very little reduction in amyloidogenesis. The time-amyloid deposition profile (Figure 2.6.2:4) of colchicine-inhibition in both protocol was similar. The inhibition of amyloidogenesis correlated with the time of colchicine administration with respect to silver nitrate, but not to amyloid enhancing factor, indicating that colchicine can suppress amyloidogenesis after inflammation has been chronically established.

Figure 2.6.2:4 is from the Applicant's Summary of published literature

Figure 2.6.2:4
Effect of Colchicine on Amyloidogenesis (Figure 1, Shtrasburg et al., 2001)



Colchicine inhibits AgNO₃ effect. The curves show the degree of amyloid in relation to the time interval between the first injection of AgNO₃ and the administration of colchicine. In the standard induction protocol (solid diamonds), AgNO₃ was initiated simultaneously with the administration of AEF. In the prolonged amyloid induction protocols, AgNO₃ was initiated 24 hours and 7 days apart from the administration of AEF (open squares and solid triangles, respectively). The two dotted lines (A and B) represent the degree of amyloid deposition in the control mice of the two prolonged amyloid induction protocols (the mean amyloid grade in the control mice of the standard protocol was 3.75; see Table 1, Shtrasburg et al., 2001). In all protocols, the study mice received one dose of 30 µg colchicine intravenously at the indicated time before (negative values on time scale), simultaneously with (0) or after the initiation of AgNO₃ injection. Control mice received the prolonged amyloid induction protocols alone (without colchicine). As can be interpreted from the similarity of the three curves, inhibition of amyloidogenesis correlates with the time of colchicine administration with respect to AgNO₃ and not to the AEF.

2.6.2.3 Secondary pharmacodynamics

Microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. They not only serve a physical role in providing the cytoskeletal structure but they also are critical conduits for communication and trafficking of various components within, into, and out of the cell, *e.g.*, vesicles, transporters, granules, organelles, nuclear receptor translocations, gene expression, and even chromosomes.

A MicroMedex search indicated the following effects of colchicine:

- 1) Colchicine has an anti-inflammatory effect in acute gouty arthritis. This is thought to be due to inhibition of granulocyte migration and the prevention of secretion of an inflammatory glycoprotein by the leukocytes (Wallace, 1974; Malawista, 1975):
- 2) Colchicine is also an antimetabolic agent by its ability to arrest cell division in the metaphase resulting in death of the cell. Cells with the highest rate of

division are affected earliest, such as gastrointestinal epithelium and "blood cells". Its antimetabolic action also prevents the polymerization of tubulin into microtubules (Hastie, 1991). Colchicine myopathy is ascribed to alterations of the microtubular network (Himmelmann & Schroder, 1992).

- 3) Colchicine inhibits platelet aggregation produced by ADP, by non-adrenaline or by collagen and reduces platelet adhesiveness (Soppitt & Mitchell, 1969).
- 4) Colchicine also inhibits the release of histamine from mast cells, the secretion of insulin from the pancreas, depresses central respiratory centers, induces hypertension by central vasomotor stimulation and enhances the patient's response to sympathomimetic agents.
- 5) It enhances gastrointestinal activity by neurogenic stimulation but also has a direct effect which depresses.

2.6.2.4 Safety pharmacology

The findings of published nonclinical studies closely match those known from the extensive clinical experience with colchicine. Adverse effects are primarily detected in gastrointestinal, muscle, and some hematopoietic cells. It is hypothesized that these are the tissues with the highest innate exposure (gastrointestinal system), which do not express P-glycoprotein (P-gp) on their membrane surface (some hematopoietic cells), or which have no clearance mechanism (muscle cells). Other tissues which express P-gp, such as brain, are relatively spared due to lack of transfer through the blood-brain barrier.

NEUROLOGICAL EFFECTS

Animal neurobehavioral or neurotoxicology studies have not been conducted with colchicine. It is generally considered that colchicine does not normally cross the blood-brain-barrier to any meaningful extent since the brain-to-blood ratio of colchicine after systemic administration is very low. [³H]-Colchicine, injected subcutaneously to male rats, distributed to the peripheral glands (anterior pituitary and adrenal glands) at high levels within 2 hours, which was 40 to 70 times the levels found in the cerebrum, cerebellum or hypothalamus (Inaba *et al*, 1979). The P-gp transporter is thought to maintain low brain concentrations of colchicine.

Despite the relatively low uptake of colchicine into the brain, high concentrations of colchicine administered peripherally can result in seizure prior to death in rodents. Whether this is a direct effect is unclear. Colchicine does not interfere with protein synthesis, but blocks axonal transport causing enzymes and organelles that are normally transported to nerve terminals to accumulate in the cell body and proximal dendrites. In addition, direct injection of colchicine into the brain causes a non-specific inflammatory response that is both dose- and species-dependent (Dasheiff and Ramirez, 1985). Based on these findings, seizures are thought to be related to colchicine induced neuronal injury rather than a lowering of the seizure threshold (Mundy and Tilson, 1990).

In animals, colchicine induced muscle weakness, changes in gait, and body positioning have been associated with alterations in skeletal muscle and peripheral neuropathy.

Markand *et al.*, (1971) found that the induced paralysis in rats was secondary to myopathic rather than neuropathic alterations. In humans, Kuncel *et al* (1987) reported muscle weakness and ascending delayed paralysis in patients with gout and altered renal function.

Neurobehavioral Effects of Colchicine (Reviewer's table derived from the Applicant's review of published studies)

Author	Species / Dose of Colchicine	Findings
Neurotoxic effect		
Dasheiff and Ramirez, 1985	anesthetized rats, 0.25 to 25 µg into the hippocampus	dose-dependent destruction of dentate granule cell (DGC) bodies 1 week later 25 µg resulted in destruction of both DGC and pyramidal cells no behavioral changes were observed
	rhesus monkeys, 5 to 200 µg in each of 4 different sites in the hippocampus (20 to 800 µg total dose)	less selective damage and more severity than in rats, 5 µg dose in 4 sites produced a large area of cystic necrosis with a surrounding zone of non-selective neuronal death and inflammation
Gorenstein <i>et al.</i> , 1985	Rat, Colchicine injected into the cerebral ventricle 10 to 100 µg Animals sacrificed at various times and brain tissue stained for lysosomal enzymes dipeptidyl peptidase II and acid phosphates	At doses known to inhibit axon transport, there was accumulation of lysosomal enzymes in cell body and dendrites within 1 hour By 5 days, a normal pattern of distribution reoccurs
Seizures		
Mundy and Tilson, 1990 (review)	mice, rats, and rabbits; direct administration into the hippocampus	ability of colchicine to cause seizures resulted in contradictory results among different studies mechanism is thought to be related to colchicine induced neuronal injury; not a lowering of the seizure threshold
Neuropathy (Peripheral Nervous System)		
Chang <i>et al.</i> 2002	Sprague-Dawley rats, females 0.2 mg/kg, i.p., daily for 5 days of the week, for either 7 months or 10 months; control rats were untreated pilot study: a dose of 0.2 mg/kg was found to result in weakness but no weight loss	gait abnormalities observed during in monthly walking-track analysis rectus femoris muscle biopsies: no differences between treated and controls seen with either phase or electron microscopy no vascular autophagic changes in the colchicine-treated group were noted. Nerve biopsies were obtained at the same time. Except for a statistical difference (P< 0.001) in the axon / myelin ratio of the sciatic nerve, no differences in the sciatic and posterior tibial nerve fibers of the two groups were seen.

		chronic colchicine administration, in doses that produce weakness and some weight loss but not diarrhea, alters neuromuscular function (as measured by changes in gait) without producing measurable changes to muscle and nerve fibers
<i>Model for sporadic dementia</i>		
Kumar <i>et al.</i> 2007	Wistar rat, males 15 µg intracerebroventricularly	Cognitive dysfunction evidenced by poor retention of memory in both Morris water maze and elevated plus-maze task paradigms
<i>Myopathies</i>		
Khan 1995		hypothesis that myopathic effects of colchicine were due strictly to block of axonal transport
Markand <i>et al.</i> 1971	rats 1.4 to 1.6 mg/kg, i.p., (dose at which approximately one-third of the animals died) or in repeated lower doses, 0.4 mg/kg/day, i.p., for 4 weeks	induced paralysis in rats was secondary to myopathic rather than neuropathic alterations In the surviving animals sacrificed 3 to 4 days after colchicine injection, extensive damage observed by light microscopy consisted of disruption and degeneration of myofibrils, large necrotic zones which lacked myofibrils but contained numerous membranous bodies, and amorphous sarcoplasmic debris. Electron microscopy revealed changes as early as 24 hours after injection of colchicine. The earliest change occurred in the sub-sarcolemmal area, followed by focal alterations in the intermyofibrillar zones. The most conspicuous change on the second and third day was the accumulation of large sarcoplasmic membranous bodies of varying size and complexity. Some contained small vesicles, osmiophilic granules, and mitochondria in the center. Others had several concentric layers of membranes and resembled myelin (some had mitochondria enclosed in the membranes). Nuclear changes were also observed. No ultrastructural changes (e.m.) were observed in the muscles of control animals or in sections of sciatic nerve or anterior horn cells of the spinal cord from treated animals. Developed hypothesis is that colchicine interferes with lysosomal degeneration.
Seiden (1973)	Sprague-Dawley rats, adult males 0.4 or 0.8 mg/kg/day, i.p.,	lower doses over a longer dosing period

	<p>daily for 2 to 22 days</p>	<p>No gross changes were observed in the muscle of the treated animals</p> <p>0.4 mg/kg/day: mild toxic effects or none at all</p> <p>0.8 mg/kg/day showed severe toxic effects including weight losses up to 27% of body weight within 4 days, diarrhea, weakness, and paralysis</p> <p>EM: no evidence of myofibrillar degeneration, but changes in myofilament orientation and the appearance of many unusual membranous structures.</p> <p>Disoriented filaments appeared in a sub-sarcolemmal position, primarily in perinuclear zones that were devoid of normally oriented myofilaments, but rich in sarcoplasm, mitochondria, and other organelles and spheromembranous bodies.</p> <p>acid phosphatase AP is found in abundance in structures that appear to be modifications of, or derived from, the SER</p>
<p>Ai <i>et al.</i>, 2003</p>	<p>rat skeletal muscle incubated in vitro with 10 µg/ml colchicine for 2 hours</p> <p>Confocal Image of Microtubules in Rat Single Muscle Fibers: Effect of Colchicine (derived from Figure 5, Ai <i>et al.</i>, 2003)</p>  <p>Confocal images of microtubules in single muscle fibers. Single fibers were teased from fixed soleus muscles that had been incubated for 2 h (A, B) in the absence (A) or presence (B) of 10 µg/ml colchicine. Then the fibers were stained with an antibody against α-tubulin and observed with a confocal microscope. In the absence of depolymerizing drugs, nuclei are surrounded by dense bundles of interlacing microtubules in their equatorial plane. Between nuclei an extensive network of microtubules can be seen. Already after 2 h of incubation with colchicine a substantial part of microtubules had disappeared (A, B). Findings are representative of ≥10 fibers from each condition.</p>	
<p>Kuncl <i>et al.</i>, 2003</p>	<p>rats 0.4 mg/kg, i.p., daily for 4 weeks</p>	<p>rat model for human myopathy blockade of receptor trafficking in the skeletal</p>

		<p>muscle is thought to be the mechanism</p> <p>Chronic proximal weakness along with skeletal muscle changes that are consistent with subacute myopathy seen in humans, <i>e.g.</i>, vacuolar changes in non-necrotic myofibers</p> <p>the effects of colchicine on receptor trafficking were monitored (used acetylcholine receptor (AChR) as a model membrane in cultured myotubes)</p> <p>Colchicine appeared to inhibit exocytosis and the overall degradation of membrane receptors The authors concluded that microtubules appear to play a functional role in the degradation of lysosomes in normal adult skeletal muscle and this underlies colchicine myopathy.</p>
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CARDIOVASCULAR EFFECTS

The Applicant conducted studies in rabbits and dogs and also provided published studies related to cardiovascular toxicology.

Based on the submitted studies, colchicine did not present any demonstrable risk of cardiovascular toxicity when tested either *in vitro*, (hERG; human ether-à-go-go-related gene) or when administered orally to dogs. The anesthetized rabbit study did find dose-dependent changes in cardiac functions, but these were with doses demonstrated to be lethal in conscious rabbits.

Table 2.6.2:2 from the Applicant's Summary

Table 2.6.2:2
Mutual Pharmaceutical-Sponsored Cardiovascular Safety Pharmacology Studies of Colchicine

Study No.	Study Title	Summary
1273HU21.001	Effects of Colchicine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	<ul style="list-style-type: none"> Up to 100 μM (40 $\mu\text{g}/\text{mL}$), hERG current not inhibited. At 10 μM (4 $\mu\text{g}/\text{mL}$), hERG inhibited $0.3\% \pm 0.0\%$. At 100 μM (40 $\mu\text{g}/\text{mL}$), hERG inhibited $0.4\% \pm 0.1\%$. In vehicle control, hERG inhibited $0.4 \pm 0.3\%$.
0406LU21.001	Dose-Range-Finding (Pyramid) Intravenous Toxicity Study of Colchicine in Rabbits	<ul style="list-style-type: none"> New Zealand White rabbits (3/sex). Regimen of 3, 3, and 3 mg/kg consecutive doses i.v. (aqueous solution) 30 min apart (well above the lethal dose) was selected in order to investigate acute onset of cardiovascular effects prior to appearance of delayed (12 to 48 hours) clinical signs & death.
1235LU21.001	Effects of Colchicine on Electrocardiogram, Heart Rate and QTc in Anesthetized Rabbit	<ul style="list-style-type: none"> Anesthetized New Zealand White rabbits (2/sex). Dosing regimen as defined in Study 0406LU21.001. Maximum increases in QTc of 7, 9, and 13% vs. 30-min vehicle. Marginal decreases in heart rate of 12, 18, and 22% compared to the 30-min vehicle control were observed. Marginal decreases in body temperature of 2, 3, and 4% compared to the 30-min vehicle control were observed. No gross electrocardiographic changes observed.
0433DU21.001	Dose-Range-Finding (Pyramid) Oral Toxicity Study in Dogs with Colchicine	<ul style="list-style-type: none"> Beagle Dog (1/sex). Oral LD₅₀ of 0.9 mg/kg/day and a max. tolerated oral dose (MTD) of 0.45 mg/kg/day estimated. Oral dose of 0.45 mg/kg/day selected so that dogs survive three doses over a 22-day period.
1259DU21.001	Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs	<ul style="list-style-type: none"> Conscious telemetered Beagle dogs (4 male). Each dog received control (distilled water) and three doses of colchicine (0.19, 0.3, and 0.5 mg/kg) with a 7-day washout between. Blood samples at pre-dose and 1 and 4 hours post-dose. All dogs survived the treatment period. Emesis, loose feces, and red color in feces on the day after 0.3 and 0.5 mg/kg doses. No biologically relevant changes in heart rate, diastolic pressure, systolic arterial pressure or mean arterial pressure observed. No gross electrocardiographic changes observed. No changes in QTc values observed.

Study title: Effects of Colchicine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Key study findings: Colchicine had no effect on the hERG channel potassium current involved in cardiac repolarization at doses up to 100 μM (40 $\mu\text{g}/\text{mL}$).

Study no.: 1273HU21.001

m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol

Conducting laboratory and location: _____

Date of study initiation: Sept 6 2006

GLP compliance: yes, except that colchicine was not characterized under GLP conditions, stability information was not provided by the Sponsor

QA report: yes

Drug, lot #, and % purity:

Colchicine, Lot COL0306003, Purity 99.7%

Vehicle: HEPES-buffered physiological saline

b(4)

The mean actual concentrations of 10 and 100 μM Colchicine test article formulations collected from the outflow of the perfusion apparatus were 97.0% and 96.3% of the calculated values and demonstrated stability between the start and end of the experimental day.

Methods

The *in vitro* effects of colchicine on the hERG (human ether-à-go-go-related gene) channel current (I_{K_r} , the rapidly activating, delayed rectifier cardiac potassium current) were investigated at near-physiological temperature in stably transfected mammalian cells that express the hERG gene. Cells were transferred to the recording chamber and superfused with HB-PS solution. Colchicine concentrations of 10 μM (4 $\mu\text{g}/\text{mL}$) and 100 μM (40 $\mu\text{g}/\text{mL}$) were tested by incubation with *in vitro* cultured human embryonic kidney cells (HEK293) expressing hERG channels. Cells stably expressing hERG were held at -80 mV. Onset and steady state block of hERG current due the test article was measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 sec; repolarizing test ramp to -80 mV (-0.5 V/s) repeated at 5 s intervals. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM), to assess the contribution of endogenous currents. The remaining unblocked current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition.

Results

Colchicine inhibited hERG current by (Mean \pm SEM) $0.3 \pm 0.0\%$ at 10 μM ($n = 3$) and $0.4 \pm 0.1\%$ at 100 μM ($n = 3$) compared to $0.4 \pm 0.3\%$ ($n = 3$) for the vehicle control. Due to the lack of effect an IC_{50} was not determined. Terfenadine (60 nM), the positive control, inhibited hERG current by 84.4% and 82.9% ($n = 2$). The results with terfenadine were consistent with _____ historical data.

b(4)

Study title: Dose-Range-Finding (Pyramid) Intravenous Toxicity Study of Colchicine in Rabbits

Key study findings: In rabbits, colchicine was administered intravenously 3 times at 15 min intervals. Three doses of 0.5 mg/kg (1.5 mg/kg total) resulted in no clinical signs or mortality. Animals administered three doses of 1.0 mg/kg or three doses comprising 1, 2, and 5.0 mg/kg each, resulted clinical signs and death. The clinical signs appeared to be more severe with increasing dose and included decreased activity, decreased body tone, loose stools, dilated pupils, abnormal gait and stance, and pale mucous membranes. Necropsy findings included fluid in the abdominal cavity, intestines, pale stomach lining, and air-filled small intestines.

Study no.: 0406LU21.001

m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol

Conducting laboratory and location: _____

Date of study initiation: Sept 19, 2006

GLP compliance: no

QA report: no

Drug, lot #, and % purity:

Colchicine (RD060075), Lot COL 0306003, Purity 99.7%

Vehicle: sterile water for injection

b(4)

Methods

Six New Zealand White rabbits (3 males and 3 females, 12-14 week of age, 2.1-2.7 kg) were administered colchicine intravenously as indicated in the table below.

Group / Treatment	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1. Colchicine	3 × 0.5	0.25	2	2	2
2. Colchicine	3 × 1.0	0.25	2	2*	2*
3. Colchicine	1.0, 2.0, and 5.0	0.5, 1.0, and 2.5	2	1	1

* The same animals were dosed after Day 19 of first regimen.

Reviewer's Comment: The concentration as listed in group 2 is not correct and likely 0.5 mg/mL if the dose volumes administered were identical for all doses.

The animals were dosed three times on one day (approximately 15 minutes apart) at an initial dose level of 0.5 mg/kg/dose. The dose level was increased based upon toxicity until the maximum tolerated dose (MTD) was determined with a minimum of at least two days (48 hours) between each dosing series. Each animal received 2 ml/kg dose based upon its most recent body weight.

Animals were observed prior to dosing, immediately following each dose, approximately 1 and 4 hours post dose, and twice daily thereafter through Day 8. Animals were observed once daily from Day 9 through 19, after which the second dosing regimen was

begun at twice the dose level. After the second regimen animals were observed twice daily through Day 6. Due to the death of these animals, a new group of animals was studied, using the third regimen; the schedule of observations was the same. All animals were subjected to a necropsy following their death or termination.

Results

In the first group of animals, no clinical signs or mortality were observed in the animals at any time point following the first regimen of 0.5 mg/kg × 3 doses.

Following the second regimen of 1.0 mg/kg × 3 doses, clinical signs of decreased activity, decreased body tone, loose stools, dilated pupils, abnormal gait and stance, and pale mucous membranes were observed in the 2 female animals through Day 5; 1 female was found dead on Day 6 and the remaining female was euthanized. Necropsy of the 2 males found dead on day 2 revealed yellow discolored lining of the abdominal cavity and yellow fluid in the abdominal cavity. Necropsy of the female found dead on Day 6 revealed a pale stomach lining. The euthanized female on Day 6 had distended intestines.

With doses of 1.0, 2.0, and 5.0 mg/kg, each 15 minutes apart, decreased activity was observed at 4 hours post dose, but both of these animals were found dead on Day 2. Necropsy revealed fluid in the abdominal cavity, intestines and stomach lining pale and air-filled small intestines.

Although well above the lethal dose, a dosing regimen of 3 mg/kg, administered three times administered 30 minutes apart was selected for the main study in order to investigate the possibility of acute onset of cardiovascular effects prior to the appearance of delayed (12 to 48 hours) clinical signs and death caused by colchicine.

Study title: Effects of Colchicine on Electrocardiogram, Heart Rate and QTc in Anesthetized Rabbits

Key study findings: The intravenous administration of colchicine to anesthetized rabbits (3.0 mg/kg/injection, three times, 30 minutes apart) resulted dose related decreases in heart rate (12%, 18%, and 22%) and increases in QTc (7%, 9%, and 13%) compared to the initial vehicle administration. There was no effect on other ECG parameters and no arrhythmias occurred. Body temperature was not affected.

Study no.: 1235LU21.001

m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol

Conducting laboratory and location: _____

Date of study initiation: Nov 21, 2006

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Colchicine, Lot COL0206002, Purity 97.5%

Vehicle: 0.9% sterile saline

Analysis of colchicine dosing solution indicated concentrations from 91.1 to 94.9% of the calculated values.

Methods

Four anesthetized (anesthetized with Pentothal® and maintained with 2-4% isoflurane) New Zealand White rabbits (2/sex, 11-13 weeks of age, 1.7 to 2.5 kg) were intravenously injected with vehicle, and then administered three consecutive 3.0 mg/kg doses of colchicine, each 30 minutes apart, for a total dose of 9.0 mg/kg. Rabbits were continuously monitored for ECG and heart rate for 30 minutes after each dose. QT intervals were measured and corrected for heart rate (HR) changes with Bazett's formula. Group mean and standard error of the percentage change from baseline were calculated for HR and QTc. Electrocardiogram (ECG) records were monitored for arrhythmias and body temperatures recorded every 15 minutes during the experiment.

The dose was based upon the results of a dose range-finding study ——— Study No.: 0406LU21.001). Data from rabbits administered vehicle followed by 3 doses of colchicine (1, 1 and 1 mg/kg each administered after a 30-minute interval) resulted in mortality of 3 of 4 animals within 48 hours. Dosing at higher doses (1, 2, and 5 mg/kg, each administered after a 30 min interval) resulted in the death of all animals within 24 hours. The dosing regimen of 3, 3 and 3 mg/kg was selected since is was well above the lethal dose and therefore was expected to produce an acute onset of clinical signs seen with the 12-48 hour delay in the dose-range finding study..

b(4)

Results

The intravenous administration of colchicine at three consecutive doses of 3.0 mg/kg produced dose related decreases in heart rate (12%, 18%, and 22%) and increases in QTc (7%, 9%, and 13%) compared to the initial 30-minute vehicle value. Arrhythmias as measured by ECGs were not observed.

Reviewer's Comment: The Applicant did not consider these changes biologically relevant since they were less than 15%. Because these were lethal doses administered to anesthetized animals with no determination of NOAEL values due to the experimental design, the findings are not useful for clinical extrapolation.

Treatment	Heart Rate (BPM)	% Change ^a	QTc (sec)	% Change ^a
Baseline	292±13.6	-	0.302 ± 0.009	-
<u>Vehicle 0.9% saline</u>				
5 min post-dose	284±13.8	-3	0.304 ± 0.008	+1
15 min post-dose	276±15.3	-5	0.305 ± 0.010	+1
30 min post-dose	269±15.2	-8	0.307 ± 0.012	+2
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	325±64.6	+21	0.290 ± 0.016	-6
15 min post-dose	249±11.9	-7	0.321 ± 0.008	+5
30 min post-dose	237±13.5	-12	0.330 ± 0.013	+7
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	236±15.3	-12	0.324 ± 0.011	+6
15 min post-dose	228±13.2	-15	0.336 ± 0.009	+9
30 min post-dose	221±11.9	-18	0.336 ± 0.013	+9
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	222±12.1	-17	0.345 ± 0.007	+12
15 min post-dose	215±12.6	-20	0.348 ± 0.010	+13
30 min post-dose	210±16.5	-22	0.344 ± 0.003	+12

Data presented as Mean ± SEM
^a%change for vehicle and test article was calculated from baseline and vehicle (30 min post-dose), respectively.

Treatment	Body Temperature(°C)	%Change
Baseline	38.30 ± 0.46	-
<u>Vehicle 0.9% saline</u>		
15 min post-dose	37.89 ± 0.52	-1
30 min post-dose	37.50 ± 0.60	-2
<u>Colchicine 3.0 mg/kg</u>		
15 min post-dose	37.14 ± 0.66	-1
30 min post-dose	36.87 ± 0.77	-2
<u>Colchicine 3.0 mg/kg</u>		
15 min post-dose	36.55 ± 0.84	-3
30 min post-dose	36.32 ± 0.92	-2
<u>Colchicine 3.0 mg/kg</u>		
15 min post-dose	36.05 ± 1.01	-4
30 min post-dose	35.82 ± 1.07	-4

Data presented as Mean ± SEM
^a%change for vehicle and test article was calculated from baseline and vehicle (30 min post-dose), respectively.

Study title: Dose-Range-Finding (Pyramid) Oral Toxicity Study in Dogs with Colchicine

Key study findings: Dogs (n=1/sex) orally administered 0.45 mg/kg colchicine had diarrhea at some time between 4 and 30 hours after dosing, and then appeared normal. Dosed 5 days after the first dose with an oral dose of 0.9 mg/kg resulted in emesis, bloody diarrhea and death in the female, and only diarrhea in the male.

Study no.: 0433DU21.001

m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol

Conducting laboratory and location: _____

Date of study initiation:

GLP compliance: no

QA report: no

Drug, lot #, and % purity:

Colchicine (RD060075), Lot COL 0306003, Purity 97.05% (potency listed as _____)

Vehicle: sterile water for injection

b(4)

Concentration verification indicated values were between 91.1 and 94.9% of the expected values.

Methods

Dogs (1 male and 1 female Beagle, 6.0-8.3 kg) each received an oral dose of colchicine of 0.45 and 0.9 mg/kg on Days 1 and 5, respectively. Animals were observed immediately post-dose on each day, 1 and 4 hours post-dose, and twice daily on non-dosing days. Heart rate was recorded prior to and 1 and 4 hours following the 0.9 mg/kg dose.

Group	Dose Level (mg/kg/day)		Concentration (mg/mL)		Dose Volume (mL/kg)	Number of Animals	
	Nominal	Actual	Nominal	Actual		Male	Female
Colchicine							
Initial Dose	0.5	0.45	0.125	0.1125	4.0	1	1
Second Dose	1.0	0.9	0.250	0.225	4.0	1	1

Results

The 2 dogs administered 0.45 mg/kg colchicine orally appeared normal through the first 4 hours. Watery feces occurred in the male by 24 hours, and in the female by 30 hours. Both dogs appeared normal 2 days following the first dose.

On day 5, the dogs received 0.9 mg/kg colchicine orally. Emesis occurred in the female by 4 hours, the male appeared normal. At approximately 23 hours, watery feces (brown in color) with mucous was present in the cage of the male while at 31 hours post-dose the female was found dead with red, watery feces present in the cage pan. Gross necropsy findings in the female consisted of red stained fur at the base of the tail with red fluid at

the rectum; the stomach and intestines appeared red-fluid-filled while the thoracic and cranial cavities appeared normal.

No significant differences in heart rate were observed during the study at either dose level that could be attributed to test article administration.

Both dogs had lost a small amount of weight after the first dose (table below).

Dog body weight (kg)

Animal Number/ Sex	Day 1 (14 Sep 2006)	Day 5 (18 Sep 2006)
3 Male	8.3	8.1
4 Female	6.9	6.8

Based on the results of the study (n=2, 1/sex), the Applicant determined an oral median lethal dose (LD₅₀) of 0.9 mg/kg and a maximum tolerated oral dose (MTD) of 0.45 mg/kg colchicine were estimated in Beagle dogs. The Applicant considered the oral dose of 0.45 mg/kg/day colchicine appropriate for the cardiovascular study in dogs, as the study design requires that the dogs survive three doses of colchicine over a 22-day period (1 week washout between doses).

A. Table 1 – Heart Rate and QTc and Percent Change

Treatment	Heart Rate (BPM)	% Change ^a	QTc (sec)	% Change ^a
Baseline	292±13.6	-	0.302 ± 0.009	-
<u>Vehicle 0.9% saline</u>				
5 min post-dose	284±13.8	-3	0.304 ± 0.008	+1
15 min post-dose	276±15.3	-5	0.305 ± 0.010	+1
30 min post-dose	269±15.2	-8	0.307 ± 0.012	+2
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	325±64.6	+21	0.290 ± 0.016	-6
15 min post-dose	249±11.9	-7	0.321 ± 0.008	+5
30 min post-dose	237±13.5	-12	0.330 ± 0.013	+7
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	236±15.3	-12	0.324 ± 0.011	+6
15 min post-dose	228±13.2	-15	0.336 ± 0.009	+9
30 min post-dose	221±11.9	-18	0.336 ± 0.013	+9
<u>Colchicine 3.0 mg/kg</u>				
5 min post-dose	222±12.1	-17	0.345 ± 0.007	+12
15 min post-dose	215±12.6	-20	0.348 ± 0.010	+13
30 min post-dose	210±16.5	-22	0.344 ± 0.003	+12

Data presented as Mean ± SEM

^a %change for vehicle and test article was calculated from baseline and vehicle (30 min post-dose), respectively.

B. Table 2 -- Body Temperature

Treatment	Body Temperature(°C)	%Change
Baseline	38.30 ± 0.46	-
Vehicle 0.9% saline		
15 min post-dose	37.89 ± 0.52	-1
30 min post-dose	37.50 ± 0.60	-2
Colchicine 3.0 mg/kg		
15 min post-dose	37.14 ± 0.66	-1
30 min post-dose	36.87 ± 0.77	-2
Colchicine 3.0 mg/kg		
15 min post-dose	36.55 ± 0.84	-3
30 min post-dose	36.32 ± 0.92	-2
Colchicine 3.0 mg/kg		
15 min post-dose	36.05 ± 1.01	-4
30 min post-dose	35.82 ± 1.07	-4

Data presented as Mean ± SEM

* %change for vehicle and test article was calculated from baseline and vehicle (30 min post-dose), respectively.

Study title: Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs

Key study findings: Colchicine (0.1, 0.3 or 0.5 mg/kg) orally administered to conscious telemetered male dogs did not result in any significant changes in blood pressure, heart rate, ECG parameters (including QTc), or arrhythmias. Clinical signs of increasing dose-related severity included emesis, bloody diarrhea, and reduced appetite.

Study no.: 1259DU21.001

m4\42-stud-rep\421-pharmacol\4213-safety-pharmacol

Conducting laboratory and location: _____

Date of study initiation:

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Colchicine (RD060075), Lot COL 0306003, Purity 97.05%

Vehicle: sterile water for injection

b(4)

The results of the analysis indicate that for days 1, 15 and 22, dosing formulations were within or just under 10% of the calculated values (0.01 mg/mL: 89.6-100.9%; 0.03 mg/mL: 92.3-99%; and 0.05 mg/mL: 92.0- 98.4%). However, the dosing solutions for day 8 were approximately 125-130% over their calculated values.

Methods

All male dogs were chosen because there was no evidence for difference in cardiovascular parameters between gender in previous studies. Four naïve male Beagle dogs (16-18 months of age, 9.3 to 11.7 kg) were implanted with telemetry transducers for monitoring of heart rate, arterial pressure (diastolic, systolic, and mean), and ECG (Lead II). Dogs were administered colchicine *via* oral gavage (10 mL/kg/dose) in a randomized

Latin-square design whereby each of the 4 dogs received control (distilled water) and three doses of colchicine (0.1, 0.3, and 0.5 mg/kg) with a washout period of at least 7 days between doses (see table below).

Treatment	Dose (mg/kg)	Dose Volume (mL/kg)	Day of Dosing			
			Dog #1	Dog #2	Dog #3	Dog #4
Control	0	10	Day 1	Day 8	Day 15	Day 22
Colchicine	0.1	10	Day 22	Day 1	Day 8	Day 15
Colchicine	0.3	10	Day 15	Day 22	Day 1	Day 8
Colchicine	0.5	10	Day 8	Day 15	Day 22	Day 1

A board-certified veterinary cardiologist also examined 1-minute tracings of the ECGs obtained 15 minutes prior to dosing and 30 minutes and 1, 2, 4, 12, and 24 hours post treatment. Plasma colchicine concentrations were determined pre-dose and 1 and 4 hours post-dose.

