CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-338

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-338**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	Original NDA 23-Sept-2003
	Second Cycle 21-Nov-2005
PRODUCT:	**IONSYS™ (fentanyl iontophoretic transdermal system)**
	40 mcg*/activation, Patient-activated
INTENDED CLINICAL POPULATION:	Adult patients with acute pain requiring opioid analgesia
SPONSOR:	ALZA CORPORATION
DOCUMENTS REVIEWED:	EDR 000 23-Sept-2003
	EDR 000 AZ 21-Nov-2005
	N000 BC 14-Mar-2006
REVIEW DIVISION:	Division of Anesthesia, Analgesia, and Rheumatology Products (DAARP)
PHARM/TOX REVIEWER:	Mamata De, Ph.D.
PHARM/TOX SUPERVISOR:	R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR:	Bob A. Rappaport, M.D.
PROJECT MANAGER:	Kimberly Compton

Date of review submission to Division File System (DFS): 19-May-2006

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology toxicology perspective, NDA 21-338 may be approved.

B. Recommendation for nonclinical studies

None

C. Recommendations on labeling

CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY

Studies in animals to evaluate the carcinogenic potential of fentanyl HCl have not been conducted. There was no evidence of mutagenicity in the Ames Salmonella mutagenicity assay, the primary rat hepatocyte unscheduled DNA synthesis assay, the BALB/c 3T3 transformation test, and the human lymphocyte and CHO chromosomal aberration in-vitro assays.

The potential effects of fentanyl on male and female fertility were examined in the rat model via two separate experiments. In the male fertility study, male rats were treated with fentanyl (0, 0.025, 0.1 or 0.4 mg/kg/day) via continuous intravenous infusion for 28 days prior to mating; female rats were not treated. In the female fertility study, female rats were treated with fentanyl (0, 0.025, 0.1 or 0.4 mg/kg/day) via continuous intravenous infusion for 14 days prior to mating until day 16 of pregnancy; male rats were not treated. Analysis of fertility parameters in both studies indicated that an intravenous dose of fentanyl up to 0.4 mg/kg/day to either the male or the female alone produced no effects on fertility (this dose is approximately 1.2 times the maximum available daily human dose on a mg/m^2 basis). In a separate study, a single daily bolus dose of fentanyl was shown to impair fertility in rats when given in intravenous doses of 0.3 times the human dose for a period of 12 days.

PREGNANCY

Teratogenic Effects—Pregnancy Category C

No epidemiologic studies of congenital anomalies in infants born to women treated with fentanyl during pregnancy have been reported.

The potential effects of fentanyl on embryo-fetal development were studied in the rat, mouse, and rabbit models.

Published literature reports that administration of fentanyl (0, 10, 100, or 500 mcg/kg/day) to pregnant female Sprague-Dawley rats from day 7 to 21 via implanted microosmotic minipumps did not produce any evidence of

teratogenicity (the high dose is approximately 1.5 times the maximum available daily human dose on a mg/m^2 basis).

In contrast, the intravenous administration of fentanyl (0, 0.01, or 0.03 mg/kg) to bred female rats from gestation day 6 to 18 suggested evidence of embryotoxicity and a slight increase in mean delivery time in the 0.03 mg/kg/day group. There was no clear evidence of teratogenicity noted.

Pregnant female New Zealand White rabbits were treated with fentanyl (0, 0.025, 0.1, 0.4 mg/kg) via intravenous infusion from day 6 to day 18 of pregnancy. Fentanyl produced a slight decrease in the body weight of the live fetuses at the high dose, which may be attributed to maternal toxicity. Under the conditions of the assay, there was no evidence for fentanyl induced adverse effects on embryo-fetal development at doses up to 0.4 mg/kg (approximately 3 times the maximum achievable human daily dose on a mg/m^2 basis).

There are no adequate and well-controlled studies in pregnant women. IONSYS™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects

Chronic maternal treatment with fentanyl during pregnancy has been associated with transient respiratory depression, behavioral changes, or seizures characteristic of neonatal abstinence syndrome in newborn infants. Symptoms of neonatal respiratory or neurological depression were no more frequent than expected in most studies of infants born to women treated acutely during labor with intravenous or epidural fentanyl. Transient neonatal muscular rigidity has been observed in infants whose mothers were treated with intravenous fentanyl.

The potential effects of fentanyl on prenatal and postnatal development were examined in the rat model. Female Wistar rats were treated with 0, 0.025, 0.1, or 0.4 mg/kg/day fentanyl via intravenous infusion from day 6 of pregnancy through 3 weeks of lactation. Fentanyl treatment (0.4 mg/kg/day) significantly decreased body weight in male and female pups and also decreased survival in pups at day 4. Both the mid-dose and high-dose of fentanyl animals demonstrated alterations in some physical landmarks of development (delayed incisor eruption and eye opening) and transient behavioral development (decreased locomotor activity at day 28 which recovered by day 50). The mid-dose and the high-dose are 0.3 and 1.5 times the maximum available daily human dose on a mg/m^2 basis.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The pharmacologic and toxicologic properties of fentanyl are well characterized. The Sponsor has right of reference to the study reports originally generated by Janssen Research Products in support of other fentanyl containing drug products. Therefore, the nonclinical development program for IONSYS consisted primarily of local tissue assessments of the final product and various components of the device.

Nonclinical toxicology studies in the IONSYS program evaluated the potential for skin irritation and sensitization associated with the system and its components. The durations of exposure and current densities were selected to be representative of human treatment with IONSYS.

The commercial E-TRANS anode and cathode hydrogel formulations were categorized as mild irritants in single-application skin irritation studies (anode, Study TR-96-1561-052; cathode, Study TR-98-1561-031). The commercial anode formulation was evaluated in New Zealand White rabbits treated for 14 hours at a current density of 0.08 mA/cm^2 (i.e., 80 uA/cm^2) resulting in a mean total fentanyl dose of 3.5 mg (0.073 mg/k/h). The commercial cathode formulation was evaluated in New Zealand White rabbits and hairless guinea pigs for approximately 13 hours at a current density of 0.07 mA/cm^2 (70 uA/cm^2).

The initial sensitization study in hairless guinea pigs evaluated an IONSYS system that had early-formulation hydrogels (TR-92-1561-022). The system was categorized as a mild to moderate sensitizer, but the findings of the study were influenced by the fact that irritation contributed to the observed responses. The systems in this study delivered fentanyl at a dose of 1.1 mg/kg over 8 hours at a maximum current density of 0.1 mA/cm^2 (100 uA/cm^2). A second sensitization study in hairless guinea pigs evaluated placebo E-TRANS systems with hydrogel formulations that were more similar to the commercial formulation (TR-97-1561-01 1). No evidence of sensitization was found in animals induced and challenged with the anode or cathode hydrogels, categorizing them as weak sensitizers.

Additional studies found no evidence of sensitization in hairless guinea pigs induced and challenged with the bactericide in the cathode hydrogel (cetylpyridinium chloride) (TR-96-1561-017) or with extracts of the skin adhesive used in the E-TRANS system (TR-96-1563-012). Extracts of the —— (polacrilin) had some potential to induce sensitization, but at the concentrations proposed for clinical use, the potential was low (TR-96-1561-016, TR-96-1561-048). Therefore, polacrilin was determined to be an acceptable —— use in E-TRANS hydrogels.

Extracts and samples of system components in contact with the hydrogels met either USP Class V-50°C or VI-50°C requirements and were therefore judged to be safe for use in the E-TRANS system.

A single dose of 0.5 mL of fentanyl (0.1 mg base/mL) was injected into the sacrospinalis muscle of the rabbit to evaluate muscle irritation potential of fentanyl. Positive controls were administered 0.5 mL doses of tetracycline (125 mg/mL) and pyralgin (500 mg/mL). The rabbits were killed 72 hours later and the degree of irritation determined by gross observation of serially sectioned muscle. The irritation responses were none to slight for fentanyl, moderate to severe for pyralgin while tetracycline showed a severe response.

The cytotoxicity of fentanyl was evaluated in the tissue culture with human keratinocytes. Cell viability was determined by using a tetrazolium dye that is reduced to a blue formazan by living cells. The resultant color is measured with a spectrophotometer. There was no cytotoxicity at concentrations below 1 mM (0.3 mg/mL).

Impurities in the fentanyl HCl are monitored in the drug substance by [redacted], and in the drug product by ALZA. Unknown impurities are also monitored routinely to ensure that their levels do not exceed the specified limits. The impurities are detectable and quantified in the drug substance by ALZA via high-performance liquid chromatography (HPLC) methods, ALZA Analytical Methods (AAMS)[redacted]. None of the impurities have been detected in the drug substance at levels close to [redacted] (the specification limit). The two impurities not specifically monitored in the drug substance are [redacted]. The [redacted] impurity is monitored in the finished product stability, and the [redacted] impurity has been monitored by the [redacted] and reported in [redacted] Certificate of Analysis. This impurity is historically below the reporting threshold of 0.05% for drug substance as defined in the International Conference on Harmonization (ICH) Q3A. ALZA does not monitor for these two impurities specifically but reports any impurities detected above [redacted] with a limit of [redacted]

In the original submission dated September, 2003 impurities of the drug substance were found to be inadequately evaluated. The Sponsor was advised to reduce the specification for the drug substance impurities to NMT [redacted] or to provide adequate qualification of the impurities via repeat-dose toxicity study in a single species and a minimal genetic toxicology screen. With the current submission dated November, 2005, the Sponsor provided acceptable specification criteria for the drug substance with the non genotoxic and genotoxic impurities. There were three potential genotoxic impurities in the drug substance namely [redacted], [redacted]. The limit of each of these impurities were set as [redacted] in the drug substance which is equivalent to [redacted] (considering the maximal daily dose of fentanyl as 3.2 mg), the total of these potential genotoxic impurities thus results into [redacted] day (<1.5 µg/day, a limit set by the current draft guidance for qualification of the genotoxic impurities). Therefore these three

potential impurities are below considered qualified in the drug substance. Among these three potential genotoxic impurities only one of them — was found as degradant. In the drug product the acceptability criteria for — is set as — which is — µg/day. In the opinion of this reviewer the limit of specification is acceptable because the actual genotoxic potential of this impurity is not known (predicted based on chemical structure). Further, the specification in the draft guidance is based on the risk of developing cancer following exposure to the compound over the entire life time, which is in contrast to what is proposed for fentanyl under the current formulation. Although this product might be used more than 72 hrs intermittently (under this submission the duration of the administration of fentanyl is 72 hrs only), chronic use of fentanyl with this formulation is highly unlikely. The other two potential genotoxic impurities are under the 1.5 µg limit in the drug product. The draft EMEA guidance for genotoxic impurity qualification is still in the draft stage and the proposed limit of NMT 1.5 µg/day is based on the theoretical values of toxicological threshold concern which is still under discussion in the scientific community. — slightly exceeds 1.5 µg/day, the proposed limit in the draft guidance for genotoxic qualification — is a — and predicted to be structural alert with genotoxic potential. No studies were done under this submission to find out whether the compound is actually genotoxic or not. So, attempt could be made for qualifying — as a non-genotoxic impurity. The amount of fentanyl/unit in this product is 10.8 mg. Therefore, with a — specification, the amount of — will account for a maximum of — µg, this is acceptable as per ICH Q3BR guidance. According to the Sponsor, fentanyl related product passes through the skin faster in this IONSYS system. Therefore, chances of passive diffusion resulting in the depot formation in skin for the impurities are minimal. From the previous experience with fentanyl (which has similar impurities) products, clearance of fentanyl after systemic absorption was quick. Therefore, fentanyl related product in the IONSYS system seem to pose minimal toxicological concern.

B. Pharmacologic activity

Fentanyl is a synthetic opioid agonist that interacts primarily with the µ-opioid receptor subtype to produced analgesia and sedation. It increases the patient's tolerance for pain and decreases the perception of suffering, although the patient may still recognize the pain itself. Opioids work to relieve nociceptive pain but are not very effective for neuropathic pain. In addition to analgesia µ-opioid agonists such as fentanyl produce drowsiness, changes in mood, respiratory depression decreased gastrointestinal motility, nausea, vomiting and alterations in the endocrine and autonomic nervous system.

High doses of fentanyl produce muscle rigidity possibly due to effects of opioids on dopaminergic transmission in the striatum. The euphoric effects of opioids are believed to be mediated in part via interaction with opioid receptors located in the

ventral tegmental area (VTA) leading to the enhancement of dopamine release in the nucleus accumbens. Opioid receptors in the locus coeruleus appear to inhibit the adrenergic neurons thought to play a role in feelings of alarm, panic, fear and anxiety. Opioids act within the hypothalamus to regulate body temperature (generally temperature decreases slightly, but at higher doses temperature may increase). Opioids inhibit neuroendocrine systems including gonadotropin-releasing hormone (GNRH) and corticotropin-releasing factor (CRF) thereby decreasing release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), and β-endorphin. This leads to decrease plasma levels of testosterone and cortisol. Opioids increase circulating levels of prolactin. Opioids such as fentanyl lead to constriction of the pupil (miosis) via increased parasympathetic nerve activity innervating the pupil. Pinpoint pupils are pathognomonic for toxic doses of μ-opioid agonists; however mydriasis can develop upon asphyxia.

C. Nonclinical safety issues relevant to clinical use

The amount of fentanyl absorbed systemically in human from a 10-minute dose from the IONSYS system increased proportionally with the applied current. The desired nominal 40 μg E-TRANS dose was delivered systemically by a 170 uA current and a 2.75 cm^2 anode surface area at Hour 23 following a standard dosing regimen of 2 sequential doses every hour for 23 hours. Mild to moderate irritation was observed with the current formulation in hairless guinea pig local tolerance studies with 70-230 uA current and comparable anode surface. The irritation is characterized by erythema. Similar local toxicity is expected in human population. Although no hypersensitivity was observed with fentanyl, polacrilin — extract at a concentration of 50% administered intradermally induced some hypersensitivity characterized by irritation and allergic reaction. These data indicate that the polacrilin has some potential to induce sensitization at high exposures, but at concentrations proposed for clinical use (—), the potential is reduced.

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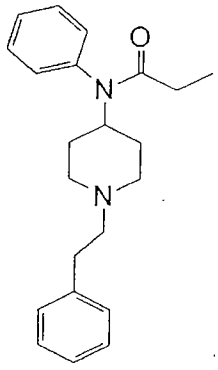
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-388
Review number: 2 (NOTE: This review incorporates the data in review 1).
Sequence number/date/type of submission: 000, 23rd September 2003
Information to Sponsor: Yes (X) No ( )
Sponsor and/or agent: ALZA CORPORATION
Manufacturer for drug substance: —
Reviewer name: Mamata De, Ph.D.
Division name: Anesthesia, Analgesia, and Rheumatology Products (DAARP)
HFD #: 170
Review completion date: 19-May-2006

Drug:

Trade name: **IONSYS™ (fentanyl iontophoretic transdermal system)**
40 mcg*/activation
Patient-activated
Generic name: Fentanyl hydrochloride
Code name: E-trans fentanyl
Chemical name: N-(l-phenethyl-4-piperidyl)propionanilide hydrochloride
Molecular formula/molecular weight: 372.93, $C_{12}H_{28}N_2O$ HCl



Structure:

Relevant INDs/NDAs/DMFs:

NDA 16-619	Sublimaze (fentanyl citrate) injection (AKORN MFG, 9/06/1967).

NDA 16-049	Innovar (fentanyl citrate + droperidol) Injection (AKORN MFG, 1/31/1968).
NDA 19-101	Fentanyl Citrate Injectable 0.05 mg base/mL (Elkins Sinn, 7/11/1984).
NDA 19-813	Duragesic@ Transdermal Fentanyl Patch (ALZA Corp, 8/7/1990).
NDA 20-195	Fentanyl Troche/Lozenge 0.1 and 0.2 mg base (ANESTA, 8/28/1991).
NDA 20-747	Actiq (oral transmucosal fentanyl citrate, Eq. 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 mg base) Lozenge (Anesta, 11/13/1997).
DMF	
DMF	
DMF	

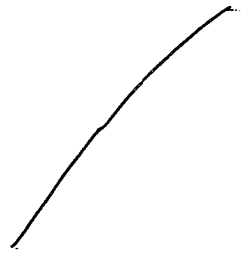
Drug class: Opioid Agonist, Narcotic.

Intended clinical population: From the final drug product label:

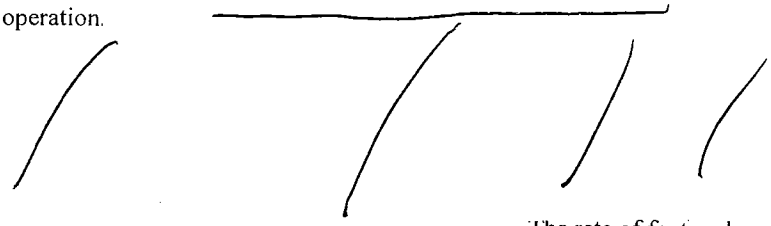
> IONSYS™ is indicated for the short-term management of acute postoperative pain in adult patients requiring opioid analgesia during hospitalization. Patients should be titrated to an acceptable level of analgesia before initiating treatment with IONSYS™. IONSYS™ is not intended for home use and is, therefore, inappropriate for use in patients once they have been discharged from the hospital. It is not recommended for patients under the age of 18 years (see **WARNINGS and PRECAUTIONS**).

Clinical formulation:

> IONSYS is a novel electrically assisted transdermal delivery system designed for the management of acute pain in patients requiring opioid analgesia. This product is recommended for use in a medically supervised setting. The system is patient activated and provides on-demand systemic delivery of fentanyl by means of a small electric current, a process known as electrotransport.
>
> A schematic of the IONSYS system's transdermal electrotransport of fentanyl cation is shown below. A source of electrical energy, such as a battery, is part of a printed circuit board assembly (PCBA) that supplies electric current to the body through two electrodes. The anode electrode and hydrogel deliver the positively charged therapeutic agent into the body. The cathode electrode and hydrogel close the electrical circuit. Each hydrogel is placed in contact with the patient's skin and contains either the drug (for the anode electrode assembly) or a pharmacologically inactive electrolyte (for the cathode electrode assembly)



During operation.



. The rate of fentanyl delivery is directly proportional to the magnitude of the applied current.

E-TRANS (fentanyl HCl) Systems were initially investigated in two dosage strengths, designed to provide nominally 25 or 40 ug of fentanyl per dose delivered on demand. The E-TRANS (fentanyl HCl) system that is the subject of this NDA provides a 40 ug dose of fentanyl (base equivalent) per activation, which is delivered over a 10-minute period with a current of 170 uA. To initiate administration of a fentanyl dose, the patient must firmly press the recessed button on the top of the system twice within 3 seconds. An audio tone (beep) indicates the start of delivery of each dose, and a red light from a light emitting diode (LED) remains on throughout the 10-minute dosing period.

Dosing may be completed up to 6 times each hour, with a maximum of 80 doses available from each system. Each system operates for 24 hours, or until the 80 doses have been administered whichever occurs first. The system becomes inoperable after this period. The maximum nominal amount of fentanyl that can be administered from a single system over 24 hours is 3.2 mg (80 doses of 40 ug each).

The E-TRANS (fentanyl HCl) System consists of a top housing assembly (the device component) and a bottom housing assembly (the drug component). The top housing assembly (THA) consists of the top housing, an plastic component that protects the electronics, and a printed circuit board assembly (PCBA) that contains the integrated circuit (IC). The IC of the E-TRANS

(fentanyl HCl) System controls system function. [redacted]. The bottom housing assembly consists of a [redacted] lastic housing with two hydrogel cavities; each cavity has an electrode and is filled with a hydrogel formulation. One cavity contains the anode hydrogel formulation with a nominal fentanyl hydrochloride content of 10.8 mg. Fentanyl hydrochloride is supplied by [redacted] The other hydrogel cavity contains a hydrogel formulation with [redacted] s.

[redacted] A release liner (protective liner) covers the skin adhesive and both hydrogel formulations and is removed before patient use. The E-TRANS (fentanyl HCl) System is packaged in a [redacted] foil pouch.

A diagram of the components of the device is provided below, reproduced from the Sponsor's submission.

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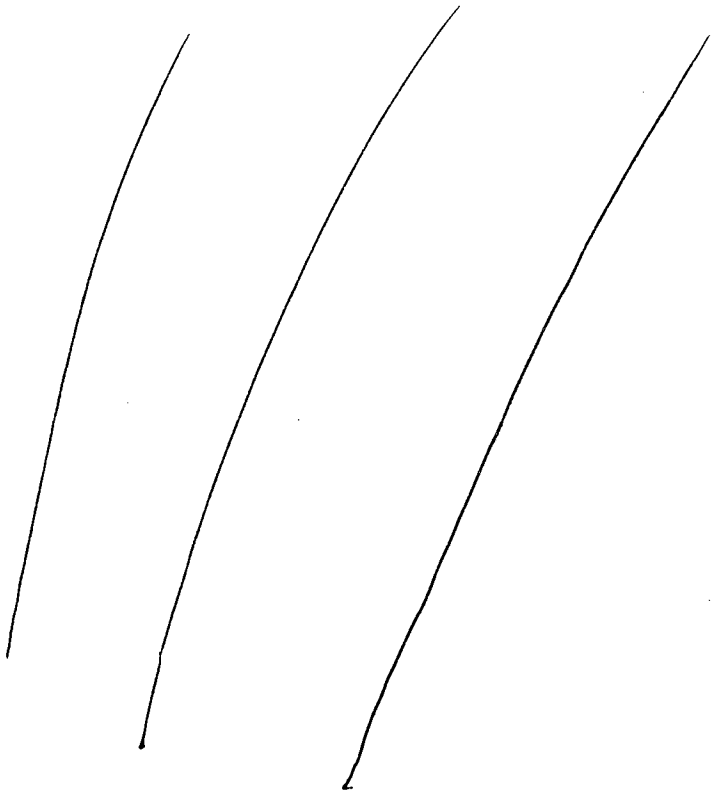
Detailed View of E-TRANS® (fentanyl HCl) System

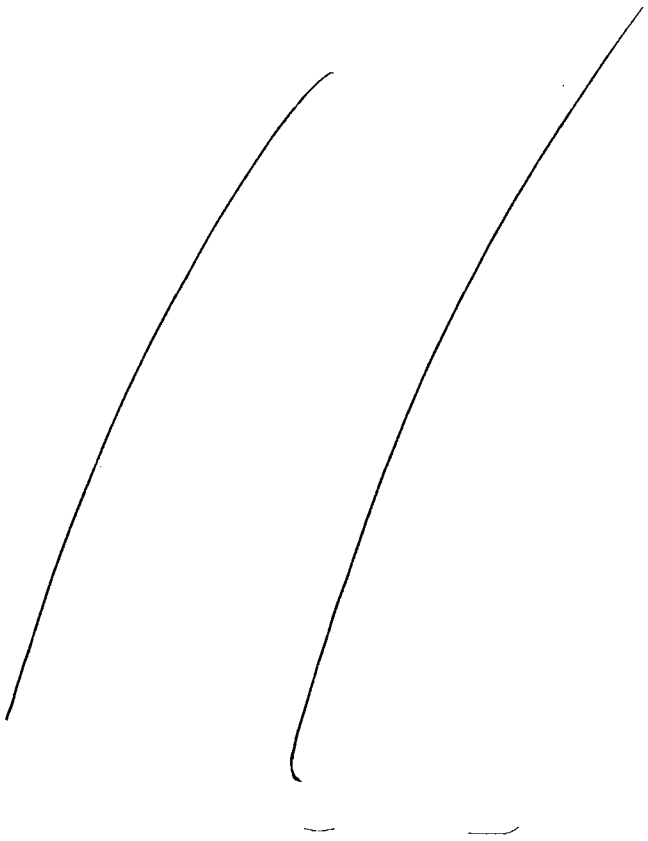


The ingredients which make up the specific components are summarized in the Sponsor's table below:

Unit Formula for E-TRANS® (fentanyl HCl) System

Names of Ingredients		Nominal Quantity	Function	Reference to Standard
Hydrogel Composition				
	Fentanyl Hydrochloride	10.8 mg	Active Drug Substance	In-house Standard
	Polacrilin			In-house Standard
				USP, Ph Eur
	Sodium Hydroxide			In-house Standard
	Polyvinyl Alcohol			In-house Standard
	Purified Water			USP, Ph Eur
	Sodium Chloride			USP, Ph Eur
				USP
				In-house Standard
	Citric Acid			USP
	Cetylpyridinium Chloride			USP, Ph Eur
Unit Components (one piece each)				
Top Housing Assembly	Assembly, Top Housing, E-TRANS 40 µg	Patient interface and electronic control		In-house Standard
Bottom Housing	Housing, Bottom, 2.78 cm^2, Red, E-TRANS	Housing for the electrodes and hydrogels		In-house Standard
Anode Electrode				In-house Standard
Cathode Electrode				In-house Standard
Skin Adhesive	Film, Adhesive	Adheres system to skin		In-house Standard
Release Liner	Film, Siliconized	Protective liner		In-house Standard

Detailed View of E-TRANS® (fentanyl HCl) System



Route of administration: Transdermal

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

Data reliance: This is a 505(b)(1) submission. Alza Corporation cross-references NDA 19-813 (Duragesic, Alza Corporation), which was also a 505(b)(1) submission.

