

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-947

PHARMACOLOGY REVIEW



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-947

SERIAL NUMBER: 000

DATE RECEIVED BY CENTER: 8/31/05

PRODUCT: OraVescent Fentanyl Citrate

INTENDED CLINICAL POPULATION: Management of breakthrough pain in opioid tolerant patients with cancer

SPONSOR: Cephalon, Inc.

DOCUMENTS REVIEWED: Pharmtox section, electronic submission

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

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From a nonclinical pharmacology toxicology perspective, NDA 21-947 may be approved.

B. Recommendation for nonclinical studies

One impurity in the drug substance, _____, exceeds ICHQ3A threshold for qualification and has not been tested for potential genetic toxicity. However, _____ does not contain a structural alert for mutagenicity, has similar pharmacodynamic and toxicologic effects as fentanyl, _____, and has been present in the fentanyl drug substance used for the Actiq product controlled with a specification of NMT _____. As such, the specification of NMT _____ does not raise significant safety concerns; however, the Sponsor should either reduce the specification to NMT _____ or provide a minimal genetic toxicology screen to confirm safety for the current specification. This can be completed post-approval.

C. Recommendations on labeling

Deletions are indicated by strikeouts
Additions are indicated by blue and underlined.

1 Page(s) Withheld

 Trade Secret / Confidential

 ✓ Draft Labeling

 Deliberative Process

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Cephalon submitted three safety pharmacology studies completed after the approval of the Actiq® NDA and genetic toxicology studies for three drug substance impurities containing structural alerts for mutagenicity. All other nonclinical information necessary to support the safety of the proposed drug product was provided via cross-reference to the data in Cephalon's NDA for Actiq.

Neurological Effects: Subcutaneous administration of 0.003 mg/kg fentanyl citrate to the rat produced no significant behavioral or physiological changes compared to the vehicle control group. The 0.03 mg/kg dose produced signs of stereotypic behavior, cage licking, 30 minutes after administration, but no other behavioral or physiological changes. The 0.3 mg/kg dose produced signs that were indicative of a generalized depression of the central nervous system. Changes in neurological function were evident from altered posture, body tone, grip strength, body carriage and gait, with the rats showing a flattened body position and extended rigid limbs, with catalepsy, paralysis and some impairment of the righting reflex. Altered sensorimotor responses included increased ease of handling and removal from cage, decreased touch response, decreased fearfulness and decreased pain response. Altered autonomic functioning included exophthalmos, abnormal pupil response, salivation, decreased pinna/corneal responses, slowed respiration rate and reduced body temperature. Some stereotyped behavior emerged by 3 h postdose, compulsive licking, probably coinciding with recovery from the initial depression of locomotor response. The effects observed following administration of 0.3 mg/kg fentanyl citrate were transient with signs of recovery having occurred approximately 5 hours postdose and complete recovery approximately 24 hours postdose.

Cardiovascular Effects: Subcutaneous administration of 0.001 and 0.01 mg/kg fentanyl citrate to conscious, telemetered beagle dogs had no effect on arterial blood pressure, heart rate or lead II ECG parameters. Administration of 0.5 mg/kg fentanyl citrate reduced heart rates between 15 and 45 minutes postdose, and increased diastolic, systolic and mean blood pressure at 5, 15, 60, 180 and 360 min postdose, with the maximal increase at 5 min. The RR interval was briefly faster at 5 min, but slower at 30 and 45 min postdose. There was no effect on PR or QRS intervals. There was an initial decrease in both corrected QT intervals (QTcF and QTcQ) at 15 and 30 min, followed by a prolonged increased corrected QT interval from 60 to 360 postdose. The mean maximal increase in corrected QT interval was +34 ms for QTcF and +30 ms for QTcQ. The high doses of fentanyl lead to altered conduction within the heart evidenced by an increased incidence of

sinus pauses >2.5 s duration, and increase incidence of escape complexes associated with the period of decrease heart rate and possibly increased escape focus excitability.

Respiratory Effects: A subcutaneous dose of 0.3 mg/kg fentanyl citrate to the rat decreased respiration rate at 30 minutes and 5 hours postdose and decreased tidal volume at 30 min postdose compared to the vehicle control group. These responses were similar to that of morphine. Lower doses of 0.003 and 0.03 mg/kg fentanyl produced no respiratory changes.

Genetic Toxicology: *In vitro* bacterial mutagenicity and *in vitro* clastogenicity studies were conducted on three impurities or degradants from fentanyl citrate supplied by _____
The three compounds are listed below:

1 1 1

All three impurities or degradants at concentrations of up to 5000 µg/plate were negative for induction of mutations in *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation. These impurities did not induce structural or numerical chromosome aberrations in the *in vitro* cytogenetic assay of human peripheral blood lymphocytes in the presence or absence of metabolic activation at concentrations that produced up to 50% cytotoxicity.

B. Pharmacologic activity

Fentanyl citrate, the active ingredient in fentanyl effervescent buccal tablets, is a potent opioid analgesic with pharmacological effects similar to morphine. This application seeks approval of an effervescent fentanyl tablet that is designed to be retained in the oral cavity during disintegration, with absorption of fentanyl across the oral mucosa. The Sponsor claims that effervescence, combined with controlled disintegration, enhances absorption of fentanyl across the oral mucosa compared the currently available formulations. There was one *in vitro* nonclinical published report to support this concept, but no *in vivo* studies.

C. Nonclinical safety issues relevant to clinical use

The most prominent adverse effect of high doses of fentanyl, as with all opioids in general, is respiratory depression, a known extension of the pharmacological action of opioids. As indicated in the table below, the maximal indicated human dose is less than 2-fold of a dose eliciting adverse respiratory, cardiovascular, or neurological effects in animal studies.

The following studies are suggested to fill knowledgeable gaps in the use of this product. They are not essential for the proposed indication of the treatment of breakthrough pain in cancer patients, since the product is critical for pain relief in this population.

				max. dose)
	Human	minimum = 100 µg Maximum [#] = 1600 µg	100 µg 1600 µg	
Neurological				
CNS Depression Altered Sensorimotor Responses Altered Autonomic Function	Rat	NOAEL = 0.03 mg/kg AE = 0.3 mg/kg	290 µg 2900 µg	0.18 1.8
Cardiovascular				
Delayed Occurrence (>60 min) of QT Prolongation Irregular rhythms Escape complexes	Dog, beagle	NOAEL = 0.01 mg/kg AE = 0.05 mg/kg	330 µg 1680 µg	0.21 1.05
Respiratory				
Decreased Respiratory Rate Decreased Tidal Volume	Rat	NOAEL = 0.03 mg/kg AE = 0.3 mg/kg	290 µg 2900 µg	0.18 1.8
Genetic Toxicology				
Cytotoxicity (<i>in vitro</i>) of Impurities	Human Lymphocytes	50% toxicity at minimum concentration = 266 µg/mL	Human <i>in vivo</i> respiratory depression: ~2-4 ng/mL	13,300 ⁺
Reproduction				
Impaired Fertility	Rat	30 µg/kg IV 160 µg/kg SC	290 µg 1550 µg	0.18 0.97

AE = dose at which adverse event occurred

[#] two 800 µg tablets, 30 min apart

* based on 60 kg person

⁺ (266 µg/mL)/(2 ng/mL)

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-947
 Review number: 1
 Sequence/date/type of submission: 000/Aug 31, 2005/Commercial, 505(b)(2)
 Information to sponsor: Yes
 Sponsor and/or agent: Cephalon, Inc., Frazer, PA 19355
 Manufacturer for drug substance:

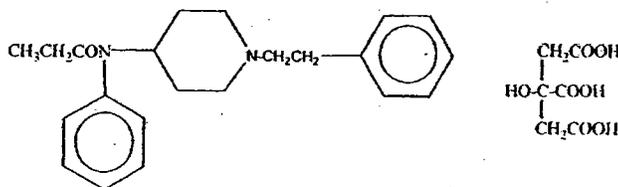
Reviewer name: L. S. Leshin
 Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
 HFD: 170
 Review completion date: June 22, 2006

Drug

Trade name: Not determined as of completion of this review.
 Generic name: **Fentanyl effervescent buccal tablets, Fentanyl Citrate**
 Chemical name: Propanamide, *N*-phenyl-*N*-[(1-(2-phenylethyl)-4-piperidinyl]-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1);
N-(1-Phenethyl-4-piperidyl) propionanilide citrate (1:1)

CAS registry number: 990-73-8
 Molecular formula: $C_{22}H_{28}N_2O \cdot C_6H_8O_7$
 Molecular weight: 336.48 (free base)
 528.59 (+citrate salt)

Structure:



increasing the hydrophobicity of the cell membranes and by thinning of the mucus layer (Eichman and Robinson, 1998).

Table 2: Quantitative Composition of Proposed Commercial Fentanyl Effervescent Buccal Tablets

Component	Reference to quality standard ^a	Function	100 µg	200 µg	400 µg	600 µg	800 µg
			(1/4 in. tablet) mg/tablet	(5/16 in. tablet) mg/tablet	(5/16 in. tablet) mg/tablet	(5/16 in. tablet) mg/tablet	(5/16 in. tablet) mg/tablet
Fentanyl Citrate	USP/Ph.Eur.	Active Ingredient					
Mannitol	USP/Ph.Eur.						
Sodium Bicarbonate	USP/Ph.Eur.		/	/	/	/	/
Citric Acid	USP/Ph.Eur.		/	/	/	/	/
Sodium Carbonate	NF/Ph.Eur.						
Sodium Starch Glycolate	NF/Ph.Eur.		/	/	/	/	/
Magnesium Stearate, Non-Bovine	NF/Ph.Eur.		/	/	/	/	/
Target Tablet Weight			100 mg	200 mg	200 mg	200 mg	200 mg

^a Product manufactured for US will be tested to USP/NF only.
^b N/A = Not Applicable

Impurities:

The potential known and unknown impurities in fentanyl citrate were described in the DMF Chemical structures provided by are shown in Figure 1, below. Three impurities

are monitored as specified impurities. All other impurities are monitored as unspecified impurities and controlled to NMT

Limits on Specified Impurities

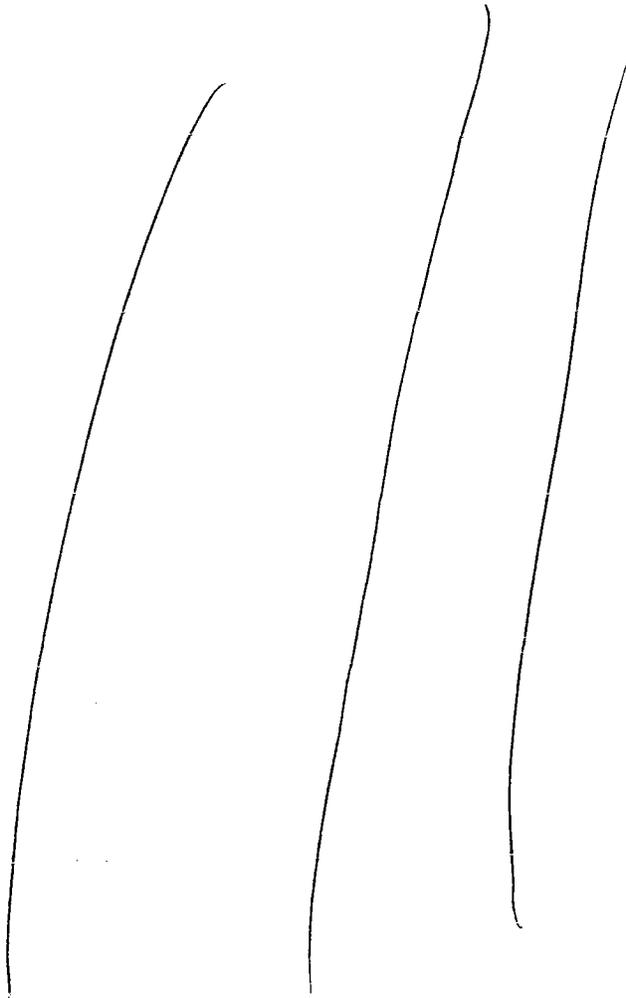
Impurity	Limits	Chemical Name
/	/	/

The Sponsor stated that they have not detected values above the current for in any batches of fentanyl citrate manufactured to date. In

this submission, they provide safety and genetic toxicology studies with Impurities

Reviewer Comment: During previous internal discussions, the three compounds studied in this NDA submission for genetic toxicity were selected for study based upon potential "structural alerts" for mutagenicity and carcinogenicity. The [redacted] were not assayed for potential genetic toxicology since they lacked "structural alerts." The pharmacodynamics and toxicity of [redacted] but not genetic toxicology, was studied under NDA 16-619 due to concerns raised associated with a manufacturing change (see Toxicology section). However, the bioactivity of the other compounds have not been studied and their toxicity characterization remains unknown.

Figure 1: Drug Substance Impurities - [redacted]



Route of administration: Oral transmucosal
(Buccal/sublingual absorption)

Regulatory History

The Referenced Drug for nonclinical information is Actiq® (oral transmucosal fentanyl citrate, NDA 20-747, Anesta, approved 11/13/1997; currently owned by Cephalon). Actiq® is currently the only opioid analgesic approved in the United States for breakthrough pain (BTP) in patients with cancer receiving opioids as their therapy for persistent pain. Fentanyl citrate is currently approved for parenteral, oral transmucosal, and transdermal administration. Parenteral fentanyl is used as a sedative and analgesic premedicant, sole and supplemental anesthetic and postoperative analgesic. Transdermal and transmucosal fentanyl is used for treating patients with chronic pain and break through cancer pain, respectively. This 505(b)(2) application seeks approval of an effervescent fentanyl tablet that is designed to be retained in the oral cavity during disintegration and absorption of the therapeutically useful amount of fentanyl across the oral mucosa. Effervescence, combined with controlled disintegration, enhances absorption of fentanyl across the oral mucosa compared the currently available formulations.

For (b)(2) applications:

Data Reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-947 are owned by Cephalon, Inc. or are data for which Cephalon, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-947 that Cephalon, 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 Cephalon, 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 21-947.

Studies reviewed within this submission:

The studies listed below were conducted under IND 65,447 (Oravescent Fentanyl; CIMA/Cephalon).

Study Number/eCTD Location	Study Title
Safety Pharmacology	
DS-2005-014 eCTD SN-000, Pharmtox, Pharm	The Characterization of Fentanyl Citrate in the Functional Observational Battery Test in Rats
DS-2005-013 eCTD SN-000, Pharmtox, Pharm	Cardiovascular Effects of Fentanyl Citrate in Conscious, Telemetered Beagle Dogs
DS-2005-015 eCTD SN-000, Pharmtox, Pharm	Effects of Fentanyl Citrate on Respiration Rate and Tidal Volume in Rats
Genotoxicity (of impurities)	
DS-2005-007 eCTD SN-000, Pharmtox, Tox	In Vitro Mammalian Chromosome Aberration Test Fentanyl, Lot RS-04019

DS-2005-008 eCTD SN-000, Pharmtox, Tox	In Vitro Mammalian Chromosome Aberration Test Fentanyl, Lot RS03027
DS-2005-009 eCTD SN-000, Pharmtox, Tox	In Vitro Mammalian Chromosome Aberration Test , Lot RS-02016-3
DS-2005-010 eCTD SN-000, Pharmtox, Tox	Bacterial Reverse Mutation Assay Fentanyl, Lot RS-04019
DS-2005-011 eCTD SN-000, Pharmtox, Tox	Bacterial Reverse Mutation Assay Fentanyl, Lot RS03027
DS-2005-012 eCTD SN-000, Pharmtox, Tox	Bacterial Reverse Mutation Assay , Lot RS-02016-3

Studies not reviewed within this submission:

All submitted studies were reviewed.

Additional studies used for review, not submitted by the Sponsor:

Cephalon has cross-referenced NDA 20-747 (Actiq) for the following nonclinical information to support the current NDA:

<p>Refer to NDA 20-747 for information concerning</p> <ul style="list-style-type: none"> Pharmacology <ul style="list-style-type: none"> Primary Pharmacodynamics Secondary Pharmacodynamics Pharmacodynamic Drug Interactions Pharmacokinetics Toxicology <ul style="list-style-type: none"> Single-Dose Toxicity Repeat-Dose Toxicity Carcinogenicity Reproductive and Developmental Toxicity Local Tolerance Literature References <p>Not Applicable (indicated by Sponsor)</p> <ul style="list-style-type: none"> Dependence Metabolites Impurities Other Toxicity Studies <ul style="list-style-type: none"> Antigenicity Immunotoxicity

NDA 20-747 (Actiq®) contained three genetic toxicology studies and a metabolism study. The remaining information was provided by cross-referenced to NDA 20-195 (Oralet®, Fentanyl lozenge, oral transmucosal fentanyl citrate; Anesta Corp.). There were no nonclinical pharmacology or toxicology studies submitted in support of NDA 20-195, which contained published scientific literature (references are indicated in the Appendix).

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

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Fentanyl is a phenylpiperidine analgesic that, depending on the species and the methods used to measure antinociception, is approximately 80 to 290 times more potent than morphine. Fentanyl is an opioid agonist, with activity at μ -, κ -, and δ -receptors. It has a higher binding affinity for μ , as compared to κ - and δ - receptors. Fentanyl may also bind to a lesser extent with central and peripheral (vascular) α -adrenergic and muscarinic (M_3) receptors. In addition to analgesia, μ -opioid agonists such as fentanyl produce drowsiness and sedation, changes in mood, respiratory depression, decreased gastrointestinal motility, muscle rigidity, euphoria, miosis, nausea, vomiting and alterations in the endocrine and autonomic nervous system. However, compared to morphine, fentanyl has a more favorable safety margin towards cardiovascular, neurological and metabolic effects. Adding other central nervous system depressants, with or without intrinsic analgesic properties can change the pharmacodynamics of fentanyl. As with all μ -opioid agonists, fentanyl has abuse potential.

Unique to this product is the rapid dissolution of the effervescent tablets with oral transmucosal absorption. Drug that is not absorbed by surfaces in the oral cavity will eventually be absorbed as it traverses the gastrointestinal tract. Theoretically, this should eliminate a substantial amount of first-pass liver metabolism, providing active drug at higher concentrations and with faster onset to pain relief than a comparable dose absorbed only after swallowing. Fentanyl is more lipid soluble than morphine, resulting in a more rapid onset but shorter duration of action than morphine. Fentanyl equilibrates rapidly between plasma and CSF. Binding sites within the central nervous system in sequentially decreasing order include the midbrain and striatum, hypothalamus, cerebral cortex, hippocampus, brainstem, spinal cord and cerebellum.

The safety concerns of fentanyl pertaining to the submitted NDA are similar to those following systemic administration of potent opioids. The major concerns are respiratory depression and the potential for abuse. The respiratory depressant effects are often of long duration and may exhibit a biphasic pattern, due to the significant amount of fentanyl sequestered in peripheral tissues, which must then reenter the plasma before elimination. The concentration of fentanyl in plasma and CSF correlates closely with the intensity of respiratory depression. Repeated injections of fentanyl lead to accumulation with increased ventilatory depression. Fentanyl is a basic drug such that an increase in plasma pH shifts the equilibrium to favor the un-ionized moiety. This increases the proportion of fentanyl available for diffusion across the blood-brain barrier. Although opioids such as fentanyl can have significant safety concerns if used improperly, the effects are well known.

2.6.2.2 Primary pharmacodynamics

Analgesia, the primary pharmacodynamic effect of fentanyl citrate was described in NDA 20-747. No additional primary pharmacodynamic studies were conducted by the Sponsor for the current NDA.

As noted in the Pharmacology/Toxicology review of NDA 20-747, the Sponsor provided no preclinical data supporting the efficacy of oral transmucosal fentanyl citrate, because there was no suitable animal model for this route of administration. However, the analgesic efficacy of fentanyl citrate by other routes in animals was well established in the literature. A summary of previously submitted studies of analgesia is presented in the Primary Pharmacodynamics section of the Pharmacology Tabulated Summary of Section 2.6.3.

2.6.2.3 Secondary pharmacodynamics

The secondary pharmacodynamics of fentanyl citrate were described in NDA 20-747. No additional secondary pharmacodynamic studies were conducted by the Sponsor for the current NDA.

As noted in the Pharmacology/Toxicology review of NDA 20-747, these secondary effects are typical μ -opioid effects and are reversible by nalorphine. In mice at doses of 10-1000 $\mu\text{g}/\text{kg}$ SC, they included dose-related increased spontaneous motor activity and response to touch, circling, Straub tail reaction, increased muscle tone, and mydriasis. In dogs, at 12.5-1000 $\mu\text{g}/\text{kg}$ IM, clinical signs included decreased motor activity, ataxia, depressed responsiveness to auditory and painful stimuli, bradycardia, respiratory depression, salivation and defecation. In humans, common fentanyl adverse effects are respiratory depression, hypotension, bradycardia, muscle and chest wall rigidity, pruritus, nausea and vomiting.

2.6.2.4 Safety pharmacology

Brief Summary

Three safety pharmacology studies were submitted in support of the current NDA submission. These studies provide additional characterization of the effects of fentanyl on the central nervous system function, cardiovascular system and respiratory system. The safety of fentanyl administration pertaining to the submitted NDA is similar to those following systemic administration of potent opioids. The major concern is respiratory depression, which can occur in humans at plasma concentrations between 2 to 4 ng/mL.

Neurological Effects: Subcutaneous administration of 0.003 mg/kg fentanyl citrate to the rat produced no significant behavioral or physiological changes compared to the vehicle control group. The 0.03 mg/kg dose produced signs of stereotypic behavior, cage licking, 30 minutes after administration, but no other behavioral or physiological changes. The 0.3 mg/kg dose produced signs that were indicative of a generalized depression of the central nervous system. Changes in neurological function were evident from altered posture, body tone, grip strength, body carriage and gait, with the rats showing a flattened body position and extended rigid limbs, with catalepsy, paralysis and some impairment of the righting reflex. Altered sensorimotor responses included increased ease of handling and removal from cage, decreased touch response, decreased fearfulness and decreased pain response. Altered autonomic functioning included exophthalmos, abnormal pupil response, salivation, decreased pinna/corneal responses, slowed respiration rate and reduced body temperature. Some stereotyped behavior emerged by 3 h

postdose, compulsive licking, probably coinciding with recovery from the initial depression of locomotor response. The effects observed following administration of 0.3 mg/kg fentanyl citrate were transient with signs of recovery having occurred approximately 5 hours postdose and complete recovery approximately 24 hours postdose.

Cardiovascular Effects: Subcutaneous administration of 0.001 and 0.01 mg/kg fentanyl citrate to conscious, telemetered beagle dogs had no effect on arterial blood pressure, heart rate or lead II ECG parameters. Administration of 0.5 mg/kg fentanyl citrate reduced heart rates between 15 and 45 minutes postdose, and increased diastolic, systolic and mean blood pressure at 5, 15, 60, 180 and 360 min postdose, with the maximal increase at 5 min. The RR interval was shortened at 5 min, but lengthened at 30 and 45 min postdose. There was no effect on PR or QRS intervals. There was an initial decrease in both corrected QT intervals (QTcF and QTcQ) at 15 and 30 min, followed by a prolonged increased corrected QT interval from 60 to 360 postdose. The mean maximal increase in corrected QT interval was +34 ms for QTcF and +30 ms for QTcQ. The high doses of fentanyl lead to altered conduction within the heart evidenced by an increased incidence of sinus pauses >2.5 s duration, and increase incidence of escape complexes associated with the period of decrease heart rate and possibly increased escape focus excitability.

Respiratory Effects: A subcutaneous dose of 0.3 mg/kg fentanyl citrate to the rat decreased respiration rate at 30 minutes and 5 hours postdose, and decreased tidal volume at 30 min postdose compared to the vehicle control group. These responses were similar to that of morphine. Lower doses of 0.003 and 0.03 mg/kg fentanyl produced no respiratory changes.

Abuse Liability: The DEA has classified fentanyl as a schedule II drug (DEA number 9801). No new nonclinical abuse liability studies were submitted with this NDA. NDA 20-747 for Actiq® contained some studies on the tolerance and dependence of fentanyl, and also relied upon findings of safety in NDA 20-195 for Oralet and published literature. The Oralet NDA also lacks drug addiction and withdrawal data, and relies on prior FDA findings. The FDA's prior findings of safety are based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection. These studies are summarized in the Safety Pharmacology Tables of the Pharmacology Tabulated Summary in Section 2.6.3.