Results

All dogs survived the treatment period. There were no unusual clinical findings following the control dose. Following the dose of 0.1 mg/kg, 1 of the 4 animals displayed immediate post-dose emesis (#1), but no other unusual clinical findings were noted on the day of dosing or the day after dosing. Following the dose of 0.3 mg/kg, 2 of the 4 animals exhibited clinical signs of emesis, loose feces and red color in feces, reduced appetite. Following the dose of 0.5 mg/kg, 3 of 4 animals had clinical signs on the day after dosing that included red mucus watery feces, soft feces, and green colored emesis, and reduced appetite.

Body Weights (kg)

Dog ID	Day 1	Day 8	Day 15	Day 22
1	_____			
2	_____			
3	_____			
4	_____			

b(4)

Clinical Observations

Week-1

Dog #	Dose (mg/kg)	Dosing Day Observations	Day After Dosing-Observations
1	0	-	-
2	0.1	-	-
3	0.3	-	-
4	0.5	-	-

Week-2

Dog #	Dose (mg/kg)	Dosing Day Observations	Day After Dosing-Observations
1	0.5	-	Red mucus, watery feces, Green colored emesis; Clear emesis in feeder 2 days after dosing
2	0	-	-
3	0.1	-	-
4	0.3	-	-

Week-3

Dog #	Dose (mg/kg)	Dosing Day Observations	Day After Dosing-Observations
1	0.3	-	Ate small amount of food, Food emesis in cage pan
2	0.5	-	Food emesis, loose feces, Red mucus in feces, Red staining on cage paper, ate small amount of food
3	0	-	-
4	0.1	-	-

Week-4

Dog #	Dose (mg/kg)	Dosing Day Observations	Day After Dosing-Observations
1	0.1	Immediate Post-dose emesis	-
2	0.3	-	Food emesis in cage pan, loose feces, red color in feces
3	0.5	-	Soft feces
4	0	-	-

- animal appeared normal.

The Sponsor table for cardiovascular parameters of the control and high dose (0.5 mg/kg) are presented below

Table 3
Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs
1259DU21.001

Effects of Oral Administration of Vehicle Upon Cardiovascular Parameters
Mean Values (n=4)

TREATMENT	TIME	HR	DAP	MAP	SAP	QTc
Vehicle	0 ^a	133	97	122	145	0.251
0 mg/kg	15 min ^b	111	81	103	124	0.253
	30 min	107	76	97	117	0.253
	45 min	99	78	99	120	0.250
	60 min	101	80	100	122	0.251
	75 min	111	84	107	128	0.254
	90 min	95	77	98	120	0.254
	105 min	90	77	98	122	0.250
	120 min	93	78	99	122	0.254
	135 min	83	76	97	121	0.252
	150 min	79	76	97	121	0.252
	165 min	82	77	98	122	0.257
	180 min	86	77	98	121	0.251
	195 min	98	83	104	127	0.255
	210 min	104	84	106	130	0.260
	225 min	97	80	102	125	0.258
	240 min	104	85	108	131	0.257
	5 hr ^c	97	79	100	122	0.250
	6 hr	98	80	102	125	0.251
	7 hr	94	80	102	125	0.252
	8 hr	92	80	101	124	0.256
	9 hr	96	81	103	125	0.260
	10 hr	97	76	97	118	0.254
	11 hr	97	77	98	119	0.253
	12 hr	100	78	99	121	0.247
13 hr	93	76	98	121	0.255	
14 hr	91	78	99	122	0.248	
15 hr	90	78	99	122	0.255	
16 hr	82	78	99	123	0.248	
17 hr	76	77	99	126	0.249	
18 hr	79	83	105	132	0.253	
19 hr	107	90	114	139	0.251	
20 hr	107	86	110	134	0.250	
21 hr	96	84	107	132	0.247	
22 hr	121	93	117	141	0.248	
23 hr	122	92	117	141	0.246	
24 hr	116	91	115	138	0.250	

HR - heart rate (beats/min)

DAP - diastolic arterial pressure (mmHg)

MAP - mean arterial pressure (mmHg)

SAP - systolic arterial pressure (mmHg)

^aRepresents the mean value for 15 minutes prior to first dose.

^bRepresents the mean value for 15 minutes prior to the indicated time from first dose.

^cRepresents the mean value for 60 minutes prior to the indicated time from first dose.

Table 6
Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs
1259DU21.001

Effects of Oral Administration of 0.5 mg/kg/dose colchicine Upon Cardiovascular Parameters
 Mean Values (n=4)

TREATMENT	TIME	HR	% Δ	DAP	% Δ	MAP	% Δ	SAP	% Δ	QTc	% Δ
Colchicine	0 ^a	132	-1	95	-1	121	0	145	0	0.251	0
0.5 mg/kg	15 min ^b	110	-2	80	-2	103	0	125	1	0.252	0
	30 min	102	-5	75	-2	95	-1	117	0	0.248	-2
	45 min	99	0	79	1	101	2	123	3	0.248	-1
	60 min	104	3	81	2	105	5	129	6	0.257	2
	75 min	109	-2	84	0	107	0	130	1	0.251	-1
	90 min	92	-3	73	-5	95	-4	119	-1	0.250	-1
	105 min	93	4	77	0	99	1	122	1	0.255	2
	120 min	93	0	79	2	101	2	125	2	0.256	1
	135 min	88	6	78	2	99	2	124	2	0.251	-1
	150 min	81	2	75	-2	95	-1	121	0	0.255	2
	165 min	91	11	79	3	102	4	127	3	0.255	0
	180 min	88	2	82	7	105	8	131	9	0.252	0
	195 min	97	-1	82	0	104	0	128	1	0.253	-2
	210 min	89	-14	80	-5	102	-4	127	-3	0.258	-1
	225 min	90	-7	79	-1	101	-1	125	0	0.255	-1
	240 min	99	-4	85	0	108	0	134	2	0.250	-3
	5 hr ^c	95	-1	81	3	103	3	125	3	0.248	0
	6 hr	94	-4	84	4	106	3	129	4	0.253	1
	7 hr	92	-2	82	2	103	1	127	2	0.251	0
	8 hr	84	-8	81	1	103	2	129	3	0.251	-2
	9 hr	91	-6	83	2	104	2	128	3	0.252	-3
	10 hr	88	-9	82	7	103	6	128	8	0.249	-2
	11 hr	91	-6	84	10	105	9	130	9	0.248	-2
	12 hr	88	-12	81	4	103	4	128	6	0.249	1
13 hr	86	-7	82	7	104	6	129	7	0.248	-3	
14 hr	85	-5	80	2	101	2	126	3	0.249	1	
15 hr	83	-8	80	3	102	3	127	4	0.249	-2	
16 hr	88	7	84	8	105	7	131	6	0.251	1	
17 hr	83	10	83	7	105	6	131	4	0.251	1	
18 hr	84	6	83	1	107	2	134	1	0.249	-1	
19 hr	100	-6	90	0	113	-1	138	-1	0.249	-1	
20 hr	113	6	89	4	112	2	137	2	0.250	0	
21 hr	103	7	89	5	112	5	139	5	0.251	2	
22 hr	112	-7	92	-1	116	-1	142	1	0.248	0	
23 hr	120	-2	90	-2	115	-2	139	-1	0.251	2	
24 hr	117	1	88	-3	110	-4	134	-3	0.247	-1	

HR - heart rate (beats/min) DAP - diastolic arterial pressure (mmHg)
 MAP - mean arterial pressure (mmHg); SAP - systolic arterial pressure (mmHg)
 % Δ - percent change from corresponding vehicle value
^aRepresents the mean value for 15 minutes prior to first dose.
^bRepresents the mean value for 15 minutes prior to the indicated time from first dose.
^cRepresents the mean value for 60 minutes prior to the indicated time from first dose.

The Sponsor tables of the ECG Parameters from controls and high dose (0.5 mg/kg) are presented below.

Table 7
Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs
1269DU21.001

Effects of Oral Administration of Vehicle Upon ECG Parameters
Mean Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	R-R	QTc
Vehicle	0 ^a	0.078	0.051	0.043	0.194	2.703	0.468	0.251
0 mg/kg	15 min ^b	0.004	0.054	0.043	0.208	2.700	0.562	0.253
	30 min	0.078	0.048	0.043	0.212	2.569	0.601	0.253
	45 min	0.002	0.052	0.043	0.214	2.602	0.643	0.250
	60 min	0.079	0.052	0.043	0.215	2.931	0.637	0.251
	75 min	0.082	0.053	0.043	0.209	2.830	0.571	0.254
	90 min	0.000	0.052	0.043	0.220	3.109	0.665	0.254
	105 min	0.078	0.051	0.043	0.223	3.147	0.751	0.250
	120 min	0.000	0.052	0.043	0.225	3.140	0.725	0.254
	135 min	0.078	0.051	0.043	0.229	3.272	0.767	0.252
	150 min	0.077	0.049	0.043	0.232	3.222	0.798	0.252
	165 min	0.076	0.050	0.043	0.235	3.204	0.790	0.257
	180 min	0.076	0.050	0.044	0.224	3.095	0.719	0.251
	195 min	0.079	0.052	0.043	0.220	3.012	0.633	0.256
	210 min	0.079	0.051	0.043	0.219	2.933	0.611	0.260
	225 min	0.078	0.049	0.043	0.225	2.897	0.678	0.258
	240 min	0.078	0.050	0.044	0.220	2.942	0.631	0.257
	5 hr ^c	0.080	0.052	0.043	0.215	2.772	0.649	0.250
	6 hr	0.077	0.050	0.042	0.216	2.819	0.636	0.251
	7 hr	0.076	0.050	0.043	0.218	2.737	0.652	0.252
	8 hr	0.077	0.051	0.042	0.225	3.023	0.675	0.256
	9 hr	0.076	0.050	0.042	0.224	2.848	0.644	0.260
	10 hr	0.077	0.049	0.042	0.218	2.666	0.632	0.254
	11 hr	0.077	0.050	0.042	0.220	2.888	0.661	0.253
	12 hr	0.077	0.050	0.043	0.214	2.565	0.648	0.247
13 hr	0.075	0.048	0.043	0.223	2.483	0.670	0.255	
14 hr	0.077	0.053	0.043	0.220	2.857	0.706	0.248	
15 hr	0.076	0.049	0.043	0.225	2.473	0.604	0.255	
16 hr	0.077	0.049	0.043	0.224	2.497	0.742	0.248	
17 hr	0.077	0.050	0.043	0.232	2.694	0.808	0.249	
18 hr	0.077	0.051	0.043	0.233	2.801	0.708	0.253	
19 hr	0.078	0.050	0.044	0.211	2.637	0.597	0.251	
20 hr	0.077	0.050	0.044	0.210	2.739	0.597	0.250	
21 hr	0.076	0.049	0.044	0.216	2.798	0.664	0.247	
22 hr	0.078	0.051	0.044	0.200	2.739	0.525	0.248	
23 hr	0.080	0.053	0.044	0.187	2.893	0.523	0.248	
24 hr	0.080	0.052	0.044	0.203	2.884	0.517	0.250	

^aRepresents the mean value for 15 minutes prior to first dose.

^bRepresents the mean value for 15 minutes prior to the indicated time from first dose.

^cRepresents the mean value for 60 minutes prior to the indicated time from first dose.

Table 10
Cardiovascular Evaluation of Colchicine in Conscious Telemetered Dogs
1269DU21.001

Effects of Oral Administration of 0.5 mg/kg/dose colchicine Upon ECG Parameters
Mean Values (n=4)

TREATMENT	TIME	PR Interval	P Duration	QRS Interval	QT	R Amplitude	R-R	QTc
Colchicine	0 ^a	0.080	0.052	0.044	0.197	2.634	0.488	0.251
0.5 mg/kg	15 min ^b	0.081	0.051	0.044	0.209	2.760	0.579	0.252
	30 min	0.081	0.050	0.044	0.211	2.603	0.628	0.240
	45 min	0.079	0.051	0.043	0.213	2.801	0.651	0.248
	60 min	0.080	0.051	0.043	0.217	2.910	0.614	0.267
	75 min	0.080	0.053	0.044	0.210	2.881	0.592	0.251
	90 min	0.080	0.051	0.043	0.222	3.004	0.708	0.250
	105 min	0.081	0.052	0.043	0.224	2.898	0.689	0.255
	120 min	0.077	0.049	0.043	0.225	2.801	0.688	0.256
	135 min	0.079	0.052	0.043	0.223	2.950	0.711	0.251
	150 min	0.078	0.050	0.043	0.234	3.047	0.772	0.255
	165 min	0.079	0.052	0.043	0.227	2.943	0.710	0.256
	180 min	0.080	0.053	0.043	0.225	3.038	0.736	0.252
	195 min	0.077	0.050	0.043	0.219	2.781	0.670	0.253
	210 min	0.076	0.051	0.044	0.231	2.968	0.726	0.258
	225 min	0.078	0.051	0.044	0.220	2.896	0.737	0.255
	240 min	0.078	0.050	0.044	0.220	2.796	0.703	0.250
	5 hr ^c	0.077	0.049	0.044	0.215	2.582	0.654	0.248
	6 hr	0.076	0.049	0.044	0.220	2.745	0.671	0.253
	7 hr	0.075	0.048	0.044	0.220	2.567	0.677	0.251
	8 hr	0.075	0.050	0.043	0.227	3.061	0.748	0.251
	9 hr	0.075	0.048	0.044	0.222	2.592	0.688	0.252
	10 hr	0.077	0.049	0.044	0.221	2.614	0.710	0.249
	11 hr	0.075	0.048	0.043	0.218	2.653	0.684	0.248
	12 hr	0.075	0.049	0.043	0.221	2.881	0.706	0.249
	13 hr	0.075	0.050	0.044	0.222	2.868	0.720	0.248
	14 hr	0.075	0.049	0.044	0.224	3.058	0.730	0.249
	15 hr	0.076	0.050	0.044	0.227	3.049	0.759	0.249
	16 hr	0.077	0.050	0.044	0.225	2.794	0.728	0.251
	17 hr	0.078	0.051	0.044	0.227	2.836	0.754	0.251
	18 hr	0.079	0.050	0.044	0.224	2.888	0.734	0.249
	19 hr	0.079	0.049	0.045	0.213	2.780	0.633	0.248
	20 hr	0.080	0.050	0.045	0.207	2.785	0.583	0.250
	21 hr	0.079	0.049	0.045	0.215	3.159	0.654	0.251
	22 hr	0.080	0.051	0.045	0.206	2.967	0.687	0.248
	23 hr	0.080	0.051	0.045	0.204	2.870	0.555	0.251
	24 hr	0.079	0.050	0.046	0.203	2.877	0.584	0.247

^aRepresents the mean value for 15 minutes prior to first dose.

^bRepresents the mean value for 15 minutes prior to the indicated time from first dose.

^cRepresents the mean value for 80 minutes prior to the indicated time from first dose.

There were no significant changes in heart rate, diastolic arterial pressure, systolic arterial pressure, mean arterial pressure, or gross electrocardiographic changes following administration of vehicle, 0.1, 0.3, or 0.5 mg/kg colchicine. QTc values also remained unaltered after the administration of 0.1, 0.3, and 0.5 mg/kg doses of colchicine.

Analysis of the plasma samples indicated a large variation between animals in the plasma concentrations for the same doses (ranging from _____ ng/mL obtained 1 hour after a dose of 0.5 mg/kg), but in general there appears to be a dose-related increase in plasma levels.

b(4)

Table 2.6.2:3 Colchicine Plasma Concentrations in Beagle Dogs

Dose (mg/kg)	Hours after dosing	Plasma Concentrations (ng/mL)			
		Animal #1	Animal #2	Animal #3	Animal #4
0.0	0	/			
	1				
	4				
0.1	0				
	1				
	4				
0.3	0				
	1				
	4				
0.5	0				
	1				
	4				

b(4)

BLQ <0.2 ng/mL

Cardiovascular Study Publications

In a chronic administration rat study by adding colchicine to the drinking water (estimated dose of ~60 µg/kg/day), there was no failure or depressed systolic function (contractile dysfunction) and no increased interstitial space or increased myocardial stiffness. In another study, rats administered 2.0 or 4.0 mg/kg, i.p., (into the lethal dose range) resulted in dose related contractile deficits 24 hours of after dosing. These included decreased maximum shortening velocity, decreased active isometric force, and decreased peak output

Cardiovascular Studies (Reviewer's table derived from the Applicant's review of published studies)

Study Author	Species / colchicine dose	Findings
Cicogna <i>et al.</i> , 1997	Spontaneously hypertensive rats (SHR) and non-hypertensive Wistar-Kyoto rats (WKY); administered colchicine in their drinking water (1 µg/mL) estimated dose of ~60 µg/kg/day from 13 months of age until signs of respiratory difficulty (17 to 26 months of age)	No treatment related effect on animal weight or overt toxicity, <i>e.g.</i> , diarrhea was observed. ~three-fold increase in the interstitial space and ~1.7-fold increase in myocardial stiffness in colchicine treated WKY rats relative to the controls but no contractile dysfunction when measured <i>in vitro</i> (<i>e.g.</i> , failure or depressed systolic function)
Mery <i>et al.</i> , 1994	Adult Wistar rats; colchicine 2.0 or 4.0 mg/kg, i.p. (4.0 mg/kg resulted in death in 2 of 10 rats)	myocardial contractility is impaired measured 24 hours post dose decreased maximum shortening velocity (-32 and -61%, respectively), decreased active isometric force (-47 and -65%, respectively), and

		<p>decreased peak output (-57 and -69%, respectively)</p> <p>Isotonic relaxation and load dependence of relaxation were also impaired which may indicate a decrease in calcium myofilament sensitivity</p> <p>The authors suggested that the cardiotoxic effect of colchicine is likely to be a factor in the fatal outcome of acute colchicine poisoning</p>
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PULMONARY EFFECTS

There were no studies conducted by the Applicant. However, in review of the literature, the Sponsor noted that during physiological testing of anesthetized cats, colchicine enhanced the lethal effects of ether or barbiturate anesthesia (Ferguson, 1952). In anesthetized cats, no acute respiratory alterations followed the i.v. injections of colchicine at 0.1 to 10 mg/kg, however some hours later the rate and depth of breathing declined abruptly (within a period of 2 to 5 minutes) and apnea ensued, leading to death.

RENAL EFFECTS

There were no studies conducted by the Applicant. They cited one study (table below) that demonstrated colchicine affects renin secretion and blocked the effects of renin inducers isoproterenol and furosemide. These effects likely involved tubulin polymerization, since lumicolchicine, a degradant resulting from exposure to ultraviolet light, that does not bind to tubulin, had no effect on renin levels. Doses of 0.4 and 0.8 mg/kg, i.p., resulted in decreased urine volume and a dehydrated state of the animal that complicated interpretation of the studies according to the study authors.

Renal Effects of Colchicine (Reviewer's table derived from the Applicant's review of published studies)

Study Author	Species / colchicine dose	Findings
Donoso <i>et al.</i> , 1982	<p>Sprague-Dawley rats, male 0.2, 0.4, and 0.8 mg/kg, i.p.; daily for 3 days</p> <p>24 h after the last injection received isoproterenol (25 µg/rat, i.p.) or saline plasma renin determined 30 min later at sacrifice</p>	<p>0.2 mg/kg: no effect on basal plasma renin concentrations, body weight, or the 24-hour urine volume no signs of dehydration or diarrhea</p> <p>0.2 mg/kg, significantly (P<0.05) reduced isoproterenol-induced plasma renin increases (effect was seen with lumicolchicine, a degradant resulting from exposure to ultraviolet light, that does not bind to tubulin)</p> <p>0.4 mg/kg: colchicine alone increased plasma</p>

		<p>renin concentrations significantly ($P < 0.05$, relative to pre-treatment) at 72 hours after administration. Diarrhea, dehydration, and a loss in body weight were evident within 48 hours.</p> <p>0.8 mg/kg, similar to 0.4 mg/kg results, the authors stated that decreased urine volume and dehydrated state of the animal complicates interpretation</p>
	<p>Similar study design but used furosemide (5 mg/kg, i.p.) as the stimulus to renin concentrations at 18, 24, and 72 hours after the first colchicine injection</p> <p>also urinary sodium concentration and hematocrit were determined in several animals from the control and the lowest dose group to check for changes in renin metabolism as a contributor to altered plasma concentrations</p> <p>0.2 mg/kg, i.p.; once daily for 3 days On the final day, rats were anesthetized, the renal artery and vein were clamped, and blood samples collected for a period of 45 minutes after clamping.</p>	<p>0.2 mg/kg, significantly ($P < 0.05$) reduced furosemide-induced plasma renin increases (effect was seen with lumicolchicine, a degradant resulting from exposure to ultraviolet light, that does not bind to tubulin)</p>

From Applicant's Summary Table 2.6.2:14

Figure 2.6.2:14 Effect of Colchicine i.p. on Plasma Renin Concentration, Body Weight and Urine Volume at Various Time Points After the First Dose in Sprague-Dawley Rats (Table 1, Donoso *et al.*, 1982)

TABLE 1. Dose-Related Effects of Colchicine in Five Rats

Dose (mg/kg/day)	Plasma renin concentration (ng/ml/hr)			Body weight (g)				Urine volume (ml/24 hrs)			
	Pretr	18 hrs	72 hrs	Pretr	24 hrs	48 hrs	72 hrs	Pretr	24 hrs	48 hrs	72 hrs
Saline	4.83 ± 1.71	5.71 ± 1.19	8.21 ± 0.94	265 ± 4	270 ± 4	265 ± 3	256 ± 4	7.6 ± 2.5	8.3 ± 2.1	8.4 ± 2.7	8.7 ± 2.4
Colchicine											
0.2	4.28 ± 1.26	4.06 ± 0.64	7.77 ± 0.20	262 ± 7	273 ± 4	260 ± 4	247 ± 3	6.2 ± 2.3	10.6 ± 2.4	8.2 ± 1.6	9.1 ± 1.9
0.4	5.53 ± 1.10	6.26 ± 1.50	11.11*† ± 0.46	263 ± 9	262 ± 5	250* ± 5	237*† ± 4	10.5 ± 2.7	10.1 ± 3.2	5.8 ± 1.7	3.9*† ± 0.8
0.8‡	4.50 ± 0.91	9.01 ± 1.58	13.30*† ± 0.86	273 ± 7	263 ± 4	247*† ± 6	235*† ± 5	7.6 ± 1.6	11.1 ± 2.0	4.5 ± 1.3	3.3*† ± 1.9

*Significantly different from saline-treated animals at the same time period.

†Significantly different from pretreatment values.

‡One of five animals died by 72 hours posttreatment.

Values are means ± SEM.

GASTROINTESTINAL EFFECTS

Gastrointestinal side effects reported clinically include abdominal cramping, abdominal pain, diarrhea, lactose intolerance, nausea, vomiting, and elevated serum enzymes AST, elevated ALT. Gastrointestinal effects are among the most prevalent adverse events observed with therapeutic doses in humans. Similar effects are observed in animal studies with vomiting and diarrhea to bloody diarrhea usually the first signs noted. These effects are due to a combination of factors, enhanced intestinal permeability, inhibition of water transport due to decreased activity of intestinal Na⁺-K⁺-ATPase, and disruption of cytoskeletal integrity in the more rapidly dividing cells of the gut and peripheral activation of central processes mediating emesis.

Gastrointestinal Effects of Colchicine (Reviewer's table derived from the Applicant's review of published studies)

Author	Species / Dose of Colchicine	Findings
Ferguson, 1952	Wistar rats, male and females 0.5, 1, 2, 4 mg/kg, iv 4 mg/kg, ip cats, males and females (1.9-4.8 kg) under anesthesia, either Dial-urethane, or pentobarbital, or inhalation ether 0.1, 0.25, 0.5, 1 mg/kg, iv or ia	altered gastrointestinal responses to colchicine were observed depending if the animals were conscious or anesthetized suggesting that the emetic actions are at least partially centrally mediated consistent emetic effects in unanesthetized animals only a fourth of the anesthetized cats vomited after a lethal dose anesthesia anesthesia eliminated the diarrhea that normally followed administration of colchicine doses of colchicine from 0.1 to 10 mg/kg had no effect on intestinal motility or tone in cats that manifested either spontaneous motility or hypermotility caused by neostigmine only in Thiry-Vell loops of the small intestines (conscious cats) were signs of increased tone and increased rate and amplitude of contractions observed. These effects appeared a few hours after injection of colchicine, persisted for hours, and could not be completely abolished by atropine. bowel responses to acetylcholine, epinephrine and histamine were unaffected by colchicine <i>in vivo</i> , and exposing isolated strips of bowel to colchicine in physiological ranges, produced no effect on normal motility
Dinsdale, 1975	Hooded Lister rats, male 0.1 to 4.0 mg/kg, s.c.	Light and electron microscopy of rapidly proliferating cells of the duodenal crypts

	<p>cytosine arabinoside hydrochloride (125 to 500 mg/kg, s.c.)</p> <p>cycloheximide (1.0 mg/kg, s.c.) 30 min prior to colchicine</p>	<p>and the mature cells of the villus population.</p> <p>less than 0.2 mg/kg : indistinguishable from controls at the light microscope level.</p> <p>at ~LD₅₀: effects restricted to the crypts of the mucosa and essentially absent from the mature cells of the villi and the adjacent pancreatic tissue.</p> <p>Cytosine arabinoside hydrochloride produced at 500 mg/kg lesions similar in morphology and location as those resulting from 2.0 mg/kg colchicine</p> <p>EM: inclusion bodies cycloheximide (protein synthesis inhibitor) greatly reduced the severity of damage to the duodenal crypts but did not prevent the arrest of mitosis by colchicine.</p> <p>The reduction in damage was attributed to interference with the cellular response to injury that requires synthesis of proteins. The nature of the damage is uncertain, but the absence of these lesions in the mature cells of the villi suggest that it is restricted to growing cells and, according to the author, possibly results from their inability to form, or maintain, microtubules in the presence of colchicine.</p>
<p>Fradkin <i>et al.</i> (1995)</p>	<p>Wistar rats, Colchicine dissolved in their drinking water 30 mg/L Studies of 8 and 23 days duration</p> <p>Rats consumed 16.4 mL in the first 8 day, and 19.2 mL in the 23 day studies.</p> <p>Calculated dose per animal 0.5 ± 0.15 mg/day Day 23 serum colchicine concentrations 3.8 ± 2.7 ng/mL (range 1.0-6.7 ng/mL)</p>	<p>colchicine increased tight junction permeability in the rat as evidenced by the sustained increase in lactulose/ mannitol excretion ratio</p> <p>The lactulose/ mannitol excretion ratio was measured periodically This type of double-probe method for measuring intestinal permeability controls for such variables as transit time, renal function and completeness of urine collection. Mean serum colchicine concentration in the rats completing 23 days of administration was 3.82 ng/mL (comparable to those recorded in FMF patients receiving 1-2 mg/day colchicine therapy).</p> <p>Except for occasional loose stools, diarrhea was not observed. Two of 5 animals were removed from the 23-day study in the last week, suffering from severe anorexia and</p>

		<p>bloody nasal discharge.</p> <p>Urinary lactulose excretion increased significantly at 8 hr post-dose compared to pretest values and was highest after 48 hours, remaining stable throughout the study period. Mannitol urinary excretion was not changed.</p>
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Diarrhea

Iacobuzio-Donahue <i>et al.</i> , 2001	humans, clinically toxic doses, distinct morphologic changes are observed in gastrointestinal mucosal biopsies of the duodenum and gastric antrum	<p>changes include metaphase mitoses, epithelial pseudo-stratification, and loss of polarity as well as abundant crypt apoptotic bodies See table 2 below.</p> <p>not observed in 5 patients without clinical colchicine toxicity</p>
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TABLE 2. Histopathologic and endoscopic features of gastrointestinal biopsy specimens from patients on oral colchicine therapy

Case no.	Biopsy site	Metaphase mitoses	Epithelial pseudostratification	Loss of polarity	Apoptosis	Villous atrophy	Endoscopic findings
Patients with clinical evidence of colchicine toxicity							
1	Duodenum bulb	++	++	++	++	-	Duodenal polyp and ulceration
	Antrum	++	++	++	++	-	Diffuse gastritis
	Body	+	+	+/-	+	-	Normal
2	Duodenum	++	-	-	++	-	Normal
	Antrum	++	+	+	++	-	Normal
	Body	+/-	-	-	-	-	Normal
3	Antrum	++	++	+	+	-	Diffuse gastritis and erosions
	Fundus	-	-	-	-	-	Normal
	Colon	++	++	+/-	-	-	Normal
4	Duodenum	++	+	+	-	+	NA
Patients without clinical evidence of colchicine toxicity							
5	Colon	-	-	-	-	-	Polyp
6	Antrum	-	-	-	-	-	Mild gastritis
	Esophagus	-	-	-	-	-	Normal
	GE	-	-	-	-	-	Normal
	Junction	-	-	-	-	-	Polyp
	Colon	-	-	-	-	-	Polyp
7	Duodenum bulb	-	-	-	-	-	NA
	Antrum	-	-	-	-	-	NA
	Esophagus	-	-	-	-	-	NA
8	Antrum	-	-	-	-	-	NA
	Colon	-	-	-	-	-	NA
9	Colon	-	-	-	-	-	Polyp

NA, not available.

many studies	rats	<p>decreased intestinal fluid transport, increased intestinal fluid secretion, and the formation of apoptotic nuclei in the crypt cells</p> <p>These effects appear to be mediated by decreased activity of intestinal Na⁺-K⁺-ATPase, increased adenylate cyclase and cAMP activities, and increased prostaglandin E2 secretion.</p>
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Rachmilewitz <i>et al.</i> , 1978, Rachmilewitz and Karmeli, 1980	<p>male rats</p> <p>5 mg/kg, i.p.</p> <p>jejunal segments obtained 1, 2, or 4 hours after injection for</p>	<p>colchicine inhibits intestinal water transport and intestinal fluid secretion mediated through decreased intestinal Na⁺-K⁺-ATPase activity, increased adenylate cyclase and cAMP activities, and increased</p>
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	<p>assays</p> <p>Histological sections from jejunum obtained 4 hours after administration</p>	<p>prostaglandin E2</p> <p>Net transport of fluid in the jejunal segments was significantly ($P < 0.01$) reduced in rats that had received colchicine (3.0 ± 0.9 g fluid/hour/g) <i>versus</i> the control rats (8.6 ± 0.7 g fluid/hour/g). Decreases in net fluid transport were paralleled by the decreases observed in $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity. Along with the decrease in net fluid transport, reduced amounts of sodium and potassium were transported.</p> <p>Histologically, the jejunum from rats treated with colchicine was relatively normal. The only unusual finding was the appearance of crypt cells undergoing mitotic arrest in metaphase.</p> <p>Four hours after administration of colchicine, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity was significantly ($P < 0.01$) decreased (18.2 ± 4.9 $\mu\text{mol/mg}$ protein/hour) as compared with control rats (40.6 ± 3.4 $\mu\text{mol/mg}$ protein/hour).</p>
<p>González <i>et al.</i>, 2005</p>	<p>C3H/S mice, adult male, 2 mg/kg, i.p., sacrifice 4 hours later</p> <p>Duodenum histopathology and TUNEL assay (nick end-labeled for DNA fragmentation)</p> <p>cell was considered to be apoptotic if cell shrinkage, intense eosinophilic cytoplasm, and a nucleus with condensed chromatin were observed</p> <p>apoptotic index was estimated as the number of apoptotic cells per 1000 nuclei</p>	<p>colchicine induced apoptosis in intestinal crypt enterocytes</p> <p>the average apoptotic indices in the whole crypt and each individual region were higher ($P < 0.0001$) in colchicine-treated mice than in saline-treated control mice</p> <p>For the whole crypt the apoptotic index in saline treated animals was 2.12 ± 0.58 and for colchicine-treated mice, it was 65.73 ± 9.09. The highest apoptotic index was seen in tiers 5-12 (transient cells) for saline (3.14 ± 0.94) and colchicine-treated (107.59 ± 10.72) animals. The lowest apoptotic index seen was in tiers 13-20 (highly differentiated cells). In this zone, the apoptotic index in the saline-treated animals was 1.01 ± 0.28 and in the colchicine-injected mice, it was 34.77 ± 10.81.</p>
<p>Duncan and Heddle (1984)</p>	<p>C57BL/6J mouse</p>	<p>apoptosis occurred in duodenal crypt enterocytes</p>

ABUSE LIABILITY

There were no studies conducted by the Sponsor. Colchicine is not considered a drug of abuse.

OTHER SYSTEMS**Metabolic and Endocrine Effects**

Functional and ultrastructural liver changes were found in some studies that examined the effects of colchicine on metabolism. Since suppression of secretory function which is dependent on microtubules for transport or storage of granules or secretory vesicles is a well characterized effect of colchicine in endocrine cells, the significance of the functional liver changes could likely be due to secondary metabolic alterations.

