CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	209819
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Product:	Sublocade (RBP-6000; Buprenorphine-ATRIGEL)
Indication:	Subcutaneous (SC) injection once monthly in treatment of moderate to severe opioid use disorder (OUD) in patients who have undergone induction to suppress opioid withdrawal signs and symptoms
Applicant:	INDIVIOR
Review Division:	Division of Anesthesia, Analgesia, and Addiction Product
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1 Executive Summary

1.1 Introduction and Regulatory Background

The Applicant, Indivior Inc., submitted a New Drug Application for SUBLOCADE™ (RBP-6000, Buprenorphine-ATRIGEL), an injectable month-long depot formulation for treatment of opioid use disorder (OUD). Sublocade delivers buprenorphine via the ATRIGEL Delivery System. The Applicant conducted their own studies and are also cross-referencing nonclinical data in their Subutex NDA (NDA 20732). However, this application is being filed as a 505(b)(2) application because the Applicant is also relying on published literature to support the safety assessment of the individual excipients N-methyl-2-pyrrolidone (NMP) and poly(lactide-co-glycolide) (PLGH), which form the Atrigel Delivery System. (b) (4) The use of NMP in this drug product is technically novel. Although NMP has been used in an FDA-approved acute-use drug product and in a drug product approved for the treatment of advanced cancer, this will be the first time NMP is used at the proposed levels in a chronic, non-oncology indication.

1.2 Brief Discussion of Nonclinical Findings

Potential systemic and local toxicity of the Sublocade drug product and the Atrigel Delivery System were evaluated in single-dose and chronic repeat-dose studies in rats and dogs and in reproductive toxicity studies in rats and rabbits. Potential genotoxicity was evaluated in vivo in rats. Generally, Sublocade and Atrigel (similar amount of NMP and PLGH as the respective Sublocade group) groups were tested. These studies lacked a saline control group, which complicates data interpretation; however, some separate studies were submitted that tested the Atrigel alone compared to a saline control arm.

Regarding local toxicity after subcutaneous dosing, the duration, frequency and severity of clinical signs and dermal observations at the injection site generally correlated with increasing volume of the Atrigel Delivery System administered. Expected effects of the injection were observed in the toxicity studies (and less frequently in the formulation development), which demonstrated swelling, abrasion, reddening and raised areas or masses, and dermal observations included edema, superficial/dermal irritation and erythema. Gross findings at injection sites in animals treated with Sublocade or the Atrigel Delivery System consisted of firm, dark or pale area/foci in the subcutaneous tissue, which correlated histologically in the single- and repeat-dose toxicology studies with subcutaneous granulomas, degenerate/necrotic cell debris, mononuclear cell infiltrate, fibroplasia and/or hemorrhage. Reversibility of local effects occurred after dosing ended over several months. The local effects are both the result of the vehicle and the buprenorphine.

No unanticipated systemic effects of acute dosing were observed. Notable single-dose effects in rats and dogs were related to buprenorphine (self-mutilation, pica, reduced

food consumption and weight loss in rats and watery feces, no feces/constipation, emesis, decreased activity, changes in food consumption such as low or no consumption, and decreased body weight in dogs. After repeated dosing in rats (monthly for 6 months), evidence of increased stress was noted by the increased adrenal and decreased thymus weights. As discussed during development, there was an increased incidence of pancreatic acinar cell apoptosis (exocrine pancreas) in the studies testing Atrigel vehicle vs Sublocade. The Applicant attributed these to a stress response. The findings were not noted in published studies with NMP (see Appendix) and were not expected to occur with PLGH. There are data to support the conclusion that decreased body weight gain, which can be a side effect of buprenorphine, can result in similar changes in the pancreas. However, based on these findings, the Applicant also included clinical monitoring for pancreatic function in their Phase 3 studies. The reader is referred to the clinical team review for further information.
We also discussed rat findings of alveolar macrophage infiltrates in lungs in both Atrigel vehicle and Sublocade groups that appeared dose responsive in incidence and reversible after discontinuation of dosing. This was intriguing given the intended route. Nevertheless, additional rat studies that tested Atrigel vehicle vs saline either did not show the alveolar infiltrates in either group or demonstrated a comparable low incidence between the saline and Atrigel vehicle groups. Therefore, the findings are likely not toxicologically significant.