Studies reviewed within this submission:

A. Studies with E-TRANS Systems Containing Fentanyl

TR-92-1561-021 Primary Skin Irritation Study of Electrotransport Therapeutic Systems Containing Fentanyl in Guinea Pigs

TR-92-1561-022 Experimental Sensitization of Fentanyl Using Electrotransport Therapeutic Systems in the Hairless Guinea Pig

TR-92-1561-023 Fourteen Day Subchronic Skin Irritation Study of Electrotransport Therapeutic Systems Containing Fentanyl in Guinea Pigs

TR-92-1561-053 Primary Skin Irritation Study of Electrotransport Therapeutic Systems Containing Fentanyl in Rabbits

TR-96-1561-052 Primary Skin Irritation Study of E-TRANS Systems with Anode Hydrogels Containing Fentanyl on Rabbits

B. Studies With E-TRANS Systems Containing No Fentanyl

TR-92-9999-014 Visual (Macroscopic) and Histological Evaluation of Guinea Pig Skin Sites Following Single and Multiple Applications of Electrotransport Therapeutic Systems

TR-93-1561-048 Primary Skin Irritation Study of Electrotransport Therapeutic Systems Placebo Anode Hydrogels in the Hairless Guinea Pig

TR-97-1561-011 Evaluation of the Sensitization Potential of Electrotransport Systems Containing Placebo Anode Hydrogels (Histidine and Polacrilir — in the Hairless Guinea Pig

TR-98-1561-031 A Primary Skin Irritation Study of E-TRANS (placebo) Systems Containing Cetylpyridinium Chloride (CPC) and — Adhesives on Hairless Guinea Pigs and Rabbits

C. Studies with E-TRANS Components

TR-90-1550-018 USP XXII: Biological Testing of —

TR-93-2420-068 USP XXII: Biological Testing of —

TR-93-8888-059 USP XXII: Biological Testing of Lower Housing, Acute System,

TR-94-1561-017 USP XXII: Biological Testing of

TR-94-1561-020 USP XXII: Biological Testing of

TR-95-1561-031 USP 23: Biological Testing of Lower Housing, Acute System, Code

TR-96-1561-011 USP 23: Biological Testing of Film, PIB Adhesive, Code

TR-96-1563-012 Evaluation of Film, Polyisobutylene (PIB) Adhesive ( ) Extract for Delayed Contact Hypersensitivity in Guinea Pigs

TR-96-1561-016 Evaluation of Polacrilin Resin Extract for Delayed Contact Hypersensitivity in Guinea Pigs

TR-96-1561-017 Evaluation of Cetylpyridinium Chloride for Delayed Contact Hypersensitivity in Guinea Pigs

TR-96-1561-044 USP 23: Biological Testing of PIB k, Code Number

TR-96-1561-048 Evaluation of Polacrilin Resir Extract Dilutions for Delayed Contact Hypersensitivity in Guinea Pigs

TR-97-1561-009 USP 23: Biological Testing of Housing, Bottom , Red, E-TRANS (Acute) 2

TR-97-1561 -01 0 USP 23: Biological Testing of Housing, Bottom,

TR-98-1561-023 In Vitro Biological Testing of (Lot No. T790, Part No.

TR-99-1561-056 ISO In Vivo Biocompatibility Testing of Housing, Top, E-TRANS (Acute), Red

TR-99-1561-057 ISO In Vivo Biocompatibility Testing of Housing, Top, E-TRANS (Acute),

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

References F through G were used to summarize this section. There were no specific pharmacology studies submitted with this NDA application.

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 but has a shorter duration of action after a single bolus administration. Antinociceptive effects can be observed after systemic, epidural, intrathecal, and transdermal treatment.

As other opioids, fentanyl binds to opiate receptors. It has a higher binding affinity for μ, as compared to κ- and opioid δ-induced secondary side effects such as respiratory depression, constipation, physical dependence and euphoria. However, as compared to morphine, fentanyl is reported to have 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. Because fentanyl can be used as a discriminative stimulus and substitutes for morphine in morphine-dependent animals, there is a risk for abuse liability.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

All clinically used opioids belong to one of two chemical classes. First, there are the morphine-skeleton-related agents such as morphine. Secondly, there are the phenylpiperidine analgesics, like meperidine (pethidine), fentanyl, sufentanil and alfentanil. All these opioids have different physicochemical properties, such as molecular weight, pKa, percentage ionization at pH 7.4, and lipophilicity. These differences may result in the opioids having different pharmacodynamic profiles.

As other opioids, fentanyl binds to opiate receptors. Fentanyl is a potent and relatively selective agonist at u opiate receptors. Its effects are readily reversed by opiate-antagonists such as naloxone.

In animals, fentanyl results in antinociceptive effects after intravenous, subcutaneous, intraperitoneal, oral, epidural, and intrathecal and transdermal administration. The potency and the duration of action of fentanyl depend on the animal species and the route of administration and the methods used to evaluate the antinociceptive properties of fentanyl.

The lowest ED_{50} values of fentanyl, meperidine and morphine after a single intravenous bolus injection to reach a pronounced antinociceptive effect in the tail withdrawal

reaction test in rats are shown in the table below. Also shown are the potency ratios of the compounds with morphine as a reference, the LD_{50} of the drugs, the safety margin (LD_{50}/ED_{50}), the maximal onset of analgesia (peak effect), and the estimated duration of effect at twice the ED_{50}.

Review of the activity of fentanyl, meperidine and morphine after a single intravenous bolus injection in the tail withdrawal reaction test in rats.

Presented are the lowest ED_{50} values to reach a pronounced antinociceptive effect, the potency ratio of the compounds with morphine as a reference, the LD_{50} of the drugs, the safety margin (LD_{50}/ED_{50}), the maximal onset of analgesia (peak effect), and the estimated duration of effect at twice the ED_{50}.

compound	ED_{50} (mg/kg)	potency	LD_{50} (mg/kg)	safety margin	peak effect (min)	duration (min)
fentanyl	0.011	291.8	3.1	281.8	4	30
meperidin e	6.04	0.5	29	4.8	4	33
morphine	3.21	1	223	69.5	30	90

After subcutaneous treatment of rats in the same test procedure, a lowest ED_{50} of 0.026 mg/kg was reported with a peak effect at 36 min and a duration of action of 97 min. Fentanyl was 196 times more potent than morphine. The subcutaneous safety margin was 365 for fentanyl versus 116 for morphine. In mice, in which the response to a noxious stimulus was assayed by a modification of Haffner's method, the corresponding safety margin following subcutaneous administration was 775 for fentanyl and 31.3 for morphine. The pharmacodynamic properties of fentanyl after passive transdermal delivery are identical to those after other route of administration is well studied. Transdermal iontophoresis of fentanyl to the abdominal skin of hairless rats also resulted in an antinociceptive effect in the tail flick test. After a single systemic administration in animals, fentanyl is approximately 40 to 292 times more potent than morphine, but with a shorter duration of action.

Drug activity related to proposed indication:

Fentanyl is used parenteral as an anesthetic and analgesic for more than 20 years. The efficacy and safety of intravenous (IV) fentanyl and patient-controlled analgesia (PCA) devices are well established, and the analgesic activity of passive transdermal delivery of fentanyl has been demonstrated in clinical trials. Electrically assisted transport (electrotransport or iontophoresis) is a means of providing controlled delivery of drugs across the skin. By the application of a small amount of current, an electrotransport system can provide relatively rapid, on-demand, noninvasive, systemic drug

administration. Numerous review articles cite iontophoresis of various compounds through the skin.

ALZA Corporation has developed the IONSYS (fentanyl HCl patient-controlled transdermal system), which is a needle-free, self-contained, on-demand, drug delivery system designed for the management of acute pain (e.g., postoperative pain). The IONSYS system is a self-adhesive unit approximately the size of a credit card and is worn on the upper outer arm or chest. The system is designed to deliver a 40-μg dose of fentanyl when the patient presses the activation button twice. Each dose is delivered over 10 minutes, and up to six doses per hour are available. IONSYS may be used for up to 24 hours, or until it delivers 80 doses, whichever comes first. The system has been shown to provide pain relief for 24 hours with a safety and efficacy profiles comparable to that of IV PCA morphine.

2.6.2.3 Secondary pharmacodynamics

Fentanyl stimulates the μ-opioid receptor, resulting in specific secondary effects such as respiratory depression, bradycardia, constipation, physical dependence and euphoria. However, it was shown to have a relatively high safety margin towards cardiovascular, metabolic and neurologic effects. All these effects are well documented in both preclinical and clinical studies.

2.6.2.4 Safety pharmacology:

No safety pharmacology studies have been submitted with the present study, following is the brief summary of the results from the safety pharmacology studies from the published literature.

Neurological effects:

Behavioral effects

In dogs, intramuscular administration of doses varying from 0.0125 to 1.0 mg/kg fentanyl citrate produced similar effects at all dose levels. Within 10 min after the administration of fentanyl citrate, the following signs were observed: decreased motor activity, ataxia, and decreased responsiveness to auditory and painful stimuli, bradycardia, respiratory depression, salivation and defecation. The intensity and duration of these effects increased with the dose used. Nalorphine, injected intravenously at the peak of the depression of the central nervous system, resulted in an immediate reversal of the central depression induced by fentanyl.

Neurological and metabolic effects

When intravenous doses of fentanyl that produce severe convulsions are compared with those required to obtain deep surgical analgesia, a safety margin for neurological toxicity can be calculated. In dogs, these ratios are 160 for fentanyl and 72 for morphine. The safety margins for metabolic toxicity, calculated as the ratio between the doses producing severe metabolic side effects such as acidosis and hypermetabolism, and those needed for deep surgical analgesia, are 60 for fentanyl and 13 for morphine.

Cardiovascular effects:

The cardiovascular effects of fentanyl have been studied extensively. The cardiovascular stability of fentanyl contributed to its safe use at high doses in cardiac surgery. In dogs, an inverse relationship was described between the cardiovascular toxicity and analgesic potency of opioid. A high dose of 5.0 mg/kg intravenous morphine, necessary for deep surgical anesthesia, produced a severe tachycardia and vasodilatation with a depression of the myocardial contractility and impairment of the lung circulation. This unfavorable cardiovascular condition does not occur after equianalgesic doses of 0.05 mg/kg intravenous fentanyl. Here the cardiovascular system remains stable, with the advantage of an increased venous return and myocardial contractility. The safety margin in dogs, expressed as the ratio between the doses producing severe cardiovascular side effects and doses necessary for deep surgical analgesia, was 5 times higher for fentanyl than for morphine.

Pulmonary effects:

Opioid receptors are found in areas involved in the regulation of respiration such as the medulla. Fentanyl interacts with these receptors and may cause respiratory depression. Respiratory depressing effects for fentanyl have been described for several species, including The degree of respiratory depression observed with opioids depends on various variables, including the opioid used, the plasma concentration reached, the route of administration and the duration of opioid intake since tolerance develops to the respiratory depressants effects of opioid. The respiratory effects of fentanyl in rats after subcutaneous and epidural injection were evaluated in different studies. In rats breathing 8% carbon dioxide, the subcutaneous dose of fentanyl that reduced minute volume by 25% as compared to control values (ventilation ID_{25}) was 0.030 mg/kg, which is 2.5 times the ED_{50} for subcutaneous analgesia (0.012 mg/kg). The ventilation ID_{25} after epidural administration was 0.056 mg/kg, which was about 18 times greater than the epidural analgesia ED_{50} of 0.0032 mg/kg. With subcutaneous morphine or meperidine, the corresponding ratios between respiratory depression and analgesia were 1.6 and 1.7, respectively.

Gastrointestinal effects:

Constipation: Opioids are known to suppress the output of fecal pellets in mice. At equi-analgesic doses, fentanyl had a less pronounced constipating effect than morphine, similar results were observed in rats.

Emesis: Fentanyl has been demonstrated to produce less nausea and vomiting than morphine. For example, in a crossover study in Mongrel dogs doses of 1.0 and 2.50 mg/kg fentanyl citrate given intramuscularly were devoid of emetic activity. Following the same doses of morphine, emesis was generally observed within 15 min in 60 to 90 % of the dogs.

Abuse Liability:

Fentanyl, like other opioids, has intrinsic discriminative stimulus properties in various species, indicating a potential for abuse. Fentanyl also suppresses the withdrawal symptoms of morphine-addicted monkeys at about 1/75 th of the morphine dose. When Innovar (fentanyl and droperidol) was injected subcutaneously twice a day for 14 days at the highest tolerated dose, signs of abstinence were very mild after abrupt withdrawal of the product.

Tolerance has been reported to develop to the antinociceptive effects of opioids after systemic administration. However, the degree of tolerance after subcutaneous administration was less with fentanyl as compared to morphine

Other:

Pharmacodynamics of the major metabolites

Fentanyl is rapidly and extensively metabolized in the liver of animal species and man. The oxidative N-dealkylation at the piperidine nitrogen, yielding phenylacetic acid and norfentanyl appears to be the main metabolic pathway. In addition, metabolites are formed via aromatic and aliphatic hydroxylations. Two minor hydroxylated metabolites, (X-hydroxy- fentanyl and para-hydroxy-fentanyl (or: 4-hydroxy-phenethyl-fentanyl) show some activity in the guinea-pig ileum bioassay. In the rat, these metabolites were recovered from brain tissue, but in small amounts relative to unchanged fentanyl.
In general, it can be stated that the metabolites of fentanyl do not contribute to the analgesic activity, as demonstrated by their weak activity in the guinea-pig ileum bioassay and in two in vivo assays, being the hot plate test in mice and the tail withdrawal test in rats.

Comparison between the results obtained with fentanyl, some of its metabolites, morphine, and meperidine in the guinea-pig ileum bioassay and in two in vivo analgesic tests.

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		

2.6.2.5 Pharmacodynamic drug interactions:

The concomitant use of other central nervous system depressants, including other opioids, sedatives or hypnotics, general anesthetics, phenothiazines, tranquillizers, skeletal muscle relaxants, sedating antihistamines, and alcoholic beverages may produce additive depressant effects. Hypoventilation, hypotension and profound sedation or coma may occur.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

There are no pharmacology tabulated summaries.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Fentanyl plasma pharmacokinetics after IV dosing are well known. In animal species and man, plasma fentanyl levels show an initial very rapid decline after an IV bolus dose, ascribed to rapid tissue uptake, followed by a much slower decrease, ascribed to tissue redistribution and metabolism. Volume of distribution is large in all species, including man. Protein binding ranges between 69 and 84% in animal species and man. Clearance is high and predominantly metabolic, with the liver being the major site of metabolism.

The main metabolic pathway of fentanyl in animals and man is the oxidative N-dealkylation to norfentanyl; in addition, various other oxidative metabolites are formed. Metabolites are excreted both in urine and in feces. None of the major metabolites contributes to the analgesic activity of fentanyl.

Various in vitro and in vivo studies on topical application in animal species have already established that transdermal dosing of fentanyl is feasible. No indications for fentanyl metabolizing activity in the skin were present, suggesting that fentanyl was absorbed through the skin unchanged. In recent studies non-ionized fentanyl showed a relatively high penetration through the lipid-rich stratum corneum, but the viable skin appeared to be a stronger barrier to absorption. With iontophoresis or electroporation (of an ionized fentanyl solution), penetration of the hydrophilic viable skin was increased compared to passive diffusion of non-ionized fentanyl. By changing the delivery mode of the current (e.g., voltage, duration and number of pulses), control of the quantity of fentanyl transported through the skin can be obtained.

Based on in vitro data, CYP3A4 is the major human P450 isoform involved in the main metabolic route for fentanyl, the oxidative N-dealkylation to norfentanyl. In vitro interaction data indicated that several drugs were able to inhibit the metabolism of fentanyl. However, the clinical relevance of these in vitro findings is probably limited to the more potent inhibitors of the fentanyl metabolism because fentanyl is a high-clearance compound, relatively insensitive to changes in intrinsic clearance. Itraconazole, for example, which is a very potent inhibitor of fentanyl metabolism in vitro, was studied in vivo in healthy subjects and resulted in no statistically significant change in the clearance of fentanyl. Therefore it is expected that only even more potent inhibitors of fentanyl metabolism, such as ketoconazole and ritonavir, may have a clinically relevant influence on fentanyl clearance.

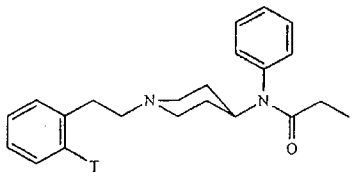
The exposure in the combined segment I/II study in the rat and in the segment II study in the rabbit was compared with human exposure data. In the rabbit segment II reproduction study, performed with fentanyl dosed via intravenous infusion, the maximal plasma fentanyl levels (C_{max}) of on average 27.8 ng/mL, were markedly higher than those expected in patients using E-TRANS (fentanyl HCl) (C_{max} expected to remain below 4 ng/mL). In the rat combined segment I/II study, in which the animals received a continuous IV infusion, the mean C_{max}, at the highest dose level was only 6.2 ng/mL, but still higher than peak levels expected in patients.

2.6.4.2 Methods of Analysis

The methods of bioanalysis used in fentanyl animal pharmacokinetic studies, performed within the Department of Pharmacokinetics of the Janssen Research Foundation.
The radiolabeled compound was prepared as described by — For the various studies, ^{3}H-fentanyl synthetic batches dissolved in ethanol were used. Specific activity ranged between 332 and 381 GBq/mmol, and radiochemical purity was > — (radio-HPLC). The ^{3}H- fentanyl in ethanol was diluted with unlabelled fentanyl at the

appropriate ratio, and then the ethanol was evaporated under a stream of nitrogen. The residue containing ^{3}H-labelled and unlabelled fentanyl was dissolved in dimethyl sulphoxide (DMSO); this solution was used in the in vitro incubations. In one study, radiochemical purity of the DMSO solution was checked by radio-HPLC one day after use, and was found to be ___

Structural formula of fentanyl (T indicates Position of the ^{3}H-label)



Radiochemical methods: In in vitro studies, mass balances for unchanged fentanyl and its major metabolites were drawn up. For the determination of total radioactivity (TR), aliquots of incubates (or incubate fractions) were mixed with appropriate scintillation cocktails and subjected to liquid scintillation counting. The percentage of radioactivity accounted for by fentanyl and its metabolites was determined based on the areas of the radioactivity peaks measured after radio-HPLC with on-line radioactivity detection. A detailed description of the radio-HPLC method used in the in vitro studies is given in the individual study reports. Metabolites were identified by co- chromatography with reference compounds using UV detection. For the detection of glucuronides and sulphate metabolites, incubates were analyzed either directly or after enzymatic hydrolysis with P-glucuronidase and/or arylsulphatase.

Assay methods for fentanyl: In in vivo studies in rats and rabbits, unlabelled fentanyl was dosed. Fentanyl levels in plasma were determined using a validated radioimmunoassay (RIA) method. Rat plasma was diluted with 2% bovine serum albumin (BSA) to a concentration within the calibration range of 1.0-100 ng/mL. The limit of quantification was 1.0 ng/mL. Independently prepared quality control samples (n=4) analyzed in the same bioanalytical runs showed mean coefficients of variation of

10.1% at 3.68 ng/mL, 13.2% at 12.3 ng/mL, and 12.3% at 30.7 ng/mL, and a mean accuracy of 98.6%, 94.4% and 92.6%, respectively.

Rabbit plasma was subjected to extraction over an [redacted] extraction column, the extracted material being dissolved (and diluted, if necessary) in 2% BSA to a concentration within the calibration range of 0.10-100 ng/mL. The limit of quantification was 0.10 ng/mL. Independently prepared quality control samples (n=2 or 4) analyzed in the same bioanalytical runs showed mean coefficients of variation of 6.7% at 0. 179 ng/mL, 4.1 % at 0.507 ng/mL, 12.4% at 0.596 ng/mL, 14.1 % at 2.98 ng/l, 6.2% at 3.17 ng/l, and 3.0% at 6.33 ng/mL. Corresponding mean accuracy values were 117.3%, 101.6%, 95.6%, 103.1%, 98.9%, and 101.2%, respectively.

2.6.4.2 Absorption

After intravenous dosing of fentanyl, the plasma concentration-time course of fentanyl was comparable in animals and in man. After an initial very rapid decline, with a corresponding half-life of 8 min in rats, of approximately 3 min in rabbits and dogs, and of <2 min in man, plasma levels of fentanyl decreased much more slowly. In rats, the elimination appeared to be a bi-exponential process, whereas in rabbits, dogs and man, it appeared to be tri-exponential. The initial rapid decline was ascribed to rapid tissue uptake. Indications for ensuing tissue redistribution were present. The volume of distribution was large in all species. The terminal plasma elimination half-life was <1 h in the rat, approximately 2 h in the rabbit, and 3-4 h in the dog and somewhat longer in man. Fentanyl elimination was predominantly metabolic, with the liver being the major metabolizing tissue. Total plasma clearance was highest in rats, lowest in man, and intermediate in rabbits and dogs. Fentanyl metabolites rapidly appeared in plasma in all species, but elimination of metabolites occurred more slowly than elimination of unchanged fentanyl. Dog data indicated dose-proportional fentanyl plasma pharmacokinetics.

Various in vitro and in vivo studies on topical application in animal species have already established that transdermal dosing of fentanyl is feasible. Non-ionized fentanyl showed a relatively high penetration through the lipid-rich stratum corneum, but the viable skin appeared to be a stronger barrier to absorption. No indications for fentanyl metabolizing activity in the skin were present, suggesting that fentanyl was absorbed through the skin unchanged.

Hairless rat skin has been used in a series of in vitro and in vivo experiments to study topical dosing of fentanyl, with emphasis on iontophoresis (application of an electrical field across the skin) and electroporation (application of high voltage external field pulses), in comparison with passive diffusion, iontophoresis or electroporation (of an ionized fentanyl solution) was examined as a means to increase penetration through the hydrophilic viable skin compared to passive diffusion of non-ionized fentanyl. Furthermore, the influence of changing the delivery mode of the current (e.g., voltage,

duration and number of pulses) on the quantity of fentanyl transported through the skin was examined.

Autoradiography of abdominal hairless rat skin after in vitro iontophoresis (fentanyl citrate at pH 3.5) compared to in vitro passive diffusion showed that iontophoresis increased the drug concentration in the part of the skin that limits molecule permeation. For the highly lipophilic compound fentanyl, this is the viable skin. In the lipid-rich stratum corneum, diffusion and iontophoresis produced similar distribution profiles; furthermore, an increase in the duration of current application did not influence the quantity of fentanyl present in the stratum corneum. In the hydrophilic environment of the viable skin, iontophoresis enhanced fentanyl penetration by a factor 10 to 40, and penetration increased with prolonged duration of iontophoresis.

Fentanyl flux through the abdominal hairless rat skin in vitro was examined in a series of experiments testing various delivery modes.

Fentanyl flux through the skin was increased with increased current density applied (direct or pulsed), with increased current application, with lower pH in the donor compartment, and with increased fentanyl citrate concentration in the donor compartment. At the same current density, direct current induced a somewhat higher flux than pulsed current. With a direct current applied for one hour, a lag time of on average 1.2-h was observed. When the direct current was switched off after one hour, fentanyl flux was maintained for at least 4.5 h. As water permeation was not influenced, it was concluded that skin lesions induced by the current did not explain the increased flux. Comparison of flux post-iontophoresis with and without replacement of the donor compartment with drug-free citrate buffer, indicated release of a built-up skin reservoir after switching off the current. For electroporation, the waveform proved to be important: exponentially decaying pulses increased fentanyl permeation more than square-wave pulses at the same applied energy. Furthermore, by changing voltage, duration and number of exponentially decaying pulses, control of the quantity of fentanyl transported through the skin could be obtained. Electroporation produced a fentanyl skin reservoir, explaining post- pulse passive transport, and lasting changes in skin permeability. Electro- osmosis appeared not to be involved in either iontophoresis or electroporation. Iontophoresis (1 h with a 0.33 mA/cm^2 continuous current and 40 ug/mL fentanyl in citrate [redacted]) was compared with passive diffusion (same application, but current remained switched off). Passive diffusion alone of ionized fentanyl did not produce detectable systemic fentanyl levels. After 1 h of iontophoresis, plasma levels were approximately 25 ng/mL. Earlier sampling times were not examined, hence no conclusion on lag-time can be drawn. C_{max} (on average 29 ng/mL) was reached 30 min after electrode removal. Fentanyl plasma levels then decreased slowly, with a secondary peak (on average 13 ng/mL) at 3 h after the start of the current. $AUC_{0-7\ h}$ averaged 68 ng.h/mL. The slow decrease of fentanyl plasma levels after the current was stopped, was ascribed to both continuing release of fentanyl from a built-up skin reservoir and redistribution of fentanyl from peripheral compartments. The iontophoretic application of fentanyl induced analgesic effects, lasting from 1h (first time point examined) up to 4 h after the start of the experiment. In electroporation experiments, high voltage pulsing

rapidly produced systemic fentanyl levels; immediately after the 5-min pulsing, plasma levels already reached 13 ng/mL on average. C_{max} (on average 32 ng/mL) was reached at 0.5-1 h after the start of the electroporation; then plasma levels slowly decreased. AUC_{0-6} averaged 68 ng.h/mL. With iontophoresis applied for a similar 5-min period, no detectable systemic fentanyl levels were reached when the current was stopped. Electroporation of fentanyl produced deep analgesia and supraspinal effects, with antinociception lasting from 5 min (first time point examined) to 1.25 h after the start of the experiment. After the 5-min iontophoresis, antinociceptive effect was observed at one time point only, namely 30 min after the start of the experiment. These combined data suggest that lag-time is shorter with electroporation than with iontophoresis.