Metabolic and Endocrine Effects (Reviewer's table derived from the Applicant's review of published studies)

Author	Species / Dose of Colchicine	Findings
Metabolic Effects		
Singh et al 1975	Mice 0.12 mg/kg, i.v	increase in serum triglycerides higher dose (but one that is not associated with gastrointestinal toxicity), there is an increase in serum and liver triglycerides, with ultrastructural changes in the liver suggestive of fat infiltration and glycogen deposits. decreased blood sugar, free fatty acid, serum amino acid, and protein decreased liver glycogen levels
Endocrine Function		
Shah and Wongsurawat (1978)	Rats 0.2 mg/kg/day, i.p.	reversible decrease in insulin secretion and increase in serum glucose
Inaba et al. (1979) Inaba and Kamata (1979)	Rats, males [³ H]-colchicine 0.5, 1.0 or 2.0 mg/kg, sc	direct and indirect effects of colchicine on the release of corticosteroids from the rat adrenal gland, <i>in vivo</i> increased serum and adrenal corticosteroid levels, evident at 1.5 hr post-dose (first sampling time) and continuing for at least 6 hr the effect of colchicine was long lasting compared to ACTH administration possibly due to pituitary ACTH secretions rather than direct stimulatory action on the adrenal gland, since <i>in vitro</i> studies found low concentrations of colchicine did not stimulate and high concentrations of colchicine suppressed the release of

		<p>corticosteroids from the adrenal gland tissue.</p> <p>when colchicine was administered 1 hr prior to the injection of ACTH there was a significant augmentation of corticosteroids in the serum and adrenal gland</p> <p>intracellular distribution of corticosteroids in the adrenal glands was similar in ACTH and colchicine treated rats</p> <p>The authors suggest that continuous stimulation of the synthesis and secretion of adrenocortical hormone (and the resultant corticosteroid response) seen with administration of colchicine may explain its effectiveness in the treatment of gout.</p>
<p><i>Adrenal Gland</i> Singh <i>et al.</i>, 1975</p>	<p>Mice, 0.5 mg/kg, i.v. Single dose, Mice followed for 60 hours</p>	<p>single low dose of colchicine to mice that did not produce diarrhea or a change in body weight or alter food intake, dramatic but reversible decreases in serum triglyceride, glucose, and free fatty acids levels</p> <p>total serum protein concentration decreased significantly ($P < 0.005$) from 4 to 20 hours after colchicine administration hematocrit values increased significantly ($P < 0.05$) from 4 to 15 hours post administration.</p> <p>Ultrastructural examination of the liver revealed an increase in the size and number of VLDL particles and a decrease in glycogen deposits. microtubules were not observed in the livers from the colchicine-treated animals in the colchicine-treated livers, numerous vesicles containing VLDL-like particles were distributed throughout. Over time, the diameter and appearance of these particles increased. The density of the larger particles closely resembled that of lipid droplets. After 11 hours, at a time when the liver triglyceride content was maximal, a huge accumulation of lipid droplets was observed in most hepatocytes. Twenty-four hours after colchicine administration, fat infiltration had practically disappeared and microtubules could again be observed. Glycogen deposits that were initially observed in large areas of the cytoplasm were reduced to only a few particles 8 hours after colchicine administration. These deposits reappeared 24 hours later. Changes in measured hepatic glycogen content mirrored these observations. Autophagic vacuoles were distributed randomly in the cytoplasm of both control and colchicine-treated livers but the number of vacuoles was much higher in the latter group.</p>

		<p>These changes were also reversible.</p> <p>blood sugar, serum free fatty acid, serum amino acid, and urea levels were also observed 11 hours after administration of colchicine. Blood sugar decreased slightly but liver glycogen content dropped dramatically. Serum free fatty levels were much lower in colchicine-treated mice but serum amino acids and urea concentrations were higher. With the exception of blood sugar concentrations, all of these alterations returned to normal levels 24 hours later</p> <p>a lower dose was administered to mice (0.12 mg/kg, i.v.; equivalent to human dose of 0.6 mg (based on BSA), changes in serum parameters were not observed but a significant increase in liver triglyceride content (controls: 8.7 ± 0.9; colchicine-treated mice: $13.0 \pm 1.3 \mu\text{mol/g}$) was seen.</p>																																																																											
<p>From Sponsor's Summary Table 2.6.2:10</p> <p style="text-align: center;">Figure 2.6.2:10 Effect of Colchicine (0.5 mg/kg, i.v) on Various Metabolic Indices (Mean \pm SEM) in Swiss Mice (Table 3, Singh <i>et al.</i>, 1975)</p> <table border="1" data-bbox="578 989 1300 1409"> <thead> <tr> <th rowspan="2"></th> <th colspan="3">Time after Administration (hours)</th> </tr> <tr> <th>0</th> <th>11</th> <th>24</th> </tr> </thead> <tbody> <tr> <td colspan="4">Serum</td> </tr> <tr> <td colspan="4">Glucose (mg/100 mL)</td> </tr> <tr> <td>Saline Controls</td> <td>--</td> <td>174 ± 11 (n=5)</td> <td>173 ± 7 (n=5)</td> </tr> <tr> <td>Colchicine</td> <td>186 ± 9 (n=10)</td> <td>$149 \pm 4^{***}$ (n=8)</td> <td>$137 \pm 5^{***}$ (n=9)</td> </tr> <tr> <td colspan="4">Free Fatty Acids (mM)</td> </tr> <tr> <td>Saline Controls</td> <td>--</td> <td>1.39 ± 0.20 (n=5)</td> <td>0.88 ± 0.10 (n=5)</td> </tr> <tr> <td>Colchicine</td> <td>0.97 ± 0.05 (n=10)</td> <td>$0.59 \pm 0.04^{***}$ (n=8)</td> <td>1.03 ± 0.08 (n=9)</td> </tr> <tr> <td colspan="4">Amino Acids (mM)</td> </tr> <tr> <td>Saline Controls</td> <td>--</td> <td>3.87 ± 0.13 (n=5)</td> <td>4.58 ± 0.31 (n=5)</td> </tr> <tr> <td>Colchicine</td> <td>3.94 ± 0.11 (n=10)</td> <td>$5.56 \pm 0.20^{***}$ (n=8)</td> <td>5.15 ± 0.14 (n=9)</td> </tr> <tr> <td colspan="4">Urea (mg/100 mL)</td> </tr> <tr> <td>Saline Controls</td> <td>--</td> <td>55 ± 2 (n=5)</td> <td>55 ± 2 (n=5)</td> </tr> <tr> <td>Colchicine</td> <td>74 ± 3 (n=10)</td> <td>$79 \pm 3^{***}$ (n=8)</td> <td>59 ± 1 (n=9)</td> </tr> <tr> <td colspan="4">Liver</td> </tr> <tr> <td colspan="4">Glycogen (mg glucose per g liver)</td> </tr> <tr> <td>Saline Controls</td> <td>--</td> <td>32.7 ± 1.5 (n=5)</td> <td>5.7 ± 0.8 (n=4)</td> </tr> <tr> <td>Colchicine</td> <td>12.1 ± 3.7 (n=7)</td> <td>$1.9 \pm 0.3^{***}$ (n=5)</td> <td>$15.5 \pm 4.6^*$ (n=5)</td> </tr> </tbody> </table> <p>* P<0,05; ** P<0,025; *** P<0,005</p>				Time after Administration (hours)			0	11	24	Serum				Glucose (mg/100 mL)				Saline Controls	--	174 ± 11 (n=5)	173 ± 7 (n=5)	Colchicine	186 ± 9 (n=10)	$149 \pm 4^{***}$ (n=8)	$137 \pm 5^{***}$ (n=9)	Free Fatty Acids (mM)				Saline Controls	--	1.39 ± 0.20 (n=5)	0.88 ± 0.10 (n=5)	Colchicine	0.97 ± 0.05 (n=10)	$0.59 \pm 0.04^{***}$ (n=8)	1.03 ± 0.08 (n=9)	Amino Acids (mM)				Saline Controls	--	3.87 ± 0.13 (n=5)	4.58 ± 0.31 (n=5)	Colchicine	3.94 ± 0.11 (n=10)	$5.56 \pm 0.20^{***}$ (n=8)	5.15 ± 0.14 (n=9)	Urea (mg/100 mL)				Saline Controls	--	55 ± 2 (n=5)	55 ± 2 (n=5)	Colchicine	74 ± 3 (n=10)	$79 \pm 3^{***}$ (n=8)	59 ± 1 (n=9)	Liver				Glycogen (mg glucose per g liver)				Saline Controls	--	32.7 ± 1.5 (n=5)	5.7 ± 0.8 (n=4)	Colchicine	12.1 ± 3.7 (n=7)	$1.9 \pm 0.3^{***}$ (n=5)	$15.5 \pm 4.6^*$ (n=5)
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<p>Pancreas Shah and Wongsurawat (1978)</p>	<p>Sprague-Dawley rats, males</p> <p>single dose study 0.5 mg/kg colchicine, i.v</p> <p>repeated dose study 0.2 mg/kg, i.p. daily for 10 days on the last day after overnight fast, administered glucose challenge (150 mg glucose pulse, iv, and</p>	<p>Both phases of insulin secretion in response to glucose were diminished by repeated clinically relevant doses of colchicine to rats <i>in vivo</i>. The effects were reversible with discontinuation of colchicine treatment.</p> <p>Following the priming glucose pulse and during the glucose infusion, mean serum glucose levels were significantly higher (P<0.05) during the time period of 10 to 45 minutes than were observed in control</p>																																																																											

	steady iv infusion, 6 mg/minute for 60 minutes)	<p>treated rats</p> <p>the acute phase of IRI secretion (between 2 and 5 minutes) and chronic phase of IRI secretion (between 10 and 60 minutes) were significantly inhibited in the colchicine-treated rats as compared with the control rats</p> <p>No difference between the two groups was observed in the serum calcium concentration</p>
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Hematopoietic Effects

Myelosuppression is a known clinical dose-related adverse event associated with colchicine due its effects on disruption of mitosis in rapidly dividing cells such as is found in the hematopoietic system. There were no animal studies indicating dose-responsiveness or that provided further insight into colchicine induced myelosuppression.

In addition, granulocytes (*e.g.*, neutrophils, eosinophils, basophils), lacking in phosphoglycoprotein (P-gp) contain concentrations of colchicine that are higher than cells that express P-gp on their surface. As noted by the Applicant these observations are consistent with hypothesis that the anti-inflammatory effect of colchicine is mediated by neutrophils. These cells also might be more susceptible to the toxic effects of colchicine and could account for adverse events observed in the hematopoietic cells in humans.

Hematopoietic Effects (Reviewer's table derived from the Applicant's review of published studies)

Author	Species / Dose of Colchicine	Findings
<i>Leukocytes</i> Klimecki <i>et al.</i> , 1994; Schinkel <i>et al.</i> , 1997).		<p>granulocytes (<i>e.g.</i>, neutrophils, eosinophils, basophils), lack in phospho-glycoprotein (P-gp) transport on membrane</p> <p>these cells may contain concentrations of colchicine that are higher than cells that do express P-gp on their surface</p>
Chappey <i>et al.</i> , 1993	Human leukocytes in vivo; 6 healthy male volunteers, administered 1 mg colchicine; concentrations of colchicine determined in cells	steady-state colchicine concentrations: mononuclear cells: ranged from 9 to 24 ng/10 ⁹ cells granulocytes: 20 to 53 ng/10 ⁹ cells for both cell types intracellular colchicine peaks appeared 48 hours after administration when plasma colchicine levels were 0.30 ng/mL
<u>Chappey <i>et al.</i> (1993)</u>	Human leukocytes in vivo; colchicine dose: 1 mg/day for 14 days and <i>In vitro</i> lymphocyte cultures	<p>similar colchicine concentrations in granulocytes and monocytes throughout the complete time course of sample collection (1 hour to 168 hours)</p> <p>After a single dose, concentrations declined slowly with a half-life of approximately 35 hours, with detectable levels present up to 240 hours post-dose</p> <p>plasma terminal half live: 49 hours granulocyte terminal half live: 41 hours</p>

		<i>In vitro</i> lymphocyte cultures results were similar, attributed to colchicine levels rather than metabolite
<u>Ben-Chetrit et al., 1998</u>	6 FMF patients, Colchicine concentrations were measured in plasma and leukocytes	Colchicine concentrations at the same measured time points were 3- to 4-fold higher in granulocytes as compared to lymphomonocytes in all FMF patients plasma colchicine levels correlated with the daily dose, but the intracellular concentrations was fairly constant possibly intracellular colchicine accumulation may be saturable.
<i>Thrombocytes</i>		
Puszkin and coworkers (1971)	human platelets <i>in vitro</i>	found that colchicine bound to a protein ("presumably derived from microtubules") that did not have direct ATPase activity but altered the cation requirements of ATPase activity for both myosin and what was known as thrombosthenin-M, a molecule thought to play a role in clot retraction.
Bouaziz et al., 2007		colchicine disruption of microtubules results in reduced thrombin-induced platelet aggregation and ATP secretion

Bone Effects

Colchicine was shown to be detrimental to bone healing in rats when administered in the drinking water (consuming approximately 1 mg/kg/day) for a 6 week period. Dudkiewicz et al 2005).

Bone (Reviewer's table derived from the Applicant's review of published studies)

Author	Species / Dose of Colchicine	Findings
Dudkiewicz et al., 2005	Wistar rats, females Colchicine administered to <i>via</i> drinking water at a dose of 1 mg/kg/day (based on water consumption) 3 groups: 1) for 1 week prior to the fracture procedure (under anesthesia, the left posterior tibia was fractured manually) and afterwards for another 6 weeks 2) colchicine treatment was started on the day of the fracture procedure and continued for 6 weeks 3) control group, did not receive colchicine	colchicine had a negative influence on fracture healing according to radiological, clinical, and mechanical (P<0.02), and pathological parameters (P<0.0001)
<i>Osteogenesis/Ablation of Hematopoiesis</i> Studies indicate that the effect of colchicine is unclear, since there is controversy surrounding the replication and interpretation of the studies.		
Arai and coworkers (1993)	1 mg/kg, i.v.	colchicine rapidly induced trabecular bone-like ectopic calcified tissue in the bone marrow cavity within 4 days of administration

		Peak growth was obtained by day 8 after which the ectopic tissue was reabsorbed within 4 days
Wlodarski and Wlodarski, 1997		could not repeat findings of Arai and coworkers (1993) in two different strains of mice or in WAG rats
Caselli and coworkers in 1999		agreed with the findings by Arai <i>et al.</i> in 1993 noted that Arai's model appears to be "a unique model of pharmacological ablation of hematopoiesis (not ablation of marrow, because the stroma is not ablated) followed by <i>de novo</i> medullary bone formation, and a unique way to probe interactions of hematopoiesis and osteogenesis in the postnatal animal <i>in vivo</i> "
Wlodarski and Wlodarski (2001)		a follow-up Letter to the Editor disagreed with the interpretation of the results obtained by both groups

2.6.2.5 Pharmacodynamic drug interactions

The Sponsor did not conduct non-clinical pharmacodynamic drug interaction studies. Some of the published literature contains multidrug studies which may involve colchicine pharmacodynamic, pharmacokinetic and toxicological effects. These are contained in the pharmacokinetic and toxicology sections.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

These are included in the specific topic sections.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The Applicant did not conduct pharmacokinetic studies of colchicine, but summarized the published nonclinical studies and included their toxicokinetic evaluation of plasma colchicine from their cardiovascular safety study in dogs. The pharmacokinetic parameters for colchicine across species is presented in Table 2.6.4:1. All the pharmacokinetic studies were single dose administration, there were no repeated dose studies found in the literature. The following information was taken from the Applicant's summary.

Applicant's Summary Table 2.6.4:1

Table 2.6.4:1 Comparison of Mean (\pm SD) Pharmacokinetic Parameter Values Following a Single Dose of Colchicine in Mice, Rats and Humans

Species (n)	Gender	Dose, mg/kg (route)	CL (mL/min·kg)	V _d (L/kg)	T _{1/2} (hr)	Comment
Wistar Rats ¹ (3)	Male	5 (oral gavage)				Samples collected for 24 hours (C _{max} = 39.7 ng/mL; T _{max} = 0.25 h)
Kunming Mice ² (5/ time pt/route)	Male	5 (i.v.)			3.9	Samples collected for 10 hours
		5 (i.p.)			7.93	2 nd peak observed at 6 hours; samples collected for 10 hours
Sprague-Dawley Rats ³ (5)	Male	10 (i.p.)	77.3 \pm 8.3	1.84 \pm 0.15	0.28 \pm 0.02	Samples collected for 2 hours
Sprague-Dawley Rats ³ (4); sham-operated controls	Male	10 (i.p.)	68.7 \pm 4.9	1.87 \pm 0.10	0.32 \pm 0.02	Samples collected for 8 hours
Hanover Wistar Rats ⁴ (4-5)	Male	1 (i.v.) bolus; followed by 2-hr i.v. infusion 12.5 (μ g/min/kg)	65.7 \pm 4.9		10.5 \pm 2.1	Values derived from post-infusion data collected (2 – 6 hours)
Human ⁵ (6)	Male	0.5 (i.v.)	10.5 \pm 1.5	6.71 \pm 1.4	30 \pm 6	Samples collected for 48 hours

Bittner *et al.*, 2002¹; QH Chen *et al.*, 2007²; Leighton *et al.*, 1990³; Desrayaud *et al.*, 1997⁴; Rochdi *et al.*, 1994⁵

In a cardiovascular safety study in conscious dogs receiving 0.5 mg/kg orally, the plasma levels 1 hour post-dose ranged from _____ ng/mL. There was no explanation for the wide variation in concentrations.

Colchicine distributes primarily into bile, liver, and kidney. Moderate levels are seen in lungs, heart, intestine, and stomach. Due to efflux by P-gp at the blood-brain barrier, only very small amounts of radioactivity are detected in the brain. Studies with

b(4)

intravenous administration of colchicine and P-gp inhibitors in rats resulted in 3- to 4-fold increases colchicine plasma concentrations and 5- to 10-fold increases in brain concentration (refer to the Pharmacokinetic Drug Interaction section). Bile duct obstruction or liver dysfunction also results in increased systemic concentrations of colchicine. Colchicine is secreted into milk.

The clearance of colchicine is mediated by liver metabolism and secretion into the bile and urine. In rats, for example, about 50% of colchicine is removed through biliary secretion and the rest through the kidneys and metabolism. Similarly, in humans, enterohepatic recirculation and biliary excretion, mediated by P-gp, is also postulated to be a major route of elimination.

In the liver, colchicine is metabolized to *O*-demethyl derivatives which, in rats and hamsters, are then glucuronidated. While the specific metabolizing enzymes have not been identified in animals, in humans CYP3A4 is the primary metabolizing enzyme. The extent of metabolism varies across species; in hamster ~40% of colchicine is converted into metabolites but in rat and mouse liver only about 11 to 12% is converted.

Excretion of colchicine into bile or urine appears to be primarily mediated through P-gp transport. Co-administration of CYP inhibitors to the rat *in vivo* results in ~3-fold increase in colchicine liver concentrations. Chemically induced liver dysfunction results in increased systemic concentrations of colchicine. Co-administration of CYP inducers to the hamster *in vivo* results in ~3- to 4-fold increase in colchicine metabolism. At high doses, in the rat, colchicine administration alters the normal function of transporters and CYP enzymes. The extent that these effects occur at lower doses and the relevance to humans is not known.

2.6.4.2 Methods of Analysis

Most studies were conducted with standard methodology available at the time of the studies.

2.6.4.3 Absorption

The absorption of oral colchicine was studied in male Wistar rats (Bittner, 2002). Colchicine (5 mg/kg) was administered isotonic sodium chloride solution. The C_{max} of colchicine was reached rapidly, at the first measured time point.

Formulation	T_{max} (h)	C_{max} (ng/mL)	AUC (h·ng/mL)	Cl/F (l/h/kg)
Colchicine alone	0.25 ± 0.01	37.9 ± 9.8	31.5 ± 12.2	139.29 ± 47.93

In humans, the absolute bioavailability is less than 50%.

2.6.4.4 Distribution

Protein Binding

Colchicine does not bind extensively to plasma proteins. The percentage of ^3H -colchicine unbound determined by equilibrium dialysis, was $59.1 \pm 1.5\%$ in rat blood, (Desrayaud *et al.*, 1997) and 23% in dog plasma (de Lannoy *et al.*, 1994) and 39% in humans (Sabouraud *et al.*, 1994).

Plasma:Red Cell Partitioning

At all concentrations tested (10, 100, and 1000 ng/mL), ~30% of ^3H -colchicine spiked into whole blood *in vitro* distributed into red blood cells; the remaining 70% was detected in the plasma (Desrayaud *et al.*, 1997).

Tissue Distribution

Colchicine distributes into multiple tissues but primarily into bile, liver, and kidney. The tissue distribution of a single dose of colchicine in mice, rats, hamsters, rabbits, dogs, and sheep is summarized in Table 2.6.4.3. In all five studies, the doses administered are similar to those used in toxicology studies. In the mouse, of the four tissues examined, radioactivity was primarily measurable in the liver followed by the kidney, intestine, and spleen (Back *et al.*, 1951). In Donrya rats, radioactivity was primarily measured in the liver and kidney followed by lower concentrations in the adrenal and pituitary glands and even lower amounts in the brain (Inaba and Kamata, 1979). In Sprague-Dawley rats and mongrel dogs, the highest levels of radioactivity were detected in the bile, followed by the liver, then the kidney. In Golden hamsters and New Zealand White rabbits, the highest levels were detected in the bile, followed by the kidney, then the liver. In all four species, moderate levels were detected in the lungs, heart, intestine, and stomach. Low levels were detected in the blood or plasma and only very small amounts of radioactivity were detected in the brain (Hunter and Klassen, 1975a). In the sheep, the highest concentration of colchicine detected was in the bone marrow followed by the heart, kidneys, lungs, and liver. Excretion of colchicine into milk was also observed.

In 10-day old rats, the tissue concentrations were much higher than were observed in 35-day old animals for a given dose (Hunter and Klassen, 1975b). The authors suggested that, in the young rats, colchicine elimination is reduced due to an immature bile duct tract. Reduced elimination, in turn, results in higher systemic and tissue levels and a lower LD_{50} (0.24 mg/kg, i.p.) than seen in 35-day old animals (LD_{50} 2.0 mg/kg, i.p.).

Applicant's Summary Table 2.6.4:3

Table 2.6.4:3
Tissue Concentrations of Colchicine in Various Species

Species	Mouse ²	Rat ¹	Rat		Rat ¹	Hamster ¹	Rabbit ¹	Dog ¹	Sheep
Reference	Back <i>et al.</i> , 1951	Inaba and Kamata, 1979	Hunter and Klassen 1973b		Hunter and Klassen 1973a				
No. of Animals	6	5			3-5	3-5	3-5	3-5	1
Strain (Gender, Age)	C-57 black (Both, Adult)	Donryu (M, Adult)	Sprague-Dawley (NS, 10 days old)	Sprague-Dawley (NS, 35 days old)	Sprague-Dawley (M, Adult)	Golden (Male, Adult)	New Zealand White (M, Adult)	Mongrel (M, Adult)	Merino (Female, Lactating, Adult)
Radio-label	¹⁴ C	³ H	³ H	³ H	³ H	³ H	³ H	³ H	Not-labeled*
Dose (route)	1 mg/mouse (s.c.)	1 mg/kg (cold) + 100 µCi/kg (s.c.)	0.1 mg/kg (i.p.)		2 mg/kg (cold) + 7 µCi/kg (i.v.)		2 mg/kg (cold) + 10 µCi/kg (i.v.)		0.16 mg/kg (oral)
Tissue Collection Time (hr)	4	2	Various (specific values not provided; estimated from graph @ 10 min. (max.))		1.5	1.5	1.5	1.5	48
Units	cpm/g tissue	cpm/g tissue	µg/g tissue		µg/g tissue or µg/mL (4 µCi/mol)				ng/g
Brain	--	--	~10	~1	0.091±0.011	0.079±0.004	0.661±0.136	--	--
Cerebrum	--	94.5±4.0	--	--	--	--	--	--	--
Cerebellum	--	115.0±17.0	--	--	--	--	--	--	--
Hypothalamus	--	136.0±12.0	--	--	--	--	--	--	--
Muscle	--	--	~60	~15	1.15±0.06	0.732±0.050	0.736±0.091	1.11±0.14	18**
Stomach	--	--	--	--	--	1.11±0.08	2.94±0.43	3.02±0.40	--
Intestine	50.1±23.1	--	--	--	--	2.16±0.31	3.92±0.54	5.84±1.05	--
Heart	--	--	~100	~35	1.50±0.08	0.863±0.086	2.61±0.42	3.1±0.63	25
Lung	--	--	~100	~35	2.02±0.12	1.10±0.15	3.17±0.44	3.84±0.70	75
Spleen	21.4±16.7	--	~200	~90	2.70±0.22	1.56±0.24	4.56±0.48	4.11±0.42	100
Kidney	65.8±22.4	10609.0±299.0	~200	~90	8.81±0.74	6.45±0.89	18.0±1.8	6.84±0.37	80
Liver	86.2±19.7	13035.0±1657.0	~370	~230	9.63±0.89	3.44±0.15	8.38±1.36	9.51±0.94	75
Plasma	--	--	--	--	0.783±0.041	1.18±0.09	3.89±0.59	1.90±0.27	--
Blood	--	--	--	--	0.836±0.045	0.670±0.086	2.97±0.53	1.92±0.31	--
Bile	--	--	--	--	542±55	198±23.1	69.8±4.5	1523±273	--
Pituitary Gland	--	7620.0±943.5	--	--	--	--	--	--	--
Adrenal Gland	--	4620.0±307.0	--	--	--	--	--	--	--
Bone Marrow	--	--	--	--	--	--	--	--	205

NS = Not Specified; ¹ mean ± SE; ² mean ± SD; *only colchicine detected during sample analysis; metabolites not measured; **Triceps femoris

Despite a relatively large volume of distribution, only small amounts of colchicine have been shown to penetrate the brain of rats, hamsters, and rabbits (Table 2.6.4:3). Limited brain uptake is thought to be due to P-gp mediated efflux at the blood-brain barrier. In rats, P-gp efflux normally reduces the penetration of colchicine into the brain and that co-administration of a P-gp inhibitor (*e.g.*, verapamil or PSC-833) can result in increased brain levels (Desrayaud *et al.*, 1997).

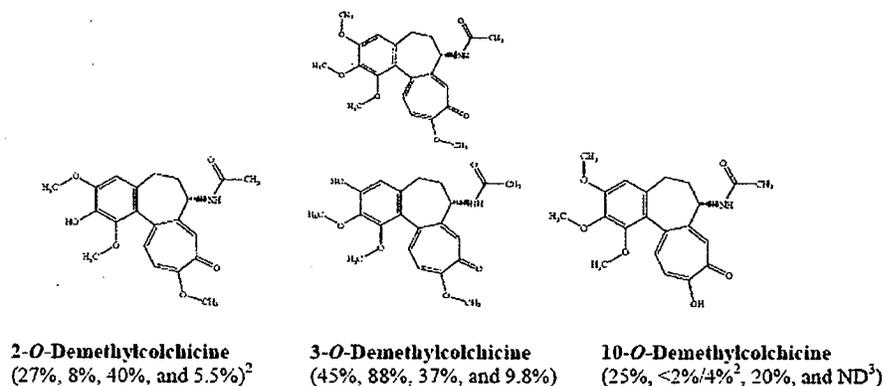
2.6.4.5 Metabolism

Colchicine is metabolized in liver microsomes. The extent varies across species; in hamster ~40% of colchicine is converted into metabolites but in rat and mouse liver only about 11 to 12% is metabolized (Schönharting *et al.*, 1974). The specific CYP450 enzymes in nonclinical species have not been defined. In human liver microsomes, the primary metabolizing CYP enzyme is CYP3A4 (Tateishi *et al.*, 1997). The primary metabolites formed in liver microsomes of all species studied are 2-DMC and 3-DMC.

Plasma concentrations of metabolites have not been measured in animals. In the Applicants' studies of the metabolite concentrations in human plasma, neither 2-*O*-Demethylcolchicine (2-DMC) nor 3-*O*-Demethylcolchicine (3-DMC), were detectable

except in isolated samples at or near the lower limit of quantitation (0.2 ng/mL). The formation rates and resulting proportions of each metabolite present vary with the species (Figure 2.6.4:2). In rats, *in vivo*, 2-DMC does not inhibit inflammation but 3-DMC is as active as colchicine (Sugio *et al.*, 1987). It is not known if these metabolites are substrates of P-gp. 10-*O*-Demethylcolchicine (colchicine) is formed in microsomes prepared from mice, rats, and 5-month old adult hamsters but not 8- to 9-week old hamsters (*Reviewer's Comment: sexual maturity in hamsters occurs about 6 weeks of age, so that does not explain the difference*). Glucuronides are present in the rat (2-DMC-glucuronide) and hamster (3-DMC-glucuronide). Other potential metabolites not found include 1-*O*-Demethylcolchicine (1-DMC), bis-demethylated metabolites, and glutathione-conjugates.

Figure 2.6.4:2
Chemical Structure of Colchicine and Its Metabolites¹



¹Metabolites (% total) formed by microsomal preparations from mouse, hamster, rat, and human microsomal preparations, respectively. Mouse, hamster, and rat data are excerpted from Schönharting *et al.*, 1974. Human data is excerpted from Tateishi *et al.*, 1997

²<2% in 8 – 9 week old hamsters, 4% in 5 month old hamsters

³ND=Not detected

Injury to the liver impairs the clearance of colchicine in rats *in vivo* (Leighton *et al.* 1990). In addition, colchicine inhibits many aspects of liver function. In rats, the administration of colchicine at doses that are ~ 0.5 LD₅₀, colchicine reduces the hematocrit, glutathione (GSH), total CYP450 content, demethylase activity and increases liver mass, serum AST, ALT, and lipid peroxidation activity.

In hamsters, CYP inducers (20-methylcholanthrene and phenobarbital) produced several fold increases in the amount of metabolism of colchicine observed *in vitro*. *In vivo* administration of colchicine also increased the metabolism of colchicine *in vitro* but to a much lower extent.

In rat hepatocytes *in vitro*, colchicine inhibits the expression of multiple CYP enzymes and associated regulatory co-factors. In primary rat hepatocytes, colchicine treatment inhibits the induction of CYP1A1 mRNA expression in a dose dependent manner (Lukić *et al.*, 1997). CYP1A1 expression is regulated by aryl hydrocarbon receptor

(AhR). To alter gene expression after binding a ligand (e.g., TCDD), AhR must translocate from the cytosol to the nucleus *via* a pathway that is microtubule dependent. The authors concluded that down regulation of CYP1A1 expression by colchicine is mediated *via* disruption of the microtubular network that translocates AhR from the cytosol to DNA. While disruption of trafficking into the nucleus is the most likely pathway, the authors noted that multiple sites in this pathway could be altered by microtubule disruption, *i.e.*, movement of AhR through the cytosol, nuclear translocation, co-activator association, or the actual binding to the DNA.