Reproduction toxicity studies conducted with Sublocade and Atrigel included fertility (female rats – 2 doses over ~1 month with last dose on Gestation Day 7; male rats – 3 doses premating over 3 months), embryo-fetal toxicity (female rats and rabbits dosed on Gestation Day 7), and pre-/post-natal toxicity (F0 maternal female rats dosed on Gestation Day 7 and Lactation Day 7). While there was no effect on mating or fertility (rats), increased post-implantation losses and a higher number of resorptions and early fetal deaths were observed. In female rats and rabbits administered Sublocade or Atrigel before mating (rats only) and again on GD 7 (rats and rabbits), increased skeletal malformations were observed. Male rat fertility and reproduction indices were lower in the high Sublocade (high buprenorphine) and high Atrigel groups as evidenced by abnormal sperm parameters (low motility, low mean number of sperm, and higher percentage of abnormal sperm). In the rat pre/post-natal study, a reduction in F1 body weights was observed in the higher dose Sublocade (buprenorphine) groups throughout the lactation and early postweaning periods only, but body weights returned to control levels during the F1 growth phase. No other adverse effects were observed in this study. Many of the adverse effects noted in these studies were present in both the Atrigel alone arm and the Sublocade arms, with a few exceptions. These data suggest a potential risk of the vehicle in this formulation but also identify the potential for cranial malformations which appear to be due to buprenorphine alone. As discussed during development, the Division was concerned that the Applicant's studies with Sublocade alone were not adequate to characterize the impact of NMP on the standard endpoints in these studies. As such, the Applicant submitted a literature-based safety justification for NMP (reviewed in the Appendix). Review of the literature suggests that there appears to be smaller safety margins for NMP than there are for buprenorphine. As such, we are recommending language be included in the drug product labeling and

post-marketing requirements (PMRs) to fully characterize the effects of NMP on these critical endpoints.

In vivo micronucleus testing of Sublocade and Atrigel in a valid assay, yielded negative results, indicating that Sublocade and Atrigel are not genotoxic.

The carcinogenic potential of NMP has been described in the published literature. Studies in rats via the inhalation and dietary routes suggest no increased risk of carcinogenicity. However, a dietary study in the mouse demonstrated increased hepatocellular adenomas and carcinomas that appear to be treatment related. The clinical significant of rodent liver tumors is not clear. Many rodent hepatocarcinogens are not believed to have relevance to humans and there are signals in this study that suggest the same may be true to the NMP-induced mouse liver tumors. However, a mode of action assessment has not been undertaken to date. Assuming a significant public health benefit potential for the drug product, a model of action assessment can be completed as a post-marketing requirement and if it supports a lack of human risk, (b) (4).

In regard to Product Quality (i.e., Drug Substance, Drug Product, Impurities, Excipients, and Extractables/Leachables), there are no nonclinical issues that preclude approval.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, Sublocade may be approved with the recommended labeling changes and with post-marketing requirements.

1.3.2 Additional Nonclinical Recommendations

Based on the review of the application, the following post-marketing requirements are recommended:

1. Conduct a fertility and early embryonic development study testing N-methyl-pyrrolidone in the rat model.

2. Conduct an embryofetal development study testing N-methyl-pyrrolidone in the rat model.

3. Conduct an embryofetal development study testing N-methyl-pyrrolidone in the rabbit model.

4. Conduct a pre- and post-natal development study testing N-methyl-pyrrolidone in the rat model.

5. Conduct a mode of action assessment for N-methyl-pyrrolidone-induced mouse hepatocellular adenomas and carcinomas to inform the human risk assessment for NMP.

1.3.3 Labeling

At the time of this review, the labeling recommendations below have not been discussed with the Applicant. The reader is referred to the approved drug product labeling for final labeling language.

Applicant's proposed label language	Reviewer's recommended changes	Comments and rationale for recommended changes
8.1 Pregnancy **Risk Summary** (b) (4) **Data**	**8.1 Pregnancy** **Risk Summary** In published animal reproduction studies with NMP, an excipient in SUBLOCADE, preimplantation losses, delayed ossification, reduced fetal weight, developmental delays and reduced cognitive function were reported at doses equivalent to the doses of NMP via SUBLOCADE. Decreased pup survival at 2 times the dose of NMP and malformation and postimplantation losses were reported at 3 times the dose of NMP via Sublocade. In animal reproduction studies with SUBLOCADE, SUBLOCADE administered subcutaneously to pregnant rats and rabbits during the period of organogenesis at a buprenorphine dose equivalent to 38 and 15 times, respectively, the maximum recommended human dose (MRHD) of 300 mg caused embryolethality, which appeared to be attributable primarily to the SUBLOCADE vehicle. In addition, reduced fetal body weights, increased visceral malformations and skeletal malformations were observed in rats and rabbits at a buprenorphine dose equivalent to 38 and 15 times, respectively, the MRHD. These effects were also observed with the SUBLOCADE vehicle alone, but the skeletal and visceral malformations in rat appear at least partially	Recommended changes include reorganizing the sentences to be in accordance with PLLR formatting and to add exposure margins based on AUC comparison, if possible. (b) (4) (b) (4) A literature-based risk assessment for NMP was also provided to help build a weight-of-evidence that the effects of NMP have been adequately characterized. Nevertheless, these studies have evaluated the potential effects of buprenorphine administered by the relevant clinical route of administration dosing regimen. Moreover, toxicokinetics were evaluated and therefore exposure margins based on buprenorphine AUC were calculated where possible. (b) (4) the recommended language does note when