NEUROLOGICAL EFFECTS

Study title: The Characterization of Fentanyl Citrate in the Functional Observational Battery Test in Rats

Key study findings: Subcutaneous administration of 0.003 mg/kg fentanyl citrate to the rat produced no significant behavioral or physiological changes compared to the vehicle control group. The 0.03 mg/kg dose produced signs of stereotypic behavior and cage licking, 30 minutes after administration, but no other behavioral or physiological changes. The 0.3 mg/kg dose produced signs that were indicative of a generalized depression of the central nervous system. Changes in neurological function were evident from altered posture, body tone, grip strength, body carriage and gait, with the rats showing a flattened body position and extended rigid limbs, with catalepsy, paralysis and some impairment of the righting reflex. Altered sensorimotor responses included increased ease of handling and removal from cage, decreased touch response, decreased fearfulness and decreased pain response. Altered autonomic functioning included exophthalmos, abnormal pupil response, salivation, decreased pinna/corneal responses, slowed respiration rate, and reduced body temperature. Some stereotyped behavior emerged by 3 h postdose, compulsive licking, probably coinciding with recovery from the initial depression of locomotor response. The effects observed following administration of 0.3 mg/kg fentanyl citrate were transient with signs of recovery having occurred approximately 5 hours postdose and complete recovery approximately 24 hours postdose.

Study no.: DS-2005-014

eCTD: SN-000, Pharmtox, Pharmacology, Module 4.2.1.3.2

Conducting laboratory and location:

Date of study initiation: Sept 23, 2004

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Fentanyl citrate, batch 67H1732, purity > —

Vehicle: saline

All fentanyl citrate formulation samples were within ± — of the theoretical. No fentanyl citrate was detected in the vehicle samples.

Methods

Male Sprague-Dawley rats (~7 weeks of age and ~200-244 grams at dosing) and were administered a single subcutaneous injection at doses of 0 (sterile saline), 0.003, 0.03 or 0.3 mg/kg fentanyl citrate (n=6/group) at a dose volume of 1.0 mL/kg. The parameters defined in the Irwin test were systematically evaluated for each rat prior to dosing and at 0.5, 5 and 24 hours post-dosing.

These parameters included measures of home cage and open field activity as well as tests of reflexive, physiologic and neuromuscular function. The observations were scored on an integer scale from 1 to 4 or 1 to 5. Factors that were present in normal animals (e.g. alertness, locomotor activity etc.) were scored as 3 (normal); potentiation or depression of these factors by the test treatment were scored as higher or lower integers, respectively. Observations that are normally absent in normal animals were arbitrarily scored from 1 (normal) to 4. Exceptions were pupil response, which was scored as 1 - present or 2 - absent, and fecal pellets, urine pools and rearing which were numerically counted. Miscellaneous observations were also noted. In addition to the observational parameters, fore and hind limb grip strength, hind limb splay and body temperature were also measured.

Results

Effect of 1 ml/kg Vehicle (Sterile Saline): Rats treated subcutaneously with 1 ml/kg vehicle (sterile saline) exhibited passivity. Other changes from normal were limited to 1 of 6 rats and were not considered to be pharmacologically relevant.

Incidence of Occurrence (n=6/dose group)

Fentanyl citrate (mg/kg, SC)	vehicle			
Predose/Time postdose (hours)	predose	0.5	5	24
Characteristic				
passivity	6	5	6	6

Effects of 0.003 mg/kg Fentanyl Citrate: Subcutaneous administration of 0.003 mg/kg fentanyl citrate produced no significant behavioral or physiological changes compared to the concurrent vehicle control group. Passivity and exophthalmos were the only changes from normal. Any other changes from normal were limited to 1 of 6 rats and were not considered to be pharmacologically relevant. Fentanyl citrate treatment at 0.003 mg/kg SC showed no significant effects on body temperature or hind limb splay at any of the time points tested. Animals from this group showed significantly higher hind limb grip strength and a significantly lower forelimb grip strength during pre-dose testing when compared to the vehicle group, but this was not thought to be pharmacologically relevant as the rats had not been dosed at this point.

Incidence of Occurrence (n=6/dose group)

Fentanyl citrate	0.003 mg/kg, SC			
Predose/Time postdose (hours)	predose	0.5	5	24
Characteristic				
passivity	4	5	6	6
exophthalmos		6	2	

Effects of 0.03 mg/kg Fentanyl Citrate: Rats treated with fentanyl citrate at 0.03 mg/kg, SC, exhibited the following changes from normal: exophthalmos, stereotyped behavior of licking the cage; and passivity. Any other changes from normal were limited to 1 of 6 rats and were not considered to be pharmacologically relevant. Fentanyl citrate treatment at 0.03 mg/kg, SC, showed no significant effects on body temperature or hind limb splay at any of the time points tested. Animals from this group showed significantly higher hind limb grip strength during pre-dose testing when compared to the vehicle group, but this was not thought to be pharmacologically relevant, as the rats had not been dosed at this point.

Incidence of Occurrence (n=6/dose group)

Fentanyl citrate	0.03 mg/kg, SC			
	predose	0.5	5	24
Characteristic				
stereotypic behavior, (cage licking)		3		
passivity	6	6	6	6
exophthalmos		6	2	

Effects of 0.3 mg/kg Fentanyl Citrate: Following administration of the high dose, 0.3 mg/kg fentanyl citrate, signs associated with a generalized depression of the central nervous system were observed. These observations included flattened posture, decreased alertness, increased ease of handling and removal from cage, decreased touch response, decreased fearfulness, decreased body tone, exophthalmos, decreased corneal reflex, salivation, piloerection, decreased locomotor activity, slowed respiration, decreased startle response, catalepsy, passivity, and decreased righting reflex. The effects observed following administration of 0.3 mg/kg fentanyl citrate were transient with signs of recovery observed approximately 5 hours post-dosing and complete recovery approximately 24 hours post-dosing. Other changes from normal were limited to 1 of 6 rats and were not considered to be pharmacologically relevant.

Rearing was reduced in all animals dosed with 0.3 mg/kg fentanyl citrate at 0.5 h post-dose but the animals showed normal rearing behavior by 5 h post-dose. There was no effect on fecal pellets or urine production. Straub tail was observed immediately post-dose in all animals treated with 0.3 mg/kg fentanyl citrate and all of these animals were licking the cage in a stereotypical manner at 3 h post-dose.

Fentanyl citrate treatment at 0.3 mg/kg, SC, showed a significant decrease in body temperature at 0.5 h postdose ($34.7 \pm 0.48^\circ\text{C}$) compared to the vehicle group ($37.8 \pm 0.08^\circ\text{C}$). This was accompanied by a decrease in fore limb grip strength (0.503 ± 0.097 kg) compared to vehicle (1.105 ± 0.037 kg) at 0.5 h postdose. There was a significant decrease in hind limb grip strength at 5 h post-dose (0.214 ± 0.018 kg) compared to vehicle (0.517 ± 0.063 kg). Hind limb splay was not measured at the 0.5 h time point, since the rats were too sedated to be dropped from any height.

Summary Text Table 1b
Fentanyl Citrate s.c. - Mean FOB Observations

Time (h)	Fentanyl Citrate (mg/kg s.c.)	Group	Hind Limb Splay (cm)	Body Temp (°C)	Grip Strength	
					Hind Limb (kg)	Fore Limb (kg)
Pre-Dose	0	B	6.9 ± 0.66	37.7 ± 0.16	0.192 ± 0.025	1.041 ± 0.027
	0.003	C	6.1 ± 0.69	37.6 ± 0.14	0.508 ± 0.034 ^{**}	0.853 ± 0.044 [‡]
	0.03	D	5.5 ± 0.47	37.4 ± 0.12	0.449 ± 0.029 ^{**}	0.869 ± 0.037
	0.3	A	5.8 ± 0.21	37.7 ± 0.13	0.254 ± 0.023	1.063 ± 0.023
0.5	0	B	6.3 ± 0.73	37.8 ± 0.08	0.248 ± 0.044	1.105 ± 0.037
	0.003	C	7.8 ± 0.67	37.4 ± 0.17	0.216 ± 0.016	1.108 ± 0.029
	0.03	D	7.2 ± 0.53	37.6 ± 0.14	0.184 ± 0.022	1.102 ± 0.016
	0.3	A	NA	34.7 ± 0.48 ^{##}	0.133 ± 0.023	0.503 ± 0.097 [‡]
5	0	B	6.3 ± 0.57	36.9 ± 0.14	0.517 ± 0.063	1.019 ± 0.065
	0.003	C	7.6 ± 0.76	37.1 ± 0.10	0.493 ± 0.036	0.977 ± 0.014
	0.03	D	6.1 ± 0.52	37.2 ± 0.13	0.387 ± 0.040	0.976 ± 0.029
	0.3	A	7.0 ± 0.72	36.4 ± 0.59	0.214 ± 0.018 ^{##}	1.038 ± 0.027
24	0	B	6.9 ± 0.44	36.8 ± 0.09	0.281 ± 0.040	1.110 ± 0.036
	0.003	C	7.3 ± 0.53	36.9 ± 0.17	0.343 ± 0.060	1.103 ± 0.016
	0.03	D	6.6 ± 0.68	37.0 ± 0.09	0.343 ± 0.052	1.040 ± 0.027
	0.3	A	7.9 ± 0.74	36.8 ± 0.12	0.352 ± 0.038	1.066 ± 0.045

Statistical analyses were performed on hind limb splay, body temperature and grip strength measurements.
 NA - Due to the heavy sedation of the animals, hindlimb splay measurements could not be obtained.
[‡] P < 0.05 and ^{##} P < 0.01 compared to vehicle (Kruskal-Wallis and Dunn's test).
^{**} P < 0.01 compared to vehicle (ANOVA and Dunnett's t-test).
 n - 6 animals per group.
 Data are mean ± SEM.

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Incidence of Occurrence (n=6/dose group)

Fentanyl citrate Predose/Time postdose (hours)	0.3 mg/kg, SC			
	predose	0.5	5	24
Characteristic				
posture (flattened)		6		
asleep			2	
abnormal carriage (flattened)		6		
decreased alertness		6	2	
increased ease of handling and removal from the cage		6		
decreased touch response		6	2	
decreased fearfulness		6	2	
decreased body tone		5	5	
exophthalmos		6	6	
decreased pinna response		6	2	
decreased corneal reflex		6	2	
increased pupil diameter		2		
decreased pupil diameter		4		
abnormal pupil response		6		
salivation		4		
piloerection		6		
decreased locomotor activity		6		
extended rigid limbs		5		
slowed respiration		6		
decreased approach response		6		
decreased startle response		6		
paralysis		6		
catalepsy		6		
passivity	6			
decreased righting reflex		6		
abnormal flexor reflex		6		
decreased pain response		6		
increased vocalization	2			

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CARDIOVASCULAR EFFECTS**Study title: Cardiovascular Effects of Fentanyl Citrate in Conscious, Telemetered Beagle Dogs**

Key study findings: Subcutaneous administration of 0.001 and 0.01 mg/kg fentanyl citrate had no effect on arterial blood pressure, heart rate or lead II ECG parameters. Administration of 0.5 mg/kg fentanyl citrate reduced heart rates between 15 and 45 minutes postdosing, and increased diastolic, systolic and mean blood pressure at 5, 15, 60, 180 and 360 min postdose, with the maximal increase at 5 min. The RR interval was briefly faster at 5 min, but slower at 30 and 45 min postdose. There was no effect on PR or QRS intervals. There was an initial decrease in both corrected QT intervals (QTcF and QTcQ) at 15 and 30 min, followed by a prolonged increased corrected QT interval from 60 to 360 postdose. The mean maximal increase in corrected QT interval was +34 ms for QTcF and +30 ms for QTcQ. The high doses of fentanyl lead to altered conduction within the heart evidenced by an increased incidence of sinus pauses >2.5 s duration, and increase incidence of escape complexes associated with the period of decrease heart rate and possibly increased escape focus excitability.

Reviewers Comment: The Sponsor stated that heart rates were lower for the -15 minute predose value in all fentanyl citrate groups than the vehicle group. They interpret this to indicate that the dogs were still acclimating to the dosing procedure. If the dogs were not yet acclimated to the procedures during the study, then the design and implementation of the study and/or analysis of the study was inappropriate. The analysis by comparison to the time-matched vehicle-treated control was not appropriate. The original data was not provided, only the mean values for each parameter for each dog. Therefore, it was not possible to accurately recalculate statistical differences. Reinterpretation of the Sponsor's results were based on estimated comparison to predose values rather than vehicle-treated values performed obtained separately, days earlier.

Study no.: DS-2005-013

eCTD: SN-000, Pharmtox, Pharmacology, Module 4.2.1.3.1

Conducting laboratory and location:

Date of study initiation: Sept 16, 2004

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Fentanyl citrate, Lot 052K1537, Purity — (HPLC)

Vehicle: Saline

All fentanyl citrate formulation samples were within ± — of the expected concentration. No fentanyl citrate was detected in the vehicle samples.

Methods

Males beagle dogs (n=4 12.2-16.7 kg, 11-12 months of age), with previously implanted telemetry transducers for arterial blood pressure and lead II ECG, were acclimated to the restraint procedure on 3 occasions, then administered escalating subcutaneous doses of 0, 0.001, 0.01 or 0.5 mg/kg fentanyl citrate (0.3 ml/kg) separated by at least 3 days.

Experimental Design (Sponsor table modified by reviewer)

Day of Treatment	1	4	8	11
Treatment	Vehicle	Fentanyl citrate		
Dose (mg/kg)	0	0.001	0.01	0.05
No. of Animals (n)	4	4	4	4

Measurements of systolic arterial blood pressure (SBP), diastolic arterial blood pressure (DBP), heart rate (HR) and the lead II ECG variables (for determination of RR interval, PR interval, QRS duration, and QT, QTcF and QTcQ intervals) were acquired continuously from approximately 30 min prior to until approximately 6 h following administration. Time points used for analysis included -15 (pre-dose), +5, 15, 30, 45, 60, 120, 180, 270 and 360 minutes post-dose. Arterial blood pressure (SBP, DBP, MAP) and heart rate at the selected time points were an average of 60 seconds of data at that time point whenever possible. Lead II ECG data at these time points were an average of at least 10 complexes of data at that time point whenever possible. All acquired ECG waveforms were inspected visually for disturbances in rhythm and waveform morphology. Mean arterial blood pressure (MAP) was calculated as $DBP + 1/3(SBP-DBP)$. QTcF interval was calculated using Fridericia's formula ($QTcF = QT/\sqrt{RR}$). In addition, QTcQ interval was calculated as $QTcQ = QT + \#(1-RR)$ where # corresponds to a correction factor specific to each individual dog as it represents the slope of the line from a plot of QT against RR interval generated over a range of heart rates on the day of vehicle administration. The data generated on the fentanyl treatment days were compared to the corresponding time point following vehicle treatment using a one-way analysis of variance (ANOVA) with repeated measures, followed by a Dunnett's post-hoc test. Data in the tables below are presented as mean \pm standard error of the mean. Changes were considered to be statistically significant when $P < 0.05$.

Results:-

General Observations: No clinical observations were noted following vehicle or 0.001 mg/kg, SC, fentanyl citrate. Doses of 0.01 mg/kg resulted in loose feces in one animal (#1849) and an unsteady gait and slight sedation in another animal (#1803). Doses of 0.05 mg/kg resulted in sedation, abnormal gait/difficulty standing, decreased respiration, cold extremities and pale gums/tongues.

Heart Rate: Heart rates were reduced at 15, 30, and 45 minutes after administration of 0.05 mg/kg fentanyl, compared to the time-matched vehicle treated group. Heart rates were also reduced compared to the pre-dose baseline value for the 0.05 mg/kg dose. The effects noted at 0.01 mg/kg fentanyl dose were probably not physiologically significant, although statistical significance was obtained. This was due to the elevated heart rates in the predose values in the vehicle and 0.001 mg/kg groups and at most post-dose time points in the vehicle group.

Table 4
The Effects of Vehicle and Fentanyl Citrate on Heart Rate in Conscious, Telemetered Beagle Dogs

Time (min)	Heart Rate (beats per minute)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	104 ± 9	84 ± 6	77 ± 6	71 ± 7*
5	102 ± 6	101 ± 10	73 ± 4	91 ± 19
15	120 ± 6	91 ± 11	76 ± 10*	50 ± 8**
30	104 ± 12	91 ± 13	78 ± 10	43 ± 4**
45	95 ± 8	89 ± 9	69 ± 5*	51 ± 4**
60	81 ± 4	88 ± 9	74 ± 8	65 ± 8
120	74 ± 5	83 ± 9	78 ± 13	87 ± 3
180	90 ± 8	71 ± 3	64 ± 12	88 ± 9
270	83 ± 4	93 ± 9	62 ± 4*	68 ± 5
360	95 ± 7	100 ± 7	69 ± 2*	69 ± 7*

Mean Arterial Pressure: In consideration of the comments above concerning the analysis, the Sponsor's findings of increased mean blood pressure at 60 and 270 minutes in the 0.05 mg/kg fentanyl dose group were due to an isolated decrease in mean blood pressure at 60 and 270 minutes in the vehicle-treated group. However, compared to the predose value, increased mean blood pressure occurred at 5, 15, 60 and 180 and 360 minutes after 0.05 mg/kg fentanyl administration, with a maximal mean increase occurring at the 5 minutes (+23 mmHg). There were no effects at lower fentanyl doses. These increases were due to increases in systolic and diastolic blood pressures at the same time points (5, 15, 60, 180, and 360 minutes), with the maximum systolic and diastolic increases at 5 minutes (systolic: +31 mm Hg, diastolic: +20 mm Hg).

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Table 3
The Effects of Vehicle and Fentanyl Citrate on Mean Arterial Blood Pressure in Conscious, Telemetered Beagle Dogs

Time (min)	Mean Arterial Blood Pressure (mmHg)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	119 ± 7	122 ± 7	119 ± 3	115 ± 5
5	133 ± 6	134 ± 8	120 ± 6	138 ± 9
15	135 ± 9	127 ± 6	124 ± 10	132 ± 8
30	126 ± 3	128 ± 7	118 ± 9	113 ± 4
45	122 ± 4	128 ± 8	118 ± 3	113 ± 7
60	111 ± 4	130 ± 8*	115 ± 3	129 ± 8*
120	123 ± 5	127 ± 9	115 ± 4	118 ± 4
180	118 ± 2	121 ± 7	110 ± 3	130 ± 4
270	108 ± 3	113 ± 5	112 ± 2	121 ± 2*
360	116 ± 3	121 ± 4	122 ± 5	132 ± 5

Table 1
The Effects of Vehicle and Fentanyl Citrate on Systolic Arterial Blood Pressure in Conscious, Telemetered Beagle Dogs

Time (min)	Systolic Arterial Blood Pressure (mmHg)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	161 ± 9	165 ± 9	164 ± 5	162 ± 7
5	180 ± 7	180 ± 11	165 ± 8	193 ± 11
15	183 ± 12	174 ± 9	173 ± 13	185 ± 11
30	172 ± 7	174 ± 9	164 ± 11	164 ± 7
45	170 ± 6	172 ± 12	166 ± 5	160 ± 14
60	155 ± 5	176 ± 12	161 ± 4	182 ± 10*
120	172 ± 9	172 ± 10	166 ± 6	167 ± 7
180	161 ± 4	164 ± 9	158 ± 7	181 ± 7*
270	151 ± 5	152 ± 3	156 ± 5	177 ± 5**
360	160 ± 1	161 ± 5	168 ± 7	177 ± 11

Table 2
The Effects of Vehicle and Fentanyl Citrate on Diastolic Arterial Blood Pressure in Conscious, Telemetered Beagle Dogs

Time (min)	Diastolic Arterial Blood Pressure (mmHg)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	98 ± 6	100 ± 6	96 ± 3	91 ± 4
5	110 ± 6	111 ± 6	97 ± 5	111 ± 9
15	111 ± 8	103 ± 5	99 ± 9	105 ± 7
30	103 ± 2	105 ± 7	94 ± 8	88 ± 4*
45	98 ± 2	105 ± 7	94 ± 3	89 ± 4
60	88 ± 3	107 ± 7	91 ± 3	103 ± 8
120	99 ± 4	104 ± 9	90 ± 4	93 ± 4
180	97 ± 1	99 ± 6	86 ± 2	105 ± 3
270	87 ± 2	93 ± 6	90 ± 2	94 ± 1
360	94 ± 4	101 ± 3	99 ± 4	109 ± 4

Lead II ECG: In consideration of the comments above concerning the heart rate analysis, the Sponsor's analysis by comparison with vehicle-treated controls was inappropriate. Predose values for the fentanyl dose groups (0.001, 0.01 and 0.05 mg/kg), had higher RR and PR intervals and QRS durations compared with the predose values obtained for vehicle-treated on the first experimental day.

RR Interval: In consideration of the comments above concerning the heart rate analysis, the Sponsor's analysis by comparison with vehicle-treated controls was inappropriate. In the 0.05 mg/kg fentanyl group, comparison to predose values indicated that the mean RR interval may have been reduced at 5 minutes, followed by an increased duration at 30 and 45 after fentanyl administration. For the RR interval, there were probably no changes in the 0.001 and 0.01 dose groups.

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Table 5
The Effects of Vehicle and Fentanyl Citrate on RR Interval in Conscious, Telemetered Beagle Dogs

Time (min)	RR Interval (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	764.4 ± 41.6	891.9 ± 94.0	932.9 ± 107.7	1033.3 ± 118.5
5	650.8 ± 23.9	693.0 ± 101.0	931.2 ± 36.0	766.7 ± 168.5
15	635.9 ± 103.1	759.3 ± 152.9	842.7 ± 124.1	1275.1 ± 240.1
30	791.0 ± 161.2	808.0 ± 120.1	908.2 ± 150.7	1448.1 ± 148.1*
45	795.9 ± 97.1	678.0 ± 85.2	999.2 ± 75.2	1313.2 ± 126.3*
60	862.7 ± 73.1	827.6 ± 85.0	990.7 ± 106.1	1069.6 ± 166.2
120	919.7 ± 146.3	905.4 ± 79.6	933.5 ± 106.7	758.8 ± 19.5
180	824.0 ± 35.2	987.6 ± 83.5	1140.2 ± 156.6	837.1 ± 82.3
270	856.1 ± 68.3	785.1 ± 101.5	1171.7 ± 88.9*	1001.3 ± 35.6
360	733.2 ± 59.0	680.8 ± 62.4	964.9 ± 28.2*	1106.9 ± 107.0**

PR Interval: In consideration of the comments above concerning the heart rate analysis, the Sponsor's analysis by comparison with vehicle-treated controls was inappropriate. There were no effects of fentanyl compared to predose values on the PR interval.

Table 6
The Effects of Vehicle and Fentanyl Citrate on PR Interval in Conscious, Telemetered Beagle Dogs

Time (min)	PR Interval (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	104.2 ± 1.9	111.4 ± 7.9	114.9 ± 9.7	116.1 ± 9.8
5	98.5 ± 4.5	106.0 ± 4.5	113.7 ± 5.2**	105.6 ± 7.3
15	100.5 ± 5.6	104.7 ± 8.6	113.9 ± 9.2	115.7 ± 7.3*
30	102.7 ± 11.5	100.6 ± 1.4	113.7 ± 6.2	116.9 ± 8.2
45	101.6 ± 3.1	111.5 ± 10.4	112.7 ± 6.6	113.9 ± 7.7
60	109.7 ± 8.3	109.6 ± 6.2	110.3 ± 5.0	107.5 ± 5.6
120	102.8 ± 5.9	104.4 ± 5.5	103.1 ± 2.8	105.6 ± 7.4
180	111.2 ± 5.6	115.2 ± 6.1	108.2 ± 5.1	103.9 ± 6.0
270	112.6 ± 9.6	111.0 ± 6.7	118.9 ± 10.3	108.7 ± 4.6
360	102.7 ± 6.1	113.7 ± 11.6	109.5 ± 4.5	107.5 ± 6.2

QRS duration: In consideration of the comments above concerning the heart rate analysis, the Sponsor's analysis by comparison with vehicle-treated controls was inappropriate. There were no effects of fentanyl compared to predose values on QRS interval.

