CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
21-306

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Anesthetic, Critical Care, and Addiction Drug Products (HFD-170)
Supplemental Review

Thomas Papoian, Ph.D.
August 31, 2001

NDA #21-306

SUBMISSION DATE: Nov. 3, 2000
CENTER RECEIPT DATE: Nov. 6, 2000
REVIEWER RECEIPT DATE: Nov. 7, 2000

SPONSOR: Purdue Pharma, L.P.; Stamford, CT

DRUG:
Generic Name: Buprenorphine Transdermal System (BTDS)

Trade Name: Norspan®

BACKGROUND:
Chronic toxicity studies conducted in animals with buprenorphine patches were generally devoid of non-dermal systemic toxicities, although some of the animals experienced signs of opioid exposure, such as reduced feces in rabbits and dogs and decreased activity in minipigs. Mean plasma levels in these animals were 1-4 ng/ml in rabbits given 30 mg/kg, 4 ng/ml in dogs given 12 mg/kg, and 0.75 ng/ml in minipigs given 8 mg/kg. The mean plasma levels achieved were 4-21X those seen in human patients given 20 mg buprenorphine patches for up to 60 days (mean steady-state plasma level of 0.192 ng/ml; Study No. BP96-0102).

To determine whether this apparent lack of systemic toxicity was due to an inadequate number of individual animals exposed to drug equal to or greater than the exposure in humans at the maximum recommended dose of 20 mg, a reassessment of animal toxicokinetic data was conducted. This reassessment is based on the findings of wide intersubject variability in the chronic animal toxicity studies.

Rabbits:

The individual and mean plasma concentrations of buprenorphine in 5 male and 5 female rabbits during the course of a 6-month toxicity study (Study No. DSE-304) are shown in Table 1 (Sponsor's Table 7.3). Results showed that generally the highest concentrations of buprenorphine occurred on Day 2, but there was variability throughout the course of the study. Levels were higher in females than in males. When compared to the mean steady-state plasma levels in humans given the 20 mg buprenorphine patch (0.192 ng/ml), plasma concentrations in all rabbits at the 60 mg dose were as high or higher during the course of the study.

Table 1 (Sponsor's Table 7.3)

Individual And Mean (±SD) Plasma Concentrations (ng/mL) of Buprenorphine In Rabbits[a] Following Dermal Application of Patches (60 mg) Every 3 Days in a 6 Month Study

Study Day	Time[b] (hr)	Animal # 50583/M	50584/M	50585/M	50586/M	50587/M	50588/M	Mean	SD
2	24						(b) (4)	3.08	2.40
23	24							0.00	
136	0							0.59	0.62
	4							0.51	0.45
	8							0.72	0.82
	12							0.77	0.92
137	24							1.08	1.14
	36							1.00	1.27
138	48							0.89	1.16
	60							0.63	0.79
139	72							0.62	0.74
170	24							0.92	0.71
171	48							0.89	0.69
172	72							0.65	0.41
		50613/F	50614/F	50615/F	50616/F	50617/F	50618/F	Mean	SD
2	24						(b) (4)	3.00	2.43
23	24							0.00	
136	0							2.13	1.08
	4							1.72	0.99
	8							2.71	1.77
	12							2.51	1.35
137	24							3.16	1.70
	36							2.97	1.04
138	48							2.93	1.58
	60							2.17	0.89
139	72							1.67	0.89
170	24							1.47	1.30
171	48							1.23	0.70
172	72							0.77	0.42

[a] M = male, F = female

[b] Time after the last dose (application of patches) was given.

NS = No sample

Note = values determined to be below the lower limit of quantitation (0.10 ng/mL) are represented as 0.00

Dogs:

The individual and mean plasma concentrations of buprenorphine in 5 male and 5 female dogs during the course of a 6-month toxicity study (Study No. DSE-214) are shown in Table 2 (Sponsor's Table 7.1). Results showed that generally the highest concentrations of buprenorphine occurred on Day 3, but there was variability throughout the course of the study. No gender differences are apparent. When compared to the mean steady-state plasma levels in humans given the 20 mg buprenorphine patch (0.192 ng/ml), plasma concentrations in all dogs at the 120 mg dose were as high or higher during the course of the study.

Table 1 (Sponsor's Table 7.1)

Individual And Mean Plasma Concentrations (ng/mL) Of Buprenorphine In Dogs Following Dermal Application Of Patches[a] For 6 Months

Group	Dose	Animal#/Sex	Day 3[b]	Day 24[b]	Day 90[b]	Day 180[b]
3	10	743/M	0.18	0	0.14	0.00
3	10	748/M	0.52	0.15	0.00	0.00
3	10	753/M	0.27	0.00	0.00	0.00
3	10	759/M	0.43	0.33	0.13	0.00
3	10	767/M	0.00	0.14	0.00	0.00
		Mean ± SD (Male)	**0.28 ± 0.21**	**0.12 ± 0.14**	**0.00 ± 0.00**	**0.00 ± 0.00**
3	10	773/F	0.00	0.25	0.23	0.00
3	10	778/F	0.6	0.00	0.14	0.00
3	10	779/F	0.00	0.00	0.00	0.00
3	10	784/F	0.00	0.00	0.00	0.00
3	10	796/F	0.00	0.19	0.39	0.00
		Mean±SD (Female)	**0.12 ± 0.27**	**0.00 ± 0.00**	**0.15 ± 0.17**	**0.00 ± 0.00**
		Mean ± SD (Combined)[c]	**0.20 ± 0.24**	**0.11 ± 0.12**	**0.10 ± 0.13**	**0.00 ± 0.00**
4	35	749/M	0.00	2.23	0.27	0.25
4	35	754/M	2.08	0.4	0.15	0.7
4	35	761/M	0.84	0.3	0.15	0.16
4	35	769/M	0.83	0.00	0.65	0.35
4	35	770/M	0.56	0.21	0.33	0.16
		Mean ± SD (Male)	**0.86 ± 0.76**	**0.63 ± 0.91**	**0.31 ± 0.21**	**0.32 ± 0.22**
4	35	771/F	1.39	0.2	0	0.46
4	35	774/F	1.66	0.25	0.22	0.28
4	35	777/F	0.53	1.11	0.76	0.22
4	35	785/F	0.34	1.32	0.46	0.7
4	35	789/F	0.46	0.55	1.25	0.63
		Mean±SD (Female)	**0.88 ± 0.60**	**0.69 ± 0.51**	**0.54 ± 0.49**	**0.46 ± 0.21**
		Mean ± SD (Combined)[c]	**0.87 ± 0.65**	**0.66 ± 0.69**	**0.42 ± 0.37**	**0.39 ± 0.22**
5	120	744/M	4.48	1.23	2.3	1.63
5	120	750/M	6.33	2.83	0.75	0.93
5	120	760/M	4.71	2.17	1.99	1.72
5	120	764/M	2.83	3.37	1.02	1.78
5	120	768/M	3.85	1.09	0.73	0.63
		Mean ± SD (Male)	**4.44 ± 1.28**	**2.14 ± 0.99**	**1.36 ± 0.74**	**1.34 ± 0.52**
5	120	772/F	8.03	3.51	2.09	4.35
5	120	775/F	2.33	0.24	0.66	0.76
5	120	781/F	1.33	5.7	3.2	0.64
5	120	786/F	6.47	2.89	1.12	0.34
5	120	794/F	2.13	4.79	0.72	0.34
		Mean±SD (Female)	**4.06 ± 2.99**	**3.43 ± 2.09**	**1.56 ± 1.08**	**1.29 ± 1.72**
		Mean ± SD (Combined)[c]	**4.25 ± 2.18**	**2.78 ± 1.68**	**1.46 ± 0.88**	**1.31 ± 1.20**

N = 5/sex/group; a = Patches were applied once every three days throughout the study.

b = Blood samples were taken one day prior to application of patches.

c = Represents combined values from male and female dogs (N = 10/group)

LOQ = 0.10 ng/mL; Any value below the LOQ was considered zero.

Minipigs:

The individual and mean plasma concentrations of buprenorphine in 5 male and 5 female minipigs during the course of a 6-month toxicity study (Study No. DSE-2142 are shown in Table 3 (Sponsor's Table 7.1). Results showed that generally the highest concentrations of buprenorphine occurred at the end of the study on Week 26, but there was variability throughout the course of the study. No gender differences are apparent. When compared to the mean steady-state plasma levels in humans given the 20 mg buprenorphine patch (0.192 ng/ml), plasma concentrations in all minipigs at the 8 mg dose ranged from lower to higher than concentrations seen in humans given the 20 mg patch.

Table 3 (Sponsor's Table 7.1)

Individual And Mean Plasma Concentrations (ng/mL)[a] Of Buprenorphine In Minipigs Following Dermal Application Of Patches Once Every Three Days For 6 Months

Group	Dose	Animal#/Sex	Day 3	Week 4	Week 13	Week 26
3	0.8	2-5/M	0.00	0.00	0.00	0.41
3	0.8	114-5/M	0.00	0.00	0.00	0.00
3	0.8	123-5/M	0.00	0.00	0.00	0.00
3	0.8	126-2/M	0.00	0.00	0.00	0.20
3	0.8	116-8/M	0.00	0.00	0.00	0.00
		Mean ± SD	**0.00**	**0.00**	**0.00**	**0.12±0.83**
3	0.8	123-1/F	0.00	0.00	0.00	0.00
3	0.8	123-2/F	0.00	0.00	0.00	0.00
3	0.8	116-2/F	0.00	3.31	0.00	0.26
3	0.8	2-4/F	0.00	0.00	0.00	0.19
3	0.8	3-4/F	0.00	0.00	0.00	0.00
		Mean±SD (Female)	**0.00**	**0.66±1.48**	**0.00**	**0.00**
		Mean ± SD (Combined)[b]	**0.00**	**0.33±0.10**	**0.00**	**0.11±0.15**
4	4	116-9/M	0.00	0.00	0.00	0.17
4	4	120-4/M	0.20	0.47	0.00	0.58
4	4	125-4/M	0.00	0.20	0.00	0.00
4	4	127-6/M	0.10	0.15	0.00	1.04
4	4	122-2/M	0.24	0.00	0.11	0.51
		Mean ± SD	**0.11±0.11**	**0.16±0.19**	**<0.10**	**0.46±0.40**
4	4	2-2/F	0.00	0.51	0.52	0.45
4	4	114-1/F	0.00	0.00	0.00	ND[c]
4	4	120-1/F	0.00	0.45	0.00	0.72
4	4	124-3/F	0.00	0.18	0.14	ND
4	4	127-3/F	0.11	0.15	<0.10	0.61
		Mean±SD (Female)	**<0.10**	**0.26±0.22**	**0.13±0.23**	**0.59±0.14**
		Mean ± SD (Combined)	**<0.10**	**0.21±0.20**	**<0.10**	**0.51±0.32**
5	8	116-6/M	0.00	0.00	0.14	0.23
5	8	119-5/M	0.10	0.33	0.14	0.63
5	8	124-5/M	0.00	0.11	0.00	0.78
5	8	125-5/M	0.21	0.21	0.21	0.33
5	8	3-9/M	0.27	0.54	0.41	2.23
		Mean ± SD	**0.12±0.12**	**0.24±0.21**	**0.18±0.15**	**0.84±0.81**
5	8	127-2/F	0.11	0.41	0.00	0.47
5	8	124-1/F	0.18	0.27	0.10	1.05
5	8	122-1/F	0.00	0.30	0.32	0.77
5	8	116-3/F	0.00	0.00	0.00	0.27
5	8	2-1/F	0.27	0.34	ND	ND
		Mean±SD (Female)	**0.11±0.12**	**0.26±0.16**	**0.11±0.15**	**0.64±0.34**
		Mean ± SD (Combined)	**0.11±0.11**	**0.25±0.17**	**0.15±0.15**	**0.75±0.62**

N = 5/sex/group; a = Blood samples were taken one day prior to application of patches.

b = Represents combined values from male and female minipigs (N = 10/group); c = ND = No data

LOQ = 0.10 ng/mL; Any value below the LOQ was considered zero.

DISCUSSION:

Although 6 month chronic toxicity studies conducted in three animal species (rabbit, dog, and minipig) did not reveal systemic toxicities unrelated to the known pharmacological properties of buprenorphine, the issue of whether this apparent lack of systemic toxicity was due to an inadequate number of individual animals exposed to plasma concentrations as high or higher than the levels found in humans was re-evaluated.

Results showed wide intersubject variability in plasma concentrations for all three species tested. However, levels in rabbits and dogs generally exceeded those found in humans at the maximum dose of 20 mg. Levels in minipigs were generally lower than those in rabbits and dogs, possibly due to the lower concentration of buprenorphine used, but were similar to human levels.

These toxicity studies were designed to address the potential toxicities in humans using the same dermal route of administration. Toxicity studies in animals are generally conducted using the same route of administration to be used to humans to better reflect the clinical situation. The dermal route was limited, however, in its ability to exposure animals to plasma concentrations that could otherwise be achieved by other routes of administration, such as buccal or intravenous.

Given that opioid-treated patients often become tolerant to the pharmacological effects of the drug, exposures in patients on long-term treatment may increase over time. Should this occur, the exposures achieved in animals by the dermal route of administration may not fully address the potential systemic toxicities of buprenorphine at higher exposures. Therefore, it would be useful to know the nondermal toxicity profile of buprenorphine at exposures up to the maximum tolerated dose to better understand the potential risk to humans who may require higher doses of buprenorphine as they become tolerant to its effects.

RECOMMENDATIONS:
To the Sponsor:

Given the wide variability in plasma drug levels in the chronic toxicity studies conducted in animals, and the fact that humans may require higher doses of buprenorphine as they become tolerant to its effects, please conduct an additional 6-month chronic toxicity study in either rabbits or dogs at a maximum tolerated dose to fully assess potential systemic toxicities that may be unrelated to its known pharmacological effects.

Thomas Papoian, Ph.D.
Supervisory Pharmacologist

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Thomas Papoian
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PHARMACOLOGIST

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Anesthetic, Critical Care, and Addiction Drug Products (HFD-170)

Thomas Papoian, Ph.D., D.A.B.T.
July 17, 2001

NDA #21-306

SUBMISSION DATE: Nov. 3, 2000
CENTER RECEIPT DATE: Nov. 6, 2000
REVIEWER RECEIPT DATE: Nov. 7, 2000

SPONSOR: Purdue Pharma, L.P.; Stamford, CT

DRUG:

Generic Name: Buprenorphine Transdermal System (BTDS)

Trade Name: Norspan®

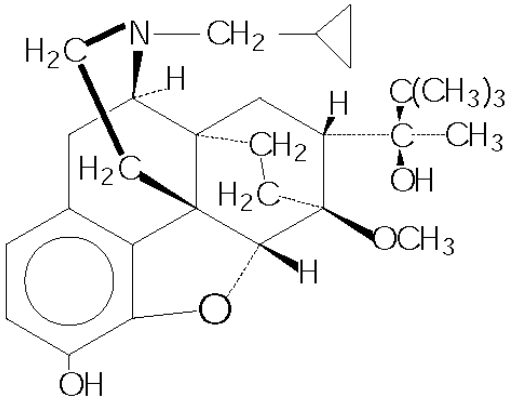
Chemical Name: 6,14-ethenomorphinan-7-methanol, 17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4, 5-epoxy-18, 19-dihydro-3-hydroxy-6-methoxy-α-methyl-, [5α, 7α, (S)].

CAS Registry Number: 52485-79-7

Molecular Formula: $C_{29}H_{41}NO_4$

Molecular Weight: 467.6

Structure:



RELATED INDs/NDAs/DMFs: IND #50,273

PHARMACOLOGIC CLASS: Opioid analgesic (partial μ-opioid agonist)

PROPOSED CLINICAL INDICATION: Management of pain in patients requiring continuous opioid analgesia.

FORMULATION (from sponsor's table):

TABLE 5.3C. BTDS Qualitative/Quantitative Composition (Production Scale Formulation)

Component	BTDS (5 mg)	BTDS (10 mg)	BTDS (20 mg)
Buprenorphine	5 mg	10 mg	20 mg
Levulinic acid	(b) (4)		
Oleyl oleate			
Povidone (PVD)			
(b) (4)			
(b) (4)			
(b) (4)			

ROUTE OF ADMINISTRATION: Transdermal

RATIONALE: The buprenorphine transdermal delivery system (BTDS) is designed to continuously release buprenorphine upon application to intact skin for the control of moderate to severe pain. Buprenorphine has partial μ-opioid agonist and κ-opioid antagonist properties. It is a full agonist at the orphinin (nociceptin) receptor. The analgesic activity of buprenorphine at low to moderate doses is 25 to 50 times that of morphine (0.3 mg of parenteral buprenorphine is about equivalent to 10 mg of parenteral morphine) with a longer duration of action (6 to 8 hours) than morphine. Since buprenorphine is a partial μ-opioid agonist, it exhibits a ceiling effect for respiratory depression as well as for its analgesic activity. Because buprenorphine is a κ-opioid antagonist it may cause less psychomimetic effects. Given the poor oral bioavailability of buprenorphine, the transdermal formulation is expected to produce a prolonged analgesic effect (up to 7 days) when compared to oral or sublingual forms.

BACKGROUND: Parenteral buprenorphine has been marketed in the U.K. since 1978. Injectable buprenorphine has been marketed in the U.S. since 1982, for various indications including management of postoperative pain, preoperative sedation and analgesia, as an adjunct to surgical anesthesia, and for the relief of moderate to severe pain associated with cancer, trigeminal neuralgia, ureteral calculi, myocardial infarction, and accidental injury. The buprenorphine transdermal delivery system is expected to provide management of pain in patients requiring continuous opioid analgesia.

Several meetings and teleconferences were held with the sponsor to discuss requirements for additional toxicity testing. Agreement was reached between the sponsor and this reviewing division that all reproductive toxicity studies and carcinogenicity studies could be conducted as Phase 4 commitments. However, it was also agreed that protocols for the reproductive toxicity studies would be submitted for review soon after the submission of the NDA so that the studies could be initiated well before the end of the NDA review period. Such protocols have been submitted and reviewed by a pharmacologist in this division, Dr. S. Thornton.

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1. OVERALL SUMMARY AND EVALUATION:

Injectable buprenorphine has been marketed in the U.S. since 1982 for various indications including management of postoperative pain, preoperative sedation and analgesia, as an adjunct to surgical anesthesia, and for the relief of moderate to severe pain associated with cancer, trigeminal neuralgia, ureteral calculi, myocardial infarction, and accidental injury. The buprenorphine transdermal delivery system (BTDS) in this NDA is designed to continuously release buprenorphine upon application to intact skin for the control of moderate to severe pain in patients requiring continuous opioid analgesia.

Buprenorphine has partial μ-opioid agonist and κ-opioid antagonist properties. It is a full agonist at the orphinin (nociceptin) receptor. The analgesic activity of buprenorphine at low to moderate doses is 25 to 50 times that of morphine (0.3 mg of parenteral buprenorphine is about equivalent to 10 mg of parenteral morphine) with a longer duration of action (6 to 8 hours) than morphine. Since buprenorphine is a partial μ-opioid agonist, it exhibits a ceiling effect for respiratory depression as well as for its analgesic activity. Because buprenorphine is a κ-opioid antagonist it may cause less psychomimetic effects. Given the poor oral bioavailability of buprenorphine, the transdermal formulation is expected to produce a prolonged analgesic effect (up to 7 days) when compared to oral or sublingual forms.

Since buprenorphine has been in clinical use for nearly 20 years, nonclinical studies submitted in this NDA were designed to mostly address the dermal toxicity and toxicokinetics of buprenorphine in patches, although complete histopathology was performed in most studies. Additional studies that were submitted included pharmacology studies on metabolism and opioid receptor binding, genetic toxicity studies, and safety toxicology studies for accidental oral ingestion and effects after immersion in warm water. Reproductive toxicity studies and carcinogenicity studies are to be submitted as Phase 4 commitments based on an agreement between the sponsor and this reviewing division.