Animal Data (b) (4)	attributable to buprenorphine [see Data]. Based on animal data, advise pregnant women of the potential risk to a fetus. **Data** *Animal Data* Preimplantation losses, delayed ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during organogenesis with NMP, an excipient in Sublocade, via inhalation at approximately equivalent doses of NMP delivered by Sublocade. Fetal malformations and resorptions have also been reported following oral administration of 3 times the MDD of NMP delivered by Sublocade at the MDD based on a body surface area comparison. Post-implantation loss and increased cardiovascular and skull malformations were demonstrated in pregnant rabbits administered oral NMP, an excipient in Sublocade, at doses 3.2 times the human MDD of NMP via Sublocade in the absence of maternal toxicity. No adverse effects were reported at an oral dose equivalent to the MDD via Sublocade based on a body surface area comparison. Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in Sublocade at doses 1.8 times the MDD. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated with NMP via inhalation during gestation at doses equivalent to the MDD of NMP via Sublocade based on a body surface area comparison. In an embryofetal development study in rats, SUBLOCADE administered subcutaneously to pregnant animals before mating and again on GD 7	findings were observed with Atrigel alone to note that the effects may be attributable to the vehicle or in combination with buprenorphine. (b) (4) As adverse effects from NMP studies alone demonstrate a smaller safety margin than the studies conducted with RBP-6000, these published studies should be presented prior to the RBP6000 studies because the report the greatest risks. (b) (4) recommended language does note when

(b) (4)	during the period of organogenesis resulted in increased post-implantation loss, which correlated with higher mean number of resorptions and decreased number of viable fetuses per litter, and decreased mean fetal body weights at 900 mg/kg (approximately 38 times the maximum recommended human dose [MRHD] of 300 mg of SUBLOCADE on an AUC basis); however, similar effects were observed with an equivalent level of ATRIGEL® Delivery System alone, indicating they may be attributable to the vehicle. Dose-related increases in incidences of skeletal malformations of the head and visceral malformations were observed with SUBLOCADE with significant changes at 900 mg/kg (approximately 38 times the MRHD on an AUC basis). Although similar effects were observed with equivalent levels of ATRIGEL Delivery System, the incidence of skeletal malformations, primarily skull malformations, was higher in the SUBLOCADE groups suggesting that buprenorphine contributed to the increased incidence. Based on these results, the NOAEL for developmental toxicity was 300 mg/kg (approximately 15 times the MRHD on an AUC basis). In an embryofetal development study in rabbits, administration of a single subcutaneous injection of SUBLOCADE to pregnant animals on Gestation Day 7 during the period of organogenesis resulted an increased litter incidence of skeletal malformations at 155 mg/kg (approximately 7 times the MRHD on an AUC basis), which appear to be buprenorphine-related adverse effects. There was also an increased litter incidence of external malformations, visceral, and skeletal malformations and variations at 390 mg/kg SUBLOCADE (approximately 15 times the MRHD on an AUC basis); however, similar effects were observed with an equivalent level of the ATRIGEL Delivery System,	adverse findings were also observed with Atrigel alone to note that the effects with RBP-6000 appear to be attributable to the vehicle or in combination with buprenorphine.