Table 7
The Effects of Vehicle and Fentanyl Citrate on QRS Duration in Conscious, Telemetered Beagle Dogs

Time (min)	QRS Duration (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	41.4 ± 1.5	42.9 ± 2.2	43.7 ± 2.7	42.9 ± 1.7
5	40.7 ± 1.9	41.6 ± 2.0	42.5 ± 2.6	41.8 ± 2.8
15	40.1 ± 1.3	42.6 ± 2.1	44.6 ± 2.8	44.3 ± 3.7
30	42.0 ± 2.5	41.4 ± 1.7	45.8 ± 2.9*	45.0 ± 3.6
45	41.0 ± 1.7	42.8 ± 2.3	45.5 ± 3.0*	45.4 ± 2.9*
60	42.4 ± 1.9	42.5 ± 2.3	45.4 ± 3.1*	45.9 ± 2.8*
120	42.8 ± 2.0	42.1 ± 2.0	45.3 ± 3.2	47.9 ± 4.3
180	43.1 ± 1.6	43.1 ± 2.0	44.5 ± 2.5	46.8 ± 3.4
270	42.9 ± 2.2	43.7 ± 2.4	43.8 ± 2.1	44.9 ± 3.1
360	42.6 ± 1.9	42.4 ± 2.7	43.1 ± 2.3	44.5 ± 2.1

QT Interval: In consideration of the comments above concerning the heart rate analysis, the Sponsor's analysis by comparison with vehicle-treated controls was inappropriate. Compared to predose values, both the 0.01 and 0.05 mg/kg fentanyl groups were longer than the predose values of the vehicle-treated group. For the 0.05 mg/kg fentanyl group, there was an initial shorter uncorrected QT interval at five minutes postdose, but was prolonged at 30 minutes to 360 minutes. A similar pattern occurred for the 0.01 mg/kg dose, although the interval was not prolonged until 45 minutes postdose.

For the 0.05 mg/kg dose, both QTcF and QTcQ correction method indicated a shortened interval at 15 and 30 minutes and lengthened interval at ≥60 minutes postdose. There was probably no effect at the 0.01 mg/kg dose with the corrected method of calculation.

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Table 8
The Effects of Vehicle and Fentanyl Citrate on QT Interval in Conscious, Telemetered Beagle Dogs

Time (min)	QT Interval (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	220.0 ± 4.6	227.8 ± 6.4	238.8 ± 5.3	235.6 ± 7.2
5	208.1 ± 8.3	206.5 ± 6.9	224.8 ± 1.5	208.9 ± 14.9
15	200.1 ± 7.1	213.4 ± 9.3	232.4 ± 3.3*	240.4 ± 7.4**
30	213.2 ± 13.6	211.0 ± 11.7	240.3 ± 5.4	250.0 ± 6.5*
45	219.5 ± 8.3	215.3 ± 10.6	253.2 ± 6.0**	252.7 ± 7.7**
60	228.2 ± 6.1	217.8 ± 8.9	252.4 ± 3.9	250.1 ± 11.5
120	229.3 ± 6.3	226.4 ± 5.4	247.8 ± 3.8	244.8 ± 7.7
180	234.3 ± 8.7	243.5 ± 3.0	253.3 ± 0.8	247.1 ± 5.0
270	231.8 ± 7.9	237.4 ± 5.0	254.2 ± 1.8*	255.1 ± 8.8*
360	223.5 ± 9.3	219.1 ± 9.9	238.5 ± 3.5	258.7 ± 2.5**

Table 9
The Effects of Vehicle and Fentanyl Citrate on QTcF Interval in Conscious, Telemetered Beagle Dogs

Time (min)	QTcF Interval (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	241.1 ± 7.0	237.9 ± 5.9	246.0 ± 7.5	234.4 ± 3.9
5	240.1 ± 8.4	235.7 ± 4.9	230.4 ± 2.7	232.6 ± 5.7
15	236.6 ± 15.6	238.3 ± 12.8	249.0 ± 9.0	225.0 ± 5.8
30	233.3 ± 3.3	228.3 ± 6.8	251.5 ± 9.4	222.2 ± 6.3
45	238.9 ± 12.3	246.3 ± 4.6	254.4 ± 9.9	231.8 ± 7.1
60	240.7 ± 7.3	232.6 ± 3.5	255.5 ± 12.0	247.4 ± 10.0
120	240.0 ± 9.4	234.8 ± 1.6	256.0 ± 10.9	268.4 ± 8.4**
180	249.9 ± 7.3	245.7 ± 7.4	246.3 ± 13.8	264.1 ± 11.5
270	244.6 ± 2.1	259.8 ± 10.0	242.1 ± 6.9	255.0 ± 7.1
360	248.2 ± 5.0	249.4 ± 3.8	241.5 ± 4.1	251.4 ± 6.0

Table 10
The Effects of Vehicle and Fentanyl Citrate on QTcQ Interval in Conscious, Telemetered Beagle Dogs

Time (min)	QTcQ Interval (ms)			
	Vehicle 0.3 ml/kg	Fentanyl Citrate 0.001 mg/kg	Fentanyl Citrate 0.01 mg/kg	Fentanyl Citrate 0.05 mg/kg
-15	237.2 ± 4.5	236.3 ± 4.4	242.3 ± 6.8	233.1 ± 4.6
5	233.8 ± 6.5	229.9 ± 2.8	229.7 ± 1.8	227.2 ± 7.2
15	225.4 ± 9.3	231.2 ± 8.7	243.2 ± 5.6	222.3 ± 7.8
30	230.7 ± 2.6	225.6 ± 4.2	246.7 ± 7.9	217.6 ± 9.5
45	234.7 ± 8.8	239.9 ± 3.6	252.4 ± 9.1	229.8 ± 9.7
60	239.2 ± 7.0	231.3 ± 2.5	252.8 ± 9.0	244.9 ± 10.4
120	234.0 ± 3.4	233.1 ± 1.1	252.0 ± 7.2*	262.7 ± 6.9**
180	247.7 ± 7.1	243.8 ± 6.7	241.7 ± 11.3	258.9 ± 7.7
270	242.4 ± 3.1	253.1 ± 6.2	240.1 ± 8.4	255.1 ± 7.2
360	242.9 ± 5.3	243.1 ± 5.5	240.9 ± 3.8	249.9 ± 6.6

ECG waveforms: A table of results from the visual inspection for disturbances in rhythm and waveform morphology is presented below. There appears to be an increase in sinus pause of >2.5 s duration with higher fentanyl doses, although none were noted for dog 1803 at 0.05 mg/kg and dog 1669 had a high incidence of events at all doses of fentanyl. There was a small increase in the incidence of bradycardia at high fentanyl doses in 2 dogs. Three dogs, 1813, 1849 and 1803, had increased numbers of escape complexes at higher fentanyl doses, mostly between 11 and 33 minutes after 0.05 mg/kg fentanyl. The escape complexes were frequently associated with non-conducted P-waves that occurred immediately before, superimposed on or following these escape beats. There was an occasional idioventricular or idiojunctional rhythm present, with non-conducted P-waves interspersed between the escape complexes giving rise to periods of apparent third degree atrioventricular block and causing complete atrioventricular disassociation.

An increase in escape complex numbers may occur as a result of either a decrease in heart rate or an increase in escape focus excitability. All three animals had lower heart rates following administration of 0.05 mg/kg, in comparison to following vehicle administration. In addition, animals 1813 and 1849 showed evidence of an increase in their escape focus rate following fentanyl administration; this escape focus rate was noted to decrease slightly over time following 0.05 mg/kg fentanyl administration. Animals 1813 and 1849 also had prolonged sinus pauses, longer than their escape focus RR interval, that were not interrupted by escape beats after this period, suggesting a further reduction in escape focus excitability had occurred. The Sponsor concludes that these animals had an increase in escape focus excitability and hence rate during this period due to the effects of fentanyl. This increase in excitability and therefore rate may represent the direct effect of fentanyl at the escape focus site or may reflect an increase in sympathetic tone.

Summary of ECG Waveform Analysis (Incidences of Occurrence; Reviewer created table)

Characteristic	Dog #	Vehicle (Day 1)		0.001 mg/kg (Day 4)		0.01 mg/kg (Day 8)		0.05 mg/kg (Day 11)	
		predose	postdose*	predose	postdose	predose	postdose	predose	postdose
Sinus Pause > 2.5 s	1803		2		1	2	76		
	1669		56	9	96	9	106	23	106
	1849		3	1	2		39	3	23
	1813								15
Sinus Tachycardia	1803		1	+	+				
	1669					1		1	
	1849		2					1	
	1813		+	++	++	+			
Sinus Bradycardia	1803								
	1669						2	1	4
	1849							1	+
	1813								
PR Interval Prolongation	1803				+				
	1669		+		+	+		+	+
	1849							+	+
	1813		++	++	++	+			
T-wave Morphology Changes	1803				+				
	1669		+		+			+	+
	1849							+	+
	1813		++	++	++	+		+	+

* for vehicle treated dogs, there was no indication if the incidences occurred predose or postdose
 + indicates an occasional or noted observation (number of incidences were not provided)
 ++ indicates a frequent observation (number of incidences were not provided)

Summary of ECG Waveform Analysis continued (Incidences of Occurrence; Reviewer created table)

Characteristic	Dog #	Vehicle (Day 1)		0.001 mg/kg (Day 4)		0.01 mg/kg (Day 8)		0.05 mg/kg (Day 11)	
		predose	postdose*	predose	postdose	predose	postdose	predose	postdose
2° AV Block	1803								
	1669		10		10	1	14		9
	1849								
	1813		61	15	180	16	38	12	10
Supraventricular Premature Complexes	1803								
	1669		5		5	2			
	1849			1			3	3	9
	1813								
Ventricular Premature Depolarization	1803								
	1669								
	1849								
	1813								1
Ventricular Escape Complexes	1803						16		207
	1669		10		4		20		21
	1849						32		190
	1813								>200

* for vehicle treated dogs; there was no indication if the incidences occurred predose or postdose

PULMONARY EFFECTS**Study title: Effects of Fentanyl Citrate on Respiration Rate and Tidal Volume in Rats**

Key study findings: A subcutaneous dose of 0.3 mg/kg fentanyl citrate to the rat decreased respiration rate at 30 minutes and 5 hours postdose, and decreased tidal volume at 30 min postdose compared to the vehicle control group. These responses were similar to that of morphine. Lower doses of 0.003 and 0.03 mg/kg fentanyl produced no respiratory changes.

Study no.: DS-2005-015

eCTD: SN-000, Pharmtox, Pharmacology, Module 4.2.1.3.3

Conducting laboratory and location:

Date of study initiation: Sept 23, 2004

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Fentanyl citrate, Lot 067H1732, Purity: —

Morphine hydrochloride, batch 02-00805 —

Vehicle: Saline

Analysis of the stock preparations of the fentanyl indicated that they were within — of the expected concentrations. The vehicle contained no detectable fentanyl.

Methods

Male Sprague-Dawley rats (265-332 g; approximately 8-10 weeks old) were acclimatized to plethysmography chambers prior to the study day. Respiration rates and tidal volumes for each animal were recorded at approximately 15 min after entering the chamber. The tidal volumes obtained from the final acclimatization period were ranked and animals randomly allocated to treatment groups in such a manner that the mean baseline tidal volumes for each group were similar. Animals with inconsistent recordings or vocalizations were excluded from the study.

Data was obtained approximately 15 minutes after placement into plethysmography chambers, corresponding to predose of -10 minutes and then 0.5, 5 and 24 hours postdose. The treatments are listed in the table below (N=8/group). Each rat received a single subcutaneous dose of fentanyl or vehicle, by injection in the dorsal cervical region (dose volume = 1 ml/kg). Morphine was used as a reference control and administered intravenously (tail vein; 2 mL/kg).

Treatments

Compound	Dose	Route
Saline vehicle	1 ml/kg	SC
Fentanyl citrate	0.003 mg/kg	SC
Fentanyl citrate	0.03 mg/kg	SC
Fentanyl citrate	0.3 mg/kg	SC
Morphine	20 mg/kg	IV

Statistical analysis consisted of either parametric tests (ANOVA and Dunnett's test) or nonparametric tests (Kruskal-Wallis and Dunn's test), dependent on the homogeneity of variance criterion evaluated by the Levene Mean test. Since the positive control, morphine, was administered by different route, all morphine postdose data were compared to predose data by paired, Student's *t*-test

Results

Respiration Rate: A subcutaneous dose of 0.3 mg/kg fentanyl citrate produced a significant decrease in respiration rate at 30 minutes and 5 hours post-dosing compared to the vehicle control group. Administration of 0.003 and 0.03 mg/kg had no effects on respiration rate. Intravenous administration of the reference substance, morphine, caused a significant decrease in respiration rate at 30 min.

Tidal Volume: A subcutaneous dose of 0.3 mg/kg fentanyl citrate caused a significant decrease in tidal volume at 30 min post-dose when compared to vehicle. Administration of 0.003 and 0.03 mg/kg fentanyl citrate did not significantly affect the tidal volume. Morphine caused a significant decrease in tidal volume at 30 min post-dose.

Effects of Fentanyl Citrate on Respiration Rate and Tidal Volume in Rats (mean ± sem)

Drug (Dose)	Respiration Rate (breaths/min)				Tidal Volume (mL)			
	Pre-Dose	30 min	5 h	24 h	Pre-Dose	30 min	5 h	24 h
Vehicle (1 mL/kg, SC)	142.71 ± 8.75	123.42 ± 5.71	117.20 ± 6.25	140.58 ± 4.91	1.83 ± 0.06	1.80 ± 0.08	1.86 ± 0.08	1.90 ± 0.06
Fentanyl Citrate (0.003 mg/kg, SC)	128.94 ± 7.31	123.25 ± 7.55	120.03 ± 6.62	136.55 ± 10.82	1.92 ± 0.11	1.81 ± 0.09	1.97 ± 0.06	1.87 ± 0.14
Fentanyl Citrate (0.03 mg/kg, SC)	145.26 ± 15.66	99.22 ± 10.04	118.45 ± 5.99	133.04 ± 6.94	1.78 ± 0.11	1.56 ± 0.13	2.11 ± 0.11	2.01 ± 0.13
Fentanyl Citrate (0.3 mg/kg, SC)	121.18 ± 3.79	76.08 ± 4.48**	93.22 ± 3.09*	144.22 ± 8.51	1.96 ± 0.09	1.35 ± 0.13*	2.00 ± 0.09	1.67 ± 0.08
Morphine (20 mg/kg, IV)	136.02 ± 9.49	77.47 ± 10.10 [#]	117.01 ± 2.59	116.10 ± 3.76 [#]	1.87 ± 0.07	0.91 ± 0.06 ^{##}	1.68 ± 0.07 [#]	2.03 ± 0.12

* $P < 0.05$ and ** $P < 0.01$ when compared to vehicle group data (ANOVA and Dunnett's test)

[#] $P < 0.05$ and ^{##} $P < 0.001$ when compared to pre-dose values (paired, Student's *t*-test)

2.6.2.5 Pharmacodynamic drug interactions

Fentanyl citrate pharmacodynamic drug interactions were described in NDA 20-747. No additional drug interaction studies were conducted by the Sponsor for this NDA submission. A table indicating drug interactions (from scientific publications) with fentanyl, from the review of NDA 20-747, is reproduced below.

Drug Interactions

Selected examples from literature reports of drug interactions with fentanyl in animals are presented below. For references, see NDA 20-747, Vol. 9/54, pp. 5-19 through 5-129.

Species	Fentanyl Dose	Coadministered Drug	Test	Results
<i>Additive/Synergistic Interactions</i>				
Rat	15 µg/kg SC	Calcium channel blockers clonidine (100 µg/kg SC) and verapamil (10 µg/kg SC)	Tail flick, hot plate tests	Synergistic increase in analgesia
Rat	Variable with dexmedetomidine, 300 µg/kg IP with other drugs	A. α ₂ -adrenergic agonists dexmedetomidine (variable) or medetomidine (200, 300 µg/kg IP) B. α ₂ -adrenergic antagonist atipamezole (1 mg/kg IP) + µ-opioid antagonist nalbuphine (2 mg/kg IP) or κ-agonist butorphanol (0.4 mg/kg IP)	A. Righting reflex, isobolographic analysis B. Righting reflex, isobolographic analysis.	A. Synergistic potentiation B. Reversal of potentiation in 'A' above
Rat	20 µg/kg SC	A. N-type calcium channel blocker KB-2796 (1, 5, 15 mg/kg SC) B. L-type calcium channel agonist Bay K 8644 (0.25, 0.5, 1 mg/kg SC)	A. Tail flick B. Tail flick	A. Potentiation B. No effect
Rabbit	15 µg/kg IV	Midazolam (700 µg/kg IV)	Antinociception, respiratory depression	Enhanced antinociception, no increase in respiratory depression
Rabbit	ED50, 2xED50, ED90 IV	Tricyclic antidepressant imipramine (1, 2.1, 3.2 mg/kg IV)	Antinociception (tooth pulp stimulation)	Supra-additive effect, no increase in PaCO ₂
Dog	IV loading dose followed by constant infusion at 0.02, 0.05 mcg/kg/min	Midazolam (loading dose and infusion 2.4, 9.6, 28.8 µg/kg/min)	Enflurane MAC reduction	MAC reduction
Cat	IV	Non-depolarizing neuromuscular blocking agent: vecuronium derivative ORG 9426	Muscle relaxing effects, time course, recovery time	No effects
Lambs (newborn)	3 mg/kg IV	Pentobarbital (4 mg/kg IV)	Analgesia, peripheral & central hemodynamic effects	Potentiation of all effects
Sheep	5 µg/kg IV	(Dopaminergic) neuroleptic drugs droperidol (5 µg/kg IV) or zuclopenthixol (100 µg/kg IV)	Nociceptive threshold	Potentiation

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Species	Fentanyl Dose	Co-administered Drug	Test	Results
PK Interactions				
Rats & Rabbits	Variable	Liposomal (phosphatidylcholine and cholesterol carriers)	Thermomodulation, analgesia	Potentiation
Antagonists				
Rat (male Sprague-Dawley)	100 µg/kg IV	A. Prazosin (α1-adrenergic blocker, 50, 250 µg/kg IV) B. Yohimbine (α2-adrenergic blocker, 1, 15, 2.3 mg/kg IV)	Antagonism of muscular rigidity	A. Antagonized fentanyl-induced rigidity B. Potentiated fentanyl rigidity
Rat	25, 100 µg/kg IV	3-hydroxymethyl-8-carboline (3-HMC, benzodiazepine antagonist, 10 mg/kg IV)	Antagonism of fentanyl induced cerebral metabolic & respiratory depression, analgesia	Enhanced analgesia, antagonized hypnotic, respiratory depressant, cardiovascular effects
Dog	40 µg/kg IV	Atropine (antimuscarinic agent, 0.1 mg/kg IV)	Antagonism of fentanyl induced blood pressure, bradycardia	Antagonized bradycardia, no effect on blood pressure
Dog	0.4 mg/kg IM	Naloxone (0.01, 0.1, 1.0, 10 mg/kg IV) Doxapram (5 mg/kg IV)	Reversal of fentanyl-droperidol-pentobarbital anesthesia	Naloxone & doxapram antagonized anesthesia better than naloxone alone
Dog	50 µg/kg IV	Naloxone (0.4 mg IV)	Hemodynamic effects	Reversal of decr in MAP, heart rate, plasma norepinephrine & epinephrine, less so with induced hypocapnia

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2.6.3 PHARMACOLOGY TABULATED SUMMARY

PRIMARY PHARMACODYNAMICS

Fentanyl Citrate Analgesic Models, ED₅₀, and Plasma Concentrations

Species/ Reference	Nociceptive Model or Effect	Analgesic ED ₅₀ / Route (µg/kg, median dose or range)	Plasma Concentrations (ng/ml)	Comments
Mouse				
	Acetic acid-induced writhing	11.5		
	Tail Immersion	94		
	Phenylquinone-induced writhing	34		
	Formalin-induced hand paw pain	50		
IND 421 Attachment 6a-5	Tail clamp	80 SC (morphine = 15000 SC)		Analgesia: SC administration: onset 4 min peak 10-15 min duration 30 min
NDA 16-049 Exhibit (1.a.iii)-A- 134, Amend No. 1	Hot plate test			Fentanyl faster onset, faster time to peak response and shorter duration than meperidine or morphine
Rat				
NDA 16-049 Exhibit (1.a.iv)-1 Janssen et al 1963	Warm water tail withdrawal			SC fentanyl 269 times more potent than morphine Oral fentanyl 400 times more potent than morphine Fentanyl faster acting and shorter acting than morphine
Carlsson et al, 1982	Tail clamp	5-50 IV		Completely abolished pain response at 25 µg/kg
	Antibradykinin	8		
Rabbit				
NDA 16-619 Exhibit (1.a.iii)-9, Vol. 2 Zattoni and Giunta 1965	Tooth pulp stimulation, (trigeminal nerve stimulation)	11.35 IV		Prevented desynchronization of EEG, increased cortical potentials interpreted to reflect depression of the cortical activating system Analgesia duration 26 min
Dog				
	Not described in review		30	For maximal analgesia
	IV Anesthetic range	70-300	30-400	

Species/ Reference	Nociceptive Model or Effect	Analgesic ED ₅₀ / Route (µg/kg, median dose or range)	Plasma Concentrations (ng/ml)	Comments
Monkey, rhesus				
	Not described in review		43.4	For maximal analgesia
	Respiratory and Analgesic Effects		3	Minimal effective dose
Lamb, neonatal				
Yaster et al. 1987	Pain (not described in summary)	Cumulative doses up to 1000 µg/kg, IV 4400 µg/kg, IV	646 ± 95	Retained normal pain response 4 of 10 fully responsive to pain

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SAFETY PHARMACOLOGY

Neurological Safety

Report, Location	Species/ Strain	Doses	Key Findings
DS-2005-014 GLP study	Rat Sprague-Dawley N=6/group	Fentanyl citrate, SC, 0.003, 0.03, 0.3 mg/kg	<p>0.003 mg/kg: No behavioral or physiological changes</p> <p>0.03 mg/kg: Stereotypic behavior: cage licking</p> <p>0.3 mg/kg: Generalized CNS depression with complete recovery between 5 to 24 hours Altered neurological function: flattened position, extended rigid limbs, catalepsy, paralysis, ↓ grip strength, ↓ righting reflex, altered gait Altered sensorimotor response: ↑ ease of handling, ↓ touch response, ↓ fearfulness, ↓ pain response Altered autonomic functioning: exophthalmos, abnormal pupil response, ↑ salivation, ↓ pinna and corneal responses, ↓ body temperature Stereotypic behavior: compulsive licking during recovery</p>

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Cardiovascular Safety

Report, Location	Species/ Strain	Doses	Key Findings
DS-2005-013 GLP study	Dog, Beagle males N=4/group conscious, telemetry study	Fentanyl citrate, SC, 0.001, 0.01, 0.05 mg/kg	<p>0.001 mg/kg: No effect on heart rate, blood pressure, or EKG parameters</p> <p>0.01 mg/kg: Slight sedation and unsteady gait No effect on heart rate or blood pressure</p> <p>0.05 mg/kg: Generalized sedation, abnormal gait, ↓ respiration, cold extremities and pale gums/tongue ↓ heart rate 15 to 45 min postdose ↑ diastolic, systolic, and mean arterial blood pressure at 5, 15, 60, 180, 360 min postdose (maximum increases at 5 min postdose) ↓ RR interval at 5 min, ↑ at 30 and 45 min No effect on PR or QRS intervals</p> <p>↓ QTcF and QTcQ interval at 15 to 30 min postdosing (mean maximal decrease = -12 for QTcF and -17 for QTcQ) ↑ QTcF and QTcQ interval at 60 to 360 min postdosing (mean maximal increase = +34 ms for QTcF and +30 ms for QTcQ)</p> <p>Altered electrical conductivity of the heart ↑ incidences of sinus pauses of >2.5 s duration ↑ incidence of bradycardia ↑ incidence of escape complexes associated the decreased heart rate and increased escape focus excitability</p>

Results from previously reviewed studies of NDA 20-747 are presented in the following table:

Previous Findings of Cardiovascular Effects of Fentanyl

Study	Species	Dose	Findings
IND 421 Attachment 6a-5 Gardocki and Yelnosky, 1964	Dog, anesthetized	Up to 5 µg/kg, IV Fentanyl 10, 20, 40 µg/kg; IV Morphine 500, 1000, 2000 µg/kg IV 10 or 50 µg/kg intraarterial 200 µg/kg	Only slight effect on heart rate and blood pressure At equianalgesic doses, vasodepressor effect of fentanyl was less than that of morphine Bradycardia produced by fentanyl reversed by atropine No effect on femoral blood flow Decreased vascular resistance
IND 421 Attachment 6a-5 Gardocki and Yelnosky, 1964	Dog		Fentanyl caused vasodepression as does morphine Cabinoxamine maleate, an antihistamine blocks morphine induced vasodepressor effect, but not that caused by fentanyl
TRR-146 NDA 16-049 Exhibit (1.a.i)-16 Amendment No. 1	Dog blood	0.05, 0.1, 0.25, 0.5, 0.75 or 1.0 mL of 0.01% Fentanyl solution Added to 1.0 ml of dog blood	<i>In vitro</i> hemolysis study At 1:1 dilution (greatest amount of fentanyl added), there was 7% hemolysis (for comparison, Nembutal at 0.75 ml per ml of blood resulted in 6% hemolysis)
Blair et al., 1986	Dog		Fentanyl prolongs cardiac action potential duration by prolonging ventricular repolarization
Arndt et al 1984	Dog	2.5, 5, 20, 40, 100 µg/kg, IV; at 5 min intervals	30 ng/mL plasma conc (cumulative dose of 67.5 µg/kg over 20 min) produced maximum cardiovascular effects on sleep-like behavior, analgesia, decreased respiratory rate, cardiac output, heart rate and arterial oxygen tension, suggesting saturation of opiate receptors
McPerson and Traystman 1984	Dog, anesthetized	25 or 100 µg/kg, IV	Fentanyl did not alter lower or upper limits of cerebrovascular autoregulation No effects on hypoxia or hypercapnia-induced alterations in cerebral vascular responsiveness and blood flow
Carlsson et al 1982	Rat	25-400 µg/kg, IV	At analgesic dose of 25 µg/kg, there was no change in blood pressure Dose-related effects: up to at 100 µg/kg (maximal effect): 35% decrease in cerebral blood flow, 50% decrease in cerebral oxygen metabolism 25% had seizures after 200-400 µg/kg Effects blocked by naloxone
Yaster et al 1987	Sheep,	cumulative dose	Induced respiratory depression

	Neonatal lamb, mechanically ventilated	of 4.4 mg/kg, IV	Decreased renal blood flow Increased arterial carbon dioxide tension No effect on: cardiac output, heart rate mean arterial blood pressure there were regional, naloxone-reversible decreases in blood flow to the spinal cord, cerebellum, medulla, diencephalon and subcortical white matter.
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Pulmonary Safety

Study	Species	Dose	Findings
DS-2005-015 GLP study	Rat, Sprague-Dawley male (N=8/group)	Fentanyl citrate, SC, 0.003, 0.03, 0.3 mg/kg positive control: Morphine, IV, 20 mg/kg	Respiration Rate ≤0.03 mg/kg: No significant effect at 0.5, 5 or 24 hours post-dose 0.3 mg/kg: ↓ in respiration rate at 0.5 and 5 hours post-dose Tidal Volume ≤0.03 mg/kg: No significant effect at 0.5, 5 or 24 hours post-dose 0.3 mg/kg: ↓ in tidal volume at 0.5 hour post-dose Morphine: ↓ in respiration rate and tidal volume

Results from previously reviewed studies of NDA 20-747 are presented in the following table:

Previous Findings of Pulmonary Effects of Fentanyl

Study	Species	Dose	Findings
Carlsson et al 1982	Rat	25-400 µg/kg, IV	At analgesic dose of 25 µg/kg, there was no change in respiration; Apneic at 50 µg/kg
IND 421 Attach. 6a-5 Gardocki and Yelnosky, 1964	Dog	10-40 µg/kg, IV	Decreased respiratory minute volume (up to 98%), (dose- dependent), max effect at 1 min, recovery over 5 min Decreased respiratory rate Decreased tidal volume
Hug and Murphy 1979	Dog	³ H-fentanyl 10 µg/kg, IV	Fast equilibration between plasma and cerebrospinal fluid Close relationship between respiratory depression and CSF and plasma concentrations Repeated injections led to accumulation and increased ventilatory depression Increased end-tidal CO ₂ from 43 to 79 torr over 10 min, complete recovery at 180 min Reversed with controlled/assisted ventilation and with naloxone administration
Arndt et al 1984	Dog	2.5, 5, 20, 40, 100 µg/kg, IV; at 5 min intervals	30 ng/mL plasma conc (cumulative dose of 67.5 µg/kg over 20 min) produced maximum cardiovascular effects on sleep-like behavior, analgesia, decreased respiratory rate, cardiac output, heart rate and arterial oxygen tension, suggesting saturation of opiate receptors
Nussmeier et al 1991	Rhesus monkeys, adult males	2, 4, 16, 64, 128 µg/kg, IV; as sequential boluses, 10 min intervals	2 µg/kg: significant reduction in respiratory rate 4 µg/kg: onset of analgesia significant increase in PaCO ₂ plasma conc 2.7 ± 0.9 ng/mL 64 µg/kg: all became apneic mean arterial blood pressure decreased cardiac output decreased maximal analgesic effect

			plasma conc 43.4 ± 26 ng/mL Plasma conc of 40 ng/mL sufficient to produce maximum CV, respiratory and analgesic effects. Effects were similar to those previously reported in humans.
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RENAL SAFETY

Results from previously reviewed studies of NDA 20-747 are presented in the following table:

Previous Findings of Renal Effects of Fentanyl

Study	Species	Dose	Findings
Hunter et al 1980	Dog anesthetized	25 µg/kg, IV infusions over 10 min	Effects lasting 30 to 90 min post-infusion: decreased mean arterial blood pressure and heart rate decreased glomerular filtration rate decreased renal blood flow decreased urine volume increased renal vascular resistance

GASTROINTESTINAL SAFETY

Results from previously reviewed studies of NDA 20-747 are presented in the following table:

Previous Findings of Gastrointestinal Effects of Fentanyl

Study	Species	Dose	Findings
IND 421 Attachment 6a-5	mouse		Fentanyl has constipating effect
Gardocki and Yelnosky 1964			
Fasoulaki et al, 1986	Rats	15.6 µg/kg; IP; Once daily for 6 days	Increased SGPT (=ALT) No effect on SGOT (=AST) No liver necrosis
IND 421 Attachment 6a-5	Dog N=4	1.0, 2.5 mg/kg IM	Fentanyl devoid of emetic activity at doses in which morphine produce emesis
Gardocki and Yelnosky, 1964			

ABUSE LIABILITY

Summary of Dependence and Tolerance of Fentanyl

Study	Species	Dose Route	Findings
NDA 16-049 (from Pharmacologist review dated May 12, 1967) Also referred to in NDA 16-619 (from Pharmacologist review dated July 19, 1967)			
IND 487 Attachment 6a-13 Dependence study	dog	200 µg/kg, IP, twice daily	fentanyl did not block abstinence syndrome produced by morphine withdrawal in morphine-addicted dogs
BRR-161 — study) NDA 16-049 Exhibit (1.a.i)-20 Amendment No. 1 Dependence study	monkey	highest tolerable dose present in Innovar (this dose was not mentioned in the reviews); subcutaneous; twice daily for 2 weeks	Withdrawal symptoms after 3.0 mg/kg of morphine were suppressed with 0.04 mg/kg of fentanyl (1/75 the morphine dose), thus fentanyl was estimated to be 75 times as potent as morphine sulfate; Abstinence signs from fentanyl were very mild when abruptly withdrawn (the term "very mild" was not explained in the reviews)
NDA 20-747 (from Pharmacologist review dated Sept 24, 1998)			
Dependence study	guinea pig <i>in vitro</i> preparation s of ileal longitudinal muscle with myenteric plexus	Guinea pigs made tolerant to fentanyl (dosing not described in review)	Withdrawal contractures induced by naloxone added to the <i>in vitro</i> preparations
Dependence study	dog	1.5-100 µg/kg; IV; every 20 min for 200 min	Increased arterial blood pressure responses to somatic nerve stimulation at 90 min after drug withdrawal
Tolerance study	rat	40 µg/kg; IP; once every 2 days for 10 days	Hot water tail-flick assay Decreased analgesia with repeated administration, By day 10, similar response between fentanyl and control treated
Tolerance study	rat	0.01 mg/kg/h for 1 week; continuous infusion to induce tolerance	Examined cross tolerance in tail flick response Cross tolerance demonstrated for buprenorphine, but not for morphine, etorphine, methadone, meperidine or levorphanol
Tolerance study	dog	4 doses 30 min apart (dosing not described in review)	Hypotension following the first dose but little or no additional hypertensive effect after subsequent doses

OTHER

Previous Findings of Muscular Effects of Fentanyl

Study	Species	Dose	Findings
NDA 16-619 Exhibit (1.a.iii)-1, Vol. 5 Canellas et al 1965	Guinea pig		Fentanyl has spasmogenic effect on the sphincter of Oddi, blocked by droperidol
Lui et al 1989	Rat	25, 50, 100 µg/kg; IV	Fentanyl produces muscular rigidity (increase in EMG activity) in unanesthetized rats: Rigidity greater in gastrocnemius muscle than rectus abdominis Hypoxemia, respiratory acidosis and hypercarbia also occurred
IND 421 Attachment 6a-5 Gardocki and Yelnosky 1964	Cats	10-160 µg/kg; IV	Fentanyl produces muscular rigidity, blocked by succinylcholine (likely centrally acting) Fentanyl has no effect on contraction of tibialis anterior muscle in response to nerve stimulation, thus unlikely fentanyl affect neuromuscular transmission
IND 487 Attachment 6a-17	Dogs		Fentanyl + d-methorphan produces muscular rigidity
IND 487 Attachment 6a-7	Rabbits	IM injection	fentanyl produced slight or no intramuscular irritation

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2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The absorption, distribution, metabolism, excretion and pharmacokinetics of fentanyl citrate were described in NDA 20-747. No additional studies were conducted by the Sponsor. A summary of the data is described here.

Absorption:

OraVescent Fentanyl Citrate is an effervescent fentanyl buccal tablet designed for placement and retention within the oral cavity for a period sufficient to allow disintegration of the tablet. Effervescent disintegration is thought to enhance absorption of fentanyl across the oral mucosa to maximize absorption. Uptake from the oral cavity would minimize first-pass metabolism, and allow delivery of therapeutically effective plasma fentanyl concentrations with administration of lower fentanyl doses.

A study to verify this hypothesis used 6 mongrel dogs in which pharmacokinetic parameters were compared between fentanyl administered intravenously and through a special drug delivery cell to the buccal mucosa. The fentanyl buccal administered formulation was tested at pH 6.6, 7.2 and 7.7 and delivery was allowed for 60 minutes. Buccal delivered fentanyl was detected in plasma at 6 minutes with the T_{max} occurring within 10 minutes for all pH formulations after terminating drug administration. Buccal fentanyl absorption, bioavailability and permeability increased 3- to 5-fold with increased pH. Bioavailability ranged from approximately 20% (pH 6.6) to 57% (pH 7.7). Another study demonstrated that the absorption of buccal fentanyl entered the systemic circulation with no depot effect, adsorption, or buccal mucosa metabolism. The terminal elimination half-life was similar after intravenous (244 ± 68 min) and buccal (205 ± 89 to 196 ± 48 min) administration.

Studies in humans compared OraVescent® Fentanyl Citrate to Actiq® formulations. Approximately 25% of the total dose of fentanyl (Actiq®) was rapidly absorbed from the buccal mucosa and became systemically available. Approximately 67% of the swallowed fentanyl (75% of the total dose) undergoes hepatic and duodenal first pass metabolism. Approximately 33% of the swallowed dose was absorbed. Thus, for Actiq®, 50% bioavailability of fentanyl was divided equally between rapid transmucosal and slower GI absorption.

The results of initial pharmacokinetic studies during the development of an effervescent fentanyl formulation indicated that the positioning of the tablet either sublingually or buccally did not alter the PK parameters. The effervescent products demonstrated an increased rate of absorption compared to Actiq® ($\uparrow 52\%$ following sublingual placement and $\uparrow 60\%$ following buccal placement). Data from these studies is presented in Section 2.6.4.10, the Comparative TK section.

Distribution:

Fentanyl was widely distributed in the body following administration. In a study in rats, intravenously administered fentanyl was detected in brain, heart, and lung at equivalent

concentrations with plasma fentanyl at 1.5 minutes. By 120 minutes, muscle and fat had equivalent concentrations of fentanyl. Additionally, fentanyl was measured in liver, gastric contents, small intestine, and kidneys. Muscle and brain affinities for fentanyl were 4:1 and 5:1 respectively, and affinity of fat for fentanyl was approximately 35:1. The high affinity of fat for fentanyl suggested that fentanyl could accumulate with repeated administration, and be reintroduced into circulation over time. Fentanyl crossed the placenta in rabbits and sheep with resulting ratios of fetal:maternal plasma fentanyl levels of 0.3 in both species.

Fentanyl is rapidly distributed after intravenous administration in humans to the brain, lung, kidney, spleen and heart. The concentration-time curve in humans was best described by a three-compartment model, with a rapid distribution phase half-life of approximately 1.7 minutes, slow distribution phase half-life of 13 minutes, and terminal elimination half-life of 219 minutes. Fentanyl crossed the human placenta resulting in a fetal:maternal plasma level ratio of 0.8. The short duration of action of intravenous fentanyl (30-60 minutes) may be due to rapid redistribution into tissues and not due to metabolism.

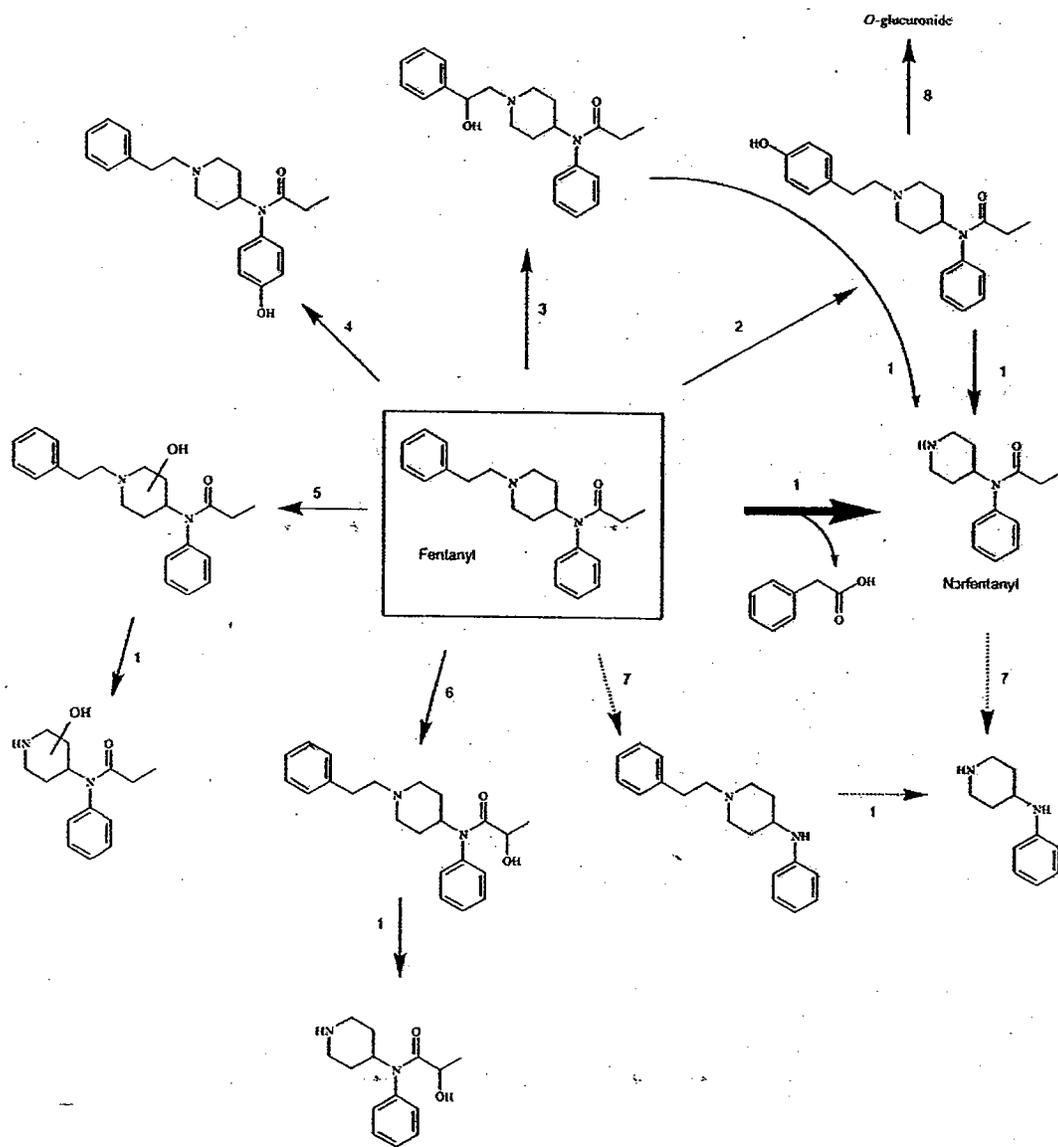
Fentanyl plasma protein binding is variable among species, representing approximately 60%-80% of unchanged drug in rabbits and dogs. In humans, 69-84% of fentanyl was protein bound with an average volume of distribution of 3-5 L/kg. In humans fentanyl binds predominantly to α -1-acid glycoprotein. Fentanyl crossed the blood-brain barrier and the placenta, and can be detected in breast milk. The total plasma clearance of fentanyl was 0.5 L/hr/kg (range: 0.3-0.7 L/hr/kg, or 10-20 ml/min/kg). The effective elimination half-life of fentanyl was about 2-4 hours, with a longer terminal elimination half-life of about 7 hours due to the slow elution from tissue sites.

Metabolism:

Fentanyl was metabolized primarily in the liver by oxidative N-dealkylation at the piperidine nitrogen, yielding phenylacetic acid and norfentanyl, and by aromatic and aliphatic hydroxylation via cytochrome P450 3A4. Fentanyl metabolism also occurred in mouse and rat kidney and the mouse adrenal gland. The major metabolites, phenylacetic acid and norfentanyl, and minor metabolites, including pharmacologically active p-hydroxy (phenethyl) fentanyl, can be detected in gastric contents, brain, heart, lung, liver, kidney, muscle and fat in rats. The major metabolic pathway for fentanyl in rats is hepatic oxidative N-dealkylation to norfentanyl and phenylacetic acid, and small amounts of pharmacologically active p-hydroxy-(phenethyl)fentanyl. Minor pathways in rats include amide hydrolysis, hydroxylation and conjugation. In humans, the primary metabolite is norfentanyl. Fentanyl metabolites lack significant analgesic activity (see table below).

Metabolic pathways of fentanyl in animals and man:

1: oxidative Ndealkylation, 2: aromatic para-phenyl hydroxylation, 3: aliphatic (α -phenylethyl oxidation, 4: aromatic aniline hydroxylation, 5: piperidine oxidation, 6: aliphatic (co-l)- propionyl oxidation, 7: amide hydrolysis (minor pathway, if present), 8: 0- glucuronidation.



Analgesic Activity of Fentanyl and Its Metabolites and other Opiates

Compound	Guinea-pig ileum bioassay IC ₅₀ (nM)	Hot-plate test (mouse) ED ₅₀ (mg/kg)	Tail-withdrawal test (rat) ED ₅₀ (mg/kg)
Fentanyl	4	0.015	0.017
Morphine	50	3.5	3.25
Meperidine	1,300	20.0	11
α-Hydroxy-fentanyl	50		
4-Hydroxy-phenethyl-fentanyl	240		1.4
Norfentanyl	3,800		inactive
Despropionyl-fentanyl	12,000		inactive
4-anilino-piperidin	120,000		

Elimination:

Fentanyl is excreted predominantly as metabolites in urine, with only 10% representing the unchanged drug, and to a lesser extent in feces, about 9% of the dose, primarily as metabolites. Neither the skin, nor oral mucosa metabolized fentanyl that was absorbed transdermally or through the oral mucosa. After intravenous administration in dogs, 32% [³H]-fentanyl was excreted in urine, with 4%-6% excreted as unchanged fentanyl, during the first 6 hours. After intravenous fentanyl administration in humans, less than 8% of the total dose was eliminated unchanged (6% in urine and 1% in feces). Approximately 75% intravenous dose was excreted in urine and 9% in feces as metabolites.

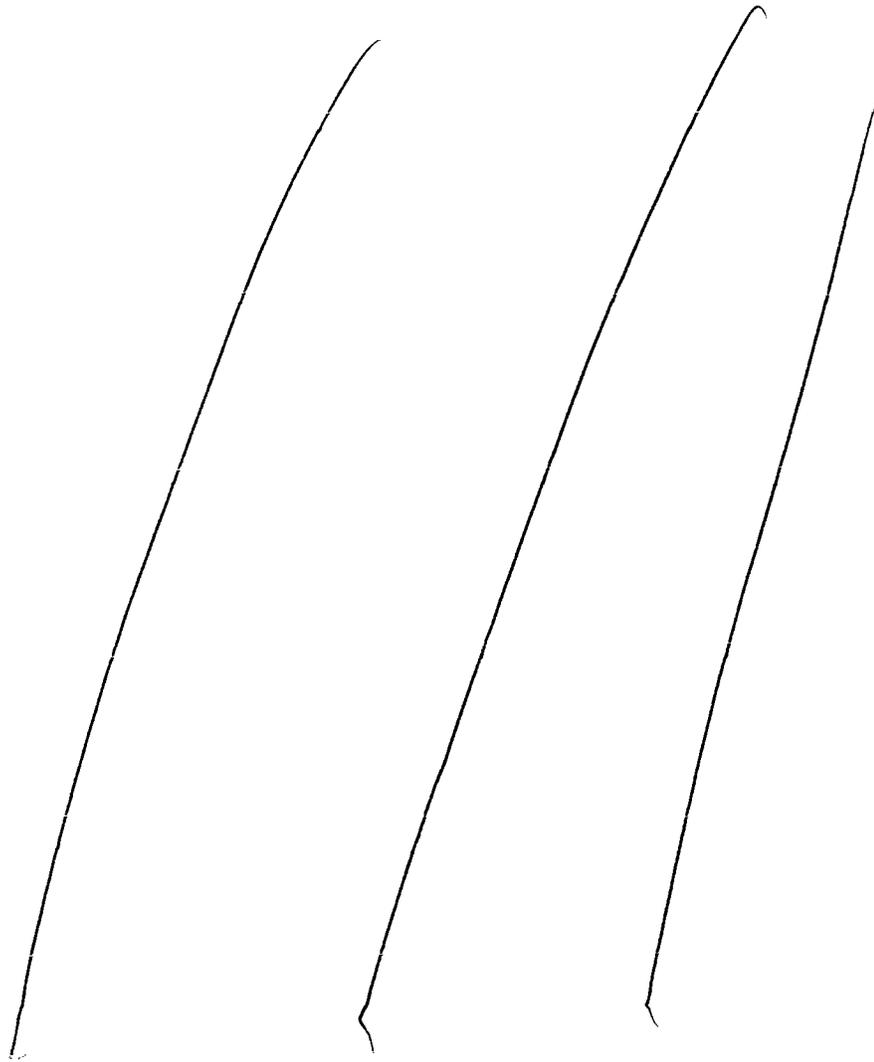
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2.6.4.10 Tables and figures to include comparative TK summary

During the development of this product, in IND 65,447, the _____ a maximal human dose of 800 µg or 13.3 µg/kg QID (3200 µg/day or 53.2 µg/kg/day). The exposure of 800 µg QID in humans had not been determined yet, but exposure had been shown to have a dose-dependent linear increase in doses of 100-800 µg. At that time, the TK data the Sponsor provided was an estimate based on dose-dependent linearity using data from a human study where 400 µg was taken QID for 5 days. This PK data was doubled to get the C_{max} and AUC_(0-24hr) for an 800 µg QID dose presented in the table below. Note that the actual PK values from study 1027 were substantially less than those obtained from extrapolation. Also in study 1029, repeated dosing at 6 hour intervals substantially increased both C_{max} and AUC. In actual use in opioid tolerant patients, a second dose could be administered 30 minutes after the first dose. These values were used to compare with repeated-dose range-finding studies in mice and rats for predicting safety margins for identifiable toxicities.

Safety Margins from TK Analysis of Rats and Mice Repeated-Dose Range-Finding Studies

Species	Dose	Day	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng-hr/mL)	Safety Margin	
					C _{max}	AUC ₀₋₂₄
Healthy volunteers (Study C25608/1029/PK/US)	800 µg qid for 5 days*	5	3.54 [#]	60.7 [#]		
Adult healthy men and women (Study 1027)	800 µg Single dose	1	1.59	7.38		
Label	800 µg		1.59	9.05 ^a		
Adult healthy men and women (Study 1028)	800 µg Single oral dose	1	0.984	4.87		
Adult healthy men and women (Study 1029)	400 µg Single dose day 1, then every 6 hours day 4 to 9	1	0.88	4.90		
		9	1.77	15.8		



HUMAN

Pharmacokinetic parameters obtained from studies to support the therapeutic doses in relation to the approved Actiq® dosing are presented in the two tables below. (Consult the Clinical Pharmacology review of NDA 20-747 for more detailed information). Study 1028 was conducted to assess the absolute and relative bioavailability of Oravescent fentanyl (OVF). The study compared bioavailability of single dose of 400 µg OVF by the proposed buccal administration against 800 µg OVF given orally, 800 µg Actiq® given by the transmucosal route and 400 µg of fentanyl given intravenously. Results of this study (Table 1 below) suggested that the fraction of

the OVF dose absorbed transmucosally is approximately 50% of the total dose (f_{TM}=0.48), and the fraction of the Actiq® dose absorbed transmucosally is approximately 25% of the total dose (f_{TM}=0.22). As a result, OVF demonstrated a higher absolute bioavailability (FOVF=0.65) when compared with Actiq® (FACTIQ=0.47). Dose normalization to equal doses (400 µg) of effervescent fentanyl demonstrated higher exposure compared with Actiq® (as measured by C_{max}, AUC_{0-∞}, and AUC_{0-tmax}).