2.6.4.3 Distribution

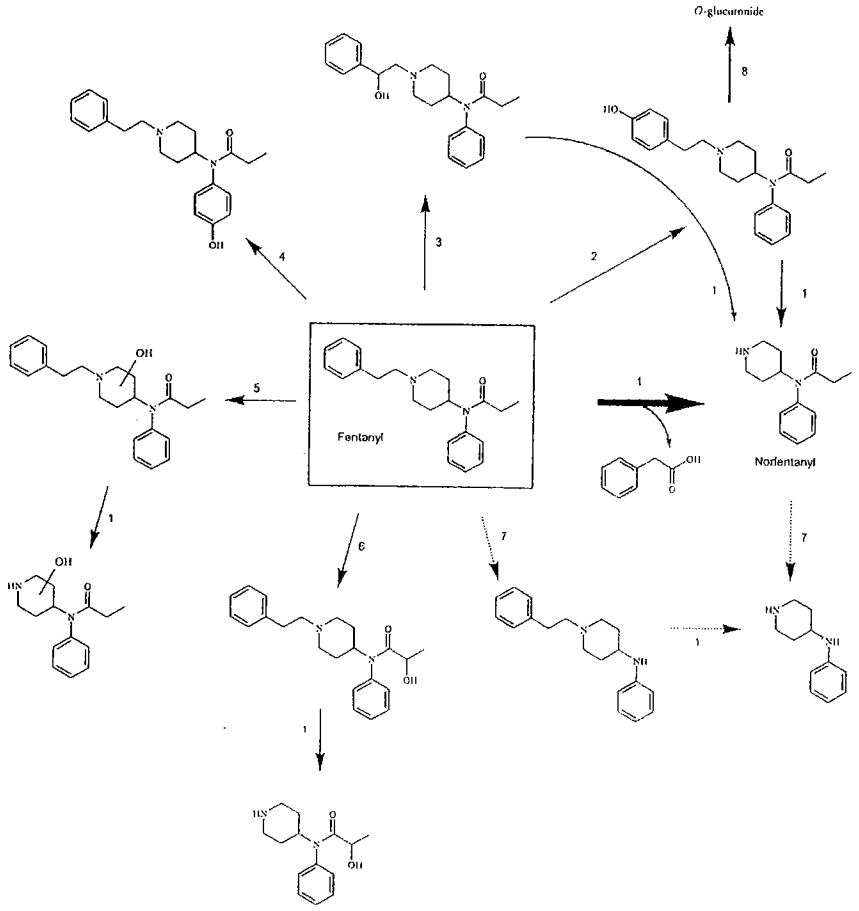
In all animal species studied, the fast onset as well as the short duration of the effect of fentanyl after intravenous administration were ascribed to the very pronounced and rapid uptake of the drug in brain followed by a rapid redistribution to sites of storage (muscle and fat) and biotransformation (liver). Rat tissue-to-plasma partition coefficients for fentanyl were >20 in fat, spleen and pancreas, >10 in lung, kidney and stomach, >5 in small intestine and testis, >2 in brain, heart, liver, large intestine, muscle and skin. In the dog, fentanyl levels in cerebrospinal fluid were approximately half those in plasma and this concentration difference was ascribed to plasma protein binding. In mouse, rabbit, sheep and man, passage of the placenta was limited. In sheep, fentanyl did not induce alterations in uterine tone or uterine blood flow.

2.6.4.4 Metabolism

Data on the in vitro metabolism of fentanyl by human liver preparations, using ^{3}H-fentanyl specifically labeled at the ortho-position of the phenylethyl group, were obtained in four in-house studies. A major metabolic pathway was the oxidative N-dealkylation leading to the formation of 2-phenylethanol, which in turn was further metabolized to phenylacetic acid. Presumably also norfentanyl was formed, but due to the position of the tritium label, this metabolite could not be detected with the radio-HPLC system. Other major metabolic pathways were aromatic hydroxylation at the phenylethyl moiety (with ensuing partial glucuronidation) and aliphatic oxidation at the opposition of the phenylethyl moiety. Indications for glucuronidation and sulphate conjugation of unidentified metabolites were present.

The main metabolic pathway of fentanyl in animals and man is the oxidative N-dealkylation to norfentanyl, with the possible exception of the dog. In addition, metabolites are formed via aromatic as well as aliphatic hydroxylation. Amide hydrolysis

appears to be a minor pathway or does not occur at all. The narcotic analgesic activity of fentanyl can be ascribed to unchanged drug.



Metabolic pathways of fentanyl in animals and ma(above diagram). 1: oxidative N-dealkylation, 2: aromatic para-phenyl hydroxylation, 3: aliphatic (x-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.

2.6.4.6 Excretion

Renal clearance of fentanyl is low. Fentanyl metabolites formed are eliminated both in urine and in feces.

2.6.4.7 Pharmacokinetic drug interactions

In-vitro data on enzymes involved and possible drug-drug interactions

In vitro data indicate that CYP3A4 is the major human P450 isoform involved in the main fentanyl metabolic route, the oxidative N-dealkylation to norfentanyl. In addition other isoforms may be involved in the overall metabolism of fentanyl. In an in vitro metabolism stud, three batches of human liver microsomes showed Km values for fentanyl metabolism of 1.43, 5.2 and 55.4 uM, respectively. These values are somewhat lower than those reported elsewhere, 82 and 117 uM, respectively. Even the lowest human Km value calculated is much higher than expected fentanyl plasma levels during application of E-TRANS (fentanyl HCl): C_{max} expected to remain below 4 ng/mL or below 0.012 uM . Assuming a liver-to- plasma ratio of 10, which is 2-5 times the ratio observed in the rat, the Km values are also higher than expected liver fentanyl levels (<40 ng/mL or <0. 1 2 uM). Therefore, a clinically relevant metabolic interaction, as a consequence of an inhibition by fentanyl of the metabolism of co- administered drugs, is not expected in patients receiving fentanyl via the E-TRANS (fentanyl HCl) system.

Two in vitro studies with human liver microsomes investigated the influence of potential co-medication on the metabolism of fentanyl. The effect of the following 55 compounds was examined:

alprazolam	indinavir	paracetamol
amitryptiline	itraconazole	prednisone
amoxicillin	ketamine	promethazine
astemizole	ketoconazole	propranolol
carbamazepine	lamivudine (3TC)	quinidine
cimetidine	meperidine	ranitidine
clarithromycin	methadone	ritonavir
clonidine	methotrexate	saquinavir
codein	metoclopramide	sulfadiazine
cyclosporin A	metoprolol	taxol
desmethylastemiz	miconazole	terfenadine ole
dexamethasone	midazolam	theophylline
doxorubicin	morphine	tolbutamide
erythromycin	naproxen	valproic acid
fluconazole	nifedipine	verapamil
fluoxetine	norfluoxetine	warfarin
fluvoxamine	omeprazole	zidovudine (AZT)

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furosemide	ondansetron
hydroxy-itraconazole	oxycodon

The data of these in vitro metabolic interaction studies indicate that several of the drugs investigated seem to be able to inhibit the metabolism of fentanyl at inhibitor concentrations that correspond to therapeutic plasma concentrations in man. The most potent inhibitors of the in vitro metabolism of fentanyl were ritonavir (IC_{50} of 0.06 ug/mL; IC_{50}/therapeutic plasma level ratio of 0.005), ketoconazole (IC50 of 0.10 ug/mL; IC_{50}/therapeutic plasma level ratio of 0.01) and itraconazole (IC_{50} of 0.10 ug/mL; IC_{50}/therapeutic plasma level ratio of 0.04). However, the clinical relevance of these in vitro findings is expected to be limited to the more potent inhibitors of the fentanyl metabolism because fentanyl is a high-clearance compound, with blood clearance approaching liver blood flow. High clearance compounds are relatively insensitive to changes in intrinsic clearance. Itraconazole, which was a very potent inhibitor of fentanyl metabolism in vitro with an IC50/therapeutic plasma level ratio of only 0.04, did not change the clearance of fentanyl in vivo in healthy human volunteers. It is expected that only compounds with an IC50/therapeutic plasma level ratio smaller than that of itraconazole (of the compounds tested, this only applies to ketoconazole and ritanovir), may possibly show a clinically relevant influence on fentanyl clearance.

2.6.4.8 Other Pharmacokinetic Studies

Plasma protein binding and distribution in blood:

There were no major species differences in plasma protein binding. The binding amounted to 69% in mice, 83% in rats, 74% in rabbits, 78% in dogs and 84% in man. In whole blood of rat, dog and man, fentanyl present in the plasma water fraction was 17%, 16% and 17%, respectively. Plasma protein binding was not concentration-dependent and appeared to be based on hydrophobic interaction. Albumin was the major, but not the only, binding protein in human plasma: binding to α1,-acid glycoprotein and to lipoproteins was also observed. Animal and human blood levels were comparable to plasma levels. The uptake of fentanyl by red blood cells appeared to be a linear function of the free drug concentration in plasma. Hemoglobin was the major binding site in human erythrocytes.

Toxicokinetics

Studies in rats

Dose range-finding for reproductive toxicity studies (24-h or 120-h infusion)

The toxicokinetics of fentanyl were studied in a single-dose Dose range-finding toxicity study in female Wistar rats after a single IV infusion of fentanyl citrate over 24 h or over 120 h. Plasma fentanyl concentrations were examined at the end of the. At the end of the 24-h infusion period, plasma levels of fentanyl were below or only scarcely higher than the limit of quantification up to a dose level of 0.05 mg/kg. Plasma levels of fentanyl

increased fairly dose-proportionally from the 0.1 to the 0.2-mg/kg-dose level and more than dose-proportionally (i.e. 3 to 4 times for each doubling of the dose) from the 0.2 to the 2 mg/kg dose level. At the 1 and 2 mg/kg dose level test article related mortality was observed. At the end of the 120-h infusion period, plasma levels were below the limit of quantification for the 0.025-mg/kg/day-dose level. The plasma level for the 0.4 mg/kg/day 5-day infusion was in good agreement with that found after 24-h infusion at 0.5 mg/kg.

Mean or median (n=3) plasma concentrations of fentanyl in female Wistar rats at the end of an IV infusion of fentanyl citrate

Dose (mg base/kg/day)	Plasma fentanyl (ng/ml)	Remarks
24-h infusion		
0.01	≤0.50	median
0.02	≤0.50	median
0.05	≤0.34	median
0.1	0.61 ± 0.13	mean ± SD
0.2	1.18	median
0.5	5.9 ± 2.4	mean ± SD
1	20 ± 11	mean ± SD
2	78	n=2
120-h infusion		
0.025	≤0.25	median
0.4	4.50 ± 0.79	mean ± SD

Five-week continuous infusion: The toxicokinetics of fentanyl were studied in male and female rats at the end (day 34) of a 5-week continuous infusion toxicity study, followed by a 4-week recovery period. Plasma fentanyl levels were examined at the end of the

infusion (day 34, 8.00 a.m.) in individual animals, and in addition for the highest dose group after a 4-week recovery period (day 65, 8.00 a.m.) in samples pooled per gender After continuous infusion of fentanyl at 0.025 mg/kg/day, all plasma levels were below the limit of quantification (<1.0 ng/mL). At 0.1 and 0.4 mg/kg/day, plasma concentrations were fairly comparable in male and female rats. Fentanyl plasma levels increased fairly dose-proportionally in females and somewhat more than dose-proportionally (about 6 times) in males. Compared to the Dose Range Finding study, plasma levels were approximately twice higher in the 5-week infusion study. After a 4-week recovery period following the 5-week infusion, all plasma concentrations of fentanyl in the rats, which had been dosed at 0.4 mg/kg/day, were below the limit of quantification (<1.0 ng/mL).

Mean (n=4, ± SD) plasma concentrations of fentanyl in male and female Wistar rats on day 34 of a 5-week continuous infusion of fentanyl and after washout (day 65)

Dose (mg base/kg/day)	Plasma fentanyl (ng/ml)	
	Male	Female
0.025	<1.0	<1.0
0.1	1.4 ± 0.3	2.0 ± 0.5
0.4	8.9 ± 0.8	9.5 ± 1.3
0.4 (day 65)	<1.0	<1.0

Combined segment I and II reproduction study

The toxicokinetics of fentanyl were studied in a combined segment I and II reproduction toxicity study in Wistar rats, in which the animals received a continuous IV infusion from at least 2 weeks prior to mating until day 16 of pregnancy. Plasma fentanyl levels were examined on day 9 after the start of the infusion and at day 16 of pregnancy. At the 0,025 mg/kg/day dose level, plasma concentrations of fentanyl were below the limit of quantification (<1.0 ng/mL) both before and during pregnancy. For the 0.1 and 0.4 mg/kg/day dose levels, plasma fentanyl levels were comparable before and during pregnancy, and increased fairly dose- proportionally. Concentrations reached were comparable or somewhat higher than those observed in the 'Dose Range Finding' study but lower than those observed in the 5-week continuous infusion study in non-pregnant animals.

Mean (n=2) plasma concentrations of fentanyl (ng/mL) in pregnant albino rabbits during the third daily 4-hour infusion of fentanyl citrate

Dose (mg base/kg/day)	t=2 h	t=4 h (end of infusion)
0.2	8.8	11.0
0.4	16.7	21.0
0.8	27.3	28.4
1.6	NS *)	NS *)

*) no sample due to mortality

Segment II reproduction study

The toxicokinetics of fentanyl were studied in a segment 11 reproduction toxicity study in albino rabbits, in which the animals received daily 4-hour infusions during days 6 through 1 8 of pregnancy. Plasma concentration-time curves were obtained after single dosing, on day 6 of pregnancy, and after repeated dosing, on day 18 of pregnancy . After repeated dosing, on day 18 of pregnancy, highest plasma levels were observed at the end of the infusion. Peak plasma levels and AUC $_{4\text{-}24h}$ values increased fairly dose-proportionally over the studied dose range. After single dosing, highest plasma levels were fairly comparable to those after repeated dosing in the 0.025 and 0.1 mg/kg/day dose groups, but lower than after repeated dosing in the 0.4 mg/kg/day dose group. Single dose AUC $_{4\text{- infinity}}$ values tended to increase more than proportionally from the 0.025 to the 0.1-mg/kg/day dose level and less than proportionally from the 0.1 to the 0.4 mg/kg/day dose level. Trough plasma levels were low, but except after the first 0.025 mg/kg/day dose, fentanyl exposure was observed throughout the dosing interval.

Mean (n=2) fentanyl pharmacokinetic characteristics in pregnant rabbits on days 6 (first dose) and 18 (last dose) of a segment II study, with dosing as a daily intravenous 4-hour infusion of fentanyl citrate

Dose (mg base/kg/day)	0.025	0.1	0.4
Day 6 (first dose)			
C_{max} (ng/ml)	1.28	8.39	18.1
t_{max} (h)	4	4	4
C_{min} (ng/ml)	<0.10	0.12	0.23
$AUC_{4-\infty}$ (ng.h/ml)	2.06	18.2	52.3
Day 18 (last dose)			
C_{max} (ng/ml)	1.48	6.85	27.8
t_{max} (h)	4	4	4
C_{min} (ng/ml)	0.11	0.17	0.62
AUC_{4-24h} (ng.h/ml)	4.18	18.7	82.0

Comparison of pharmacokinetics and exposure in animals and man

After intravenous dosing of fentanyl, the general plasma concentration-time course, the tissue distribution, the routes of metabolism and of excretion are similar in animal species and man.

E-TRANS (fentanyl HCl) is designed to deliver fentanyl in on-demand doses of ~40 ug over 10 min. Patients may self-administer at the highest possible dose rate, i.e. one delivery every 10 min. Fentanyl plasma levels reached with E-TRANS (fentanyl HCl) delivering one dose every 10 min, with this high dose rate specifically studied, are only available from volunteers blocked with naltrexone. In one volunteer study, a 25-ug system was applied to 9 male subjects. They were dosed 75 ug in the first 30 min of every hour for 25 h, with an estimated total fentanyl dose of 1875 ug. In a similar volunteer study in 12 subjects an E-TRANS (fentanyl HCl)-like system as applied delivering approximately 47-48 ug in 20 min with one dose delivered every hour for 25 h. Maximal fentanyl plasma levels were below 4 ng/mL in all individuals. In a pilot efficacy and safety study in 253 post-operative patients, fentanyl plasma levels were measured in subjects on either a 25ug system (50 values) or a 40 ug system (52 values), with fentanyl IV supplements administered when required. The majority of subjects had plasma levels around 1-2 ng/mL. It was not reported whether and, if so, when, IV supplements were administered to the patients of which plasma levels were presented.

Maximal fentanyl plasma levels reached in male volunteers with E-TRANS (fentanyl HCl) (-like) systems administering fentanyl at a high dose rate.

Study design	Mean C_{max} (ng/ml)	Range C_{max} (ng/ml)
75 µg in 30 min every h for 25 h (n=9)	2.57	—
47-48 µg in 20 min every h for 25 h (n=12)	2.69	—

In the rabbit reproduction study, performed with fentanyl dosed via intravenous infusion, the maximal plasma fentanyl levels of on average 27.8 ng/mL (highest dose level, were markedly higher than those expected in patients using E-TRANS (fentanyl HCl). In the rat combined segment I/II study, the mean C_{max} at the highest dose level was only 6.2 ng/mL, but still higher than the peak levels expected in-patients. However, a 'Dose Range Finding' study indicated that higher rat dose levels were not feasible because of increased mortality.

2.6.4.9 Discussion and Conclusions

Fentanyl interacts with opioid receptors to produced analgesia and sedation. It increases the patient's tolerance for pain and decreases the perception of suffering, although the patient may still recognize the pain itself. Opioids work to relieve nociceptive pain but are not very effective for neuropathic pain. In addition to analgesia u- opioid agonists such as fentanyl produce drowsiness, changes in mood, respiratory depression decreased gastrointestinal motility, nausea, vomiting and alterations in the endocrine and autonomic nervous system. Fentanyl is a synthetic opioid agonist that interacts primarily with the u-opioid receptor subtype.

High doses of fentanyl produce muscle rigidity possibly due to effects of opioids on dopaminergic transmission in the striatum. The euphoric effects of opioids are believed to be mediated in part via interaction with opioid receptors located in the ventral tegmental area (VTA) leading to the enhancement of dopamine release in the nucleus accumbens. Opioid receptors in the locus coeruleus appear to inhibit the adrenergic neurons thought to play a role in feelings of alarm, panic, fear and anxiety. Opioids act within the hypothalamus to regulate body temperature (generally temperature decreases slightly, but at higher doses temperature may increase). Opioids inhibit neuroendocrine systems including gonadotropin-releasing hormone (GNRH) and corticotropin-releasing factor (CRF) thereby decreasing release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and adrenal corticotrophic hormone (ACTH), and P-endorphin. This leads to decrease plasma levels of testosterone and cortisol. Opioids increase circulating levels of prolactin. Opioids such as fentanyl lead to constriction of the pupil (miosis) via increased parasympathetic nerve activity innervating the pupil.

Pinpoint pupils are pathognomonic for toxic doses of u-opioid agonists, however mydriasis can develop upon asphyxia.

The safeties of fentanyl administration pertaining to the submitted NDA are similar to those following systemic administration of potent opioids. The major concern is respiratory depression, which can occur at plasma concentrations between 2 and 4 ng/mL. In addition, fentanyl administration may produce sedation, nausea and vomiting, bradycardia, urinary retention and constipation. Although opioids such as fentanyl can have significant safety concerns, the effects are well known. Therefore, given careful clinical monitoring, especially for respiratory depression, the proposed application does not appear to pose significant concerns regarding safety pharmacology.

Opioids are readily absorbed from the gastrointestinal tract and the rectal mucosa. More lipophilic agents are also absorbed through the nasal or buccal mucosa and those with the greatest lipophilicity can be absorbed transdermally. An increase in temperature from 32°C to 37°C has been shown to double the rate of fentanyl delivery.

Fentanyl is widely distributed in the body following administration. There is some evidence that it can accumulate in skeletal muscle and fat. Fentanyl demonstrates approximately 69-84% protein binding and an average volume of distribution of 6 L/kg. Fentanyl crosses the placenta and can also be detected in breast milk. N-dealkylation and hydroxylation via cytochrome P450 3A4 metabolize fentanyl primarily in the liver. In humans, the primary metabolite is norfentanyl. Fentanyl is not considered to have any active or toxic metabolites.

The metabolites of fentanyl and unchanged drug are primarily eliminated via the urine with only 10% representing the unchanged drug. About 9% of the dose is eliminated in the feces, primarily as metabolites. The skin does not appear to metabolize fentanyl that is absorbed transdermally. The short duration of action of intravenous fentanyl (30-60 minutes) is probably due to rapid redistribution into tissues and not due to metabolism. The elimination half-life is about 4 hours. Although there are always concerns regarding the potential for respiratory depression following fentanyl administration, careful clinical monitoring and co-treatment with naltrexone should prevent any unnecessary adverse events related to fentanyl administration.

Toxicokinetic data have been provided from the reproductive toxicity studies performed in rat and rabbit after intravenous administration of fentanyl. A comparison of pharmacokinetics and exposure in animals and man after intravenous dosing of fentanyl resulted in similar observations regarding the general plasma concentration-time course, the tissue distribution, the routes of metabolism and of excretion are in animal species and man. E-TRANS(fentanyl HCl) is designed to deliver fentanyl in on-demand doses of [redacted] 40 ug over 10 min. Patients may self-administer at the highest possible dose rate, i.e.

one delivery every 10 min. Fentanyl plasma levels reached with E-TRANS (fentanyl HCl) delivering one dose every 10 min, with this high dose rate specifically studied, are only available from volunteers blocked with naltrexone. In one volunteer study, a 25-ug system was applied to 9 male subjects. They were dosed 75 ug in the first 30 min of every hour for 25 h, with an estimated total fentanyl dose of 1875 ug. In a similar volunteer study in 12 subjects an E-TRANS (fentanyl HCl)-like system as applied delivering approximately 47-48 ug in 20 min with one dose delivered every hour for 25 h . Maximal fentanyl plasma levels were below 4 ng/mL in all individuals. In a pilot efficacy and safety study in 253 post-operative patients, fentanyl plasma levels were measured in subjects on either a 25 ug system (50 values) or a 40ug system (52 values), with fentanyl IV supplements administered when required. The majority of subjects had plasma levels around 1-2 ng/mL. It was not reported whether and, if so, when, IV supplements were administered to the patients of which plasma levels were presented.

In the rabbit reproduction study, performed with fentanyl dosed via intravenous infusion, the maximal plasma fentanyl levels of on average 27.8 ng/mL (highest dose level) were markedly higher than those expected in patients using E-TRANS (fentanyl HCl). In the rat combined segment I/II study, the mean C_{max} at the highest dose level was only 6.2 ng/mL, but still higher than the peak levels expected in patients. However, a 'Dose Range Finding' study indicated that higher rat dose levels were not feasible because of increased mortality.

2.6.4.10 Tables and figures to include comparative TK summary

There are no summary tables for comparative TK.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

There are no summary tables for pharmacokinetics.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Single dose toxicity

Single dose toxicity studies were performed with fentanyl following intravenous administration to adult mice. Doses of 0, 10 and 20 mg/kg were administered to mice and of 0, 1.25, 2.5 and 5 mg/kg to rats. LD50-values, calculated 14 days after the intravenous injection, were 12 mg/kg (mice) and 2.3 mg/kg (rats).

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. 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. All surviving mice completely recovered within 6 hours after injection.

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. The surviving rats recovered within 1 day after dosing. No abnormalities were found at autopsy in mice or rats. In previously conducted single dose toxicity studies, fentanyl was tested in Mice, dogs (s.c.), intramuscular (i.m.), intra-arterial (i.a.) and intragastric (i.g.) administration. Clinical responses were of central nervous system (CNS) nature, i.e. decreased activity or decreased activity followed by increased activity in rodents, and convulsions, tremors, loss of righting reflex, sedation and respiratory depression in dogs.