(b) (4)	indicating they may be attributable to the vehicle In addition, increased post-implantation loss, which correlated with increased mean number of resorptions and decreased mean number of viable fetuses, and decreased fetal body weights were observed at 390 mg/kg (approximately 15 times the MRHD on an AUC basis); however, similar findings were also observed with an equivalent level of the ATRIGEL Delivery System alone. Based on these results, the NOAEL for developmental toxicity for SUBLOCADE was 78 mg/kg (approximately 3 times the MRHD on an AUC basis). In a pre- and postnatal development study in rats, SUBLOCADE was administered subcutaneously to pregnant animals once during implantation (on Gestation Day 7) and once during weaning (on Lactation Day 7). There were no adverse effects on offspring survival, sexual maturation, behavioral assessment, or reproductive performance at up to 300 mg/kg (approximately 10 times the MRHD on an mg/m^2 basis)	The Applicant did not propose language to describe the pre- and postnatal development study results. We recommend this language here.
8.3 Females and Males of Reproductive Potential **Animal Data**	**8.3 Females and Males of Reproductive Potential** Human Data	Human Class labeling language should go first in the label.
(b) (4)	Chronic use of opioids may cause reduced fertility in females and males of reproductive potential. It is not known whether these effects on	

(b) (4)	fertility are reversible *[see Adverse Reactions]*. **Animal Data** *Infertility* Male Male fertility may be reduced based on animal data demonstrating adverse effects of SUBLOCADE on sperm parameters [see Nonclinical Toxicology (13.1)].	There were no effects on female fertility indices; therefore, no nonclinical female information is needed here. (b) (4) .
12.1 Mechanism of Action (b) (4)	**12.1 Mechanism of Action** Buprenorphine is a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor.	(b) (4) The recommended language is consistent with the reference product label.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility (b) (4)	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** **Carcinogenicity** Long term studies in animals to evaluate carcinogenic potential of SUBLOCADE have not been conducted. However, the carcinogenic potential of buprenorphine has been evaluated in Sprague-Dawley rats and CD-1 mice. In the carcinogenicity study conducted in Sprague-Dawley rats, buprenorphine was administered in the diet at doses of 0.6, 5.5, and 56 mg/kg/day (approximately 0.5, 5, and 50 times the recommended human monthly SC dose of 300 mg of buprenorphine based on body surface area comparisons) for 27 months. A statistically significant dose-related increase in Leydig cell tumors occurred. In an 86-week study in CD 1 mice, buprenorphine was not carcinogenic at dietary doses up to 100 mg/kg/day (approximately 45 times the recommended human monthly SC dose of 300 mg of buprenorphine based on body surface area comparisons). NMP, an excipient in SUBLOCADE, produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 6 and 8 times the maximum daily dose (MDD) of NMP via Sublocade. No tumors were noted at 1 and 1.3 times the MDD. The clinical significance of these findings is unclear. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity. **Mutagenicity** No evidence of mutagenic potential for subcutaneous SUBLOCADE was found in in vivo subcutaneous micronucleus test using rats' marrow.	The Applicant's proposed exposure margins take into consideration that the 300 mg MRHD is given monthly and so the calculation compares the human equivalent doses of the rat doses to a human dose of 300 mg divided by 28. As the human PK profile showed similar daily concentrations over the monthly dosing period, the Applicant's approach is acceptable. A literature-based risk assessment on the potential carcinogenicity of NMP was submitted with the NDA. Therefore, information from the published literature on NMP are recommended here. (b) (4)

(b) (4)

Mutagenic potential for buprenorphine was studied in a series of tests utilizing gene, chromosome, and DNA interactions in both prokaryotic and eukaryotic systems. Results were negative in yeast (S. cerevisiae) for recombinant, gene convertant, or forward mutations; negative in Bacillus subtilis "rec" assay, negative for clastogenicity in CHO cells, Chinese hamster bone marrow and spermatogonia cells, and negative in the mouse lymphoma L5178Y assay. Results were equivocal in the Ames test: negative in studies in two laboratories, but positive for frame shift mutation at a high dose (5 mg/plate) in a third study.

Results were positive in the Green-Tweets (E. coli) survival test, positive in a DNA synthesis inhibition (DSI) test with testicular tissue from mice, for both in vivo and in vitro incorporation of [3H]thymidine, and positive in unscheduled DNA synthesis (UDS) test using testicular cells from mice.