Table 1: Mean (+/- SD) Pharmacokinetic Parameters of Fentanyl in Healthy Volunteers (N= 26) Administered a Single Dose of 400 µg OVF or 800 µg Actiq® Transmucosally, 400 µg Fentanyl Intravenously, or 800 µg OVF Orally

Parameter	400 OVF	400 IV	800 OVF (po)	800 ACTIQ
C _{max} (ng/mL)	1.020 ± 0.424	3.000 ± 1.112	0.984 ± 0.542	1.257 ± 0.414
t _{max} (hr) ^a	0.78 [0.33-4.00]	0.17 [0.08-0.75]	1.50 [0.68-4.00]	1.51 [0.58-4.00]
AUC _{0-tmax} (ng·hr/mL)	0.398 ± 0.178	1.43 ± 0.39	0.110 ± 0.136	0.280 ± 0.101
AUC ₀₋₂₄ (ng·hr/mL)	5.00 ± 1.74	7.79 ± 1.95	4.87 ± 3.01	7.51 ± 2.57
AUC ₀₋₄ (ng·hr/mL)	5.52 ± 2.43	9.01 ± 2.79	5.52 ± 4.15	8.47 ± 3.73
AUC ₀₋₃₂ (ng·hr/mL)	5.79 ± 2.50	9.31 ± 2.76	5.76 ± 4.15	8.79 ± 3.69
AUC _{0-∞} (ng·hr/mL)	6.48 ± 2.98	10.29 ± 2.88	6.60 ± 4.47	9.58 ± 3.91
λ _z (hr ⁻¹)	0.0568 ± 0.0364	0.0411 ± 0.0153	0.0703 ± 0.0527	0.0438 ± 0.0195
t _{1/2} (hr) ^b	12.2	16.9	9.87	15.8
CL or CL/F (L/hr)	77.0 ± 42.8	41.7 ± 11.3	174 ± 108	95.0 ± 31.7
V _z or V _d /F (L)	1481 ± 493	1102 ± 332	2696 ± 769	2345 ± 780
AUC Extrap. (%)	13.2 ± 4.9	11.5 ± 5.3	13.5 ± 6.7	12.5 ± 5.5
F _{ORAL}	NA	NA	0.311 ± 0.131	NA
F _{OVF}	0.648 ± 0.200	NA	NA	NA
F _{ACTIQ}	NA	NA	NA	0.465 ± 0.105
f _{TM}	0.477 ± 0.318	NA	NA	0.224 ± 0.173
f _O	0.523 ± 0.318	NA	NA	0.776 ± 0.173
F _{OVF/ACT}	1.34 ± 0.39	NA	NA	NA
F _{OVF/po}	2.32 ± 1.07	NA	NA	NA
F _{ACT/po}	NA	NA	NA	1.80 ± 0.75

^a Median [range]; ^b Harmonic mean; NA = Not Applicable.

F_{OVF/ACT}, F_{OVF/po}, F_{ACT/po} = Relative bioavailability of OVF to ACTIQ, OVF to oral OVF, and ACTIQ to oral OVF, respectively.

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Summary of Single Dose Pharmacokinetics from Dose-Proportionality Study 1027

Variable	ORAVESCENT fentanyl			
	100 mcg (N=31)	200 mcg (N=31)	400 mcg (N=31)	800 mcg (N=31)
AUC _{0-inf} ^a (ng hr/mL)	0.98±0.37	2.11±1.13	4.72±1.95	9.05±3.72
AUC _{0-t} (ng hr/ml)	0.80±0.26	1.39±0.46	2.90±0.92	5.27±1.85
AUC ₀₋₂₄ (ng hr/ml)	0.96±0.41	1.85±0.80	3.98±1.37	7.38±2.71
AUC ₀₋₇₂ (ng hr/ml)	0.99±0.46	1.93±0.90	4.39±1.80	8.39±3.59
AUC _{0-max} (ng hr/ml)	0.09±0.06	0.13±0.09	0.34±0.23	0.52±0.38
AUC _{0-t} (ng hr/ml)	0.91±0.42	1.79±0.82	4.17±1.72	8.11±3.63
C _{max} (ng/mL)	0.25±0.14	0.40±0.18	0.97±0.53	1.59±0.90
t _{max} ^b (min)	45.0 (25.0, 181.0)	40.0 (20.0, 180.0)	35.0 (20.0, 180.0)	40.0 (25.0, 180.0)
t _{1/2} ^{a,b} (hr)	2.63 (1.47, 13.57)	4.43 (1.85, 20.76)	11.09 (3.44, 20.59)	11.70 (4.63, 28.63)

^a Not all subjects' data were extrapolated. For 100 mcg, n=25; for 200 mcg, n=27; for 400 mcg, n=29; and for 800 mcg, n=30.

^b Median (range) is presented for these variables. Mean ± SD is presented for all other variables.

AUC_{0-t} = AUC from time zero to the last time point at which at least 75% of the subjects within all dose groups had a measurable plasma concentration.

The following table is the sponsor's summary of the first experiment PK parameters:

Table 8-3: PK Data Generated by CIMA's Fentanyl Protocol 099-08

Means and Ratios of LS Means	AUC (0-t) (ng/mL)*hr (N=12)	AUC (0-inf) (ng/mL)*hr (N=12)	C _{max} ng/mL (N=12)	T _{1/2} Hr (N=12)	T _{max} Hr (N=12)
A: OraVescent [®] Fentanyl Citrate Buccal	2.656	3.037	0.6412	3.94	0.501
B: NE [*] Buccal	2.041	2.672	0.3986	3.44	2.0
C: Actiq [®]	1.809	2.116	0.4073	2.77	2.0
A/B (90% CI)	135.0 (114.3-159.3)	129.4 (103.2-162.2)	152.0 (131.3-175.8)	-	-
A/C (90% CI)	159.5 (135.1-188.2)	153.0 (122.6-190.8)	155.6 (134.5-180.0)	-	-
B/C (90% CI)	118.1 (100.1-139.5)	118.2 (93.9-148.8)	102.4 (82.5-118.4)	-	-

* NE = non-extended release.

Fentanyl Protocol 099-08 (Project 26609). This study also compared the effects of placement of the lozenge on PK parameters. A total of 24 healthy study participants were administered 200 µg of fentanyl in the form of OraVescent® sublingually, OraVescent® buccally, or the Actiq® formulation buccally.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

No new toxicology studies were submitted for the current NDA. The toxicity of fentanyl is fairly well characterized. Based upon extensive human experience with this drug, additional repeat-dose toxicology studies were not required for this 505(b)(2) submission. During early meetings, the Sponsor indicated "to the best of their knowledge, no suitable model for examining the tolerability and toxicity of repeated administration via the oral transmucosal route has been developed." In animal studies, doses were usually administered by subcutaneous administration to attempt to mimic transmucosal absorption while avoiding first pass liver metabolism.

General Toxicology, Single Dose Studies:

NDA 20-747 for Actiq® did not contain single-dose toxicology data, but relied upon findings of safety in NDA 20-195 for Oralet, and published literature. The Oralet NDA also lacked single-dose toxicology data, and relied on prior FDA findings. The FDA's prior findings of safety were based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection. Those studies are summarized below:

Intravenous doses of 0, 10 and 20 mg/kg were administered to mice. The LD₅₀ value, calculated from survival data 14 days after intravenous injection, was 12 mg/kg. Intravenous injections of fentanyl were lethal for 1 out of 5 mice at 10 mg/kg and for all five mice at 20 mg/kg. Mortality occurred within the first hour after dosing. The fentanyl mortality dose response curve in mice was biphasic following both subcutaneous and intravenous dosing. The mechanism for this biphasic response is not known, but the mortality observed at the low end of the dose-response curve was considered associated with respiratory depression. Fentanyl produced some central opioid-like actions, e.g. excitation, exophthalmos, Straub tail on arched back, corneal opacity, dyspnea, loss of righting reflex, hypertonia and spasms. Mice also exhibited decreased activity or decreased activity followed by increased activity. All surviving mice completely recovered within 6 hours after injection.

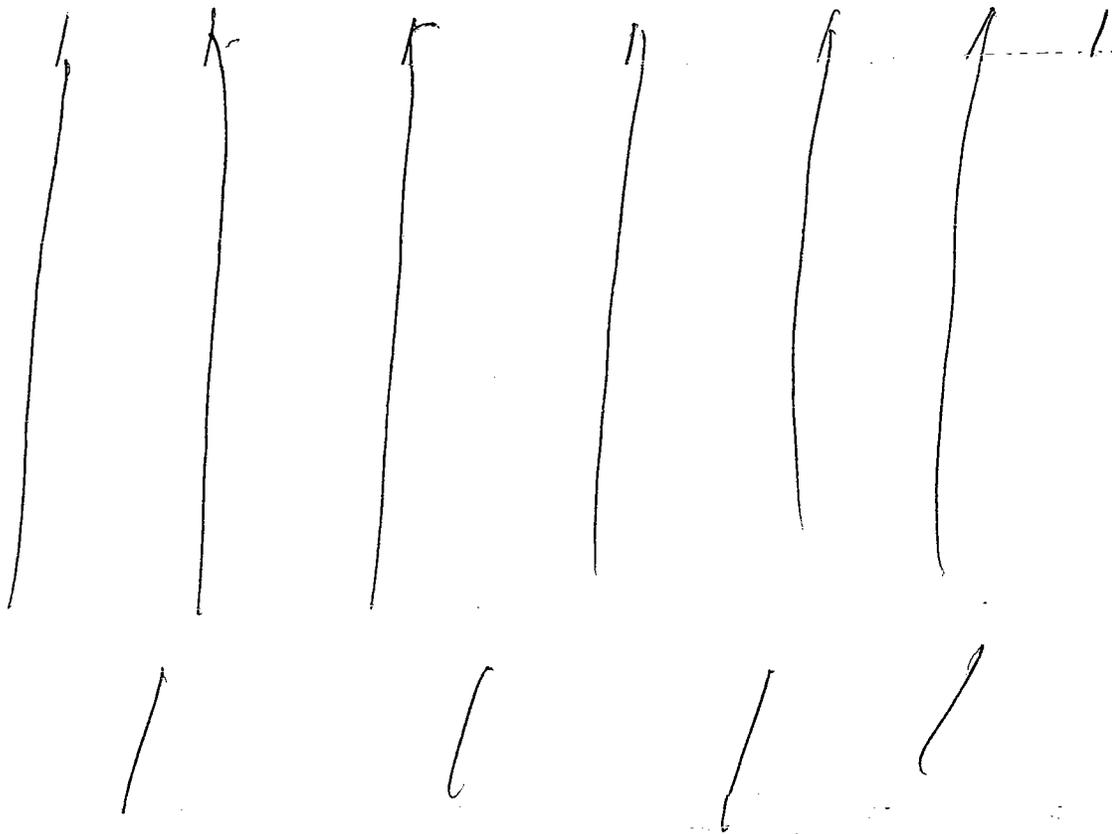
Intravenous doses of 0, 1.25, 2.5 and 5 mg/kg were administered to rats. The LD₅₀ value, calculated from survival data 14 days after intravenous injection, was 2.3 mg/kg. In rats, no mortality occurred at a dosage of 1.25 mg/kg. At a dose of 2.5 mg/kg, three out of five rats died immediately after dosing. At a dose of 5 mg/kg, all rats died immediately after administration of fentanyl. Apart from mortality, the following clinical observations were noted at all doses: blockade of cornea and pinna reflexes, dyspnea, loss of righting reflex, muscular rigidity, hypertonia, exophthalmos and salivation. Rats also exhibited decreased activity or decreased activity followed by increased activity. The surviving rats recovered within 1 day after dosing. No abnormalities were found at autopsy in mice or rats.

Fentanyl was tested in dogs by subcutaneous (SC), intramuscular (IM), intra-arterial (IA) and intragastric (IG) administration. Clinical responses were of central nervous system nature, i.e. convulsions, tremors, loss of righting reflex, sedation and respiratory depression in dogs.

In a study of the potential manufacturing impurity or degradant, both fentanyl and had similar pharmacodynamic and toxicity profiles in rats and mice.

General Toxicology, Repeated-Dose Studies:

The Pharmacology-Toxicology review of NDA 20-747 stated, that at the time of its submission (Nov 1996) there were no long-term studies to assess fentanyl subchronic and chronic toxicity. The NDA 20-747 for Actiq® did not contain repeat-dose toxicology data, but relied upon findings of safety in NDA 20-195 for Oralet, and published literature. The Oralet NDA also lacks repeat-dose toxicology data, and relies on prior FDA findings. The FDA's prior findings of safety are based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection.



Genetic toxicology: *In vitro* bacterial mutagenicity and *in vitro* clastogenicity studies were conducted on three impurities or degradants from fentanyl citrate supplied by

— The three compounds were:

The three impurities listed above were identified by the Agency as containing a structural alert for mutagenicity. All three impurities or degradants at concentrations of up to 5000 µg/plate were negative for induction of mutations in *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation. These impurities did not induce structural or numerical chromosome aberrations in the *in vitro* cytogenetic assay of human peripheral blood lymphocytes in the presence or absence of metabolic activation at concentrations that produced up to 50% cytotoxicity.

Two additional impurities or degradants that do not contain the structural alert for mutagenicity are monitored as specified impurities. One of the two compounds, _____ had pharmacodynamic and toxicologic profile similar to fentanyl in acute studies in rats and mice. The pharmacologic and toxicity of the other four compounds were not examined in this or previous NDAs.

Carcinogenicity: Long-term studies in animals to assess the carcinogenic potential of fentanyl have not been completed.

Reproductive toxicology: Limited data describing the developmental and reproductive toxicity of fentanyl have been provided by the Sponsor. The current drug label lacks data describing the potential effect of fentanyl on male fertility, teratogenicity in a second species, and peri- and post-natal development.

Special toxicology: Local irritation and sensitization studies were not conducted in animals because the Sponsor was unable to obtain a suitable animal model for oral transmucosal delivery of fentanyl. Local irritant effects and sensitization were to be evaluated in the clinical studies.

2.6.6.2 Single-dose toxicity

Single-dose (acute) toxicity studies with fentanyl citrate were described in NDA 20-747. No additional single-dose toxicity studies were conducted for this submission. NDA 20-747 for Actiq® does not contain single-dose toxicology data, but relies upon findings of safety in NDA 20-195 for Oralet and published literature. The Oralet NDA also lacks single-dose toxicology data, and relies on prior FDA findings. The FDA's prior findings of safety are based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection. These studies are summarized in the table below:

Studies in Mice:

Doses of 0, 10 and 20 mg/kg were administered to mice. The LD₅₀ value, calculated 14 days after the intravenous injection, was 12 mg/kg. Intravenous injections of fentanyl were lethal for 1 out of 5 mice at 10 mg/kg and for all five mice at 20 mg/kg. Mortality occurred within the first hour after dosing. The fentanyl mortality dose response curve in mice was biphasic following both

subcutaneous and intravenous dosing. The mechanism for this biphasic response is not known, but the mortality observed at the low end of the dose-response curve was considered to be associated with respiratory depression. Fentanyl produced some central opioid-like actions, e.g. excitation, exophthalmos, Straub tail on arched back, corneal opacity, dyspnea, loss of righting reflex, hypertonia and spasms. Mice also exhibited decreased activity or decreased activity followed by increased activity. All surviving mice completely recovered within 6 hours after injection.

Studies in Rats:

Doses of 1.25, 5, and 10 mg/kg fentanyl were administered via the intravenous route to rats. The LD₅₀ values, calculated 14 days after the intravenous injection was 2.3 mg/kg. In rats, no mortality occurred at a dosage of 1.25 mg/kg. At a dose of 2.5 mg/kg, three out of five rats died immediately after dosing. At a dose of 5 mg/kg, all rats died immediately after administration of fentanyl. Apart from mortality, the following clinical observations were noted at all doses: blockade of cornea and pinna reflexes, dyspnea, loss of righting reflex, muscular rigidity, hypertonia, exophthalmos and salivation. Rats also exhibited decreased activity or decreased activity followed by increased activity. The surviving rats recovered within 1 day after dosing. No abnormalities were found at autopsy in mice or rats.

Studies in Dogs:

Fentanyl was tested in dogs (SC), intramuscular (IM), intra-arterial (IA) and intragastric (IG) administration. Clinical responses consistent with central nervous system toxicity, included convulsions, tremors, loss of righting reflex, sedation and respiratory depression.

Summary of Single Dose Toxicity of Fentanyl

Study	Species and Number of Animals	Dose Route	Findings
NDA 16-049 (from Pharmacologist review dated May 12, 1967)			
TRR 143 Exhibit (I.a.i)-18 Amendment No. 1	Dogs n=4	0.3 mg/kg Intra-arterial	Convulsion (n=1) ↓ activity Bradycardia Loss of righting reflex Recovery within 24 hours
TRR 104	Newborn rat pups n=9/dose	4, 8, 16, 32, 75, 100 mg/kg intragastric	LD ₅₀ = 20 mg/kg (95% confidence interval = 14-29 mg/kg) ↓ weight of survivors (dose related)
NDA 16-619 (from pharmacologist review dated July 19, 1967)			
TRR-143			See NDA 16-049
TRR-18	rabbit n=4	0.05 mg; intramuscular (0.5 ml of 0.1 mg/ml)	None to slight irritation at injection site after 72 hours
BRR-172	monkey n=5	intravenous, dose not provided in review	Marked analgesia and apnea produced, Reversed within 2 minutes by intravenous Nalline, (nalorphine) in 4 monkeys, and intramuscular Nalline in one monkey

LD₅₀ (mg/kg) base (from NDA 16-619)

Species	Route of Administration				
	IV	IM	SC	PO	IG
Mouse	7.1		39.5	120	
Rat	2.3	1	9.5	18	
Dog	14	30	1.2		
Hamster		8			
Guinea pig	3*	65			
Cat	1				
Monkey	.03				
Newborn rat pup					20

* LD₁₀₀
 from NDA 16-619, Exhibit (1.a.i)-1, Vol. 2

Single-dose studies of impurities or degradants:

The Chemistry review (7/5/91) of NDA 16-619, Suppl 21, (submitted 9/27/88), and the PharmTox review (3/21/96) of Supplement 30 (Annual Report, submitted 4/20/95), both included review of 4 nonclinical reports of _____, and fentanyl (identified as R 4263) conducted by Janssen Research Foundation, Beerse, Belgium, in association with changes in manufacturing.

Both fentanyl and _____ had similar pharmacodynamic and toxicity profiles in rats and mice. A summary table of the findings is included below.

Structure of _____

Comparison of Acute Toxicity of Fentanyl (R 4263) and _____ in Rats

Study	Species	Dose	Findings
100503 100506	Rat, Wistar; adult males; n=5/dose	0, 1.25, 2.5, 5 mg/kg; intravenous; single dose; monitor for 14 days	Toxicity of _____ See Table 4 below Opiate-like effects: blockade of cornea and pinna reflexes, dyspnea loss of righting reflex muscular rigidity hypertonia exophthalmos salivation all surviving animals recovered completely within 1 day deaths occurred immediately after injection (2.5 mg/kg: n=3; 5 mg/kg: n=5) no gross pathological abnormalities were observed LD ₅₀ mean (95% confidence interval) Fentanyl (R 4263) = 2.3 (1.7-3.2) mg/kg = 3.1 (2.1-4.6) mg/kg

Rat toxicity studies of R 4263 (fentanyl) and _____

Table 4: Comparative data for the 2 compounds: incidence of behavioral abnormalities

Behavioral abnormalities	Compound	Test dose (mg/kg, iv)			ED ₅₀ (95% C.I.) (mg/kg, iv)
		1.25	2.5	5.0	
Blockade pinna reflex	R 4263	5	5	5	< 1.25
		5	5	5	< 1.25
Blockade cornea reflex	R 4263	5	5	5	< 1.25
		5	5	5	< 1.25
Exophthalmos	R 4263	5	0	0	< 1.25
		3	0	0	≤ 1.25
Muscular hypertonia or rigidity	R 4263	5	5	5	< 1.25
		5	5	5	< 1.25
Loss of righting reflex	R 4263	5	5	5	< 1.25
		5	5	5	< 1.25
Dyspnea	R 4263	5	3	5	< 1.25
		0	3	5	2.3 (1.7 - 3.2)
Salivation	R 4263	1	2	0	-
		0	1	0	-
Mortality	R 4263	0	3	5	2.3 (1.7 - 3.2)
		0	2	4	3.1 (2.1 - 4.6)

Study	Title
100503	A comparison of the acute IV toxicity of the narcotic analgesic fentanyl (R 4263) and its potential impurity _____ in male rats. A. The toxicity of fentanyl (R 4263)
100506	A comparison of the acute IV toxicity of the narcotic analgesic fentanyl (R 4263) and its potential impurity _____ in male rats. B. The toxicity of _____

Comparison of Acute Toxicity of Fentanyl (R 4263) and _____ in Mice

Study	Species	Dose	Findings
100504 100505	Mouse, Swiss; adult males; n=5/dose	0, 10, 20 mg/kg; intravenous; single dose; monitored for 14 days	Toxicity of _____ See Table 4 below Opiate-like effects: Excitation exophthalmos Straub tail on arched back dyspnea loss of righting reflex hypertonia (only with fentanyl) spasms (only with fentanyl) corneal opacity all surviving animals recovered completely within 6 hrs deaths occurred immediately after injection (20 mg/kg: n=4) no gross pathological abnormalities were observed LD ₅₀ mean (95% confidence interval) Fentanyl (R 4263) = 12 (9.1-17) mg/kg _____ = 16 (12-22) mg/kg

Mouse toxicity studies of R 4263 (fentanyl) and

Table 4: Comparative data for the 2 compounds: incidence of behavioral abnormalities

Behavioral abnormalities	Compound	Test dose (mg/kg, iv)		ED ₅₀ (95% C.L.) (mg/kg, iv)
		10	20	
Corneal opacity	R 4263	3	0	≤ 10
		4	1	< 10
Dyspnea	R 4263	1	5	12 (9.1 - 17)
		0	5	14 (11 - 18)
Excitation	R 4263	4	0	< 10
		5	5	< 10
Exophthalmos	R 4263	5	0	< 10
		5	5	< 10
Hypertonia	R 4263	4	0	< 10
		0	0	> 20
Loss of righting reflex	R 4263	5	5	< 10
		0	5	14 (11 - 18)
Arched back	R 4263	4	0	< 10
		5	1	< 10
Straub phenomenon	R 4263	4	0	< 10
		5	1	< 10
Spasms	R 4263	2	0	-
		0	0	-
Mortality	R 4263	1	5	12 (9.1 - 17)
		0	4	16 (12 - 22)

Study	Title
100504	A comparison of the acute IV toxicity of the narcotic analgesic fentanyl (R 4263) and its potential impurity in male mice. B. The toxicity of
100505	A comparison of the acute IV toxicity of the narcotic analgesic fentanyl (R4263) and its potential impurity in male mice. A. The toxicity of fentanyl (R 4263)

2.6.6.3 Repeat-dose toxicity

Repeat-dose toxicity studies with fentanyl citrate were described in NDA 20-747. No additional repeat-dose toxicity studies were conducted.

The PharmTox review of NDA 20-747 stated, that at the time of its submission (Nov 1996) there were no long-term studies to assess fentanyl subchronic and chronic toxicity. The NDA 20-747 for Actiq® does not contain repeat-dose toxicology data, but relies upon findings of safety in NDA 20-195 for Oralet and published literature. The Oralet NDA also lacks repeat-dose toxicology data, and relies on prior FDA findings. The FDA's prior findings of safety are based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection. These studies are summarized in the table below.