Repeat dose toxicity

Evaluation of the subchronic toxicity potential in SPF Wistar rats: R004263 Janssen Research Foundation (Final report issued July 3rd 1997, full data set of summarized results (page 1-127), submitted in the link Vol 1-10)

80 male and 80 female SPF Wistar rats (4 groups: 20 males and 20 females per group) were used for this study. Route of administration was intravenous infusion
Dosage groups 0-(2) - 0.025 (2) - 0.1 (2)- 0.4-(2) mg/kg b.w. /day (mL/kg b.w/hour)
Duration of dosing period was 5 weeks with a recovery period of 4 weeks
Parameters studied were mortality; Clinical observations (including ophthalmoscopy); Body weight; Food consumption; Hematology; Serum analysis; Urinalysis; Organ weights; Gross pathology; Histopathology

From the data, it can be conclude that fentanyl, when administered continuously by intravenous infusion to rats up to a dose of 0.4 mg/kg body weight/day for a period of five weeks, was well tolerated and did not result in mortality, changes in hematological parameters and urinary parameters nor in macroscopic or histological changes. No effects on the other observed parameters (behavior and appearance, body weight, food consumption, serum parameters and organ weights) were present when animals were dosed at 0.025-mg/kg-body weight/day.

When animals were dosed at 0.1mg/kg body weight/day, a few females became slightly excited and slight changes in some serum parameters (an increase in glucose in both sexes and an increase in inorganic phosphate in females) were noted.
At the dose of 0.4-mg/kg-body weight/day, excitation was seen in both males and females, food consumption decreased slightly and slight changes in some serum parameters were seen in both sexes. In males, a slight decrease in body weight towards the end of the dosing period and a pronounced decrease were seen in the first week of the recovery period. A slight decrease in the weight of the liver was also noted. All changes were reversible within a four-week recovery period.

Safety evaluation of McN-JR-4Z63 Administered Intravenously to Dogs for Thirty Days. Toxicological Research Report No. 108 (650802); Data set (page 1-8) of summarized results submitted

Twenty-four purebred beagle dogs supplied by ——— were used in this thirty-day study. McN-JR-4263 as a ——— (Batch No. 1049, Series A) was administered intravenously in doses of 0.1, 0. 3, and 1 mg/kg/day, which in approximately 3, 10 and 30 times the maximum clinical dose in humans, Vehicle (Batch No. 1050, Series A) was also given intravenously in a 5 cc. volume to control animals. All dosages are calculated as the base (fentanyl). There were no deaths. Nor were there significant gross lesions noted after thirty days of dosing. At the termination of the study, a light weight loss was noted in most dosed animals. This loss apparently was dose related. The control animals lost little to no weight. McN-JR-4263 sedated the animals at all three dose levels, hyperpnea and reduced food consumption was seen in all dosed animals. Convulsions occurred in some of the dogs of each experimental group but like the other behavioral effects, these convulsions were more frequent in the high dose animals. Emesis occurred in two of the high dose dogs.
There were only minor histopathological differences between controls and those animals given 0.1 and 0.3 mg/kg, IV of McN -JR -4263. Four high dose (1 mg/kg, IV) dogs had some liver and kidney pathology that may be drug related, but none of these alterations were severe or irreversible.

Toxicity Study of McN-.TR-4263 in Rats and Dogs: Toxicological Research Report No. 9 (630529); Data set of summarized results (page 1-41)

One hundred adult rats were used in a 4 week experiment designed to determine the toxicity of McN-JR-4263 after intramuscular administration. The doses administered were 0.1 and 0.4 mg/kg/day which is about 15 and 60 times respectively of the amounts recommended for clinical use.

The following conclusions and observations were made from the data included in-the report. Deaths occurred in 4 low dose males and in 7 males and 1 female in the high dose group. The low dose animals gained weight at a rate slightly less than the control while the high dose animals lost weight. There was no significant change in hematology. The ratio of the weight of certain organs to body weight of the rate showed no significant change with the exception of the testes, which were somewhat smaller than the controls. Observation at gross necropsy revealed hemorrhage areas at the site of intramuscular injection of several animals of each sex and done level including the controls. This was probably due to repeated injection at the same location. Histology did not reveal any drug-related lesions.

II. Twenty-two beagle dogs were used for subacute toxicity study of McN-JR-4263 after intramuscular administration. The doses administered we re 0.1 and 0.4 mg and 1 mg/kg/ day as in the case of the rats. There was no significant change in hematology, body weight, or organ weights. Gross observation at necropsy and histopathology did not reveal any drug related lesions.

Subject- Safety Evaluation of McN-.TR-4263-49 Given Intravenously to -Rats for Thirty Days. Toxicological Research Report No. I26 (650922); Data set (page 1-19) of summarized results submitted

One hundred and forty CFN rats obtained from ____ were used in an experiment designed to determine the safety for use of McN-JR- 4263-49 given intravenously over a period of thirty days. The animals were given injections of McN-JR-4263-49 in doses of 0. 1.0. 075, 0.05, 0. 03, 0.02 and 0. 01 mg/kg. The citrate salt of McN-JR-4263 was used in this study; however, all calculations are in terms of the base. Results indicate no appreciable deviation from control values for the McN-JR-4263-49 rats in parameters of organ weight, food consumption or hematology.
Deaths occurred at all dose levels except the lowest (0. 01 mg/kg, iv. Some of these deaths were accidental and therefore were not considered in the percent mortality. Eighty-three per cent of the high dose (0.1 mg/kg) animals, sixty-seven per cent of the 0. 075 mg/kg seventy-one per cent of the 0. 05 mg/kg rate and forty-five per cent of the 0. 03 mg/kg animals died.

All the animals increased in body weight. The high dose, male rate (0.1 mg/kg, iv) exhibited a very slight increase in weight when compared to the controls. But none of the males of this dose group survived the 30-day period.
SGPT, Cl, glucose and BUN values for the experimental rats did not differ significantly from those of the control rats. Group III (McN-JR-4263-49, 0. 075 mg/kg) had elevated glucose and BUN levels which exceeded those of the controls. The SGOT values of almost all the rats, control, as well an experimental, were abnormally high. SGOT values

of the rats receiving 0. 075,0. 05 and 0. 02 mg/kg of McN-JR-4263-49 were higher than those of the control rats.

There were no gross abnormalities among any of the surviving, experimental animals. In the control rats, three animals had congested or consolidated lungs; one, a spotted liver and another, a small testis.

With the exception of cardiac lesions, there were no obvious histopathological differences between dosed animals and controls. Since all the McN-JR-4263-49 rats were bled by cardiac puncture, it is quite probable that the punctures were the cause of these lesions. Furthermore, the fact that the lesions are not dose related seems to lessen the probability of drug response.

Genetic toxicology:

Fentanyl was investigated for its potential to induce point and/or gene mutation chromosome aberrations in vitro and in vivo test systems. The transforming potential of fentanyl was also evaluated in an in vitro transformation assay. Furthermore, an unscheduled DNA synthesis test was performed. On special request of the — — additional mutagenicity studies were conducted (two Ames tests and one micronucleus test.

In conclusion, mutagenicity studies on DNA-damage, point and/or gene mutations, and chromosome aberrations and on the transforming potential of fentanyl indicated no mutagenic potential.

Carcinogenicity:

No carcinogenicity testing has been performed with fentanyl, nor are they required for this drug product. This decision was made based upon considerations related to the maximum duration of patient treatment and any perceived cause for concern, as described in the ICH-SIA-topic. Since fentanyl is expected to be administered to patients over a limited period (up to 72 hours), evaluation of the carcinogenic potential is not required as part of the normal drug development, unless there would be a special concern for the patient population.

Based upon the available preclinical data, there is no special concern. Fentanyl has no chemical structure that raises suspicion for a carcinogenic potential. An extensive set of mutagenicity studies indicated that fentanyl was not genotoxic. The adverse effects found in the repeated dose toxicity studies indicated an absence of possible pre- and/or hyperplastic changes that could raise a suspicion for a carcinogenic potential. Pharmacokinetic data have demonstrated that there is no undue tissue accumulation of fentanyl or its metabolites.

Reproductive toxicology:

Reproductive Function:

The effects of fentanyl on male fertility were investigated in rats following intravenous infusion (continuously) to males 4 weeks before mating and throughout mating with undosed females. The doses were 0.025, 0.1 and 0.4 mg/kg. The dose selection was based upon information from a previously conducted 'Dose Range Finding Study'.

Fentanyl dosed up to 0.1 mg/kg in male rats did not lead to mortality or clinical symptoms. Parental toxicity was only evidenced in male rats dosed at 0.4 mg/kg, by decreases in body weight, in weight gain and in food consumption.

None of the maternal or litter parameters were adversely affected in any of the groups, indicating that up to 0.4 mg/kg there was no primary adverse effect on male fertility. Histopathology of the male tract organs did not indicate drug-related changes.

Reproductive function and embryo-fetal development:

The effects of fentanyl on female fertility and embryo-fetal development were investigated in rats, when administered continuously by intravenous infusion for 14 days prior to mating until day 16 of pregnancy at doses of 0.025, 0.1 and 0.4 mg/kg.

Fentanyl dosed up to 0.4 mg/kg did not lead to mortality nor resulted in relevant clinical signs. There were no relevant adverse effects at any dosage on body weight, weight gain or food consumption during the pre- copulation period.

Dosing up to 0.025 mg/kg did not affect any parameter. A decrease in body weight was seen towards the end of pregnancy period at 0.4 mg/kg and a dose-dependent decrease in corrected maternal weight gain was seen at 0.1 and 0.4 mg/kg. There was a slight decrease in food consumption towards the end of the pregnancy period in females dosed at 0.1 mg/kg. At 0.4 mg/kg, food consumption was decreased throughout the pregnancy period. These changes evidenced maternal toxicity in females dosed at 0.1 and 0.4 mg/kg.

There were no adverse effects on the weight of the gravid uterus and of the ovaries, on the copulation and fertility rates as well as on the pre-coital intervals in any of the dosage groups. Additionally, there were no adverse effects on any of the litter data (number of corpora lutea, number of live and dead fetuses, mean litter size, number of implantations, number of resorptions and pre- and post-implantations). No test article related teratogenic effects were seen.

It can be concluded that fentanyl, when dosed up to 0.4 mg/kg, had no adverse effects on the fertility of female rats and that embryo-fetal development was not adversely affected.

Embryo-fetal and perinatal toxicity embryotoxicity and teratogenicity

In a Dose Range Finding study, the potential effects of fentanyl were evaluated when administered once daily by intravenous infusion during 4 hours/day to female non-pregnant rabbits at doses of 0.2, 0.4, 0.8 and 1.6 mg/kg for a period of 3 days.

All rabbits survived the study up to 0.8 mg/kg. Since one rabbit of the 1.6 mg/kg dosage group died after the first administration, dosing was ended on day 0 in the other rabbit of this dosage group. Therefore, no parameters, except for clinical observations on day 0, could be evaluated for the 1.6 mg/kg dosage group, Clinical abnormalities at all doses consisted of dark colored eyes and dose-related sedation during the fentanyl dosed up to 0.4 mg/kg did not lead to mortality nor resulted in relevant clinical signs. There were no relevant adverse effects at any dosage on body weight, weight gain or food consumption during the pre- copulation period.

Dosing up to 0.025 mg/kg did not affect any parameter. A decrease in body weight was seen towards the end of pregnancy period at 0.4 mg/kg and a dose-dependent decrease in corrected maternal weight gain was seen at 0.1 and 0.4 mg/kg. There was a slight decrease in food consumption towards the end of the pregnancy period in females dosed at 0.1 mg/kg. At 0.4 mg/kg, food consumption was decreased throughout the pregnancy period. These changes evidenced maternal toxicity in females dosed at 0.1 and 0.4 mg/kg.

There were no adverse effects on the weight of the gravid uterus and of the ovaries, on the copulation and fertility rates as well as on the pre-coital intervals in any of the dosage groups. Additionally, there were no adverse effects on any of the litter data (number of corpora lutea, number of live and dead fetuses, mean litter size, number of implantations, number of resorptions and pre- and post-implantations). No test article related teratogenic effects were seen.

It can be concluded that fentanyl, when dosed up to 0.4 mg/kg, had no adverse effects on the fertility of female rats and that embryo-fetal development was not adversely affected.

Embryo-fetal and perinatal toxicity Embryotoxicity and teratogenicity:

In a Dose Range Finding study, the potential effects of fentanyl were evaluated when administered once daily by intravenous infusion during 4 hours/day to female non-pregnant rabbits at doses of 0.2, 0.4, 0.8 and 1.6 mg/kg for a period of 3 days.

All rabbits survived the study up to 0.8 mg/kg. Since one rabbit of the 1.6-mg/kg dosage group died after the first administration, dosing was ended on day 0 in the other rabbit of this dosage group. Therefore, no parameters, except for clinical observations on day 0, could be evaluated for the 1.6 mg/kg dosage group, Clinical abnormalities at all doses consisted of dark colored eyes and dose-related sedation during the consumption throughout the pregnancy and the lactation period. Fertility and gestation rate, duration of gestation and the number of implantations were comparable between all groups of the dosed females. For the F1- generation, there were no adverse effects on any of the parameters in the 0.025- or 0. 1 -mg/kg dosage groups during the lactation period. The parameters studied in these periods included the number of live and dead pups, mean

litter size, birth rate, fetal observations, body weight, survival rate, physical, sexual and behavioral development and an object discrimination test. At 0.4 mg/kg, a slight decrease in body weight of the pups was noted on days 4 and 7 and the survival rate was decreased at 4 days after birth. The survival rate was normalized from day 7 onwards, the body weight of the pups from day 14 onwards. In the second, undosed generation, the reproductive performance was normal and comparable between rats of the vehicle group and rats originating from the 0.025, 0.1 and 0.4 mg/kg-dosed dams. The examined parameters included: copulation and fertility rates, weight of the gravid uterus, pre-coital interval, number of corpora lutea, number of live and dead F2 fetuses, implantations and resorptions and pre-and post-implantation loss. These findings indicate that fentanyl does not result in primary effects on peri-and postnatal parameters on the Fl -generation and it is not considered to be a behavioral teratogen in rats at doses up to 0.4 mg/kg.

In addition, reproduction studies that were formerly conducted to support currently approved formulations with fentanyl are briefly summarized below. Fentanyl, administered as fentanyl citrate, was tested in rats in fertility and developmental toxicity studies and in a 3-generation study.

Special toxicology:

STUDIES WITH E-TRANS COMPONENETS CONTAINING FENTANYL

Primary Skin Irritation Study of Electro- transport Therapeutic Systems Containing Fentanyl in Guinea Pigs TR-92-1561-021

Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious hairless guinea pigs (n=6) for 16 hours to evaluate skin irritation. The current density of each system was 0.1 mA/cm^2 for a total current of about 70 uA. The ETS (fentanyl) disposable component was comprised of a Fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel, which was used in conjunction with a zero-order disposable controller. The systems were secured with [redacted] bandage, [redacted] tape, and [redacted] tape. Observations for retention of systems were made periodically during the wearing period. After the completed wearing period, the systems were removed and the anode and cathode sites were scored for erythema and eschar formation and edema at 0.5 and 48 hours post system removal. Primary irritation indexes calculated for the fentanyl gel (anode) and saline gel (cathode) (1.9 and 0.7) indicate that both were mild irritants on hairless guinea pig skin. Residual drug analysis showed a mean fentanyl release for the ETS (fentanyl) to be 2.24 mg/kg/16 hour or drug flux of 111 $ug/cm^2/h$. Histological assessment indicated that the treatments resulted in minimal to moderate changes in parakeratosis, acanthosis, and hyperkeratosis for intact hairless guinea pig skin sites following a 16 hour exposure to fentanyl (anode) and saline (cathode). The lesions described would be expected to be reversible following cessation of the respective topical treatments.

Fentanyl Using Electrotransport Therapeutic Systems in the Hairless Guinea Pig TR-92-1561-022

Five groups of conscious hairless guinea pigs received different treatments. Group I received saline gel (non-drug containing) + current, Group II received fentanyl gel + current, Group III received fentanyl gel passively (no current), Group IV was untreated (sham), and Group V was the positive control (0.1% DNCB in acetone). The Electrotransport Therapeutic Systems (ETS), current density 0.1 mA/cm2 for a total current of about 70 uA anodic delivery with a maximum dose of 1.1 mg/kg/8 h, were secured on the guinea pigs with — bandage and — tape. Groups I-IV received nine occluded topical induction applications (8 h) of the respective test article over a period of 3 weeks. Group V received six induction applications of 0.1% DNCB in acetone over a period of 2 weeks. Induction sites were scared for erythema and edema 0.5 and 24 hours post- removal of the various induction applications. No treatments were performed on any of the animals for the 2 weeks following the ninth induction. The irritation indexes for the anode or treatment sites for Groups II III, V, and I after the last induction were 1.3 (mild), 2.1 (moderate), 0 (none), and 3.5 (moderate), respectively.

Primary Skin Irritation Study of Electro- transport Therapeutic Systems Containing Fentanyl in Rabbits TR-92-1561-053

Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious New Zealand White rabbits (n=3) for is hours to evaluate skin irritation. The current density of each system was 0.1 mA/cm^2 for a total current of about 70 uA. The ETS (fentanyl) disposable component was comprised of a Fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel, which was used in conjunction with a zero-order disposable controller. The systems were secured with — bandage, — tape, and an orthopedic stockinet. After the completed wearing period, the systems were removed and the anode and cathode sites were scored for erythema and eschar formation and edema at 0.5 and 48 hours post system removal. Primary irritation indexes calculated for the fentanyl gel and saline gel (1.2 and 0.2) indicate that the fentanyl and saline gels were a mild and negligible irritant, respectively.

Fourteen Day Subchronic Skin Irritation Study of Electrotransport Therapeutic Systems Containing Fentanyl in Guinea Pigs TR-92-1561-023

Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious hairless guinea pigs (n=10) for 8 hours to evaluate skin irritation. There were a total of fourteen applications (alternated between two sites) for each guinea pig. The current density of each system was 0.1-mA/ cm^2 for a total current of 70 uA. The ETS (fentanyl) disposable component was comprised of a Fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel, which was used in conjunction with a zero-order disposable controller. The systems were secured with — bandage. — tape, and — tape. Observations for retention of systems were made periodically during the wearing period. After each application, the systems were removed, the anode and cathode sites were scored for erythema and eschar formation and

edema at 0.5 hour post system removal, and additional observations were made at 24 and 48 hours post system removal of final applications (13 and 14). Cumulative irritation indexes calculated for the fentanyl gel (anode sites II and IV) and saline gel (cathode sites I and III) (1.9 and 0.6) indicate that both were mild irritants on hairless guinea pig skin. Residual drug ' analysis showed a mean fentanyl release for the ETS (fentanyl) to be 1.21 mg/kg/8 hour or drug flux of 110 mg/cm^2/h. Histological assessment indicated that the treatments resulted in minimal to moderate changes for intact hairless guinea pig skin sites following fourteen 8-hour exposures to fentanyl (anode) and saline (cathode). The lesions described would be expected to be reversible following cessation of the respective topical treatments.

Primary Skin Irritation Study of E-TRANS Systems with Anode Hydrogels Containing Fentanyl on Rabbits TR-96-1561-052

E-TRANS systems with anode hydrogels containing fentanyl were applied to intact dorsal areas of conscious rabbits (n=6) for 14 hours to evaluate skin irritation. The current density was approximately 80 uA/cm^2 based on a total current of approximately 230 uA and a skin contact area of approximately 2.8 cm^2. Each anode and cathode hydrogel application site was scored for erythema and eschar formation and edema at approximately 0.5, 24, and 48 hours after system removal, and Primary Irritation Indices (PIIs) were calculated.

The system for rabbit No. 425 was removed approximately 11.6 hours after system application due to adverse clinical observations. In addition, the application sites for rabbit No. 425 were scored approximately 1 hour after system removal instead of 0.5 hours after system removal. The PIIs for the anode and cathode hydrogels were 1.5 and 1.6, respectively which categorize them as mild irritants. Based on residual fentanyl content of the worn anode hydrogels, the hydrogels released a mean fentanyl dose of approximately 0.073 mg/kg/h or a total mean dose of approximately 3.522 mg.

STUDIES WITH E-TRANS COMPONENETS CONTAINING NO FENTANYL

Visual (Macroscopic) and Histological Evaluation of Guinea Pig Skin Sites Following Single and Multiple Applications of Electrotransport Therapeutic Systems: TR-92-9999-014

Electrotransport Therapeutic Systems (ETS) containing saline hydrogels were applied to conscious hairless guinea pigs and secured with — bandage and → or — tape. After the completed wearing period, the systems were removed and the anode and cathode skin sites were scored visually (macroscopically) and histologically to determine the skin response to electrically assisted transdermal delivery of sodium and chloride ions from saline hydrogels. The first phase of the study with current density of 0.1 mA/cm^2 was evaluated in Groups I - III and VII with wearing periods of 6 and 24 hours for 1, 2, 3 and 7 applications, respectively to the same or alternate site. For this second phase of the study, various repeat applications alternated between two sites from 1 through 14 days were evaluated for Groups IV-VI and VIII. Cumulative irritation seen at

some of the anode and cathode sites precluded certain animals from completing the intended duration of wearing or application. For data analysis purposes, the sites of each animal were organized into groupings that reflected similar number of applications (1 through 4 and 7) to sites and were re-designated as Group A through Group I when comparing irritation indices.

Two current densities (0. 1 mA/cm^2 and 0.2 $mA/cm^{2)}$ and two total currents (200 uA and 100 uA) were evaluated. The anode and cathode sites were scored for erythema and edema at 0.5 and 24 hours after removal of each application and at 0.5, 24, and 48 hours post-removal of the last application, and an irritation index was calculated. Additional observations after the 48-hour observation were made to observe the resolution of the irritated skin sites. At the end of the last observation, the treated sites (anode and cathode) and one untreated site (control) were excised and submitted for histological evaluation.

The skin irritation scores categorized the cathode as mild to moderate irritant (1.7- 4.4) and the anode as mild to moderate irritant (2.1-5.2). The additional observations made after the 48-hour observation to follow the resolution of the irritated skin sites showed mild irritation (1.0-2.0) for the cathode and mild to low moderate irritation (1.2-3.4) for the anode. Some animals scratched their site after system removal, usually after the second application to the same site, and this self- inflicted injury resulted in eschar formation and contributed to the high irritation scores. For four groups, as the number of applications increased from one to four, (with one day between applications to the same site at current density of 0. 1 mA/cm^2), there was no increase in irritation at the anode and cathode sites. In addition, there was no consistent decrease in irritation as the number of days (one to five) between applications increased. In general, comparison between the two current densities (0.1 mA/cm^2 and 0.2 mA/cm^2) for two applications (one day between applications) showed that the higher current density resulted in higher anode mean scores of 2.3 and 3.3 at 48 hour, respectively. Three applications, one day between applications, showed no difference in irritation at the anode site.

Histological assessment by light microscopy indicated that the topical applications of the Electrotransport Therapeutic System (ETS) containing saline hydrogels to non- abraded (intact) hairless guinea pig skin sites, resulted in biologically meaningful increases in incidence and severity of dermatitis (lymphohistiocytic (LHC) and polymorphonuclear (PMN) cellular infiltrates), parakeratosis, acanthosis, hyperkeratosis, inflammatory crusts (serocellular crusts), and epidermal necrosis (ulceration), when compared to the respective untreated control sites. LHC cellular infiltrates, acanthosis, and hyperkeratosis by incidence were the three major changes or lesions that were observed in 100% of the animals in nine of the eleven groups of cathode and anode treatment sites.

Comparing different cathode treatment sites, LHC cellular infiltrates, hyperkeratosis acanthosis, and parakeratosis were unrelated to the number of applications (increased applications did not consistently result in increased severity). An increase of LCH cellular infiltrates and parakeratosis appeared to be related to an increase in current density but this was not apparent for hyperkeratosis and acanthosis. When comparing different anode treatment sites only parakeratosis appeared to be related to an increase in

current density. For both the cathode and anode, the incidence and severity of the PMN cellular infiltrates and serocellular crusts could not be attributed to any particular dosing schedule or current density, although it was obviously related to treatment. Histopathologically, the anode sites were slightly more irritated or inflamed than the cathode sites. All the skin lesions described in this study ranged from minimal to marked in severity and would be expected to be reversible following cessation of treatment.

Evaluation of Polacrilin Resin — , Extract Dilutions for Delayed Contact Hypersensitivity in Guinea Pigs: TR-96-1561-048

Eighty male Hartley guinea pigs (20 per group) were used to evaluate the intradermal sensitization potential of polacrilin resin — , extract. Fresh aqueous or saline extracts of the resin (4 g resin: 20 mL) were prepared for each administration. During intradermal induction, Group 1 animals received sterile water emulsified 1:1 by volume with Freund's Complete Adjuvant (FCA). Group 2 animals received saline. Group 3 animals received the aqueous resin extract (2%) emulsified 1: 1 by volume with FCA. Group 4 animals received the resin extract (1%) in saline. Each group received five injections of the respective test or control article intra-dermally in the shoulder region every two to three days for a total of five 0.1 mL doses within a 10 day period. For animals in Groups 1 and 3, the induction dose was administered as two 0.05 mL injections. At the time of the second and fourth injection doses, topical applications (0.1 mL) of the respective test or control article were also applied to abraded skin; sterile water to Group 1 and 2 animals and resin extract in sterile water to Group 3 and 4 animals. Each application was occluded for 24 hours. No treatments were made for approximately two weeks after the last induction dose.