Pharmacology:

Published studies have shown that buprenorphine is metabolized by two different routes, N-dealkylation to norbuprenorphine and conjugation with glucuronic acid. Norbuprenorphine is also subsequently conjugated with glucuronic acid. Studies with human liver microsomes demonstrated that CYP3A4 is the primary enzyme responsible for catalyzing norbuprenorphine formation from buprenorphine.

A study was conducted to determine the extent of buprenorphine metabolism and nature of the metabolites under conditions similar to those used in the mutagenicity studies (rat S9 liver fraction), and to determine the extent to which CYP3A, CYP1A and UDPGT are involved in the first pass metabolism of buprenorphine. Results showed that buprenorphine was metabolized to norbuprenorphine under conditions similar to those used in the bacterial mutation assay (Ames test), and that buprenorphine was not metabolized to either norbuprenorphine or buprenorphine glucuronide when incubated with rat skin microsomes. These results suggested that buprenorphine is not metabolized when applied as a transdermal patch. Clinical pharmacokinetic data may be used to confirm these experimental observations found with rat skin microsomes.

A study was conducted to examine the binding capabilities of buprenorphine and its analogs (present as impurities) against delta, kappa, mu, and orphanin receptors. Results showed

that buprenorphine had highest affinity for kappa receptors (Ki = 0.157 nM) with high affinity for both delta (Ki = 1.9 nM) and mu receptors (Ki = 1.33 nM). (b) (4) showed highest affinity for mu receptors, equal to that seen with buprenorphine. (b) (4) had highest affinity for mu receptors as well. These results indicated that buprenorphine possesses antinociceptive activity by behaving as a mu opioid partial agonist, and treatment with buprenorphine may result in less psychomimetic effects by virtue of its strong kappa antagonist properties.

Toxicology:

Acute Toxicity:

Preliminary dermal irritation studies were conducted to examine the practicality of applying the patches intended for human use without the active drug substance buprenorphine to the skin of rats, rabbits, and dogs. Patches of various sizes were applied for 14 days, and the skin site examined for erythema, edema, desquamation, and other dermal signs of irritation. Results showed that irritation was seen in each animal at the site of the binder tape, although the patches had to be reapplied several times in each animal during the course of the study due to disruption of the adhesion. In the rabbit and dog, the application site, the area of the patch that would contain the active drug, appeared normal. In the rat, focal and/or pinpoint areas of eschar were noted at the application site. It was concluded that the patch manufactured for human use was practical to apply to rats, rabbits, and dogs for toxicity testing.

Following this preliminary study, various strength buprenorphine patches were applied to the dorsal trunk of rats, rabbits, and dogs for 3 days at doses up to 40 mg/kg in rats, 30 mg/kg in rabbits, and 12 mg/kg in dogs, which constituted up to 10% of body surface area. Results showed that minimal dermal findings were noted, most of which were attributed to the adhesive and not to burprenorphine exposure. Body weights in the high dose groups were reduced 7-8% in dogs and rabbits, respectively. No significant clinical signs were noted.

Two additional single application 3-day studies were conducted in two strains of pigs, SPF Yorshire pigs and Hanford minipigs, using doses up to 5 mg/kg with a 4-day follow-up. Results showed minor clinical signs (soft stools) and dermal findings that included binder irritation, moderate to severe erythema and/or focal pinpoint areas of eschar on Days 4-8. Other dermal findings included slight to well-defined erythema, slight edema, and slight to moderate desquamation and/or dermal irritation in the binder tape area, but not in the area with drug. No other drug-related findings were noted.

Subchronic Toxicity:

Dermal toxicity and toxicokinetic studies were conducted in rabbits, dogs, and minipigs from 7 days to 3 months duration.

In rabbits, plasma concentrations were measured 7 days after patch application of doses up to 30 mg/kg. Results showed a wide variability in most parameters examined. A clear dose-response relationship was difficult to establish. $AUCs_{(0\text{-}72\ hrs)}$ were from 10.6 ng/ml/hr at 2.5 mg/kg, 155.0 ng/ml/hr at 15 mg/kg, and 88.7 ng/ml/hr at 30 mg/kg. The time to peak plasma drug levels (Tmax) was between 24-72 hours.

A separate study was conducted in rabbits given buprenorphine patches at doses up to 24 mg/kg for 28 days, where patches were replaced every 3 days. Results showed a decrease in fecal output, a pharmacologic effect found with other opioids. When compared to the no-patch and placebo-patch controls, significant decreases in food consumption of about 50% were noted in the 12 and 24 mg/kg buprenorphine-treated groups that occurred early on about Days 1-2. However, these decreases returned to normal values by about Day 8. No drug-related changes were found in clinical chemistry, hematology, gross necropsy, organ weights, or non-dermal histopathology. Dermal findings consisted dose-related effects on increasing the severity of dermatitis. The dermal findings were slightly more severe in the buprenorphine groups, but were found in all patch groups, including placebo. According to the histopathology report, the dermal changes were essentially due to the patch application and shaving procedures, and were expected to quickly reverse following cessation of treatment. However, confirmation of this by inclusion of a reversibility group was not performed in this study.

In dogs, 28-day and 3-month dermal toxicity studies were conducted. In the first study, dogs were given placebo patches or buprenorphine patches at doses up to 12 mg/kg for 28 days. Patches were replaced every 3 days. Results showed decreased activity and low fecal output were reported, findings similar to the known pharmacological effects of opioids. Dermal findings consisted of slight to moderate irritation, erythema and edema that was more intense during the first 2-3 weeks of exposure. By study termination, drug exposed sites showed pinpoint eschar and redness and swelling. A dose response was not obvious, but there was a higher incidence of effects in the high dose (12 mg/kg) groups. Body weight changes due to buprenorphine were minimal. Food consumption in both male and female dogs was markedly affected by drug treatment. However, food consumption returned to normal levels by the end of the study. There were no drug-related changes in hematology, coagulation, or urinalysis. At necropsy, no additional gross or microscopic abnormalities were reported other than the dermal findings reported above. Toxicokinetic analysis showed that the highest concentration of plasma buprenorphine levels occurred the day after application of a new patch, and a general dose-related increase in systemic absorption. The highest average plasma concentrations during the course of the study showed a wide range of variability.

In the 3-month study, dogs were given placebo patches or buprenorphine patches at doses up to 12 mg/kg for 3 months. Patches were replaced every 3 days. Results showed low fecal output, as before, and dermal effects consisting of edema and erythema that were markedly increased in the high dose (12 mg/kg) females, but not in the high dose males. Food consumption in the high dose (12 mg/kg) groups during the first week of the study was about half that consumed in the control groups, and body weights showed dose-dependent decreases during the first week of the study. However, weight gains returned to normal levels by the 3rd week of the study. There were no apparent drug-related effects on hematology or coagulation parameters, urinalysis, ECG, blood pressure, respiratory rates, or effects on gross necropsy or organ weights. Microscopic examination did not report any drug-related findings, except for the dermal application sites that were similar between the dogs receiving buprenorphine patches and those receiving placebo patches. Toxicokinetic analysis showed no gender differences. Systemic exposure was generally increased with increasing dosage, but there was wide variability in exposures between individual dogs. Also, accumulation of buprenorphine was not demonstrated over time.

In minipigs, 28-day and 3-month dermal toxicity studies were conducted. In the first study, Hanford minipigs were given placebo patches or buprenorphine patches at doses up to 8 mg/kg for 28 days. Patches were replaced every 3 days. No mortality or drug-related clinical signs were reported. Results of dermal findings consisted of slight erythema and yellow staining. Microscopic examination of the dermal application sites reported minimal to mild parakeratosis and exudate, findings interpreted by the sponsor as expected in skin shaved and wrapped for 4 weeks. Toxicokinetic analysis showed only one minipig out of six with a measurable level (0.20 ng/ml) during the last two weeks of the study with an $AUC_{0\text{-}144\ hr}$ of 18.7 ng/ml/hr.

In the 3-month study, minipigs were given placebo patches or buprenorphine patches at doses up to 8 mg/kg for 3 months. Patches were replaced every 3 days. Clinical signs consisted of decreased activity in the high dose (8 mg/kg) group, and was attributed to the known pharmacological effect of opioids. Gross dermal findings consisted of changes that were difficult to attribute to exposure to buprenorphine and not to the patching precedure per se. Final body weights in the high-dose (8 mg/kg) animals were 7-13% lower than either control group. Clinical chemistry measurements were sporadic and were not able to determine a dose-related effect. The same was true for organ weights. Microscopic examinations did not report any drug-related findings other than those observed in the skin. Plasma drug levels generally showed a dose-response relationship, but there was a rather wide variability between the animals. Values ranged from below the limit of detection for the low-dose (0.8 mg/kg) group, 0.11-0.24 ng/ml for the mid-dose (4 mg/kg) group, and 0.18-0.38 ng/ml for the high-dose (8 mg/kg) group.

Chronic Toxicity:

Six-month dermal toxicity and toxicokinetic studies were conducted in rabbits, dogs, and minipigs. In rabbits, placebo patches or buprenorphine patches were applied for 6 months at doses up to 30 mg/kg. Patches were replaced every 3 days. The only clinical signs that were drug-related were reduced feces in the high dose (30 mg/kg) groups. All other clinical observations were observed to be of equal frequency between the groups. Dermal observations included an increased frequency of dermal irritation in the buprenorphine-treated groups when compared to placebo-patch controls. These included slight to well-defined erythema, slight edema, and desquamation. Microscopic examination of non-dermal tissues did not show any drug-related findings. Plasma buprenorphine levels generally increased with increasing dose, but showed wide variability. Drug levels were markedly higher in female rabbits than in male rabbits.

In dogs, placebo patches or buprenorphine patches were applied for 6 months at doses up to 12 mg/kg. Patches were replaced every 3 days. The only clinical sign reported was an increased incidence of no feces in the mid and high dose groups, an effect known to occur with exposure to opioids. In the skin, microscopic findings of squamous epithelial hyperplasia and hyperkeratosis were similar in both the buprenorphine-treated groups and placebo-patch controls, indicating an effect to the patch rather than to the drug. Microscopic examination of non-dermal tissues did not find any effects that could be attributed to drug exposure.Toxicokinetic analysis did not show a gender effect. There appeared to be increased absorption and exposure with increasing dosage. In general, levels at the end of the study were lower than those earlier in the study.

In minipigs, placebo patches or buprenorphine patches were applied for 6 months at doses up to 8 mg/kg. Patches were replaced every 3 days. The only drug-related clinical signs noted were decreased activity in several minipigs in the mid and high dose groups. This finding was considered to be a known effect of opioid treatment. Microscopic examination of various tissues did not report any drug-induced changes except in the treated dermal sites. Two minipigs in the high dose group had severe dermal irritation consisting of eschar on up to 10% of the dermal test site and blanching on 10-25% of the test site. Overall, dermal findings did not exhibit a clear dose-response relationship, and many of the dermal findings were found to some extent in the placebo-patch groups as well. Most of the noted effects, such as changes in body weights and at the dermal test sites, were attributed to the patching procedure rather than to the drug itself. Systemic exposures generally showed increased absorption and exposure with increasing dosage, but also showed high variability.

Genetic Toxicity:

Several genetic toxicity studies were conducted with buprenorphine. In the bacterial reverse mutation (Ames) assay, buprenorphine was not found to be mutagenic when tested at concentrations that produced significant cytotoxicity. In the mouse lymphoma L5178Y cell assay, buprenorphine was not found to be mutagenic when tested at concentrations that produced up to 80% cytotoxicity. Clastogenic potential of buprenorphine (induction of small colonies) was not determined due to technical difficulties. When tested with human peripheral blood lymphocytes, buprenorphine was not found to be clastogenic at concentrations that produced ≥50% inhibitions in mitotic indexes. Finally, in the mouse micronucleus assay, an *in vivo* assay for clastogenicity, buprenorphine was not found to induce micronuclei at doses that produced evidence of bone marrow cytotoxicity. Overall, these results indicate that buprenorphine does not possess genotoxic potential.

Safety/Special Toxicology:

Several studies were conducted to address the concern for reversal of respiratory depression by buprenorphine, potential of buprenorphine to induce dermal sensitization, accidental oral ingestion, and effects after immersion in warm water.

In rats, a study was conducted to examine whether nalfemene, an opioid antagonist and analog of naltrexone, could reverse or attenuate respiratory depression induced by buprenorphine. In animals pretreated with buprenorphine, nalfemene at doses up to 1 mg/kg did not attenuate the decrease in respiratory rate induced by doses of buprenorphine up to 10 mg/kg. Even 10 mg/kg of nalfemene did not attenuate the respiratory depressive effect of a low dose (1 mg/kg) of buprenorphine. These results showed that nalfemene was unable to reverse the respiratory depression induced by buprenorphine in the rat. [Note: In the labeling for Buprenex (buprenorphine HCl), it is stated that naloxone, another opioid antagonist, may not be effective in reversing respiratory depression produced by buprenorphine.]

A study was conducted in rabbits to examine the potential irritant and/or corrosive effects of buprenorphine patches when exposed to the skin for up to 3 days. Results showed that buprenorphine exposure for 4 hours produced slight irritation that was similar to that seen with the control (placebo) patches. After exposure for 3 days, however, buprenorphine patches

produced moderate irritation that was manifested by areas of blanching and/or eschar formation, erythema, and some edema.

In guinea pigs, a study was conducted to examine the potential of buprenorphine patches to induce dermal sensitization (delayed contact-type hypersensitivity) when given by multiple topical applications. Results indicated that 10 mg buprenorphine patches did not induce contact sensitization (delayed-type hypersensitivity reactions) in guinea pigs.

A study was conducted in beagle dogs to examine the potential safety issue of buprenorphine patches if accidentally swallowed. Dogs were given a single oral dose of gelatin capsules containing buprenorphine patches cut up into small pieces at 5 or 20 mg/dog (= 0.5 or 2.0 mg/kg). Other dogs were given capsules containing buprenorphine patches pounded with a meat tenderizer to mimic mastication (chewing). Results found decreased fecal output, a known pharmacological effect of opioids, was noted in most animals. No other apparent toxicological findings were noted in dogs given buprenorphine patches orally in capsules. This may be related to the very low (< 1 ng/ml) measured plasma drug concentrations. The results of this study performed in beagle dogs suggest that accidental ingestion (swallowing) of 20 mg buprenorphine patches may not result in significant clinical effects.

An additional study was conducted on beagle dogs to examine the potential safety issue of buprenorphine patches if accidentally chewed with subsequent buccal absorption of buprenorphine. Buprenorphine patches (5 mg and 20 mg) were applied to the buccal areas of female beagle dogs and left in place for 30 min by taping close the mouths of the animals. After 30 min, the patches were removed. Results showed decreased activity and red and swollen cheek mucosal areas in high dose groups within a few hours after dosing. No microscopic lesions were observed in any section of the buccal membrane from any dog, although red and swollen cheeks were noted grossly. Toxicokinetic data showed that buprenorphine was well absorbed after buccal administration in dogs given patches with holes. In this group, the mean Cmax within 30 min was 174 ng/ml and the mean AUC was 245±242 ng/ml/hr. The apparent $t_{1/2}$ was 2.5 hours. These results indicate that accidental buccal absorption may be a potentially significant safety issue, particularly if children were to chew used (discarded) or unused patches.

A final safety study was conducted in mini-swine to examine whether immersion in a warm water bath (41° C) for 15 min would alter the plasma levels of buprenorphine in animals wearing the patches. Results showed that there did not appear to be any effect of warm water immersion on increasing dermal absorption of buprenorphine from applied dermal patches in minipigs. However, given the low levels of drug absorbed before immersion (< 1 ng/ml), it is difficult to draw any conclusions.

Conclusions:

Since buprenorphine has been marketed in Europe and the U.S. since the early 1980's by the intravenous and intramuscular routes of administration, the nonclinical studies submitted in the current NDA focused on the dermal route of administration and the possible adverse effects of Buprenorphine Trans-Dermal System (BTDS) on the skin of animals. However, most of the animal dermal toxicity studies did perform complete histopathology of all the tissues as well.

Results of acute, subchronic, and chronic dermal toxicity studies in several animal species showed minimal dermal toxicity after exposure to buprenorphine-containing patches. Although some of the dermal irritation, such as edema and erythema, was attributed to drug, many of the

effects were also found in placebo patches, indicating that the skin reactions were attributable as much to the patching procedure as to the drug itself. The majority of clinical signs, such as reduced activity and low fecal output, could be attributed to effects seen with other opioids. Other than the skin, no drug-related histopathological changes were reported in any of the tissues examined.

In human patients given 5, 10, and 20 mg buprenorphine patches for up to 60 days (Study No. BP96-0102), mean steady-state plasma levels on Day 60 were reported to be 0.057, 0.120, and 0.192 ng/ml, respectively. In 6 month chronic dermal toxicity studies in animals, plasma levels were 1-4 ng/ml in rabbits given 30 mg/kg, 4 ng/ml in dogs given 12 mg/kg, and 0.75 ng/ml in minipigs given 8 mg/kg. The plasma levels achieved in animals were 4-21X those seen in human patients. Therefore, the lack of significant toxicities can not be attributed to lack of comparable systemic exposure to those seen in humans. Toxicokinetic data (plasma drug levels) from these animal studies showed a wide range of variability, but plasma levels generally increased with increasing dosage. Cmax was generally found to occur just after patch replacement, which was performed every 3 days in these studies (Note: the clinical duration is currently 7 days per patch).

Buprenorphine HCl was found to be negative for genotoxicity (mutagenicity and clastogenicity) in all the assays conducted.

Immersion in warm water did not increase the dermal absorption of buprenorphine in minipigs. Similar studies were conducted in humans with similar results.

Given the large first-pass effect of buprenorphine, accidental swallowing of buprenorphine patches may not pose a significant safety issue. However, the markedly increased systemic absorption that occurred in beagle dogs after buccal administration, as can occur by accidental chewing of used or unused patches by children with subsequent respiratory depression, may pose a significant safety issue.

Overall, results of nonclinical studies conducted both *in vivo* and *in vitro* showed that the Buprenorphine Trans-Dermal System is relatively safe for its intended clinical use of managing pain in patients requiring continuous opioid analgesia.

2. REVIEW OF INDIVIDUAL STUDIES:

2.1. Pharmacology:

2.1.1. In vitro metabolism of buprenorphine by rat liver and skin subcellular fractions (BupDM001):

Purpose: Published studies have shown that buprenorphine is metabolized by two different routes, N-dealkylation to norbuprenorphine and conjugation with glucuronic acid. Norbuprenorphine is also subsequently conjugated with glucuronic acid. Studies with human liver microsomes demonstrated that CYP3A4 is the primary enzyme responsible for catalyzing norbuprenorphine formation from buprenorphine.

In this study, the metabolism of buprenorphine in rat liver microsomes and S9 fractions, and rat skin microsomes was examined to determine the extent of buprenorphine metabolism and nature of the metabolites under conditions similar to those used in the mutagenicity studies (rat S9 liver fraction), and to determine the extent to which CYP3A, CYP1A and UDPGT are involved in the first pass metabolism of buprenorphine.

Results: Incubation studies with buprenorphine and Aroclor 1254-treated rat liver S9 fractions found that buprenorphine was primarily metabolized to norbuprenorphine. No other metabolite peaks were detected under the experimental conditions used. These results indicated that buprenorphine was metabolized to norbuprenorphine under conditions similar to those used in the bacterial mutation assay (Ames test; DSE-232).

Incubation studies with buprenorphine and rat skin microsomes showed that buprenorphine was not metabolized to either norbuprenorphine or buprenorphine glucuronide. Similar results were also obtained with human skin microsomes that lacked CYP3A4 activity. These results suggested that buprenorphine is not metabolized when applied as a transdermal patch. Clinical pharmacokinetic data may be used to confirm these experimental observations.