Summary of Repeat Dose Toxicity of Fentanyl

Study	Species and Number of Animals	Dose Route	Findings
NDA 16-049 (from Pharmacologist review dated May 12, 1967)			
TRR 9 Also in IND 487 Attachment 6a-4	Rats, n=30/dose	0, 0.1, 0.4 mg/kg; intramuscular once daily for 30 days	↑ Deaths (dose related) 0.4 mg/kg: 27% (7 males, 1 female) 0.1 mg/kg: 13% (4 males, 0 female) ↓ Body weight (dose related) Hematology: normal Organ weight and histology: normal
TRR 126 Also in NDA 16-049 Exhibit (I.a.i)-10 Amendment No. 1	Rats; n=20/dose; males and females	0, 0.01, 0.02, 0.03, 0.05, 0.075, 0.10 mg/kg; intravenous; once daily for 30 days	Dose-related increase in deaths (0.02 mg/kg = 10%, 0.10 mg/kg = 83%) ≥0.02 ↑ SGOT (=AST) No gross abnormalities among survivors (Cardiac lesion not dose related, thought to be due to bleeding by cardiac puncture)
TRR 9 Also in IND 487 Attachment 6a-14	Dogs, n=10 control n=12 fentanyl	0, 0.1, 0.4 mg/kg intramuscular once daily for 30 days	↓ Body weight (dose related) Hematology: normal Organ weight and histology: normal
TRR 108 Also in IND 487 Attachment 6a-14	Dogs n=3/sex/dose	0, 0.1, 0.3, 1.0 mg/kg; intravenous; once daily for 30 days	No deaths Dose related convulsions (incidence not stated) Sedation ↓ Body weight at 1.0 mg/kg ↓ spleen and gonad weight (dose related) Gross necropsy: normal Histopathology, 1.0 mg/kg: liver: possibly mild cholestasis kidney: few cast in lumen of collecting tubules of and vacuolar alteration
NDA 16-619 (from pharmacologist review dated July 19, 1967)			
BRR-130 Also in IND 487 Attachment 6a-3	Rats n=2/sex/dose	5, 10, 20, 40, 80, 160, 320, mg/kg/day; Oral (mixed with food); once daily for 14 days	Deaths at dose ≥10 mg/kg/day Body weight loss ≥20 mg/kg/day Surviving rats at 40 and 160 mg/kg/day had blood around mouth, bloody urine, and bloody diarrhea during the first week
TRR-9			See NDA 16-049, above
TRR-108			See NDA 16-049, above

5 Page(s) Withheld

Trade Secret / Confidential

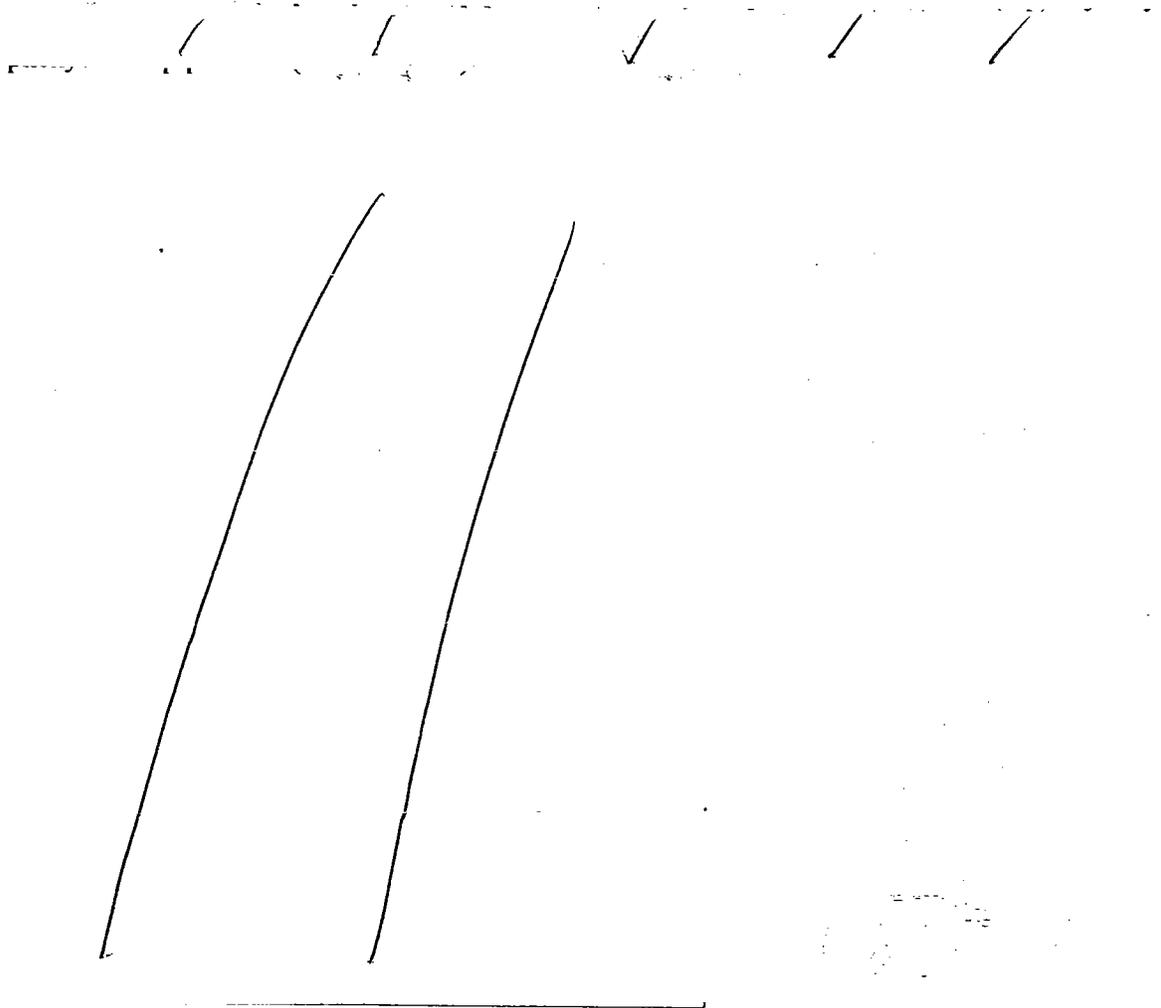
Draft Labeling

Deliberative Process

2.6.6.4 Genetic toxicology

Fentanyl was previously studied for genotoxic potential in NDA 20-747 for Actiq®. It was found to be non-mutagenic at concentrations up to 5000 µg/plate with or without metabolic activation in the bacterial reverse mutation assay using *S. typhimurium* and *E. coli* strains. Fentanyl was also non-mutagenic in either the presence (at 100-500 µg/mL) or absence (at 50-600 µg/mL) of metabolic activation in the in vitro L5178Y/TK+ mouse lymphoma mutagenesis assay. Fentanyl was found to be negative for clastogenicity at doses up to 48 mg/kg, IP, in the in vivo mouse micronucleus assay.

In vitro bacterial mutagenicity and in vitro clastogenicity studies were conducted on three impurities from fentanyl citrate supplied by _____ . The three isolated impurities were identified as _____



All three of these isolated impurities at concentrations of up to 5000 µg/plate were found to be negative for induction of mutations in *Salmonella typhimurium* and *Escherichia coli* with or without metabolic activation. These impurities did not induce structural or numerical

chromosome aberrations in the *in vitro* cytogenetic assay of human peripheral blood lymphocytes in the presence or absence of metabolic activation at concentrations that produced up to 50% cytotoxicity. Note that these concentrations are at least 10,000-fold higher than analgesic concentrations of fentanyl in the 10 ng/mL range.

Impurity/Degradant Cytotoxicity to Human Lymphocytes

Impurity or Degradant	Dose at 50% Human Lymphocyte Cytotoxicity		
	Incubation time ± metabolic activation		
	20 hr -S9	4 hr -S9	4 hr +S9
—	~528 µg/mL		
—	~280 µg/mL	840 µg/mL	840 µg/mL
—	~266 µg/mL	2660 µg/mL	798 µg/mL

Genetic Toxicology Studies of Fentanyl

In NDA 20-747, there were three genotoxicity studies performed summarized in the table below.

Genetic Toxicology Studies of Fentanyl (from NDA 20-747)

Study Type and Number	Assay or Animal	Dose of Fentanyl citrate	Findings
Bacterial Reverse Mutation Assay #RA/FC/96/002 (from IND 65,447 in support of NDA 20-747 for Actiq)	<i>S. typhimurium</i> TA98 TA100 TA1535 TA1537 <i>E. coli</i> WP2 <i>uvrA</i>	Up to 5000 µg/plate ± S9 (Batch B0030-950701, purity)	Not mutagenic
<i>In vitro</i> mouse (L5178Y TK +/-) lymphoma assay #RA/FC/96/001	mice thymidine kinase locus of L5178Y lymphoma cells	100 to 500 µg/mL - S9 50 to 600 µg/mL + S9 (Batch B0030-950701, purity)	Not mutagenic
<i>In vivo</i> micronucleus cytogenetic assay #RA/FC/96/003	Mice, ICR, male and females n=5/sex/dose, bone marrow erythrocytes	0 to 48 mg/kg, IP	Not clastogenic Clinical signs (during initial 48 hrs): at MTD = 48 mg/kg hyperactivity, prostration, lethargy

In a 1993 review of NDA 16-049 Suppl 27 (submitted 6/11/92) for Innovar, studies were genotoxicology studies were performed by Janssen Research Foundation. They were also

mentioned in the Review of the Annual Report for NDA 16-619 (1991-1992). These studies, an Ames bacteria mutation assay #N79258 and a micronucleus assay in mice #N80793, both gave negative results. Without obtaining the original study reports, no further information was available about these studies.

BACTERIAL REVERSE MUTATION ASSAYS

Studies DS-2005-010, DS-2005-011 and DS-2005-012

The following methods were common to the three bacterial reverse mutation assays.

Methods

Strains/species/cell line: *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537
Escherichia coli WP2 *uvrA*

Doses used in definitive study: 50, 150, 500, 1500 and 5000 µg/plate

Negative controls: dimethyl sulfoxide (DMSO)

Positive controls:

S9 activation	Strain	Positive Control	Dose (µg/plate)	
yes	<i>Salmonella typhimurium</i>			
	TA98	2-aminoanthracene	1.0	
	TA100	2-aminoanthracene	1.0	
	TA1535	2-aminoanthracene	1.0	
yes	<i>Escherichia coli</i>			
	WP2 <i>uvrA</i>	2-aminoanthracene	10.0	
	no	<i>Salmonella typhimurium</i>		
		TA98	2-nitrofluorene	1.0
TA100		sodium azide	1.0	
TA1535		sodium azide	1.0	
no	TA1537	9-aminoacridine	75	
	<i>Escherichia coli</i>			
no	WP2 <i>uvrA</i>	methyl methanesulfonate	1000	

Incubation and sampling times:

An initial assay established a dose-range for the confirmatory mutagenicity assay and provided a preliminary mutagenicity evaluation. Vehicle control, positive controls and eight dose levels of the test article were plated, two plates per dose, with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2 *uvrA* on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9.

The confirmatory mutagenicity assay evaluated and confirmed the mutagenic potential of the test article. Five dose levels of test article along with appropriate vehicle control and positive controls

were plated with overnight cultures of TA98, TA100, TA1535, TA1537 and WP2-uvrA on selective minimal agar in the presence and absence of Aroclor-induced rat liver S9 metabolic activation system.

The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. Each bulk preparation of S9 was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

The plates were inverted and incubated for approximately 48 to 72 hours at $37\pm 2^\circ\text{C}$. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate using the codes shown in the table below. For each replicate, the mean and standard deviation of the number of revertants per plate were determined.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 uvrA were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2-times the mean vehicle control value. An equivocal response was a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response was evaluated as negative, if it was neither positive nor equivocal.

**APPEARS THIS WAY
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Plate Scoring of Toxicity and Degree of Precipitation

Code	Description	Characteristics
1	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over greater than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., greater than 3 particles on a plate with 30 revertants). These plates are counted manually.

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

The following criteria must have been met for a valid assay:

All *Salmonella* strains must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvrB* gene.

- 1) Strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- 2) All WP2 *uvrA* cultures must demonstrate the deletion in the *uvrA* gene.
- 3) All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21 ; WP2 *uvrA*, 10 - 60.
- 4) Strain culture titers must be greater than or equal to 0.3×10^9 cells/mL, to ensure that appropriate numbers of bacteria are plated.
- 5) The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- 6) A minimum of three non-toxic dose levels is required to evaluate assay data.

A dose level was considered toxic if one or both of the following criteria were met:

- 1) A >50% reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count.
- 2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

For the test article to be evaluated positive:

- 1) It must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article.
- 2) Strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3-times the mean vehicle control value. Strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2-times the mean vehicle control value.
- 3) An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited.
- 4) A response will be evaluated as negative, if it is neither positive nor equivocal.

Study title: Bacterial Reverse Mutation Assay

for _____

Key findings: _____

_____, up to 5000 µg/plate, did not induce mutations in the reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of metabolic activation with rat liver S9.

Reviewer's Comment: This was a valid study.

Study no.: DS-2005-010

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location: _____

Date of study initiation: June 20, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: _____

Lot RS-04019,

Purity _____

Vehicle: dimethylsulfoxide (DMSO)

The concentration of _____ stock solution was _____ of the expected concentration (see Table 1 below).

Basis of dose selection:

The results of the initial toxicity-mutation assay are summarized in Table 21, below. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate. Precipitate was observed at 5000 µg/plate, but no appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg/plate.

Results

Study outcome:

The test article formed a soluble and clear solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 100 mg/mL. No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions and the S9 and Sham mixes.

The results of the initial toxicity-mutation assay were summarized in Table 21, below. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation up to 5000 µg/plate. Precipitate was observed at 5000 µg/plate, but no appreciable toxicity was observed. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg/plate.

The results of the confirmatory mutagenicity assay were summarized in Table 22. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation up to 5000 µg/plate. Precipitate was observed at 5000 µg/plate. No appreciable toxicity was observed.

**Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay**

Table 21

Test Article Id	/ / /									
Study Number	: AA84CK.503.BTL					Experiment No : B1				
Average Revertants Per Plate ± Standard Deviation										
Liver Microsomes: None										
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 4	128 ± 12	17 ± 7	9 ± 1	17 ± 1	16 ± 4	128 ± 12	17 ± 7	9 ± 1	17 ± 1
1.5	18 ± 6	132 ± 11	13 ± 4	4 ± 4	12 ± 5	18 ± 6	132 ± 11	13 ± 4	4 ± 4	12 ± 5
5.0	18 ± 0	138 ± 24	15 ± 1	4 ± 4	13 ± 6	18 ± 0	138 ± 24	15 ± 1	4 ± 4	13 ± 6
15	15 ± 6	131 ± 10	15 ± 5	7 ± 4	12 ± 2	15 ± 6	131 ± 10	15 ± 5	7 ± 4	12 ± 2
50	17 ± 4	125 ± 13	20 ± 8	6 ± 1	13 ± 8	17 ± 4	125 ± 13	20 ± 8	6 ± 1	13 ± 8
150	21 ± 7	143 ± 6	18 ± 1	7 ± 3	11 ± 7	21 ± 7	143 ± 6	18 ± 1	7 ± 3	11 ± 7
500	15 ± 4	136 ± 6	21 ± 8	5 ± 0	11 ± 4	15 ± 4	136 ± 6	21 ± 8	5 ± 0	11 ± 4
1500	23 ± 5	131 ± 42	21 ± 4	7 ± 4	16 ± 7	23 ± 5	131 ± 42	21 ± 4	7 ± 4	16 ± 7
5000	25 ± 8	119 ± 17	35 ± 2	7 ± 3	11 ± 1	25 ± 8	119 ± 17	35 ± 2	7 ± 3	11 ± 1
Positive	166 ± 10	428 ± 27	324 ± 16	549 ± 26	122 ± 3	166 ± 10	428 ± 27	324 ± 16	549 ± 26	122 ± 3
Liver Microsomes: Rat liver S9										
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	25 ± 2	130 ± 25	8 ± 4	9 ± 1	15 ± 1	25 ± 2	130 ± 25	8 ± 4	9 ± 1	15 ± 1
1.5	25 ± 1	136 ± 13	10 ± 3	10 ± 0	15 ± 1	25 ± 1	136 ± 13	10 ± 3	10 ± 0	15 ± 1
5.0	30 ± 4	120 ± 1	13 ± 1	6 ± 1	14 ± 1	30 ± 4	120 ± 1	13 ± 1	6 ± 1	14 ± 1
15	30 ± 2	159 ± 2	15 ± 1	3 ± 0	15 ± 1	30 ± 2	159 ± 2	15 ± 1	3 ± 0	15 ± 1
50	30 ± 11	134 ± 22	9 ± 4	3 ± 3	16 ± 1	30 ± 11	134 ± 22	9 ± 4	3 ± 3	16 ± 1
150	27 ± 6	160 ± 30	12 ± 1	8 ± 2	17 ± 1	27 ± 6	160 ± 30	12 ± 1	8 ± 2	17 ± 1
500	31 ± 5	158 ± 25	8 ± 0	6 ± 1	10 ± 1	31 ± 5	158 ± 25	8 ± 0	6 ± 1	10 ± 1
1500	32 ± 4	135 ± 7	11 ± 5	8 ± 2	12 ± 2	32 ± 4	135 ± 7	11 ± 5	8 ± 2	12 ± 2
5000	28 ± 6	136 ± 1	15 ± 3	8 ± 2	10 ± 1	28 ± 6	136 ± 1	15 ± 3	8 ± 2	10 ± 1
Positive	571 ± 57	711 ± 34	113 ± 1	85 ± 1	368 ± 39	571 ± 57	711 ± 34	113 ± 1	85 ± 1	368 ± 39
Vehicle = Vehicle Control										
Positive = Positive Control (50 µL plating aliquot)										
Plating aliquot: 100 µL										

Bacterial Mutation Assay
 Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Test Article Id : _____

Study Number : AA84CK.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	14 ± 2	125 ± 19	15 ± 4	5 ± 2	12 ± 1
50	12 ± 2	124 ± 6	20 ± 6	6 ± 1	14 ± 3
150	11 ± 2	130 ± 12	16 ± 2	4 ± 2	18 ± 2
500	14 ± 4	132 ± 13	19 ± 3	4 ± 2	12 ± 2
1500	13 ± 4	130 ± 13	19 ± 2	5 ± 2	13 ± 3
5000	9 ± 7	130 ± 2	22 ± 6	6 ± 2	12 ± 3
Positive	160 ± 35	483 ± 52	380 ± 14	637 ± 118	180 ± 22

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	21 ± 6	151 ± 4	10 ± 3	6 ± 1	16 ± 5
50	25 ± 4	156 ± 22	15 ± 4	9 ± 2	16 ± 3
150	26 ± 13	146 ± 30	14 ± 7	6 ± 2	15 ± 1
500	18 ± 6	148 ± 12	13 ± 4	5 ± 2	13 ± 2
1500	30 ± 5	139 ± 17	14 ± 2	4 ± 1	13 ± 3
5000	23 ± 4	151 ± 5	15 ± 5	7 ± 4	11 ± 5
Positive	899 ± 186	948 ± 235	117 ± 28	65 ± 29	505 ± 108

Vehicle = Vehicle Control

Positive = Positive Control (50 µL plating aliquot)

Plating aliquot: 100 µL

APPEARS THIS WAY
 ON ORIGINAL

Study title: Bacterial Reverse Mutation Assay
for _____

Key findings:

_____, up to 5000 µg/plate, did not induce mutations in the reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of metabolic activation with rat liver S9.

Reviewer's Comment: This was a valid study.

Study no.: DS-2005-011

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location: _____

Date of study initiation: June 20, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: _____

Lot RS-03027, Purity _____
Vehicle: dimethylsulfoxide (DMSO)

The concentration of _____ stock solution were _____
of the expected concentration.

Basis of dose selection:

In the initial toxicity-mutation assay, no positive mutagenic response was observed (see Sponsor's table 21 reproduced below). The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate. No precipitate was observed but toxicity was observed beginning at 1500 or at 5000 pg per plate. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg/plate.

Results

Study outcome:

The test article formed a soluble and clear solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 100 mg/mL. No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions and the S9 and Sham mixes.

The results of the confirmatory mutagenicity assay are summarized in Table 22, reproduced from the study report. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation up to 5000 µg/plate. No precipitate was observed, but toxicity was observed beginning at 1500 or at 5000 µg/plate with most test conditions.

Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay

Table 21

Test Article Id : _____

Study Number : AAB4CL.503.BTL Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 0	128 ± 8	17 ± 1	6 ± 1	16 ± 4
1.5	17 ± 1	101 ± 11	25 ± 0	3 ± 1	16 ± 4
5.0	20 ± 6	118 ± 11	22 ± 1	8 ± 0	14 ± 4
15	16 ± 4	115 ± 8	18 ± 2	6 ± 4	12 ± 3
50	17 ± 5	117 ± 8	19 ± 2	6 ± 1	13 ± 6
150	17 ± 0	126 ± 8	19 ± 1	7 ± 0	16 ± 5
500	14 ± 4	128 ± 23	20 ± 7	6 ± 3	9 ± 4
1500	26 ± 0	122 ± 10	16 ± 1	4 ± 1	2 ± 1
5000	0 ± 0	0 ± 0	2 ± 2	0 ± 0	1 ± 1
Positive	196 ± 48	463 ± 28	272 ± 22	764 ± 9	124 ± 4

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	37 ± 3	165 ± 8	17 ± 2	10 ± 8	14 ± 1
1.5	27 ± 0	131 ± 5	14 ± 4	4 ± 0	17 ± 3
5.0	31 ± 4	140 ± 10	12 ± 4	11 ± 3	15 ± 1
15	25 ± 11	135 ± 17	10 ± 1	5 ± 0	15 ± 2
50	30 ± 11	123 ± 4	12 ± 4	7 ± 2	14 ± 6
150	26 ± 4	123 ± 9	13 ± 4	7 ± 2	18 ± 4
500	30 ± 0	149 ± 2	10 ± 1	5 ± 2	10 ± 4
1500	23 ± 6	162 ± 34	8 ± 3	7 ± 2	5 ± 1
5000	5 ± 7	37 ± 52	2 ± 2	2 ± 1	2 ± 2
Positive	297 ± 62	630 ± 6	69 ± 2	46 ± 5	352 ± 8

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 100 µL

Bacterial Mutation Assay
 Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Test Article Id : _____

Study Number : AA84CL.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation
 Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	15 ± 3	113 ± 22	21 ± 6	8 ± 3	17 ± 1
15		127 ± 13	17 ± 5	7 ± 3	16 ± 4
50	14 ± 3	138 ± 10	21 ± 5	8 ± 2	13 ± 2
150	16 ± 2	136 ± 26	22 ± 5	9 ± 3	13 ± 1
500	10 ± 2	121 ± 8	16 ± 5	6 ± 2	10 ± 3
1000	12 ± 3				
1500	13 ± 5	104 ± 8	15 ± 2	6 ± 1	6 ± 2
1800	13 ± 4				
5000	6 ± 2	62 ± 7	10 ± 2	2 ± 1	3 ± 3
Positive	142 ± 14	504 ± 35	408 ± 71	444 ± 29	166 ± 24

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	19 ± 1	143 ± 17	13 ± 3	4 ± 1	17 ± 3
15	22 ± 2	148 ± 18	14 ± 4	7 ± 4	16 ± 3
50	17 ± 7	138 ± 9	11 ± 4	6 ± 1	19 ± 8
150	24 ± 5	113 ± 8	13 ± 6	5 ± 2	15 ± 3
500	17 ± 4	135 ± 24	11 ± 1	5 ± 1	9 ± 2
1500	21 ± 4	144 ± 21	8 ± 6	4 ± 4	5 ± 1
5000	17 ± 1	84 ± 10	9 ± 4	3 ± 2	1 ± 1
Positive	502 ± 106	817 ± 155	111 ± 12	50 ± 13	428 ± 42

Vehicle = Vehicle Control

Positive = Positive Control (50 µL plating aliquot)

Plating aliquot: 100 µL

Study title: Bacterial Reverse Mutation Assay
for impurity

Key findings: up to 5000 µg/plate, did not induce mutations in the reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of metabolic activation with rat liver S9.

Reviewer's Comment: This was a valid study.

Study no.: DS-2005-012

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location:

Date of study initiation: June 20, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Vehicle: dimethylsulfoxide (DMSO)

Lot RS-02016-3, Purity

The concentration of concentration.

stock solution was of the expected

Methods

Basis of dose selection:

The results of the initial toxicity-mutation assay are summarized in Table 21. The maximum dose tested was 5000 µg/plate. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate. No precipitate was observed, but toxicity was observed beginning at 1500 or at 5000

µg/plate. Based on the findings of the initial toxicity-mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg/plate.