For the first challenge, all groups received (0.1 mL) intradermal injections of 0.1% and I% resin saline extract and saline alone. Each site was evaluated at approximately 2, 24, 48 and 72 hours post injection. There were no positive responders (combined erythema and edema score >2) at 72 hours in any of the groups to the test or control articles at Challenge No. 1. For the second challenge, all groups received intradermal injections of 1 % and 50% resin saline extract and saline alone. There were no sensitization responders in Groups 1, 2, or 4. Positive responders at the 72-hour observation in Groups 1 and 2 were considered to be due to irritation and not sensitization. Two animals in Group 3 had positive responses to both challenge concentrations of polacrilin extract.

This sensitization study repeated some work performed in an earlier sensitization study (TR-96-1561-016) with polacrilin extracts at more clinically relevant doses and evaluated the doses in animals with and without coadministration of FCA. Induction with polacrilin extract in FCA enhanced the immune response of animals to polacrilin extract. Polacrilin extract at 1 % and 50% was classified as a mild sensitizer in this study in FCA-treated animals induced with 1 % polacrilin extract and as a weak sensitizer without coadministration of FCA. Polacrilin has some potential to induce sensitization, but at concentrations proposed for clinical use, the potential is reduced. Polacrilin was determined to be an acceptable — for use in electrotransport hydrogels.

Evaluation of the Sensitization Potential of Electrotransport Systems Containing Placebo Anode Hydrogels (Histidine and Polacrilin —) in the Hairless Guinea Pig: TR-97-1561 -011

Hairless guinea pigs (— GOHI-hr) were used to evaluate the sensitization potential of an E-TRANS system composed of a placebo anode hydrogel (containing histidine and polacrilin —, and a citrate — , cathode hydrogel. The female guinea pigs were divided into four groups; Group I (n-=10) was the negative-control group (histidine with no polacrilin —, for the anode test group, Group II (n = 10) was the anode test group (histidine and polacrilin —,, Group III (n=5) was the anode negative-control group (histidine with no polacrilin — for the positive control group, and Group IV (n = 5) was the anode positive control group (tetracaine). The cathode hydrogel formulation was the same for all four E-TRANS systems with varying anodes.

Animals in Groups I and II received nine induction applications over 21 days (three applications per week) of their respective test or control articles. Each application was for 16 hours (13 hours activated and 3 hours passive). The direct current density at the anode was approximately 71.4 uA/cm^2 (based on a total current of approximately 100 uA and an electrode area of 1.4 cm^2). The direct current density at the cathode was approximately 50 uA/cm^2 (based on a total current of approximately 100 uA and an electrode and hydrogel area of 2.0 cm^2). Groups III and IV received nine induction applications over 21 days, each with a 6-hour wearing duration. The direct current density at the anode and cathode was approximately 30 uAcm^2 (based on a total current of approximately 60 uA and an electrode area of 2 cm^2) . Each anode and cathode application site was evaluated for primary and cumulative skin irritation after removal of the first and last induction applications, respectively, approximately 2 and 24 hours after system removal.

Approximately 10 days after the last induction application, each guinea pig in Groups I and II was challenged with two E-TRANS systems: one with a placebo anode hydrogel containing histidine and polacrilin —and a — cathode hydrogel and one with a placebo anode hydrogel containing histidine with no polacrilin — and a citrate — cathode hydrogel. E-TRANS Systems for Groups I and II ran at an anode direct current density of 71.4 uA cm^2 and a cathode current density of 50 uA/cm^2 for 16 hours (13 hours activated and 3 hours passive). Each guinea pig in Groups III and IV was also challenged with two E-TRANS systems; one with a placebo anode hydrogel containing histidine with no polacrilin — and a citrate — cathode hydrogel and one with a tetracaine anode hydrogel and a citrate — cathode. E-TRANS systems for Groups III and IV ran at an anode and cathode direct current density of 30 uA/cm^2 for 6 hours. Approximately 15 days after the first challenge application, each guinea pig in Groups III, IV and V (naive) were challenged with an E-TRANS system with a tetracaine anode hydrogel and with hydroxyethylcellulose (HEC) placebo and HEC tetracaine gels (not an E-TRANS system). The E-TRANS system and gels were applied for 6 hours. For both challenge phases, all application sites were scored for irritation approximately 2, 24, 48, and 72 hours after system removal.

No evidence of sensitization occurred in the animals induced and challenged with the anode placebo hydrogel containing histidine with polacrilin, thereby categorizing it as a weak sensitizer. No evidence of sensitization occurred in the animals induced and challenged with the cathode hydrogel (at either 50 uA/cm^2 or 30 uA/cm^2), thereby categorizing it as a weak sensitizer. Animals induced with tetracaine were considered to be sensitized upon electrical and passive challenge with tetracaine; these results confirmed that a sensitization reaction could be elicited in the animals.

A Primary Skin Irritation Study of E-TRANS (placebo) Systems Containing Cetylpyridinium Chloride (CPC) and — Adhesives on Hairless Guinea Pigs and Rabbits, TR-98-1561-031:

The objective of this study was to evaluate the potential degree of dermal inflammation produced by three different E-TRANS (placebo) systems containing the — cetylpyridinium chloride (CPC) in the cathode hydrogel and — adhesives to adhere the systems to the skin. Twelve female hairless guinea pigs and six females New Zealand White rabbits were used. Each guinea pig wore one or two E-TRANS systems and each rabbit wore three E-TRANS systems on intact dorsal areas. The following three systems were evaluated: (1) E-TRANS (placebo) with cathode hydrogel containing 0.08% CPC and — adhesive, (2) E-TRANS (placebo) with cathode hydrogel containing 0.2% CPC and — adhesive, and (3) E-TRANS (placebo) with cathode hydrogel containing 0.2% CPC and — adhesive. All systems had the same histidine- based anode. This resulted in either two or four intact skin sites for guinea pigs (one or two anode hydrogel and one or two cathode hydrogel sites) and six intact skin sites for rabbits (three-anode hydrogel and three cathode hydrogel sites). The total wearing time was approximately 13.3 hours. The direct current density was approximately 71.4 uA/cm^2 at both the anode and cathode (based on a hydrogel area of 1.4 cm^2 and a total current of approximately 100 uA). The anode and cathode application sites were scored for erythema and eschar formation and edema 0.5, 24, and 48 hours after system removal, and PIIs were calculated. No treatment-related changes occurred in body weights or clinical conditions. No mortality occurred. The PIIs are presented below. At time of removal, the Light Emitting Diode (LED) on some of the E-TRANS (placebo) systems indicated that they might have operated improperly. These systems were submitted to the E-TRANS group at ALZA-MN for analysis.

Analysis indicated that one 0.08% CPC and — adhesive guinea pig system, three 0.2% CPC and — adhesive rabbit systems, and two 0.2% CPC and — rabbit systems did not operate properly. The irritation scores from these application sites were not used in the Primary Irritation Index calculation.

Histopathology revealed an increased incidence rate of acanthosis, hyperkeratosis, and dermal inflammation at all sites in guinea pigs when compared to untreated control. Based on severity and incidence rates, treatment sites without CPC produced the least changes, while those with CPC were the most affected. Rabbits had an increased incidence of one or more of the following lesions noted at all treatment sites: dermal inflammation, epidermal necrosis, acanthosis, parakeratosis, and/or hyperkeratosis.

Treatment sites without CPC produced the least changes, while those with CPC were the most affected.

In conclusion, the cathode hydrogel containing 0.08% CPC and the cathode hydrogel containing 0.2% CPC were mild irritants in guinea pigs and rabbits. The corresponding anode was a mild irritant in guinea pigs and a non to negligible irritant in rabbits. The adhesive was a mild irritant in guinea pigs and a moderate irritant in rabbits. The adhesive was a mild irritant in guinea pigs and a moderate irritant in rabbits.

STUDIES WITH E-TRANS COMPONENTS

USP XXII: Biological Testing of

Extracts and samples of , were used in three assays: The Systemic Injection, Intracutaneous Injection, and Implantation Tests. All procedures necessary to meet Class VI requirements of the USP XXII to establish biological suitability of plastic materials were followed. Extracts of the plastic prepared at 50°C met the requirements of the Systemic Injection Test and Intracutaneous Injection Test. The Implantation Test evaluated the reaction of tissue to the plastic after implantation for 7 days and requirements of this test were met , met the requirements for Class VI-50°C plastics as described in the United States Pharmacopeia XXII.

USP XXII: Biological Testing of TR-93-2420-068

The test article. , and , was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline, Polyethylene Glycol, and Cottonseed Oil USP. These extracts were evaluated for systemic toxicity in accordance with the guidelines of the current USP. A single dose of the appropriate test article extract was injected into five mice per extract by either the intravenous or 3.ntraperitoneal routs. Similarly, five mice were dosed with each corresponding blank vehicle. The animals ware observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of significant systemic toxicity from the extracts. Each test article extract met the USP requirements.

USP XXII: Biological Testing of Lower Housing, Acute System, Code Number TR-93-8888-059

The test article, Lower Housing, Acute system. , was extracted in 0.9% sodium Chloride USP solution, Alcohol in saline, polyethylene glycol, and cottonseed oil NF. These extracts were evaluated for systemic toxicity in accordance

with the guidelines of the current USP. A single dose of the appropriate test article extract was injected into five mice per extract by either the intravenous or intraperitoneal routs. Similarly, five mice were dosed with each corresponding blank vehicle. The animals were observed immediately and at 4, 24, 40, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of significant systemic toxicity from the extracts. Each tent article extract met the USP requirements.

USP XXII: Biological Testing of [redacted]
[redacted] TR-94-1561-020

The test article, [redacted] was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline Polyethylene Glycol, and Cottonseed Oil NF. These extracts were evaluated for intracutaneous toxicity in accordance with the guidelines of the current USP. A 0.2-mL dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding blank vehicle was injected at the left side of the back of each rabbit. Two rabbits were used for each pair of extracts. Observations for erythema and edema were conducted at 24, 48, and 72 hours after intracutaneous injection.

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the USP requirements.

USP 23: Biological Testing of Lower Housing, Acute System, Code Number [redacted] TR-95-1561-031

The test article, Lower Housing, Code: [redacted] was implanted in living muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP. Nonsterile implant samples (supplied by the Sponsor) were aseptically loaded into needles. USP negative control strips were sterilized by steam in similar needles. Rabbits were implanted and were then euthanatized 7 days later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the USP negative control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a nonirritant as compared to the USP negative control article.

USP 23: Biological Testing of Film, ——— PIB Adhesive, Code No. ——— TR-96-1561-011:

The test article, polyisobutylene, Code ——— , was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline, Polyethylene Glycol, and Cottonseed Oil, NF. These extracts were evaluated for intracutaneous toxicity in accordance with the guidelines of USP 23.

A 0.2-mL dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding blank vehicle was injected on die left side of the back of each rabbit. Two rabbits were used for each pair of extracts. Observations for erythema and edema were conducted at 24, 48, and 72 hours after intracutaneous injection.

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the requirements of USP 23.

Evaluation of Film, ——— Polyisobutylene (PIB) Adhesive (Code No. ——— 5) extract for Delayed Contact Hypersensitivity in Guinea Pigs, TR-96-1563-012:

The test was designed to evaluate the allergenic potential or sensitizing capacity of a test article. The test is used as a procedure for screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization. Based on the standards set by the study protocol, Polyisobutylene (PIB) ——— Ethanol in Sodium Chloride extract), exhibited no reaction to the intradermal or topical challenges (0% sensitization). Therefore, as defined by Kligman, this is a Grade I reaction and the test article is classified as having weak allergic potential. A Grade I sensitization rate is not considered significant according to standard Magnusson and Kligman test.

Evaluation of Polacrilin Resin ——— Extract for Delayed Contact Hypersensitivity in Guinea Pigs. TR-96-1561-016:

The objective of this study was to determine the sensitization potential of a resin extract when applied to guinea pigs using a modification of Freund's Complete Adjuvant Test. A Category V classification (extreme sensitizer 515 positive responders) was obtained for the positive control article, DNCB, when challenged intradermally (0.05% DNCB in 5% ethanol/95% sterile saline) and topically (0.05% DNCB in acetone). These results confirmed that a skin sensitization reaction could be elicited in the animals.

Intradermal challenge injections of 25% to 1 00% polacrilin extracted 4 g: 20 mL saline elicited a sensitization reaction in 60% to 85% of the animals; categorizing polacrilin as

having a moderate to extreme sensitization potential. intra- dermal challenge injections with 1% polacrilin extracted 4 g:20 mL and polacrilin extracted 0.03 mg:20 mL in saline did not elicit a sensitization response, indicative of a weak sensitization potential. Topical challenge with undiluted polacrilin did not elicit a positive sensitization response. Portions of this study were repeated in another sensitization study (TR-96- 1561-048) with polacrilin extracts at more clinically relevant concentrations.

The second study evaluated the potential of 1 % polacrilin extract in eliciting a sensitization response with and without coadministration of FCA. Induction with 1 % polacrilin extract in FCA enhanced the immune response of animals to polacrilin extract. Polacrilin extract at 1% and 50% was classified as a mild sensitizer in the second study in animals treated with FCA and as a weak sensitizer without coadministration of FCA.

Polacrilin has some potential to induce sensitization, but at concentrations proposed for clinical use, the potential is reduced. Polacrilin was determined to be an acceptable for use in electrotranspart hydrogels.

Evaluation of Cetylpyridinium Chloride for Delayed Contact Hypersensitivity in Guinea Pigs: TR-96-1561-017

Forty-five male Hartley guinea pigs were used to evaluate the sensitization potential of cetylpyridinium chloride (CPC). During induction, Group 1 (n = 20) received 1% w/v CPC in sterile water emulsified 1: 1 by volume with Freund's Complete Adjuvant (FCA). Group 2 (n = 20) received sterile water emulsified 1:1 by volume with FCA. Group 3 (n = 5) received the positive control article, 0.05% 1-chloro 2, 4-dinitrobenzene (DNCB), in 5% ethanol/95% water.

For the first challenge, the vehicle control and test groups were intradermally injected (0. 1 mL) with 0.005% and 0.01 % CPC in saline and with saline alone. The DNCB group was challenged with an intradermal injection (0.1-mL) of 0.05% DNCB in 5% ethanol/95% saline and with vehicle alone. The sites were evaluated approximately 2, 24, 48 and 72 hours after injection.

For the second challenge, 2 weeks after the first challenge, both the vehicle control and test group received 0.4 mL topical applications of 0.1% CPC in sterile water and of vehicle alone to abraded skin sites. The positive control group was challenged with 0.05% w/v DNCB in acetone and with acetone alone, each applied topically to abraded skin sites occluded for 24 hours. The sites were evaluated at approximately 2, 24, 48, and 72 hours post removal.

No animals induced with CPC were considered to be sensitized upon intradermal or topical challenge with CPC, categorizing CPC as a weak sensitizer under the conditions of this study.

The positive control material, DNCB, was shown to be a sensitizer using the Freund's Complete Adjuvant Test (FCAT).

Evaluation of Polacrilin Resin — xtract Dilutions for Delayed Contact Hypersensitivity in Guinea Pigs. TR-96-1561-048:

The sensitization potential of polacrilin resin — , extract dilutions (4 g:20 mL) was evaluated when intradermally administered to male Hartley albino guinea pigs using a modification of Freund's Complete Adjuvant Test (FCAT). During induction, four groups, each containing 20 guinea pigs, received intradermal injections of either water (Group 1) or 2% polacrilin resin aqueous extract (Group 3) emulsified 1: 1 with Freund's Complete Adjuvant (FCA), saline (Group 2), or 1% polacrilin resin saline extract (Group 4) for a total of five 0.1 mL doses over a 10-day period (on Days 1, 3, 5, 8, and 10). For Groups 1 and 3, each FCA induction injection was administered as two 0.05-mL intradermal injections. For Groups 2 and 4, each induction injection was administered as one 0.1 mL injection. Topical application (abraded skin; 24-hr intervals) of 0. 1 mL of the undiluted aqueous extract of the test article (Groups 3 and 4) or of water (Groups 1 and 2) was also performed at the time of the second and fourth induction injections (Days 3 and 8).

Intradermal Challenge # 1 injections (saline vehicle, 0. 1%, and 1% of the test article) were administered to both the test and vehicle control groups approximately 2 weeks after the last induction dose (Day 22); intradermal Challenge #2 injections (saline vehicle, 1%, and 50% of the test article) were administered approximately two weeks later (Day 36). Treatment sites were scored for erythema and eschar formation and edema at 2, 24, 48, and 72 hours ± 1 hr after injection. In addition to scores, the diameter of erythema or edema responses with a score >2 was recorded to more clearly indicate the intensity of the responses. A scale of 0 (not present) to 4 (severe) was used at each scoring of erythema and edema. An animal was considered to have demonstrated a positive sensitization response if the combined erythema and edema score was >2 at 72 hrs.

Study title: USP 23: Biological Testing of — — TR-96-1561-044:

The test article. Electrode, — Code Number: was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline, Polyethylene Glycol, and Cottonseed Oil, NF. These extracts were evaluated for systemic toxicity in accordance with the guidelines of USP 23.

A single dose of the appropriate test article extract was injected into five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding blank vehicle. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of significant systemic toxicity from the extracts. Each test article extract met the requirements of USP 23.

USP 23: Biological Testing of Housing, Bottom, 2.78 cm^2, Red, E-TRANS (Acute), Code Number [redacted] 12702:TR-97-1561-009

The test article, Housing, Bottom, 2.78 cm^2, Red, E-TRANS (Acute), Code [redacted], was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline, Polyethylene Glycol, and Cottonseed Oil, NF. These extracts were evaluated for systemic toxicity in accordance with the guidelines of USP 23.

A single dose of the appropriate test article extract was injected into five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding blank vehicle. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of significant systemic toxicity from the extracts. Each test article extract met the requirements of USP 23.

USP 23: Biological Testing of Housing, Bottom, 1.39 cm^2, [redacted] E-TRANS (Acute), Code Number [redacted] TR-97-1561-010:

The test article, Housing, Bottom, 1.39 cm^2, [redacted] E-TRANS (Acute), Code #: [redacted], was extracted in 0.9% Sodium Chloride USP solution, Alcohol in Saline, Polyethylene Glycol, and Cottonseed Oil, NF. These extracts were evaluated for systemic toxicity in accordance with the guidelines of USP 23.

A single dose of the appropriate test article extract was injected into five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding blank vehicle. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.

Under the conditions of this study, there was no mortality or evidence of significant systemic toxicity from the extract. Each test article extract met the requirements of USP 23.

In Vitro Biological Testing of [redacted] (Lot No. T790, Part No. [redacted] . USP Elution and MTT Assay: TR-98-1561-023:

The biocompatibility of a mixture of [redacted] (Lot No. T790 Part No. [redacted] and [redacted] was evaluated using two in vitro tests: (1) the Elution test method described in United States Pharmacopoeia (USP) 23, and (2) the MTT (3-[4,5-di- methylthiazol-2-2, 5-diphenyltetrazolium bromide) assay. Due to the noxious vapors emitted from the [redacted] (Lot No. T790, Part No [redacted]), the USP Elution test and MTT assay were unable to be performed using the [redacted] alone. To test [redacted] (Lot No.

T790, Part No. — in a state similar to the final product, a — — (Lot No. T790, Part No .) and — (Lot No. LF1436037, — Part No. —) was used to perform the in vitro tests. The Housing, Bottom, 2.78 cm^2, Red, Code Number — met the requirements of the Systemic Injection Test, Intracutaneous Injection Test, and the Muscle Implantation Test. The test material is therefore classified as Class VI-50°C as described in the USP 23, and is suitable for use as a housing material.

ISO In Vivo Biocompatibility Testing of Housing, Top, E-TRANS (Acute), — , — Code Number — TR-99-1561-056:

The test article, Housing, Top, E-TRANS (Acute), — , Code Number — was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for systemic toxicity in accordance with the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part 11: Tests for Systemic Toxicity.
A single dose of the appropriate test article extract was injected into each of five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding reagent control. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection.
Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements.

ISO In Vivo Biocompatibility Testing of Housing, Top, E-TRANS (Acute) — , — Code Number — TR-99-1561-057:

The test article, Housing-, Top, E-TRANS (Acute) — , Code Number — was extracted in to 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for systemic toxicity in accordance with the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices, Part II: Tests for Systemic Toxicity. A single dose of the appropriate test article extract was injected into each of five mice per extract by either the intravenous or intraperitoneal route. Similarly, five mice were dosed with each corresponding reagent control. The animals were observed immediately and at 4, 24, 48, and 72 hours after systemic injection. Under the conditions of this study, there was no mortality or evidence of systemic toxicity from the extracts. Each test article extract met the test requirements.

USP XXII: Biological Testing of — Control Number — TR-94-1561-017

The test article, — Code# — , Control # — , was implanted in living muscle tissue of the rabbit. Muscle tissue was evaluated for evidence

of irritation or toxicity in accordance with the guidelines of the current USP.

Non sterile implant samples were aseptically loaded into needles. USP reference control strips were sterilized by steam in similar noodles. Rabbits were implanted and were then euthanized 7 days later. Muscle tissues were excised and the implant sites were examined macroscopically. A microscopic evaluation of representative implant sites from each rabbit was conducted to further define any tissue response.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the negative control implant material. The implanted test article met the USP requirements. Microscopically, the test article was classified as a slight irritant as compared to the reference control article.

2.6.6.2 Single-dose toxicity

No full study report for the single dose toxicity study with fentanyl has been submitted with the present study. Following is a summary table from the previous data referred by the Sponsor.

Summary of the single dose toxicity with fentanyl:

Species	Route of administration	Duration of observation	Sex	LD_{50} (mg/kg)	95% confidence limits (mg/kg)
Mouse	intravenous	14 days	M	12	9.1 - 17
		3 days	M	11.2	7.4 - 16.8
		5 days	-	13	10 - 17
	oral	7 days	M	105	75 - 147
			F	100	75 - 133
	subcutaneous	3 days	M	62	27 - 142
		5 days	-	70	38 - 127
Rat	intravenous	14 days	M	2.3	1.7 - 3.2
		5 days	-	2.3	1.5 - 3.5
	oral	7 days	M	18	29 - 113
			F	10 - 20	-
	intramuscular	5 days	M	1	-
			F	1 - 8	8 - 11
	subcutaneous	5 days	-	9.5	4.9 - 19
New-born	intragastrically	16 days	-	20	14 - 29
Dog	intravenous	5 days	M	15	10 - 22.5
			F	14	9.3 - 21
	intramuscular	7 days	M	30 - 40	-
			F	>40	-
	intramuscular	7 days	M	>0.35	-
			F	>0.35	-
	subcutaneous	5 days	-	>1.25	-
	intra-arterially	7 days	M	>0.3	-
			F	>0.3	-
Cat	intravenous	5 days	-	>0.16	-
Hamster	intramuscular	5 days	M	<8	-
			F	9.7	6 - 15

Continued

Preclinical Summary on E-TRANS™ (fentanyl HCl)

Species	Route of administration	Duration of observation	Sex	LD_{50} (mg/kg)	95% confidence limits (mg/kg)	Ref.
Guinea-pig	intravenous	0 days	-	3 [a)]		A 10
	intramuscular	5 days	M	50 - 55	40 - 63	A 6
			F	82	66 - 102	
Monkey	intravenous	1 day	M	>0.03	-	A 10

a) LD_{100}
- not known

2.6.6.3 Repeat-dose toxicity:

Repeat-dose toxicology studies were not completed for this NDA, however, the Sponsor summarized the reports from subchronic toxicity studies with fentanyl administered via IV (bolus and infusion), IM and linked the submission to the summarized raw data.

A Dose Range Finding study was performed to determine suitable doses for use in subsequent longer-term studies. Fentanyl, as fentanyl citrate, was administered once by an intravenous infusion over 24 hours up to a dose of 2 mg/kg. In addition, the effect of two doses, 0.025 and 0.4 mg/kg were evaluated after a continuous infusion for five days.

No abnormalities were noted when fentanyl was given as a single dose over 24 hours up to a dose of 0.1 mg/kg. At a dose of 0.2 mg/kg, given over 24 hours, one animal was slightly sedated while at 0.5 mg/kg, three out of five animals were sedated, resulting in a decrease in body weight and food consumption. A slight increase in aspartate aminotransferase and a slight increase in the weight of the adrenals were also noted after a single dose of 0.5 mg/kg. Single doses of 1and 2 mg/kg, given over 24 hours were lethal. Mortality was noted in two out of five rats after a single dose of 1 mg/kg and in three out of five rats after a single dose of 2 mg/kg. The animals died during the dosing period as a result of asphyxia. Various changes observed in the serum parameters of the surviving rats at these doses were a reflection of the bad condition of the animals.