2.1.2. Opioid receptor binding (d, k, m and nociceptin) activity of buprenorphine, (b) (4)

Purpose: This study examined the binding capabilities of buprenorphine and its analogs (present as impurities) against delta, kappa, mu, and orphanin receptors.

Results: Binding data for buprenorphine and the analogs present as impurities (b) (4) are shown in Table 1 (Sponsor's Table). As shown, buprenorphine had highest affinity for kappa receptors (Ki = 0.157 nM) with high affinity for both delta (Ki = 1.9 nM) and mu receptors (Ki = 1.33 nM). (b) (4) showed highest affinity for mu receptors, equal to that seen with buprenorphine. (b) (4) and (b) (4) had highest affinity for mu receptors as well.

Table 1 (Sponsor's Table)

Summary of Binding Data

Compound	Ki values (nM)			
	Delta	Kappa	Mu	Orphanin
Buprenorphine	1.9	0.157	1.33	128
[redacted] (b) (4)				

ND, not determined

Conclusions: These results indicated that buprenorphine possesses antinociceptive activity by behaving as a mu opioid partial agonist, and treatment with buprenorphine may result in less psychomimetic effects by virtue of its strong kappa antagonist properties.

2.2. Toxicology:

2.2.1. Acute toxicity:

2.2.1.1. A preliminary dermal patch tolerance study in rats, rabbit and dog (DSE-167):

Testing Facility: [redacted] (b) (4)
Study Number: DSE-167
Study Date(s): Sept. 15, 1995 to Sept. 29, 1995
GLP Compliance: No
QA Report: Yes

Purpose: This study examined the practicality of applying the patches intended for human use to the skin of rats, rabbits, and dogs. Results of these studies will be used to design subsequent toxicity studies with patches containing the active drug substance buprenorphine.

Methods: Placebo patches corresponding to the following strength of buprenorphine patches were used: 5 mg (4 cm X 4 cm), 10 mg (6.8 cm X 4.5 cm), and 20 mg (7 cm X 7 cm). One 10 mg placebo patch was applied to the dorsal trunk of a male rat, one 5 mg and one 20 mg placebo patch was applied to the dorsal trunk of a female rabbit, and one 5 mg and one 20 mg placebo patch was applied to the dorsal trunk of a female dog. The patches were covered with an expandable stocking and tape, and left in place for 14 days. Collars were placed on each animal to prevent biting of the patch. After 14 days, the patch was removed and the skin site examined for erythema, edema, desquamation, and other dermal signs of irritation.

Results: The patches had to be reapplied several times in each animal during the course of the study due to disruption of the adhesion. In each animal irritation was seen at the site of the binder tape. In the rabbit and dog, the application site, the area of the patch that would contain the active drug, appeared normal. In the rat, focal and/or pinpoint areas of eschar were noted at the application site.

Conclusions: It was concluded that the patch manufactured for human use was practical to apply to rats, rabbits, and dogs for toxicity testing.

2.2.1.2. A preliminary single application dermal toxicity study in rats, rabbits and dogs with buprenorphine patches (DSE-107):

Testing Facility: (b) (4)
Study Number: DSE-107
Study Date(s): Dec. 19, 1995 to Jan. 14, 1996
GLP Compliance: No
QA Report: Yes

Purpose: This study examined the dermal and clinical effects of a single application of a maximum number of buprenorphine patches to rats, rabbits, and dogs.

Methods: Various strength buprenorphine patches of 5 mg (lot no. Ch.B.8/28042/5), 10 mg (lot no. Ch.B.8/28043/5), or 20 mg (lot no. Ch.B.8/28044/5) per patch were applied to the dorsal trunk of rats, rabbits, and dogs at according to the following treatment groups (Table 2; Sponsor's Table):

Table 2 (Sponsor's Table)

Species	No. of Animals	Nominal Dose Level*
Rat	2 Males	20 mg/kg (5 mg/rat)
	2 Males	40 mg/kg (10 mg/rat)
Rabbit	1 Male, 1 Female	5 mg/kg (10 mg/rabbit)
	1 Male, 1 Female	7.5 mg/kg (15 mg/rabbit)
	1 Male, 1 Female	15 mg/kg (30 mg/rabbit)
	1 Male, 1 Female	30 mg/kg (60 mg/rabbit)
	1 Male, 1 Female (Toxicokinetic Phase)	30 mg/kg (60 mg/rabbit)
Dog	1 Female	3 mg/kg (30 mg/dog)
	1 Female	6 mg/kg (60 mg/dog)
	1 Female	12 mg/kg (120 mg/dog)
	1 Female (Toxicokinetic Phase)	12 mg/kg (120 mg/dog)

*mg/kg body weight dose levels are approximate.

The patches were wrapped and secured with tape for 3 days. Body weights and food consumption were recorded during the course of the study. On Day 4, the patches were removed and the and skin observed on Day 4 and Day 8 for signs of erythema, edema, desquamation and other dermal signs. Blood was collected for toxicokinetics at various times each day for up to 8 days from the two rabbits and one dog that received, according to the sponsor, the maximum tolerated dose of 60 mg/rabbit and 120 mg/dog.

Results: No deaths or remarkable clinical signs were reported. Decreased food consumption was noted in some rabbits and dogs on Days 2 and 3.

Dermal scores were negative in the rat. In rabbits, erythema was noted on Day 4 in the 7.5 mg/kg and 30 mg/kg groups. In dogs, a slight erythema was noted on Day 4 in the 12 mg/kg dog. Dermal findings in both species were attributed to the adhesive portion of the patch, and not to the area of active drug.

Decreases in body weight gain were found in rabbits and dogs. Body weight gain in the 30 mg/kg rabbit decreased 8% when compared to the predose weight. In the 12 mg/kg dog, body weight gain decreased 7% when compared to the predose weight.

Results of toxicokinetic data were not provided in this study report.

Conclusions: In rats, rabbits, and dogs given buprenorphine patches at doses up to 10 mg/rat, 60 mg/rabbit, and 120 mg/dog, which constituted up to 10% of body surface area,

minimal dermal findings were noted, most of which were attributed to the adhesive and not to burprenorphine exposure. Body weights in the high dose groups were reduced 7-8% in dogs and rabbits, respectively. No significant clinical signs were noted. The sponsor concluded that it was practical to use the rabbit and dog for further toxicity testing of buprenorphine patches.

2.2.1.3. A preliminary single application dermal toxicity study in SPF Yorkshire pigs with buprenorphine patches (DSE-179):

Testing Facility: (b) (4)
Study Number: DSE-179
Study Date(s): Completed Oct. 1, 1996
GLP Compliance: No
QA Report: Yes

Purpose: This study explored the acute toxic effects of buprenorphine patches after a single application to SPF Yorshire pigs for 3 days.

Methods: Male SPF Yorshire pigs (1/group; 20-32 kg) were each given buprenorphine patches (lot no. of 10 mg patch was 8/28043/5; lot no. of 20 mg patch was 8/28044/6) applied to the dorsal trunk at the following doses (Table 3; Sponsor's Table):

Table 3 (Sponsor's Table)

No. of Males	Dose Level	Approximate Dose Level (mg/kg)
1	120 mg/pig	6.3
1	60 mg/pig	2.8
1	120 mg/pig	5.0
4 (Toxicokinetic Phase)	120 mg/pig	4.1

The patches were held in place for 3 days. After 3 days, the patches were removed and the application site examined daily for the next 4 days for the following dermal signs: erythema, edema, desquamation, and any other dermal reactions. Also, blood was taken for up to 7 days after dosing from 4 pigs given 120 mg (=4.1 mg/kg) for toxicokinetic analysis.

Results: There were only minor clinical signs (soft stools) and no changes in body weight gains. The dermal test site showed yellow staining that may have been due to the buprenorphine. Dermal reactions consisted of slight to well-defined erythema, slight edema, and slight to moderate desquamation and/or dermal irritation in the binder tape area. Even though there were no placebo patches, it was concluded from the location of the dermal reactions that the findings

were due to the patch application and not to the test article. Toxicokinetic data was not presented.

Conclusions: The SPF Yorshire pig was considered a suitable test system for evaluation of buprenorphine patches in longer-term studies.

2.2.1.4. A preliminary single application dermal toxicity study in Hanford minipigs with buprenorphine patches (DSE-185):

Testing Facility: (b) (4)
Study Number: DSE-185
Study Date(s): April 16, 1996 to May 10, 1996
GLP Compliance: No
QA Report: Yes

Purpose: This study explored the acute toxic effects of buprenorphine patches after a single application to Hanford minipigs (Minipig-HA) for 3 days.

Methods: Male Hanford minipigs (1/group; 5.6-8.8 kg) were each given buprenorphine patches (lot no. of 20 mg buprenorphine TDS patch was 8/28044/5) applied to the dorsal trunk at the following doses (Table 4; Sponsor's Table):

Table 4 (Sponsor's Table)

No. of Males	Dose Level	Approximate Dose Level (mg/kg)
1	60 mg/minipig	10.7
1	120 mg/minipig	16.8
4 (Toxicokinetic Phase)	120 mg/minipig	15.8

According to the sponsor, six 20-mg patches (=120 mg/pig) represent the maximum number that could be applied to ≤10% of the total body surface area of a minipig. The patches were held in place for 3 days. After 3 days, the patches were removed and the application site examined daily for the next 4 days for the following dermal signs: erythema, edema, desquamation, and any other dermal reactions. Also, blood was taken for up to 8 days after dosing from 4 pigs given 120 mg (=4.1 mg/kg) for toxicokinetic analysis.

Results: No significant clinical signs were noted. Yellow staining was noted at the test site of each animal that may have been due to the buprenorphine. In the pig receiving 60 mg, dermal findings included scabs around the test area and desquamation on Day 4-5. In the pig

receiving 120 mg, dermal findings included binder irritation, moderate to severe erythema and/or focal pinpoint areas of eschar on Days 4-8. Toxicokinetic data was not presented.

Conclusions: The Hanford minipig was considered a suitable test system for evaluation of buprenorphine patches in longer-term studies. However, difficulty in retaining 6 patches was noted. It was recommended that no more than 5-20 mg patches be used in the minipig.

2.2.2. *Subchronic Toxicity:*

2.2.2.1. A 7-day dermal toxicokinetic study in rabbits with buprenorphine patches (+toxicokinetic report and analytical report) (DSE-258):

Testing Facility: (b) (4)
Study Number: DSE-258
Study Date(s): May 8, 1997 to June 10, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study measured plasma concentrations of buprenorphine after dermal application of the buprenorphine TDS patch to rabbits for 7 days.

Methods: Male and female New Zealand White rabbits (3/sex/group; about 3 kg) were given dermal buprenorphine patches (5 mg lot no. = 7/00499/6; 10 mg lot no. = 7/00499/6A; 20 mg lot no. = 7/00499/6B) to the dorsal trunk area at 0, 2.5, 15, or 30 mg/kg according to the following schedule (Sponsor's Table):

Table 5 (Sponsor's Table)

Study Design and Dosing

Group	No. of Animals		Dosage Material	Nominal Dosage Level		No. of Patches Applied x Patch Size	Actual Dosage Range (mg/kg)[2]
	Male	Female		(mg/kg)[1]	(mg/rabbit)		
1	3	3	Placebo patch	0	0	3 x 20 mg	0
2	3	3	Buprenorphine	2.5	5	1 x 5 mg	1.5 - 1.8
3	3	3	Buprenorphine	15.0	30	3 x 10 mg	9.4 - 10.6
4	3	3	Buprenorphine	30.0	60	3 x 20 mg	19.3 - 22.9

[1]Approximate level based on mg/kg body weight.
[2]Based on the total mg administered to the animals divided by the actual body weights.

Patches were replaced every three days. Body weights were recorded and clinical signs monitored. Dermal irritation was not recorded in this study.

Blood was collected predose and at 1, 2, 4, 8, and 24 hours after dosing on Day 1, and from one rabbit per dose group in sequence on Days 3 through 7. Plasma was frozen and subsequently analyzed for drug concentrations.

Results: There were no significant effects of buprenorphine on body weights or clinical signs for the 7 days of observation.

Plasma drug concentrations are summarized in Table 5 (Sponsor's Table 7.4). As shown, there was wide variability in most parameters examined. A clear dose-response relationship was difficult to establish. Males has higher exposures at the two lower doses, while females had higher exposures at the high dose. The time to peak plasma drug levels (Tmax) was between 24-72 hours.

Table 5 (Sponsor's Table 7.4)

Pharmacokinetics Of Buprenorphine In Rabbits Administered 5, 30 Or 60 mg Doses By Dermal Application (Patches) Once Every Three Days: Pharmacokinetics After The First Dosing Interval

Dose (mg)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-72} (ng•hr/mL)
5	Male	0.3	48	10.6
	Female	0	0	0
30	Male	4.27	48	155
	Female	0.15	4	2.85
60	Male	2.95	72	88.7
	Female	5.07	24	237

Conclusions: Given the wide variability and lack of a dose-response relationship, it was difficult to draw any conclusions from this study.

2.2.2.2. A 28-day dermal toxicity study in rabbits with buprenorphine patches (DSE-168):

Testing Facility: (b) (4)
Study Number: DSE-168
Study Date(s): March 19, 1996 to April 17, 1996
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity of buprenorphine patches when placed dermally on rabbits for 27 days. Since the intended clinical duration at the time of this study was 3 days, patches in this rabbit study were replaced every 3 days. [Note: Current intended clinical duration is 7 days per patch.]

Methods: Male and female New Zealand White rabbits (5/sex/group; 2.3-2.8 kg) were assigned to receive buprenorphine patches (5 mg lot no. = 8/28042/5; 10 mg lot no. = 8/28043/5; 20 mg lot no. = 8/28044/5) on the dorsal trunk at 0, 2, 12, or 24 mg/kg according to the following schedule:

Table 6 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)[1]	Dosage Level (mg/rabbit)	Patches To Be Applied	Actual Dosage Range (mg/kg)[2]
	Male	Female					
1	5	5	Control (collar)	0	0	0	0
2	5	5	Placebo patch	0	0	3 x "20 mg" size	0
3	5	5	Buprenorphine	2.0	5	1 x 5 mg	1.5 - 2.0
4	5	5	Buprenorphine	12.0	30	3 x 10 mg	9.8 - 13.0
5	5	5	Buprenorphine	24.0	60	3 x 20 mg	20.6 - 27.1

[1]From the protocol.
[2]Based on the total mg administered to the animals divided by the actual body weights.

Patches were applied every 3 days. The area covered by the patches was about 10% of the body surface area. The patches were wrapped, secured with double-stick tape, and covered with a stockinet jacket.

Rabbits were observed daily for clinical signs. When patches were replaced every 3 days, the application sites were examined for dermal irritation. Body weights were recorded weekly. Food consumption was recorded daily. On study termination, blood was collected for clinical chemistry and hematology. Rabbits were euthanized and organ weights taken. All tissues (41) and organs taken at necropsy were examined microscopically.

Results: No deaths were reported. Fecal output was decreased in the 12 and 24 mg/kg males, a pharmacologic effect found with other opioids.

When compared to the no-patch controls, body weights in all patch groups were decreased up to 12%. However, there were no significant differences in body weights between the placebo and buprenorphine patch groups.

When compared to the no-patch and placebo-patch controls, significant decreases in food consumption of about 50% were noted in the 12 and 24 mg/kg buprenorphine-treated groups that occurred early on about Days 1-2. However, these decreases returned to normal values by about Day 8.

No drug-related changes were found in clinical chemistry, hematology, gross necropsy, organ weights, or non-dermal histopathology.

There were dermal findings that consisted of slight to severe erythema, slight to moderate edema and desquamation, and focal/pinpoint eschar, eschar exfoliation, and fissuring. Also, minimal to moderate chronic dermatitis was found that was characterized as focal or diffuse mononuclear cell infiltrates in the superficial dermis. The dermal findings were slightly more severe in the buprenorphine groups, but were found in all patch groups, including placebo, and were higher in females than in males. According to the histopathology report, the dermal changes were essentially due to the patch application and shaving procedures, and were expected to quickly reverse following cessation of treatment. However, confirmation of this by inclusion of a reversibility group was not performed in this study.

Conclusions: Drug-related effects consisted of decreased fecal output and decreased food consumption, effects found with other opioids. The main findings were dose-related effects on increasing the severity of dermatitis, but these findings were also found in the rabbits that received placebo patches, indicating that these effects were likely due to the patch application and shaving procedures. The minimal degree of irritation in these studies indicated that the dermal effects may easily resolve following patch removal. However, reversibility in this study was not demonstrated.

2.2.2.3. A 28-day dermal toxicity study in dogs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-169):

Testing Facility: (b) (4)
Study Number: DSE-169
Study Date(s): March 26, 1996 to April 25, 1996
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity of buprenorphine patches when applied to Beagle dogs for 27 days. Also, plasma drug levels of buprenorphine (toxicokinetics) were reported.

Methods: Male and female Beagle dogs (6.3-9.2 kg; 4/sex/group) were given buprenorphine patches (placebo patch lot no. = 8/28040/5; 5 mg patch lot no. = 8/28042/5; 10

mg patch no. = 8/28043/5; 20 mg patch lot no. = 8/28044/5) at 3 (3.5-4.8), 6 (6.4-9.8), or 12 (14.0-18.7) mg/kg according to the dosing schedule below (Table 7; Sponsor's Table). The ranges in parentheses reflect the actual dosage levels. Placebo controls received placebo patches, and untreated controls received no patches. Patches were held in place with a jacket wrapped with tape. Patches were replaced every 3 days, and the dermal sites rotated as much as possible.

Table 7 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)[1]	Dosage Level (mg/dog)	Patches To Be Applied	Actual Dosage Range (mg/kg)[2]
	Male	Female					
1	4	4	Control (collar)	0	0	0	0
2	4	4	Placebo patch	0	0	6 x Placebo 20 mg size	0
3	4	4	Buprenorphine	3	30	6 x 5 mg	3.5 - 4.8
4	4	4	Buprenorphine	6	60	6 x 10 mg	6.4 - 9.8
5	4	4	Buprenorphine	12	120	6 x 20 mg	14.0 - 18.7

[1]From the protocol.
[2]Based on the total mg the animals were administered divided by the actual body weights.

Animals were observed for clinical signs of toxicity, and the dermal sites examined and graded for irritation each time the patch was replaced. Food consumption was recorded daily and body weights were recorded weekly. Blood and urine samples were collected predose and at sacrifice for clinical pathology parameters. ECGs (leads I, II, III, A_{vr}, A_{VL}, and A_{VF}) and indirect blood pressure measurements were taken predose and before sacrifice on Day 29. Blood samples were also collected for toxicokinetic analysis from one dog per sex per group predose and 4 hours after dosing every 3rd day before patch replacement.

On Days 30-31, all dogs were euthanized and a gross necropsy conducted that consisted of evaluation of external surfaces and all viscera. A total of 6 dermal sites of drug application were collected from each dog as follows:

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Weights of selected organs were collected. Specimens from 42 tissues were fixed, stained, and examined microscopically.

Results: No deaths were reported. In the 6 mg/kg and 12 mg/kg groups, decreased activity and low fecal output were reported. These findings may be interpreted as similar to the known pharmacological effects of opioids.

Dermal findings are summarized in Table 8A (males; Sponsor's Table 2) and Table 8B (females; Sponsor's Table 2). In male dogs, exposure of dermal sites to buprenorphine produced slight to moderate irritation, erythema and edema that was more intense during the first 2-3 weeks of exposure. By study termination, drug exposed sites showed pinpoint eschar and redness and swelling. A dose response was not obvious, but there was a higher incidence of effects in the high dose (120 mg) groups. Dermal sites exposed to placebo patches showed slight to mild erythema and edema. No irritation was noted in untreated (no patches) dogs. Findings in female dogs were similar to those of males, but the incidences of dermal effects were similar in all dose groups, including placebo patch controls. Dermal findings present in all groups, including placebo-patch controls, included minimal to moderate acanthosis and minimal to moderate hyperkeratosis.