Colchicine Metabolism Studies (Reviewer's table derived from the Applicant's review of published studies)

Author	Methods	Findings															
Leighton <i>et al.</i> 1990	<p>Sprague-Dawley Rats, colchicine 10 mg/kg, i.p. for controls and CYP-inhibited rats, i.v. for rats with liver dysfunction (to prevent galactosamine from interfering with i.p. absorption)</p> <p>liver dysfunction induced diffuse hepatocellular injury by galactosamine (400 or 1000 mg/kg, i.p. 24 hours prior to dosing)</p> <p>a CYP inhibition with cimetidine (120 mg/kg, i.p.)</p> <p>blood samples were collected for 2 hours</p>	<p>CYP-inhibition (cimetidine pretreatment): reduced clearance (32%) increased half-life (37%) (authors noted that cimetidine also inhibits kidney transport so that the extent to which this small effect was accounted for by metabolism <i>versus</i> transporter inhibition is not known).</p> <p>hepatic dysfunction by 1000 mg/kg galactosamine: clearance reduced to one-third that of controls ans substantial prolongation in elimination half-life</p>															
Ulrichová <i>et al.</i> , 1992	<p>rats colchicine 1 mg/kg, i.p.</p> <p>liver removed 24 hours after colchicine administered</p>	<p>Colchicine reduced: liver demethylase activity to 26% of control CYPP450 content to 46% of control</p> <p>reduced demethylase activity was most likely a consequence of the reduction in the CYP protein content</p> <p>effects were most significant for colchicine</p>															
Lukić <i>et al.</i> , 1997	<p>male Wistar rats colchicine or its metabolites, 2-DMC, 3-DMC, or 10-DMC (colchicine) administered 2 µmol/kg, i.p. (equivalent to 1 mg/kg colchicine, ~one half the LD₅₀)</p> <p>at 24 hours after dosing determined liver mass, liver protein, CYP content, various blood parameters, and the <i>in vitro</i> activity of various liver enzymes (e.g., lipid peroxidation (LP), GSH, superoxide dismutase (SOD))</p>	<p>Of the metabolites tested, 10-DMC (colchicine) had the most pronounced effects</p>															
		<table border="1"> <thead> <tr> <th></th> <th>colchicine</th> <th>2-DMC</th> <th>3-DMC</th> <th>Colchicine</th> </tr> </thead> <tbody> <tr> <td>liver mass</td> <td>↑</td> <td>-</td> <td>↑</td> <td>↑</td> </tr> <tr> <td>AST</td> <td>↑</td> <td>↑</td> <td>-</td> <td>↑</td> </tr> </tbody> </table>		colchicine	2-DMC	3-DMC	Colchicine	liver mass	↑	-	↑	↑	AST	↑	↑	-	↑
	colchicine	2-DMC	3-DMC	Colchicine													
liver mass	↑	-	↑	↑													
AST	↑	↑	-	↑													

	ALT LP	↑ ↑	↑ ↓ in vitro	↑ ↓ in vitro	↑ ↑
	total liver protein	↓	-	-	↓
	CYP450 content	↓	↓	↓	↓
	hematocrit	↓	↓	↓	↓
	GSH concentration	↓	↓	↓ (↑ in erythrocytes)	↓ in vivo, in vitro and in erythrocytes
	SOD in vivo in vitro	↓ ↑	↑ -	↓ ↑	↓ ↑
Dvorák <i>et al.</i> , 2006	primary rat hepatocytes, colchicine doses: 0.01, 0.1, and 1 μM; (equivalent to 3.99, 39.9, and 399 ng/mL) co-administered with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 5 nM) to induce CYP1A1 histology: primary rat hepatocytes with colchicine (1 μM; 399 ng/mL) for 3 hours. Microtubules were tagged with monoclonal anti-tubulin antibody and visualized by fluorescence			Colchicine inhibited the induction of CYP1A1 mRNA expression by TCDD in a dose dependent and time dependent manner 6-, 32-, and 18-fold at 6, 24, and 48 hours post treatment, respectively disruption of the microtubular network plays a role in the CYP1A1 down-regulation colchicine clearly disrupts the microtubule network.	
YJ Chen <i>et al.</i> , 2007	rat nonspecific P450 inhibitor proadifen was administered i.v. 10 minutes prior to administering colchicine (1 mg/kg, i.v.) Concentrations of unbound colchicine were determined in blood, bile, and liver using microdialysis probes			Presumably due to inhibition of CYP metabolism, proadifen coadministration significantly increased colchicine liver concentrations (Cmax) (Table 2.6.4:9) significant changes in bile or blood concentrations were not observed. However, at higher concentration of colchicine (3 mg/kg and 10 mg/kg, i.v.), in addition to the increased concentration of colchicine measured in the liver, the total amount of colchicine in the bile increased 40% to 50% (P<0.05) over time (AUC min·μg/mL), relative to the controls..	
	Table 2.6.4.9 from Applicant's Summary				

Table 2.6.4:9		
PK Parameters (mean \pmSE; n=6) in Sprague-Dawley Rat Blood, Liver, and Bile Following Administration of Colchicine Either Alone or Together with Proadifen (CYP inhibitor) (derived from Table 4, YJ Chen et al., 2007)		
Drug Treatment	Colchicine only	With Proadifen
Dose	1 mg/kg, i.v.	10 mg/kg, i.v.
Blood		
AUC (min- μ g/mL)	14.8 \pm 1.7	16.2 \pm 2.3
C_{max} (μ g/mL)	1.3 \pm 0.4	1.0 \pm 0.2
CL (mL/kg-min)	72.9 \pm 9.9	67.1 \pm 7.8
Liver		
AUC (min- μ g/mL)	23.1 \pm 6.8	75.9 \pm 15.6*
C_{max} (μ g/mL)	0.5 \pm 0.2	1.7 \pm 0.4*
Bile		
AUC (min- μ g/mL)	1756 \pm 441	1716 \pm 94
C_{max} (μ g/mL)	48.5 \pm 9.1	43.7 \pm 2.5
AUC_{bile} / AUC_{blood}	1.8 \pm 0.6	5.0 \pm 1.0*
AUC_{bile} / AUC_{liver}	121.6 \pm 24.7	115.2 \pm 15.2

P < 0.05 significantly different from colchicine only group

Schönharting <i>et al.</i> , 1974	hamsters Syrian Golden hamsters administered repeated doses of either phenobarbital (80 mg/kg, i.p. for 4 days), a single dose of 20-methylcholanthrene (2 mg, i.p. 48 hours prior to sacrifice), or repeated doses of colchicine (200 mg/kg, i.p., for 14 days) Microsomes isolated from the liver	The percentage of all colchicine metabolites formed after 1.5-hour incubation with colchicine (relative to the amount of substrate added) : 10.5% in microsomes isolated from control hamsters, 40% in microsomes isolated from phenobarbital-treated hamsters, 35% in microsomes isolated from 2-methylcholanthrene-treated hamsters, and 15% in microsomes isolated from colchicine-treated hamsters.
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2.6.4.6 Excretion

Colchicine is excreted primarily into the feces (70%) and, to a lesser extent, into the urine (14%). In rats, renal clearance was determined to account for approximately 16% of overall clearance. Colchicine is actively secreted into the urine of rats and dogs in addition to filtration. In rats, net secretion appears to be mediated by one or more transporters including P-gp, while in dogs, P-gp does not appear to be involved. Biliary excretion is one of the main elimination pathways for colchicine and it is actively excreted into the bile by one or more transporters. Demethyl metabolites, and other unidentified polar metabolites are also excreted in the bile. Colchicine is excreted into milk.

In humans, enterohepatic recirculation and biliary excretion, mediated by P-gp, are also postulated to be a major route of elimination. However, in contrast to the rat, phase I biotransformation does not appear to be a major factor in its disposition.

Studies of Colchicine Excretion (Reviewer's table derived from the Applicant's review of published studies)

Author	Methods	Findings																												
Excretion in feces and urine																														
Hunter and Klaassen, 1975a	Sprague-Dawley rats Other species in Table 2.6.4:5 ³ H-colchicine 0.2 mg/kg, i.v.	Rat: During the first 24 hours 56% of the dose was excreted in the feces and 11% in the urine Three days after dosing, 83% of the total radiolabel had been excreted, with 70% recovered in the feces and 14% in the urine																												
Table 2.6.4:5 from the Applicants Summary Table 2.6.4:5 Percent of Dose Collected in Bile within 2 Hours of Intravenous (2 mg/kg) Administration of Colchicine (Hunter and Klaassen, 1975a)																														
<table border="1"> <thead> <tr> <th rowspan="2">Species</th> <th rowspan="2">% Radioactivity Excreted</th> <th colspan="3">Fraction of Radioactivity Excreted As:</th> </tr> <tr> <th>Parent</th> <th>Demethylcolchicine</th> <th>Polar metabolites</th> </tr> </thead> <tbody> <tr> <td>Sprague-Dawley rat</td> <td>50%</td> <td>53%</td> <td>15%</td> <td>32%</td> </tr> <tr> <td>New Zealand rabbit</td> <td>16%</td> <td>72%</td> <td>0%</td> <td>28%</td> </tr> <tr> <td>Mongrel dog</td> <td>20%</td> <td>34%</td> <td>35%</td> <td>31%</td> </tr> <tr> <td>Hamster</td> <td>32%</td> <td>45%</td> <td>10%</td> <td>45%</td> </tr> </tbody> </table>			Species	% Radioactivity Excreted	Fraction of Radioactivity Excreted As:			Parent	Demethylcolchicine	Polar metabolites	Sprague-Dawley rat	50%	53%	15%	32%	New Zealand rabbit	16%	72%	0%	28%	Mongrel dog	20%	34%	35%	31%	Hamster	32%	45%	10%	45%
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Mongrel dog	20%	34%	35%	31%																										
Hamster	32%	45%	10%	45%																										
Milk secretion																														
Panariti, 1996	Merino Sheep, lactating 0.16 mg/kg colchicine orally	highest amount of colchicine detected in the milk was 1.25% of dose occurred at 9 hours post administration																												
Henderson and Peaker, 1983	Goats [¹⁴ C]-colchicine, intra-mammary treatment with colchicine	less than 20% of the infused colchicine is secreted in the milk inhibition of secretion in one mammary gland was similar following at weeks 6, 12, 18, 24, and 30 of lactation.																												
Biliary excretion																														
Hunter and Klaassen (1975a)	anesthetized rats, hamsters, rabbits, and dogs with bile duct cannula radiolabeled colchicine 2.0 mg/kg, i.v.	rat: peak biliary excretion rate was achieved within 5 minutes of dosing. Species differences in secretion amount, range 16 to 50%																												
Leighton <i>et al.</i> , 1990	male Sprague-Dawley rats colchicine 10 mg/kg, i.p., at 48 hours after bile duct ligation surgery. Chemically induced cholestasis with ANIT (25 mg/100gm BW)	Bile duct ligation decreased colchicine clearance by 84% Chemically induced cholestasis: colchicine clearance was decreased 55%, and the half life was increased 56%																												
YJ Chen <i>et al.</i> (2007)	Sprague-Dawley rats anesthetized Colchicine (1, 3, or 10 mg/kg, i.v., n=6) administered alone or 10 minutes following administration of the transporter inhibitor, cyclosporine A (CsA; 20 mg/kg, i.v.) Blood, bile, and liver samples were	P-gp transport plays in the excretion of colchicine into the bile PK of colchicine in rat blood, liver, and bile was dose dependent over the dose range of 1 to 10 mg/kg results for the lowest dose are closest to human doses (1 mg/kg, i.v. in rats																												

	<p>obtained over time using microdialysis probes and individual microdialysate samples were analyzed for colchicine concentrations using LC/MS/MS</p> <p>Unbound concentrations of colchicine (Cu) were calculated from the microdialysate concentration (Cm) after adjusting for recovery</p>	<p>corresponds to 0.16 mg/kg, i.v. human equivalent dose).</p> <p>Co-administration of CsA resulted in a significantly lower concentration of colchicine in the bile and thereby increased concentration of colchicine in the blood (Table 2.6.4:8)</p>																																										
<p>Table 2.6.4:8 from the Applicant's Summary</p> <p style="text-align: center;">Table 2.6.4:8 PK Parameters (mean ±SE; n=6) in Sprague-Dawley Rat Blood, Liver, and Bile Following Administration of Colchicine Either Alone or Together with Cyclosporin A (Transporter Inhibitor) (derived from Table 4, YJ Chen et al., 2007)</p> <table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th>Drug Treatment</th> <th>Colchicine only</th> <th>With Cyclosporin A</th> </tr> <tr> <th>Dose</th> <th>1 mg/kg, i.v.</th> <th>20 mg/kg, i.v.</th> </tr> </thead> <tbody> <tr> <td colspan="3">Blood</td> </tr> <tr> <td>AUC (min-µg/mL)</td> <td>14.8 ± 1.7</td> <td>24.7 ± 2.2*</td> </tr> <tr> <td>C_{max} (µg/mL)</td> <td>1.3 ± 0.4</td> <td>0.9 ± 0.2</td> </tr> <tr> <td>CL (mL/kg-min)</td> <td>72.9 ± 9.9</td> <td>42.2 ± 4.3*</td> </tr> <tr> <td colspan="3">Liver</td> </tr> <tr> <td>AUC (min-µg/mL)</td> <td>23.1 ± 6.8</td> <td>14.1 ± 1.3</td> </tr> <tr> <td>C_{max} (µg/mL)</td> <td>0.5 ± 0.2</td> <td>0.4 ± 0.1</td> </tr> <tr> <td colspan="3">Bile</td> </tr> <tr> <td>AUC (min-µg/mL)</td> <td>1756 ± 441</td> <td>315 ± 53*</td> </tr> <tr> <td>C_{max} (µg/mL)</td> <td>48.5 ± 9.1</td> <td>5.4 ± 0.8</td> </tr> <tr> <td>AUC_{liver} / AUC_{blood}</td> <td>1.8 ± 0.6</td> <td>0.6 ± 0.05</td> </tr> <tr> <td>AUC_{bile} / AUC_{blood}</td> <td>121.6 ± 24.7</td> <td>12.9 ± 1.8*</td> </tr> </tbody> </table> <p>* P < 0.05 significantly different from colchicine only group</p>			Drug Treatment	Colchicine only	With Cyclosporin A	Dose	1 mg/kg, i.v.	20 mg/kg, i.v.	Blood			AUC (min-µg/mL)	14.8 ± 1.7	24.7 ± 2.2*	C _{max} (µg/mL)	1.3 ± 0.4	0.9 ± 0.2	CL (mL/kg-min)	72.9 ± 9.9	42.2 ± 4.3*	Liver			AUC (min-µg/mL)	23.1 ± 6.8	14.1 ± 1.3	C _{max} (µg/mL)	0.5 ± 0.2	0.4 ± 0.1	Bile			AUC (min-µg/mL)	1756 ± 441	315 ± 53*	C _{max} (µg/mL)	48.5 ± 9.1	5.4 ± 0.8	AUC _{liver} / AUC _{blood}	1.8 ± 0.6	0.6 ± 0.05	AUC _{bile} / AUC _{blood}	121.6 ± 24.7	12.9 ± 1.8*
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<p>Renal Excretion</p>																																												
<p>Speeg <i>et al.</i>, 1992</p>	<p>male Sprague-Dawley rats anesthetized, bile-duct and ureter cannulated</p> <p>administered an intravenous bolus (5.5 mg/kg) of colchicine followed by a constant infusion (231 µg/min/kg)</p> <p>After achieving steady state, serial samples of urine and blood collected</p> <p>a second drug administered cyclosporine (CsA), cremophor, p-aminohippurate (PAH), ranitidine, tetraethylammonium (TEA) bromide, N-methylnicotinamide (NMN), probenecid</p> <p>Additional serial urine and blood samples collected</p> <p>Renal clearance was calculated CL_r = (urine concentration × urine volume) / (plasma concentration).</p>	<p>renal clearance accounted for ~16% of overall clearance</p> <p>CL_r = 6.92 ± 0.45 mL/min·kg</p> <p>Average urine flow = 63.24 ± 3.84 µL/min</p> <p>GFR was 4.49 ± 0.13 mL/min·kg Average secretory ratio (CL_r / GFR) = 1.482 ± 0.095</p> <p>Thus, there was net secretion of colchicine into urine.</p> <p>Co-administration of organic ions, e.g., TEA and NMN or the OATP inhibitor, probenecid, had no effect on this ratio.</p> <p>With CsA co-administration, the renal clearance of colchicine was reduced to approximately GFR</p>																																										

de Lannoy <i>et al.</i> , 1994	dogs, similarly conducted experiment as Speeg <i>et al.</i> , 1992	colchicine was actively secreted when CsA was co-administered with colchicine, no changes were observed
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2.6.4.7 Pharmacokinetic drug interactions

They reviewed the literature and together with their clinical studies of CYP enzyme substrate interaction studies indicated that drugs that are substrates of P-gp transporters and CYP3A4 may affect colchicine pharmacokinetics and therefore the safety of using colchicine. The Sponsor noted that synergistic pharmacologic effects could contribute to the reported interactions involving reported cases of myotoxicity. This information is presented in the label and further information can be found in the Clinical Pharmacology Review.

P-glycoprotein

Colchicine is the classical substrate of the P-gp transporter as it was used for isolating the 170 kD protein from Chinese hamster ovary (CHO) that is now known as P-g (Juliano and Ling, 1976). At least four different P-gp binding sites are known and colchicine binds to the H site (Shapiro and Ling, 1997).

Brain: In rats, P-gp efflux normally reduces the penetration of colchicine into the brain and that co-administration of a P-gp inhibitor (*e.g.*, verapamil or PSC-833) can result in increased brain levels.

Liver/Bile: In rats, P-gp efflux normally increases the secretion of colchicine into the bile. Co-administration of a P-gp inhibitor (*e.g.*, Cyclosporin A) can result in decreased bile and increased liver levels.

Kidney: Co-administration of the transporter inhibitor, CsA, with colchicine reduced the renal secretion of colchicine (Speeg *et al.*, 1992). This effect appears to be P-gp dependent because the co-administration of organic ions, *e.g.*, tetraethylammonium (TEA) bromide, N-methylnicotinamide (NMN), or the OATP inhibitor, probenecid, had no effect.

Other Transporters

In rats, colchicine has been shown to block the recruitment of ABC transporters to the bile duct cannicular membrane (Misra *et al.*, 2003). Colchicine administration increases *mdr* gene expression and P-gp protein (the encoded product of *mdr*) levels in the liver of rats and mice *in vivo* (Vollrath *et al.*, 1994)

CYP450

Co-administration of cimetidine with colchicine intraperitoneally to rats resulted in decreased colchicine clearance and increased half-life (Leighton *et al.*, 1990). As cimetidine is an inhibitor of inorganic anionic and cationic transporter (OAT and OCT2) as well as a weak inhibitor of CYP3A and CYP1A2, the basis for this potential

interaction is not clear.

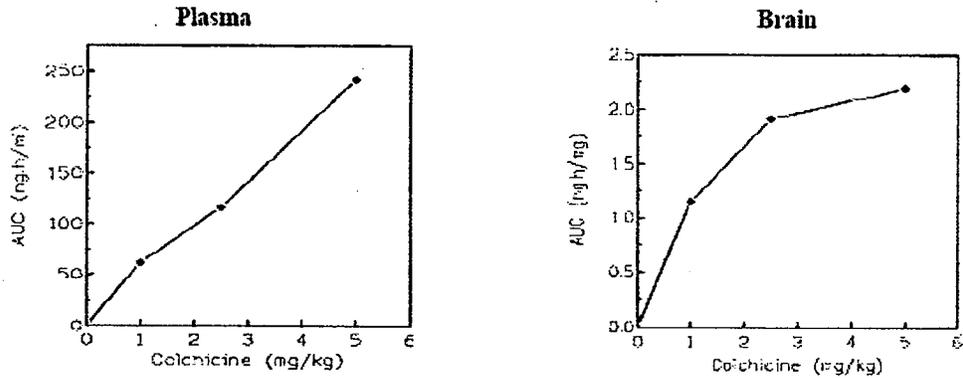
In hamsters, the metabolism of colchicine is increased following the administration of CYP inducers. Compared with other species, in hamsters, colchicine is more extensively metabolized and has a different metabolic profile (Schönharting *et al.*, 1974).

P-gp Transporter Effects on Colchicine Concentrations in Brain (Reviewer's table derived from the Applicant's review of published studies)

Author	Methods	Findings												
<p>Desrayaud <i>et al.</i>, 1997</p>	<p>male Hanover Wistar rats,</p> <p>Colchicine administered iv (femoral vein) alone or together with the P-gp inhibitor, PSC-833,</p> <p>simultaneous microdialysis of blood (jugular vein) and brain (front cortex) of conscious, freely moving rats</p> <p>vehicle or PSC-833 bolus dose (2.3 mg/kg, i.v.), followed by a 7-hour infusion (16.7 µg/min/kg). One hour after starting the PSC-833 treatment, colchicine was administered initially as a bolus dose (1 mg/kg, i.v.), followed by a 2-hour infusion (12.5 µg/min/kg).</p> <p>Blood and brain dialysate samples collected every 20 minutes during the infusion and for 4 hours after initiating the infusion.</p> <p>Unbound colchicine concentrations were measured in brain and blood dialysate samples <i>via</i> RIA</p>	<p>control group: brain colchicine concentrations were below the limit of detection.</p> <p>PSC-833 pretreatment: brain colchicine concentrations were significantly greater for the first hour after administration. Thereafter, no significant differences were observed between the two groups</p> <p>Relative to the control group, intravenous PSC-833 co-administration increases the brain exposure of colchicine (AUC₀₋₆, brain) at least 10-fold and blood exposure of colchicine (AUC₀₋₆, blood) increased ~2.5-fold</p>												
<p>Table 2.6.4:6 from the Applicants Summary</p> <p style="text-align: center;">Table 2.6.4:6 Effect of a Constant Rate Intravenous Infusion of the P-gp Inhibitor, PSC-833, on Blood and Brain Exposure of Free Colchicine (Table II, Desrayaud <i>et al.</i>, 1997)</p> <table border="1" data-bbox="537 1419 1330 1524"> <thead> <tr> <th>PK Parameter</th> <th>Control group (n=4)</th> <th>PSC-833 treated group (n=3)</th> </tr> </thead> <tbody> <tr> <td>AUC_{0-6 hr, blood} (µg/mL·hr)</td> <td>0.51 ± 0.02</td> <td>1.28 ± 0.24*</td> </tr> <tr> <td>AUC_{0-6 hr, brain} (µg/mL·hr)</td> <td>≤0.02 ± 0.01</td> <td>0.20 ± 0.05*</td> </tr> <tr> <td>K_{brain/blood}</td> <td>≤0.04 ± 0.01</td> <td>0.15 ± 0.06*</td> </tr> </tbody> </table> <p>Mean ± SE; * P<0.05; K_{brain/blood} = (AUC_{0-6 hr, brain} / AUC_{0-6 hr, blood})</p>			PK Parameter	Control group (n=4)	PSC-833 treated group (n=3)	AUC _{0-6 hr, blood} (µg/mL·hr)	0.51 ± 0.02	1.28 ± 0.24*	AUC _{0-6 hr, brain} (µg/mL·hr)	≤0.02 ± 0.01	0.20 ± 0.05*	K _{brain/blood}	≤0.04 ± 0.01	0.15 ± 0.06*
PK Parameter	Control group (n=4)	PSC-833 treated group (n=3)												
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K _{brain/blood}	≤0.04 ± 0.01	0.15 ± 0.06*												
<p>Drion <i>et al.</i>, 1996</p>	<p>Male Sprague-Dawley rats anesthetized throughout the procedure</p> <p><i>in situ</i> brain perfusion technique was used to isolate P-gp inhibition to the blood brain barrier only and to minimize the</p>	<p>distribution of colchicine into the brain of rats was also found to be P-gp dependent</p> <p>Inhibition of P-gp efflux resulted in ~ 4- to 8-fold increase in the amount of colchicine detected in all areas of the brain with a blood-brain barrier.</p>												

	<p>perfusion time so that first passage uptake of the drug into the brain could be studied..</p> <p>either PSC-833 (a P-gp inhibitor; 10 mg/kg), i.v. verapamil (also a P-gp inhibitor; 1 mg/kg), i.v. or vehicle i.v.</p> <p>5 minutes later perfuse with heparinized blood containing ¹⁴C-colchicine or ¹⁴C-vinblastine through a catheter in the right carotid artery</p> <p>After 10, 20, or 60 seconds, the animal was sacrificed by decapitation and the brain removed</p> <p>Samples were dissected from nine different areas of the right cerebral hemisphere, counted with a scintillation counter and the distribution volume (μL/g) calculated</p>	<p>the brain distribution volume of colchicine after 20 seconds in the control group was $0.67 \pm 0.41 \mu\text{L/g}$</p> <p>with co-administration of P-gp inhibitors, verapamil or PSC-833, the brain distribution volume increased to 2.48 ± 0.26 or $5.64 \pm 0.70 \mu\text{L/g}$, respectively.</p> <p>the uptake of colchicine into the choroid plexus (which has no blood-brain barrier and does not express P-gp) was higher (~200 – 400 μL/g; estimated from Figure 5, specific values not provided) and was not P-gp dependent</p>
<p>Drion <i>et al.</i>, 1997</p>	<p>male Sherman rats anesthetized for the entire procedure colchicine was administered to rats at three doses (1, 2.5, and 5 mg/kg, i.v.) either alone or together with verapamil (0.5 mg /kg, i.v.)</p> <p><i>in vivo</i> experiment: Blood and brain samples were collected at 1, 2, and 3 hours (n=3 to 5 animals per time point) and measured for colchicine concentrations by RIA</p>	<p>the brain uptake of colchicine in the rat was studied using both an <i>in vivo</i> method and an <i>in situ</i> brain perfusion technique</p> <p>In the <i>in vivo</i> experiment, after administering colchicine only, brain, colchicine concentrations over this same time period are much lower and appear to saturate with increasing dose</p> <p>plasma concentrations of colchicine increased proportionally with dose over time (Figure 2.6.4:8).</p>
<p>Figure 2.6.4:8 from the Sponsor's Summary</p>		

Figure 2.6.4:8
Effect of Increasing Intravenous Colchicine Dose on Exposure (AUC_{0-3 hr}) in Plasma (left) and Brain (right) (Insets of Figure 1 and 2, Drion *et al.*, 1997)



When verapamil was co-administered with colchicine (time point of sample collection not specified), both brain and plasma colchicine concentrations increased with dose (Figure 2.6.4:9; Table 2.6.4:7).

Table 2.6.4:7 from the Applicant's Summary

Table 2.6.4:7
Colchicine Concentration (mean ± SE) in Plasma and Brain after Administering Colchicine Intravenously Either Alone or Together with Verapamil (0.5 mg/kg, i.v.) (Drion *et al.*, 1997)

Colchicine Dose (mg/kg)	Tissue**	Units	Colchicine Alone	Colchicine with Verapamil	Fold Increase *
2.5	Plasma	ng/mL	96.44 ± 2.53	112.05 ± 0.54	1.16
	Brain	ng/μg	1.21 ± 0.10	2.11 ± 0.01	1.75
5	Plasma	ng/mL	208.71 ± 7.83	343.67 ± 19.15	1.65
	Brain	ng/μg	1.372 ± 0.061	6.179 ± 0.155	4.5

*Fold Increase calculated as: $\frac{\text{Mean Colchicine Concentration after Administration of Colchicine Alone}}{\text{Mean Colchicine Concentration after Co-administration of Colchicine - Verapamil}}$

** Time collected was not specified.

Colchicine Effects on Transporters (Reviewer's table derived from the Applicant's review of published studies)

Author	Methods	Findings
Colchicine Inhibits Transporter Function		
Misra <i>et al.</i> , 2003	Rats Colchicine 2.5 mg/kg, i.p. (dose equivalent to the LD ₅₀ ; the extent to which these effects translate to a lower dose is not known)	colchicine blocks the recruitment of ABC transporters to the bile duct cannicular membrane Colchicine blocked the recruitment/trafficking of transporters (bile salt efflux protein [bsep] and multi-drug resistance protein 2 [mrp2]) to the cannicular membrane Also blocked bile acid secretion
Colchicine Induces Transporter Expression		
Vollrath <i>et al.</i> , 1994	male Wistar rats male CFI mice A single dose of colchicine (2 mg/kg, i.p.) Rats: At various time points, the liver, kidney, adrenal gland, and small bowel (all of which express the <i>mdr</i> gene in rats) were removed. Mice: at 24 hours, the liver was removed. For analysis, the probe used for analysis of the rat samples was derived from human cDNA; therefore, the specific <i>mdr</i> gene class-member was not identified. In mice, mouse-specific probes for <i>mdr1a</i> , <i>mdr1b</i> , and <i>mdr2</i> genes were used.	Colchicine administration increases <i>mdr</i> gene expression and P-gp protein (the encoded product of <i>mdr</i>) levels in the liver Rat liver within 24 hours, <i>mdr</i> mRNA levels increased 5- to 6-fold. by 48 hours, expression levels returned to background. Similar changes in expression were not observed in kidney, adrenal gland, or small bowel. At 48 and 72 hours after dosing, P-gp protein levels were higher in the colchicine-treated animals than in the controls. Mouse liver: Only increased <i>mdr2</i> gene expression in the liver; the other <i>mdr</i> family members were not affected. Overall, the authors concluded that regulation of <i>mdr</i> mRNA levels is complex and varies across species.

2.6.4.8 Other Pharmacokinetic Studies

These were incorporated into other sections.

2.6.4.9 Discussion and Conclusions

Early toxicology studies lacked the toxicokinetic information to correlate blood concentrations with specific toxicities. More recent studies that did obtain toxicokinetic data, lacked toxicity information and were only single dose studies. Some metabolites of colchicine have biological activity, but whether they are present in blood has not been determined. Colchicine has been instrumental in identifying and understanding the role of transporters especially P-gp in its absorption, distribution and excretion, especially in maintaining its low brain concentration, the secretion of colchicine into bile, and its secretion into kidney tubules.

2.6.4.10 Tables and figures to include comparative TK summary

Tables of Pharmacokinetic Parameters from Human Studies conducted by the Applicant

Table 1
Mean (CV%) Pharmacokinetic Parameter Values Following Administration of Single Oral Doses of Colchicine in Healthy Male Adult Subjects (Studies Sponsored by Mutual)

Study No. or Ref (Country)	N	Dose (mg)	C _{max} (ng/mL)	AUC _{0-∞} (ng·hr/mL)	t _{1/2} (hr)	T _{max} ¹ (hr)	CL/F (L/hr)	Vd/F (L)
MPC-004-07-1001 (U.S.)	24	0.6 mg	2.5 (28.9)	14.1 (39.7)	6.4 (73.9)	1.4 (0.5-2.0)	48.8 (36.8)	379 (44.5)
MPC-004-07-1004 (U.S.)	13	0.6 mg	2.5 (28.7)	12.3 (36.0)	5.0 (89.5)	1.5 (1.0-3.0)	54.1 (31.0)	342 (54.4)
MPC-004-07-1006 (U.S.)	23	0.6 mg	2.8 (31.0)	15.5 (49.6)	8.9 (126.4)	1.3 (0.5-2.0)	46.8 (43.7)	432 (56.1)

¹ T_{max} is reported as the mean (range)

Table 3
Mean (%CV) Pharmacokinetic Parameter Values Following Administration of Multiple Oral Doses of Colchicine (0.6 mg Dose b.i.d., Mutual) in Healthy Adult Subjects (Studies MPC-004-07-1004 and MPC-004-07-1005)

Mutual Study Number	N / Duration	C _{max} (ng/mL)	T _{max} ¹ (hr)	AUC _{0-∞} (ng·h/mL)	C _{av,∞} (ng/mL)	C _{min,∞} (ng/mL)	CL/F (L/hr)	Vd/F (L)	t _{1/2} (hr)
MPC-004-07-1004	13 / 10 days	3.6 (23.7)	1.3 (0.5-3.0)	20.4 (16.3)	1.7 (16.3)	0.9 (23.5)	30.3 (19.0)	1151 (18.7)	26.6 (16.3)
MPC-004-07-1005	27 / 14 days	3.1 (27.8)	1.4 (1.0-3.0)	18.4 (22.5)	1.5 (22.6)	0.8 (23.5)	34.2 (22.4)	738 (40.1)	14.7 (27.2)

¹ T_{max} mean (range)

Table 4
Mean (± SD) Pharmacokinetic Parameter Values in Otherwise Healthy Adults with FMF and Those with Renal Impairment (With and Without Hepatic Impairment)

	Adults with FMF		
	Healthy (N=4)	Renally Impaired (N=5)	
		Normal Liver Function (n=4)	Cirrhosis (n=1)
C _{max} (ng/mL)	5.8 ± 2.1	7.8 ± 2.6	4.5
T _{max} (hr)	1.5 ± 0.6	1.8 ± 0.5	1.0
t _{1/2} (hr)	4.4 ± 1.0	18.8 ± 1.2	50.0
CL/F (L/hr/kg)	0.727 ± 0.110	0.168 ± 0.063	0.078
Vd/F (L/kg)	4.87 ± 2.05	4.56 ± 1.64	5.77

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

These are included in the specific topic sections.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

The Sponsor did not conduct single or repeat dose toxicology studies. They summarized the extensive nonclinical published studies concerning toxicology of colchicine.

Historical information provided by the Applicant and obtained from published animal studies and human use indicates similar toxicities with increasing doses. The published nonclinical literature contains limited information for chronic treatment studies and most toxicology studies are only a few weeks duration. Furthermore, almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely collected such as clinical pathology and histopathology findings.

GENERAL TOXICOLOGY

Single Dose: On an acute basis, the LD₅₀ in rats is approximately 1.7 mg/kg i.v., not dissimilar following i.p. injection. The appearance of clinical effects at lethal doses are delayed, taking 3 to 6 hours to appear after the highest doses (up to 4 mg/kg) and up to 24 hours to appear after the lower doses of from 0.5 to 2 mg/kg (both routes). The LD₅₀ is less than 1.0 mg/kg, i.v., in rabbits and 0.9 mg/kg, p.o., in dogs. These values are similar to doses that have been reported to be fatal in human overdose (some fatalities resulted from dose levels between 0.5 to 0.8 mg/kg with 100% fatality at doses greater than 0.8 mg/kg). The hamster, a species in which the extent of metabolism appears higher than in other species, apparently is more resistant to the usual toxic effects of colchicine *in vivo*, as evidenced by surviving single i.p. injections of colchicine as high as 70 mg/kg without adverse effects.

Repeated Dose: The calculated LD₅₀ values for male and female rats, dosed orally, have been reported to be 51 and 26 mg/kg, respectively. Increasing doses of colchicine caused decreased body weight and food consumption up to 4 days after administration; there was no statistically significant difference in body weight between controls and treated animals after 10 days. Rats that died or were sacrificed moribund showed necrosis of liver, heart, lymphocytes, and bone marrow upon histopathological examination.