Dosing at 0.025 and 0.4 mg/kg for five days was well tolerated. All rats survived the study. No adverse effects were noted except for a slight sedation of nearly all animals at 0.4 mg/kg. Based upon these data the doses for the continuous intravenous infusion toxicity study in rats were selected.

In the 5-week toxicity study, fentanyl was administered continuously by intravenous infusion to Wistar rats. The dosing period was followed by a 4-week recovery period in

order to examine the reversibility of any adverse effects. The doses were 0, 0.025, 0.1 and 0.4 mg/kg.

All rats survived the study. Dosing rats up to a dose of 0.4 mg/kg did not result in ophthalmologic abnormalities, changes in hematological and urinary parameters nor in macroscopic or histological changes.

Fentanyl was well tolerated and did not result in any adverse effects when animals were dosed at 0.025 mg/kg. When rats were dosed at 0.1 mg/kg, a few females became slightly excited in the last week of dosing. In addition, slight increases in serum glucose in both sexes and in serum inorganic phosphate in females were present. At a dose of 0.4 mg/kg, excitation was seen in several males and females. Decreases in food consumption, especially in males, as well as slight changes in some serum parameters were present in both sexes (increases in calcium and glucose in both sexes and increases in potassium and inorganic phosphate in females). In males, a slight decrease in body weight towards the end of the dosing period and a pronounced decrease in the first week of the recovery period were present, resulting in a slight decrease in the weight of the liver. All changes were reversible within a four-week recovery period.

Rats were given an intravenous injection of fentanyl, which was administered as the citrate salt, at doses of 0, 0.01, 0.02, 0.03, 0.05, 0.075 and 0.1 mg/kg during 4 weeks.

Intravenous dosing in rats resulted in mortality at all doses except the lowest dosage of 0.01 mg/kg, which was determined as the no toxic effect dose. No relevant changes in food consumption, hematology and organ weights were noted. Apart from mortality, an increase in serum aspartate aminotransferase was present from 0.02 mg/kg onwards. With the exception of cardiac lesions, which were seen at 0.01 and 0.02 mg/kg, no histological changes were observed after autopsy. These myocardial changes, however, were considered to be related to the euthanasia procedure i.e. cardiac puncture. The animals that died after dosing were not necropsied as their deaths were likely due to respiratory arrest and were generally peracute.

Fentanyl citrate was administered in, the diet to rats for 14 days. The doses were 0, 5, 10, 20, 40, 80, 160 and 320 mg/kg. Four rats (2 males and 2 females) were tested at each dosage.

Mortality occurred at doses from 10 mg/kg onwards in males and from 20 mg/kg onwards in females. The no toxic effect dose was 5 mg/kg.

The surviving rats at 40 mg/kg and 160 mg/kg had blood around the mouth and lower abdomen. The urine appeared bloody and there was bloody diarrhea. These complications only appeared during the first week. At higher doses (20, 40 and 160 mg/kg), decreased body weight was noted in the surviving rats even though food consumption increased. No other parameters were examined in this study.

Rats were evaluated in a 4-week toxicity study, designed to determine the toxicity of fentanyl after intramuscular administration. The doses administered were 0, 0.1 and 0.4 mg/kg. Deaths occurred in 4 out of 15 males dosed at 0.1 mg/kg and in 7 males and one female out of 15 in the high dose group. Decreased body weight was noted in the 0.1 mg/kg dosage group and became more pronounced in the 0.4 mg/kg dosage group. Testicular weight was slightly decreased in the 0.4 mg/kg-dosage group. Histology did not reveal any drug-related lesions. Repeated dose toxicity in dogs

Fentanyl, as fentanyl citrate, was administered to dogs intravenously by injection at doses of 0, 0.1, 0.3 and 1mg/kg. All dogs survived the study.

All the animals receiving fentanyl were sedated immediately after the administration of the compound, resulting in occasionally exhibited hyperpnea decreased food intake and lack of defecation. Upon recovery from sedation, excitement was occasionally observed in the dogs. Convulsions were seen in all dosage groups. These effects, sedation as well as convulsions were much more pronounced at the highest dose (1 mg/kg) than in the 0.1 and 0.3 mg/kg dosage groups. In addition, emesis and loss of righting reflex were observed in the animals of the 1 mg/kg dosage group.

A slightly decreased body weight was present at 0.1 mg/kg whereas a moderately to severely decreased body weight occurred at 0.3 and 1 mg/kg. Histological changes in the liver and the kidneys were related to the test article. The liver changes included hepatocellular changes, which were only present at 1 mg/kg and, at light microscopic level, focal cholestasis, which was present at all, doses. Vacuolar alterations in the kidney were noted at 0.3 and 1 mg/kg whereas granular casts in the lumen of the collecting tubules were only present in one dog at 1 mg/kg.

A second 4-week toxicity study was performed in dogs. In this study, fentanyl was administered by an intramuscular injection. The doses were 0, 0.1 and 0.4 mg/kg.

Fentanyl up to 0.4 mg/kg was well tolerated and did not lead to mortality. No effects on behavior and appearance body weight and food consumption was noted and no alterations in hematology, organ weights and histology were seen in any dosage group.

The following table is from Sponsor and summarizes the NOTEL values from the rat and dog subchronic studies:

Species	Duration /Route	Doses of fentanyl in mg/kg/day										
		0	0.01	0.02	0.025	0.03	0.05	0.075	0.1	0.3	0.4	1
Rat	5 weeks i.v.	X			X				X		X	
	4 weeks i.v.	X	X	X		X	X	X	X			
	4 weeks i.m.	X							X		X	
Dog	4 weeks i.v.	X							X	X		X
	4 weeks i.m.	X							X		X	

No toxic effect level

2.6.6.3 Genetic toxicology

Ames Test: Tested fentanyl at 8.4-2 1 00 mg/plate both with and without metabolic activation. The results were negative.

Mouse Lymphoma Assay: in the presence of metabolic activation, positive findings were reported at a concentration of 37 ug/mL.

Unscheduled DNA Synthesis Test: Tested fentanyl from 0.4-84 ug/ mL in primary rat hepatocytes. The results were negative.

Mammalian Cell Transformation Assay: Tested fentanyl at 2.5 to 50 ug/mL without S9 activation and 10-250 pig/mL with S9 activation in BALB/c-3T3 cells. The results were negative.

Chromosomal Aberrations Test: Tested fentanyl at 0.62 to 650 ug/mL without metabolic activation and 0.62-2500 ug/mL with metabolic activation in Chinese Hamster Ovary cells. The results were negative for both conditions.

In Vivo Mouse Micronucleus Test: These data are summarized from previously reviewed data submitted to NDA 19-813 (Serial number 209.1, for ALZA's Duragesic

product distributed by Janseen). This data was submitted on October 22, 1999, presumably as part of Phase IV commitments. According to the review by Dr. Kathleen Haberny, "Male and female mice received IV injection of fentanyl at 2.5, 5, 1 0, and 20 mg/kg. No increase in number of micronucleated polychromatic and normochromatic erythrocytes were observed in bone marrow. It was concluded that fentanyl did not induce structural and/or numerical chromosome aberrations in erythrocytes of bone marrow.

Mutagenicity test	*In vitro* or *in vivo*	Test system	Dose - route - frequency	Results
Point and/or gene mutations				
Ames test	*in vitro*	Salm. typhimurium	25 up to 2500 µg/plate +/- S9	no induction of gene mutations
			25 up to 2500 µg/plate +/- S9	no induction of gene mutations
			8.4 up to 2100 µg/plate +/- S9	no induction of gene mutations
Mouse lymphoma test	*in vitro*	Mouse lymphoma cells	13 up to 126 µg/ml +/- S9	increase in mutations only at cytotoxic conc. in the presence of S9 (false-positive finding)
		Mouse lymphoma cells	-S9: 200 up to 500 µg/ml +S9: 100 up to 600 µg/ml	no increase in gene mutations
Chromosome aberrations				
Micronucleus test	*in vivo* (i.v.)	Mouse	0.63, 2.5, 10 mg/kg	no increase in PCE with micronuclei
Chromosome aberrations	*in vitro*	Chinese hamster ovary cells	-S9: 0.06 up to 2050 µg/ml +S9: 0.62 up to 2050 µg/ml	no increase in cells with chromosome aberrations
		Human peripheral lymphocytes	-S9: 1.1×10^{-6} - 7.5×10^{-6} mol/l	no increase in cells with chromosome aberrations
Mammalian Cells Transformations				
Transformation	*in vitro*	BALB/C-$3T_3$ cells	25 up to 250 µg/ml +/- S9	no increase in transformed cells
Primary DNA damage				
Unscheduled DNA	*in vitro*	Rat hepatocytes	0.4 up to 84 µg/ml +/- S9	no increase in UDS

2.6.6.5 Carcinogenicity

Carcinogenicity summary: Carcinogenicity studies have not been completed for fentanyl. The current labeling for Janssen's Duragesic Transdermal product states that "Because long-term animal studies have not been conducted, the potential carcinogenic effects of DURAGESIC are unknown."

Carcinogenicity conclusions: Carcinogenicity studies are not required for this drug product due to the acute indication.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Male Fertility Study In SPF Wistar Rats (Segment 1): Experiment No. 3899

Key study findings: Fentanyl was administered to male rats (0, 0.025, 0.1, or 0.54 mg/kg/day) via continuous intravenous infusion for 4 weeks prior to and during mating with female rats (untreated). The following key findings were noted:

1. There were no mortalities in males at any dose tested.
2. Clinical signs included excitation in HD males beginning at week 4.
3. There were no clear changes in body weight and weight gain in males at the LD and MD. However, rats treated with the HD fentanyl demonstrated a significant decrease in weight gain noted from week 3 onwards.
4. There were no clear findings in either food consumption
5. The male reproductive fertility is not adversely affected by fentanyl administration (iv) upto 0.4 mg/kg/day.

Study no.: 3899
Volume #1 and page #: 233-270
Conducting laboratory and location: Janssen Lab, Belgium
Date of study initiation: 10- 27- 97
GLP compliance: Yes
QA reports: yes (x) no ()
Drug, lot #, and % purity: batch number B0046-950502, It passed the specifications of the chemical quality control department of that company and analysis revealed an assay value of —— (HPLC).

Methods

Doses: 0, 0.025, 0.1, 0.4 mg/kg/day
Species/strain: SPF Wistar rats
Number/sex/group: 20/sex/group
Route, formulation, volume, and infusion rate: IV infusion (48 mL/kg/day)

R004263 (batch number B0046-950502) was synthesized at ——. The test article was formulated as an isotonic, sterile aqueous solution at 3 concentrations: 0.52, 2.08 and 8.33 mg/mL. The ingredients of the solutions were R004263, pyrogen free water, citric acid and NaCl. The control solution consisted of pyrogen free water and NaCl.
Satellite groups used for toxicokinetics: N/A
Study design: The males were dosed 4 weeks prior to mating and females were left untreated

Parameters and endpoints evaluated: Please see the result section

Results

Adult rat data (dosed males and females)

Mortality: No test article-related mortality was observed in male rats dosed up to 0.4-mg/kg-body weight. No mortality was observed in the untreated females.
Clinical observations: Excitation was seen from week 4 onwards in 6 out of 19 males dosed at 0.4-mg/kg-body weight/day. No clinical signs were observed in the undosed female rats.

Body weight, weight gain and corrected maternal weight gain: There were no relevant changes in body weight and weight gain in males dosed at 0.025 and 0.1 mg/kg body weight/day. At 0.4 mg/kg body weight/day, a significant decrease in weight gain was noted from week 3 onwards, resulting in a decreased body weight. In the undosed females, body weight and corrected maternal weight gain were comparable between all groups.

Food consumption: There were no relevant changes in food consumption in males dosed at 0.025 and 0.1 mg/kg body weight/day. At 0.4 mg/kg body weight/day, a significant decrease in food consumption was noted throughout the dosing period. Food consumption was not adversely affected in the undosed females.

Copulation and fertility rate: Copulation and fertility rate was comparable between groups.

Pre coital interval: The pre-coital interval was comparable between all groups.

Weight of the gravid uterus: The weight of the gravid uterus was not adversely affected in rats dosed up to 0.4 mg/kg body weight/day.

Number of implantation and number of corpora lutea: The number of implantations and of corpora lutea were not adversely affected.

Pre- and post-implantation loss: There were no relevant adverse effects in pre- or post-implantation loss.

Offspring: There were no relevant adverse effects on the number of live and dead fetuses, the mean litter size or the number of resorptions.

Embryofetal development

Study title: Intravenous developmental toxicity study in the rabbit (Exp. No. 4397).

Key study findings: No teratogenic effect noticed after fentanyl administration (IV) upto 0.4 mg/kg/day; 3 /18 animals at high dose, 0.4 mg/kg died after the first dose in sedation.

Study no.: 4379
Volume #1 and page #: 133-231
Conducting laboratory and location: Janssen Laboratories, Belgium
Date of study initiation: 09-30-1997
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, and % purity: B0046, 960401

Methods

Doses: 0, 0.025, 0.1, 0.4 mg/kg
Species/strain: New Zealand rabbits
Number/sex/group: 18 female/group
Route, formulation, volume, and infusion rate:
In the present study, R004263 was formulated as a sterile, isotonic, aqueous solution at 3 concentrations: 3.13, 12.5 and 50 ug/mL. The ingredients of the solutions were R004263, citric acid and physiological saline. The test article formulations were administered by intravenous infusion at a volume of 8-mL/kg-body weight/day during 4 hours/day. The animals were dosed from day 6 until day 18 of pregnancy. The volumes to be administered were not adapted for changes in body weight. The control group was infused with a physiological saline solution at the same dosage volume during the same treatment period.
Satellite groups used for toxicokinetics: None

Study design:

There were 4 groups, each consisting of 18 females as follows:

Dosage group	mg/kg bwt/day
C: Control	0
L: Low	0.025
M: Medium	0.1
H: High	0.4

The 72 rabbits were artificially inseminated on 4 separate days. On each insemination day, 20 or 16 rabbits entered the study and were allotted to 4 groups (4 to 5 rabbits per group).

Parameters and endpoints evaluated: Please see the result section

Results

Mortality: One rabbit of the 0.025 mg/kg dosage group (animal No. 32) was sacrificed on day 21 of pregnancy because it was aborting. Animal No. 51 of the 0.1 mg/kg dosage group had a hind leg paralysis as the result of an accident on day 10 and was sacrificed that day. Three rabbits of the 0.4 mg/kg dosage showed severe sedation and died in the beginning of the dosing period (on days 8, 7 and 6, respectively). Necropsy of these animals did not reveal specific findings.

It is concluded that there was no test article-related mortality in rabbits dosed up to 0.1 mg/kg. The death of 3 rabbits dosed at 0.4 mg/kg in the beginning of the dosing period was considered test article-related.

Clinical Observations: Clinical observations consisted of sedation throughout the dosing period. Slight sedation was seen in all rabbits of the 0.025 mg/kg dosage group. The rabbits dosed at 0.1 mg/kg showed slight to moderate sedation. All rabbits dosed at 0.4 mg/kg showed severe sedation. Other clinical observations were only observed occasionally and their incidences were comparable between groups. They were therefore not considered test article-related.

Body weight and corrected maternal weight gain: Body weight was determined individually on days 0, 6, 9, 12, 15, 19, 22, 25 and 28 of the dams presumed pregnancy. Average body weight at the various time intervals and corrected mean maternal weight gain (maternal body weight on day 28 minus body weight on day 0 minus gravid uterus weight) is listed per dosage group in table 1.

Body weight was comparable between the control and the low dosage group. Body weight of rabbits dosed at 0.1 mg/kg was slightly decreased ($p<0.05$) at the end of the dosing period, but was comparable towards the end of the pregnancy period. Body weight of rabbits dosed at 0.4 mg/kg was decreased ($p<0.01$) towards the end of the dosing period and remained somewhat lower until the end of the pregnancy period ($p<0.05$, not statistically significant). A dose-dependent decrease in corrected mean maternal weight gain was seen in rabbits dosed at 0.1 mg/kg (not statistically significant) and 0.4 mg/kg ($p<0.01$).

Food consumption: There were no adverse effects on food consumption in rabbits dosed at 0.025 mg/kg. A dose-dependent decrease ($p<0.01$-0.001) in food consumption was seen during the dosing period in rabbits dosed at 0.1 and 0.4 mg/kg.

Weight of gravid uterus: The mean weight of the gravid uterus was increased in rabbits dosed at 0.025 mg/kg (not statistically significant) and 0.1 mg/kg ($p<0.05$). The increases, however, were not considered relevant since there was no dose-relationship. It is concluded that there were no test article-related adverse effects on the mean weight of the gravid uterus.

Number of fetuses and resorptions: The number of live fetuses and the mean litter size in rabbits of all fentanyl-dosed groups were higher ($p<0.05$, not statistically significant)

compared to the control group. The low number of live fetuses in the control group was mainly due to 3 females containing only early resorptions and no live fetuses. Moreover, since they were not dose-related, these differences were not considered test article-related. It is concluded that there were no test article-related adverse effects on the number of live and dead fetuses, the mean litter size or the number of early and late resorptions in rabbits dosed up to 0.4 mg/kg.

Number of implants and corpora lutea of pregnancy: There were no test article-related adverse effects on the number of implantation and the number of corpora lutea of pregnancy.

Terminal and necroscopic evaluations-section data (implantation sites, pre- and post-implantation loss, etc.):

Offspring (malformations, variations, etc.):

Pre- and post-implantation loss: The slight decrease ($p<0.05$) in pre-implantation loss in rabbits dosed at 0.1 mg/kg was not considered relevant since there was no dose-relationship. It is concluded that there was no increase in pre- or post-implantation loss in any of the fentanyl dosed groups.

Body weight and sex ratio of live fetuses: The sex ratio of the fetuses was comparable between all groups. There were no adverse effects on the body weight of the fetuses of rabbits dosed up to 0.1 mg/kg. At 0.4 mg/kg, the body weight of the fetuses was slightly decreased ($P<0.05$).

Fetal observations: After external examination, fetal skeletal examination (using the Alizar in Red staining technique) and fetal sectioning, a number of observations were made. Malformations were seen in 1, 2 and 2 fetuses of the control, low and medium dosage group, respectively. One fetus of the control group showed fused ribs. In the 0.025-mg/kg-dosage group, one of the fetuses had a bifurcated rib and one fetus had an eye filled with brown mass. One fetus of the 0.1 mg/kg dosage group had a. bifurcated rib. Another fetus of this dosage group had no gonads, and sex could not be determined. All these malformations were seen in only few fetuses and there was no dose-dependent increase in the incidence of these malformations. Therefore, they are not considered test-article-related. The slightly increased incidence ($p<0.05$) of a rudimentary 13th pair of ribs in the 0.025 mg/kg dosage group was not dose-related and therefore not considered relevant. The incidences of all other minor abnormalities and variations were comparable between all groups. In conclusion, fetal evaluation revealed no teratogenic effects in rabbits dosed up to 0.4 mg/kg.

Prenatal and postnatal development

Study title: Intravenous pre- and postnatal developmental toxicity study in the Wistar rat Report No. 4055

Key study findings:

- In the pre and post natal development study in rat (IV infusion, Segment III) body weight of male and female pups on day 4 ($p<0.05$) and day 7 (not statistically significant) was slightly decreased in the 0.4 mg/kg dosage group. At 0.4 mg/kg/day, survival rate after 4 days was decreased ($P<0.001$).
- Statistical analysis revealed that incisors erupted and eye opened somewhat later in some animals of the mid (0.1 mg/kg/day) and high dosage groups (0.4mg/kg/day).
- The activity parameters examined comprised static and mobile movements, rearing, active and mobile time. The number of static as well as mobile movements were slightly increased ($p<0.05$ -0. 0 1) in the mid (0.1 mg/kg/day) and high dose (0.4 mg/kg/day) group at 28 days of age. These effects were transient since all activity parameters were comparable between all groups at 50 days of age.

Study no.: 4053
Volume #1 and page #: 1-181
Conducting laboratory and location: Janssen Lab, Belgium
Date of study initiation: January 20th, 1997
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R004263, —.

Methods

Doses: 0.025, 0.1, 0.4 mg/kg
Species/strain: 96 female Wistar rats were used
Number/sex/group: 24 female/group
Route, formulation, volume, and infusion rate: IV infusion at the rate of 1mL/hr, the compound was formulated in 0.9% saline, the animals were dosed 48 mL/kg body wt/day from day 6 of pregnancy upto 3 weeks of lactation
Satellite groups used for toxicokinetics:
Study design: The dams and the F1 generation were evaluated for the parameters described in the result section. The F1 generation was also mate to evaluate the fertility index.
Parameters and endpoints evaluated refer to the result section.

Results

F_0 in-life:

Clinical observations during pregnancy and lactation occurred only occasionally and their incidences were comparable between all groups. It is therefore concluded that there were no relevant adverse clinical symptoms in any of the groups during the pregnancy or the lactation period.

Mortality: No mortality was noted in the female rats dosed at 0.025 and 0. 1 mg/kg. One female (Animal No. 16) of the control group and 1 female (Animal No. 92) of the high dosage group had not delivered pups. These animals were sacrificed on day 26 and proved to be not pregnant. Animal No. 93 of the high dosage group died on day 24 of pregnancy. Shortly before death, food consumption was decreased and the animal showed a rough haircoat and a bad condition. Necropsy revealed a swollen spleen and multiple white foci on the kidneys. These findings were, not considered test article-related and the death was considered incidental. The animal had 7 implantations. It is concluded that there was no test article-related mortality in this study.

Body weight: The females were weighed individually on days 1, 6, 11, 17 and 22 of their presumed pregnancy and further on the day of delivery and on days 4, 7, 14 and 21 of lactation. Body weight and weight gain (body weight day 22 body weight day 6) recorded for the various groups during pregnancy. Body weight during pregnancy and at delivery and weight gain during pregnancy were comparable between all groups. During the lactation period, body weight of females dosed at 0.025 and 0.1 mg/kg was comparable to that of the control group. At 0.4 mg/kg, body weight was marginally decreased (not statistically significant) throughout the lactation period.

Food consumption: There were no adverse effects on food consumption in females dosed up to 0.1 mg/kg. Food consumption was slightly decreased (not statistically significant) throughout the pregnancy period and throughout the lactation period in the females dosed at 0.4 mg/kg.

Fertility rate and gestation rate: Fertility and gestation rate was comparable between all groups.

F_0 necropsy:

Duration of gestation: The increase ($p<0.05$) in duration of gestation at 0.1 mg/kg was marginal, not dose-related and therefore not considered relevant. It is concluded that the duration of gestation was comparable between all groups.

Number of implantations: The number of implantations was recorded after natural delivery. The number of implantations was comparable between all groups.

F_1 generation data: lactation period

Number of live and dead pups and mean litter size: The mean litter size and the number of live and dead pups per pregnant female were recorded after natural delivery. The mean litter size and the number of live and dead pups per pregnant female were comparable between all groups.

Birth rate: The birth rate is given in the following table.

Dosage group (mg/kg)	Birth rate
Control	298/326
0.25	328/362
0.1	316/337
0.4	308/342

Significance computed by Chi Square test (two-tailed): * P<0.05-, P<0.01; P<0.001
Birth rate was comparable between all groups.

F_1 physical development:

Fetal and litter observations: In this study only the presence of external anomalies was evaluated. Fetal observations were only observed occasionally and their incidences were comparable between all groups. No teratogenic effects were evidenced in the fentanyl-dosed groups.

It is therefore concluded that there were no relevant adverse clinical symptoms or teratogenic abnormalities in the pups of the dosed groups.

Body weight: The pups were weighed individually at delivery and also on days 4, 7, 14 and 21 of age. Body weight of pups at birth was comparable between all groups. Body weight of male and female pups on day 4 ($p<0.05$) and day 7 (not statistically significant) was slightly decreased in the 0.4 mg/kg dosage group. Body weight of the pups on days 14 and 21 was comparable between all groups.

Survival rate: Survival of pups was recorded on days 4, 7, 14 and 21 of age. The survival rate of pups after 4 days was comparable between the control and the 0.025 and 0. 1 mg/kg dosage group. At 0.4 mg/kg, survival rate after 4 days was decreased ($p<0.001$). After 7, 14 and 21 days, the survival rate of the pups was comparable between all groups.

Physical development: F_1 pups were further examined for physical landmarks of development at various time-periods. Statistical analysis revealed that incisors erupted and eye opened somewhat later in some animals of the medium and high dosage groups compared to the control group. These slight differences, however, were not considered to be relevant. It is concluded that physical landmarks of development were normal and comparable between groups.