Table 8A (Sponsor's Table)

Dermal Findings in Male Dogs Treated with Buprenorphine Patches for 28 Days

----- M A L E -----

GROUP: LEVEL (MG/DOG):	1 0 CONTROL	2 0 PLACEBO PATCH	3 30 BUP PATCH	4 60 BUP PATCH	5 120 BUP PATCH
EXPOSURE SITE					
-ERYTHEMA - GRADE 0	44/ 4	25/ 4	11/ 4	15/ 4	5/ 4
-ERYTHEMA - GRADE 1	0/ 0	12/ 3	4/ 2	15/ 4	10/ 2
-ERYTHEMA - GRADE 2	0/ 0	6/ 3	24/ 3	9/ 3	14/ 4
-ERYTHEMA - GRADE 3	0/ 0	1/ 1	5/ 3	0/ 0	11/ 4
-ERYTHEMA - GRADE 4	0/ 0	0/ 0	0/ 0	0/ 0	4/ 1
-MAXIMIZED GRADE 4	0/ 0	0/ 0	0/ 0	5/ 1	0/ 0
-EDEMA - GRADE 0	44/ 4	39/ 4	21/ 4	29/ 4	19/ 4
-EDEMA - GRADE 1	0/ 0	5/ 1	20/ 3	10/ 3	8/ 4
-EDEMA - GRADE 2	0/ 0	0/ 0	3/ 1	4/ 2	17/ 3
-EDEMA - GRADE 3	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
-ESCHAR - FOCAL/PINPOINT	0/ 0	14/ 3	24/ 3	16/ 3	30/ 4
-ESCHAR - MILD	0/ 0	0/ 0	0/ 0	4/ 1	0/ 0
-ESCHAR - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
-DESQUAMATION	0/ 0	2/ 2	2/ 2	0/ 0	3/ 3
-ESCHAR EXFOLIATION	0/ 0	7/ 3	9/ 3	13/ 3	16/ 4
-TEST SITE STAINING - BROWN	0/ 0	4/ 2	9/ 3	17/ 3	24/ 4
OTHER					
-RED RAISED AREA(S) INSIDE DERMAL TEST SITE(S)	0/ 0	8/ 2	18/ 3	9/ 3	24/ 4
-YELLOWISH COLORED FLUID EXUDING FROM TEST SITE	0/ 0	0/ 0	0/ 0	4/ 2	0/ 0
-APPARENT THICKENING OF SKIN INSIDE DERMAL TEST SITE(S)	0/ 0	0/ 0	1/ 1	0/ 0	6/ 2

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Table 8A (Sponsor's Table)

Dermal Findings in Male Dogs Treated with Buprenorphine Patches for 28 Days (cont)

----- M A L E -----

GROUP: LEVEL (MG/DOG):	1 0 CONTROL	2 0 PLACEBO PATCH	3 30 BUP PATCH	4 60 BUP PATCH	5 120 BUP PATCH
OTHER					
-APPARENT BINDER IRRITATION	0/ 0	9/ 3	10/ 3	9/ 2	5/ 3
-PATCHES WERE FOUND BUNCHED TOGETHER	0/ 0	0/ 0	3/ 2	0/ 0	0/ 0
-PATCHES FOUND IN CAGE/TRAY	0/ 0	0/ 0	10/ 3	0/ 0	5/ 2
-BINDER WAS FOUND DESTROYED	0/ 0	0/ 0	2/ 1	1/ 1	8/ 3
-WRAP FOUND LOOSENED	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
-PATCH FOUND IN CAGE/TRAY	0/ 0	0/ 0	7/ 2	0/ 0	0/ 0
-COLLAR DESTROYED	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0
-PATCH FOUND IN DOG RUN	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Table 8B (Sponsor's Table)

Dermal Findings in Female Dogs Treated with Buprenorphine Patches for 28 Days

----- F E M A L E -----

GROUP: LEVEL (MG/DOG):	1 0 CONTROL	2 0 PLACEBO PATCH	3 30 BUP PATCH	4 60 BUP PATCH	5 120 BUP PATCH
EXPOSURE SITE					
-ERYTHEMA - GRADE 0	44/ 4	5/ 4	7/ 4	16/ 4	14/ 4
-ERYTHEMA - GRADE 1	0/ 0	16/ 3	19/ 4	19/ 4	22/ 4
-ERYTHEMA - GRADE 2	0/ 0	16/ 4	13/ 4	5/ 3	7/ 2
-ERYTHEMA - GRADE 3	0/ 0	7/ 2	1/ 1	4/ 2	1/ 1
-MAXIMIZED GRADE 4	0/ 0	0/ 0	4/ 1	0/ 0	0/ 0
-EDEMA - GRADE 0	44/ 4	16/ 4	22/ 4	35/ 4	25/ 4
-EDEMA - GRADE 1	0/ 0	25/ 4	21/ 4	5/ 3	12/ 4
-EDEMA - GRADE 2	0/ 0	2/ 1	0/ 0	4/ 2	5/ 2
-EDEMA - GRADE 3	0/ 0	1/ 1	1/ 1	0/ 0	2/ 1
-ESCHAR - FOCAL/PINPOINT	0/ 0	24/ 4	27/ 4	14/ 3	18/ 4
-ESCHAR - MILD	0/ 0	0/ 0	4/ 1	0/ 0	0/ 0
-DESQUAMATION	0/ 0	1/ 1	3/ 2	4/ 2	2/ 1
-ESCHAR EXFOLIATION	0/ 0	11/ 4	13/ 3	6/ 3	14/ 4
-TEST SITE STAINING - BROWN	0/ 0	24/ 3	10/ 3	13/ 3	8/ 2
OTHER					
-RED RAISED AREA(S) OUTSIDE DERMAL TEST SITE(S)	0/ 0	2/ 1	0/ 0	0/ 0	1/ 1
-RED RAISED AREA(S) INSIDE DERMAL TEST SITE(S)	0/ 0	27/ 4	20/ 4	4/ 2	3/ 1
-YELLOWISH COLORED FLUID EXUDING FROM TEST SITE	0/ 0	0/ 0	1/ 1	0/ 0	1/ 1
-APPARENT THICKENING OF SKIN INSIDE DERMAL TEST SITE(S)	0/ 0	0/ 0	4/ 2	0/ 0	3/ 1

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Table 8B (Sponsor's Table)

Dermal Findings in Female Dogs Treated with Buprenorphine Patches for 28 Days (cont)

----- F E M A L E -----

GROUP: LEVEL (MG/DOG):	1 0 CONTROL	2 0 PLACEBO PATCH	3 30 BUP PATCH	4 60 BUP PATCH	5 120 BUP PATCH
OTHER					
-APPARENT BINDER IRRITATION	0/ 0	5/ 2	14/ 4	19/ 4	7/ 3
-PATCHES FOUND IN CAGE/TRAY	0/ 0	0/ 0	6/ 3	0/ 0	4/ 2
-BINDER WAS FOUND DESTROYED	0/ 0	2/ 2	0/ 0	0/ 0	6/ 2
-PATCH FOUND IN CAGE/TRAY	0/ 0	0/ 0	0/ 0	0/ 0	2/ 2
-FOUND COLLAR OFF	2/ 2	0/ 0	0/ 0	0/ 0	0/ 0
-COLLAR DESTROYED	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Body weight changes due to buprenorphine were minimal. When compared to the untreated group, body weights in the placebo groups decreased about 5%, and in the drug-treated groups body weights decreased 7-10%.

Food consumption in both male and female dogs was markedly affected by drug treatment. During the first few days of the study (Days 2-5), food consumption decreased in a dose-dependent manner with a complete lack of food intake in the high dose (12 mg/kg) groups during this period. However, food consumption returned to normal levels by the end of the study.

There were no drug-related changes in hematology, coagulation, or urinalysis. However, there were some terminal biochemistry changes in the buprenorphine-treated dogs when compared to placebo patch controls. In the 6 mg/kg and 12 mg/kg male groups, calcium was significantly decreased (up to a 7% decrease). Also, total bilirubin was decreased in the high dose (12 mg/kg) males. In female dogs, calcium and urea nitrogen were decreased when compared to placebo-patch controls. It should be noted that the differences between placebo-patch controls and high dose buprenorphine dogs were similar to the differences between untreated controls and placebo-patch controls, indicating that much of the effect was due to the patch, although presence of drug was a contributing factor.

At necropsy, no additional gross or microscopic abnormalities were reported other than the dermal findings reported above.

Toxicokinetic analysis showed that the highest concentration of plasma buprenorphine levels occurred the day after application of a new patch. The highest average plasma concentrations during the course of the study showed a wide range of variability and are summarized in Table 9.

Table 9

Summary of Peak Plasma Concentrations
in Dogs Treated with Buprenorphine Patches for 28 Days

Dose		**Range of Peak Ave. Plasma Drug Conc. (ng/ml)**
mg/dog	**mg/kg**	
30	3	0.13 to 2.92
60	6	1.24 to 4.73
120	12	2.62 to 8.71

Conclusions: Application of buprenorphine patches on the skin of dogs resulted in drug-related effects. In the 6 mg/kg and 12 mg/kg groups, decreased activity and reduced fecal output were reported, effects known to occur with other opioids. Food consumption showed marked drug-related effects with near complete lack of food intake in the high dose groups early in the study (Days 2-5). However, food consumption returned to normal levels by the end of the study.

Dermal findings were found in all patch groups, with and without buprenorphine. These included minimal to moderate acanthosis and minimal to moderate hyperkeratosis. However, buprenorphine groups showed additional effects including slight to moderate irritation, erythema

and edema that was more intense during the first 2-3 weeks of exposure. By study termination, drug exposed sites showed pinpoint eschar and redness and swelling. Although a clear dose relationship was difficult to establish, there was a higher incidence in the high dose (12 mg/kg) groups, particularly in the male dogs.

When compared to placebo-patch controls, there were some drug-related decreases in certain biochemistry parameters, such as decreased calcium bilirubin, and urea nitrogen. However, similar effects were also seen in the placebo-patch groups when compared to untreated controls.

Toxicokinetic analysis showed that the highest concentration of plasma buprenorphine levels occurred the day after application of a new patch. Also, there was a general dose-related increase in systemic absorption, but the range between the animals in the average peak levels over the course of the study was wide.

2.2.2.4. A 3-month dermal toxicity study in dogs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-213):

Testing Facility: (b) (4)
Study Number: DSE-213
Study Date(s): Nov. 18, 1996 to Feb. 18, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity of buprenorphine patches when applied to Beagle dogs for 3 months. Also, plasma drug levels of buprenorphine (toxicokinetics) were reported.

Methods: Male and female Beagle dogs (5.9-8.0 kg; 3/sex/group) were given buprenorphine patches (placebo patch lot no. = 7/00500/6B; 5 mg patch lot no. = 7/00499/6; 10 mg patch no. = 7/00499/6A; 20 mg patch lot no. = 7/00499/6B) at 1 (range 1.0-1.6), 3.5 (range 4.1-5.5), or 12 (range 13.8-18.5) mg/kg according to the dosing schedule below (Table 10; Sponsor's Table). The ranges in parentheses reflect the actual dosage levels. Placebo controls received placebo patches, and untreated controls received no patches. Patches were held in place with a jacket wrapped with tape. Patches were replaced every 3 days, and the dermal sites rotated as much as possible.

Table 10 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)[1]	Dosage Level (mg/dog)	Patches Applied	Actual Dosage Range (mg/kg)[2]
	Male	Female					
1	3	3	Untreated Control	0	0	0	0
2	3	3	Placebo Patch	0	0	6 x Placebo 20 mg size	0
3	3	3	Buprenorphine Patch	1	10	1 x 10 mg	1.0 - 1.6
4	3	3	Buprenorphine Patch	3.5	35	1 x 5 mg 1 x 10 mg 1 x 20 mg	4.1 - 5.5
5	3	3	Buprenorphine Patch	12	120	6 x 20 mg	13.8 - 18.5

[1]From the protocol.
[2]Based on the total mg applied divided by the sex-group mean body weight.

Dogs were observed daily for clinical signs. Dermal patch sites were examined for irritation every 3 days during patch replacements. Body weights were recorded weekly, and food consumption was recorded daily. Blood and urine samples were collected predose and at sacrifice for clinical pathology parameters. ECGs (leads I, II, III, A_{vr}, A_{VL}, and A_{VF}), indirect blood pressure measurements, and respiratory rate determinations were taken predose, and on Days 1, 2, 4, 25, 43, and 89. Blood samples were also collected for toxicokinetic analysis from each dog at predose on Days 1, 2, 3, 22, 23, 24, 52, 53, 54, 88, 89, and 90 prior to patch replacement.

On Day 89, all dogs were euthanized and a gross necropsy conducted that consisted of evaluation of external surfaces and all viscera. A total of 6 dermal sites of drug application were collected from each dog as follows:

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Weights of selected organs were collected. Specimens from 42 tissues were fixed, stained, and examined microscopically.

Results: No deaths were reported. Clinical signs consisted of low fecal output in the 3.5 mg/kg and 12 mg/kg groups, an effect seen with other opioids.

Most of the dermal effects were seen common to all patch groups, including placebo. These included minimal edema, erythema, and red raised areas. However, the incidences of edema and erythema were markedly increased in the high dose (12 mg/kg) females (Table 11B), but not in the high dose males (Table 11A). There were no other gross dermal effects that could be related specifically to exposure to buprenorphine.

Table 11A

Dermal Findings in Male Dogs Treated with Buprenorphine Patches 3 Months

----- M A L E -----

GROUP: LEVEL (MG/DOG):	1 0 CONTROL	2 0 PLACEBO PATCH	3 10 BUP PATCH	4 35 BUP PATCH	5 120 BUP PATCH
EXPOSURE SITE					
-NO ERYTHEMA	93/ 3	38/ 3	52/ 3	55/ 3	62/ 3
-NO EDEMA	93/ 3	56/ 3	77/ 3	78/ 3	78/ 3
-ERYTHEMA - GRADE 1	0/ 0	23/ 3	31/ 3	28/ 3	17/ 2
-ERYTHEMA - GRADE 2	0/ 0	20/ 3	10/ 3	10/ 3	11/ 1
-ERYTHEMA - GRADE 3	0/ 0	11/ 2	0/ 0	0/ 0	3/ 2
-ERYTHEMA - GRADE 4	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-EDEMA - GRADE 1	0/ 0	30/ 3	16/ 3	15/ 3	14/ 1
-EDEMA - GRADE 2	0/ 0	6/ 1	0/ 0	0/ 0	1/ 1
-EDEMA - GRADE 3	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-ESCHAR - FOCAL/PINPOINT	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
-DESQUAMATION	0/ 0	0/ 0	6/ 1	3/ 1	2/ 2
-ESCHAR EXFOLIATION	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
-RED RAISED AREA(S) WITHIN TEST SITE	0/ 0	18/ 3	10/ 2	16/ 3	16/ 2

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.
REFER TO PROTOCOL APPENDIX A FOR DEFINITION OF DERMAL CODES.

Table 11B

Dermal Findings in Female Dogs Treated with Buprenorphine Patches 3 Months

---- F E M A L E -----

GROUP:	1	2	3	4	5
LEVEL (MG/DOG):	0 CONTROL	0 PLACEBO PATCH	10 BUP PATCH	35 BUP PATCH	120 BUP PATCH
EXPOSURE SITE					
-NO ERYTHEMA	93/ 3	52/ 3	78/ 3	51/ 3	11/ 3
-NO EDEMA	93/ 3	77/ 3	85/ 3	73/ 3	30/ 3
-ERYTHEMA - GRADE 1	0/ 0	26/ 3	12/ 3	36/ 3	35/ 3
-ERYTHEMA - GRADE 2	0/ 0	11/ 3	3/ 1	6/ 2	34/ 3
-ERYTHEMA - GRADE 3	0/ 0	1/ 1	0/ 0	0/ 0	11/ 1
-MAXIMIZED GRADE 4	0/ 0	3/ 2	0/ 0	0/ 0	0/ 0
-EDEMA - GRADE 1	0/ 0	15/ 3	7/ 1	20/ 3	57/ 3
-EDEMA - GRADE 2	0/ 0	0/ 0	1/ 1	0/ 0	4/ 2
-EDEMA - GRADE 3	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-ESCHAR - MILD	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-ESCHAR - MODERATE	0/ 0	2/ 1	0/ 0	0/ 0	0/ 0
-DESQUAMATION	0/ 0	2/ 2	1/ 1	1/ 1	0/ 0
-ESCHAR EXFOLIATION	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0
-DISCHARGE FROM TEST SITE	0/ 0	1/ 1	0/ 0	0/ 0	1/ 1
-RED RAISED AREA(S) WITHIN TEST SITE	0/ 0	19/ 3	5/ 1	18/ 3	66/ 3

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.
REFER TO PROTOCOL APPENDIX A FOR DEFINITION OF DERMAL CODES.

Food consumption in the high dose (12 mg/kg) groups during the first week of the study was about half that consumed in the control groups. Food consumption in the high dose groups returned to normal levels by the 3rd week of the study.

Body weights showed dose-dependent decreases during the first week of the study. Both male and female dogs in the mid and high dose groups lost significant weight (up to 350 grams) during the first week of the study. However, weight gains returned to normal levels by the 3rd week of the study.

There were no apparent drug-related effects on hematology or coagulation parameters. However, albumin/globulin (A/G) ratios were statistically increased in the low dose females on day 93. Urinalysis did not reveal any drug-related effects.

No drug-related effects were reported on ECG, blood pressure, or respiratory rates.

There were no drug-related effects on gross necropsy or organ weights.

Microscopic examination did not report any drug-related findings, except for the dermal application sites. Dermal effects consisted of minimal to moderate acanthosis, minimal or mild acute or chronic dermatitis, minimal or mild parakeratosis, and minimal or mild chronic folliculitis. However, there were no apparent differences in either the incidences or severity of these microscopic changes between dogs receiving buprenorphine patches and those receiving placebo patches.

Toxicokinetic analysis showed no gender differences. Mean plasma concentrations are summarized in Table 12 (Sponsor's Table). As shown, there was wide variability in exposures between individual dogs: 0 to 0.23 mg/ml, 0.31 to 2.14 ng/ml, and 1.1 to 5.07 ng/ml for the 1.0, 3.5, and 12.0 mg/kg groups, respectively. Also, accumulation of buprenorphine was not demonstrated over time.

Table 12 (Sponsor's Table)

Summary of Mean Plasma Concentrations
in Dogs Treated with Buprenorphine Patches for 3 Months

Study Day	Male	Female	Combined M & F
	Mean±SD	Mean±SD	Mean±SD
10 mg Dose			
22	0.00	0.00	0.00
23	0.35±0.25	0.00	0.17±0.25
52	0.00	0.00	0.00
53	0.18±0.16	0.23±0.10	0.21±0.12
88	0.00	0.00	0.00
89	0.20±0.18	0.25±0.33	0.23±0.24
35 mg Dose			
22	0.29±0.19	0.33±0.14	0.31±0.15
23	0.72±0.68	0.81±0.53	0.77±0.55
52	0.31±0.12	0.36±0.06	0.34±0.09
53	0.58±0.35	0.84±0.36	0.71±0.35
88	0.48±0.20	0.44±0.35	0.46±0.26
89	2.26±0.54	2.02±1.40	2.14±0.96[a]
120 mg Dose			
22	0.72±0.39	1.48±0.37	1.10±0.54
23	4.07±2.26	3.77±2.77	3.92±2.27
52	1.82±0.57	1.37±0.41	1.60±0.51
53	3.67±0.84	5.26±2.68	4.46±1.98
88	1.84±0.14	1.62±0.27	1.73±0.23
89	4.99±1.17	5.14±2.75	5.07±1.89

N = 3/sex/dose

[a] Statistically significant from respective day 23 and 53 total mean value ($p<0.05$)

Note = Values that resulted in a mean concentration of <0.10 are considered to be below the LOQ.