In a rising-dose study that was performed in rats given i.p. injections of colchicine (five times weekly with weekly escalation of dose) the maximum tolerated dose (MTD) was 0.4 mg/kg/day. In the final week of the study, at a dose of 1.6 mg/kg/day, serious toxicity such as severe diarrhea, bloody staining of the nose, and hind-leg paralysis appeared in a few rats. After only 3 days at this dose level (approximately 9% had died to this point),

35% of the remaining animals died.

In a rising-dose study performed in cats given i.p. injections of colchicine five times weekly, with the dose being doubled each week beginning at 0.025 mg/kg/day, to a final level of 0.2 mg/kg/day in the fourth week, two cats died in both the second and third weeks. During the second week (0.05 mg/kg) signs of diminished food consumption, weight loss, and lethargy began to appear. By the third week, all cats had developed ascites and extreme wasting atrophy of the hindquarters.

GENETIC TOXICOLOGY

The Applicant conducted assays to determine the mutagenic and clastogenic potential of purified colchicine which contains a conformational isomer impurity that exists in equilibrium with colchicine at approximately a 1% concentration and cannot be eliminated. The bacterial reverse mutation (Ames) assay was negative at doses up to 5000 µg/plate, with or without the presence of metabolic enzymes. A chromosomal aberration assay with human white blood cells (enriched lymphocytes) was negative at doses up to those that disrupted mitosis. At the highest concentrations, 860 and 2000 ng/mL, an increased proportion of cells exhibited mitotic disruption in the form of elevated mitotic index (mitotic arrest) and centromeric disruption (dissociated chromatids). These are not considered clastogenic effects.

Published studies reported colchicine was positive in mutagenic (*in vitro* mouse lymphoma thymidine kinase [TK] assay) and clastogenic (*in vitro* mammalian cell micronucleus assay *in vivo* in mice, hamsters, and rats) assays. In retrospect, these were probably false positives, a result of the cellular proliferation essential for these assays. The study of Honma et al 2001 determined that the mutagenic effects in the mouse lymphoma TK assay was due to loss of a functional *tk* allele generated by the loss of the entire chromosome 11 where the *tk* gene resides. There were no mutants with structural changes such as deletions or translocations involving chromosome 11. The mutations described in these assay arose through mitotic nondisjunction without structural DNA changes. In the published clastogenicity assays, cells with micronuclei were counted, but the chromosomes were not examined for signs of clastogenicity, rather only micronuclei were counted. The study of Jie and Jia (2001) examined the micronuclei and found they were composed mainly of whole chromosomes. These findings are consistent with colchicine's well characterized induction of aneuploidy. The conformational isomer did not alter the genotoxic results compared to literature reported effects. In retrospect, all colchicine preparations probably contained this isomer. Micronuclei can arise from acentric fragments induced by substances causing chromosomal breakage (clastogens) as well as from whole lagging chromosomes induced by those causing aneuploidy.

CARCINOGENICITY

No carcinogenicity studies have been reported for colchicine. There are two publications that utilize cellular assays to predict carcinogenic potential and that evaluated colchicine, and a third that investigated the temporal effects of colchicine administration as a pre-

initiating stimulus, in a two-stage initiation-promotion carcinogenicity study. Both studies suggest that colchicine could have carcinogenic potential, a conclusion that (together with the lack of 2-year assays) will be reflected in the proposed labeling.

Carcinogenicity studies have not been requested due to the long history of clinical experience, although specific documentation of any relationship between colchicine use and carcinogenicity is lacking. They are still possible options if clinical findings from expanded safety surveillance result in signals for further study. From the few repeated dose studies reported in the literature and (possibly the same studies conducted for the original approval of colchicine/probencid combination in 1961, original reviews were not found), there was a low threshold between adverse effects and the lethal dose in studies of less than 1 month duration in rats. With low doses of colchicine administered in drinking water of spontaneously hypertensive and nonhypertensive rats, respiratory difficulties developed within 4 to 13 months (Cicogna et al 1997). While there may be greater susceptibility in this strain, overall studies do not provide much confidence that a 2 year oral study in rats or mice would be productive. A study was conducted with dermally applied colchicine twice weekly in mice for 6 months, and this could be conducted with transgenic mice, but those mice would not be an appropriate model for an orally ingested drug since they have a high spontaneous background rate of internal tumor formation that may confound and mask colchicine induced effects.

REPRODUCTIVE TOXICOLOGY

Colchicine disruption of microtubule formation results in reproductive and developmental toxicity by cells involved in meiosis and mitosis. The effects are species and dose dependent, with the timing of exposure also critical for the effects on embryonic development. In general, published nonclinical studies indicated adverse effects on sperm development and fertility, early embryonic development and implantation, organogenesis (teratology), and late-stage embryonic development. In males, colchicine interfered with seminiferous tubule fluid secretion, testosterone production/release from rat Leydig cells, and disrupted microtubules in Sertoli cells in the epididymides, resulting in abnormal sperm production. In females, colchicine administration can result in eggs with Y chromosomes, and interfere with sperm penetration, the second meiotic division, and normal cleavage, and produce triploid and mosaic embryos. Published nonclinical studies demonstrated that colchicine is embryo-lethal early in development, is associated with the production of skeletal abnormalities during organogenesis, and causes slow embryofetal development. Published studies indicate that colchicine-mediated microtubular disruption inhibits the secretion of various hormones, including progesterone from luteal cells does inhibit the biosynthesis of progesterone. It also can reduced milk yield and alter milk composition (reduced fat content).

LOCAL TOLERANCE

Colchicine is an eye irritant.

2.6.6.2 Single-dose toxicity

Applicant' Summary Table

2.6.7.5 Single-Dose Toxicity

Mutual did not sponsor any single-dose toxicity studies. The information presented below is a summary of the publicly available literature.

<u>Species / Strain</u>	<u>Method of Administration</u>	<u>Doses (mg/kg)</u>	<u>Approximate Lethal Dose (mg/kg)</u>	<u>Noteworthy Findings</u>	<u>Source</u>
Sprague-Dawley rats	Oral	10, 20, 30	n/a	Female rats were more sensitive to single, oral dose of colchicine. Pretreatment with LPS increased the oral toxicity of colchicine; more so in males. The matrix did not cause significant effect on toxicity. Dose-related decreased body weight and food consumption up to 4 days after administration. Rats that died or were sacrificed moribund showed necrosis of liver, heart, lymphocytes, and bone marrow.	<u>Wissenfeld et al., 2007</u>
Wistar rats	Intravenous Intraperitoneal	0.5, 1, 2, 4 4.	1.7	Delayed effects appearing 3 to 6 hr post-dose included lethargy, weakness, decreased appetite, diarrhea, reactivity to noise, unkempt appearance, and weight loss. Also seen: reversible paralysis of the hind quarters about 48 hours after treatment, and ascites 1 week post-dose. The LD ₅₀ for i.p. administration was slightly less than the i.v. LD ₅₀ .	<u>Ferguson, 1952</u>
Golden hamsters	Intraperitoneal	1.2 to 70 mg/kg	n/a	Hamsters showed normal weight gain during study period, and were observed to be fertile as early as 5 days after treatment with colchicine. The authors claim that this study indicates that the hamster possesses a natural resistance to the usual toxic effects of colchicine	<u>Orsini and Pansky, 1952</u>
Rats (female)	Intraperitoneal	0.4 mg	n/a	Two of 3 rats killed 24 hours after injection had developed diarrhea. The remaining 19 treated rats developed diarrhea (within 24 to 48 hours), slowness of reaction, and moderate to severe muscle weakness.	<u>Markand et al., 1971</u>
Cats	Intravenous	0.12, 0.25, 0.5, 1	0.25	Vomiting, diarrhea, lethargy, anorexia appeared 22 h after dosing, and were severe and prolonged.	<u>Ferguson, 1952</u>

2.6.6.3 Repeat-dose toxicity

There were no studies conducted by the Applicant. They provided published studies and summaries of the literature.

Applicant' Summary Table

2.6.7.6 Repeat-Dose Toxicity

Mutual did not sponsor any repeated-dose toxicity studies. The information presented below is a summary of the publicly available literature.

<u>Species / Strain</u>	<u>Method of Administration</u>	<u>Duration of Dosing</u>	<u>Doses (mg/kg/day)</u>	<u>Number of animals (sex)</u>	<u>Noteworthy Findings</u>	<u>Source</u>
Sprague-Dawley rats	Intraperitoneal	For 2 to 22 days	0.4, 0.8	23 (males)	Low-dose rats exhibited mild toxic effects or none at all. High-dose animals showed severe toxic effects including weight loss, diarrhea, weakness, and paralysis.	<u>Sciden, 1973</u>
Wistar rats	Intraperitoneal	5 times / week	0.1 - 1.6	69	Clinical findings included weight loss, ascites in 50% of the animals, and mortality in 5% of the population at 0.4 mg/kg. At 0.8 mg/kg, diarrhea became prominent and another 4% died. At 1.6 mg/kg, serious toxicity including 35% mortality (within 3 days), severe diarrhea, bloody staining of the nose and hind-leg paralysis, appeared in a few rats. The maximum tolerated dose (MTD) was 0.4 mg/kg.	<u>Ferguson, 1952</u>
Golden hamsters	Oral in diet	9 days	est. 125 mg/kg/day for mice, 45 for hamsters and 31 for rats, from <i>C. aurumale</i> seed in diet.	2	Hamsters were studied in comparison to 2 mice, 2 rats and 2 rabbits. Both mice died on Day 7, exhibiting substantial weight loss. One rat decreased in weight by nearly 50% by Day 6, when it died; the other rat's weight decreased by approximately 30% before being sacrificed at study termination on Day 9. The hamsters gained 1.5 and 6 g respectively during the 9 day period.	<u>Orsini and Pansky, 1952</u>
Rabbits	Subcutaneous	Twice weekly for 15 weeks	1.5, 3	Adult males	Post mortem examination of the organs revealed no pathological lesions with the exception of the testes. The average weight of the testes was 1.5 g in the rabbits receiving colchicine and 4.5 g in the controls.	<u>Barsoum, 1955</u>
Cats	Intraperitoneal	5 times / week	0.025 - 0.2	12	Two cats died at 0.05 mg/kg and signs of decreased food consumption, weight loss, and lethargy began to appear. By the third week (third dose level), all cats had developed ascites and extreme wasting atrophy of the hindquarters.	<u>Ferguson, 1952</u>

2.6.6.4 Genetic toxicology

The Applicant conducted assays to determine the mutagenic and clastogenic potential of purified colchicine. In addition, the Applicant provided summaries of the published *in vitro* and *in vivo* assays and the Mutual-sponsored assays which are presented in the tables at the end of this section. The bacteria reverse mutation assay was negative at doses up to 5000 µg/plate, with or without the presence of metabolic enzymes. A chromosomal aberration assay with human white blood cells was negative at doses that disrupted mitosis, an essential aspect of the assay. At the highest concentrations, 860 and 2000 ng/mL, an increased proportion of cells exhibited mitotic disruption in the form of elevated mitotic index (mitotic arrest) and centromeric disruption (dissociated chromatids). Colchicine did not cause any significant increase in clastogenicity.

Reviewer Comment: There was no confirmatory assay for the mutagenic assays, rather two different treatment protocols were used, and each conducted once. There are numerous of other genetic toxicology studies cited to support the conclusions reached with these two assays, that lack of replication would not compromise the overall interpretation.

Previous studies reported in the literature indicated colchicine was both positive and negative for mutagenic and clastogenic assays (refer to Applicant's Summary Tables at the end of this section). Positive mutagenic effects were reported for the *in vitro* mouse lymphoma tk assay at doses of 10 to 50 ng/mL. Positive clastogenic results were reported for in the *in vitro* mammalian cell micronucleus assay (0.1 µg/mL), and *in vivo* micronucleus in mice (0.35 to 5 mg/kg), hamsters (3 mg/kg), and rats (2.5 to 5 µg/kg). The Sponsor included this information in the label. In general positive chromosomal aberration assays were obtained with higher doses than utilized in studies that had negative results.

The assays conducted with mammalian cells require cellular proliferation (mitosis) as part of the methodology. The study of Honma et al 2001 determined that the mutagenic effects in the *in vitro* mouse lymphoma tk assay was due to loss of a functional tk allele generated by the loss of the entire chromosome 11 where the tk gene resides. There were no mutants with structural changes such as deletions or translocations involving chromosome 11. The mutations observed in the assay arose through mitotic nondisjunction without structural DNA changes. In the published clastogenic assays, cells with micronuclei were counted, but not examined at the chromosome level for signs of clastogenicity. The study of Jie and Jia 2001 examined the micronuclei and found they were composed mainly of whole chromosomes. These findings are consistent with colchicine's well characterized induction of aneuploidy. Micronuclei can arise from acentric fragments induced by substances causing chromosomal breakage (clastogens) as well as from whole lagging chromosomes induced by those causing aneuploidy.

Study title: Colchicine: Bacterial Mutation Test

Key findings: Colchicine was not mutagenic the bacteria reverse mutagenicity assay of *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* at concentrations up to 5000 µg/plate in the presence or absence of S9 liver metabolic enzymes.

Reviewer Comments: There were no confirmatory assays, rather two different treatment protocols were used, each conducted once. However, within each method, there are no indications, such as dose trends, which might suggest the conclusions are tentative, thus the lack of replication does not compromise the overall interpretation. There is a possible discrepancy in the purity of the colchicine used, since the certificate of analysis associated with this study indicates a purity value different from that listed in the Applicant's Summary Table of the drug substance. When an inquiry was submitted to the Sponsor they addressed the difference in lot number, but did not address the difference in purity.

Study no.: MPC-004-07-0002

m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro

Conducting laboratory and location: _____

Date of study initiation: Feb 14, 2008

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Colchicine, Lot CCU-117153, Purity 99.64% (potency _____),

Reviewer Comment: In section 2.6.7.4 Toxicology, table of the Drug Substance There is a potential discrepancy in that the drug substance indicates Batch Number RD10016 98.77, with Conformational isomer: 1.1%, Any unspecified impurity: not detected, and Total impurities: ____ .
Vehicle: sterile water, USP

Analysis of doses indicated concentrations were within $\pm 10\%$ of calculated for stock solutions and $\pm 15\%$ of calculated for dosing solutions.

Reviewers Comment: A correction factor was applied for expressing doses in terms of pure material, however it is not clear if this factor was based on purity or potency or both factors.

Methods**Strains/species/cell line:**

S. typhimurium TA1535 *hisG46 rfa ΔuvrB*

S. typhimurium TA1537 *hisC3076 rfa ΔuvrB*

S. typhimurium TA98 *hisD3052 rfa ΔuvrB* pKM101

S. typhimurium TA100 *hisG46 rfa ΔuvrB* pKM101

E. coli WP2 *trp uvrA*

Doses used in definitive study: 1.58, 5, 15.8, 50, 158, 500, 1581, 5000 µg/plate

Basis of dose selection:

There were no preliminary tests conducted. Only the 5 highest doses not demonstrating toxicity were selected for analysis. There were no confirmatory assays, rather two different treatment protocol were used, each conducted once.

Negative controls: sterile water

Positive controls:

Absence of S9	Dose (µg/plate)	Strain
Sodium azide (NaAz)	0.5	TA1535, TA100
9-aminoacridine (9AC)	50	TA1537
2-nitrofluorene (2NF)	1	TA98
4-nitroquinoline N-oxide (NQO)	0.5	WP2 <i>uvrA</i>
Presence of S9		
2-aminoanthracene (2AA)	5 15	TA1535 WP2 <i>uvr</i>
Benzo[a]pyrene (BaP)	5	TA1537, TA98, TA100

Incubation and sampling times: Assays were conducted using both the plate incorporation method (48-72 hrs at 37 C) and pre incubation method (30 min at 37 C, followed by addition of agar and incubation for 48-72 hrs at 37 C) as indicated in the following table:

Text Table 1 Study Design for Plate Incorporation and Pre-incubation Assays

Material	Formulation conc. (µg/mL)	Final conc. (µg/plate)	Number of replicates		Number of strains
			0S9	+S9	
Vehicle	-	-	3	3	5
Test article	6.32	1.58	3	3	5
	20.0	5.0	3	3	5
	63	15.8	3	3	5
	200	50	3	3	5
	632	158	3	3	5
	2000	500	3	3	5
	6325	1581	3	3	5
	20000	5000†	3	3	5
Positive control	‡	‡	3	3	5

‡ Depends on the test organism, positive control agent and methodology used.

† Test article was tested at levels up to 5000 µg/plate, which is the standard limit dose recommended by regulatory guidelines.

Plates were evaluated for the quality of the background lawn and the number of revertant colonies. Colony numbers were enumerated visually if precipitation or other artifacts interfered. The mean number of revertant colonies for all treatment groups was

compared with those obtained for the concurrent vehicle control group. Where five (relatively non-toxic) dose levels were analyzed, the remaining plates from lower dose levels may not have been evaluated, and are not reported.

Results

Study validity:

If available, the plates from at least five non-toxic dose levels of the test article were assessed in each experiment, *i.e.* the five highest levels below the toxic level. Toxic effects of the test article are normally indicated by the partial or complete absence of a background lawn (colony counts, if any, are not reported) or a substantial dose-related reduction in revertant colony counts compared with lower dose levels and concurrent vehicle controls (*i.e.* fold response < 0.6) taking into account the laboratory historical control range.

The mutagenic activity of the test article was assessed by applying the following criteria:

- *Positive*: If treatment with the test article produced a dose-related increase in revertant colony numbers to at least twice the concurrent vehicle control levels with any bacterial strain (1.5× for Strain TA100), either in the presence or absence of S9 mix. The historical control range was also taken into consideration.
- *Negative*: If treatment with the test article did not produce a dose-related increase of at least 1.5 (strain TA100) or 2 (other strains) times the concurrent vehicle controls, it was concluded that there was not sufficient evidence of mutagenic activity.
- *Equivocal*: If the results obtained failed to satisfy the criteria for a clear positive or negative response, the results were considered equivocal. Equivocal may also apply if no clear conclusion can be made. The reproducibility of any apparent effect is taken into account in making any clear conclusion.

For an assay to be considered valid, the mean revertant colony counts of the vehicle controls for each strain should lie close to or within the current historical control range of the laboratory. All positive control articles (with S9 mix where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5× for strain TA100).

Reviewer's Comment: The Applicant did not explain the reason for the 1.5X lower threshold for a positive response with strain TA100.

Study outcome:

The mean revertant colony counts for the vehicle controls were close to or within the laboratory historical control range. Positive controls produced at least a 2-fold increase in revertant colonies compared to vehicle controls, except for strain TA100 in which a 1.5-fold increase was obtained. In the presence of colchicine, there was no precipitation observed and there was no toxicity indicated by the visible thinning of the bacteria background lawn. Colchicine, with or without S9 liver metabolic enzymes did not substantially increase the number of revertant colonies for any bacteria strain.

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation Test				
			Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 <i>inv-d</i>
Without Activation	Water	-	28 ± 3	13 ± 1	29 ± 1	162 ± 10	46 ± 7
	Colchicine	50	23 ± 4	14 ± 4	33 ± 5	153 ± 12	57 ± 3
		158	24 ± 1	16 ± 8	25 ± 1	143 ± 3	53 ± 4
		500	23 ± 5	12 ± 5	29 ± 5	164 ± 6	53 ± 8
		1581	26 ± 5	9 ± 1	29 ± 5	143 ± 10	60 ± 5
		5000	24 ± 4	12 ± 4	27 ± 0	133 ± 13	49 ± 8
	NaAz	0.5	322 ± 19			637 ± 16	
	9AC	50		564 ± 68			
	2NF	1			189 ± 22		
	NQO	0.5					290 ± 49

Concentrations expressed in terms of pure colchicine

DMSO = dimethyl sulfoxide, NaAz = Sodium azide, 9AC = 9 Aminoacridine, 2NF = 2 Nitrofluorene, NQO = 4 Nitroquinoline N-oxide

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation Test				
			Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 <i>inv-d</i>
With Activation	Water	-	24 ± 4	17 ± 4	47 ± 8	171 ± 12	62 ± 3
	Colchicine	50	30 ± 8	16 ± 2	41 ± 14	169 ± 8	66 ± 5
		158	18 ± 7	15 ± 6	32 ± 9	152 ± 8	65 ± 7
		500	28 ± 9	17 ± 5	42 ± 12	166 ± 11	67 ± 7
		1581	23 ± 7	21 ± 6	39 ± 11	166 ± 8	65 ± 8
		5000	21 ± 3	20 ± 7	37 ± 3	160 ± 10	63 ± 13
	2AA	5	363 ± 10				
	2AA	15					367 ± 19
	BaP	5		113 ± 21	435 ± 11	1331 ± 142	

Concentrations expressed in terms of pure Colchicine

DMSO = dimethyl sulfoxide, 2AA = 2 Aminoanthracene, BaP = Benzo[a]pyrene

Metabolic Activation	Treatment	Dose Level (µg/plate)	Pre-incubation Test				
			Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 <i>inv-d</i>
Without Activation	Water	-	24 ± 9	15 ± 2	32 ± 12	149 ± 10	49 ± 11
	Colchicine	50	25 ± 5	13 ± 2	34 ± 3	153 ± 4	51 ± 6
		158	29 ± 7	16 ± 2	27 ± 2	152 ± 18	52 ± 10
		500	22 ± 7	12 ± 1	22 ± 5	150 ± 19	50 ± 2
		1581	20 ± 2	16 ± 7	27 ± 5	154 ± 7	40 ± 10
		5000	25 ± 6	13 ± 5	27 ± 2	146 ± 14	52 ± 6
	NaAz	0.5	330 ± 23			483 ± 21	
	9AC	10		2240 ± 224			
	2NF	1			122 ± 19		
	NQO	0.5					1409 ± 62

Concentrations expressed in terms of pure Colchicine

DMSO = dimethyl sulfoxide, NaAz = Sodium azide, 9AC = 9 Aminoacridine, 2NF = 2 Nitrofluorene, NQO = 4 Nitroquinoline N-oxide

Metabolic Activation	Treatment	Dose Level (µg/plate)	Pre-incubation Test				
			Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 <i>inv-d</i>
With Activation	Water	-	24 ± 4	17 ± 4	41 ± 9	152 ± 11	55 ± 2
	Colchicine	50	33 ± 3	18 ± 6	39 ± 10	162 ± 11	56 ± 7
		158	23 ± 6	19 ± 1	39 ± 2	148 ± 10	67 ± 1
		500	22 ± 2	18 ± 2	37 ± 4	125 ± 7	60 ± 10
		1581	25 ± 4	13 ± 1	48 ± 7	155 ± 12	44 ± 2
		5000	20 ± 6	14 ± 2	43 ± 10	169 ± 3	68 ± 10
	2AA	5	307 ± 4				
	2AA	15					511 ± 8
	BaP	5		137 ± 16	375 ± 23	1140 ± 96	

Concentrations expressed in terms of pure Colchicine

N/A = Not applicable, DMSO = dimethyl sulfoxide, 2AA = 2 Aminoanthracene, BaP = Benzo[a]pyrene

Study title: Colchicine: Chromosome Aberration Test

Key findings: Colchicine, up to levels that caused substantial mitotic disruption in *in vitro* preparations of human peripheral blood lymphocytes, did not produce a significant increase in chromosomal aberrations. The highest one or two levels, 860 and 2000 ng/mL, had an increased proportion of cells showing mitotic disruption in the form of elevated mitotic index (mitotic arrest) and centromeric disruption (dissociated chromatids). Centromeric disruption is not classified as a chromosomal aberration and the biological significance of this *in vitro* finding is unknown. Colchicine did not cause any significant increase in chromosome breakage in this *in vitro* test when tested at levels causing substantial mitotic disruption.

Study no.: MPC-004-07-0003

m4\42-stud-rep\423-tox\4233-genotox\42331-in-vitro

Conducting laboratory and location: _____

b(4)

Date of study initiation: report date May 27, 2008, study initiation date not provided

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Colchicine, Lot CCU-117153, Purity 99.64%; (potency _____ ; a correction factor was applied and all dose levels throughout this report are expressed in terms of pure material.

b(4)

Vehicle: Sterile water, USP

Reviewer Comment: In section 2.6.7.4 Toxicology, table of the Drug Substance There is a potential discrepancy in that the drug substance indicates Batch Number RD10016 98.77, with Conformational isomer: 1.1%, Any unspecified impurity: not detected, and Total impurities: _____.

b(4)

Analysis of the dose formulations indicated that concentrations were within $\pm 10\%$ of calculated values for stock solutions and within $\pm 15\%$ of calculated values for dosing solutions.

(*Reviewer comment.* It is not clear if the correction factor was derived from purity or potency or both factors.)

Methods

Strains/species/cell line: Human peripheral blood lymphocytes obtained by venipuncture from a healthy, non-smoking, male donor (n=1 donor).

Doses used in definitive study: 1.00, 2.33, 5.41, 12.6, 29.3, 68.2, 159, 369, 860, 2000 ng/mL

Basis of dose selection:

The colchicine was tested at dose levels of 1.00 ng/mL to 2000 ng/mL based on published information. The low levels had no effect on spindle function, while the high level had very clear effects. The highest dose level chosen for detailed examination was that showing clear evidence of mitotic arrest, as indicated by at least a doubling in the relative mitotic index. This dose and the next 3 lower biologically relevant dose levels of test article were subjected to detailed analysis.

Reviewer Comment: References for this "published information" were not provided in the report.

Negative controls: sterile water

Positive controls:

In the absence of S9 Mix: Mitomycin C (MMC), 0.05, 0.10, and 0.20 µg/mL

In the presence of S9 Mix: Cyclophosphamide monohydrate (CP), 8, 12, and 16 µg/mL

Incubation and sampling times: The blood sample was taken directly into blood collecting tubes containing sodium heparin then held at room temperature until cultured within 2 hours. Culture medium was RPMI 1640 supplemented with 10% fetal calf serum, 50 µg/mL gentamycin and 4 units of heparin/mL. Whole blood was mixed with medium and phytohemagglutinin to stimulate lymphocyte division. Treatments were performed approximately 48 hours after culture initiation. Cultures were treated for 4 hours in the absence and presence of rat S9 mix and for 21 hours in the absence of rat S9 as indicated in the table below. Appropriate concurrent negative and positive controls were included for each treatment regime. The medium was changed for the 4 hour incubation cultures and cultured fresh complete medium for an additional 17 hours. Colcemid was added to all cultures at a final concentration of 0.1 µg per mL approximately two hours prior to harvesting. Cells were harvested, fixed, dried onto slides and Giemsa stained.

Text Table 1 Study Design

Dose number	Material	Formulation conc. (ng/mL or µg/mL [†])	Final conc. (ng/mL or µg/mL [†])	Number of cultures		
				4 Hours (0S9)	4 Hours (+S9)	21 Hours (0S9)
0	Vehicle	-	-	2	2	2
1	Colchicine	100	1.00	2	2	2
2		233	2.33	2	2	2
3		541	5.41	2	2	2
4		1260	12.6	2	2	2
5		2930	29.3	2	2	2
6		6820	68.2	2	2	2
7		15900	159	2	2	2
8		36900	369	2	2	2
9		86000	860	2	2	2
10		200000	2000*	2	2	2
1	MMC‡	5.00	0.05	2		2
2		10.0	0.10	2		2
3		20.0	0.20	2		2
1	CP‡	800	8.0		2	
2		1200	12		2	
3		1600	16		2	

† µg/mL for positive controls Mitomycin C and Cyclophosphamide only.

‡ Please note that units were incorrectly listed in the final protocol for both positive controls.

Dose concentration however were prepared as per standard procedures and consequently no impact was considered.

* Where 2000 ng/mL (high level) was a level expected to show clear effects.

Results

Study validity:

The mitotic index was determined by examination of at least 500 cells per culture for selected treatment groups, i.e. relevant dose levels not showing extreme toxic effects. Lymphocyte toxicity is primarily indicated by a decreased mitotic index compared to the concurrent control group. The relative mitotic index for each treatment group was calculated as a percentage ratio compared with the appropriate concurrent vehicle control group.

For each treatment regime and phase, the highest dose level selected for examination of aberrations is the highest dose level tested in the case of test articles showing relatively low toxicity. Routinely the highest level examined is the lowest concentration which causes a reduction in the RMI to below 50%. However, in this particular study, the highest dose level chosen for detailed examination was that showing clear evidence of mitotic arrest, as indicated by at least a doubling in the RMI. In addition, one relatively non-toxic dose level of positive control agent was selected for examination from each of the three treatment regimes/phases.

Slides selected for examination were randomized then encoded to minimize potential operator bias. They were examined by light microscopy, and (where practical) a total of 200 readable metaphases per experimental point were examined for the presence of chromosome aberrations using oil-immersion optics. Usually, only one slide was examined per culture, the remaining slide(s) being held temporarily in reserve. if for

example there were insufficient metaphases available to complete analysis with the first slide.

Readable metaphases are identified by the following criteria:

- Chromosome number between 44 and 48 in a single stage of condensation
- Well-spread with minimal overlap of chromosomes and chromosome arms
- Chromatids separate with centromere intact
- Structure of chromosomes clear and well-defined

The International System for Chromosome Aberration Nomenclature (1995) was followed to designate the observed aberrations. Since the nature of chromosomal and chromatid gaps is uncertain (they may or may not represent true breaks in chromatid structure), these two types of aberration were recorded but were not included in statistical analysis of aberrations. The incidences of numerical types of aberration, such as polyploidy and endoreduplication, were recorded.

Results from replicate cultures were combined to facilitate interpretation and maximize the power of statistical analysis. The results obtained for each treatment group were compared with the results obtained for the concurrent vehicle control group from the same treatment regime using Fisher's Exact Test.

- A positive response is normally indicated by a statistically significant (dose-related, if applicable) increase in the incidence of aberrant cells for the treatment group compared with the concurrent control group ($p \leq 0.01$); individual and/or group mean values should exceed the laboratory historical control range (99% limit).
- A negative result is indicated where group mean incidences of aberrant metaphase cells for the group treated with the test article are not significantly greater than incidences for the concurrent control group ($p > 0.01$) and where these values fall within or close to the historical control range.
- An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response.

For an assay to be considered valid, the vehicle/negative control results should lie within or close to the historical control range, while the positive control should produce a significant increase in the incidence of aberrant cells compared with the concurrent control.

Study outcome:

Colchicine did not cause any statistically significant increases in the incidence of cells with chromosome breakage at any experimental point. The proportion of aberrant metaphases for all vehicle and test article groups was within the laboratory historical control range. The positive control treatment resulted in significant increases in the proportion of aberrant metaphases.

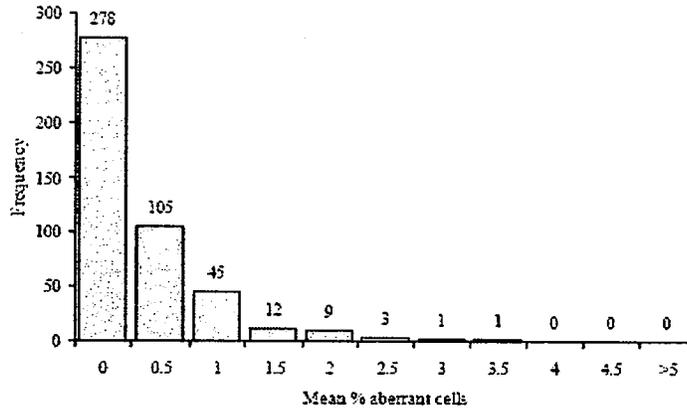
There were no substantial increases in the incidence of chromatid or chromosome gaps or polyploidy were observed at any experimental point. No precipitation or visible change in culture medium color was observed. The highest one or two levels had an increased

proportion of cells showing mitotic disruption in the form of elevated mitotic index (mitotic arrest) and centromeric disruption (dissociated chromatids). A high incidence of centromeric disruption was observed at concentrations of ≥ 29.3 ng/mL for the 4-hour with or without S9 regimes and at concentrations of ≥ 12.6 ng/mL for the 21-hour without S9 regime. Centromeric disruption is not classified as a chromosomal aberration and the biological significance of this *in vitro* finding is unknown. Colchicine did not cause any significant increase in chromosome breakage in this *in vitro* test when tested at levels causing substantial mitotic disruption.