F_1 generation data: growth period:

During a 3-month growth period, two F_1 males and two F_1 females (reduced to 1 male and 1 female per litter after the conduct of the object discrimination test) per litter were followed for clinical observations and body weight.

Clinical observations: There were no adverse clinical observations in any of the groups.

Body weight and weight gain: The body weights and weights gain of F_1 parental animals were measured and reported on a weekly basis. Body weight and weight gain was comparable between all groups.

Sexual and behavioral development: F_1 pups were further examined for sexual landmarks of development at various time-periods. Statistical analysis revealed that sexual landmarks of development were normal and comparable between groups.
Locomotor activity was examined at the age of approximately 28 and 50 days with an activity monitoring equipment (Benwick Electronics). The activity parameters examined comprised static and mobile movements, rearing, active and mobile time. The number of static as well as mobile movements were slightly increased ($p<0.05$-0.01) in the medium and high dosage groups at 28 days of age. These effects were transient since all activity parameters were comparable between all groups at 50 days of age. It is therefore concluded that there were no adverse effects on the locomotor activity of the offspring.

Object discrimination test: The discrimination index was not altered by fentanyl treatment. No major deficit observed.

F_1 reproduction:

Clinical observation: There were no adverse clinical observations in any of the groups.

Mortality: No mortality occurred in any group.

Body weight and corrected weight gain: The females that were sacrificed on day 15 of pregnancy, were weighed individually on days 1, 9 and 15 of there presumed pregnancy. Body weight and corrected weight gain were comparable between all groups.

Food consumption: During pregnancy, food consumption was recorded individually for the periods day 1- 8 and day 9 - 14. Food consumption was comparable between all groups.

Copulation and fertility rate: Copulation and fertility rates were comparable between all groups.

Pre-coital interval: The pre-coital interval was comparable between all groups.

Weight of gravid uterus: The weight of the gravid uterus was comparable between all groups.

F_2 findings:

Number of fetuses and resorptions: The number of live fetuses and the number of resorptions were comparable between all groups.

Number of implantations and corpora lutea of pregnants: The number of implantations and the number of corpora lutea of pregnancy were comparable between all groups.

Pre- and post-implantation loss: There were no adverse effects on pre- or post-implantation loss.

Summary

The current submission referred to 3 new reproductive studies with fentanyl as follows:

Species		Doses (mg/kg/day)	Batch-number	Type of study
Reproductive function				
Rat	i.v. (infusion)	0, 0.025, 0.1, 0.4	B0046-950502	male fertility
	i.v. (infusion)	0, 0.025, 0.1, 0.4	B0046-950502	female fertility + developmental
Developmental toxicity		Embryotoxicity and teratogenicity		
Rabbit	i.v. (infusion)	0, 0.025, 0.1, 0.4	B0046-960401	developmental
Developmental toxicity		Pre- and postnatal toxicity		
Rat	i.v. (infusion)	0, 0.025, 0.1, 0.4	B0046-950502	pre- and postnatal developmental

Male Fertility Study in SPF Wistar Rats (Segment 1): Experiment No. 3899

Fentanyl dosed up to 0.1-mg/kg-body weight/day in male rats did not lead to mortality or clinical symptoms. In male rats dosed at 0.4-mg/kg-body weight/day, parental toxicity was evidenced by the presence of excitation in some animals and by decreased body weight, weights gain and food consumption.

None of the maternal or litter parameters studied were adversely affected in any of the groups, indicating that up to 0.4 mg/kg there were no primary adverse effects on male fertility. Furthermore, histopathology of the male genital tract organs (Exp. 3831) did not indicate drug-related changes.

Intravenous developmental toxicity study in the rabbit (Exp. No. 4397).

The potential effects of R004263 on the embryo-fetal development were evaluated when administered by intravenous infusion from day 6 to day 18 of pregnancy at dosages of 0.025, 0.1 and 0.4 mg/kg.

R004263 dosed up to 0.1 mg/kg body weight/day in pregnant female rabbits did not lead to mortality. The death of 3 out of 18 rabbits dosed at 0.4 mg/kg in the beginning of the

dosing period was considered test article related. Clinical observations consisted of sedation throughout the dosing period. Slight sedation was seen in all rabbits of the 0.025 mg/kg dosage groups. The rabbits dosed at 0.1 mg/kg showed slight to moderate sedation. All rabbits dosed at 0.4 mg/kg showed severe sedation. Body weight, corrected mean maternal weight gain, food consumption and weight of the gravid uterus were not adversely affected in rabbits dosed at 0.025 mg/kg. A dose-dependent decrease in body weight and food consumption during the dosing period and in corrected mean maternal weight gain was seen in rabbits dosed at 0.1 and 0.4 mg/kg.

As for the litter parameters, there were no adverse effects on weight of the gravid uterus or on most of the other parameters studied (number of fetuses, resorptions, implantations and corpora lutea, pre- and post- implantation loss, sex ratio of live fetuses) in any of the groups. Only a slight decrease in body weight of the live fetuses of the 0.4 mg/kg dosage group was seen, which was considered to be related to maternal toxicity. Fetal evaluation revealed no test article related teratogenic effects in any group.

Intravenous pre- and postnatal developmental toxicity study in the Wistar rat Report No. 4055:

The potential effects of fentanyl (R004263) on the pre- and post-natal development in rats were assessed when administered continuously by intravenous infusion from day 6 of pregnancy through a 3-week lactation period at dosages of 0.025, 0.1 and 0.4 mg/kg body weight/day.

Fentanyl dosed up to 0.4 mg/kg body weight/day did not lead to mortality nor resulted in relevant clinical signs. There were no relevant adverse effects on body weight, weight gain or food consumption during the pregnancy or the lactation period in females dosed up to 0. 1 mg/kg. A marginal decrease in body weight throughout the, lactation period and a slight decrease in food consumption throughout the pregnancy and the lactation period in the females dosed at 0.4 mg/kg evidenced slight maternal toxicity. Fertility and gestation rate, duration of gestation and the number of implantations were comparable between all groups of dosed females.

As for the F_1 generation, there were no adverse effects on any of the parameters in the 0.025 or 0.1 mg/kg dosage groups during the lactation and growth period. The parameters studied in these periods included the number of live and dead pups, mean litter size, birth rate, fetal observations, body weight, survival rate, physical, sexual and behavioral development and object discrimination test. In the 0.4 mg/kg dosage group, there was a slight decrease in body weight of the pups on days 4 and 7, and the survival rate was decreased at 4 days after birth. The survival rate was comparable to the control values from day 7 onwards, the body weight of the pups from day 14 onwards. There were no adverse effects on any of the other parameters in this dosage group.

The Reproductive performance of the undosed F_1 generation (copulation and fertility

rates, weight of gravid uterus, pre-coital interval, number of corpora lutea of pregnancy, number of live and dead F2 fetuses, implantations and resorptions, pre- and post-implantation loss) was comparable between all groups. These findings indicate that fentanyl does not result in primary adverse effect in perinatal and postnatal parameters on the F_1 generation and it is not considered to be a behavioral teratogen.

The present submission also referred to the previous reproductive and developmental toxicology summary (reviewed by the Division already by Dr. Dan Mellon): Janssen referenced a total of 5 reproductive toxicology studies in rats to support the NDA for Duragesic. Four of these studies were initially conducted by McNeil Laboratories in support of NDA 16-619 (Sublimaze (fentanyl citrate) Injection).

Design of Reproductive Toxicity Tests Performed with Fentanyl in Rats

Study	Number of females (per dosing level)	Mode of administration	Dose (mg/kg/day)	Duration of treatment
1	25	intravenous	0, 0.01, 0.03	Day 6 – 18
2	G_1 = 100, 11, 11, 12, 6 G_2 = 50, 6, 6, 3 G_3 = 200, 3	subcutaneous	G_1 = 0, 0.16, 0.32, 0.64, 1.25 G_2 = 0, 0.16, 0.32 G_3 = 0, 0.16	Day 0 – 21 gestation, all groups
3	100, 5, 5, 6	subcutaneous	0, 0.04, 0.08, 0.16	Day 0 – 21
4	200, 20, 20, 20, 20	subcutaneous	0, 0.16, 0.32, 0.64, 1.25	Day 0 – 21
5	43, 28, 28, 28, 28	subcutaneous infusion	0, 0.01, 0.1, 0.5	2 weeks prebreeding, during breeding and Day 1 – 21

Study 2 above was also conducted by McNeil Laboratories to support NIDA 16-619 (Sublimaze (fentanyl citrate) Injection). The results of study 2 demonstrated a significant decrease in pregnancies in the treated groups, an increase in the number of still born, but no change in litter size or significant malformations.

Study 3 above was also conducted by McNeil Laboratories to support NDA 16-619 (Sublimaze (fentanyl citrate) Injection). The results of study 3 demonstrated one or two deaths at each dose tested. The study involved 100 animals in the control group and 5-6 per test dose. The results indicated 80 litters born to the control group, 4 litters at the low dose and 1 litter each at the higher doses. There were no fetal abnormalities noted and no decrease in litter size of successful pregnancies. There were decreased birth weights and increased resorptions at higher doses.

Study 4 above was also conducted by McNeil Laboratories to support NIDA 16-619 (Sublimaze (fentanyl citrate) Injection). The results of study 4 demonstrated a decrease in pregnancies and average weight of pups. There were no changes in litter size, but the number of resorptions increased with dose.

Study 5 listed above is a published report by Fujinaga et al. (1986). This study concluded, "fentanyl is devoid of adverse reproductive effects in this strain of rats [Sprague-Dawley] up to dosages of 500 ug/kg/day administered by osmotic minipumps."

Reproductive and developmental toxicology conclusions: Fentanyl has been listed as Pregnancy Category C. The current labeling for Duragesic reads as follows:
"Fentanyl has been shown to impair fertility and to have an embryocidal effect in rats when given in intravenous doses 0.3 times the human dose for a period of 12 days. No evidence of teratogenic effects has been observed after administration of fentanyl to rats. There are no adequate and well- controlled studies in pregnant women. DURAGESIC should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.'

2.6.6.7 Local tolerance

Study title: Primary Skin Irritation Study of Electro- transport Therapeutic Systems Containing Fentanyl in Guinea Pigs

Key study findings:

- Primary Irritation Indexes calculated for the fentanyl gel (anode) and saline gel (cathode) (1.9 and 0.7) indicate that both were mild irritants on hairless guinea pig skin. Residual drug analysis showed a mean fentanyl release for the ETS (fentanyl) to be 2.24-mg/kg/16 hour. Histological assessment indicated that the treatment resulted in minimal to moderate changes in parakeratosis, acanthosis, and hyperkeratosis for intact hairless guinea pig skin sites following a 16 hour exposure to fentanyl (anode) and saline (cathode).
- The continuous administration of 70 uA total current (current density 0.1 mA/ cm^2) and the drug flux of 111 ug/ cm^2 /h for 16 hours resulted in clinical observed systemic effects which can be attributed to the analgesic activity of the compound. The intended maximum dose for human is 3.6 mg/day. For a 70 kg human, this would be a dose of 0.051 mg/ kg/day. The dose administered for this study in guinea pigs was 2.2-mg/kg/16 hour, which is about 40 times the human daily dose.

Study no.: TR-92-1561-021
Volume # 1 and page #: 1-46
Conducting laboratory and location: ALZA CORPORATION, Palo Alto, CA
Date of study initiation: 12-17-92
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, and % purity: — , ETS Fentanyl system and — , zero order controller system

The ETS (fentanyl) 5 mg disposable component contained an anode hydrogel and a cathode hydrogel, each approximately 1.6 cm in diameter and 0.16 cm thick which were in contact with a anode electrode and a cathode electrode, respectively. The overall dimensions of the disposable component were 6.9 cm by 8.5 cm. The anode of each component was masked using a release liner that had an opening of about 0.7 cm^2.

The formulation was 1.31% fentanyl HCl, 0.09% fentanyl base. USP grade water. polyvinyl alcohol, USP grade water. sodium chloride, citric acid, glycerin, sodium citrate, and

The zero-order disposable controller was used in conjunction with the ETS (fentanyl) 5-mg disposable component. It contained the power source set to 100 uA/ cm^2, current density, for a total current of about 70 uA (electrode area 0.7 cm^2).

Formulation/vehicle: The ETS system with saline in the cathode gel was used as control vehicle.

Methods: Six male hairless guinea pig were used for the present study. The ETS patch were used on either side of the head on the intact skin (please seed the study design for detailed orientation of the fentanyl, saline and the controls area which are actually the untreated area).

Doses

Fentanyl Doses in Hairless Guinea Pigs

Current Density ($\mu A/cm^2$)	Electrode Area cm^2	Wearing Time (h)	Rate of Fentanyl Delivered[a] ($\mu g/cm^2/h$)	Total Dose (mg/h)	Total Dose (mg/kg[b]/h)
32.5	2.0	3	58	0.348	0.696
32.5	2.0	6	58	0.696	1.39
100	1.0	6	122	0.732	1.46
32.5	2.0	16	58	0.928	1.86
100	0.7	16	122	1.37	2.74
32.5	2.0	24[c]	58	2.78	5.56
100	1.0	24[c]	122	2.93	5.86

a Based an residual analysis of fentanyl hydrogels
b Based on a 0.5 kg guinea pig
c After a wearing period of 24 hours, deaths were seen in two guinea pigs/group 48 and 72 hour post- removal of fentanyl-containing hydrogels

Study design:

Six hairless guinea pigs with two intact dorsal skin sites

HEAD

I II

A = Fentanyl anode hydrogel site
B = Saline cathode hydrogel site

Guinea Pig #	365	366	367
	B A	B A	B A
	368	369	370
	B A	B A	B A

Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious hairless guinea pigs (n-6) for 16 hours to evaluate skin irritation. The current density of each system was 0.1 mA/ for a total current of about 70 uA. The ETS (fentanyl) disposable component was comprised of a fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel, which was used in conjunction with a zero-order disposable controller. The systems were secured with [redacted] bandage [redacted] tape, and [redacted] tape. After the completed wearing period, the systems were removed and the anode and cathode sites were scored for erythema and eschar formation and edema at 0.5 and 48 hours post system removal. Residual drug analysis showed a mean fentanyl release for the ETS (fentanyl) to be 2.24 mg/kg/16 hour or drug flux of 111 ug/ cm^2 /h.

Results: The animals were first scored visually for primary and measured in the Draize scale. The primary observation was followed by histological evaluation of the skin for the assessment of the parakeratosis, hyperkeratosis and acanthosis.

Primary Irritation Scores
16 hour Application in Guinea Pigs

Treatment	Guinea Pig Number	Intact Sites 0.5 hr[a] ery	0.5 hr[a] ed	48 hr ery	48 hr ed	Primary Irritation Index[b]
	365	2	0	2	0	
Fentanyl	366	2	0	2	0	
anode	367	2	0	2	0	1.9
hydrogel	368	2	0	1	0[e]	
	369	2	0	2	0	
	370	2	0	2	0	
Means		2.0		1.8		
	365	1	0[c]	1	0	
Saline	366	1	0[c]	0	0	
cathode	367	1	0	0	0	0.7
hydrogel	368	1	0[c]	0	0[e]	
	369	1	0[d]	1	0	
	370	1	0[c]	0	0	
Means		1.0		0.3		

ery = erythema and eschar formation

ed = edema formation

[a] Hour(s) after removal of test and control articles

[b] PII = sum of erythema and edema scores divided by # observations

[c] Less than ten small raised areas (<0.1 mm) resembling "goose-bumps"

[d] Raised focal area (2 mm in diameter) on perimeter of site

[e] This animal was euthanized at 24 hour post removal of application not 48 hour post removal of application

Histology: Histological assessment indicated that the treatments resulted in average minimal to moderate changes for intact hairless guinea pig skin sites following a 16 hour exposure to fentanyl (anode) and saline (cathode). There were biologically meaningful increases in the incidences, but not severities of parakeratosis, acanthosis, and hyperkeratosis when compared to the respective saline control skin sites. There were no biologically meaningful differences in severities of parakeratosis, acanthosis, and hyperkeratosis in the fentanyl-exposed sites when compared to the saline control sites. No lesions were observed in any of the untreated control sites.

Macroscopic observations for two animals led to their necropsy and tissues submission for histological evaluation. Histopathology evaluation showed that in one animal (#368) the inflammation of the terminal rectum (observed during macroscopic evaluation) was apparently due to the rectal prolapse seen grossly. The underlying cause of the prolapse could not be determined on the basis of histopathology. Rectal prolapse usually results from hypermotility associated with acute enteritis and proliferative ileitis (Harkness et al 1989).

Histopathology evaluation showed that in animal #367, the kidney lesions were characterized as acute tubular necrosis or nephrosis. Nephrosis is produced by exogenous or endogenous toxic substances that reach the kidney via the bloodstream (Casey et al 1978a). Endogenous nephrosis may result from a combination of toxic metabolites produced by the existing diseases combined with reduced renal blood flow (Casey et al 1978b). For animal #367, an endogenous type of necrosis is suspected to be the cause for the nephropathy, coupled with reduced renal blood flow which may occur in states of dehydration as seen in guinea pig #367. The necrosis of the liver is most likely related to the metabolic disturbances from the primary nephropathy. The urinary changes of #367 are most likely artifacts secondary to the intraperitoneal injection of Beuthanasial. It was suspected it was inadvertently injected into the lumen of the urinary bladder.
For another guinea pig #368, moderate to mark diffuse lymphoid cell depletion was seen in the spleen of this animal. This most likely represents lympholysis due to stress-induced hyperadrenocorticism. Inflammatory conditions and inanition often result in depletion of the erythroid cellular elements of the marrow. All remaining system tissue changes for both guinea pigs were considered to be within the range of normality for the guinea pig.

Summary and conclusion

Primary Irritation Indexes calculated for the fentanyl gel (anode) and saline gel (cathode) (1.9 and 0.7) indicate that both were mild irritants on hairless guinea pig skin. Residual drug analysis showed a mean fentanyl release for the ETS (fentanyl) to be 2.24-mg/kg/16 hour. Histological assessment indicated that the treatment resulted in minimal to moderate changes in parakeratosis, acanthosis, and hyperkeratosis for intact hairless guinea pig skin sites following a 16 hour exposure to fentanyl (anode) and saline (cathode).

The continuous administration of 70 uA total current (current density 0.1 mA/ cm^2) and the drug flux of 111 ug/ cm^2 /h for 16 hours resulted in clinical observed systemic effects

which can be attributed to the analgesic activity of the compound The intended maximum dose for human is 3.6 mg/day. For a 70 kg human, this would be a dose of 0.051 mg/ kg/day. The dose administered for this study in guinea pigs was 2.2-mg/kg/16 hour, which is about 40 times the human daily dose.

Study title: Fourteen Day Subchronic Skin Irritation Study of Electrotransport Therapeutic Systems Containing Fentanyl in Guinea Pigs

Key study findings:

- The continuous administration of 70 uA total current (current density 0.1 mA/cm^2) and the drug flux of 110 ug/ cm^2/h for 8 hours resulted in clinical observed systemic effects which can be attributed to the analgesic activity of the drug. The intended maximum dose for humans is 3.6 mg/day. For a 70 kg human, this would be a dose of 0-0.051 mg/kg/day. The dose administered for this study in guinea pigs was 1.21 mg/kg/8 hour, which is about 25 times the human daily dose.

- Histological assessment indicated that the treatments resulted in minimal to moderate changes in parakeratosis, acanthosis, and hyperkeratosis for intact hairless guinea pig skin sites following fourteen 8-hour exposures to fentanyl (anode) and saline (cathode).

Study no.: TR-92-1561-023
Volume # 1 and page #: 1-45
Conducting laboratory and location: ALZA CORPORATION, Palo Alto, and Ca
Date of study initiation: TR-92-1561-023
GLP compliance: yes
QA reports: yes (x) no ()
Drug, lot #, and % purity: — / ETS Fentanyl system and ' — ; zero order controller system

The ETS (fentanyl) 5 mg disposable component contained an anode hydrogel and a cathode hydrogel, each approximately 1.6 cm in diameter and 0.16 cm thick which were in contact with a — anode electrode and —— cathode electrode, respectively. The overall dimensions of the disposable component were 6.9 cm by 8.5 cm. The anode of each component was masked using a — release liner that had an opening of about 0.7 cm^2.

formulation was 1.31% fentanyl HCl, 0.09% fentanyl base. . USP grade water. —— polyvinyl alcohol, ——
—— nine.

— . USP grade water, ——
citric acid ——
— polyvinyl alcohol. —— % sodium citrate, and —
——

The zero-order disposable controller was used in conjunction with the ETS (fentanyl) 5-mg disposable component. It contained the power source set to 100-uA/ cm^2, current density, for a total current of about 70 uA (electrode area 0.7 cm^2).

Formulation/vehicle: The ETS system with saline in the cathode gel was used as control vehicle.

Methods

Doses:

Fentanyl Doses in Hairless Guinea Pigs

Current Density ($\mu A/cm^2$)	Electrode Area cm^2	Wearing Time (h)	Rate of Fentanyl Delivered[a] ($\mu g/cm^2/h$)	Total Dose (mg/h)	Total Dose ($mg/kg^b/h$)
32.5	2.0	3	58	0.348	0.696
32.5	2.0	6	58	0.696	1.39
100	1.0	6	122	0.732	1.46
32.5	2.0	16	58	0.928	1.86
100	0.7	16	122	1.37	2.74
32.5	2.0	24[c]	58	2.78	5.56
100	1.0	24[c]	122	2.93	5.86

a Based an residual analysis of fentanyl hydrogels
b Based on a 0.5 kg guinea pig
c After a wearing period of 24 hours, deaths were seen in two guinea pigs/group 48 and 72 hour post- removal of fentanyl-containing hydrogels.

Study design:

Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious hairless guinea pigs (n=10) for 8 hours to evaluate skin irritation. There were a total of fourteen applications (alternated between two sites) for each guinea pig. The current density of each system was 0.1-mA/ cm^2 for a total current of 70 uA. The ETS (fentanyl) disposable component was comprised of a Fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel, which was used in conjunction with a zero-order disposable controller. The systems were secured with [redacted] bandage [redacted] tape, and [redacted] tape. Observations for retention of systems were made periodically during the wearing period. After each application, the systems were removed, the anode and cathode sites were scored for erythema and eschar formation and edema at 0.5 hour post system removal, and additional observations were made at 24 and 48 hours post system removal of final applications (day 13 and 14). Application site map is provided below:

Application Sites

Ten hairless guinea pigs with fourteen consecutive applications rotated among four sites

HEAD

I II = Applications 1,3,5,7,9,11,13

III IV = Applications 2,4,6,8,10,12,14

I, III = Saline cathode hydrogel site
II, IV = Fentanyl anode hydrogel site

Results: All guinea pigs were observed to be normal and healthy prior to application of the electrotransport therapeutic systems containing fentanyl. Upon daily removal (at the end of 8 hours), all animals appeared lethargic. The following morning prior to the next application, all animals appeared to have normal activity and relatively normal body temperature. However, there were two guinea pigs that had a cooler than expected body temperature when compared to the other recovered guinea pigs when handled. This occurred at 0.5-hr post-removal of application #1 and 8-hour post ap. Animal temperatures were not quantified with the use of a thermometer at any time during the study. These were the only incidences where cool body temperature was observed. All remaining guinea pigs had normal body temperature when handled for the duration of the study.

In addition, other clinical signs were also observed. A total of five guinea pigs showed rectal bleeding (pink-red fluid) as follows: Guinea pig #371 and #379 at 0.5 hour post removal of application #1, #374 at 0.5 hour post removal of application #2, #372 at 8 hour post- application, #376 at 8 hour post-application #6. Within a half-hour later, no signs of bleeding were seen. This bleeding does not appear to be uncommon. This bleeding was previously reported by McNeil Labs (1967b) in rats that received 40 and 160 mg/kg fentanyl for 14 days in their diet (McNeil Labs 1967b). The surviving animals had bloody urine, bloody diarrhea, and blood around the mouth and lower abdomen during the first week of treatment.

All guinea pigs lost weight during the study. The weight loss for guinea pigs #374 and #376 were quite apparent by observation. Guinea pigs #374 and #376 appeared to be dehydrated and skinny in appearance by the 10th application and remained the same until the end of the study. At the time of the 14th application, the head of guinea pig #376 was slightly tilted. The cause may be attributed to a possible ear infection. The average percentage weight lost was 26% for all guinea pigs. The range of percentage lost was 16%-39%. A frequently observed side effect of analgesics in the guinea pig is inappetence or anorexia (Flecknell 1987). This side effect most probably caused the biologically meaningful decrease in body weight over the course of the study.

Guinea Pig Body Weights (g)

Guinea Pig Number	Week 1	2	3	3[a]	Weight Loss A	% Weight Loss
371						23
372						35
373						16
374						33
375						22
376						39
377						16
378						31
379						27
380						21
Mean	694	541	506	511	183	26
SD	51.3	41.7	53.2	53.4	59.5	8.0

[a] Weight recorded after final observation

Cumulative Irritation Indexes calculated for the fentanyl gel (anode sites II and IV) and saline gel (cathode sites III and I) (1.9 and 0.6) indicate that both were mild irritants on hairless guinea pig skin.