Conclusions: Treatment of dogs with buprenorphine patches for up to 3 months resulted in some drug-related effects. These included low fecal output and reduced food consumption and body weights, effects known to occur with other opioids.

Dermal findings consisted of minimal edema, erythema, and red raised areas in all patch groups, including placebo. However, the incidences of edema and erythema were markedly increased in the high dose (12 mg/kg) females, but not in the high dose males. Microscopically, these findings consisted of minimal to moderate acanthosis, minimal or mild acute or chronic dermatitis, minimal or mild parakeratosis, and minimal or mild chronic folliculitis. No apparent

differences were noted in either the incidences or severity of these microscopic changes between dogs receiving buprenorphine patches and those receiving placebo patches.

Toxicokinetic analyses showed no gender-related differences or drug accumulation over time. Systemic exposure was generally increased with increasing dosage. However, there was wide variability in exposures between individual dogs.

2.2.2.5. A preliminary dose range finding dermal toxicity study in male Hanford minipigs with buprenorphine patches (+toxicokinetic report and analytical report) (DSE-210):

Testing Facility: (b) (4)
Study Number: DSE-210
Study Date(s): Sept. 28, 1996 to Oct. 11, 1996
GLP Compliance: No
QA Report: Yes

Purpose: This dose range-finding study examined the potential toxicity of buprenorphine patches when applied to the skin of Hanford minipigs for 14 days.

Methods: Male Hanford minipigs (7-12 kg; 1 minipig/group) had buprenorphine patches (5 mg patch lot no. = 7/00499/6; 10 mg patch lot no. = 7/00499/6A; 20 mg patch lot no. = 7/00499/6B) applied to the shaved dorsal trunk at 10, 25, 50, or 100 mg/minipig (= 0.5, 1.5, 3.0, or 5.6 mg/kg). Two sets of controls received either placebo patches (lot no. 7/00500/6B) or no patch. New patches were reapplied every 3 days.

Minipigs were observed daily for clinical signs. The application site on the skin was examined for signs of erythema, edema, desquamation, or irritation every 3 days when the patch was replaced. Body weights were taken weekly. ECGs and blood pressures were recorded predose and on Day 12. Blood was collected for measurement of plasma drug concentrations on Days 1, 4, and 7-13. On Day 16 all animals were sacrificed and a gross necropsy performed.

Results: Dermal findings consisted of slight well-defined erythema and edema in the 50 mg and 100 mg minipigs. No other findings were noted on body weights, clinical signs, ECGs, blood pressures, or presence of drug-related gross lesions.

Toxicokinetic data showed that steady-state plasma concentrations of buprenorphine were detectable only in the 100 mg minipig (5.6 mg/kg). Values ranged from 0.11 to 0.14 ng/ml on Days 4 to 13. The plasma drug concentrations in the lower dose groups were below the limit of detection.

Conclusions: Minimal toxic effects were found when buprenorphine patches were applied to the skin on minipigs at doses up to 5.6 mg/kg for 14 days, and consisted of only minimal dermal irritation in the 3.0 and 5.6 mg/kg minipigs. Plasma drug levels were above the limit of detection only in the high dose (5.6 mg/kg) minipig.

2.2.2.6. A 28-day dermal toxicity study in Hanford minipigs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-251):

Testing Facility: (b) (4)
Study Number: DSE-210
Study Date(s): May 21, 1997 to June 18, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity and toxicokinetics of single dose buprenorphine patches when applied to the skin of Hanford minipigs for 28 days using 7 day patching intervals.

Methods: Male Hanford minipigs (16.0-22.1 kg; 6/group) had about 6 to 10 twenty-mg buprenorphine patches (20 mg patch lot no. = 7/00499/6B) applied to the shaved dorsal trunk to equal a dose of 8 mg/kg (actual dose equaled 7.5-8.5 mg/kg). Patches were covered with bandage and secured with tape. No control groups were used in this study. New patches were reapplied every 7 days.

All minipigs were observed daily for clinical signs. Dermal application sites were examined and graded for dermal irritation when new fresh patches were reapplied every 7 days. Body weights were taken weekly.

On Day 29, animals were sacrificed and a gross necropsy performed that consisted of organ weight collection and microscopic examination of the dermal application sites as shown below.

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Also, blood was collected predose and on Days 2-7 and Days 16-21 for determination of plasma drug concentrations.

Results: No mortality or drug-related clinical signs were reported. Dermal effects consisted of slight erythema in 4 out of 6 minipigs and yellow staining in all animals. One minipig lost weight during Days 8-15, whereas the other 5 minipigs gained weight during the course of the study.

No gross changes or relative organ weights (brain weight to final body weight) were noted at necropsy. Microscopic examination of the dermal application sites reported minimal to mild parakeratosis and exudate, findings interpreted by the sponsor as expected in skin shaved and wrapped for 4 weeks.

Plasma drug concentrations were generally below the limit of detection (< 0.10 ng/ml). Only one minipig had a measurable level (0.20 ng/ml) during the last two weeks of the study that resulted in an $AUC_{0\text{-}144\ hr}$ of 18.7 ng/ml/hr.

Conclusions: Minimal toxic effects were found when buprenorphine patches were applied to the skin on minipigs at doses of 8.0 mg/kg (range of 7.5-8.5 mg/kg) for 28 days, and consisted of slight erythema and yellow staining. Microscopic findings of the application sites consisted of minimal to mild parakeratosis and exudate. Toxicokinetic analysis showed only one minipig out of six with a measurable level (0.20 ng/ml) during the last two weeks of the study with an $AUC_{0\text{-}144\ hr}$ of 18.7 ng/ml/hr.

2.2.2.7. A 3-month dermal toxicity study in Hanford minipigs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-211):

Testing Facility: (b) (4)
Study Number: DSE-211
Study Date(s): Oct. 21, 1996 to Jan. 22, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity and toxicokinetics of buprenorphine patches when applied to the skin of Hanford minipigs for 3 months.

Methods: Male and female Hanford minipigs (13.5-22.3 kg; 4/sex/group) had buprenorphine patches (5 mg patch lot no. = 7/00499/6; 10 mg patch lot no. = 7/00499/6A; 20 mg patch lot no. = 7/00499/6B) applied to the shaved dorsal trunk at 5, 10, or 20 mg/minipig (= 0.8, 4.0, or 8.0 mg/kg, respectively) according to the schedule in Table 13 (Sponsor's Table). Two sets of controls received either placebo patches (lot no. 7/00500/6B) or no patch. Patches were covered with bandage and secured with tape. New patches were reapplied every 3 days.

Table 13 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)*	Size of Patches Applied**	Actual Dosage Range (mg/kg)***	Average Actual Dosage (mg/kg)***
	Male	Female					
1	4	4	Control (Untreated)	0	0	0	0
2	4	4	Placebo patch	0	Placebo (20 mg size)	0	0
3	4	4	Buprenorphine	0.8	5 mg	0.70 - 0.91	0.8
4	4	4	Buprenorphine	4.0	10 mg	3.77 - 4.26	4.0
5	4	4	Buprenorphine	8.0	20 mg	7.58 - 8.48	8.0

*From the protocol.
**The number of patches was varied based on sex-group mean body weight and rounded to the nearest whole number. The number of patches applied to each sex-group during the study is included in Appendix C of this report.
***Actual level based upon total mg applied divided by the sex-group mean body weight.

All animals were observed daily for clinical signs. Dermal application sites were examined and graded for dermal irritation when new fresh patches were reapplied every 3 days. Body weights were taken weekly. Blood was taken predose and once a month for clinical pathology measurements (hematology, coagulation, and biochemistry). Blood was also taken throughout the study for measurement of plasma drug concentrations. Ophthalmological exams were performed predose and on Day 79. Six lead ECGs and indirect blood pressure measurements were taken generally just after patch removal at predose and several times throughout the study.

On Days 92-94, all minipigs were sacrificed and a necropsy performed that consisted of an examination of external body surfaces and all viscera. Dermal application sites were collected for gross and microscopic examination as shown below.

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Several organs were weighed, and 41 tissues from all animals were fixed and examined microscopically.

Results: No deaths were reported. Clinical signs consisted of decreased acitivy in the high dose (8 mg/kg) group, and was attributed to the known pharmacological effect of opioids. Gross dermal findings consisted of slight to severe erythema, yellowing of the skin, slight to moderate edema, mild blanching, focal/pinpoint to severe eschar, and yellow pustules. It was difficult to distinguish the dermal effects between the placebo and buprenorphine patch groups, and therefore, it was not possible to attribute these gross dermal findings to exposure to buprenorphine and not to the patching precedure per se.

Overall body weight gains in the high-dose (8 mg/kg) groups were 32% lower than the no-patch controls and 21-23% lower than the placebo patch controls. Final body weights in the high-dose (8 mg/kg) animals were 7-13% lower than either control group. There were no differences in body weights between the lower dose (0.8 and 4.0 mg/kg) groups and controls.

Clinical chemistry changes were noted but the results were difficult to interpret due to the lack of a dose-response relationship. Also, statistically significant differences were noted, but these values were within historical limits for these values.

Results of ophthalmology and cardiology tests were neagtive. Gross necropsy did not report any findings. Organ weight measurements found some changes, such as increased thyroid weights in drug-treated animals, but there was no dose-relationship.

Microscopic examinations did not report any drug-related findings other than those observed in the skin. The dermal findings consisted of minimal to mild parakeratosis, minimal to moderate microabscessation of the startum corneum of the epidermis, and minimal to mild chronic dermatitis. However, these dermal findings occurred with equal incidence and severity in both placebo-patch and buprenorphine-patch groups, indicating that they were probabaly due to the patching procedure rather than an effect of buprenorphine.

Toxicokinetic data did not show a gender effect. Plasma levels of buprenorphine were not detectable until around Day 22. Steady-state concentrations are summarized in Table 14 and Figure 1 (Sponsor's Figure 8.3). As shown, values ranged from BLD (below limit of detection) for the low-dose (0.8 mg/kg) group, 0.11-0.24 ng/ml for the mid-dose (4 mg/kg) group, and 0.18-0.38 ng/ml for the high-dose (8 mg/kg) group.

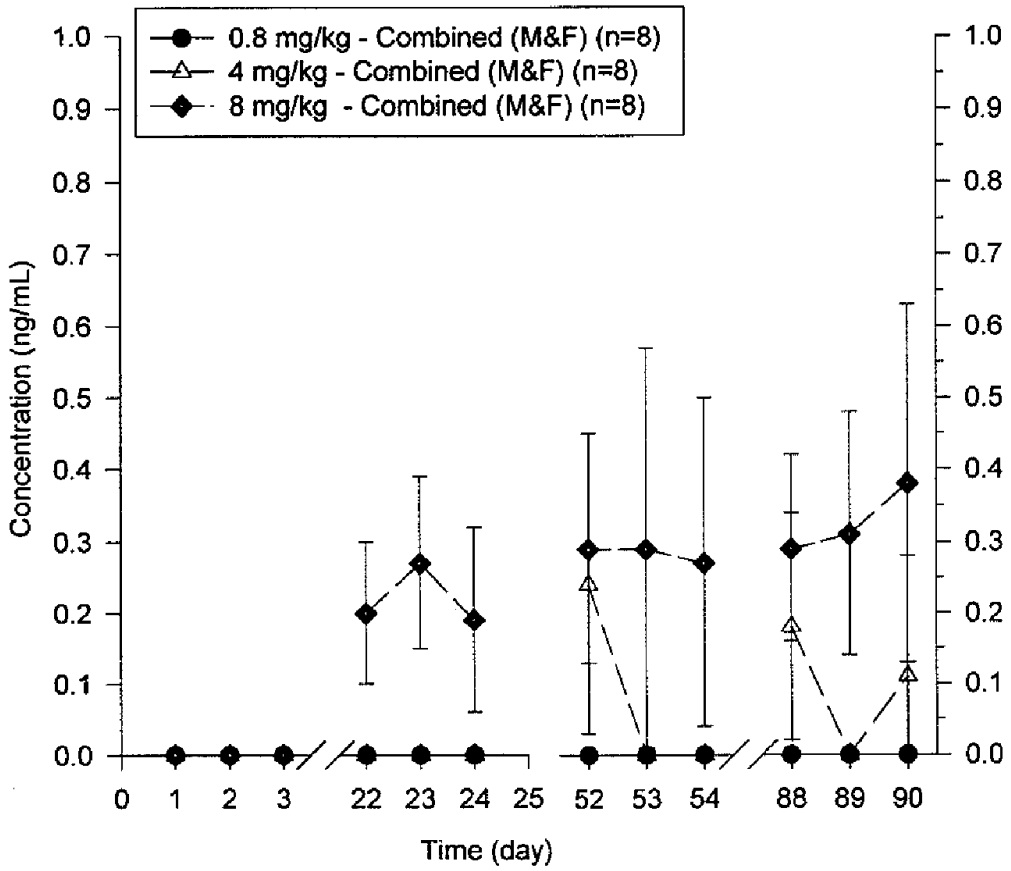
Table 14

Mean Steady-State Plasma Buprenorphine Concentrations
In Minipigs Given Buprenorphine Patches Every 3 Days for 3 Months

Dose (mg/kg)	**Range of Steady-State Plasma Drug Conc. (ng/ml)**
0.8	BLD
4.0	0.11-0.24
8.0	0.18-0.38

Figure 1 (Sponsor's Figure 8.3)

Mean (±SD) Plasma Concentrations (ng/mL) of Buprenorphine in Male + Female Hanford Minipigs Administered a 0.8, 4 and 8 mg/kg Dose of Buprenorphine by Dermal Application of Patches Once Every Three Days for 3 Months



Conclusions: Clinical signs consisting of decreased activity were noted, but these effects are known to occur with exposure to opioids. When compared to no-patch and placebo controls, body weight gains and final body weights were decreased 20-30% in the high-dose (8 mg/kg) minipigs. No body weight decreases were found in the lower dose groups.

Clinical chemistry measurements were sporadic and were not able to determine a dose-related effect. The same was true for organ weights.

Gross dermal findings consisted of slight to severe erythema, yellowing of the skin, slight to moderate edema, mild blanching, focal/pinpoint to severe eschar, and yellow pustules. Microscopically, these were defined as minimal to mild parakeratosis, minimal to moderate microabscessation of the startum corneum of the epidermis, and minimal to mild chronic dermatitis. However, the incidence and severity of these findings were similar between the placebo-patch and buprenorphine patch groups, indicating that they were probabaly due to the patching procedure rather than an effect of buprenorphine.

Plasma drug levels generally showed a dose-response relationship, but there was a rather wide variability between the animals.

2.2.3. *Chronic Toxicity:*

2.2.3.1. A 6-month dermal toxicity study in rabbits with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-304):

Testing Facility: (b) (4)
Study Number: DSE-304
Study Date(s): March 30, 1998 to Sept. 28, 1998
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity and toxicokinetics of buprenorphine patches when applied to the skin of New Zealand White rabbits for 6 months.

Methods: Male and female New Zealand White rabbits (2.2-2.7 kg; 6/sex/group) had buprenorphine patches (5 mg patch lot no. = 7/01508/6A; 10 mg patch lot no. = 7/01508/6B; 20 mg patch lot no. = 7/10508/6C) applied to the dorsal trunk area at doses of 2.5, 15, or 30 mg/kg according to the schedule below (Table 15; Sponsor's Table). Two groups of controls received either no patch or placebo patches.

Table 15 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)[1]	Dosage Level (mg/rabbit)	Actual Dosage Range (mg/kg)[2]
	Male	Female				
1	6	6	Control (collar)	0	0	0
2	6	6	Placebo Patch	0	0	0
3	6	6	Buprenorphine Patch	2.5	5.0	1.3 - 2.9
4	6	6	Buprenorphine Patch	15.0	30.0	8.1 - 12.9
5	6	6	Buprenorphine Patch	30.0	60.0	17.1 - 25.3

[1]From the protocol.
[2]Based on the total mg applied divided by the sex-group mean body weight. Based on practical consideration of patch retention for the 20 mg size patch, a maximum of four patches of each size was used.

The adhesive normally used for human use was removed before application to the skin of rabbits, and the patches were held in place with wrapping and jackets. Patches were replaced every 3 days.

Rabbits were observed daily for clinical signs. Dermal application sites were observed and graded for signs of irritation each time the patch was replaced. Food consumption was recorded daily, and body weights were recorded weekly. Blood was collected predose and at Days 25, 86, and 183 for clinical chemistry analysis (hematology, coagulation, and biochemistry). Ophthalmoscopic exams were performed predose and at study termination. Blood was also collected for toxicokinetic analysis on Days 2, 23, 89, 170, 171, and 172. On Day 136, blood was collected for toxicokinetics at several time points for up to 72 hours after patch replacement.

At study termination (Days 183-185) rabbits were euthanized and a necropsy performed that consisted of a gross exam of external surfaces and collection of the treated skin sites from the following areas:

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Several organ weights were collected, and 41 tissues from all animals were fixed, stained and examined microscopically.

Results: No deaths were reported. The only clinical signs that were drug-related consisted of reduced feces in the high dose (30 mg/kg) groups. All other clinical observations were observed to be of equal frequency between the groups.

Dermal observations included an increased frequency of dermal irritation in the buprenorphine-treated groups when compared to placebo-patch controls. These included slight to well-defined erythema, slight edema, and desquamation.

Body weights in all the patch groups, including placebo patches, were 10-20% less than the no-patch controls, indicating an effect of the patching procedure on body weight reduction. However, body weights in the high-dose (30 mg/kg) group were 10% lower than the placebo-patch controls, indicating a drug-related effect at the high dose. Similarly, food consumption was also decreased in all patch groups when compared to no-patch controls, and food consumption in the mid and high dose groups was decreased when compared to placebo-patch controls.

Clinical pathology results did not disclose any consistent drug-related findings. Although there were some statistically significant effects, the changes were neither time nor dose-dependent. No drug-related effects were noted in ophthalmoscopy exams, gross necropsy observations, or organ weights.

Microscopic examination of non-dermal tissues did not show any drug-related findings. In samples from the drug-treated skin sites, there was a minimal increase in the thickness of the overlying squamous epithelium in drug-treated rabbits. Other dermal effects were found in similar frequency and severity between the drug-treated and placebo-patch groups.

Plasma drug levels 24 hours after application of the first patch were generally low. Toxicokinetic data from Day 136 are summarized in Table 16 (Sponsor's Table 7.7). As shown, the elimination half-life was 17-18 hours at the mid dose (15 mg/kg) and 39-40 hours at the high dose (30 mg/kg). Plasma buprenorphine levels generally increased with increasing dose, but showed wide variability. Drug levels were markedly higher in female rabbits than in male rabbits.

Table 16 (Sponsor's Table 7.7)

Mean (±SD) Toxicokinetic Metrics of Buprenorphine In Rabbits Following Dermal Application of Patches on Day 136 of a 6 Month Study

Dose (mg)	Gender	C_{max} (ng/mL)	t_{max} (hour)	AUC_{0-72} (ng•hr/mL)	AUC/Dose	t½ (hour)
5	Male	0.356 ±0.274	36.0 ±26.8	11.8 ±9.93	4.72 ±3.97	ND
	Female	0.738 ±0.276	39.0 ±11.5	34.0[a] ±14.7	13.6[b] ±5.89	ND
30	Male	0.687 ±0.185	13.3 ±14.2	30.0 ±9.68	2.00 ±0.645	16.8 (N=1)
	Female	1.19 ±0.620	26.0 ±20.7	53.4 ±33.3	3.56 ±2.22	18.2 (N=1)
60	Male	1.18 ±1.24	26.0 ±15.9	59.1 ±68.7	1.97 ±2.29	40.2 (N=2)
	Female	3.70 ±1.76	36.0 ±10.7	187[a] ±82.9	6.23 ±2.76	39.0 (N=2)

ND = Not determined

N = 4 – 6 rabbits/sex/dose group, unless otherwise noted.