Impairment of Fertility
In a fertility study in rats, female mating, fertility, and fecundity indices were unaffected by the SC administration of SUBLOCADE up to 900 mg/kg buprenorphine (approximately 38 times the maximum recommended human dose [MRHD] of 300 mg on an AUC basis). However, higher mean post-implantation loss was observed with SUBLOCADE at 900 mg/kg and at an equivalent level of ATRIGEL alone, which correlated with higher mean number of resorptions and reduced mean number of viable fetuses/litter. Mean gravid uterine weight and mean final body weight were lower with SUBLOCADE at 900 mg/kg and at an equivalent level of ATRIGEL alone,

(b) (4)	and correlated with higher mean number of resorptions and lower fetal body weights. The NOAEL for female fertility was 900 mg/kg and the NOAEL for female-mediated developmental parameters was 600 mg/kg (approximately 25 times the MRHD on an AUC basis). Male fertility and reproduction indices were lower as evidenced by abnormal sperm parameters (low motility, low mean number of sperm, and higher percentage of abnormal sperm) with SUBLOCADE at 600 mg/kg and with an equivalent level of ATRIGEL alone. The NOAEL for male fertility, including sperm analysis, and male-mediated developmental parameters was 300 mg/kg (approximately 32 times the MRHD on an AUC basis). Adverse effects on testes and male fertility were noted in published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in SUBLOCADE at greater than 11.6 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 3.5 times the MDD of NMP delivered by Sublocade. No adverse effects were noted at oral doses equivalent to the dose of NMP delivered by SUBLOCADE.	

		Exposure margins were changed to AUC_{0-24h} basis. (b) (4)

		Fertility information for NMP from published literature are added here.

The following table summarizes the exposure margins calculated based on a comparison of AUC levels achieved in animal reproductive and development studies compared to AUC levels reported from Clinical Study RB-US-12-0005 in which treatment-seeking opioid-dependent patients were inducted and stabilized over a 13-day period on doses of Subutex at 8 to 24 mg prior to being administered 300 mg of RBP-6000. Note that steady-state in humans administered 300 mg RBP-6000 was reached after the 4th injection and are represented in the table below as the multiple-dose (MD) human $AUC_{0\text{-}24h}$. For safety margins, this MD human AUC value was used to compare to the last animal $AUC_{0\text{-}24h}$ value when multiple doses were administered to animals. In contrast, human AUC after a single-dose (SD) was used for safety margin calculations when the animal study only employed a single-dose (e.g., rabbit EFD study). It's also worth noting that the exposure margins based on the rabbit EFD study were calculated using animal $AUC_{0\text{-}336h}$ values and human SD $AUC_{0\text{-}672h}$ values since the rabbit study report did not include $AUC_{0\text{-}24h}$ or $AUC_{0\text{-}672h}$ values. Therefore, these exposure margins represent more conservative margins.

Exposure Margins for Reproductive and Developmental Toxicology Studies

Animal Doses of RBP-6000 (mg/kg)	Animal $AUC_{0\text{-}24h}$ (ng.h/mL)	Animal $AUC_{0\text{-}336h}$ (ng.h/mL)	MD Human $AUC_{0\text{-}24h}$ of 178.1	SD Human $AUC_{0\text{-}672h}$ of 1268	Human Exposure Margins [c]

			(ng.h/mL) [a]	(ng.h/mL) [b]	
Fertility and Early Embryonic Development – Rat					
Females					
300	2720				15.3
600	4430				24.9
900	6710				37.7
Males					
300	5690				32
600	10100				56.7
900	14200				79.7
Embryofetal Development – Rat					
300	2720				15.3
600	4430				24.9
900	6710				37.7
Embryofetal Development – Rabbit [d]					
78	N/A	3010			2.4
155	N/A	7880			6.2
390	N/A	18700			14.7

a. Human $AUC_{0\text{-}24h}$ observed after 4th injection (Day 85) of RBP-6000 (300 mg buprenorphine) in multicenter, multiple-dose Study RB-US-12-0005
b. Human $AUC_{0\text{-}672h}$ observed after 1st injection of RBP-6000 (single 300 mg buprenorphine dose) in multicenter, multiple-dose Study RB-US-12-0005
c. Exposure margins were calculated using $AUC_{0\text{-}24h}$ values when available. In addition, AUC values from single-dose nonclinical studies were compared to single-dose human AUC, and AUC values from repeat-dose nonclinical studies were compared to multi-dose human AUC.
d. $AUC_{0\text{-}336h}$ is presented because $AUC_{0\text{-}24h}$ was not calculated for this study; Exposure margins were calculated using human $AUC_{0\text{-}672h}$ since $AUC_{0\text{-}336h}$ were not evaluated, and therefore represent more conservative margins.