Cumulative Skin Irritation Scores[j]

SITE II: Fentanyl (Anode)

Observation (h)	Application	Guinea Pig Number 371	372	373	374	375	376	377	378	379	380	Sum
0.5	1	2	2	2	2	2	2	2	2	2	1	19
0.5	3	2	2	2	2	2	2	2	2	2	2	20
0.5	5	2[a]	2[a]	2[a]	2[a]	2[ab]	2[a]	2[a]	2[ab]	2[a]	2[a]	20
0.5	7	2	2[a]	2[a]	2[a]	2[bc]	2[ac]	2[ac]	2[abc]	2[a]	2[ac]	20
0.5	9	2[a]	2[a]	2	2[a]	2[ab]	3[a]	1[c]	2[abc]	2[a]	2	20
0.5	11	2[a]	2[a]	2[a]	2[a]	2[ab]	2[ab]	2[ab]	2[ab]	2[ab]	2[ab]	20
0.5	13	2[a]	3[a]	2	1[d]	2[ab]	2[ab]	2[ab]	2[ab]	2[ab]	2[a]	20
24	13	2	2	2	1[e]	2[ab]	2	2	2[ab]	1[b]	2	18
48	13	2	2	2	1[g]	1[ab]	2	1	1[ab]	0[b]	2	14
											Total =	171
											CII[k] =	1.9

[a] Red markings seen on guinea pig back due to adhesive from system; seen on perimeter of system

[b] Bruise on center of back of guinea pig due to system

[c] Flaky epidermis seen on sides and ventral portion of body

[d] 48 hour observation for application #11

[e] 72 hour observation for application #11

[f] 48 hour observation for application #12

[g] 96 hour observation for application #11

[h] 72 hour observation for application #12

[i] 96 hour observation for application #12

[j] Irritation scores are combined erythema and edema scores for each observation

[k] Cumulative Irritation Index = Sum/(No. Observations)

Histology : Histological assessment indicated that the treatments resulted in minimal to moderate changes for intact hairless guinea pig skin sites following fourteen 8-hour exposures to fentanyl (anode sites II and IV) and saline (cathode sites I and III). There were biologically meaningful increases in the incidences, but not the severities of parakeratosis, acanthosis, and hyperkeratosis, when compared to the respective saline and untreated control skin sites. There were no biologically meaningful differences in severities of parakeratosis, acanthosis, and hyperkeratosis in the test article treatment sites, when compared to the saline and untreated control treatment sites.
The very minimal degree of lymphohistocytic cellular infiltrate and dermal fibrosis seen in one animal in the untreated control group was considered an incidental finding. In addition, the presence of a follicular cyst in one animal in the saline control group was also considered incidental. None of these incidental findings were considered to have any meaningful impact on the study results.

The current measurements recorded after application and before removal of systems indicate that all systems were functioning properly.

Summary and conclusion

Cumulative Irritation Indexes calculated for the fentanyl gel (anode sites II and IV) and saline gel (cathode sites I and III (1.9 and 0.6) indicate that both were mild irritants on hairless guinea pig skin. Residual drug analysis showed a mean fentanyl release for the ETS (fentanyl) to be 1.21 mg/kg/8 hour. Histological assessment indicated that the treatments resulted in minimal to moderate changes in parakeratosis, acanthosis, and hyperkeratosis for intact hairless guinea pig skin sites following fourteen 8-hour exposures to fentanyl (anode) and saline (cathode).

The continuous administration of 70 uA total current (current density 0.1 mA/cm^2) and the drug flux of 110 $ug/ cm^2/h$ for 8 hours resulted in clinical observed systemic effects which can be attributed to the analgesic activity of the drug. The intended maximum dose for humans is 3.6 mg/day. For a 70 kg human, this would be a dose of 0-0.051 mg/ kg/day. The dose administered for this study in guinea pigs was 1.21 mg/kg/8 hour, which is about 25 times the human daily dose.

Study title: Primary Skin Irritation Study of E-TRANS Systems with Anode Hydrogels Containing Fentanyl on Rabbits

Key study findings:

- The primary irritation index for the anode and cathode hydrogels were 1.5 and 1.6, respectively which categorize them as mild irritants. Based on residual fentanyl content of the worn anode hydrogels, the hydrogels released a mean fentanyl dose of approximately 0.073 mg/kg/h or a total mean dose of approximately 3.522 mg.

Study no.: TR-96-1561-052
Volume #1 and page #: 1-33
Conducting laboratory and location: ALZA CORPORATION, Palo Alto, and Ca
Date of study initiation: August 16, 1996
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

A substrate with an anode and a cathode hydrogel cavity was used. The controller was attached via the snap connectors on the substrate. To supply and regulate the electrical current, the zero-order disposable controller was attached via snap connectors to the controller substrate. The power source was set to 230 uA (± 5%), for a current density of approximately 80-uA/ cm^2 (based on a skin contact area of approximately 2.8 cm^2).

. Hydrogels is approximately 2.8 cm^2. The anode and cathode hydrogels were housed in hydrogel cavities of the lower housing of the clinical system designated as "E-TRANS (fentanyl) acute 40 ug/dose, 140 uA system (code number '). The lower housings were cut in half such that the anode and cathode hydrogels in the lower housings were separated prior to delivery to the Toxicology department. The anode and cathode ' electrodes were in the anode and cathode hydrogel cavities of the lower housing, respectively. The pHs of the cathode and anode hydrogel are respectively. The lot and code # for the hydrogel components are provided in the formulation section.

Formulation/vehicle: Saline was used as vehicle.

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Formulation of Hydrogels

Hydrogel Component	Weight Percentage	Code Number	Control Number
Purified water, USP			
Polyvinyl alcohol,			
Citric acid, USP			
Sodium chloride, USP			
Cetylpyridinium chloride			
Fentanyl hydrochloride	1.74		
Polacrilin			

[a] Notebook Reference: 4740:10-11.

Methods

Doses: Based on in vitro flux data through human epidermis with a similar anode hydrogel formulation and similar electrical conditions the fentanyl steady state flux was approximately 100.44 ug/ cm^2 /hour. Thus, the total estimated fentanyl dose was approximately 3.94 mg or 1.97 mg/kg during the 14-hour wearing period (based on a 2 kg rabbit). This was a fentanyl dose of approximately 0.28 mg/hour. A total fentanyl dose of approximately 3.522 mg was released during this study for a fentanyl release rate of approximately 0.073 mg/kg/h.

Study design:

The dosing plan is represented diagrammatically as follows. All rabbits wore the system for about 14 hours. The rabbit's body weights and health, visual evaluation of application sites, electrical measurements, pH measurements, and residual fentanyl analysis was assessed.

Dosing Plan

A = Fentanyl anode hydrogel
B = Cathode hydrogel

		HEAD	
		I	
		II	
Rabbit No.:	340	410	425
	A	A	A
	B	B	B
Rabbit No.:	426	428	436
	B	B	B
	A	A	A

Results: Individual rabbit body weights, weight changes from initiation to termination of the study, and the appropriate means and standard deviations are presented in the following table. The rabbits lost an average of 0.03 kg during the study.

Rabbit Body Weights (kg)

Rabbit Number	Initial Weight	Final Weight	Δ
340			0.0
410			-0.1
425			-0.1
426			0.0
428			0.0
436			0.0
Mean	3.67	3.63	-0.03
SD	0.45	0.47	0.05

All rabbits were observed to be normal and healthy prior to test article application. The first clinical observations were performed approximately 0.6 to 1.1 hours after system application and all the rabbits appeared lethargic. Approximately 1.6 to 2.1 hours after system application all the rabbits appeared lethargic and had muscle rigidity. The

sedative effect of fentanyl may have affected food and water intake during the 14 hour wearing period.

Additional adverse clinical observations included lacrimation and nasal discharge in 2/6 rabbits at approximately 10.7 and 12.7 hours after system application.
In addition, two rabbits became cool to the touch (animal temperatures were not quantified with a thermometer during the study) prior to system removal.

Individual irritation scores, means of total erythema and edema scores at each scoring observation, are presented in the following table. The primary irritation index for the anode and cathode hydrogels was 1.5 and 1.6, respectively, which categorize them as mild irritants.

SUMMARY AND CONCLUSIONS

The primary irritation index for the anode and cathode hydrogels were 1.5 and 1.6, respectively which categorize them as mild irritants. Based on residual fentanyl content of the worn anode hydrogels, the hydrogels released a mean fentanyl dose of approximately 0.073 mg/kg/h or a total mean dose of approximately 3.522 mg.

Primary Irritation Scores

Treatment	Rabbit No.	Scores for Application Sites: 0.5 hours ery	0.5 hours ed	24 hours ery	24 hours ed	48 hours ery	48 hours ed	Primary Irritation Index (Category)[a]
fentanyl anode hydrogel	340[b]	2	0	2	0	2	0	
	410	2	0	3	0	2	0	
	425[c]	1	0	1	0	1	0	1.5
	426	1	0	0	0	0	0	(mild)
	428	2	0	2	0	2	0	
	436	2	0	2	0	1	0	
Mean		1.7		1.7		1.3		
cathode hydrogel for fentanyl anode hydrogel	340[b]	2	2	2	3	2	2	
	410	2	0	2	0	2	0	
	425[c]	0	0	0	0	0	0	1.6
	426	1	0	0	0	0	0	(mild)
	428	2	0	2	0	1	0	
	436	2	0	0	0	1	0	
Mean		1.8		1.5		1.3		

NOTES: hours = hours after removal of system
ery = erythema and eschar formation, ed = edema formation
Mean = sum of erythema and edema scores divided by the number of observations (n=6)

[a] The Primary Irritation Index was calculated by adding all erythema and edema scores for a particular hydrogel at 0.5 and 48 hour observations and dividing by the number of observations for that particular hydrogel (n=12).

[b] System did not appear to be functioning at system removal (battery voltage was 0 volts).

[c] System was removed after 11.6 hours due to adverse clinical observations. The application sites for rabbit No. 425 were scored approximately 1 hour (instead of 0.5 hours) after system removal (see Appendix 2 for protocol deviation).

Study title: Primary Skin Irritation Study of Electro- transport Therapeutic Systems Containing Fentanyl in Rabbits

Key study findings:

- The Primary Irritation Indices calculated for the ETS containing fentanyl (anode) and saline (cathode) were 1.2 and 0.2, indicating that the fentanyl and saline gels were mild and negligible irritants, respectively, on rabbit skin

Study no.: TR-92-1561-OS3

Volume # 1, and page #: 1-20
Conducting laboratory and location: ALZA CORPORATION. Palo Alto, Ca
Date of study initiation: December 2, 1992
GLP compliance: Yes

QA reports: yes (x) no ( )
Drug, lot #, and % purity:
Electrotranspart Therapeutic Systems (ETS) with fentanyl 5 mg disposable component, Code Number: [redacted] contained an anode hydrogel and a cathode hydrogel, each approximately 1.6 cm in diameter and 0.16 cm thick which were in contact with [redacted] anode electrode and a [redacted] cathode electrode, respectively. The overall dimensions of the disposable component were 6.9 cm by 8.5 cm. The anode of each component was masked using a [redacted] release liner that had an opening of about 0.7 cm^2. [redacted] formulation is 1.31% fentanyl HCl, 0.09% fentanyl base, [redacted] USP grade water, [redacted] and polyvinyl alcohol [redacted] The anticipated rate of delivery is approximately 85 ug/h or 1.4 mg/16 hours. Based on a 3.0 kg rabbit, a total dose of 0.9 mg/kg/16 hours was administered for an application of two systems per rabbit. [redacted]

Zero-order disposable controller Lot Number: 2651-80,81 was used in conjunction with the ETS (fentanyl) 5 mg disposable component. It contained the power source set to 100 uA/ cm^2 current density, for a total current of about 70 uA (electrode area 0.7 cm^2).

Formulation/vehicle: Saline was used as a vehicle

Methods

Doses: The anticipated rate of delivery is approximately 85 ug/h or 1.4 mg/16 hours. Based on a 3.0 kg rabbit, a total dose of 0.9 mg/kg/16 hours was administered for an application of two systems per rabbit. Residual fentanyl was not measured to confirm the dosing.

Study design: Electrotransport Therapeutic Systems (ETS) containing Fentanyl were applied to intact dorsal areas of conscious New Zealand White rabbits (n=3) for 16 hours to evaluate skin irritation. The current density of each system was 0.1 mA/ cm^2 for a total current of about 70 uA. The ETS (fentanyl) disposable component was comprised of a Fentanyl anode hydrogel in the anode cup and a saline cathode hydrogel which was used in conjunction with a zero-order disposable controller. The systems were secured with [redacted] bandage, [redacted] tape, and an orthopedic stockinet. After the completed wearing period, the systems were removed and the anode and cathode sites were scored for erythema and eschar formation and edema at 0.5 and 48 hours post system removal. Primary Irritation was calculated for the fentanyl gel and saline gel.

Figure 1

Application Sites

Three New Zealand White rabbits with four intact dorsal skin sites

HEAD

I II

IV III

A = Fentanyl anode hydrogel site
B = Saline cathode hydrogel site

Rabbit #	744		810		811	
	B	B	B	B	B	B
	A	A	A	A	A	A

Results: All rabbits appeared normal and healthy prior to application of the electrotransport therapeutic systems. Upon removal of the systems (16 hours post application), all animals appeared slightly relaxed.
The Primary Irritation Indices for the fentanyl anode hydrogel and the saline cathode hydrogel (1.2 and 0-2) indicate that the fentanyl gel and saline gel are mild and negligible irritants, respectively, on rabbit skin. The PIIs are presented below

Summary and conclusion

The Primary Irritation Indices calculated for the ETS containing fentanyl (anode) and saline (cathode) were 1.2 and 0.2, indicating that the fentanyl and saline gels were mild and negligible irritants, respectively, on rabbit skin. The adhesive adhering to the skin and the lack of system-to-skin contact due to hair growth suggests that the rabbit is not an optimal animal model for ETS evaluation. These observations were not seen in the hairless guinea pigs (TR-92-1561- 021, TR-92-1561-023), indicating that the hairless guinea pig is the preferred animal model of choice.

Primary Irritation Scores
16 hour Application in Rabbits

Treatment	Rabbit Number	Site	Intact Sites 0.5 hr[a] ery	0.5 hr[a] ed	48 hr ery	48 hr ed	Primary Irritation Index[b]
	744	III	1	0	0	0	
Fentanyl	744	IV	2	0	2	0	
anode	810	III	1	0	1	0	1.2
hydrogel	810	IV	2	0	1	0	
	811	III	2	0	0	0	
	811	IV	2	0	0	0	
Means				1.7		0.7	
	744	I	0	0	0	0	
Saline	744	II	1	0	0	0	
cathode	810	I	1	0	0	0	0.2
hydrogel	810	II	0	0	0	0	
	811	I	0	0	0	0	
	811	II	0	0	0	0	
Means				0.3		0.0	

ery = erythema and eschar formation

ed = edema formation

[a] Hour(s) after removal of test and control articles

[b] PII = sum of erythema and edema scores divided by # observations

2.6.6.8 Special toxicology studies

Study title: Experimental Sensitization Evaluation of Fentanyl Using Electrotransport Therapeutic Systems in the Hairless Guinea Pig

Key study findings:

- This study in hairless guinea pigs indicate that electrically-assisted delivery of fentanyl (anode) at a maximum dose of 1.1 mg/kg/8 h and the maximum current density of 0.1 mA/cm^2 be placed in the mild to moderate sensitizer category.

Study no.: TR-92-1561-022
Volume #1 and page #: 1-147
Conducting laboratory and location: ALZA CORPORATION, Palo Alto, Ca
Date of study initiation: October 30, 1992
GLP compliance: Yes
QA reports: yes (x) no ()

Drug, lot #, and % purity: Electrotransport Therapeutic Systems (ETS), (fentanyl) Code Name: ETS disposable component (fentanyl) 5 mg Code Number: 091109 was used for the present study. The ETS (fentanyl) 5 mg disposable component contained an anode hydrogel and a cathode hydrogel, each approximately 1.6 cm in diameter and 0.16 cm thick, which were in contact with a — anode electrode and a — cathode electrode, respectively. The overall dimensions of the disposable component are 6.9 cm by 8.5 cm. The anode of each component was masked using a — release liner that had an opening of about 0.7 cm^2.
The pH of the anode was recorded prior to and after application of induction of #8. Measurements before application were on average 6.8. Final measurements were 7.1 (Group II) and 6.7 (Group III).

Electrotransport Therapeutic Systems (ETS), (Placebo); Lot Number: 2690-1
The Placebo build disposable placebo component contained an anode hydrogel and a cathode hydrogel, each approximately 1.6 cm in diameter and 0.16 cm thick, which were in contact with a — anode electrode and a — cathode electrode, respectively. The overall dimensions of the disposable component are 6.9 cm by 8.5 cm. The anode of each component was masked using a — release liner that had an opening of about 0.7 cm^2.

Zero-order disposable controller Lot Number: 2651-80,81, 2651-115; The zero-order disposable controller was used in conjunction with the ETS (fentanyl) 5 mg disposable component. For inductions and Challenges #1-3, the power source was set, to 0.1 mA/cm2 current density, for a total current of about 70 uA (electrode area 0.7 cm^2). For Challenges #5 and #6, the power source was set to 0.060 mA/cm^2 current density, for a total current of about 42 uA (electrode area 0.7 cm^2). The controller provided use up to 7 days.
Chlora-2, 4 dinitro-benzene (DNCB) Toxicology Code Number: — Manufacturer: —

Acetone Code Number: —
Code Name: Fentanyl base Code Number: —
Code Name: Alcohol (— USP) Code Number —
Code Name: Purified Water Code Number: —

Formulation/vehicle: Drug system: The formulation was 1.31% fentanyl HCl, 0.09% fentanyl base. — USP grade water — polyvinyl alcohol, — sodium chloride, — citric acid, — sodium citrate.

/ / / / /

Methods

Doses:

Residual analysis of fentanyl in the patches assessed that compared to the unworn fentanyl systems (control), ETS (GROUP II) released approximately 600 ug/ 8 hours (75 ug/h) at current density 0.1 mA/cm^2. Based on a mean weight of 573 gm for ten guinea pigs, 1.1 mg/kg was the apparent dose delivered in 8 hours.

Study design: Five groups of conscious hairless guinea pigs received different treatments. Group I received saline gel (non-drug containing) + current,

Group II received fentanyl gel + current, Group III received fentanyl gel passively (no current), Group IV was untreated (sham), and Group V was the positive control (0.1% DNCB in acetone). The Electrotransport Therapeutic Systems (ETS), current density 0.1 mA/cm2 for a total current of about 70 uA anodic delivery with a maximum dose of 1.1 mg/kg/8 h, were secured on the guinea pigs with [redacted] bandage and [redacted] tape. Groups I-IV received nine occluded topical induction applications (8 h) of the respective test article over a period of 3 weeks. Group V received six induction applications of 0.1% DNCB in acetone over a period of 2 weeks. Induction sites were scored for erythema and edema 0.5 and 24 hours post- removal of the various induction applications. No treatments were performed on any of the animals for the 2 weeks following the ninth induction. The irritation indexes for the anode or treatment sites for Groups II III, V, and I after the last induction were 1.3 (mild), 2.1 (moderate), 0 (none), and 3.5 (moderate), respectively.

For the first and second challenge, Groups I-IV were challenged with the ETS containing fentanyl gels for 8 hours. All treatment sites were scored 0.5, 24, 48, and 72 hours after removal. A third challenge with saline gel (non-drug containing) and current occurred approximately 2 weeks after the second challenge. A fourth challenge with passive fentanyl in ethanol occurred approximately 6 weeks after the third challenge. A fifth challenge with fentanyl gels and current occurred approximately 3 weeks after the fourth challenge. The sixth and last challenge, with saline gel (non-drug containing) and current occurred approximately 5 weeks after challenge #5. Group V received three challenges of DNCB and during the fourth and fifth challenges served as a control group for the test article. The controllers were set at current density of 0.1 mA/cm^2, total current of 70 uA for Challenges 1-3 and 0-06 mA/, total current of 42 uA for Challenges 5 and 6.

For Challenge #1, the anode responses to fentanyl gel and current might reflect cumulative irritation, which was seen in all four groups. However, comparing fentanyl

and current (Group II) to saline and current (Group 1), the Passive (Group III), or sham (Group IV) would place it in the mild to moderate sensitizer category. For Challenge #2, the number of responders increased in all groups, most likely indicative of irritation. Challenge #3 with saline and current suggests that animals induced with current (Groups I and II) are more hyperreactive since similar responses are seen in these groups and not seen in Groups III and IV. Subsequent challenges with fentanyl at a lower current density (0.06 mA/ cm^2) places it in the mild to moderate sensitizer category.

Results: All guinea pigs were observed to be normal and healthy prior to applications of the electrotransport therapeutic systems containing fentanyl or saline. At the end of each 8 hour application, animals treated with fentanyl appeared lethargic, watery eyes, rectal bleeding and in some instances cool body temperature and decreased respiration (labored breathing). In some instances, some of the animals had died or were euthanized. These observed systemic effects can be attributed to the analgesic and pharmacological activity of the drug (dose = 1.08 mg/kg/8-hour. During the course of the study, all groups exhibited an average mean weight gain.

The following table summarizes the average scores for inductions 1, 4, 6, 7, and 9 and the average scores for challenges #1 and #2 at 48 hours. Group I (saline + current) after the first induction treatment was categorized as negligible irritants. For Group II (fentanyl + current), the cathode (saline) was categorized as a negligible irritant and treatment for the anode was a mild irritant. For Group III, passive treatment of fentanyl resulted in no irritation and Group V, positive control of 0.1@. DNCB was categorized as a mild irritant. Cumulative irritation indices for Groups II, III, and I show slight increases in average scores over the nine applications. Group V (DNCB) responses increased to moderate after six induction applications at which time induction applications were terminated.

Group	Treatment	Irritation Index for Inductions and Challenges 1 and 2						
		1	4	6	7	9	Ch 1	Ch 2
I (n=10)	saline + current							
	(cathode)	0.2	0.6	---	1.0	1.2		
	(anode)	0.6	1.1	---	1.7	1.3	1.8	2.1
II (n=10)	fentanyl + current							
	(cathode)	0.2	0.6	---	0.3	0.2		
	(anode)	1.7	1.7	---	1.9	2.1	2.0	2.0
III (n=10)	fentanyl passive							
	(cathode)	0	0.2	---	0	0		
	(anode)	0	0.2	---	0.1	0	1.9	2.1
IV (n=10)	untreated	---	---	---	---	---	1.4	2.0
V (n=5)	0.1% DNCB	0.9	3.5	3.5		---		

Challenge #1

Groups I-IV were challenged at the anode with fentanyl and current. The systems were set at current density 0.1 mA/ cm^2, total current 70 uA, with an approximate flux rate of 100 ug/cm^2 hour (dose of 0.7 mg/kg/8 h). At 72 hours, corrected for the 10% responders seen in the passive (Group III) or sham (Group IV) animals, the fentanyl/current (Group II) has 50% positive responders. Consideration of the saline/current (Group I) reduces it to 40%, placing ETS delivery of fentanyl in the potentially moderate sensitizer category. However, the differences between scores for Group I (induced with saline/ current), Group II (induced with fentanyl/ current) and Group III (induced with passive fentanyl) were not statistically significant ($p < 0.05$) at any time. This absence of significant differences among Groups I, II and III indicates that the reactions seen may be attributed to irritation and not sensitization. DNCB challenges to Group V resulted in moderate to severe response in 100% of the animals.

Challenge #2

Groups I-IV were challenged at the anode with fentanyl and current of 70 uA. The systems were set at current density 0.1 mA/cm^2 with an approximate flux rate of 100 ug/cm^2 hour (dose of 0.7 mg/kg/8 h).

During this application, the number of responders increased in all groups, most likely indicative of irritation since two groups (II and III) were seeing drug/current for the second time. Statistical analysis showed a significant difference in scores at the 72 hour observation ($p < 0.05$) only between Group III (induced with passive fentanyl) and Group IV (sham) when challenged with ETS (fentanyl).

Correcting for irritation at the 72 hour observation by Group I, 111, and IV gives the following % responders for Group II: 22%, 0%, and 44%, respectively. This spans the weak to moderate categories. Group V (DNCB) animals had moderate responses in 100% of animals.

Challenge #3

Groups I-IV were challenged at the anode with saline and current of 70 UA. The systems were set at current density 0.1 mA/cm^2. The percent of positive responders at 72 hours follows:

Group I	44% (4/9)
Group II	38% (3/8)
Group III	0% (0/10)
Group IV	0% (0/9)
Group V (DNCB)	100% (5/5)

These data suggest that animals induced with current (Groups I and II) are more hyperreactive. This occurrence may be explainable as "excited-skin syndrome" (Anderson, 1985), since Groups I and II gave similar responses not seen in Groups III and IV.

Challenge #4