Table 1 Results and Statistical Analysis

Treatment	Conc. (ng/mL or ug/mL [‡])	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations					Incidental observations †			
						b	e	B	E	other	g	G	P	C
<i>4 hours treatment in the absence of S9 (0S9)</i>														
Water	-	8.5	100	200	0.0	0	0	0	0	0	2	0	0	0
Colchicine	5.41	10.3	121	200	0.0	0	0	0	0	0	0	0	0	0
	12.6	7.5	89	200	1.0	2	0	0	0	0	3	0	0	1
	29.3	12.4	146	200	1.0	1	0	2	0	0	1	0	0	14
	68.2	17.8	210	200	2.5	5	0	0	0	0	0	0	1	69
MMC	0.10	6.9	82	200	20.5**	34	3	13	0	0	9	1	0	0
<i>4 hours treatment in the presence of S9 (+S9)</i>														
Water	-	7.7	100	200	2.0	4	0	0	0	0	2	0	0	1
Colchicine	5.41	8.7	114	200	0.5	1	0	0	0	0	4	0	0	0
	12.6	7.7	101	200	1.0	2	0	0	0	0	1	0	0	3
	29.3	9.2	120	200	2.5	5	0	0	0	0	0	0	0	25
	68.2	17.9	234	200	1.5	3	0	0	0	0	1	0	0	44
CP	8.0	2.2	28	200	28.5**	62	8	5	0	0	6	1	0	0
<i>21 hours treatment in the absence of S9 (0S9)</i>														
Water	-	5.5	100	200	0.5	1	0	0	0	0	2	0	0	0
Colchicine	1.00	4.3	77	200	2.0	3	0	2	0	0	2	0	0	0
	2.33	5.7	103	200	0.0	0	0	0	0	0	1	0	0	0
	5.41	8.2	148	200	0.5	1	0	0	0	0	2	0	0	5
	12.6	22.7	411	200	1.0	2	0	0	0	0	1	0	0	14
MMC	0.05	4.9	89	200	12.5**	19	2	6	0	0	5	1	0	0
MI, RMI	Mitotic Index, Relative Mitotic Index (vehicle = 100%)													
b, e, g	Chromatid break, exchange, gap													
B, E, G	Chromosome break, exchange, gap													
other	Includes pulverized chromosomes and cells with > 8 aberrations													
P	Polyploidy and endoreduplication													
C	Centromeric disruption													
†	g, G, P and C are excluded from the calculation of % aberrant cells													
‡	ug/mL for Mitomycin C and Cyclophosphamide only													
Results of statistical analysis using one-tailed Fisher's exact test														
	*	p ≤ 0.01 (significant)												
	**	p ≤ 0.001 (highly significant)												
otherwise		p > 0.01 (not significant)												

Figure 1 Historical Control Values



The laboratory historical mean incidence of aberrant metaphase cells for negative/vehicle control cultures for the human lymphocyte chromosome aberration test is 0.32% (SD 0.53) for 454 treatments. These QA audited results were collected from GLP compliant studies performed from 05 February 2003 prior to 15 November 2007.

The historical positive control values (for QA-audited and GLP compliant studies) are listed below:

- Mitomycin C (4 hour OS9): mean 10.4%, SD 5.1, 112 treatments
- Mitomycin C (21 hour OS9): mean 12.0%, SD 5.1 116 treatments
- Cyclophosphamide (4 hour -S9): mean 19.5%, SD 7.6, 115 treatments

Additional Genetic Toxicology Studies (published literature)

A summary of many of the published *in vitro* and *in vivo* assays is presented in the following tables.

Applicant's Summary Tables

2.6.7.7.G Genotoxicity: *In Vitro*

The following table summarizes the publicly available studies evaluating the *in vitro* genotoxicity of colchicine.

<u>Reference</u>	<u>Test System</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
Ames Tests				
<u>Brice and Heddle, 1979</u>	AMES test with the following <i>S. typhimurium</i> strains: TA1535, TA1537, TA98, and TA100.	0.05, 0.5, 5, 50, and 500 µg/plate with and without S9 activation. Each strain was irradiated at 10, 100, 1000, 10000, and 100000 rads without S9.	The criterion of a positive response was a 50% increase above the spontaneous frequency obtained at the same time.	Negative for mutagenicity
National Toxicology Program, <u>Study ID: 62896</u> (1986)	AMES test with the following <i>S. typhimurium</i> strains: TA1535, TA1537, TA98, and TA100.	0, 100, 333, 1000, 3333, and 10000 dose units. With two species of S9 activation (rat and hamster) and without activation.	A test tube containing the suspension of <i>S. typhimurium</i> and S9 or buffer was incubated at 37° C with colchicine for 20 minutes. Agar was added and placed onto Petri dishes and incubated.	The number of histidine-independent colonies was not significantly higher than that of the control; therefore colchicine was negative for mutagenicity with and without S9 activation.
<u>Hemmerly and Demerec, 1955</u>	<i>E. coli</i> (Sd-4, Sd-4-73, WP-14, and WP-2)	5, 10, 20 mg/ml	Bacteria were allowed to grow for 24 h before treatment at 37 °C.	Colchicine was shown to be weakly mutagenic to the WP-14 strain.
Chromosomal Aberration Tests				
National Toxicology Program, <u>Study ID: 103168</u> (1987)	CHO cells	0, 100, 500, 1600, 5000 µg/mL without S9 activation and 0, 500, 1600, 3000, 4000, and 5000 µg/mL with S9 activation.	Cells were harvested after a 14 hour incubation period, 100 cells were examined for the following aberrations: simple (breaks and terminal deletions), "complex" (rearrangements and translocations), and "other" (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).	Analyses were conducted to assess the presence of a dose-response and the significance of the individual dose points compared to the vehicle control. Only colchicine without activation was positive for chromosomal aberrations.
<u>Reference</u>	<u>Test System</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
National Toxicology Program, <u>Study ID: 103168</u> (1987)	CHO cells	0, 0.005, 0.016, 0.05, and 0.16 without activation, 0, 0.1, 0.3, 0.4, 0.5, and 1.6 with activation.	Chinese hamster embryo cells were incubated with colchicine in McCoy's 5A medium supplemented with fetal calf serum, L-glutamine, and antibiotics. 5-Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation (serum was not used with S9 activation). <u>Without activation:</u> cells were incubated for 26 hours, medium with colchicine was removed and replaced by fresh medium, BrdU, and colcemid. Cells were incubated for an additional 2 hours and then harvested. <u>With activation:</u> After the initial 2 hour incubation, medium with colchicine was removed and replaced by medium containing serum and BrdU. Cells were incubated for 26 hours (with colcemid for the final 2 hours) and then harvested.	SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. Colchicine showed a negative response with and without S9 activation.
<u>Anni and Hermer, 1997</u>	CHO cells	62.5, 125, 250, 500, and 1000 µg/ml	A series of glass slides was seeded with CHO cells at a density of at least 1 x 10 ⁵ cells/ml (18 h experiment) or 4 x 10 ⁵ cells/ml (42 h experiment). Cells were preincubated for 24 h before treatment. Quadruplicate cultures were prepared for each group in the assay. Colcemid at 0.4 µg/ml was added to arrest cells in metaphase 2 hours prior to harvesting for slide preparation. One to two hundred well-spread metaphase figures were scored for structural chromosomal aberrations.	After 18 h treatment, an increase in % of metaphase cells with aberrations over control was observed at 500 and 1000 µg/ml. After 24 h treatment, induction of tetraploid cells was observed at all concentrations and a high incidence of all types of chromosomal aberrations was found at 1000 µg/ml.
Micronucleus Tests				
<u>Jie and Jia, 2001</u>	Mouse NIH 3T3 cells	0.0, 0.1 µg/ml	The chromosomal composition of micronuclei was analyzed by multicolor fluorescence <i>in situ</i> hybridization with DNA probes for the centromere repeated minor satellite and the hexamer repeat.	Micronuclei (MN) were shown to be composed mainly of whole chromosomes with 39.0 MN/1000 nuclei.

<u>Reference</u>	<u>Test System</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Schmuck et al., 1988</u>	Syrian hamster embryo cells, 13 days old	10^{-8} to 10^{-6} M	1.5×10^6 cells in culture were incubated for 24 h, and then treated with colchicine diluted in DMSO (final concentration of 0.1%). After 5 h of incubation with colchicine, the medium was changed, and further incubated for 12 h. Cells were fixed and scored for number of micronuclei in 2000 cells.	The activity of colchicine was calculated to be 140 MN/ μ mole of colchicine, which is considered to be a strong increase in MN frequency.
<u>Lynch and Parry, 1993</u>	Chinese hamster Luc2 cells	0.0, 0.01, 0.025, 0.05, 0.1 and 1.0 μ g/ml	An assay capable of detecting numerical and structural chromosome changes was developed. Chromosome loss was inferred by indirect visualization of human CREST antikinetochore antibodies bound to centromeres in chemically induced micronuclei of cytochalasin-B arrested cells. The day before treatment, cells were seeded into 25 cm ² flasks and allowed to grow overnight. Duplicate cultures were treated with 100 μ l of colchicine diluted to the appropriate concentration. Cytochalasin-B was added simultaneously to a concentration of 3.5 μ g/ml. Cells were incubated for 24 h at 37 °C. Slides were immunofluorescence stained.	Colchicine induced highly significant ($P < 0.001$) increases in all 4 endpoints (micronuclei frequency, frequency of cells with disorganized nuclei, % cells containing kinetochore-labeled micronuclei, and % micronuclei with labeled kinetochores).
<u>Kiffs et al., 2003</u>	CHO K5 cells	0.0, 62.5, 125, 250, 500, and 1000 μ g/ml	The standard comet assay and the all-cell comet assay were performed on CHO K5 cells, which were placed in 12-well culture cluster plates (~300,000 cells/well flask). Colchicine was dissolved directly into the medium. Cells were treated for 3 h and 24 h at 37 °C. A maximum of 25 cells were scored for each concentration.	An increase in tail movement and length was observed in the standard comet assay (3 h). In the all-cell comet assay after 3 h colchicine was ineffective at altering DNA migration, however at 24 h, colchicine (62.5, 500, and 1000 μ g/ml) showed a significant positive effect.
<u>Natarajan et al., 1993</u>	Chinese hamster primary embryonic cells	9 doses ranging from 1.25 μ g/mL to 11.25 μ g/mL	Primary cell cultures of 13-day old male or female hamster embryos were frozen. Cells were exposed by replacing the medium with fresh medium containing the drug at specific concentrations. Cells were fixed after 24 hours of treatment.	A dose-dependent increase, 2-3 fold over controls, in the frequencies of aneuploid cells was observed. Concentrations below 3.75 μ g/mL did not show significant inhibition of mitosis. There were high frequencies of c-mitoses and polyploidy cells.
<u>Vian et al., 1995</u>	Human peripheral lymphocyte cultures	0, 5, 7.5, 10, and 25 ng/ml	Human peripheral lymphocytes were isolated from healthy volunteers <36 years old, supplemented with fetal calf serum, and stimulated with phytohemagglutinin. Colchicine was added 24 h later. Lymphocytes were then exposed to cytochalasin B (6 μ g/ml) after 44 h. Lymphocytes were harvested by centrifugation at 72 h.	A statistically significant ($p < 0.05$) induction of micronuclei was noted at 3 consecutive doses (5, 7.5, and 10 ng/ml). A marked increase in the micronuclei in binucleated lymphocytes was observed at a dose (10 ng/ml) where the mitotic ratio decreased by 70%.
<u>Lotfi and Maclachado-Santelli, 1996</u>	Human skin, bovine skin, and bovine bladder fibroblasts. Human skin epithelial cells	0.002, 0.02, 0.2, and 1 μ g/ml	Colchicine was dissolved in culture medium and added to exponentially growing cell cultures to the final concentration (0.002, 0.02, 0.2, and 1 μ g/ml) for 72 h and 96 h.	Human skin and bovine bladder fibroblasts treated at all concentrations during both 72 and 96 h treatment showed increased number of micronucleated cells over control.
Lymphoma Cell Assay				
<u>Honma et al., 2001</u>	Mouse lymphoma L5178Y tk +/- 3.7.2c cells.	0-150 ng/ml (est.)	The mouse lymphoma assay was conducted by the microwell method. Colchicine was tested with a single culture at 3 h and 24 h. Relative Survival (RS) and Relative Total Growth (RTG) were calculated. Mutation frequencies (MF) were also calculated based on Poisson distribution.	Colchicine was found to be cytotoxic, with RTG being more greatly affected than RS. At 3 hours the MF was unaffected. Colchicine clearly induced mutations in a dose-dependent manner with the 24 h treatment.

<u>Reference</u>	<u>Test System</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Yuan et al., 2003</u>	Mouse lymphoma L5178Y tk cells.	10-50 ng/ml	Determination of cytotoxicity, RS, suspension growth rate (SGR), and MF were performed.	The RS and SGR decreased significantly with increasing doses. The MF of the tk gene was 3 times higher than that of spontaneous mutations.

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
Micronucleus Test					
<u>Tschimoto and Matter, 1979</u>	CD1 mice	Oral	0.625, 1.25, 2.5 mg/kg	2 mice/sex were treated with test article with an interval of 24 h. Bone marrow smears were prepared 6 h after the second treatment. Only polychromatic erythrocytes were analyzed.	Colchicine was found positive for induction of micronuclei.
<u>Tschimoto and Matter, 1979</u>	CD1 mice	Intraperitoneal	0.625, 1.25, 2.5 mg/kg	2 mice/sex were treated with test article with an interval of 24 h. Bone marrow smears were prepared 6 h after the second treatment. Only polychromatic erythrocytes were analyzed.	Colchicine was found positive for induction of micronuclei.
<u>Canmerer et al., 2007</u>	CD-1 mice	Intraperitoneal	0.25, 0.5, 1 mg/kg	Peripheral blood experiment: blood collection was performed at 48 hours after the treatment	The highest dose showed a clear statistically significant increase in MN-PCE%
<u>Matter and Grauwiler, 1974; Matter et al., 1974</u>	CD Albino mice	Intraperitoneal	0, 0.625, 1.25, 2.5, 5.0 mg/kg	Mice were injected i.p. with various doses on 2 consecutive days. Bone marrow was prepared 6 h after the second treatment.	Colchicine produced bone-marrow depression and induction of micronuclei.
<u>Bruce and Heddle, 1979</u>	Female hybrid mice	Intraperitoneal	Dose level not specified	Female mice were treated for five consecutive days and were sacrificed 4 h after the last dose. Bone marrow cells were prepared from the femur.	Colchicine was negative for micronucleus induction.

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Garriott et al., 1994</u>	Fisher 344 rats	Oral gavage	0, 0.06, 0.6, 6.0 mg/kg	Colchicine was administered for 14 days. Three treatment groups and one vehicle control group of 5/sex/group were evaluated. 24 h after the last treatment, a sample of bone marrow was taken.	Colchicine was negative for micronucleus induction, thought to be because of the wide dose interval used since colchicine is only active in a narrow dose range. Also, the dose was selected based on the results of a previous study that showed positive results but was dosed intraperitoneally.
<u>Cammerer et al., 2007</u>	Wistar rats	Intraperitoneal	0.7, 1, 1.3 mg/kg	<u>Peripheral blood experiment:</u> A single i.p. treatment was administered to rats. Blood sampling was performed at 24, 30, and 48 h following treatment. <u>Bone marrow experiment:</u> A single i.p. treatment was administered to rats. Bone marrow samples were collected 48 h following treatment. <u>Splenectomized rat experiment:</u> Rats were treated with a single i.p. dose of colchicine. Samples were collected at 24, 30, 48, and 72 h after treatment.	The high-dose group was not analyzed in any experiments due to severe toxicity and animal mortality. <u>Peripheral blood experiment:</u> No statistically significant increase in micronucleus frequency was found at any time-point. <u>Bone marrow experiment:</u> Marginal increase in the average MN-PCE after treatment with 1 mg/kg, but not statistically significant. <u>Splenectomized rat experiment:</u> Low- and mid-dose rats showed statistically significant increase in MN reticulocytes at 48 h after treatment.
<u>Kallio et al., 1995</u>	Male Sprague-Dawley rats	Intraperitoneal	0.1, 0.2, 0.4, 0.8, 1.5	Animals were injected with colchicine and killed 24 h after treatment for the preparation of slides of the stage I spermatids.	The highest induction after 24 hr was seen at 0.8 mg/kg colchicine (statistically significant). No increases compared to controls were observed in MN frequencies after 6, 18 or 48 h treatments at doses of 0.2 or 0.8 mg/kg. Colchicine was unable to induce spermatid MN in seminiferous tubules <i>in vivo</i> .

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Tschimoto and Matter, 1979</u>	Chinese Hamsters	Intraperitoneal	0.625, 1.25, 2.5 mg/kg	Colchicine was administered twice over an interval of 24 h to adult hamsters. 6 hours following the second treatment, the animals were killed and femoral bone marrow was used for MNT scoring and for chromosome preparations which were incubated with colcemid for 90 min. at 37°C	Colchicine was positive for induction of micronuclei, but was negative in the Chromosome Analysis test.
Sister Chromatid Exchange					
<u>Tschimoto and Matter, 1979</u>	Chinese Hamsters	Intraperitoneal	10 mg/kg	Colchicine was injected in four animals 24 h after FUDR administration. Animals were killed and chromosome preparations made from femoral marrow cells two hours after colchicine treatment.	Colchicine was found negative for sister chromatid exchange.
Aneuploidy and Heteroploidy Tests					
<u>Albanese, 1988</u>	Alpk:APFSD mice	Intraperitoneal	2 mg/Lg	Ovulation was synchronized by the administration of exogenous gonadotrophins. Females were then dosed with colchicine 3 or 12 h prior to ovulation i.e. immediately after the HCG injection or 9 h after the HCG. Dosed females were caged with proven fertile males (2 females/male), and left overnight, then checked for the presence of a vaginal plug, which was considered indicative of successful mating.	No toxic signs in the dosed females. When dosed 12 h prior to ovulation, colchicine was seen to completely inhibit both meiotic divisions but did not interfere with the ovulatory process. All the oocytes ovulated had either a polyploidy number of chromosomes or showed signs of degeneration (condensed chromatin or failure of post-fertilization DNA synthesis). When colchicine was dosed 3 h prior to ovulation, a small number of oocytes had completed both meiotic divisions and the first-cleavage embryos derived from these females appeared chromosomally normal.
Reference					
<u>Leopardi et al., 1993</u>	Male mice	Intraperitoneal	1, 3, 6 mg/kg	Bone marrow cells were sampled 18 or 24 h after treatment; germ cells were sampled 6, 8, or 18 h after treatment.	Colchicine yielded significant increases in hyperloid spermatocytes over the controls, at all 3 dose levels and both a dose- and time-dependent increases were seen in hyperploidy index. Treatment of marrow cells resulted in strong mitotic arrest.
Metaphase Arrest					
<u>Liang et al., 1985</u>	Male Swiss mice	Intraperitoneal	2, 20, 40 mg/kg colchicine	This study was designed to demonstrate the dose-response of metaphase accumulation following colchicine treatment. Animals were treated with intraperitoneal doses and sacrificed 3 h after injection. Testes were removed, and spermatocyte preparations were made. The frequencies of M-I and M-II figures in the preparations were determined by counting at least 4000 interphase cells for each sample.	The frequency of M-II figures was increased in a dose-proportional manner at all exposure levels and M-I figure frequency did not increase appreciably at the low- and mid-dose treatments. At 40 mg/kg, the frequencies of both the M-I and M-II figures were increased to 4 x control levels.
<u>Liang et al., 1985</u>	Male Swiss mice	Intraperitoneal	40 mg/kg colchicine	This experiment was designed to determine the time-response of metaphase accumulation following colchicine treatment. Animals were sacrificed 1, 2, or 3 h after injection. Testicular preparations were made and the frequencies of M-I and M-II figures were determined.	Upon consideration of the increase in frequency of the M-I and M-II figures and the quality of the cells for optimal counting, it was determined that the optimal duration of colchicine treatment was between 2 and 3 h.
<u>Liang et al., 1985</u>	Male Swiss mice	Intraperitoneal	10 ⁻⁴ M, equivalent to 40 mg/kg colchicine, 37 mg/kg Colcemid and 90 mg/kg vinblastine	A comparison of the effectiveness of colchicine, Colcemid and vinblastine in arresting metaphases was performed. Animals were sacrificed 2 h after injection, and the frequencies of M-I and M-II figures were determined in the testicular preparations.	All 3 metaphase arrestants were effective at these doses, in producing accumulation of meiotic metaphases but colchicine was the most effective in increasing the yield of M-II spreads.

Reference	Test System	Route of Admin.	Dose	Study Design	Results
Liang et al., 1985	Male Swiss mice	Intraperitoneal	40 mg/kg colchicine	This experiment was designed to compare the yield of acceptable M-I and M-II figures between testicular preparations of colchicine-treated and untreated mice. Three animals were injected with colchicine 3 h prior to sacrifice, and three were sacrificed untreated. The number of M-I and M-II figures acceptable for chromosome analysis was counted.	Colchicine treatment at 40 mg/kg for 3 h produced an approximately 8-fold increase in the frequency of acceptable M-I figures and a 50-fold increase in the frequency of acceptable M-II figures for cytogenetic evaluation, as compared to control animals.
Fourreman, 1988	<i>D. melanogaster</i>	Dietary	0, 1, 5, 10 ppm	Adult exposure: 1-4 day old adult virgin females were fed for 3 days on concentrations of colchicine in 5% sucrose solution. At the end of the exposure period, females were mated <i>en masse</i> . A total of 850 females were exposed to 10 ppm, 800 exposed to 5 ppm, 1875 exposed to 1 ppm, and 1750 controls. Larval exposure: 72 h old larvae were collected and transferred to synthetic medium containing either 0, 1, 5, or 10 ppm colchicine. Virgin adult females were collected twice daily from exposure bottles. Matings were done <i>en masse</i> . A total of 1000, 1100, 775, and 1725 females exposed to 10, 5, 1, and 0 ppm colchicine, respectively, were tested.	An exposure-related decrease in fertility was observed in treated females. Significant increases in chromosome gain products were obtained for the two high-dose exposure groups in those broods representing early oocytes and oogonia. Non-significant increases in chromosome gain products were noted for the 5 and 10 ppm adult exposure groups in broods 1 and 2, due to small sample sizes. It was concluded that most female germ cell stages are sensitive to induction of chromosome gain by colchicine exposure, but that early oocytes and oogonia are either more sensitive to the induction of chromosome gain by colchicine, receive a higher dosage because of the increased time of exposure, or are less sensitive to the killing effect of the colchicine exposure than later oocyte stages.
National Toxicology Program, Study ID: 733667	<i>D. melanogaster</i>	Oral, Thoracic injection (under the wing)	Oral: 0, 3, 6 ppm; Injection: 0, 10 ppm	A description of the standard NTP protocol and a copy of the study data is included section 4.3, literature references.	Colchicine tested negative for a sex-linked recessive lethal mutation in <i>D. melanogaster</i> .

Additional Genetic Toxicology Study (Reviewer's table derived from the Applicant's review of published studies)

Lee et al., 2003	comet assay of L5178Y tk+/- cells	Cells cultured for 18 h to a concentration of 1.8 x 10 ⁶ cells/ml prior to treatment with colchicine (0.4, 4, and 40 µg/ml) for 3 or 24 h	<p>at 3 h without S9 activation, there was a significant tail moment (positive result), which lacked a pattern of bimodality</p> <p>Cells with increased DNA damage display an increased migration of genetic material in the direction of the electrophoresis current.</p> <p>The extent of damage is evaluated by measuring the displacement of genetic material between the cell nucleus and the resulting tail</p> <p>Tail moment is known to be able to identify an apoptotic population, because the population displays an essentially bimodal distribution of DNA damage)</p>
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2.6.6.5 Carcinogenicity

As discussed with the Applicant at the pre-NDA meeting, no carcinogenicity studies would be required for this application. They provided a review of the relevant published literature. No carcinogenicity studies have been performed with colchicine.

Reviewer Comment: These studies do not directly support a carcinogenic potential for colchicine, but contribute to a weight of evidence that suggests colchicine may contribute to the carcinogenic initiation induced by other agents. The mechanism for this initiation is unknown but may relate to the aneuploidic properties of colchicine, since aneuploidy can result in tumor development.

Carcinogenicity Studies (Reviewer's table derived from the Applicant's review of published studies)

<p>Berenblum and Armuth (1977)</p>	<p>Female ICR mice about 6 weeks old, in a two-stage initiation-promotion carcinogenicity study</p> <p>Groups of mice received 2.0 mg/kg colchicine, sc, (table below) at various times prior to <i>tumor initiation</i> by a single s.c. injection of urethane (25 mg/mouse, s.c.) on Day 0, followed by <i>tumor promotion</i> with dermal application of the phorbol ester TPA (0.1 mL of a 0.02% solution in acetone) twice weekly for 25 weeks, to clipped dorsal skin.</p> <p>Animals were observed for tumor formation during and for an additional 12 months beyond the treatment period, and skin tumors other tumor types were recorded.</p> <p>Additional control groups for each of the materials were included in the study design as well.</p> <table border="1" data-bbox="495 1218 1006 1438"> <thead> <tr> <th>Group</th> <th>Time of colchicine pretreatment (before urethane initiation)</th> <th>% of Mice with tumors at week 25</th> </tr> </thead> <tbody> <tr> <td>1, n=75</td> <td>no colchicine</td> <td>42%</td> </tr> <tr> <td>2, n=40</td> <td>5 hr</td> <td>55%</td> </tr> <tr> <td>3, n=40</td> <td>9 hr</td> <td>77%</td> </tr> <tr> <td>4, n=40</td> <td>12 hr</td> <td>45%</td> </tr> </tbody> </table>	Group	Time of colchicine pretreatment (before urethane initiation)	% of Mice with tumors at week 25	1, n=75	no colchicine	42%	2, n=40	5 hr	55%	3, n=40	9 hr	77%	4, n=40	12 hr	45%	<p>Colchicine augmented urethane tumor induction and this was time dependent with the peak effect at 9 hour prior to tumor initiation and corresponds to the peak of metaphase arrest following the colchicine injection.</p> <p>The authors commented that it was fairly well accepted that the initial step in neoplastic transformation is the result of a somatic cell mutation and that the initiating phase of carcinogenesis must, therefore, be associated with the mitotic apparatus of the cell</p>
Group	Time of colchicine pretreatment (before urethane initiation)	% of Mice with tumors at week 25															
1, n=75	no colchicine	42%															
2, n=40	5 hr	55%															
3, n=40	9 hr	77%															
4, n=40	12 hr	45%															
<p>Kowalski <i>et al.</i> (2001)</p>	<p><i>in vitro</i> assay (T1) to predict carcinogenicity using a bovine papillomavirus DNA-carrying C3H/10T½ cell line</p> <p>Colchicine doses of 0.001, 0.01, 0.1, and 1.0 ng/ml. were added with each medium change twice weekly for 21 days.</p> <p>solvent control, and a positive control (mezerein at 0.5 ng/ml) were included</p> <p>doses consisted of triplicate cultures</p>	<p>0.01 and 0.1 ng/ml colchicine produced a significant (p<0.05) increase in the number of foci (predictor of carcinogenicity). A dose of 1.0 ng/ml caused significant cytotoxicity (p<0.05).</p> <p>although this assay correctly predicted 77% of the chemicals for which rodent carcinogenicity was reported, the predictive values of 77% is likely too low for regulatory usefulness</p>															

2.6.6.6 Reproductive and developmental toxicology

There were no reproductive and developmental toxicology studies conducted by the Applicant, rather they provided a summary of colchicine's effects on reproductive and development from studies in the published literature. Nonclinical studies indicated that colchicine has adverse effects on sperm development (fertility), early embryonic development and implantation, organogenesis (teratology), and late-stage embryonic development. These effects are consistent with its pharmacodynamic effect as an inhibitor of microtubule formation and resulting in the disruption of cytoskeletal functions, cell movement, and cell division. Studies and case reports of patients on therapeutic doses of colchicine for FMF or gout, in general have not revealed this severity of adverse effects. Clinical cases of azoospermia or oligospermia, miscarriage or spontaneous abortion, and genetic abnormalities have been reported, but it is difficult to attribute these effects to colchicine therapy since they appear to be rare events or could not be easily separated from the potential detrimental effects of FMF disease progression.

Human Genetic Abnormalities and Teratogenicity

Human reproductive and developmental findings are discussed in the Medical Officer's Review. For comparison with the nonclinical findings a brief summary is presented here. Although nonclinical studies indicate colchicine has very detrimental effects on reproduction and is teratogenic, in general at the therapeutic doses used in humans there is no clear relationship between colchicine therapy and female infertility and malformations. Azoospermia has been reported, but is possibly rare. As reviewed in Kallinich et al, 2007, a lack of colchicine treatment may bear a greater risk of infertility in females, by the development of ovarian amyloidosis with subsequent ovarian dysfunction (Ismajovich 1973), having a higher rate of spontaneous abortion (20% without colchicine treatment versus 12% with colchicine treatment; Rabinovitch et al, 1992), and aggravation of amyloid nephropathy (Cabili et al, 1992) with a significant risk for adverse maternal and fetal outcomes (Sanders and Lucas, 2001)

Several case series in patients with familial Mediterranean fever (FMF) from a registry of FMF patients in Israel suggest that colchicine does not cause harm to the fetus (birth defects, growth or development disorders) or mother if used during pregnancy. Although miscarriages (spontaneous abortions) and infertility were reported among these patients, the incidence appears to be similar to women with FMF not receiving colchicine (Rabinovitch et al, 1992; Ehrenfeld et al, 1987).

Effects on Fertility

Interference with gonadal hormones

Colchicine altered testosterone production by rat Leydig cells in vitro. It stimulated cAMP production and testosterone secretion in a dose and time-dependent manner when measured immediately upon addition, but at one hour after administration, colchicine inhibited cAMP production, testosterone secretion, and lutenizing hormone stimulated

testosterone secretion. In ovine corpus luteal cells, colchicine disrupted normal intracellular transport of secretory granules and progesterone secretion, but not the biosynthesis of progesterone. In rat luteinized ovarian cells, colchicine attenuated cholesterol production by high density lipoproteins *in vitro* or *in vivo*, even though the rate limiting enzyme of cholesterol biosynthesis (HMG CoA) was stimulated three-fold.

from the Applicant's Summary Table

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
Male Fertility					
<u>Saltarelli et al., 1984</u>	Rat Leydig cells isolated from adult Wistar rats	<i>In vitro</i> in cell suspension	10 μ M	Testosterone and cAMP were measured in suspensions of Leydig cells or plasma membranes isolated from the testes of untreated male rats, before and after the addition of Luteinizing hormone (LH).	Leydig cells develop a rapid rise in testosterone synthesis when exposed to colchicine. In cells pretreated with colchicine, both the steroidogenic effects of LH and LH-dependent cAMP accumulation were inhibited.
Female Fertility					
<u>Koch and Spitzer, 1983</u>	<i>Drosophila melanogaster</i>	Culture medium	0.5 mL colchicine mixed with 10g medium	Effects of colchicine were evaluated by percent survival of exposed adults, mean number eggs laid per female per day and percent emergent adults (number adult flies produced x 100 divided by total number of eggs laid).	Dosage-related effects on oogenesis were produced in females exposed to colchicine included the production of eggs which did not hatch and transformation of oocytes into a nurse cell. Exposure of males for up to 7 days did not appear to be a major factor in decreased percent emergent adults.