[a] Significantly higher than corresponding value in males

[b] Significantly higher than AUC/dose values in females at both higher doses

Conclusions: Minimal toxic effects were observed in this study. There were slight dermal effects in drug-treated rabbits that were defined grossly as slight to well-defined erythema, slight edema, and desquamation. Microscopically, dermal effects included a minimal increase in the thickness of the overlying squamous epithelium in drug-treated rabbits.

Food consumption and body weights decreased as much from the patching procedure as from exposure to buprenorphine. No other toxic effects that could be attributed to drug treatment were noted.

Plasma drug exposures generally increased with increasing dose, but showed wide variability. Drug levels were markedly higher in female rabbits than in male rabbits.

2.2.3.2. A 6-month dermal toxicity study in dogs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-214):

Testing Facility: (b) (4)
Study Number: DSE-214
Study Date(s): April 7, 1997 to Oct. 10, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity and toxicokinetics of buprenorphine patches when applied to the skin of Beagle dogs for 6 months.

Methods: Male and female Beagle dogs (5.9-9.6 kg; 5/sex/group) had buprenorphine patches (5 mg patch lot no. = 7/00499/6; 10 mg patch no. 7/00499/6A; 20 mg patch lot no. = 7/00499/6B) applied to the dorsal trunk area at doses of 1, 3.5, or 12 mg/kg according to the schedule below (Table 17; Sponsor's Table). The actual ranges were 1.1-1.4, 3.7-6.3, and 12.6-18.3 mg/kg for the low, mid, and high doses, receptively. Two groups of controls received either no patch or placebo patches. Patches were trimmed free of adhesive, and were replaced every 3 days. The high dose was stated to represent the maximum number of patches that could be applied to this species.

Table 17 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)[1]	Dosage Level (mg/dog)	Type of Patches Applied	Actual Dosage Range (mg/kg)[2]
	Male	Female					
1	5	5	Untreated Control	0	0	0	0
2	5	5	Placebo Patch	0	0	6 x Placebo 20 mg size	0
3	5	5	Buprenorphine Patch	1	10	1 x 10 mg	1.1 -1.4
4	5	5	Buprenorphine Patch	3.5	35	1 x 5 mg 1 x 10 mg 1 x 20 mg	3.7 - 5.3
5	5	5	Buprenorphine Patch	12	120	6 x 20 mg	12.6 - 18.3

[1]From the protocol.
[2]Based on the total mg applied divided by the sex-group mean body weight.

Dogs were observed daily, and a detailed clinical exam was performed each month. Dermal application sites were examined and graded for signs of irritation when the patch was replaced every 3 days. Body weights were recorded weekly. Blood was collected predose and on Days 25, 87 and on days of sacrifice (Days 185-187) for clinical pathology measurements

(hematology, coagulation, and biochemistry). Urinalysis was conducted on samples obtained predose and at sacrifice. Blood was also collected for toxicokinetic analysis on several days throughout the study. Ophthalmoscopic exams were performed predose and near study conclusion (Day 183). Six lead ECGs and indirect blood pressure measurements were taken predose and on Days 1, 2, 4, 22, 88, and 179.

On Days 185-187, dogs were sacrificed and a necropsy performed that consisted of gross exam of external surfaces and viscera, and collection of treated skin sites from the following areas:

Shoulders

1L	1R
2L	2R
3L	3R

Hips

Weights of selected organs were taken, and 41 tissues from all animals were collected, fixed and examined microscopically.

Results: No deaths were reported. The only clinical sign reported was an increased incidence of no feces in the mid and high dose groups, an effect known to occur with exposure to opioids. Gross dermal findings were similar in all groups, including placebo-patch controls, and included slight to well-defined erythema, slight edema, desquamation, red raised areas, and a low incidence of moderate to severe erythema and slight to moderate edema. Because of the lack of a consistent dose-related effect and similar incidence between drug and placebo controls, it was difficult to attribute these gross dermal findings to exposure to buprenorphine, and not to the patching precedure itself.

Body weights did not show statistically significant differences between the groups. There was not more than a 10% difference between the groups. Food consumption was reduced in both the buprenorphine-treated groups and the placebo-patch controls when compared to the no-patch controls, indicating an effect of the patching procedure. However, the drug-treated groups showed a greater decrease than the placebo-patch groups, indicating a drug-related effect.

Clinical pathology (hematology, coagulation, and biochemistry) and urinalysis did not show a consistent dose-related or drug-induced effect. Most of the values were within historical limits. The same was true for absolute and relative organ weight values.

Microscopic examination did not find any effects that could be attributed to drug exposure. In the skin, the findings of squamous epithelial hyperplasia and hyperkeratosis were similar in both the buprenorphine-treated groups and placebo-patch controls, indicating an effect to the patch rather than to the drug.

Toxicokinetic analysis did not show a gender effect. Mean plasma concentrations are shown in Table 18 (Sponsor's Table 7.2). There appeared to be increased absorption and expsoure with increasing dosage. In general, levels at the end of the study were lower than those earlier in the study.

Table 18 (Sponsor's Table 7.2)

Mean (±SD) Plasma Concentrations Of Buprenorphine In Dogs Following Dermal Application Of Patches[a] For 6 Months

Group	Dose (mg)	Days	Concentration (ng/mL)[b]		
			Males	Females	Combined[c]
3	10	3	0.28 ± 0.21	0.12 ± 0.27	0.20 ± 0.24[d]
3	10	24	0.12 ± 0.14	0.00 ± 0.00	0.11 ± 0.12[d]
3	10	90	0.00 ± 0.00	0.15 ± 0.17	0.10 ± 0.13[d]
3	10	180	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	35	3	0.86 ± 0.76	0.88 ± 0.60	0.87 ± 0.65
4	35	24	0.63 ± 0.91	0.69 ± 0.51	0.66 ± 0.69
4	35	90	0.31 ± 0.21	0.54 ± 0.49	0.42 ± 0.37
4	35	180	0.32 ± 0.22	0.46 ± 0.21	0.39 ± 0.22
5	120	3	4.44 ± 1.28	4.06 ± 2.99	4.25 ± 2.18
5	120	24	2.14 ± 0.99	3.43 ± 2.09	2.78 ± 1.68
5	120	90	1.36 ± 0.74	1.56 ± 1.08	1.46 ± 0.88[e,f]
5	120	180	1.34 ± 0.52	1.29 ± 1.72	1.31 ± 1.20[e,f]

N = 5/sex/group

a = Patches were applied once every three days (days 0, 4, 7, 10 etc throughout the study)

b = Blood samples were taken one day prior to application of patches.

c = Represents combined values from male and female dogs (N = 10/group)

d = Significantly higher than value from the corresponding dogs on day 180 ($p \leq 0.05$).

e = Significantly lower than value from the corresponding dogs on day 3 ($p \leq 0.05$).

f = Significantly lower than value from the corresponding dogs on day 24 ($p \leq 0.05$).

LOQ = 0.10 ng/mL; any value below the LOQ was considered to be zero.

Conclusions: Application of buprenorphine patches to the skin of Beagle dogs for 6 months resulted in minimal toxicity. Most of the noted effects were attributed to the patching procedure rather than to the drug itself. Systemic exposures showed increased absorption and exposure with increasing dosage, but were limited by the maximum number of patches that could be applied to the skin of dogs.

2.2.3.3. A 6-month dermal toxicity study in Hanford minipigs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-212):

Testing Facility: (b) (4)
Study Number: DSE-212
Study Date(s): March 4, 1997 to Sept. 5, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential toxicity and toxicokinetics of buprenorphine patches when applied to the skin of Hanford minipigs for 6 months.

Methods: Male and female Hanford minipigs (6.6-16.1 kg; 5/sex/group) had buprenorphine patches (5 mg patch lot no. = 7/00499/6; 10 mg patch no. 7/00499/6A; 20 mg patch lot no. = 7/00499/6B) applied to the dorsal trunk area at doses of 0.8, 4.0, or 8.0 mg/kg according to the schedule below (Table 19; Sponsor's Table). The actual ranges were 0.68-0.94, 3.68-4.27, and 5.00-8.42 mg/kg for the low, mid, and high doses, receptively. Two groups of controls received either no patch or placebo patches. Patches were replaced every 3 days. The high dose was stated to represent the maximum number of patches that could be applied due to lack of practical dosing area.

Table 19 (Sponsor's Table)

Dosing Schedule

Group	No. of Animals		Dosage Material	Nominal Dosage Level (mg/kg)*	Type of Patches Applied**	Actual Dosage Range (mg/kg)***	Average Actual Dosage (mg/kg)***
	Male	Female					
1	5	5	Control (Untreated)	0	0	0	0
2	5	5	Placebo patch	0	Placebo (20 mg size)	0	0
3	5	5	Buprenorphine	0.8	5 mg	0.68 - 0.94	0.8
4	5	5	Buprenorphine	4.0	10 mg	3.68 - 4.27	4.0
5	5	5	Buprenorphine	8.0	20 mg	5.00 - 8.42	7.0

*From the protocol.
**The number of patches was varied based on sex-group mean body weight and rounded to the nearest whole number.
***Actual level based on total mg applied divided by the sex-group mean body weight.

Animals were observed daily for clinical signs. Dermal application sites were examined and graded for signs of irritation when the patch was replaced every 3 days. Body weights were

recorded weekly. Blood was collected predose and on Days 24, 87, and 187 for clinical pathology parameters (hematology, coagulation, and biochemistry). Six lead ECGs and indirect blood pressure measurements were recorded predose, and on Days 1, 2, 22, 85, and 178. Blood was also collected for toxicokinetic analysis on Days 3, 24, 87, and 177.

On Days 183-186, all animals were sacrificed and a necropsy performed that consisted of a gross exam of all external surfaces and all viscera. The drug-treated dermal sites were divided into six areas and collected. Weights of several organs were taken, and 41 tissues from all animals were fixed, stained with H&E, and examined microscopically.

Results: No drug-related deaths were noted. However, one female in the 4 mg/kg group and one female in the 8 mg/kg group were euthanized due to a prolapsed rectum, a finding that was considered to be a spontaneous low-frequency occurrence at the animal facility. One additional female in the 4 mg/kg group was euthanized due to dehydration and subsequent development of salt toxicity after it was discovered that the water line was detached. Treatment with lactated Ringer's solution s.c. for 5 days was unsuccessful.

The only drug-related clinical signs noted were decreased activity in several minipigs in the mid and high dose groups. This finding was considered to be a known effect of opioid treatment.

Dermal findings are summarized in Table 20A for the males (Sponsor's Table 2) and Table 20B for the females (Sponsor's Table 2). Grossly, the treated areas showed slight erythema, yellow staining, and well-defined to moderate to severe erythema, very slight edema, and focal/pinpoint to mild eshar, and focal/pinpoint to mild blanching. Two minipigs in the high dose group had severe dermal irritation consisting of eschar on up to 10% of the dermal test site and blanching on 10-25% of the test site. They were not dosed on Days 55 and 58. Overall, dermal findings did not exhibit a clear dose-response relationship, and many of the dermal findings were found to some extent in the placebo-patch groups as well.

Table 20A (Sponsor's Table 2)

Dermal Findings in Male Minipigs Treated with Buprenorphine Patches for 6 Months

----- M A L E -----

GROUP: LEVEL:	1 UNTREATED CONTROL	2 PLACEBO CONTROL	3 0.8 MG/KG BUP	4 4.0 MG/KG BUP	5 8.0 MG/KG BUP
EXPOSURE SITE					
-ERYTHEMA - GRADE 0	310/ 5	183/ 5	235/ 5	149/ 5	96/ 5
-EDEMA - GRADE 0	310/ 5	308/ 5	290/ 5	295/ 5	294/ 5
-ERYTHEMA - GRADE 1	0/ 0	128/ 5	49/ 5	137/ 5	168/ 5
-ERYTHEMA - GRADE 2	0/ 0	0/ 0	25/ 2	20/ 4	33/ 4
-ERYTHEMA - GRADE 3	0/ 0	0/ 0	0/ 0	4/ 2	6/ 1
-MAXIMIZED - GRADE 4	0/ 0	0/ 0	0/ 0	0/ 0	5/ 1
-EDEMA - GRADE 1	0/ 0	0/ 0	19/ 1	14/ 3	13/ 1
-ESCHAR - GRADE 1	0/ 0	0/ 0	3/ 1	1/ 1	20/ 1
-ESCHAR - GRADE 2	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
-ESCHAR - GRADE 4	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1
-BLANCHING - GRADE 1	0/ 0	0/ 0	6/ 1	3/ 3	2/ 1
-BLANCHING - GRADE 2	0/ 0	0/ 0	0/ 0	0/ 0	4/ 1
-TEST SITE STAINING YELLOW	0/ 0	244/ 5	220/ 5	267/ 5	298/ 5

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Table 20B (Sponsor's Table 2)

Dermal Findings in Female Minipigs Treated with Buprenorphine Patches for 6 Months

----- F E M A L E -----

GROUP: LEVEL:	1 UNTREATED CONTROL	2 PLACEBO CONTROL	3 0.8 MG/KG BUP	4 4.0 MG/KG BUP	5 8.0 MG/KG BUP
EXPOSURE SITE					
-ERYTHEMA - GRADE 0	309/ 5	234/ 5	204/ 5	165/ 5	152/ 5
-EDEMA - GRADE 0	309/ 5	305/ 5	305/ 5	269/ 5	261/ 5
-ERYTHEMA - GRADE 1	0/ 0	51/ 5	75/ 5	82/ 5	99/ 5
-ERYTHEMA - GRADE 2	0/ 0	22/ 1	28/ 4	26/ 4	10/ 4
-ERYTHEMA - GRADE 3	0/ 0	3/ 1	2/ 1	0/ 0	4/ 1
-ERYTHEMA - GRADE 4	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
-MAXIMIZED - GRADE 4	0/ 0	0/ 0	0/ 0	2/ 1	5/ 1
-EDEMA - GRADE 1	0/ 0	4/ 1	4/ 3	3/ 1	9/ 1
-ESCHAR - GRADE 1	0/ 0	2/ 1	1/ 1	10/ 2	10/ 1
-ESCHAR - GRADE 2	0/ 0	0/ 0	0/ 0	1/ 1	1/ 1
-BLANCHING - GRADE 1	0/ 0	2/ 2	5/ 2	3/ 2	1/ 1
-BLANCHING - GRADE 2	0/ 0	0/ 0	0/ 0	1/ 1	5/ 1
-TEST SITE STAINING YELLOW	0/ 0	239/ 5	233/ 5	240/ 5	249/ 5

NOTE: DATA REFLECT THE TOTAL OCCURRENCE OF EACH DERMAL FINDING OVER THE NUMBER OF ANIMALS EXHIBITING THE FINDING.

Overall mean body weights in high dose (8 mg/kg) males were decreased by 29% when compared to no-patch controls. However, body weights in the placebo-patch controls were decreased by 24% when compared to no-patch controls, indicating an effect of the patching procedure rather than from the drug itself. Similar body weight changes (15% decrease) were found in the high dose females when compared to no-patch controls, and body weights in the placebo-patch female controls were decreased by 11% when compared to no-patch controls, again indicating an effect of the patching procedure rather than from the drug itself. Body weights in the lower dose groups (0.8 or 4.0 mg/kg) were not significantly different from either of the control groups.

Clinical pathology tests (hematology, coagulation, and biochemistry) did not show any dose-related alterations that could be attributed to drug treatment. Most of the changes were either within historical limits or were not consistent with regards to dose.

Other than the gross findings of prolapsed rectum and signs of dehydration mentioned above for the three euthanized minipigs, no other gross findings that could be attributed to drug treatment were reported.

Changes in organ weights were reported, but these changes could not be attributed to drug treatment since similar changes were found in placebo-patch controls.

Microscopic examination of various tissues did not report any drug-induced changes except in the treated dermal sites. Changes seen in the dermally exposed sites consisted of mild parakeratosis and moderate micropustule formation. These changes were found in skin from buprenorphine-treated minipigs as well as from placebo-patch controls, indicating an effect of the patching procedure and not from the drug itself.

Toxicokinetic analysis showed that mean plasma drug concentrations were below the limit of detection in the low dose groups (0.8 mg/kg), but were detectable with wide variability in the mid and high dose groups (Table 21; Sponsor's Table 7.2). No gender differences were apparent. Combined mean plasma drug levels at Week 26 were 0.11±0.15, 0.51±0.32, and 0.75±0.61 ng/ml for the low, mid and high dose groups, respectively.

Table 21 (Sponsor's Table 7.2)

Mean[a] (±SD) Plasma Concentrations (ng/mL) of Buprenorphine in Hanford Minipigs Administered a 0.8, 4 and 8 mg/kg Dose of Buprenorphine by Dermal Application of Patches[b] Once Every Three Days for 6 Months

Dose Given (mg/kg)	Sampling Day[b]	Male Mean±SD	Female Mean±SD	Combined M & F[c] Mean±SD
0.8	Day 3	0.00	0.00	0.00
	Week 4	0.00	0.66±1.48	0.33±0.10
	Week 13	0.00	0.00	0.00
	Week 26	0.12±0.18	<0.10	0.11±0.15
4	Day 3	0.11±0.11	0.00	0.00
	Week 4	0.16±0.19	0.26±0.22	0.21±0.20
	Week 13	0.00	0.13±0.23	0.00
	Week 26	0.46±0.40	0.59±0.14[d]	0.51±0.32[d]
8	Day 3	0.12±0.12	0.11±0.12	0.11±0.11
	Week 4	0.24±0.21	0.26±0.16	0.25±0.17
	Week 13	0.18±0.15	0.11±0.15	0.15±0.15
	Week 26	0.84±0.81	0.64±0.34[d]	0.75±0.61[d]

[a] Mean ± standard deviation from 5 sex/group

[b] Blood samples were taken one day prior to application of patches.

[c] Mean ± standard deviation from the combined total of male + female minipigs

[d] Significantly higher than corresponding values on day 3 and week 13

LOQ = 0.10 ng/mL

Conclusions: Application of buprenorphine patches to the skin of Hanford minipigs for 6 months resulted in minimal toxicity. Most of the noted effects, such as changes in body weights and at the dermal test sites, were attributed to the patching procedure rather than to the drug itself. Systemic exposures generally showed increased absorption and exposure with increasing dosage, but also showed high variability.

2.2.4. Genetic Toxicity:

2.2.4.1. Mutagenicity test with buprenorphine hydrochloride in the S*almonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay (DSE-232):

Testing Facility: (b) (4)
Study Number: DSE-232
Study Date(s): May 14, 1997 to July 13, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This test examined the ability of buprenorphine and/or its metabolites to induce reverse mutations in the histidine locus in *Salmonella typhimurium* bacterial strains and at the tryptophan locus in *E. coli* with and without metabolic activation. This assay detects reverse mutations in the test strains that restore the functional capability of the bacteria to synthesize the essential amino acid histidine. The revertant or mutated bacteria are detected by their ability to grow in the absence of histidine required by the parent test strain.

Methods: The following bacterial tester strains were used: *S. thyphimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* strain WP2*uvr*A.

A preliminary dose range-finding study was used to determine the doses for the mutagenicity test. Tester strains *S. typhimurium* TA100 and *E. coli* WP2*uvr*A were incubated with buprenorphine at concentrations from 6.67 µg/plate to 5 mg/plate in molten top agar (45^0 C) with or without S9 (± metabolic activation), then overlaid onto agar and incubated at 37^0 C for 48 hours. Control cultures were treated with either vehicle (DMSO) or appropriate positive control. Cytotoxicity was determined by evaluation of the bacterial lawn when compared to vehicle-treated cultures. Results showed significant cytotoxicity at ≥333 µg/plate with S9 and at ≥66.7 µg/plate without S9.

Once the dose range was determined, a mutagencity test was conducted with the procedure similar to the cytotoxicity test, except that the number of revertant colonies in the plates after incubation were counted either manually for the buprenorphine-treated plates or by an automated colony counter for the positive control-treated plates. A positive response was defined as a 2-fold increase over vehicle controls in mean number of revertants per plate for tester strains TA98, TA100, and WP2*uvr*A, and a 3-fold increase over controls for strains TA1535 and TA1537. The mutagenicity test was repeated in an confirmatory assay.