2 Drug Information

2.1 Drug - Buprenorphine

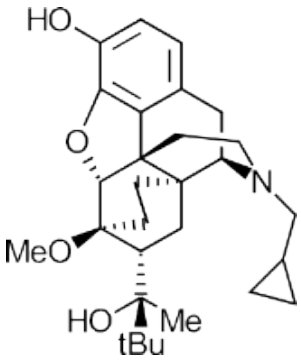
CAS Registry Number - 52485-79-7

Code Name - RX6029M

Chemical Name - (2*S*)-2-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-6-methoxy-6α,14-ethano-14α-morphinan-7α-yl]-3,3-dimethylbutan-2-ol

Molecular Formula/Molecular Weight - $C_{29}H_{41}NO_4$/467.6

Structure



Pharmacologic Class - partial opioid agonist (FDA EPC)

2.2 Relevant INDs, NDAs, and DMFs

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
18401	Buprenex (Buprenorphine)	DAAAP	0.3 mg base/mL (injection)	Approved	12/29/1981	Pain	INDIVIOR
20732	Subutex (Buprenorphine)	DAAAP	Up to 8 mg base (sublingual tablet)	Approved (Discontinued)	10/8/2002	Opioid Addiction	INDIVIOR
20733	Suboxone (Buprenorphine:Naloxone)	DAAAP	Up to 8 mg:2 mg base (sublingual tablet)	Approved (Discontinued)	10/8/2002	Opioid Addiction	INDIVIOR
22410	Suboxone (Buprenorphine:Naloxone)	DAAAP	Up to 12 mg:3 mg base (sublingual buccal film)	Approved	8/30/2010	Opioid Addiction	INDIVIOR

IND#	Drug	Status	Division	Indication	Stamp Date	Sponsor
107607	RBP-6000	active	DAAAP	For the treatment of opioid dependence	9/17/2010	INDIVIOR

DMF#	Subject of DMF	Holder	Submit Date	Reviewer's Comment
				(b) (4)

The application does not rely upon a DMF for drug substance information.

2.3 Drug Formulation

RBP-6000 drug product is a sterile, non-aqueous solution for subcutaneous injection indicated for the treatment of opioid use disorder. It contains 100 mg or 300 mg of buprenorphine (buprenorphine base) in the ATRIGEL Delivery System, which is

designed to deliver buprenorphine over a minimum of 28 days after subcutaneous injection. The ATRIGEL Delivery System is composed of a biodegradable 50:50 poly(DL-lactide-coglycolide) polymer (PLGH), (b) (4), and *N*-methyl-2-pyrrolidone (NMP) as a solvent.

Table 1. Drug Product Components and Function

List of Components for RBP-6000

Component	Reference to Quality Standards	Function
Buprenorphine	Ph. Eur.	API
50:50 Poly(DL-lactide-co-glycolide) (PLGH)	In-House	(b) (4)
N-methyl-2-pyrrolidone (NMP)	Ph. Eur.[a]	Solvent
(b) (4)		

API Active Pharmaceutical Ingredient
US-NF United States-National Formulary
Ph. Eur. European Pharmacopoeia
[a] Pharmaceutical Grade
[b] (b) (4)

Table 2. Quantitative Drug Product Composition

Nominal Composition of Delivered RBP-6000 Drug Product

Component	Nominal (% w/w)	100 mg Syringe (mg)	300 mg Syringe[a] (mg)
Buprenorphine	18	100	300
50:50 Poly(DL-lactide-co-glycolide)	(b) (4)	178	533
N-methyl-2-pyrrolidone		278	833
(b) (4)			
Approximate Delivered Volume (mL[b])	-	0.5	1.5

[a] Delivered mass does not equal the sum of the components due to rounding
[b] Approximate volume (b) (4)

2.4 Comments on Novel Excipients

Technically, NMP has been used in other FDA-approved drug products; however, one product is for acute use only and the second product is for an advanced cancer indication. PLGH has been used in numerous FDA-approved depot drug products via other routes of administration and is not considered novel for the SC route.

Poly(DL-lactide-co-glycolide) 50:50 (PLGH): PLGH is present in the drug product at 533 mg and at a concentration of (b) (4)% (w/w) based on the maximum recommended human dose (MRHD) of 300 mg, which may be administered not more frequently than once monthly.

PLGH 50:50 is listed in the FDA Inactive Ingredient Database (IID) for the intravitreal route with a max potency of 350 mg and a concentration of 35%, which is equivalent to the total daily intake to this excipient based on the MRHD of the approved product. PLGA 75:25 form is listed for the intramuscular route with a maximum potency of 33.1 mg and a concentration of 3.31% for a chronic use drug product, which translates to 99.3 mg based on an MRHD of 3 mL, or 11.25 mg leuprolide acetate.