Spermatogenesis

In mice and rats, colchicine administration resulted in abnormally shaped sperm, primarily by altering early primary spermatocytes, rather than spermatids or spermatogonia, and altering necessary shape changes of Sertoli cells to accommodate the changing morphology of spermatid development. Sloughing of seminiferous epithelium occurred in most of the tubules, and a complete blockage in seminiferous tubule fluid secretion. In the rat, germ cell mitoses and meioses was arrested, Sertoli cell microtubules depleted, and sloughing of Sertoli and closely related germ cells, all within 6 hours of intra-testicular administration. Intraperitoneal administration of colchicine to mice in several studies resulted in sperm abnormalities and changes in chromosome cycles and chromosome arrangements. Intra-testicular administration of colchicine in mice results in degradation of microtubules and increased abnormalities of the epididymidal spermatid head structures. Five days of intraperitoneal dosing of colchicine (0.6 to 5.0 mg/kg/day) in mice resulted in an increase in sperm abnormalities that were observable at 1 week and still apparent a month after dosing.

from the Applicant's Summary Table

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
Male Fertility					
<u>Bergner, 1950</u>	CF1 male albino mice, weighing 15-35 g	Intraperitoneal	0.35, 0.17, and 3.5 mg/kg	Mice were sacrificed 6, 24, and 48 h after dosing. The left testis was fixed, seminiferous tubules mounted, and stained on slides. Length, arrangement within the cell, and size of metaphase were compared to chromosomes in the spermatogonia from the control.	Colchicine at 3.5 mg/kg showed an increase in areas of cells at metaphase, shortened chromosomes, and a change in chromosome arrangement.
<u>Wvrobek and Bruce, 1975</u>	(C57B1 X C3H)F ₁ male mice (11-14 weeks)	Intraperitoneal	0.6 and 1.0 mg/kg (est.)	4 mice per dose were injected over 5 consecutive days, cauda epididymides were, suspended, filtered, mounted, and 1000 sperm per slide were examined at 400-fold magnification with blue-green filters. Sperm abnormalities were examined 1, 4, and 10 weeks following exposure.	An increase in sperm abnormalities observed at dose levels, 1 and 4 wks post-exposure.
<u>Bruce and Heddie, 1979</u>	(C57B1 X C3H)F ₁ male mice (11-14 weeks)	intraperitoneal	0.75-5.0 mg/kg (est.)	3 mice per dose were injected over 5 consecutive days and killed 35 days later, cauda epididymides were, suspended, filtered, mounted, and 333 sperm per slide were examined at 400-fold magnification.	An increase in sperm abnormalities observed at all treatment levels.
<u>Schmid et al., 2001</u>	(102-EL X C3H/E1) F ₁ male mice aged 10-14 weeks, weighing 25-29 g	Intraperitoneal	3 mg/kg	25 mice were dosed with 100 mg/kg BrdU (fluorescent label for the last S-phase before meiosis), and 13 days later the mice were dosed with colchicine. Between 20-24 days after dosing, BrdU labeled sperm were identified using a FITC-labeled anti-BrdU antibody and green fluorescent sperm were scored using a laser scanning cytometer.	Colchicine treatment prolonged the duration of meiotic divisions by about 48 h. On days 21 and 22, the frequencies of labeled sperm were 11.7 and 9.4% respectively, while the controls were 28.4 and 30.6%.
<u>Handel, 1979</u>	Sexually mature male ICR mice	Testicular injection	0 M, 10 ⁻⁴ M, and 10 ⁻⁶ M colchicine; 20 µL of solution.	At least 6 mice were dosed with each concentration. Controls were either injected or not with 20 µL of vehicle (PBS). Animals maintained >3 days were re-injected on the third day. 500-900 sperm (with pooling from different mice) per dose were scored. Epididymides were suspended, filtered, mounted, and scored for abnormal sperm. Light and electron microscopy were performed on prepared tubules.	An increase in abnormal head structure observed in epididymal sperm. Evident starting at 3 days in the higher and 5 days for the lower concentration. Microtubules were degraded in the testis and abnormalities of the head causing acrosome of testicular spermata.
<u>Russel et al., 1981</u>	Male adult Sprague-Dawley rats	Testicular injection	0.00005 mg/250 g	Injections were made into the central area of the testis. Animals in long term experiments had the right testis removed 12 h after treatment. All rats were sacrificed 60 days later at which time the left testis was removed.	Animals treated with colchicine and vincristine showed identical responses; the Sertoli microtubules were notably absent from the Sertoli cytoplasm of treatment animals 6 hours after treatment. Mitotic and meiotic divisions were arrested. Portions of tubules showing Sertoli and closely related germ cells were sloughed leaving a variably denuded seminiferous epithelium. These effects were seen due to the disruption of microtubules.

<u>Allard et al., 1993</u>	Adult CD rats	Testicular injection	0.004, 0.04, 0.4, 4, 40 µg	Efferent duct ligation, long term recovery, histopathology time course, seminiferous tubule fluid (STF) secretion, testis weight, and tubulin immuno-histochemistry were evaluated.	No effect on STF secretion or change in testis weight at up to 0.4 µg colchicine/testis, while 4 and 40 µg testis, respectively, decreased the volume of or blocked STF secretion entirely. A dose-related decrease in testis weight was seen with doses of 4 and 40 µg. At the higher doses, most seminiferous tubules were atrophic or had undergone miceralization. In the histopathology time-course study, sloughing of seminiferous tubules was observed at 1 h after exposure. Immuno-histochemistry showed distribution of tubulin staining localized to the Sertoli cells in the seminiferous epithelium and the sloughed material in the lumen.
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Female Fertility

The mouse appears to be more sensitive to colchicine's effects than the rabbit. Doses of 0.5, 1.5, and 3 mg/kg, i.p. to mice interfered with sperm penetration, the second meiotic division, and normal cleavage in the mouse. In rabbits, doses of 0.5 and 1.0 mg/kg, i.p. before or after induced ovulation had no effect, but 4.0 mg/kg, i.v., inhibited ovulation induced by human chorionic gonadotropins.

Clinically, no clear relationship between female infertility and colchicine therapy has been established. A lack of colchicine treatment may bear a greater risk of infertility in females (reviewed in Kallinich et al, 2007) since colchicine treatment prevented the development of ovarian amyloidosis with subsequent ovarian dysfunction (Ismajovich 1973), reduced the spontaneous abortion rate (12%) compared with 20% in untreated women (Rabinovitch et al 1992), and pregnancy could aggravate amyloid nephropathy with a significant risk for adverse maternal and fetal outcomes (Sanders and Lucas 2001).

from the Applicant's Summary Table

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>McGaughey and Chang, 1969</u>	Female Swiss Webster mice	Intraperitoneal	0.5, 1.5, 3.0 µg/g	Effects of colchicine, administered near ovulation, on fertilization, pronuclear development, and first cleavage were evaluated. Female mice were injected w/ colchicine before or after induced ovulation and artificially inseminated. Recovered eggs were examined for sperm penetration 1.5 to 6.5 hr after ovulation.	Chromosome counts of eggs at the first cleavage showed heteroploidy ranging from hypo-haploid to triploid and absence of normal diploid eggs. Colchicine interfered with sperm penetration, the second meiotic division, and normal cleavage. Only 7% of eggs recovered from dosed females (all 3 levels) earlier than 3.5 hr after ovulation were penetrated, but 35% from untreated females and 41% from females given colchicine at 3.5 hrs after ovulation were penetrated.
<u>Epstein et al., 1972</u>	ICR/Ha Swiss mice (8-10 weeks)	Intraperitoneal	1.1, 2.5 mg/kg	Male mice were dosed singly with subtoxic concentrations of colchicine, and then mated during sequential weekly periods with groups of untreated virgin females. After treatment, each male was caged with 3 untreated virgin female mice, which were replaced weekly for 8 consecutive weeks. 7 and 9 males were used at the lower and higher dose levels, respectively. Female mice were dissected 13 days following the mid-week of presumptive mating. At autopsy, mice were scored for pregnancy, and for numbers of implants.	Compared to the control, colchicine caused a significant reduction in the number of implants, but no increase in early fetal deaths. It also produced postmeiotic effects that extended to meiotic stages, and there was also a reduction in pregnancy that roughly paralleled the reduction in total implants.
<u>Mailhes et al., 1988</u>	CD1 mice, 8-12 weeks old, weighing 26-34 g.	Intraperitoneal	0, 0.1, 0.2, and 0.3 mg/kg	Super ovulation was induced using pregnant mare's serum followed by human chorionic gonadotrophin (HCG) given 48 h later. Colchicine was administered immediately after HCG. Animals were sacrificed 17 h after treatment, and the oocytes were analyzed for chromosome number.	Colchicine treatment resulted in a significant ($P < 0.01$) increase in hyperploid ($N > 20$) oocytes over controls.
<u>Azhar et al., 1986</u>	Female Sprague-Dawley rats	Intravenous	2.5 mg/kg	Animals were treated with colchicine, killed 4 h later, and ovaries were assayed for their cholesterol content, ability to synthesize cholesterol endogenously, or ability to utilize lipoprotein-delivered cholesterol for the production of progesterone.	Animals treated with colchicine show a 60% decline in stored cholesterol in luteinized ovary cells, 3-fold increase in activity of cholesterol synthesizing enzyme HMG CoA reductase, 3-fold increase in capacity of cells to incorporate precursor [14 C]acetate into cholesterol, but secreted much less progesterone than the saline-treated rats, due to a reduced uptake of HDL-cholesterol.

<u>Piko and Bomiel-Helmreich, 1960</u>	Female Wistar CF, Long-Evans, and Sherman rats. Male Wistar CF rats.	Intraperitoneal	0.25 to 0.5 mg/kg	Female Wistar rats were dosed at various times (1 to 2 hrs or 1 1/2 hrs) after mating and eggs were examined for abnormalities. In a second experiment 24 female rats were given colchicine to induce polyspermy and were killed 8 to 15 days after copulation.	When 13 females were dosed 1-2 hours after delayed matings, polar bodies were suppressed in about 70 % of the eggs. Other abnormalities included: eggs with a single pronucleus (male), and subnuclear in place of a female pronucleus. In a second experiment, resulting embryos showed triploid and mosaic mitoses, 8 triploid embryos were highly retarded and clearly abortive.
<u>Sugawara and Mikamo, 1980</u>	Virgin female Chinese hamsters, 5-8 months	Intraperitoneal	5 mg/kg	Colchicine was administered on the day of proestrus after the termination of germinal vesicle breakdown; therefore eggs were exposed to colchicine at the onset of spindle formation. Eggs were collected from the ampullar region of the oviducts. Morphological features were examined under a dissecting microscope.	Morphologically abnormal secondary oocytes with one or two extremely large first polar bodies occurred in 11.5% of 416 oocytes. The overall incidence of aneuploids increased significantly (P<0.001, 15.9%) compared to controls.
<u>Esey et al., 1982</u>	NZW rabbits	Intravenous	0, 4.0 mg/kg	Ovulation was induced using human chorionic gonadotrophin (hCG) at a dose of 50 IU/kg by i.v. Colchicine (and a long list of other anti-inflammatory agents) was administered within 1 hour of hCG. Laparotomies were routinely performed 20 to 24 hours after dosing with hCG. The ovaries were examined under a microscope to determine the number of ruptured follicles.	Colchicine (and vincristine) significantly reduced the number of ovulating follicles.
<u>McGaushey and Chang, 1969</u>	Female rabbits	Intraperitoneal	0.5, 1.0 mg/kg	Female rabbits were artificially inseminated and treated with colchicine 3 h before or 1 h after expected time of ovulation and eggs were examined at 11 to 22.5 h after ovulation.	These dose levels did not cause a decrease in the proportions of penetrated eggs. Penetrated eggs displayed no distinct abnormalities.

Early and Late Embryonic Development and Teratology

<u>Gemmell and Stacy, 1976</u>	Female sheep	Intravenous	1 mg/kg	Ewes close to Day 10 of the cycle were treated and ovaries were sampled at 1 h intervals (up to 4 hr) following treatment. Serum progesterone was also measured at the same time-points	Morphology of luteal cells had changed 45 min after injection. By 2 h there was no sign of the granules being secreted and were not detectable within the cell. Peripheral plasma levels of progesterone fell to 86% of control values at 1 hr post-dose. Colchicine disrupted the microtubular system in the luteal cell and thereby inhibits the intracellular transport of granules. Cells were still able to synthesize steroids after treatment.
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Nonclinical studies demonstrated that colchicine is embryotoxic, fetotoxic, and teratogenic. Aneuploidy during germ cell meiosis can result in spontaneous abortion and congenital abnormality. Interruptions of cell division and/or migration during embryonic development and organogenesis can result in resorptions and terata.

Colchicine has a lethal effect on young embryos, produces skeletal abnormalities during organogenesis, and retards development of advanced embryos (Van Dyke and Ritchey, 1947). Pregnant mice appeared to be more sensitive to colchicine than non-pregnant mice, since the intravenous toxicity, assessed by LD₅₀, was lower in colchicine treated mice (nonpregnant: 4.13 mg/kg; pregnant: 1.54 mg/kg; Beliles, 1972). Colchicine treatment on Day-8 of gestation (GD-8) in Swiss albino mice at levels of 1.0 mg/kg, i.p., resulted in an increased incidence (p<0.005) of intrauterine deaths, and at 0.5 and 1.0 mg/kg dose levels, an increased incidence of fetal malformations. Other toxic effects observed in the mouse were changes in the live birth and viability index, cytological changes.

For mice, colchicine was found to be teratogenic when administered just before or around the time of implantation in mice, and early in gestation (now considered the period of organogenesis). Colchicine treatment resulted in specific craniofacial malformations exencephaly, hydrocephalus, anophthalmia and microtia, as well as major skeletal malformations. Craniofacial abnormalities were also observed by Shoji and Makino (1967), and Shoji (1968) in mice treated with colchicine at different dose levels (0.5, 1.0, 1.5, or 2.0 mg/kg, single s.c. injection) and at different gestational stages. Other findings included developmental abnormalities in the urogenital system and craniofacial area (including nose and tongue). Rabbits were less susceptible to the teratogenic effect of colchicine and mainly skeletal and visceral defects were produced (Szabo *et al.*, 1969, 1971). Administration of colchicine to pregnant rats (0.6 mg/kg, i.p.) as a single dose on either gestation day 6 or 8 resulted in partial resorptions, but if dosing occurred on days 10, 12, or 14, the pups survived but with retarded development.

The resistance of the hamster to colchicine was evaluated in a teratology model (Ferm, 1963). Colchicine injected intravenously into pregnant golden hamsters on the eighth day of gestation day 8 at levels of 10 mg/kg/day produced 50% mortality in the fetuses and gross congenital craniofacial defects, umbilical hernias and some skeletal anomalies (mainly fused ribs) in the survivors.

In the rat, mouse, and hamster, colchicine caused specific developmental abnormalities in the central nervous system resulting in behavioral changes, changes in growth and cytological changes (including somatic cell genetic material) that were detected in the newborn. Offspring from pregnant rats injected with 0.4 mg/kg colchicine on gestation days 18, 19, and 20 had reduced sizes of isocortical and hippocampal structures when examined at birth, which was followed by behavioral and learning retardation as they matured (Petit and Isaacson, 1976). In the hamster, abnormalities were also noted in the eye, ear, body wall and musculoskeletal system of the newborn.

Applicant's Summary Tables

Effects on Embryofetal Development

<u>Ingalls <i>et al.</i>, 1968</u>	Albino female mice	Intraperitoneal	1.0, 1.5, 2.0mg/kg	A single injection given 3.5 to 7.5 days after mating.	Pregnancy rates were decreased in the groups treated with 1.0 mg/kg, 7.5 days after mating compared to controls and groups treated 3.5 days after mating. Increases in fetal malformations and resorptions were seen in groups treated later. Colchicine was embryolethal at 2.0 mg/kg administered around the time of implantation (5.5 days after mating). A sizeable reduction in successful pregnancies was observed to be dose-related in mice treated with 1, 1.5, or 2 mg/kg of colchicine on Day 6.5 after mating.
<u>Sieber <i>et al.</i>, 1978</u>	Swiss albino mice (pregnant)	Intraperitoneal	0.5, 1.0, and 1.5 mg/kg	Colchicine was administered once on Days 6, 7, or 8 of gestation.	Colchicine showed an (P < 0.005) increase in number of dead (except for 0.5 mg/kg dose level) and abnormal fetuses, when compared to controls on Day 7 for both doses. On Day 8 of gestation, an increase in the number of dead fetuses (P < 0.005) was observed, when compared to controls.

<u>Shoji and Makino, 1967</u>	Female mice (60 to 90 days old)	Subcutaneous	0.0, 0.5, 1.0, 2.5 mg/kg	A single dose of colchicine was administered between the second and fourteenth day of gestation. Mice were sacrificed on GD-13.	There was a significant (P<0.05) increase in the number of dead fetuses between the 2.5 mg/kg and control groups, and the incidence of abortions and maternal death increased with an increase in dose. The number of deformed mice also showed a significant (P<0.05) increase for the 1.0 mg/kg and 2.5 mg/kg dose when compared to controls.
<u>Shoji 1968</u>	Female mice (60 to 90 days old)	Subcutaneous	0.5, 1.0, 1.25, 1.5, 2.5 mg/kg	Colchicine was administered by a single dose between the second and fourteenth days of gestation	Statistically significant teratogenic effects were seen in the 1.25 and 1.5 mg/kg groups. The highest frequency of malformed fetuses was seen from dams treated on the gestation Day 4. Fetal mortality showed dose-related increases.
<u>Szabo et al. 1969 and 1971</u>	Random-bred female mice	Subcutaneous or intravenous	Dose not specified	Single dose of colchicine was administered to pregnant mice between Gestation Days 5 and 14 of pregnancy.	Colchicine was abortifacient and teratogenic just before or around the time of implantation (near GD 6), resulting in highly specific craniofacial malformations.
<u>Van Dyke and Richey, 1947</u>	Wistar rats (pregnant)	Intraperitoneal	0.6 mg/kg	Embryos from pregnant rats given a single dose of colchicine on Day 6, 8, 10, 11, or 14 of gestation and sacrificed 3 days later were studied.	Embryos from GD 6 and GD 8 treatments were dead and partially resorbed. Embryos from GD 10 to 14 were living but showed general developmental retardation.
<u>Fern, 1963</u>	Virgin female golden hamsters	Intravenous	10, 20, 50 mg/kg	Colchicine was injected as a single dose into the femoral vein on Day 8 of gestation.	The low-dose of colchicine produced a 50% mortality in hamster embryos and a number of gross congenital malformations. Only 2/50 fetuses from the 20 mg/kg group survived, and no high-dose fetuses survived treatment.
<u>Szabo et al 1969 and 1971</u>	Random bred female mice and rabbits	Subcutaneous or intravenous	Doses not specified	Colchicine was provided as a single s.c. or i.v. administration between Days 5 and 14 of pregnancy.	Colchicine was abortifacient in both species.. was teratogenic in the mouse, only just before or around the time of implantation. Rabbits were less susceptible and showed mainly skeletal and visceral defects.

Additional Nonclinical Studies of Colchicine Administration During Pregnancy
 (Reviewer's table derived from the Applicant's review of published studies and additional publications)

Author	Species	Dose	Findings
Mouse			
Didcock et al. (1956)	mouse	1.5 to 18 mg/kg, oral and SC on various single days during organogenesis	produced interruption of pregnancy
Bellies, 1972	mouse		Pregnant mice were more sensitive to the toxicity of colchicine than non-pregnant mice
Rat			
Tuchmann-Duplessis and Mercier-Parot, 1958	rat		embryocidal effects
Thiersch, 1958	rat		embryocidal effects
Rabbit			
Chang (1944)	rabbit	0.1% colchicine	sperm suspended in 0.1% colchicine solution and artificially inseminated female rabbits 33 young resulted: 1 - open fontanelle and very small philtrum 1 - one was otocephalic 1 - microcephaly with enlarged eyes. Doses of similar origin were artificially inseminated: resulted in 425 normal young

Didcock et al. (1956)	rabbit	1.5 to 18 mg/kg, oral and SC on various single days during organogenesis	produced interruption of pregnancy
Adams et al. (1961)	rabbit	2 to 8 mg/kg	arrested cleavage in ova degeneration of rabbit blastocysts
Morris et al. (1967)	rabbit	0.1 to 5.0 mg/kg	high dosages after the 9th day were highly lethal to fetuses, death within 2-4 hrs 5 mg/kg, 50% of dams died from toxicity
	rabbit	0.1 to 0.5 mg per kg	teratogenic effects: small incidence of gastroschisis failure of neural tube closure
Morris et al. (1967)	monkey	1 to 2 mg per kg single administration on days 24, 45, 66, and 84 of pregnancy	four normal fetuses, no adverse effects detected

Pre- and Post-natal Development

Offspring from pregnant rats injected with 0.4 mg/kg colchicine on embryonic days 18, 19, and 20 had reduced size of isocortical and hippocampal structures when examined at birth, which was followed by behavioral and learning retardation as they matured (Petit and Isaacson, 1976). The mechanisms responsible for these anomalies are not completely clear but may be due to an interruption of cell division or to cell death before or during migration.

from Applicant's Summary Table

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Petit and Isaacson, 1976</u>	Long Evans Hooded rats	Subcutaneous	0, 0.4 mg/kg	Colchicine was injected in pregnant rats on embryonic Days 18, 19, and 20.	Offspring were found to have isocortical and hippocampal structures greatly reduced in mass. Cells with pyknotic nuclei were found in layers 4, 5, and 6 of the cerebral isocortex, the habenula, and anterior medial nuclei of the thalamus. Brains taken at postnatal days 22 and 32 were smaller, and had a 20-30% reduction of cells at the vertex of the neocortex with up to 50% reduction in the thickness of the corpus callosum.

Lactation

Nonclinical studies indicate that colchicine administration affects both the rate of milk production and its composition. Colchicine disrupts the cytoskeletal system necessary for differentiation of the mammary epithelium, the organization and polarization of the endoplasmic reticulum, and the translational movement of secretory vesicles and milk fat globules. In lactating mammary tissues of rats, goats and cows, colchicine treatment resulted in ultrastructural alterations characterized by a reduction in size, greater dispersion, and accumulation of secretory vesicles that were blocked from exocytosis,

and the accumulation of large lipid droplets.

Milk Composition: In goats, colchicine resulted in decreased fat content 36 hours after administration, the time point of peak lactation inhibition. Fat globules within the cells continued to grow in size during the time that the secretion of fat globules and non-fat exocytosis decreased. Following intramammary infusion of [¹⁴C]-colchicine in goats, less than 20% of the infused colchicine was secreted in the milk. It was associated with fat globules because colchicine binds to the cytoplasmic, but not the exterior surface of the globule membrane. Colchicine inhibited lactose production in mammary gland slices from lactating guinea pigs.

Applicant's Summary Tables

<u>Reference</u>	<u>Test System</u>	<u>Route of Admin.</u>	<u>Dose</u>	<u>Study Design</u>	<u>Results</u>
<u>Loizzi et al. 1975</u>	Lactating guinea pig mammary gland	<i>In vitro</i>	10 ⁻⁶ M (approx 0.004 mg/ml)	Mammary gland slices were exposed to colchicine in culture	Inhibition of lactose production in lactating mammary gland tissues caused by increased in cellular level of cyclic AMP by hormonal stimulation of its product or inhibition of its intracellular breakdown. Lactose production was significantly reduced to 74.7% of controls.
<u>Knudson et al. 1978</u>	Lactating Brown Norwegian and Buffalo hybrid rats	Intramammary Injection	0.2 mg	Rats were sacrificed 1, 2, and 4 hours after colchicine was injected through one abdominal teat; mammary cells were obtained.	Extensive accumulation of secretory vesicles apical to the Golgi complex caused by 2-hr exposure. Casein micelles were enlarged, cisternal swelling of endoplasmic reticulum, and shrunken Golgi apparatus was observed after 4-hr exposure. Microtubules were not detected after treatment.
<u>Morales et al. 1982</u>	Lactating albino rats; Cows	Intramammary Injection	0.2 mg (rats); 25 mg (cows)	Rats: Colchicine was injected through one abdominal teat 3 hours prior to sacrifice and tissue removal. Cows: Colchicine was administered through one half of lactating mammary gland 24 hours prior to mammary tissue biopsy	It was observed that fat droplet does not always have a peripheral secretory vesicle and displaces the plasma membrane.
<u>Knudson et al. 1978</u>	Lactating goats	Infusion	5 mg	Goats infused with colchicine into one half of udder once or after each of 3 sequential 12-hour milkings. Lactating tissue from infusion side was biopsied and prepared for investigation.	Endoplasmic reticulum and whole cells were swollen. Intracellular orientation and compartmentalization were lost. Single-dose caused reduction in size and greater dispersion of secretory vesicles blocked from exocytosis and accumulation of lipid droplets. Microtubules rarely detected after treatment.
<u>Akers and Nickerson 1983; Nickerson and Akers 1983</u>	Holstein heifers	Infusion	30, 20 mg	Pregnant females were treated on Day 1 with 30 mg/day and with 20 mg/day every 2 days during the last week of pregnancy.	Reduced rates of milk component biosynthesis were seen in the early weeks. Cells from untreated quarters showed large percentages of rough endoplasmic reticulum and Golgi components. Majority of the cells from treated quarters were undifferentiated and lacked secretory vesicles, structured Golgi apparatus and limited rough endoplasmic reticulum.

Additional Nonclinical Studies: Effects of Colchicine on Lactation (Reviewer's table derived from the Applicant's review of published studies)

Burvenich and Peeters, 1980; Burvenich, 1980	A reversible decrease in milk flow is consistently observed when colchicine was infused into the udder of the goat <i>via</i> the teat canal. Mammary blood flow has been shown to increase from the 8 th to the 24 th hour after intramammary infusion of colchicine, as well as fever that is not related to pyrogens in the infusate, hardness of the gland and positive California mastitis test (CMT) scores of the milk
<i>Effects on Milk Yield</i>	
Patton (1975)	<p>In a single-dose study performed by, depression in milk yield from the infused side peaked at 36 hours following a single administration and had substantially reversed by 72 to 96 hours. Milk from the infused and non-infused sides were essentially normal in composition, however, globulins and riboflavin were elevated in milk from the infused side only</p> <p>a decrease in fat content occurred 36 hours following colchicine, the time point of peak lactation inhibition. Yields and fat content did not change on the un-infused side. Histopathologic examination revealed that fat globules within the cells continued to grow in size during the time that the secretion of fat globules and non-fat exocytosis decreased. The authors postulated that the drawing to or interaction with the plasma membrane in the secretion of both fat globules and secretory vesicles may depend upon a common structural functional unit that was perturbed by colchicine, which could be the microtubule.</p>
Henderson and Peaker, 1983	The magnitude of the temporary inhibition of secretion in one mammary gland of goats was similar following intramammary treatment with colchicine at Weeks 6, 12, 18, 24, and 30 of lactation (
<u>Sokka and Patton, 1983</u>	Analysis of milk for radioactivity following intramammary infusion of [¹⁴ C]-colchicine indicated that that less than 20% of the infused colchicine is secreted in the milk, and is associated with fat globules because the drug binds to the cytoplasmic, but not the exterior surface of the globule membrane

2.6.6.7 Local tolerance

The Applicant did not conduct local tolerance studies. They provided a published study describing the effects of colchicine on eye irritation.

Eye irritation

Estable (1948) evaluated colchicine applied to the rabbit eye conjunctiva. Single or repeated application of a dilute solutions of colchicine (1:2000, 1:1000, 1:500 or 1:100 dilution of a saturated solution, concentration not provided in the paper) resulted in increased irritation (hyperemia, inflammation, hyperemia, blepharospasm) with increasing concentration or increasing days of application.

Eye Irritation Toxicity of Colchicine (Reviewer's table derived from the Applicant's review of published studies)

Colchicine Form and Dose	Effect	
Solution (dilutions of a saturated solution of unknown purity; also the concentration of a saturated solution was not provided)		
1:2000	slight and reversible hyperemia of the conjunctiva	
1:1000	slight conjunctival congestion that disappeared within 24 hrs	
1:500	vascular conjunctival inflammation (hyperemia, vasodilatation) that is apparent 24 hours after application and increased with additional dosing	returned almost to normal within 4 days after the last application
1:100	vascular conjunctival reaction appears in a few hours and after the third application, the cornea turns opaque and a vascular corneal invasion begins in a few days.	changes were reversible, unless more daily applications were administered; corneal vascularization, only if it was applied daily for several days.
Dry powdered	immediate lacrimation, blepharospasm, and acute conjunctivitis In 24 hours, the conjunctival congestion increased and extended to the iris, and slight edema of the palpebral conjunctiva developed. Conjunctival congestion persisted for 48 hours only	corneal vascularization, only if it was applied daily for several days

2.6.6.8 Special toxicology studies

There were no Special Toxicology studies.

2.6.6.9 Discussion and Conclusions

Historical information provided by the Applicant and obtained from published animal studies and human use indicates similar toxicities with increasing doses. The published nonclinical literature contains limited information for chronic treatment studies and most toxicology studies are only a few weeks duration. Furthermore, almost all the studies were conducted prior to GLP regulations and lack much of the information now routinely collected such as clinical pathology and histopathology findings. Single and repeated dose animal studies of at most a few weeks, cited in the literature (and possibly the same studies conducted for the original approval of colchicine/probencid combination in 1961, original reviews were not found), indicated a low threshold between adverse effects and mortality which is consistent with human toxicology findings. In general, the acute toxic signs in animals (rats, dogs, rabbits, cats) with short-term colchicine administration are gastrointestinal tract-related and include emesis, distended intestines, diarrhea (bloody in more severe cases), lack of appetite and lethargy. With increasing doses these signs become more severe, and there is a loss of body tone, abnormal gait and hindlimb paralysis and wasting atrophy, ascites and eventually death. For comparison, in humans, at sufficient doses, colchicine can produce gastrointestinal disorders, profound muscle weakness, respiratory insufficiency, and peripheral neuropathy.

Comparing human lethality with the limited nonclinical data at nonlethal doses, indicated that human deaths have been reported at doses lower than those that affect rodents, implying that NOAEL determinations may not provide a useful margin of safety. A direct NOAEL comparison could not be conducted since for the most part, nonclinical studies were not conducted to identify a NOAEL, but to identify toxicities. These factors together with the extensive clinical experience and clinical toxicity of colchicine contributed to the decision early in the developmental program that further nonclinical studies were not necessary.

Genetic toxicology studies indicate that colchicine treatment results in aneuploid cells through mitotic or meiotic non-disjunction, but colchicine is not considered mutagenic or clastogenic although results from these assays often result in positive results (a false positive finding, from different mechanism leading to a similar result). The significance of aneuploidy toward carcinogenic potential in comparison with a pure clastogenic mechanism cannot be quantitatively assessed. However, most tumors consist of aneuploid cells and both mechanism can result in tumors (Weaver et al 2007, *Cancer Cell* 11:25-36; Torres et al 2008, *Genetics* 179:737-746).

Carcinogenicity studies were not requested due to the long history of clinical use of colchicine in gout (for over 100 years) and more recently, in FMF (since 1972), although specific documentation of any relationship between colchicine use and carcinogenicity is lacking. From the few repeated dose nonclinical studies reported in the literature there was a low threshold between adverse effects and the lethal dose. With low doses of colchicine administered in drinking water of spontaneously hypertensive and normotensive rats, respiratory difficulties developed within 4 to 13 months (Cicogna et al 1997). While there may be greater susceptibility in this strain, overall the studies do not

provide much confidence that a 2 year oral study in rats or mice would be productive. A study was conducted with dermally applied colchicine twice weekly in mice for 6 months (Berenblum and Armuth, 1977), and this could be conducted with transgenic mice, but those mice would not be an appropriate model for an orally ingested drug since they have a high spontaneous background rate of internal tumor formation that may confound and mask colchicine induced effects. They are still possible options if clinical findings from expanded safety surveillance result in signals for further study.

Studies listed in the Table, below, were selected, if possible, for non-lethal doses, a method of administration that would result in distribution throughout the body, and repeated dosing. Most studies were not designed to determine a NOAEL, but to characterize the effects of colchicine, thus the listed animal dose was the lowest dose administered. It is important to note that the animal doses were not the LOAEL, since lower doses that may have resulted in a NOAEL were not administered. Thus the table provides hazard identification and cannot be used for risk evaluation. The exception was the cardiovascular study in conscious dogs conducted by the Applicant in which a NOAEL dose was determined and which is indicated as such in the Table.

Nonclinical Findings and Human Equivalent Dose* (Reviewer's Table)

Effect	Lowest Dose at which effect was observed (unless indicated otherwise)	Human Equivalent Dose [#] (based on body surface comparison)	
		mg/kg	mg/day (based on 60 kg subject)
Neurobehavioral (studies of colchicine not administered directly into the CNS)			
gait abnormalities (with no detectable LM or EM changes in nerve or skeletal muscle; Chang et al 2002)	rats, females 0.2 mg/kg/day i.p. , 5 days/week, for 7 or 10 months	0.032	1.92
myopathy (disorientated filaments; Seiden 1973)	rats, males 0.4 mg/kg/day, i.p. for 2 to 22 days	0.064	3.84
Cardiovascular (nonlethal studies)			
No changes in QTc values, or other ECG parameters, heart rate, MAP, systolic and diastolic pressure (conscious dogs using telemetry; Applicant study 1259DU21.001)	beagle dogs, 0.5 mg/kg, po, NOAEL single dose	0.28	16.7
Gastrointestinal			
enhanced intestinal permeability (Fradkin et al (1995))	rats (~200 g) ~0.5 mg/day, p.o. , (or ~2.5 mg/kg/day) for up to 3 weeks (dissolved in drinking water; estimated from volume consumed) serum colchicine on day 23 3.89 ng/mL (range 1.0- 6.7 ng/mL)	0.45	27.1
Reproductive and Developmental			
Fertility			
sperm abnormalities (Wyrobek and Bruce 1975; Bruce and Heddle 1979)	mice 0.6 and 0.75 mg/kg, i.p. over 5 days, sacrificed at 1, 4, and 10 weeks post-treatment	0.097 0.12	5.8 7.2
triploid and mosaic embryos androgenetic eggs (formed by the combination of two male pronuclei (Piko and Bomsel-Helmreich, 1960)	Wistar CF rat 0.25 to 0.5mg/kg, i.p. at 2 to 2.5 hours after mating in the rat	0.04	2.4

interfered with sperm penetration, the second meiotic division, and normal cleavage in mouse, but no effect in rabbit (McGaughey and Chang, 1969)	mice 0.5 mg/kg, i.p. administered before or after induced ovulation and artificial insemination, eggs recovered 1.5 to 6.5 hr after ovulation	0.04	2.4
	rabbits 1.0 mg/kg, i.p. administered before or after ovulation and eggs examined at 11 to 12.5 h after ovulation	0.32	19.2
inhibition of hCG-induced ovulation (Espey <i>et al.</i> 1982)	rabbit 4.0 mg/kg, iv	- ^a	- ^a
Embryo and Fetal Development			
fetal death, teratogenicity (Sieber <i>et al.</i> 1978)	mice, 0.5 mg/kg, i.p. administered once, on either gestation day 6, 7, or 8	0.04	2.4
fetal death, teratogenicity (Shoji 1968)	mice, 0.5 mg/kg, s.c., administered once, between gestation days 2 to 14	0.04	2.4
fetal death, growth inhibition, teratogenicity (Van Dyke and Ritchev, 1947)	rat, 0.6 mg/kg, i.p. single dose on gestation day 6, 8, 10, 12 or 14, examined 3 days later	0.97	58.2
Postnatal Development			
embryo and fetal death, growth inhibition, and postnatal behavioral and learning retardation (Petit and Isaacson, 1976)	rat, 0.6 mg/kg, i.p. single dose on either gestation day 6, 8, 10, 12, or 14	0.97	58.2

*Studies were selected for non-lethal doses, a method of administration that would result in distribution throughout the body, and repeated dosings, if possible)

The maximum proposed human dose is 2.4 mg/day (0.6 mg tablets QID)

^a the animal was administered intravenously, but there were no measurements of blood colchicine concentrations, so the HED was not calculated

2.6.6.10 Tables and Figures

Refer to the individual topic sections

2.6.7 TOXICOLOGY TABULATED SUMMARY

Refer to the individual topic sections

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The doses of colchicine for the proposed indication are at or below those for which there is extensive clinical pharmacological and toxicological knowledge and clinical experience. Colchicine has been used extensively in laboratories as a research tool to study cell division, cellular and intracellular particle movement, cytoskeletal form and function, and more recently inflammasomes, an intracellular structure involved in the formation and release of inflammatory cytokines. The nonclinical pharmacology is well characterized, but the nonclinical toxicology lags behind the knowledge of clinical toxicology. The vast majority of previous studies were not conducted to meet today's regulatory standards nor performed according to GLP. Repeated dose studies of at most a few weeks, cited in the literature, indicated a low threshold between adverse effects and mortality, although the colchicine used in these older studies was unlikely to be as pure as the Applicants drug. These factors together with the more widely known clinical experience and clinical toxicity of colchicine contributed to the decision that further nonclinical studies, including carcinogenicity studies would be of limited usefulness or difficult to accomplish.