Results: In the initial mutagenicity test, dose ranges for the *S. thyphimurium* strains were 3.33-1000 µg/plate with S9 and 1.3-333 µg/plate without S9. No increase in the number of mean revertants per plate was found when compared to vehicle controls. The positive controls showed the expected responses. Similarly, the dose ranges used for the initial *E. coli* assay were 10-3330 µg/plate with S9 and 3.33-1000 µg/plate without S9. Again, no increase in the number of mean revertants per plate was found when compared to vehicle controls.

In the confirmatory repeat assay, doses were slightly increased in the cultures without S9 to 3.33-1000 µg/plate for the *S. thyphimurium* strains and 10-3330 µg/plate for the *E. coli* cultures. Concentrations of buprenorphine with S9 remained the same (3.33-1000 µg/plate for *S.*

thyphimurium strains and 10-3330 µg/plate for the *E. coli* strain). Again, no increase in the number of mean revertants per plate was found when compared to vehicle controls.

Conclusions: Buprenorphine was not found to be mutagenic in the bacterial reverse mutation (Ames) assay when tested at concentrations that produced significant cytotoxicity.

2.2.4.2. L5178Y TK +/- Mouse lymphoma forward mutation assay with buprenorphine Hydrochloride with a confirmatory assay (DSE-233):

Testing Facility: (b) (4)
Study Number: DSE-233
Study Date(s): June 3, 1997 to July 31, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This assay evaluated the ability of buprenorphine HCl to induce forward mutations at the thymidine kinase (TK) locus in TK+/- mouse lymphoma cells either in the presence or absence of metabolic activation (±S9). Cells deficient in TK due to the forward mutation TK+/- to TK-/- are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). Thymidine kinase proficient cells (TK+/-) are sensitive to TFT, which causes the inhibition of cellular metabolism and halts further cell division. Thus, mutant cells (TK-/-) are able to proliferate in the presence of TFT, whereas normal heterozygous cells (TK+/-), which contain thymidine kinase, are not.

Methods: A preliminary cytotoxicity assay was conducted to determine the dose range for the mutagenicity assay. Mouse lymphoma L5178Y cells that were heterozygous at the thymidine kinase locus ($TK^{+/-}$) were treated with 1.97-1000 µg/ml buprenorphine with or without metabolic activation (±S9) for 4 hours at 37 C. Control cultures were treated with either vehicle (DMSO) or appropriate positive control. Cells were washed free of drug and incubated overnight. Surviving cells were counted (method of determining cell viability was not mentioned). Results showed >90% cytotoxicity when compared to vehicle-treated cultures at concentrations ≥31.3 µg/ml for both the +S9 and -S9 cultures.

Once the dose range was determined from the preliminary cytotoxicity assay, two mutation assays was conducted. Concentrations of buprenorphine HCl tested were 10-35 µg/ml without S9, and 7.5-30 µg/ml with S9. Control cultures were treated with either vehicle (DMSO) or appropriate positive control. Cells were treated for 4 hours, washed, then incubated overnight. Cells counts were made and cloning efficiency was determined by seeding the cells in soft agar, followed by incubation for 10-14 days. Surviving colonies were counted using a colony counter. Both large and small colonies were counted (large colonies represent cells with forward mutations at the $TK^{+/-}$ locus, while small colonies represent cells with larger deletions of genetic material in and around the $TK^{+/-}$ locus and can be used as an index of clastogenicity). A significant response was defined as a 2-fold increase in mutant frequency above the vehicle control. According to ICH guidelines, cells should be exposed to drug concentrations that produce levels of cytotoxicity up to 80%.

Results: There were no significant increases in mutant frequencies, when compared to vehicle control cultures, at drug concentrations that produced up to 80% cytotoxicity in either of the two mutagenicity assay when conducted either in the presence or absence of metabolic activation. The highest concentrations tested produced significant (70-88%) cytotoxicity. The positive controls showed the expected responses.

However, there were technical difficulties associated with colony sizing for small and large colonies.

Conclusions: Buprenorphine was not found to be mutagenic in the mouse lymphoma L5178Y cell assay when tested at concentrations that produced up to 80% cytotoxicity. Clastogenic potential of buprenorphine (induction of small colonies) was not determined due to technical difficulties.

2.2.4.3. Mutagenicity test on buprenorphine hydrochloride in a chromosome aberrations study in human whole blood lymphocytes with a confirmatory assay with multiple harvests (DSE-234):

Testing Facility: (b) (4)
Study Number: DSE-234
Study Date(s): May 22, 1997 to Aug. 4, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the ability of buprenorphine to induce chromosomal aberrations (clastogenic potential) in cultured human whole blood lymphocytes either in the presence or absence of metabolic activation (±S9).

Methods: In the initial dose rang-finding study, human peripheral blood lymphocytes were cultured in the presence of 1% phytohemagglutinin (PHA) and then treated with buprenorphine dissolved in DMSO at concentrations ranging from 0.01 to 35 µg/ml for 24 hours, at which time Colcemid was added to arrest cells in metaphase. Cells were then stained with Giemsa stain and chromosomes evaluated for number of mitotic cells per 1000 cells (mitotic index) as an measure of cell viability/cytotoxicity. Results are summarized in Table 22. As shown, there was extensive (≥90%) cytotoxicity at buprenorphine concentrations of ≥117 µg/ml in the absence of metabolic activation (-S9), and at 350 µg/ml in the presence of metabolic activation (+S9).

Table 22

Results of Range-Finding Assay for Cytotoxicity
(Percent Reduction in Mitotic Index Compared to Solvent Controls)

Buprenorphine Conc. (µg/ml)	S9 (±)	% Reduction in Mitotic Index
1.17	Absent (-S9)	43
3.5		18
35.0		39
117		100
350		100
11.7	Present (+S9)	29
35.0		40
117		75
350		90

Based on these results, buprenorphine concentrations were selected for the clastogencity assay of 5-60 µg/ml in the absence of activation (-S9), and 15-120 µg/ml in the presence of activation (+S9).

For the clastogenicity assay, proliferating cultures were incubated with drug for 24 and 48 hours in the absence of S9 and for 4 hours in the presence of S9 before metabolic arrest with Colcemid. Negative controls were treated with vehicle (DMSO), and appropriate positive controls were included. Cells were fixed, stained, and examined for chromosome aberrations as performed in the cytotoxicity assay. A significant increase ($P < 0.01$) in the numbers of aberrations, when compared to vehicle controls, was considered a positive response. An initial clastogenicity study (±S9) was repeated in a confirmatory study.

Results: Absence of metabolic activation (-S9): In the initial study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 25 µg/ml. The 25 µg/ml concentration produced a 76% reduction in mitotic index (Note: ICH Guideline S2A recommends that drug concentrations be tested in lymphocyte cultures at levels that produce a ≥50% decrease in mitotic index).

In the 24 hour confirmatory study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 42.5 µg/ml. The 42.5 µg/ml concentration produced a 66% reduction in mitotic index.

In the 48 hour confirmatory study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 42.5 µg/ml. The 42.5 µg/ml concentration produced a 59% reduction in mitotic index.

The positive control, mitomycin C, produced the expected increase in chromosome aberrations.

Presence of metabolic activation (+S9): In the initial study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 80 µg/ml. The 80 µg/ml concentration produced a 74% reduction in mitotic index.

In the 24 hour confirmatory study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 100 µg/ml. The 100 µg/ml concentration produced a 56% reduction in mitotic index.

In the 48 hour confirmatory study, no significant increases in cells with chromosome aberrations, polypoidy, or endoreduplication were observed at concentrations up to 100 µg/ml. The 100 µg/ml concentration also produced a 56% reduction in mitotic index, a reduction similar to that found after 24 hours of treatment.

The positive control, cyclophosphamide, produced the expected increase in chromosome aberrations.

Conclusions: Buprenorphine HCl was not found to be clastogenic when tested with human peripheral blood lymphocytes in the presence or absence of metabolic activation (±S9) at concentrations that produced ≥50% inhibitions in mitotic indexes.

2.2.4.4. Mutagenicity test on buprenorphine hydrochloride in the *in vivo* mouse micronucleus assay – Amended final report (DSE-235):

Testing Facility: (b) (4)
Study Number: DSE-235
Study Date(s): May 28, 1997 to June 27, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This *in vivo* study examined the clastogenic potential of buprenorphine to induce micronuclei in bone marrow polychromatic erythrocytes of mice. Normally, when a bone marrow erythroblast matures into a polychromatic erythrocyte, the nucleus is completely extruded. However, if the animal is exposed to an agent that causes chromosomal damage (clastogencity), a piece of chromosome (micronuclei) will remain in the erythrocyte cytoplasm after the nucleus is extruded. An increase in the frequency of micronucleated polychromatic erythrocytes is an index of drug-induced chromosomal damage or clastogenicity.

Methods: Outbred male and female Crl:CD-1(ICR) BR mice were used to maximize genetic heterogeneity and to minimize strain-specific responses. For the dose selection study, mice (3/sex/group) were given a single oral dose of buprenorphine by gavage at 50, 250, 750, 2000, or 5000 mg/kg. Controls received vehicle (corn oil). Animals were observed for 3 days, and mortality recorded. Results showed mortality in the 2000 mg/kg groups (4/6) and in the 5000 mg/kg groups (5/6). No deaths were recorded in the lower dose (≤ 750 mg/kg) groups. Therefore, the dose selected for the micronucleus study were 400, 800, and 1600 mg/kg.

Mice (5/sex/dose/harvest timepoint) were given a single oral dose of buprenorphine by gavage at 400, 800, and 1600 mg/kg. Vehicle controls received corn oil, and positive controls received 80 mg/kg cyclophosphamide. After either 24, 48, or 72 hours, 5 mice/sex were

sacrificed and slides of bone marrow prepared, fixed, and stained for microscopic examination. Slides were scored for micronuclei (measure of clastogenicity) and for the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio (measure of bone marrow cytotoxicity). At least 1000 PCEs were counted per slide. According to the sponsor, the historical background frequency of micronuclei in this strain on mouse is 0.0-0.4%. A positive response was defined as a statistically significant dose-related increase in micronucleated PCEs and a statistically significant increase at any dose level above concurrent controls.

Due to the high mortality in the 1600 mg/kg groups (7/10) of the first assay, only 24 hour timepoint mice were used for that dose, but the study data were not provided. Also, based on the high mortality (7/10) at the 1600 mg/kg dose in the first assay, a second micronucleus study was performed with lower doses at 200, 400, and 800 mg/kg. All other parameters in the second assay were the same as in the first study.

Results: Only data from the second study (200-800 mg/kg) were reported. In that study, no statistically significant increases in micronuclei from buprenorphine-treated mice were noted at any of the doses tested (Table 23; Sponsor's Table 1). The positive control showed the expected increase in micronuclei. Also, there was a statistically significant decrease in the PCE:NCE ratio (an index of drug-induced cytotoxicity) at the 800 mg/kg dose (Table 23; Sponsor's Table 1).

Table 23 (Sponsor's Table 1)

Micronucleus Data Summary Table

ASSAY: 18582

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
CONTROLS							
VEHICLE	Corn Oil	24 hr	0.24 ± 0.05	0.08 ± 0.02	0.16 ± 0.04	0.48 ± 0.06	0.73 ± 0.07
		48 hr	0.08 ± 0.04	0.04 ± 0.02	0.06 ± 0.02	0.66 ± 0.09	0.77 ± 0.03
		72 hr	0.20 ± 0.06	0.04 ± 0.02	0.12 ± 0.04	0.70 ± 0.10	0.80 ± 0.05
POSITIVE	CP 80.0 mg/kg	24 hr	4.14 ± 0.59*	2.10 ± 0.28*	3.12 ± 0.46*	0.59 ± 0.05	0.67 ± 0.03
TEST ARTICLE	200 mg/kg	24 hr	0.08 ± 0.06	0.02 ± 0.02	0.05 ± 0.03	0.52 ± 0.07	0.70 ± 0.03
		48 hr	0.08 ± 0.02	0.12 ± 0.02	0.10 ± 0.01	0.73 ± 0.13	0.84 ± 0.05
		72 hr	0.04 ± 0.02	0.06 ± 0.04	0.05 ± 0.02	0.67 ± 0.05	0.53 ± 0.02**
	400 mg/kg	24 hr	0.16 ± 0.07	0.02 ± 0.02	0.09 ± 0.04	0.62 ± 0.08	0.55 ± 0.04
		48 hr	0.10 ± 0.04	0.12 ± 0.02	0.11 ± 0.02	0.65 ± 0.08	0.86 ± 0.07
		72 hr	0.18 ± 0.04	0.12 ± 0.04	0.15 ± 0.03	0.47 ± 0.07	0.77 ± 0.07
	800 mg/kg	24 hr	0.08 ± 0.04	0.12 ± 0.06	0.10 ± 0.03	0.35 ± 0.08**	0.73 ± 0.06
		48 hr	0.20 ± 0.13	0.16 ± 0.08	0.18 ± 0.07	0.68 ± 0.09	0.52 ± 0.05**
		72 hr	0.14 ± 0.05	0.00 ± 0.00	0.07 ± 0.03	0.33 ± 0.03**	0.82 ± 0.10

* Significantly greater than the corresponding vehicle control, $p<0.01$.
** Significantly less than the corresponding vehicle control, $p<0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

Conclusions: Under the conditions of this *in vivo* assay for clastogenicity, buprenorphine HCl was not found to induce micronuclei in mice at doses that produced evidence of bone marrow cytotoxicity.

2.3. Safety/Special Toxicology:

2.3.1. Postmortem distribution of buprenorphine in rats following subcutaneous administration (DSE-267):

Testing Facility: (b) (4)
Study Number: DSE-267
Study Date(s): March 31, 1998 to April 3, 1998
GLP Compliance: Yes
QA Report: Yes

Purpose: This study determined the tissue distribution of ^{3}H-buprenorphine and its metabolites in rats for up to 72 hours after euthanasia. The reasons for such a study were not made clear.

Methods: Male Sprague-Dawley rats (3/time point) were given a single subcutaneous injection of ^{3}H-buprenorphine at 10 mg/kg (59,700 dpm/µg). Two hours after injection, rats were euthanized by CO_2 administration. At several time points afterwards for up to 72 hours, 3 rats per time point were euthanized and radioactivity measured from several tissue samples, including blood (heart), blood (inferior vena cava), bone marrow, brain, eye, heart, kidney, liver, lung, muscle (psoas), stomach with contents, and skin from the injection site.

Results: Measurements of radioactivity in any tissue did not display any particular trends up or down over the course of 72 hours. The only finding of note was the approximately 200-fold higher concentrations of radioactivity in the skin than in any other tissue examined.

Conclusions: These results indicated that the majority of drug did not significantly redistribute from the skin to other tissues after death. The clinical implications of these results were not immediately apparent.

2.3.2. Effects of nalmefene on buprenorphine-induced respiratory depression in rats (DSE-239):

Testing Facility: (b) (4)
Study Number: DSE-239
Study Date(s): May 21, 1997 to May 30, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined whether nalfemene, an opioid antagonist and analog of naltrexone, could reverse or attenuate respiratory depression induced by buprenorphine in the rat.

Methods: Male Sprague-Dawley rats (6/group) were given a single subcutaneous injection of either buprenorphine and/or nalfemene at doses between 0.1 and 10 mg/kg according to the schedule below (Table 24; Sponsor's Table). Groups 22-28 examined whether nalfemene

could reverse the respiratory depressive effects of buprenorphine, a situation with important clinical implications in cases of buprenorphine overdose.

Table 24 (Sponsor's Table)

Group Assignments

Group	Number	Test Article	Dose Level (mg/kg)	Dose Volume (ml/kg)	Endpoints
1	6	Vehicle	0	1.0	Res/pTV/BT
2	6	Buprenorphine	0.1	1.0	Res/pTV/BT
3	6	Buprenorphine	0.3	1.0	Res/pTV/BT
4	6	Buprenorphine	1.0	1.0	Res/pTV/BT
5	6	Buprenorphine	3.0	1.0	Res/pTV/BT
6	6	Buprenorphine	10	1.0	Res/pTV/BT
7	6	Vehicle	0	1.0	Res/pTV/BT
8	6	Nalmefene	0.1	1.0	Res/pTV/BT
9	6	Nalmefene	0.3	1.0	Res/pTV/BT
10	6	Nalmefene	1.0	1.0	Res/pTV/BT
11	6	Nalmefene	3.0	1.0	Res/pTV/BT
12	6	Nalmefene	10	1.0	Res/pTV/BT
13	6	Veh/Veh	0/0	1.0/1.0	Res/pTV/BT
14	6	Nal/Veh	1/0	1.0/1.0	Res/pTV/BT
15	6	Nal/Bup	1/0.1	1.0/1.0	Res/pTV/BT
16	6	Nal/Bup	1/0.3	1.0/1.0	Res/pTV/BT
17	6	Nal/Bup	1/1.0	1.0/1.0	Res/pTV/BT
18	6	Nal/Bup	1/3.0	1.0/1.0	Res/pTV/BT
19	6	Nal/Bup	1/10	1.0/1.0	Res/pTV/BT
20	6	Veh/Veh	0/0	1.0/1.0	Res/pTV/BT
21	6	Veh/Nal	0/1	1.0/1.0	Res/pTV/BT
22	6	Bup/Veh	10/0	1.0/1.0	Res/pTV/BT
23	6	Bup/Nal	0.1/1	1.0/1.0	Res/pTV/BT
24	6	Bup/Nal	0.3/1	1.0/1.0	Res/pTV/BT
25	6	Bup/Nal	1.0/1	1.0/1.0	Res/pTV/BT
26	6	Bup/Nal	3.0/1	1.0/1.0	Res/pTV/BT
27	6	Bup/Nal	10/1	1.0/1.0	Res/pTV/BT
28	6	Nal/Bup	1/10	1.0/1.0	Res/pTV/BT

Veh = Vehicle Nal = Nalmefene Bup = Buprenorphine HCl

After injection, respiratory rates and pseudo tidal volumes were measured in each animal for one hour using a plethysmograph. Body temperatures were recorded pretest and one hour after dosing using an implanted telemetric temperature monitor.

Results: When compared to controls, buprenorphine alone in the first 15 minutes reduced respiratory rates at all doses tested (0.1 to 10 mg/kg). Pseudo tidal volumes did not show a drug-

related effect. Respiratory rates from 15-80 minutes were decreased in all groups, including controls, indicating that the initial rates were elevated probably due to stress from the handling and injections. Body temperatures were slightly increased (about one degree) when buprenorphine was given at doses from 0.3 to 10 mg/kg.

Nalfemene alone had no effect on respiratory rate, pseudo tidal volume, or body temperature when tested at doses up to 10 mg/kg. When animals were pretreated with nalfemene, respiratory rates were decreased by vehicle similar to low doses buprenorphine (0.1-0.3 mg/kg). In nalfemene-pretreated animals, higher doses of buprenorphine (1-10 mg/kg) increased the respiratory rate to pretreated values. Nalfemene pretreated had no effect on pseudo tidal volume in buprenorphine-treated animals.

In animals pretreated with buprenorphine, nalfemene at doses up to 1 mg/kg did not attenuate the decrease in respiratory rate induced by doses of buprenorphine up to 10 mg/kg (Table 25; Sponsor's Table 8). Even 10 mg/kg of nalfemene did not attenuate the respiratory depressive effect of a low dose of buprenorphine (1 mg/kg).

Table 25 (Sponsor's Table 8)

Effects of Nalfemene Post-Treatment
on Buprenorphine-Induced Respiratory Depression

RESPIRATORY RATE (breaths/minute)
MEAN ± S.D.