PLGH is not listed in the IID as being used in a subcutaneously injected product; however, a Mercado search does indicate that this excipient is also used in an FDA-approved SC hormone delivery product. Nonetheless, this NDA included chronic repeat-dose SC toxicity studies in rat and dog which can be used to support the safety of the drug product formulation.

(b) (4)

N-Methyl-2-pyrrolidone (NMP): NMP is included in the drug formulation as a solvent with the highest level of 833 mg and at a concentration of (b) (4)% w/w based on the MRHD of 300 mg, which may be administered not more frequently than once monthly.

NMP is listed in the FDA IID for the SC and periodontal routes with a maximum potency of 26% for the SC route and no level reported for the latter. The approved SC product containing (b) (4)% NMP is indicated for an advanced cancer indication. Though considered a chronic indication, adequate nonclinical safety support for a chronic program cannot be assumed due to the risk:benefit profile for that product. Therefore, we have required that development programs proposing chronic indications provide data to fully qualify NMP in accordance with the excipient guidance.

This NDA included chronic repeat-dose SC toxicity studies in rat (Study RBLS-R04-60-09) and dog (Study RBL-C03-60-09) that tested NMP through its inclusion in the RBP-6000 formulation with monthly dosing.

Compendial
99.7% pure
Impurities – Individual (b) (4)%), Total (b) (4)%)

The following table was submitted by the Applicant. An updated version is included in Section 11 Integrated Summary and Safety Evaluation.

Table 3. Applicant's Safety Margins for RBP-6000, NMP, and PLGH

Safety Margins for ATRIGEL Delivery System and Individual Components

Study	Species	Highest Dose Qualified in Animal Studies (mg/kg)			Safety Factor Based on Human Equivalent Dose[a]		
		ATRIGEL Delivery System	NMP	PLGH	ATRIGEL Delivery System	NMP	PLGH
Micronucleus assay	Rat	2000	1200	800	14.1	13.9	14.5
Single-dose Toxicology	Rat	1992	996	996	14.1	11.6	18.1
	Rabbit	1143	720	423	16.2	16.7	15.4
	Dog	196	98	98	4.8	3.9	6.1
Repeat-dose Toxicology	Rat	1290	645	645	9.1	7.5	11.7
	Rabbit	1290	645	645	18.3	15.0	23.4
Fertility and embryo-foetal toxicity[b]	Rat (M)	1415[c]	863	552	10.0	10.0	10.0
	Rat (F)	2829	1726	1103	20.0	20.0	20.0
Pre- and post-natal development	Rat (P)	1415	863	552	10.0	10.0	10.0
	Rat (F_1)	1415	863	552	10.0	10.0	10.0
Embryo-foetal development	Rabbit	713	435	278	10.1	10.1	10.1

DART: Development and reproductive toxicity; F: Female; F_1: First generation litter; M: Male; NMP: *N*-methyl-2-pyrrolidone; P: Parenteral generation; PLGH: poly(lactide-co-glycolide) with carboxylic acid endgroup

[a] Safety factor is calculated by dividing the human equivalent dose from toxicity studies by the maximum human dose; human equivalent dose calculations based on FDA Guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." Maximum human dose of ATRIGEL Delivery System in RBP-6000 is 1366 mg or 22.8 mg/kg. Maximum human dose of NMP and PLGH in RBP-6000 is estimated to be 833 mg (13.9 mg/kg) and 533 mg (8.9 mg/kg), respectively.

[b] NOAEL is presented for each species tested.

[c] NOAEL for male reproductive and fertility indices was 2829 mg ATRIGEL/kg body weight and represent 20-fold safety margins for both NMP and PLGH; however, the NOAEL of 1415 mg/kg was due to adverse effects on sperm function (percent abnormal sperm).

2.5 Comments on Impurities/Degradants of Concern

Drug Substance: The buprenorphine drug substance (DS) is manufactured by Indivior UK Limited. No DMF is referenced. All DS information has been submitted to the NDA.

The original NDA submission proposed DS specifications of NMT (b) (4)% for 3 specified impurities, which the Applicant justified as being within the Ph.Eur. monograph limits. However, we informed the Applicant in the 60-day filing letter and in subsequent information requests that the specifications needed to be (b) (4) NMT 0.15%, in accordance with the ICH Q3A(R2) qualification threshold. The Applicant submitted revised DS specifications, which included limits of NMT (b) (4)% for (b) (4) and (b) (4), which are acceptable. The limit for (b) (4)

(b) (4) Therefore, the DS impurity specifications are acceptable.

The proposed specification for the residual solvent (b) (4) of NMT (b) (4) %, which corresponds to (b) (4) ppm, is acceptable as it is within the concentration limit indicated for this Class 3 solvent per the ICH Q3C guidance.