Unresolved toxicology issues

The photodegradant impurities contain structural alerts for mutagenicity and therefore need specifications set to maintain daily intake at less than 1.5 µg/day. If that is not possible, qualification studies (genetic toxicology and a 28-day repeated dose study) are necessary. These components are not in the clinical product (*at the current levels of impurity detection*), due to package protection from light. The currently marketed *approved* generics of *Colbenemid*, and the numerous marketed, but *unapproved colchicine only products* have the same potential of containing these impurities, and should all be limited with regards to these impurities. Since these have been on the market for years, this reviewer recommends the lowering of specifications or qualification studies could be done postmarketing.

GLP studies of reproductive and developmental toxicology and carcinogenicity have not been conducted. For reproductive and developmental toxicology, there is sufficient information from published literature to convey the risk in the label. Furthermore there are recent clinical epidemiology studies of pregnancies in colchicine treated women with

FMF that have not found detrimental effects that could be attributed to colchicine, but there were limited number of pregnancies studied.

Carcinogenicity studies have not been requested due to the long history of clinical experience, although specific documentation of any relationship between colchicine use and carcinogenicity is lacking. They are still possible options if clinical findings from expanded safety surveillance result in signals for further study. From the few repeated dose studies reported in the literature and (possibly the same studies conducted for the original approval of colchicine/probencid combination in 1961, original reviews were not found), there was a low threshold between adverse effects and the lethal dose in studies of less than 1 month duration in rats. With low doses of colchicine administered in drinking water of spontaneously hypertensive and nonhypertensive rats, respiratory difficulties developed within 4 to 13 months (Cicogna et al 1997). While there may be greater susceptibility in this strain, overall studies do not provide much confidence that a 2 year oral study in rats or mice would be productive. A study was conducted with dermally applied colchicine twice weekly in mice for 6 months, and this could be conducted with transgenic mice, but those mice would not be an appropriate model for an orally ingested drug since they have a high spontaneous background rate of internal tumor formation that may confound and mask colchicine induced effects.

Recommendations

The application may be approved from the nonclinical pharmacology and toxicology perspective.

Suggested labeling: Refer to the Executive Summary Section I-C.

Signatures (optional):

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

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

APPENDIX/ATTACHMENTS**Applicant provided References**

- Ai H, Ralston E, Lauritzen HP, *et al.* Disruption of microtubules in rat skeletal muscle does not inhibit insulin- or contraction-stimulated glucose transport. *Am Physiol Endocrinol Metab* 2003;285:E836-44.
- Akers RM, Nickerson SC. Effect of prepartum blockade of microtubule formation on milk production and biochemical differentiation of the mammary epithelium in Holstein heifers. In *t J Biochem.* 1983;15:771-75
- Albanese R. Chemically induced aneuploidy in female germ cells. *Mutagenesis* 1988;3:249-55.
- Allard E, Johnson KJ, Boekelheide K. Colchicine disrupts the cytoskeleton of rat testis seminiferous epithelium in a stage-dependent manner. *Biol Reproduction* 1993;48:143-53.
- Arai N, Ohya K, Ogura H. Osteopontin mRNA expression during bone resorption: An *in situ* hybridization study of induced ectopic bone in the rat. *Bone Mineral* 1993;22:129-45.
- Arni P, Hertner T. Chromosomal aberrations in vitro induced by aneugens. *Mutation Res* 1997;379:83-93.
- Azhar S, Yii-Der I, Reaven C, *et al.* The effect of colchicine on cholesterol processing by the progesterone-producing cells of the luteinized ovary. *J Steroid Biochem* 1986;24:739-45.
- Back A, Walaszek E, and Uyeki E. Distribution of radioactive colchicine in some organs of normal and tumor-bearing mice. *Proc Soc Exp Biol Med* 1951;77:667-9.
- Balci-Peynircioglu B, Waite AL, Hu C, *et al.* Pyrin, product of the *MEFV* locus, interacts with poapoptotic protein, siva. *J Cell Physiol* 2008.
- Bar-Eli M, Wilson L, Peters RS, *et al.* Microtubules in PMNs from patients with familial Mediterranean fever. *Am J Med Sci* 1982;284(2):2-7.
- Barsoum H. The effect of colchicine on the spermatogenesis of rabbits. *J Pharmacol Exp Ther* 1955;115:319-22.
- Beliles RP. Influence of pregnancy on the acute toxicity of various compounds in mice. *Toxicol Appl Pharm* 1972;23:537-40.
- Berenblum I, Armuth V. Effect of colchicine injection prior to the initiating phase of two-stage skin carcinogenesis in mice. *Br J Cancer* 1977;35:615-20.
- Bergner AD. Studies on colchicine derivatives: III. Effect on mitotic activity of mouse spermatogonia. *Cancer* 1950;3:134-41.
- Bittner B, Guenzi A, Fullhardt P, *et al.* Improvement of the bioavailability of colchicine in rats by co-administration of D- α -tocopherol polyethylene glycol 1000 succinate and a polyethoxylated derivative of 12-hydroxy-stearic acid. *Arzneim Forsch (Drug Res)* 2002; 52:684-688.
- Bouaziz A, Amor NB, Woodard GE, *et al.* Tyrosine phosphorylation / dephosphorylation balance is involved in thrombin-evoked microtubular reorganisation in human platelets. *Thromb Haemost* 2007;98:375-84.
- Brandwein SR, Sipe JD, Skinner M, Cohen AS. Effect of colchicine on experimental amyloidosis in two CBA/J mouse models: Chronic inflammatory stimulation and administration of amyloid-enhancing factor

during acute inflammation. *Lab Invest* 1985;52:319-25.

Brossi A. Bioactive alkaloids. 4. Results of recent investigations with colchicine and physostigmine. *J Med Chem* 1990;33(9):2311-9.

Brossi A, Yeh H JC, Chrzanowska M, *et al.* Colchicine and its analogues: recent findings. *Med Res Rev* 1988;8:77-94.

Bruce RW, Heddle JA. The mutagenic activity of 61 agents as determined by the micronucleus, salmonella, and sperm abnormality assays. *Can J Genet Cytol* 1979;21:319-34.

Buchanan JF, Davis LJ. Drug-induced infertility. *Drug Intell Clin Pharmacy* 1984;18:122-32.

Burvenich C. Influence of colchicine on mammary blood flow in goats. *Arch Int Pharmacodyn* 1980;246:165-66.

Burvenich C, Peeters G. Effect of intramammary infusion of colchicine on mammary blood flow in lactating goats. *Z Tierphysiol Tiernahr Futtermittelkd* 1980;44:211-17.

Cammerer Z, Elhajouji A, Kirsch-Volders M, *et al.* Comparison of the peripheral blood micronucleus test using flow cytometry in rat and mouse exposed to aneugens after single-dose applications. *Mutagenesis* 2007;22:129-34.

Caselli GF, Fiorentino S, Pellegrini L, *et al.* Does Colchicine really induce bone formation in the rodent bone marrow? Yes, it does. *Calcif Tiss Int* 1999;65:414-15.

Cerquaglia C, Diaco M, Nucera G, La Regina M, Montalto M, Manna R. Pharmacological and clinical basis of treatment of familial Mediterranean fever (FMF) with colchicine or analogues: An update. *Current Drug Targets - Inflammation & Allergy* 2005;4:117-24.

Chang E, Dellon AL, Dellon ES, Henderickson ME. Developing a model of colchicine neuropathy. *Microsurgery* 2002;22:46-48.

Chen QH, Hou S, Gan LC, *et al.* Determination of Colchicine by High Performance Liquid-chromatographic method with UV detection and its application to pharmacokinetic studies. *Yakugaku Zasshi* 2007a; 127(9):1485-90.

Chen YJ, Huang SM, Liu CY, *et al.* Hepatobiliary excretion and enterohepatic circulation of colchicine in rats. *Int J Pharm*, 2007(b). Doi:10.1016/j.jpharm.2007.08.052.

Cicogna AC, Brooks WW, Haycs JA, *et al.* Effect of chronic colchicine administration on the myocardium of the aging spontaneously hypertensive rat. *Mol Cell Biochem* 1997;166:45-54.

Dasheiff RM, Ramirez LF. The effect of colchicine in mammalian brain from rodents to rhesus monkeys. *Brain Res Rev* 1985;10:47-67.

de Lannoy IA, Mandin RS, Silverman M. Renal secretion of vinblastine, vincristine and colchicine *in vivo*. *J Pharmacol Exp Ther* 1994;268:388-95.

Desrayaud S, Guntz P, Scherrmann JM, Lemaire M. Effect of the P-glycoprotein inhibitor, SDZ PSC 833, on the blood and brain pharmacokinetics of colchicine. *Life Sci* 1997; 61:153-163.

Dinareello CA, Chusid MJ, Fauci AS, *et al.* Effect of prophylactic colchicine therapy on leukocyte function in patients with familial Mediterranean fever. *Arthritis and Rheumatism* 1976;19:618-22.

- Donoso VS, Bailie MD. Effect of colchicine on drug-induced changes in plasma renin concentration in rats. *Hypertension* 1982;4:676-80.
- Drion N, Risede P, Cholet N, *et al.* Role of P-170 glycoprotein in colchicine brain uptake. *J Neurosci Res* 1997;49:80-8.
- Drion N, Lemaire M, Lefauconnier J-M, Scherrman J-M.** Role of P-glycoprotein in blood-brain transport of colchicine and vinblastine. *J Neurochem* 1996; 67(4): 1688-1693.
- Dudkiewicz I, Brosh T, Perelman M, Salai M. Colchicine inhibits fracture union and reduces bone strength: *In vitro* study. *J Orthopaedic Res* 2005;23:877-81.
- Duncan AM, Heddle JA. The frequency and distribution of apoptosis induced by three non-carcinogenic agents in mouse colonic crypts. *Cancer Letters* 1984;23:307-311.
- Dvorák Z, Vrzal R, Ulrichova J, *et al.* Involvement of cytoskeleton in AhR-dependent CYP1A1 expression. *Cur Drug Metab* 2006;7:301-13
- Epstein SS, Arnold E, Andrea J, *et al.* Detection of chemical mutagens by the dominant lethal assays in the mouse. *Toxicol Appl Pharmacol* 1972;23:288-25
- Espey LL, Stein VI, Dumitrescu J. Survey of antiinflammatory agents and related drugs as inhibitors of ovulation in the rabbit. *Fertil Steril* 1982;38:238-47.
- Estable JJ. The ocular effect of several irritant drugs applied directly to the conjunctiva. *Am J Ophthalmol* 1948;31:837-44.
- Ferguson FCJ. Colchicine: General pharmacology. *J Pharma Exp Ther* 1952;106:261-70.
- Ferm VH. Colchicine teratogenesis in hamster embryos. *Proc Soc Exp Biol Med* 1963;112:77579
- Fisher HK. Effect of colchicine *in vivo* on elastic behavior of rat lungs. *Clin Res* 1977;25:163A
- Foureman PA. The TX₂ Y test for the detection of nondisjunction and chromosome breakage in drosophila melanogaster II. Results of female exposures. *Mutat Res* 1988;203:309-16.
- Galloway SM, Armstrong MJ, Reuben C, *et al.* Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ Mol Mutagen* 1987;10(S10):1-175 (NTP Study ID: 103168)
- Garriott ML, Brunny JD, Kindig DEF, *et al.* The *in vivo* rat micronucleus test: Integration with a 14-day study. *Mutat Res* 1995;342:71-76.
- Gemmell RT, and Stacy BD. Effects of colchicine on the structure and function of the corpus luteum in the sheep. *J Reprod Fertil* 1976;46:525-26.
- González NV, Badrán AF, and Barbeito CG. Daily variations in colchicine-induced apoptosis in duodenal crypts. *Chronobiology International*, 2005; 22(1):79-88.
- Gorenstein C, Bundman MC, Lew PJ, Olds JL, Ribak CE. Dendritic transport: I. Colchicine stimulates the transport of lysosomal enzymes from cell bodies to dendrites. *J Neuro Sci* 1985;5:2009-17.
- Handel MA. Effects of colchicine on spermiogenesis in the mouse. *J Embryol Exp Morph* 1979;51:73-83.
- Hastie SB. Interactions of colchicine with tubulin. *Pharm Ther* 1991;51:377-401.

- Hemmerly J, Demerec M. XIII. Tests of chemicals for mutagenicity. *Cancer Res Supplement* 1955;3:69-75.
- Henderson AJ, Peaker M. Compensatory increases in milk secretion in response to unilateral inhibition by colchicine during lactation in the goat. *J Physiol* 1983;334:433-440.
- Honma M, Momose M, Sakamoto H, *et al.* Spindle poisons induce allelic loss in mouse lymphoma cells through mitotic non-disjunction. *Mutat Res* 2001;493:101-14.
- Hunter AL and Klaassen CD. Biliary excretion of colchicine. *J Pharmacol Exp Ther* 1975a;192:605-17.
- Hunter AL and Klaassen CD. Biliary excretion of colchicine in newborn rats. *Drug Metab Disp* 1975b;3(6): 530-535.
- Iacobuzio-Donahue CA, Lee EL, Abraham SC, *et al.* Colchicine toxicity: distinct morphologic findings in gastrointestinal biopsies. *Am J Surg Pathol* 2001;8:1067-73.
- Inaba M, Kamata K. Effect of colchicine on steroid secretion from rat adrenal gland. *Jpn J Pharmacol* 1979;29:631-638.
- Ingalls TH, Curley FJ, Zappasodi P. Colchicine-induced craniofacial defects in the mouse embryo. *Arch Environ Health* 1968;16:326-32.
- Jie YM, Jia C. Chromosomal composition of micronuclei in mouse NIH 3T3 cells treated with acrylamide, extract of tripterygium hypoglaucom (level) hutch, mitomycin C and colchicine, detected by multicolor FISH with centromeric and telomeric DNA probes. *Mutagenesis* 2001;16:145-49.
- Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976;455(1):152-62.
- Kallinich T, Haffner D, Niehues T, *et al.* Colchicine use in children and adolescents with Familial Mediterranean Fever: literature review and consensus statement. *Pediatr* 2007;119:474-483.
- Kallio M, Sjöblom T, Lähdetie J. Effects of vinblastine and colchicine on male rat meiosis in vivo: Disturbances in spindle dynamics causing micronuclei and metaphase arrest. *Environ Mol Mutagen* 1995;25:106-17.
- Kiffé M, Christen P, Arni P. Characterization of cytotoxic and genotoxic effects of different compounds in CHO K5 cells with the comet assay (single cell gel electrophoresis assay). *Mutation Res* 2003;537:151-68.
- Khan MA. Effects of myotoxins on skeletal muscle fibers. *Prog Neurobiol* 1995;46:541-60.
- Klimecki WT, Futscher BW, Grogan TM *et al.* P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* 1994;84:2451-58.
- Knudson CM, Stemberger BH, Patton S. Effects of colchicine on ultrastructure of the lactating mammary cell: Membrane involvement and stress on the golgi apparatus. *Cell Tiss Res* 1978;195:169-81.
- Koch EA, and Spitzer RH. Multiple effects of colchicine on oogenesis in *Drosophila*: Induced sterility and switch of potential oocyte to nurse-cell developmental pathway. *Cell Tiss Res* 1983;228:21-32.
- Kowalski LA, Assi KP, Wee RKH, *et al.* In vitro prediction of carcinogenicity using a bovine papillomavirus DNA-carrying C3H/10T1/2 Cell line (T1). II: Results from the testing of 100 chemicals. *Environ Mol Mutagen* 2001;37:231-40.
- Kumar A, Seghal N, Naidu PS, *et al.* Colchicines-induced neurotoxicity as an animal model of sporadic

- dementia of Alzheimer's type. *Pharmacological Reports* 2007;59:274-83.
- Kuncl RW, Bilak MM, Craig SW, Adams R. Exocytotic "constipation" is a mechanism of tubulin/lysosomal interaction in colchicine myopathy. *Exp Cell Res* 2003;285:196-207.
- Lee M, Kwon J, Chung MK. Enhanced prediction of potential rodent carcinogenicity by utilizing comet assay and apoptotic assay in combination. *Mutat Res.* 2003;541:9-19.
- Leighton JA, Bay MK, Maldonado AL, *et al.* The effect of liver dysfunction on colchicine pharmacokinetics in the rat. *Hepatology*, 1990;11(2):210-5.
- Leopardi P, Zijno A, Bassani B, *et al.* In vivo studies on chemically induced aneuploidy in mouse somatic and germinal cells. *Mutat Res* 1993;287:119-30.
- Liang JC, Hsu TC, Gay M. Response of murine spermatocytes to the metaphase-arresting effect of several arrestants. *Experientia* 1985;41:1586-88.
- Lidar M, Livneh A. Familial Mediterranean fever: Clinical, molecular and management advancements. *Netherland J Med* 2007;65:318-24.
- Loizzi RF, de Pont JJ, Bonting SL. Inhibition by cyclic AMP of lactose production in lactating guinea pig mammary gland slices. *Biochim Biophys Acta* 1975;392:20-25.
- Lotfi CFP, Machado-Santelli GM. Comparative analysis of colchicine induced micronuclei in different cell types in vitro. *Mutat Res* 1996;349:77-83.
- Lothman EW, Stein DA, Wooten GF, Zucker DK. Potential mechanisms underlying the destruction of dentate gyrus granule cells by colchicine. *Exp Neurol* 1982;78:293-302.
- Lukic V. *In vitro* and *in vivo* effects of colchicine and its metabolites on selected enzyme systems in rats. *Acta Pharm* 1997; 47:83-91.
- Lynch AM, Parry JM. The cytochalasin-B micronucleus/kinetochore assay in vitro: Studies with 10 suspected aneuploids. *Mutat Res* 1993;287:71-86.
- Mailhes JB, Preston RJ, Yuan ZP, *et al.* Analysis for mouse metaphase II oocytes as an assay for chemically induced aneuploidy. *Mutat Res* 1988;198:145-52.
- Markand ON, D'Agostino AN. Ultrastructural changes in skeletal muscle induced by colchicine. *Arch Neurol* 1971;24:72-82.
- Matter BE, Grauwiler J. Micronuclei in mouse bone-marrow cells, a simple in vivo model for the evaluation of drug-induced chromosomal aberrations. *Mutat Res* 1974;23:239-49.
- Matter BE, Jaeger I, Grauwiler J. The relationship between the doses of various chemical mutagens required to induce micronuclei in mouse bone marrow and their lethal doses. *Proceedings of the European Society for the Study of Drug Toxicity* 1974;15:275-80
- McGaughey RW, Chang MC. Inhibition of fertilization and production of heteroploidy in eggs of mice treated with colchicine. *J Exp Zoology* 1969;171:465-80.
- Mery P, Riou B, Chemla D, Lecarpentier Y. Cardiotoxicity of colchicine in the rat. *Intensive Care Med* 1994;20:119-23.
- Midgely AR, Pierce B, Dixon FJ. Nature of colchicine resistance in Golden Hamster. *Science* 1959;130:40-41.

- Misra *et al.*, Mechanism by which cAMP increases bile acid secretion in rat liver and canalicular membrane vesicles. *Am J Physiol Gastrointest Liver Physiol* 2003;285: G316-G324
- Molad Y. Update on colchicine and its mechanism of action. *Curr Rheumatol Rep* 2002;4:252-56.
- Morales CR, Domitrovic HA, Sampietro J. Influence of colchicine on lactating mammary gland of the cow and the rat with special reference to the exocytosis and to the milk fat globule secretion. *Zbl Vet Med Anat Histol Embryol* 1982;11:56-64.
- Mortelmans K, Haworth S, Lawlor T, et al. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* 1986;8(S11):1-119. (NTP Study ID:62896)Mundy WR, Tilson HA. Neurotoxic effects of colchicine. *NeuroToxicology* 1990;11:539-48.
- Muzaffar A, Bossi A. Chemistry of colchicines. *Pharmac Ther* 1991;49:105-9.
- Natarajan AT, Duivenvoorden WCM, Meijers M, *et al.* Induction of mitotic aneuploidy using Chinese-hamster primary embryonic cells: Test results of 10 chemicals. *Mutat Res* 1993;287:4756.
- Nickerson SC, Akers RM. Effect of prepartum blockade of microtubule formation on the ultrastructural differentiation of the mammary epithelium in Holstein heifers. *Int J Biochem* 1983;15(6):777-88.
- Orsini M, Pansky B. The natural resistance of the Golden hamster to colchicine. *Science* 1952;115:88-89
- Padeh S. Peiodic fever syndromes. *Pediatr Clin N Am* 2005;52:577-609.
- Panariti E. Tissue distribution and milk transfer of colchicine in a lactating sheep following a single dose intake. *Dtsch tierarztl Wschr* 1996;103:128-129.
- Patton S. Mechanisms of secretion: Effects of colchicine and vincristine on composition and flow of milk in the goat. *J Dairy Sci.* 1975;59:1414-19.
- Petit TL, Isaacson RL. Anatomical and behavioral effects of colchicine administration to rats late in utero. *Dev Psychobiol* 1976;9:119-29.
- Piko L, Bomsel-Helmreich O. Triploid rat embryos and other chromosomal deviants after colchicine treatment and polyspermy. *Nature* 1960;186:737-38.
- Puzkin E , Puskin S, Aledort LM. Colchicine-binding protein from human platelets and its effect on muscle myosin and platelet myosin-like thrombosthenin-M. *J Biol Chem* 1971;246:271-76.
- Potten CS. Stem cells in gastrointestinal epithelium: Numbers, characteristics and death. *Phil Trans R Soc Lond B* 1998;353:821-30.
- Rachmilewitz D, Fogel R, Karmeli F. Effect of colchicine and vinblastine on rat intestinal water transport and Na-K-ATPase activity. *Gut* 1978;19:759-64.
- Rachmilewitz D, Karmeli F. Effect of colchicine on jejunal adenylate cyclase activity, PGE2 and CAMP contents. *Eur J Pharmacol* 1980;67:235-39.
- Rao A, Haywood J, Craddock AL, *et al.* The organic solute transporter α - β , Osta -Ost β , is essential for intestinal bile acid transport and homeostasis. *PNAS* 2008;105:3891-96.
- Ravelli RB, Gigant B, Curmi PA, *et al.* Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature* 2004;428:198-202.
- Reynolds AF, Oakley JC. The colchicine experimental epileptic focus: An intracellular study. *Brain Res*

1984;322:326-28.

Rochdi M, Sabouraud A, Girre C, *et al.* Pharmacokinetics and absolute bioavailability of colchicine after i.v. and oral administration in healthy human volunteers and elderly subjects. *Eur J Clin Pharmacol* 1994;46:351-4.

Rosner M, Capraro HG, Jacobson AE, *et al.* Biological effects of modified colchicines. Improved preparation of 2-demethylcolchicine, 3-demethylcolchicine, and (+)-colchicine and reassignment of the position of the double bond in dehydro-7-deacetamidocolchicines. *J Med Chem* 1981;24:257-61.

Russel LD, Malone JP, MacCurdy DS. Effect of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat. *Tissue Cell* 1981;13:349-67.

Sabouraud A, Chappey O, Dupin T, *et al.* Binding of colchicine and thiocolchicoside to human serum proteins and blood cells. *Int J Clin Pharm Th* 1994 ;32(8) :429-32.

Saltarelli D, Llosa-hermier MP, Tertrin-Clary C. Effects of antimicrotubular agents in cAMP production and in steroidogenic response of isolated rat leydig cells. *Biol Cell* 1984;52:259-66.

Samuels J, Aksentijevich I, Torosyan Y, *et al.* Familial Mediterranean fever at the millenium clinical spectrum, ancient mutations, and a survey of 100 America referrals to the National Institutes of Health. *Medicine* 1998;77:268-97.

Schinkel AH, Mayer U, Wagenaar E, *et al.* Normal viability and altered pharmacokinetics in mice lacking *mdr1*-type (drug-transporting) P-glycoproteins. *Proc Nat Acad Sci. USA* 1997;94:4028-33.

Schmid TE, Attia S, Baumgartner A, *et al.* Effect of chemicals on the duration of male meiosis in mice detected with laser scanning cytometry. *Mutagenesis* 2001;16:339-43.

Schmuck G, Lieb G, Wild D, *et al.* Characterization of an in vitro micronucleus assay with Syrian hamster embryo fibroblasts. *Mutation Res* 1988;203:397-404.

Schönharting M, Mende G, Siebert G. Metabolic transformation of colchicine, II: The metabolism of colchicine by mammalian liver microsomes. 1974. *Hoppe-Seyler's Z Physiol Chem.* 355: 1391-1399.

Seiden D. Effects of colchicine on myofilament arrangement and the lysosomal system in skeletal muscle. *Z. Zellforsch* 1973; 144:467-473.

Shah JH, Wongsurawat N. Impairment of glucose-induced insulin secretion and glucose tolerance during colchicine treatment. *Diabetes* 1978;27:925-30.

Shapiro AB, Ling V. Positively cooperative sites for drug transport by P-glycoprotein with distinct drug specificities. *Eur J Biochem* 1997;250:130-137.

Shoji R. Some account on teratogenic and embryocidal effects of colchicine on mouse embryos. *J Faculty Sci Hokkiado Univ* 1968;16:514-24

Shoji R, Makino S. Teratogenic and embryocidal effects of colchicine on mouse embryos. *Proceedings of the Congenita; Abnormalities Association of Japan* 1967;(7):61.

Shtrasburg S, Prs M, Gal R, Salai M, Livneh A. Inhibition of the second phase of amyloidogenesis in a mouse model by asingle-dose colchicine regimen. *J Lab Clin Med* 2001;138:107-11.

Sieber SM, Whang-Peng J, Botkin C, and Knutson T. Teratogenic and cytogenetic effects of some plant-derived antitumor agents (Vincristine, colchicine, maytansine, VP-16-213 and VM26) in mice. *Teratology* 1978;18:31-48.

- Simon A, van der Meer JWM. Pathogenesis of familial periodic fever syndromes or hereditary autoinflammatory syndromes. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R86-R98.
- Singh A, Le Marchand Y, Orci L, Jeanrenaud B. Colchicine administration to mice: A metabolic and ultrastructure study. *Eur J Clin Invest* 1975;5:495-505.
- Sokka TK, Patton S. *In vivo* effects of colchicine on milk fat globule membrane. *Biochim Biophys Acta* 1983;731:1-8.
- Speeg KV, Maldonado AL, Liaci J, *et al.* Effect of cyclosporine on colchicine secretion by the kidney multidrug transporter studied *in vivo*. *J Pharmacol Exp Ther* 1992;261:50-55.
- Sugawara S, Mikamo K. An experimental approach to the analysis of mechanisms of meiotic nondisjunction and anaphase lagging in primary oocytes. *Cytogenet Cell Genet* 1980;28:251-64
- Sugio K, Maruyama M, Tsurufuji S, Sharma PN, Bossi A. Separation of tubulin-binding and anti-inflammatory activity in colchicine analogs and congeners. *Life Sci* 1987;40:35-39.
- Szabo KT, Free SM, Birkhead HA, *et al.* The embryotoxic and teratogenic effects of various agents in the fetal mouse and rabbit. *Toxicol Appl Pharmacol* 1971;19:371-72.
- Szabo KT, Kang JY. Comparative teratogenic studies with various therapeutic agents in mice and rabbits. *Teratology* 1969;2:270.
- Tang-Wai DF, Bossi A, Arnold LD, Gros P. The nitrogen of the acetamido group of colchicine modulates p-glycoprotein-mediated multidrug resistance. *Biochem* 1993;32:6470-6476
- Tateishi T, Soucek P, Caraco Y, *et al.* Colchicine biotransformation by human liver microsomes: Identification of CYP3A4 as the major isoform responsible for colchicine demethylation. *Biochem Pharmacol* 1997;10:111-116
- Territo MC, Peters RS, Cline MJ. Leukocyte function in familial Mediterranean fever. *Am J Hematol* 1976;1(3):307-11.
- Tsuchimoto T, Matter BE. *In vivo* cytogenetic screening methods for mutagens, with special reference to the micronucleus test. *Arch Toxicol* 1979;42:239-48.
- Ulrichová J *et al.* Biochemical evaluation of colchicine and related analogs. *Planta Med.*, 1993;59(2):144-7.
- Valencia R, Mason JM, Woodruff RC, *et al.* Chemical mutagenesis testing in *Drosophila*. III. Results of 48 coded compounds tested for the National Toxicology Program. *Environ Mutagen* 1985;7:325-48. (NTP Study ID: 733667)
- Van Dyke JH, Ritchey MG. Colchicine influence during embryonic development in rats. *Anat Rec* 1947;97:375.
- Vian L, Van Hummelen P, Bichet N, *et al.* Evaluation of hydroquinone and chloral hydrate on the *in vitro* micronucleus test on isolated lymphocytes. *Mutat Res* 1995;334:1-7.
- Vollrath V *et al.* Effect of colchicine and heat shock on multidrug resistance gene and P-glycoprotein expression in rat liver. *J Hepatol* 1994;21(5):754-63.
- Wiesenfeld PL, Garthoff LH, Sobotka TJ, *et al.* Acute oral toxicity of colchicine in rats: Effects of gender,

vehicle matrix and pre-exposure to lipopolysaccharide. *J Appl Toxicol* 2007;27:42133.

Williams JP. Selective effects of colchicine on foetal nucleotide synthesis. *Biochem Soc Trans* 1974;2:699-702.

Wilson L. Microtubules as drug receptors: Pharmacological properties of microtubule protein. *Ann N Y Acad Sci* 1975;253:213-31.

Wisniewski H, Terry RD. Experimental colchicine encephalopathy: I. Induction of neurofibrillary degeneration. *Lab Invest* 1967;17:577-87.

Wlodarski KH, Wlodarski P. Colchicine-induced osteogenesis: Demonstration versus proof. *Calcif Tissue Int* 2001;69:58-59.

Wlodarski KH, Wlodarski PK. Does colchicine really induce bone formation in the rodent bone marrow? *Calcif Tissue Int* 1997;61:165-67.

Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. *Cell Biol* 1975;72:4425-29.

Yu JW, Femandes-Alnemri T, Datta P, *et al.* Pypin activates the ASC pyroptosome in response to engagement by autoinflammatory PSTPIP1 mutants. *Mol Cell* 2007;28:214-17.

Yuan J, Liu S, Cao J. Spindle poisons induce tk gene mutation in mouse lymphoma cells. *Acta Academiae Medicinae Militaris Tertiae* 2003;25:1688-91.

Zhang L, Strong JM, Qui S, *et al.* Scientific perspective on drug transporters and their role in drug interactions. *Mol Pharmacol* 2006;3:62-69.

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PHARMACOLOGIST

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PHARMACOLOGIST
I concur with Dr. Leshin that this NDA may
be approved from the nonclinical standpoint. Please see
Supervisory Memo for further details.