Time Post-Dosing (min)	Buprenorphine/Nalmefene (mg/kg)								
	0/0	0/1	1/0	0.1/1	0.3/1	1/1	3/1	10/1	1/10 **
0-5	243 ± 49	246 ± 31	156 ± 34**	164 ± 30**	157 ± 47**	156 ± 34**	168 ± 34**	158 ± 41**	239 ± 38
6-10	217 ± 43	185 ± 24	145 ± 17**	144 ± 16**	151 ± 16*	151 ± 22*	153 ± 22*	164 ± 27*	165 ± 23*
11-15	177 ± 30*	140 ± 24	151 ± 22	144 ± 16	152 ± 10	136 ± 22	136 ± 24	145 ± 24	150 ± 20
16-20	148 ± 33	127 ± 18	140 ± 16	125 ± 27	140 ± 18	138 ± 22	133 ± 25	139 ± 41	126 ± 21
21-25	140 ± 32	117 ± 13	133 ± 25	129 ± 37	134 ± 24	133 ± 21	134 ± 38	132 ± 20	137 ± 44
26-30	140 ± 10	122 ± 22	122 ± 19	119 ± 35	130 ± 22	155 ± 33	134 ± 29	138 ± 19	155 ± 40
31-35	128 ± 22	118 ± 15	132 ± 17	129 ± 40	133 ± 20	135 ± 9	128 ± 33	134 ± 17	154 ± 31
36-40	135 ± 43	115 ± 17	134 ± 25	142 ± 33	131 ± 23	145 ± 12	135 ± 38	138 ± 21	140 ± 39
41-45	124 ± 30	115 ± 14	132 ± 12	152 ± 12*	136 ± 15	139 ± 14	142 ± 25	134 ± 23	150 ± 35*
46-50	123 ± 15	117 ± 13	131 ± 11	148 ± 38	137 ± 19	139 ± 12	136 ± 35	145 ± 47	153 ± 33
51-55	119 ± 8	115 ± 10	130 ± 15	129 ± 34	132 ± 22	152 ± 15	144 ± 35	137 ± 28	153 ± 35
56-60	133 ± 32	120 ± 13	136 ± 16	126 ± 24	140 ± 28	140 ± 7	145 ± 22	140 ± 20	147 ± 25

Conclusions: These results showed that nalfemene was unable to reverse the respiratory depression induced by buprenorphine in the rat. However, in the labeling for Buprenex (buprenorphine HCl), it is stated that naloxone, another opioid antagonist, may not be effective in reversing respiratory depression produced by buprenorphine.

2.3.3. A primary dermal irritation study in rabbits with buprenorphine patches (DSE-170):

Testing Facility: (b) (4)
Study Number: DSE-170
Study Date(s): Feb. 12, 1996 to Feb. 29, 1996
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential irritant and/or corrosive effects of buprenorphine patches in rabbits when exposed to the skin for up to 3 days.

Methods: Male New Zealand White rabbits (2.3-2.7 kg; 3/group) were each given one test patch at either 5, 10, or 20 mg of buprenorphine (lot nos. 8/28042/5, 8/28043/5, 8/28044/5) per patch and one control patch. Concentrations of buprenorphine in the patches were stated to be equal, implying that the patches differed in size, but this was not made clear. The patches were held to the skin with a semi-occlusive binder for either 3 hours or 4 days. After either 3 hours or 4 days of application, the patches were removed and the animals examined for signs of erythema and edema at 1, 24, and 72 hours and 7, 10, and 14 days after patch removal according to the Macroscopic Dermal Grading System based on the method of Draze.

Results: Dermal scoring was summarized as follows (Table 26; Sponsor's Table):

Table 26 (Sponsor's Table)

Results of Dermal Scoring of Buprenorphine Patches in Rabbits

Group	Exposure	Dose Level	Calculated P.I.I.	Irritation Rating
1	4 Hours	5 mg test patch	1.08	Slight Irritant
		5 mg placebo patch	0.42	Slight Irritant
		10 mg test patch	1.58	Slight irritant
		10 mg placebo patch	0.33	Slight Irritant
		20 mg test patch	1.17	Slight Irritant
		20 mg placebo patch	0.25	Slight Irritant
2	3 Days	5 mg test patch	4.00	Moderate Irritant
		5 mg placebo patch	1.25	Slight Irritant
		10 mg test patch	4.58	Moderate Irritant
		10 mg placebo patch	1.83	Slight Irritant
		20 mg test patch	5.00	Moderate Irritant
		20 mg placebo patch	1.33	Slight Irritant

As shown, buprenorphine in the 4 hour test produced slight irritation that was similar to that seen with the control (placebo) patches. In the 3 day test, buprenorphine patches produced moderate irritation that was manifested by areas of blanching and/or eschar formation, erythema, and some edema.

Conclusions: These results showed that buprenorphine patches produced some irritation that could be attributed to the excipients of the patches, but that prolonged exposure (3 days) to buprenorphine produced a greater degree of irritation.

2.3.4. A dermal sensitization study in guinea pigs with buprenorphine patches using a modified Buehler design (DSE-163):

Testing Facility: (b) (4)
Study Number: DSE-163
Study Date(s): Jan. 12, 1996 to Feb. 28, 1996
GLP Compliance: Yes
QA Report: Yes

Purpose: This study examined the potential of buprenorphine patches to induce dermal sensitization (delayed contact-type hypersensitivity) when given to guinea pigs by multiple topical applications.

Methods: Male and female Hartley-derived albino guinea pigs (339-455 gms) were divided into the following groups (Table 27; Sponsor's Table):

Table 27 (Sponsor's Table)

Treatment Groups

Group	No. of Animals	
	Males	Females
Range-Finding	2	2
Test	10	10
Placebo Control	5	5
Challenge Control	5	5
Rechallenge Control[a]	5	5
DNCB Test	5	5
DNCB Control	5	5

[a]A rechallenge control group was maintained for the study; however, the rechallenge procedure was not required.

The test guinea pigs were topically treated with 10 mg buprenorphine patches once per week for three weeks. The dose was calculated to be 3.2 mg/patch (= 1.3 mg/kg). Placebo controls

received placebo patches. A positive control consisting of 0.1% (w/v) 1-chloro-2,4-dinitrobenzene (DNCB) for induction and 0.1% and 0.05% DNCB for challenge was used.

Following a 2-week rest period, a challenge was performed where the test and previously unexposed (naïve) control guinea pigs were challenged with 10 mg topical buprenorphine patches. Dermal responses to the challenge were compared between the two groups, previously sensitized and previously unsensitized, and recorded 24 and 48 hours after challenge using a dermal grading system for erythema, edema, and other dermal lesions.

Results: After buprenorphine challenge in previously sensitized animals, dermal scores were minimal and ranged from 0 to ±. Similar results were noted with the placebo patch controls. In the DNCB positive controls, dermal scores ranged from 1.5 to 2.9, indicating the expected positive response.

Conclusions: Results were interpreted as indicating that 10 mg buprenorphine patches did not induce contact sensitization (delayed-type hypersensitivity reactions) in guinea pigs.

2.3.5. A single dose oral administration (capsule) safety study in beagle dogs with buprenorphine patches (+ toxicokinetic report and analytical report) (DSE-244):

Testing Facility: (b) (4)
Study Number: DSE-244
Study Date(s): March 10, 1997 to April 28, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: This study used dogs to examine the potential safety issue of buprenorphine patches if accidentally swallowed.

Methods: Male beagle dogs (7-10 kg; 3/group) were given a single oral dose of gelatin capsules containing buprenorphine patches cut up into small pieces at 5 or 20 mg/dog (= 0.5 or 2.0 mg/kg) according to the following schedule (Table 28; Sponsor's Table). Group 4 dogs were given capsules containing buprenorphine patches pounded with a meat tenderizer to mimic mastication (chewing). Control dogs received capsules containing placebo patches.

Table 28 (Sponsor's Table)

Treatment Schedule

Group	No. of Males	Dosage Material	Dosage Level (mg/dog)	Nominal Dosage Level (mg/kg)[1]	Actual Dosage Level (mg/kg)[2]
1	3	Placebo Patch[3]	0	0	0
2	3	Buprenorphine Patch	5	0.5	0.6
3	3	Buprenorphine Patch	20	2.0	2.2
4	3	Buprenorphine Patch	20	2.0	2.3

[1]Approximate level from the protocol.
[2]Based on the mg applied divided by the sex-group mean body weight.
[3]20 mg size placebo patch.

After dosing, dogs were closely examined for clinical signs during the first few hours, then daily for 14 days. Stools were examined for presence of patch material. Body weights were recorded several times during the study, and food consumption was recorded daily. Blood was collected predose and at study termination for clinical pathology measurements (hematology, coagulation, and biochemistry). Blood was also collected several times during the study for determination of plasma drug concentrations (toxicokinetics). Six lead ECGs and indirect blood pressure measurements were performed several times during the course of the 14 day study period.

On Day 14, dogs were sacrificed and a complete gross necropsy performed that consisted of a gross examination of organs, and microscopic examination of selected tissues from the gastrointestinal tract, including esophagus, stomach, duodenum, ileum, jejunum, cecum, colon, rectum, and anus.

Results: No deaths were reported. No obvious clinical signs were noted. Decreased fecal output, a known pharmacological effect of opioids, was noted in most animals. Body weights and food consumption were similar between drug-treated and placebo-control groups. There were no consistent drug-related findings in any of the clinical pathology parameters measured. ECGs were normal. Gross necropsy observations and microscopic examination of tissues from the GI tract did not note any drug-related effects.

Toxicokinetic analysis found very low levels of buprenorphine in the plasma of dogs given drug-containing patches orally in capsules (Table 29; Sponsor's Table 7.2). The maximum concentration detected was 0.78 ng/ml in dogs 4 hours after receiving 20 mg patches.

Table 29 (Sponsor's Table 7.2)

Mean (±SD) Plasma Concentrations (ng/mL) Of Buprenorphine In Dogs Given Capsules Filled With Either A 5 Or 20 mg Buprenorphine Patch

Time (hr)	5 mg (patches cut in half)	20 mg (patches cut in half)	20 mg (patches cut & pounded)
0	0.00	0.00	NS
0.25	NS	NS	0.00
0.5	NS	NS	0.00
1	NS	NS	0.20 ± 0.17
2	NS	NS	0.46 ± 0.48
4	0.25 ± 0.29	0.78 ± 0.62	0.14 ± 0.13
24	0.11 ± 0.09	0.26 ± 0.13	0.00
48	0.00	0.00	0.00
72	0.00	0.00	0.00

NS = no sample taken

Conclusions: No apparent toxicological findings were noted in dogs given buprenorphine patches orally in capsules. This may be related to the very low (< 1 ng/ml) measured plasma drug concentrations. The results of this study performed in beagle dogs suggest that accidental ingestion of 20 mg buprenorphine patches may not result in significant clinical effects.

2.3.6. A single dose buccal administration safety study in beagle dogs with a buprenorphine patch (+ toxicokinetic report and analytical report) (DSE-245):

Testing Facility: (b) (4)
Study Number: DSE-245
Study Date(s): March 11, 1997 to April 29, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: Similar to the preceding study, this study used dogs to examine the potential safety issue of buprenorphine patches if accidentally chewed with subsequent buccal absorption of buprenorphine.

Methods: Buprenorphine patches (5 mg and 20 mg) were applied to the buccal areas of female beagle dogs (6-9 kg; 3/group) and left in place for 30 min by taping close the mouths of the animals. After 30 min, the patches were removed. Control dogs received placebo patches.

Group 4 dogs were given 20 mg buprenorphine patches with holes poked in them to mimic mastication (chewing) (Table 30; Sponsor's Table).

Table 30 (Sponsor's Table)

Treatment Schedule

Group	No. of Females	Dosage Material	Dosage Level (mg/dog)	Nominal Dosage Level (mg/kg)[1]	Actual Dosage Level (mg/kg)[2]
1	3	Placebo Patch[3]	0	0	0
2	3	Buprenorphine Patch	5	0.5	0.7
3	3	Buprenorphine Patch	20	2.0	2.6
4	3	Buprenorphine Patch	20	2.0	2.8

[1]Approximate level from the protocol.
[2]Based on the mg applied divided by the sex-group mean body weight.
[3]20 mg size placebo patch.

After dosing, dogs were closely examined for clinical signs during the first few hours, then daily for 14 days. Body weights were recorded several times during the study, and food consumption was recorded daily. Blood was collected predose and at study termination for clinical pathology measurements (hematology, coagulation, and biochemistry). Blood was also collected several times during the study for determination of plasma drug concentrations (toxicokinetics). Six lead ECGs and indirect blood pressure measurements were performed several times during the course of the 14 day study period.

On Day 15, dogs were sacrificed and a complete gross necropsy performed that consisted of a gross examination of organs, and microscopic examination of the buccal membrane.

Results: No deaths were reported. Decreased activity was noted in one high dose dog given the patch with the holes within a few hours after dosing. Also, red and swollen cheek mucosal areas were noted in other animals in both (holes and no holes) high dose groups within a few hours after dosing. Red and swollen cheek mucosal areas were noted in the placebo patch controls, but only after 3-10 days.

Decreased body weights and food consumption were noted in the high dose groups, but by the end of the study, body weights were similar to those of control dogs (8.0 vs 8.1 kg, respectively).

No significant drug-related findings were noted in clinical pathology (hematology, coagulation, and biochemistry) or cardiology (ECG, BP) parameters.

No drug-related gross lesions were described. According to the histopathology report, no microscopic lesions were observed in any section of the buccal membrane from any dog, although red and swollen cheeks were noted grossly.

Toxicokinetic data showed that buprenorphine was well absorbed after buccal administration in dogs given patches with holes (Table 31; Sponsor's Table 7.2). In this group, the mean Cmax within 30 min was 174 ng/ml and the mean AUC was 245±242 ng/ml/hr. The apparent $t_{1/2}$ was 2.5 hours.

Table 31 (Sponsor's Table 7.2)

Mean (±SD) Plasma Concentrations (ng/mL) Of Buprenorphine In Dogs Following A 5 Or 20 mg Buccal Administration With A Buprenorphine Patch

Time (hr)	5 mg (patches cut in half)	20 mg (patches cut in half)	20 mg (patches cut & pierced)
0	0.00	0.00	NS
0.25	NS	NS	67.7 ± 61.3
0.5	NS	NS	174 ± 233
1	NS	NS	76.1 ± 87.1
2	NS	NS	32.3 ± 34.3
4	0.50 ± 0.03	3.03 ± 1.08	4.91 ± 1.80
24	0.00	0.15 ± 0.03	0.23 ± 0.15
48	0.00	0.00	0.00
72	0.00	0.00	0.00

NS = no sample taken

Conclusions: Dogs given buprenorphine patches by the buccal route of administration showed drug-related effects. These included decreased activity, an effect seen with other opioids, and a temporary decrease in body weight. Red and swollen cheek mucosal areas were noted in animals in both (holes and no holes) high dose groups within a few hours after dosing, although no microscopic lesions in the buccal mucosa were observed. Toxicokinetic data showed that buprenorphine was well absorbed after buccal administration in dogs given patches with holes with a mean Cmax within 30 min of 174 ng/ml. These results indicate that accidental buccal absorption may be a potentially significant safety issue.

2.3.7. Evaluation of the effects of bath immersion on plasma buprenorphine levels in mini-swine (+ toxicokinetic report and analytical report) (DSE-243):

Testing Facility: (b) (4)
Study Number: DSE-243
Study Date(s): April 5, 1997 to May 14, 1997
GLP Compliance: Yes
QA Report: Yes

Purpose: The study examined whether immersion of mini-swine in a warm water bath (41° C) for 15 min altered the plasma levels of buprenorphine in animals wearing the patches.

Methods: Four 20 mg buprenorphine patches (= 80 mg per minipig) were applied to the skin of male and female minipigs (9-12 kg) for 7 days. Patches were replaced every 3 days. Blood was collected periodically during the first 7 days for determination of plasma drug concentrations. One day after application of the third set of patches, animals (n=4) were immersed in a hot tub at 41° C for 15 min. Control animals (n=4) were kept at room temperature. Blood was again taken for up to 24 hours to determine the effect of hot water on dermal absorption of buprenorphine, and levels compared to both preimmersion levels and to animals kept at room temperature.

Results: When compared to preimmersion levels or to levels found in animals kept at room temperature, immersion in hot water resulted in essentially no change in plasma buprenorphine concentrations, except for one animal #2/M whose Cmax levels increased to 5.53 ng/ml at 24 hours from a baseline of 0.18 ng/ml (Table 32; Sponsor's Table 7.2). Given the long delay of 24 hours after the initial 15 min immersion in detection of increased levels in this animal, it is difficult to draw any overall conclusions from these results.

Table 32 (Sponsor's Table 7.2)

Plasma Concentrations (ng/mL) Of Buprenorphine In Minipigs Following Immersion In A Hot Water Bath

		Immersed in Water Bath for 15 min.					
Time (hr)	Animal #:	1/F	2/M	13/F	14/F	MEAN	SD
0[a]		0.1	0.18	0.52	0.52	0.33	0.22
0.5		0.12	0.23	0.6	0.58	0.38	0.24
1		0.00[b]	0.17	0.56	0.72	0.36	0.33
2		0.12	0.11	0.4	0.44	0.27	.018
4		0.00	0.27	0.43	0.13	0.21	0.18
6		0.1	0.15	0.44	0.52	0.30	0.21
8		0.35	0.24	0.43	0.4	0.36	0.08
12		0.00	0.17	0.43	0.34	0.24	0.19
24		0.2	5.53	0.29	0.41	1.61	2.62
		Housed in Individual Runs					
Time (hr)	Animal #:	3/M	11/F	12/M		MEAN	SD
0[a]		NA	0.00	0.14		0.00	(n=2)
0.5		NA	0.1	0.19		0.15	(n=2)
1		NA	0.11	0.18		0.15	(n=2)
2		NA	0.12	0.18		0.15	(n=2)
4		NA	0.11	0.16		0.14	(n=2)
6		0.11	0.11	0.31		0.18	0.12
8		0.14	0.11	0.21		0.15	0.05
12		NA	0.13	0.15		0.14	(n=2)
24		NA	0.12	0.15		0.14	(n=2)

[a] = corresponds to value at 168 hr in Table 1 and also to 24 hrs after last dose was given.

[b] = zero was used for any value that was below the limit of quantitation (0.10 ng/mL).

NA = data not available

Conclusions: There did not appear to be any effect of warm water immersion on increasing dermal absorption of buprenorphine from applied dermal patches in minipigs. However, given the low levels of drug absorbed before immersion (< 1 ng/ml), it is difficult to draw any conclusions.

3. LABELING (Package Insert):

Proposed Labeling:

The labeling proposed by the sponsor is as follows:

(b) (4)

Revised Labeling:

The **Carcinogenesis, Mutagenesis, and Impairment of Fertility** section is acceptable. However, according to 21 CFR 201.57 (Specific requirements on content and format of labeling for human prescription drugs), if there are no animal reproduction studies and no adequate and well-controlled studies in humans, the labeling for this section should follow more closely the specific language as follows:

Pregnancy - Pregnancy Category C

Animal reproduction studies have not been conducted with Norspan™. It is also not known whether Norspan™ can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Norspan™ should be given to a pregnant woman only if the potential benefit justifies the potential risk to the fetus. Buprenorphine has been shown to cross the placenta in humans. Buprenorphine has been detected in newborn blood, urine, and meconium and also in the mother's milk at low concentrations.

4. RECOMMENDATIONS:

From a nonclinical safety perspective, it is recommended that the application NDA #21-306 (Norspan®; Buprenorphine Transdermal System) be approvable with the recommended changes in labeling.

Agreement was reached between the sponsor and this reviewing division that all reproductive toxicity studies and carcinogenicity studies could be conducted as Phase 4 commitments.

Also, the possibility of respiratory depression in children after accidental chewing of used (discarded) or unused patches may need to be addressed as a potential abuse liability issue.

Thomas Papoian, Ph.D., D.A.B.T.
Supervisory Pharmacologist

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/s/

Thomas Papoian
7/18/01 08:37:45 AM
PHARMACOLOGIST