APPEARS THIS WAY
ON ORIGINAL

Results

Study outcome:

The test article formed a soluble and clear solution in dimethyl sulfoxide (DMSO) at a maximum concentration of 100 mg/mL. No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions and the S9 and Sham mixes.

The results of the confirmatory mutagenicity assay were summarized in Table 22. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation up to 5000 µg/plate. No precipitate was observed, but toxicity was observed beginning at 1500 or at 5000 µg/plate.

**Bacterial Mutation Assay
Summary of Results - Initial Toxicity-Mutation Assay**

Table 21

Test Article Id	_____									
Study Number	AA84CM.503.BTL					Experiment No : B1				
Average Revertants Per Plate ± Standard Deviation										
Liver Microsomes: None										
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA				
Vehicle	21 ± 5	122 ± 6	22 ± 1	9 ± 2	14 ± 1					
1.5	16 ± 4	130 ± 4	17 ± 0	4 ± 1	14 ± 1					
5.0	12 ± 3	133 ± 11	19 ± 4	9 ± 2	15 ± 3					
15	25 ± 4	132 ± 13	25 ± 4	7 ± 3	19 ± 4					
50	22 ± 4	138 ± 18	24 ± 1	7 ± 6	10 ± 3					
150	20 ± 1	140 ± 16	20 ± 4	5 ± 3	13 ± 5					
500	22 ± 6	138 ± 8	21 ± 10	6 ± 2	13 ± 3					
1500	16 ± 3	119 ± 3	14 ± 2	6 ± 4	11 ± 4					
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0					
Positive	193 ± 18	411 ± 45	356 ± 51	376 ± 44	130 ± 13					
Liver Microsomes: Rat liver S9										
Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2	uvrA				
Vehicle	45 ± 5	161 ± 1	18 ± 1	11 ± 1	21 ± 13					
1.5	31 ± 6	168 ± 16	16 ± 1	5 ± 6	15 ± 1					
5.0	32 ± 4	172 ± 30	10 ± 4	8 ± 3	17 ± 4					
15	36 ± 3	176 ± 18	12 ± 1	11 ± 8	15 ± 5					
50	38 ± 13	178 ± 11	11 ± 1	7 ± 4	17 ± 8					
150	32 ± 1	185 ± 19	10 ± 1	9 ± 0	18 ± 8					
500	39 ± 2	152 ± 8	14 ± 6	5 ± 2	12 ± 1					
1500	31 ± 6	155 ± 35	13 ± 5	8 ± 4	11 ± 2					
5000	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0					
Positive	401 ± 62	510 ± 22	124 ± 14	97 ± 2	452 ± 255					
Vehicle = Vehicle Control										
Positive = Positive Control (50 µL plating aliquot)										
Plating aliquot: 100 µL										

Bacterial Mutation Assay
Summary of Results - Confirmatory Mutagenicity Assay

Table 22

Test Article Id. :		Study Number :		Experiment No :	
		AA84CM.503.BTL		B2	
Average Revertants Per Plate \pm Standard Deviation					
Liver Microsomes: None					
Dose (μ g/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	26 \pm 13	140 \pm 17	33 \pm 9	9 \pm 6	20 \pm 3
15	28 \pm 2	126 \pm 8	22 \pm 5	9 \pm 2	19 \pm 6
50	30 \pm 3	133 \pm 22	22 \pm 2	7 \pm 1	21 \pm 2
150	29 \pm 4	129 \pm 6	20 \pm 3	6 \pm 3	18 \pm 3
500	25 \pm 6	118 \pm 12	21 \pm 4	9 \pm 5	17 \pm 3
1500	10 \pm 3	147 \pm 20	24 \pm 8	8 \pm 2	14 \pm 2
5000	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Positive	195 \pm 15	588 \pm 22	387 \pm 48	706 \pm 212	185 \pm 30
Liver Microsomes: Rat liver S9					
Dose (μ g/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	30 \pm 3	133 \pm 16	16 \pm 2	6 \pm 1	16 \pm 3
15	19 \pm 6	122 \pm 19	11 \pm 3	6 \pm 2	21 \pm 2
50	36 \pm 5	115 \pm 11	15 \pm 4	7 \pm 3	19 \pm 4
150	31 \pm 3	104 \pm 11	14 \pm 3	8 \pm 1	21 \pm 1
500	25 \pm 3	127 \pm 14	11 \pm 6	7 \pm 1	18 \pm 3
1500	26 \pm 6	124 \pm 18	11 \pm 5	6 \pm 1	12 \pm 1
5000	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Positive	615 \pm 139	552 \pm 49	82 \pm 8	42 \pm 5	533 \pm 99
Vehicle = Vehicle Control					
Positive = Positive Control (50 μ L plating aliquot)					
Plating aliquot: 100 μ L					

APPEARS THIS WAY
ON ORIGINAL

IN VITRO MAMMALIAN CHROMOSOME ABERRATION ASSAYS

Studies DS-2005-007, DS-2005-008 and DS2005-009

The following methods were common to all three studies using the *in vitro* mammalian chromosome aberration assay:

Strains/species/cell line: Peripheral blood lymphocytes were obtained from a healthy non-smoking 28-year old adult female for the preliminary toxicity assay and from the same donor for the definitive assay. The donor had no recent history of radiotherapy, viral infection or the administration of drugs.

Reviewer Comments: Blood lymphocytes were obtained from 1 or 2 individuals, although not stated, it seems this individual provided cells for all lymphocyte assays for each impurity.

Negative controls:

Dimethyl sulfoxide, solvent vehicle for the test articles; was used as the solvent control at the same concentration as that found in the test article-treated groups.

Positive controls:

Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.3 and 0.6 µg/mL. Cyclophosphamide (CPa) was used as the positive control in the S9-activated study at final concentrations of 20 and 40 µg/mL. For both positive controls one dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Incubation and sampling times:

Human peripheral blood lymphocytes were treated in the absence and presence of rat liver S9 activation system for 4 hours, or treated in the absence of S9 activation for 20 hours.

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. Each bulk preparation of S9 was assayed for its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

A toxicity test was performed to evaluate the test article effect on mitotic index. The cells were cultured in RPMI-1640 complete medium supplemented with 1% phytohemagglutinin (PHA) and exposed to solvent alone and to nine concentrations of the test article for 4 hours in both the presence and absence of S9 activation, and for 20 hours continuously in the absence of S9 activation. The cells were incubated at 37±1°C in a humidified atmosphere of 5±1% CO₂ in air. At the completion of the 4-hour exposure period, the treatment medium was removed, the cells washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS), refed with RPMI-1640 complete medium and incubated an additional 16 hours. Two hours prior to the scheduled cell harvest, Colcemid® was added to the cultures at a final concentration of 0.1 µg/mL. Cells were collected by centrifugation, treated with hypotonic potassium chloride (0.075

M KCl), fixed (methanol:glacial acetic acid, 3:1 v/v) and stained (5% Giemsa). The number of cells in mitosis per 500 cells scored was determined to evaluate test article effect on mitotic index.

The chromosome aberration assay was performed by exposing duplicate cultures of human peripheral blood lymphocytes (HPBL) to at least 4 concentrations of the test article, and positive and solvent controls. In the non-S9-activated study, the cells were exposed for 4 or 20 hours. In the S9-activated study, the cells were exposed for 4 hours. The dividing cells were harvested at approximately 20 hours from the initiation of treatment, two hours after Colcemid® treatment.

Study validity:

The selection of dose levels for analysis of chromosome aberrations in HPBL was based upon toxicity of the test article in the non-activated 20-hour exposure group. The highest dose level selected for evaluation was the dose which induced at least 50% toxicity, as measured by mitotic inhibition, relative to the solvent control, with a sufficient number of scorable metaphase cells.

Metaphase cells with 46 centromeres were examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask was reduced when the percentage of aberrant cells reached a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome).

Fragments observed with an exchange figure were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (2-10 aberrations) also were recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted. The percent polyploid and endoreduplicated cells were evaluated per 100 cells.

The toxic effects of treatment are based upon mitotic inhibition relative to the solvent-treated control and are presented for the preliminary toxicity test and the chromosome aberration assay. The number and types of aberrations per cell, the percentage of structurally and numerically damaged cells (percent aberrant cells), and the frequency of structural aberrations per cell (mean aberrations per cell) in the total population of cells examined was calculated and reported for each treatment group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell.

Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test to compare pairwise the percent aberrant cells of each treatment group with that of the solvent

control. In the event of a positive Fisher's exact test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

The test article was considered to induce a positive response when the percentages of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.05$). A reproducible significant increase at the high dose only with no dose response or a reproducible significant increase at one dose level other than the high dose with no dose response will be considered positive. The test article was concluded to be negative if no statistically significant increase was observed relative to the solvent control.

For a valid test the frequency of cells with structural chromosome aberrations in the solvent controls must be within the historical range for solvent controls. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Study title: In Vitro Mammalian Chromosome Aberration Test

Lot RS-04019

Key findings: _____ did not induce structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration test with human peripheral blood lymphocytes, in the presence and absence of metabolic activation. Thus this impurity was not clastogenic.

Reviewer's Comment: This was a valid study.

Study no.: DS-2005-007

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location: _____

Date of study initiation: June 17, 2005

GLP compliance: yes

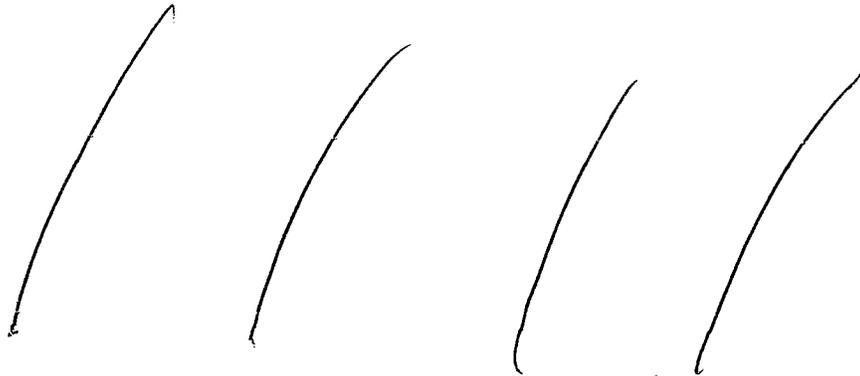
QA reports: yes

Drug, lot #, and % purity: _____

, Lot RS-04019,

Purity _____

Vehicle: dimethylsulfoxide (DMSO)



The stock solution from which dilutions were prepared was — of the expected concentration (see Table 1 below).

Methods

Strains/species/cell line: Peripheral blood lymphocytes were obtained from a healthy non-smoking 28-year old adult female for the preliminary toxicity assay and from the same donor for the definitive assay. The donor had no recent history of radiotherapy, viral infection or the administration of drugs.

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	220, 440, 880, 1760
	20 hr	0 hr	100, 200, 400, 450, 500, 550, 600
S9-activated	4 hr	16 hr	220, 440, 880, 1760

Basis of dose selection:

In a preliminary study, the maximum dose tested was 1760 µg/mL (10 mM) produced at least 50% reduction in mitotic index relative to the solvent control; however, this reduction in mitotic index was not observed at any dose in either the non-activated or S9-activated 4-hour exposure groups. Substantial toxicity was observed at dose levels > 528 µg/mL in the non-activated 20-hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 220 to 1760 µg/mL for both the non-activated and the S9-activated 4-hour exposure groups, and from 100 to 600 µg/mL for the non-activated 20-hour exposure group.

Treatment Time (hours)	Recovery Time (hours)	Harvest Time (hours)	S9	Mitotic Index Reduction ¹ at highest dose scored ($\mu\text{g/mL}$)	LED ² for Structural Aberrations ($\mu\text{g/mL}$)	LED ² for Numerical Aberrations ($\mu\text{g/mL}$)
4	16	20	-	40% at 1760	None	None
20	0	20	-	55% at 550	None	None
4	16	20	+	40% at 1760	None	None

¹ relative to the solvent control at high dose evaluated for chromosome aberrations

² LED = lowest effective dose level

Results

Study outcome:

Lymphocytes were first exposed to nine concentrations of _____ ranging from 0.176 $\mu\text{g/mL}$ to 1760 $\mu\text{g/mL}$ (10 mM), as well as solvent controls, in both the absence and presence of an Aroclor-induced S9 activation system for 4 hours, or continuously for 20 hours in the absence of S9 activation. The test article was soluble in DMSO and in the treatment medium at all concentrations tested. At the conclusion of the treatment period, hemolysis was observed at dose levels ≥ 528 $\mu\text{g/mL}$ in all treatment groups. The osmolality in treatment medium of the highest concentration tested, 1760 $\mu\text{g/mL}$, was 407 mmol/kg. The osmolality of the solvent DMSO in the treatment medium was 426 mmol/kg. In order to maintain neutral pH, the pH of dose levels 176, 528 and 1760 $\mu\text{g/mL}$ was adjusted to approximately 7.5 with 1 N HCl.

Non-S9-activated 4-hour exposure group: At the highest test concentration evaluated microscopically for chromosome aberrations, 1760 $\mu\text{g/mL}$, mitotic inhibition was 40%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 440, 880 and 1760 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (19.0%).

S9-activated group: At the highest test concentration evaluated microscopically for chromosome aberrations, 1760 $\mu\text{g/mL}$, mitotic inhibition was 40%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 440, 880 and 1760 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) group was statistically significant (23.0%).

Non-activated 20-hour exposure group: At the highest test concentration evaluated microscopically for chromosome aberrations, 550 $\mu\text{g/mL}$, mitotic inhibition was 55%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 100, 450 and 550 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose

level $p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (20.0%). The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test).

**TABLE 7
SUMMARY**

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean ± SD)		Cells With Aberrations Numerical Structural (%)	
				Numerical	Structural				
DMSO	-S9	4	10.8	200	200	0.000	±0.000	0.0	0.0
440	-S9	4	10.0	200	200	0.000	±0.000	0.0	0.0
880	-S9	4	9.3	200	200	0.000	±0.000	0.0	0.0
1760	-S9	4	6.5	200	200	0.005	±0.071	0.0	0.5
MMC, 0.6	-S9	4	9.1	200	100	0.190	±0.394	0.0	19.0**
DMSO	+S9	4	10.7	200	200	0.000	±0.000	0.0	0.0
440	+S9	4	9.3	200	200	0.000	±0.000	0.0	0.0
880	+S9	4	9.2	200	200	0.000	±0.000	0.0	0.0
1760	+S9	4	6.4	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	3.5	200	100	0.290	±0.574	0.0	23.0**
DMSO	-S9	20	10.9	200	200	0.000	±0.000	0.0	0.0
100	-S9	20	11.0	200	200	0.000	±0.000	0.0	0.0
450	-S9	20	7.5	200	200	0.000	±0.000	0.0	0.0
550	-S9	20	4.9	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	5.9	200	100	0.220	±0.484	0.0	20.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p < 0.05$; **, $p < 0.01$; using the Fisher's exact test.

Study title: In Vitro Mammalian Chromosome Aberration Test

Lot RS03027

Vehicle: dimethylsulfoxide (DMSO)

Key findings: _____, did not induce structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration test with human peripheral blood lymphocytes, in the presence and absence of metabolic activation. Thus, this impurity was not clastogenic.

Reviewer's Comment: This was a valid study.

Study no.: DS-2005-008

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location: _____

Date of study initiation: June 17, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: _____

Lot RS-03027, Purity _____

Methods

Strains/species/cell line:

Peripheral blood lymphocytes were obtained from a healthy non-smoking 28-year-old adult female on 20 June 2005 for the preliminary toxicity assay and from a healthy non-smoking 24-year-old adult female on 28 June 2005 for the definitive assay.

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels ($\mu\text{g/mL}$)
Non-activated	4 hr	16 hr	100, 150, 200, 250, 300, 350, 400, 450, 500
	20 hr	0 hr	12.5, 25, 50, 100, 150, 200, 250, 300
S9-activated	4 hr	16 hr	100, 150, 200, 250, 300, 350, 400, 450, 500

Basis of dose selection:

The test article was soluble in DMSO at a concentration of 280 mg/mL, the maximum concentration prepared in the assay. In the preliminary toxicity assay, the doses tested ranged from 0.28 to 2800 $\mu\text{g/mL}$ (10 mM). Visible precipitate was observed in treatment medium at 2800 $\mu\text{g/mL}$ and dose levels $\leq 840 \mu\text{g/mL}$ were soluble in treatment medium at the beginning and conclusion of the treatment period. At the conclusion of the treatment period, hemolysis was observed at dose levels $\geq 280 \mu\text{g/mL}$ in all treatment groups.

Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was observed at doses $\geq 840 \mu\text{g/mL}$ in the non-activated and S9-activated 4-hour exposure groups. Substantial toxicity was observed at dose levels $\geq 280 \mu\text{g/mL}$ in the non-activated 20-hour exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 100 to 500 $\mu\text{g/mL}$ for both the non-activated and the S9-activated 4-hour exposure groups, and from 12.5 to 300 $\mu\text{g/mL}$ for the non-activated 20-hour exposure group.

Results

Study outcome:

In the chromosome aberration assay, the cells were treated for 4 or 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test).

At the conclusion of the treatment period, hemolysis was observed at dose levels $\geq 300 \mu\text{g/mL}$ in all treatment groups. The osmolality in the treatment medium at the highest concentration tested, 500 $\mu\text{g/mL}$, was 411 mmol/kg. The osmolality of the solvent (DMSO) in the treatment medium was 402 mmol/kg. The pH of the highest concentration of test article in treatment medium was approximately 7.0.

Non-activated 4-hour exposure group: At the highest test concentration evaluated, 300 $\mu\text{g/mL}$, mitotic inhibition was 56%, relative to the solvent control. The dose levels selected for analysis of

chromosome aberrations were 100, 200 and 300 $\mu\text{g}/\text{mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (14.0%).

S9-activated group: At the highest test concentration, 300 $\mu\text{g}/\text{mL}$, mitotic inhibition was 55%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 100, 200 and 300 pg/mL . The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) group was statistically significant (17.0%).

Non-activated 20-hour exposure group: The highest test concentration evaluated, 100 $\mu\text{g}/\text{mL}$, mitotic inhibition was 52%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 25, 50 and 100 pg/mL . The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (15.0%).

**APPEARS THIS WAY
ON ORIGINAL**

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	10.8	200	200	0.000	±0.000	0.0	0.0
100	-S9	4	10.9	200	200	0.000	±0.000	0.0	0.0
200	-S9	4	9.9	200	200	0.000	±0.000	0.0	0.0
300	-S9	4	4.8	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	10.1	200	100	0.140	±0.349	0.0	14.0**
DMSO	+S9	4	11.2	200	200	0.000	±0.000	0.0	0.0
100	+S9	4	11.3	200	200	0.000	±0.000	0.0	0.0
200	+S9	4	10.3	200	200	0.000	±0.000	0.0	0.0
300	+S9	4	5.0	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	9.3	200	100	0.170	±0.378	0.0	17.0**
DMSO	-S9	20	10.1	200	200	0.000	±0.000	0.0	0.0
25	-S9	20	10.9	200	200	0.000	±0.000	0.0	0.0
50	-S9	20	10.0	200	200	0.000	±0.000	0.0	0.0
100	-S9	20	4.8	200	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-S9	20	9.1	200	100	0.160	±0.395	0.0	15.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's exact test.

APPEARS THIS WAY
ON ORIGINAL

Study title: In Vitro Mammalian Chromosome Aberration Test
for impurity

Key findings: Impurity _____, did not induce structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration test with human peripheral blood lymphocytes, in the presence and absence of metabolic activation. Thus, this impurity was not clastogenic.

Reviewer's Comment: This was a valid study.

Study no.: DS2005-009

e-submission: N_000\2005-08-31\pharmtox\toxicology

Conducting laboratory and location: _____

Date of study initiation: June 17, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Vehicle: dimethylsulfoxide (DMSO)

Lot RS-02016-3, Purity _____

The concentration of _____ concentration.

_____ stock solution was _____ of the expected

Methods

Strains/species/cell line:

Peripheral blood lymphocytes were obtained from a healthy non-smoking 24-year-old adult female on 20 June 2005 for the preliminary toxicity assay and from the same donor on 28 June 2005 for the definitive assay. The donor had no recent history of radiotherapy, viral infection or the administration of drugs.

Doses used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	31.3, 62.5, 125, 250, 500, 750, 1000, 1250, 1500
	20 hr	0 hr	12.5, 25, 50, 75, 100, 125, 150, 175, 200
S9-activated	4 hr	16 hr	50, 100, 200, 250, 300, 350, 400, 500

Basis of dose selection:

The selection of dose levels for analysis of chromosome aberrations in HPBL was based upon precipitation of the test article in treatment medium and toxicity. In the presence of test article precipitation in the treatment medium, the highest dose level evaluated was the lowest precipitating dose level. In the absence of test article precipitation in the treatment medium, the highest dose level selected for evaluation was the dose that induced at least 50% toxicity, as measured by mitotic inhibition, relative to the solvent control, with a sufficient number of scorable metaphase cells. At least two additional lower dose levels were included in the evaluation.

In the preliminary toxicity assay, the maximum dose tested was 2660 µg/mL (10 mM). Human peripheral blood lymphocytes were treated in the absence and presence of an Aroclor-induced S9 activation system for 4 hours and continuously for 20 hours in the absence of S9 activation. The test article was soluble in DMSO at all concentrations tested. Visible precipitate was observed in treatment medium at dose levels ≥ 266 µg/mL and dose levels ≤ 79.8 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period in all treatment groups, except at the conclusion of the non-activated 20-hour treatment period, dose levels ≤ 266 µg/mL were soluble in the treatment medium. Hemolysis was observed at dose levels ≥ 266 µg/mL in all treatment groups.

Selection of dose levels for the chromosome aberration assay was based on a reduction in the mitotic index relative to the solvent control. Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was observed at 2660 µg/mL in the non-activated 4-hour exposure group and at dose levels ≥ 798 in the S9-activated 4-hour exposure group. Substantial toxicity was observed at dose levels ≥ 266 µg/mL in the non-activated 20-hour exposure group.

Results

The test article was soluble in DMSO at a concentration of 266 mg/mL, the maximum concentration prepared in the assay. In the chromosome aberration assay, the test article was soluble in DMSO at all dose levels tested. Visible precipitate was observed in treatment medium at dose levels ≥ 250 µg/mL and dose levels < 200 µg/mL were soluble in treatment medium at the beginning and conclusion of the treatment period. At the conclusion of the treatment period,

hemolysis was observed at dose levels ≥ 500 $\mu\text{g/mL}$ in the non-activated and the S9-activated 4-hour exposure groups. The osmolality in treatment medium of the highest concentration tested, 1500 $\mu\text{g/mL}$, was 413 mmol/kg. The osmolality in treatment medium of the lowest precipitating concentration, 250 $\mu\text{g/mL}$, was 398 mmol/kg. The osmolality in treatment medium of the highest soluble concentration, 200 $\mu\text{g/mL}$, was 385 mmol/kg. The osmolality of the solvent (DMSO) in the treatment medium was 427 mmol/kg. The pH of the highest concentration of test article in treatment medium was 7.5.

In the chromosome aberration assay, the cells were treated for 4 and 20 hours in the nonactivated test system and for 4 hours in the S9-activated test system. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test).

Non-activated 4-hour exposure group: At the highest test concentration evaluated microscopically for chromosome aberrations, 250 $\mu\text{g/mL}$, mitotic inhibition was 15%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 62.5, 125 and 250 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (14.0%).

S9-activated group: At the highest test concentration evaluated microscopically for chromosome aberrations, 250 $\mu\text{g/mL}$, mitotic inhibition was 10%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 50, 100 and 250 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) group was statistically significant (14.0%).

Non-activated 20-hour exposure group: At the highest test concentration evaluated 175 $\mu\text{g/mL}$, mitotic inhibition was 53%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 50, 100 and 175 $\mu\text{g/mL}$. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (16.0%).

**TABLE 7
SUMMARY**

Treatment µg/ml	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	8.6	200	200	0.000	±0.000	0.0	0.0
62.5	-S9	4	6.5	200	200	0.000	±0.000	0.0	0.0
125	-S9	4	6.8	200	200	0.000	±0.000	0.0	0.0
250	-S9	4	7.3	200	200	0.000	±0.000	0.0	0.0
MBC, 0.6	-S9	4	6.3	200	100	0.140	±0.349	0.0	14.0**
DMSO	+S9	4	9.3	200	200	0.000	±0.000	0.0	0.0
50	+S9	4	8.9	200	200	0.000	±0.000	0.0	0.0
100	+S9	4	9.6	200	200	0.000	±0.000	0.0	0.0
250	+S9	4	8.4	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	8.2	200	100	0.140	±0.349	0.0	14.0**
DMSO	-S9	20	10.8	200	200	0.000	±0.000	0.0	0.0
50	-S9	20	7.8	200	200	0.000	±0.000	0.0	0.0
100	-S9	20	6.7	200	200	0.000	±0.000	0.0	0.0
175	-S9	20	5.1	200	200	0.000	±0.000	0.0	0.0
MBC, 0.3	-S9	20	5.7	200	100	0.180	±0.435	0.0	18.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p<0.05; **, p<0.01; using the Fisher's exact test.

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2.6.6.5 Carcinogenicity

The Sponsor has not performed long-term studies in animals to evaluate the carcinogenic potential of fentanyl. Likewise, carcinogenicity studies were not performed in support of the Referenced Fentanyl Product of NDA 20-747 at the time of its approval. Although carcinogenicity data are not available for any fentanyl drug product, the policy in the Office of New Drugs (OND) is to request such data only in the event that the Sponsor's to complete these studies these studies in support of the safety of the drug product

Previous Division Advice and Sponsor Submissions

During the November 2001 pre-IND 65,447 meeting for OraVescent fentanyl citrate, the sponsor was informed that

The Division later informed the Sponsor that carcinogenicity studies would not be required to support the proposed indication of **breakthrough cancer pain**.

2.6.6.6 Reproductive and developmental toxicology

Reproductive and developmental toxicity studies with fentanyl citrate were described in NDA 20-747. No additional studies were conducted in support of the current NDA.

NDA 20-747 for Actiq® does not contain reproductive toxicology data, but references information in NDA 20-195 for Oralet. The Oralet NDA also lacks reproductive toxicology data, and refers to published literature. The information included in the Oralet and Actiq labels appear to be based on studies conducted by McNeil Laboratories (Fort Washington, PA) in support of NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection. These studies are summarized in the table below.

As mentioned to the Sponsor at the time of the NDA for Actiq® (same Sponsor as this NDA 21-947), studies characterizing the effects of fentanyl on male fertility are lacking. Since ICH S5A recommends that Segment II studies be completed in two mammalian species (generally with the

rabbit being the second species), therefore, an embryofetal development study in a second species, and a segment III (peri- and post-natal development) study are also lacking from the current label.

Summary of Reproductive Toxicity of Fentanyl in Pregnant Rats

Study	Dose Route	Findings						
NDA 16-049 (from pharmacologist review, 5/12/67)								
TRR-142	0, 0.5, 2 mg/kg intravenous; once daily; day 6-18 of gestation n=10	<i>(Current Reviewer's Comments: There was no separate study of only the fentanyl component of Innovar, therefore this study was not relevant to NDA 21-947).</i>						
NDA 16-619 (from pharmacologist review, 7/19/67, and medical officer review,)								
TRR-111	0, 0.001, 0.03 mg/kg; intravenous; once daily; day 6-18 of gestation n=25/dose	<p>One-half of the animals were sacrificed and examined on Day 20. One-half were allowed to deliver naturally and were killed on postpartum day 30.</p> <p>Day 20 rats: No effect of fentanyl on pregnancy or litters</p> <p>For rats allowed to deliver and raise pups: 0.03 mg/kg: Increased deaths for the first 24 hours after birth Higher number of resorptions than other doses or groups</p> <p>There was no effect of fentanyl on occurrence of malformations</p> <p><i>(Current Reviewer's Comments: The expression of data in absolute numbers, rather than per litter, makes interpretation of the data problematic).</i></p>						
Dose (mg/kg/day)	N	Pregnant	Number of Pups	Pup Weight (average)	Pup Deaths		Resorptions	Malformations
					24 hours	30 days		
Day 20 Data								
0	13	10	109	1.92	-	-	6	0
0.01	13	10	109	2.01	-	-	11	0
0.03	13	10	106	2.08	-	-	5	0
Natural Delivery data								
0	12	11	93	-	3	14	0	0
0.01	12	10	116	-	2	5	0	0
0.03	12	9	86	-	13	19	26	0

Study		Dose Route		Findings						
TRR-44		0, 0.04, 0.08, 0.16, 0.31 mg/kg; subcutaneous; once daily; day 6-18 of gestation n=/dose		The dosing regimen was repeated for the second and third successive generations (G ₂ and G ₃). Decreased number of pregnancies Increased number of stillborns No effect on litter size No effect on the incidence of malformations <i>(Current Reviewer's Comments: The expression of data in absolute numbers, rather than per litter, makes interpretation of the data problematic).</i>						
Dose (mg/kg/day)	N	Pregnant	Deaths	Litter Size (ave.)	Birth Weight (ave.)	Stillborn	Resorptions	Malformations		
G₁										
0	100	80	0	8.7	5.7	60/703	0	0		
0.04	11	6	1	9.0	5.8	11/54	0	0		
0.08	11	2	4	10.0	5.4	11/20	1	0		
0.16	12	3	5	14.0	5.0	0/42	1	0		
0.31	6	0	2	-	-	-	-	-		
G₂										
0	50	44	0	10.2	5.6	24/449	0	0		
0.04	6	4	2	13.0	5.7	5/52	0	0		
0.08	6	3	2	12.0	5.6	3/36	0	0		
0.16	3	2	0	11.5	5.2	1/23	0	0		
G₃										
0	200	180	3	11.3	5.1	13/2041	79	7		
0.04	3	3	0	9.7	3.9	0/29	0	0		
TRR-45		0, 0.04, 0.08, 0.16, 0.31 mg/kg; subcutaneous; once daily; day 1-21 of gestation n=/dose		On day 22, pups were delivered by cesarean section. Decreased number of pregnancies (dose-related) Decreased average birthweight of pups (dose-related) Greater percentage of resorptions (dose-related) No effect on litter size No effect on the incidence of malformations <i>(Current Reviewer's Comments: The expression of data in absolute numbers, rather than per litter, makes interpretation of the data problematic).</i>						
Dose (mg/kg/day)	N	Pregnant	Deaths	Weight change /dam	Litter Size (ave.)	Birth Weight (ave.)	Still-born	Resorp-tions	Malform-ations	
0	200	180	3	62	11.3	5.1	13/2041	79	7	
0.04	20	13	3	61	11.6	4.5	0/151	16	0	
0.08	20	9	1	48	11.6	4.3	0/105	21	0	
0.16	20	10	2	42	10.6	3.7	0/106	13	0	
0.31	20	1	3	52	12.0	2.7	0/12	0	0	

Study	Dose Route	Findings
TR-45 Janssen	0, 0.16, 0.32, 0.64, 1.25 mg/kg; subcutaneous; once daily; day 1-21 of gestation n=200/control n=20/fentanyl dose	Decreased number of pregnancies Decreased average weight of pups Increased number of resorptions (doses-related) No effect on litter size <i>(Current Reviewer Comment: No data in review, but this study may have been the basis for label statements in Oralet label NDA 20-195, especially the 1.25 mg/kg dose..)</i>
Fujinaga et al. (1986)	0, 0.01, 0.1, 0.5 mg/kg; subcutaneous; once daily; n=43/control n=28/fentanyl dose	Doses were administered 2 weeks prebreeding, during breeding, and Days 1-21 of pregnancy for all groups <i>(Current Reviewer Comments: The results of this study were not presented in previous reviews.)</i>

Published scientific studies:

Study/Reference	Methods	Findings/Conclusions
Fujinaga et al. (1986) Would satisfy Segment I (fertility) study in females and the Segment II (Embryo-fetal development) requirements	female Sprague-Dawley rats 10, 100 or 500 µg/kg/day via mini osmotic pumps Treated beginning 2 weeks prior to breeding and during the entire period of pregnancy	"fentanyl is devoid of adverse reproductive effects in this strain of rats [Sprague-Dawley] up to dosages of 500 µg/kg/day administered by osmotic minipumps"
Fujinaga and Mazze, (1988) adjunct to a Segment 2 study	postnatal mortality of a single dose of fentanyl (500 µg/kg/day), subcutaneously via the mini-osmotic pumps, continuously (24 hr/day) treated from day 5 of gestation to day 20, when cesarean sections were performed.	There were no effects of fentanyl treatment on postnatal mortality No teratogenic effects
Mazze et al, (1987)	500 µg /kg/day continuously infused via miniosmotic pumps from day 7 to 21 of gestation, when cesarean sections were performed	No reproductive or teratogenic effects in the fentanyl only treated group
Bruce et al. 1983	Sea urchin eggs exposed to fentanyl at concentrations of 3.3 and 33 nM	No adverse effects on fertilization or cell division
Martin and Jurand, 1992 Anesthesia 47:473-476	Fetal mice exposed to fentanyl Maternal doses 12-16 mg/kg, IP On gestation day 9	No teratogenic effects
Craft et al. 1983 Anesthesia Analgesia 62:894-8.	Sheep 50, 75, 100 ug IV on gestation days 124 to 138 Cardiovascular parameters measured for 2 hrs post-drug administration	No effect on uterine blood flow and uterine tone No cardiovascular and acid-base effects in maternal or fetal sheep

2.6.6.9 Discussion and Conclusions

No new toxicology studies were submitted in support of NDA 21-947. The toxicity of fentanyl is fairly well characterized. Based upon extensive human experience with this drug, additional repeat-dose toxicology studies were not required for this 505(b)(2) submission. During early meetings, the Sponsor indicated "to the best of their knowledge, no suitable model for examining the tolerability and toxicity of repeated administration via the oral transmucosal route has been developed." In animal studies, doses were usually administered by subcutaneous administration to attempt to mimic transmucosal absorption while avoiding first pass liver metabolism.

General toxicology, Single Dose Studies:

No additional single-dose toxicity studies were conducted. NDA 20-747 for Actiq® did not contain single-dose toxicology data, but referenced information contained in NDA 20-195 for Oralet and published literature. The Oralet NDA also lacked single-dose toxicology data, and therefore referenced data found in the published literature, including information found in the product labels for NDA 16-619 for Sublimaze (fentanyl citrate) Injection and NDA 16-049 for Innovar (fentanyl and droperidol) Injection.

Intravenous doses of 0, 10 and 20 mg/kg were administered to mice. The LD₅₀ value, calculated from survival data 14 days after intravenous injection, was 12 mg/kg. Intravenous injections of fentanyl were lethal for 1 out of 5 mice at 10 mg/kg and for all five mice at 20 mg/kg. Mortality occurred within the first hour after dosing. The fentanyl mortality dose response curve in mice was biphasic following both subcutaneous and intravenous dosing. The mechanism for this biphasic response is not known, but the mortality observed at the low end of the dose-response curve was considered associated with respiratory depression. Fentanyl produced some central opioid-like actions, e.g. excitation, exophthalmos, Straub tail on arched back, corneal opacity, dyspnea, loss of righting reflex, hypertonia and spasms. Mice also exhibited decreased activity or decreased activity followed by increased activity. All surviving mice completely recovered within 6 hours after injection.

Intravenous doses of 0, 1.25, 2.5 and 5 mg/kg were administered to rats. The LD₅₀ value, calculated from survival data 14 days after intravenous injection, was 2.3 mg/kg. In rats, no mortality occurred at a dosage of 1.25 mg/kg. At a dose of 2.5 mg/kg, three out of five rats died immediately after dosing. At a dose of 5 mg/kg, all rats died immediately after administration of fentanyl. Apart from mortality, the following clinical observations were noted at all doses: blockade of cornea and pinna reflexes, dyspnea, loss of righting reflex, muscular rigidity, hypertonia, exophthalmos and salivation. Rats also exhibited decreased activity or decreased activity followed by increased activity. The surviving rats recovered within 1 day after dosing. No abnormalities were found at autopsy in mice or rats.

Fentanyl was tested in dogs by subcutaneous (SC), intramuscular (IM), intra-arterial (IA) and intragastric (IG) administration. Clinical responses were of central nervous system nature, i.e. convulsions, tremors, loss of righting reflex, sedation and respiratory depression in dogs.

In a study of the potential manufacturing impurity or degradant, both fentanyl and had similar pharmacodynamic and toxicity profiles in rats and mice.

General Toxicology, Repeated-Dose Studies:

Repeat-dose toxicity studies with fentanyl citrate were described in NDA 20-747. No additional repeat-dose toxicity studies were submitted in support of NDA 21-947.

The Pharmacology-Toxicology review of NDA 20-747 stated, that at the time of its submission (Nov 1996) there were no long-term studies to assess fentanyl subchronic and chronic toxicity. The NDA 20-747 for Actiq® did not contain repeat-dose toxicology data, but referenced information contained in NDA 20-195 for Oralet, and published literature. The Oralet NDA also lacks repeat-dose toxicology data, and references published literature, including the product labels for Sublimaze (fentanyl citrate) Injection and Innovar (fentanyl and droperidol) Injection.

Genetic toxicology: *In vitro* bacterial mutagenicity and *in vitro* clastogenicity studies were conducted on three impurities or degradants from fentanyl citrate supplied by

Safety qualification for the three impurities was requested due to the presence of a structural alert for mutagenicity. The three compounds and specifications are listed below:

All three impurities or degradants at concentrations of up to 5000 µg/plate were negative for induction of mutations in *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation. These impurities did not induce structural or numerical chromosome aberrations in the *in vitro* cytogenetic assay of human peripheral blood lymphocytes in the presence or absence of metabolic activation at concentrations that produced up to 50% cytotoxicity. As such, these three impurities are considered adequately qualified.

Two other drug substance impurities are listed by _____ that do not contain structural alerts for mutagenicity. These two are listed below:

— NMT —
 — NMT —

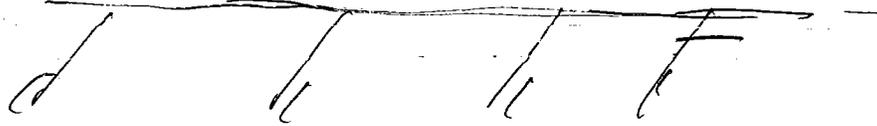
The impurity _____ exceeds ICHQ3A threshold for qualification. However, of the five impurities listed in the summary table below, only _____ has been subjected to some characterization. _____ has been shown to have a pharmacodynamic and toxicologic profile similar to fentanyl in acute studies in rats and mice.

_____ The compound does not contain a structural alert for mutagenicity.

Impurity Name	Chemical Name	Specification	Comment
/	/	NMT	Above ICHQ3A; / /
		NMT	Below ICHQ3A
		NMT	Structural Alert; Negative genetic toxicity data
		NMT	Structural Alert; Negative genetic toxicity data
		NMT	Structural Alert; Negative genetic toxicity data

Carcinogenicity: No long-term animal studies have been completed to date to characterize the carcinogenic potential of fentanyl. However, the compound has tested negative in the standard battery of genetic toxicology assays, and a search of the published literature (reviewer search), did not produce evidence of hyperplasia following chronic administration.

Reproductive toxicology: As mentioned to the Sponsor at the time of the NDA for Actiq® (same Sponsor as this NDA 21-947), there is still no evidence that male fertility studies have been completed.



Special toxicology: Local irritation and sensitization studies were not conducted in animals because the Sponsor was unable to obtain a suitable animal model for oral transmucosal delivery of fentanyl. Local irritant effects were evaluated in the clinical studies, particularly in patients who have preexisting mucositis due to chemotherapy.

2.6.6.10 Tables and Figures

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2.6.7 TOXICOLOGY TABULATED SUMMARY

Name	Fentanyl	Impurity
Chemical Name	N-phenyl-N-[(1-(2-phenylethyl)-4-piperidinyl]-2-hydroxy-1,2,3-propanetricarboxylate (1:1)	
Assigned Limits		
Detected Amounts		
Bacterial Reverse Mutation Assay	Up to 5000 µg/plate	Up to 5000 µg/plate
<i>S. typhimurium</i> TA98 TA100 TA1535 TA1537 <i>E. coli</i> WP2 <i>uvrA</i>	Negative*	Negative
<i>In Vitro</i> Chromosomal Assay	* from IND 65,447 in support of NDA 20-747 for Actiq <i>In vitro</i> mouse (L5178Y TK +/-) lymphoma assay 50 to 600 µg/mL ±S9	Cytotoxic effects: 4-hour incubation ±S9: >50% at > 840 µg/mL 20 hour incubation -S9: > 50% at > 280 µg/mL
Human Peripheral Blood Lymphocytes (not used in fentanyl testing)	Negative* <i>In vivo</i> micronucleus cytogenetic assay 0 to 48 mg/kg, IP Negative*	Cytotoxic effects: 4-hour incubation -S9: >50% at > 2660 µg/mL 4-hour incubation +S9: >50% at 798 µg/mL 20 hour incubation -S9 > 50% at > 266 µg/mL Negative

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Fentanyl citrate, the active ingredient in fentanyl effervescent buccal tablets, is a potent opioid analgesic with pharmacological effects similar to morphine. This application seeks approval of an effervescent fentanyl tablet that is designed to be retained in the oral cavity during disintegration, with absorption of fentanyl across the oral mucosa. The Sponsor claims that effervescence, combined with controlled disintegration, enhances absorption of fentanyl across the oral mucosa compared the currently available formulations. There was one *in vitro* nonclinical published report to support this concept, but no *in vivo* studies.

The most prominent adverse effect of high doses of fentanyl, as with all opioids in general, is respiratory depression, a known extension of the pharmacological action of opioids. As indicated in the table below, the maximal indicated human dose is less than 2-fold of a dose eliciting adverse respiratory, cardiovascular, or neurological effects in animal studies.

Cardiovascular studies in conscious dogs suggest that fentanyl does alter conductivity of heart tissues and lengthens the QT interval, but not until 60 minutes after administration, and only at the highest dose examined 0.05 mg/kg. A prolonged QT interval may lead to severe rhythm abnormalities and death. The cardiovascular effects of fentanyl are not well characterized in nonclinical studies. Most Sponsors relied on older approved fentanyl submissions for much of the basic drug safety information. Those submissions for fentanyl were in settings that involved anesthesia and constant patient monitoring. As new approaches to pain relief have expanded the use of fentanyl in non-hospital settings, the cardiovascular effects need to be more thoroughly elucidated, since the patient population for this product are frequently not under direct observation during the use of this product. Although there does not appear to be a clinical safety signal at this time, the data do not appear to raise concern to such a level that would prevent approval of the NDA.

A genotoxicity program, consisting of an *in vitro* reverse bacterial mutagenicity study and an *in vitro* mammalian chromosome aberration study, was performed on three isolated impurities or degradation products found in the fentanyl citrate API and/or fentanyl effervescent buccal tablets. Each impurity was found to be negative for mutagenicity and for clastogenicity when tested at concentrations up to the limit of the assay. The chromosomal aberration assay used human lymphocytes, and it was found that each of the impurities was toxic to the cultured cells, greatly limiting the maximal dose of impurity that could be tested.

Genetic Toxicology: *In vitro* bacterial mutagenicity and *in vitro* clastogenicity studies were conducted on three impurities or degradants from fentanyl citrate supplied by _____

— The three compounds were

All three impurities or degradants at concentrations of up to 5000 µg/plate were negative for induction of mutations in *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation. These impurities did not induce structural or numerical chromosome

aberrations in the *in vitro* cytogenetic assay of human peripheral blood lymphocytes in the presence or absence of metabolic activation at concentrations that produced up to 50% cytotoxicity.

Unresolved toxicology issues:

One impurity in the drug substance, _____ exceeds ICHQ3A threshold for qualification and has not been tested for potential genetic toxicity. However, _____ does not contain a structural alert for mutagenicity, has been shown to have similar pharmacodynamic and toxicologic effects as fentanyl, _____, and has been present in the fentanyl drug substance used for the Actiq product. As such, the specification of NMT _____ does not raise significant safety concerns, however, the Sponsor should either reduce the specification to NMT _____ or provide a minimal genetic toxicology screen to confirm the safety of the current specification. This can be completed post-approval.

Cardiovascular studies in conscious dogs suggested that fentanyl does alter conductivity of heart tissues and lengthens the QT interval, but not until 60 minutes after administration, and only at the highest dose examined 0.05 mg/kg. The Sponsor's original analysis of the data was inappropriate. Since only mean data was provided for each animal, a complete reanalysis was not possible.

At a pre-IND meeting in Nov 2002 the Division raised concerns that the drug product may produce local tissue irritation _____

Although the local tissue reaction has been addressed with clinical studies, it is not known if there could be any drug product-related change in the rate of healing of these sites. Should there be concerns raised regarding local tissue reactions in the future, preclinical models of wound healing may be considered.

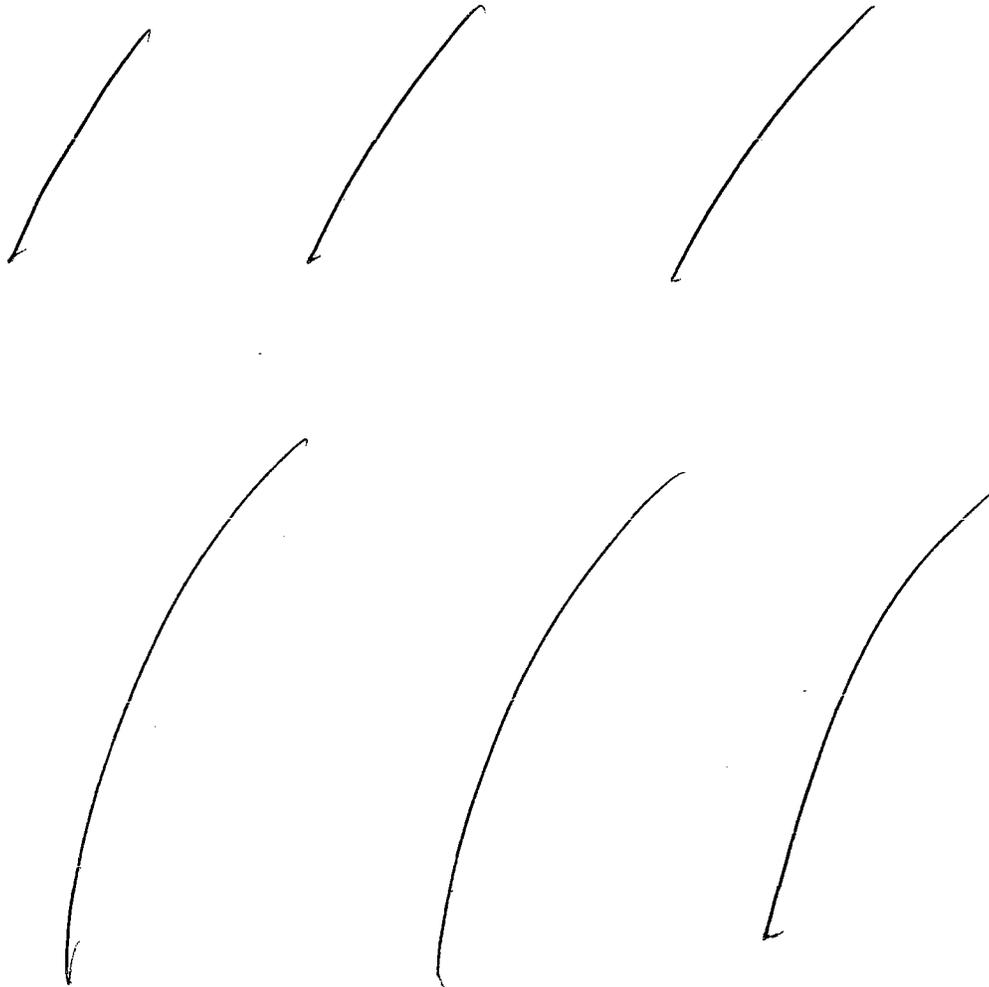
Although fentanyl has been used clinically for many decades, reproductive studies described in the label are incomplete. Studies of male fertility, embryofetal development in a second species, and peri- and post-natal development have not been performed. _____

Recommendations:

From the nonclinical pharmacology and toxicology perspective, the NDA may be approved.

Suggested labeling:

Deletions are indicated by strikeouts
Additions are indicated by blue.



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 Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

Reviewer Signature L.S. Leshin
L.S. Leshin DVM, Ph.D.

Supervisor Signature R. Daniel Mellon Concurrency Yes X No
R. Daniel Mellon Ph.D.

APPENDIX/ATTACHMENTS

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/s/

R. Daniel Mellon
6/22/2006 05:53:32 PM
PHARMACOLOGIST

I concur with Dr. Leshin's recommendation that from the nonclinical pharmacology toxicology perspective, NDA 21947 may be approved. I have submitted Dr. Leshin's review at his request and am signing on his behalf and as the PharmTox Supervisor for DAARP.