Table 4. Drug Substance Specifications

Attribute	Analytical Procedure	Acceptance Criteria	
		Min	Max
Physical description	Visual assessment	White to pale cream powder, free from any visible particulate contamination	
Identification	FTIR Ph. Eur. 2.2.24	Shall be positive	
Identification	HPLC	Shall be positive	
Appearance of solution	Ph. Eur. 2.2.1 Ph. Eur. 2.2.2	Clear and colourless	
Specific optical rotation (with respect to dried substance)	Ph. Eur. 2.2.7	(b) (4)	
Assay % w/w (b) (4)	HPLC		
Related substances[1] (% w/w) (b) (4) Any individual unspecified impurity Total	HPLC		
Water content (% w/w)	Coulometric Ph. Eur. 2.5.32		
Residual solvent – (b) (4) (% w/w)	GC		
Residue on ignition (% w/w)	Ph. Eur. 2.4.14		0.1
Bioburden Total aerobic microbial count (CFU/g) Total yeasts and moulds count (CFU/g)	Ph. Eur. 2.6.12	(b) (4)	
Bacterial endotoxin (EU/g)	Ph. Eur. 2.6.14 Method C		

CFU = colony forming unit
EU = endotoxin unit
FTIR = Fourier transform Infrared spectroscopy
GC = gas chromatography
HPLC = high performance liquid chromatography
w/w = weight/weight

[1] Assignment of related substances against Ph. Eur. monograph (b) (4)

Drug Product: The proposed drug product specifications are summarized in the Applicant's tables below for nominal composition and specifications. Note that the proposed DP degradant specifications for (b) (4) at NMT (b) (4) % and for (b) (4) at NMT (b) (4) % (b) (4) the appropriate ICH

Q3B(R2) qualification threshold of NMT 0.2%, or 3 mg total daily intake (whichever is lower), based on the MRHD of RBP-6000 at 300 mg buprenorphine.

Table 5. Drug Product Specifications

RBP-6000 Drug Product Specification (100 mg and 300 mg)

Attribute	Acceptance Criteria Min Max	Analytical Procedure
Colour	Colourless to Amber[a]	Visual
Appearance and Description	Clear, viscous solution.	Visual
Identification[b]	Matches standard reference scan	^{1}H-NMR Ph. Eur. 2.2.33
Buprenorphine Content (% of label claim in mg of buprenorphine in dispensed product)	(b) (4)	HPLC
Related Substances (Relative Weight %) (b) (4) Other Individual Total Report any value (b) (4) %		HPLC
Content Uniformity (% of label claim in mg of buprenorphine in dispensed product)[b]	Meets Ph. Eur. 2.9.40 Content Uniformity Criteria	HPLC
Molecular Weight (M_w) (kDa)	(b) (4)	GPC
Polydispersity (M_w/M_n)		GPC
NMP Content (% w/w)		HPLC
(b) (4)		(b) (4)
Bacterial Endotoxins (EU/unit)[b]		Ph. Eur. 2.6.14 Method C
Sterility		Ph. Eur. 2.6.1

(b) (4)

To justify the proposed specification of NMT (b) (4)% for (b) (4) the Applicant noted that (b) (4) concentrations of (b) (4) are significantly higher after sublingual (SL) buprenorphine administration than after SC administrations of RBP-6000. This was demonstrated in Clinical Study RB-US-12-0005 where subjects exhibited a C_{max} of (b) (4) ng/mL after the fourth monthly SC dose of 300 mg RBP-6000 compared to a C_{max} of (b) (4) ng/mL after multiple doses of 24 mg SL buprenorphine. The Applicant also provided (Q)SAR data, using expert rule-based and statistically-based methodology in accordance with ICH M7, indicating that (b) (4) is

not predicted to be genotoxic via the Ames and it also predicted [redacted] would be no more toxic [redacted]

The Applicant also noted that [redacted] was tested in single-dose and multi-dose nonclinical SC toxicity studies in rats and dogs at up to [redacted]%. From a systemic safety perspective, the human equivalent dose (HED) level of the degradant tested at the NOAEL of the dog chronic study provides adequate coverage for the potential level that could be exposed to humans based on the proposed specification as illustrated in the Applicant's table below.

Table 6. Comparison of Impurity [redacted] Levels in Nonclinical Batches and Proposed Clinical Specifications

Toxicology Study	Species	BUP NOAEL (mg/kg)	[redacted] Level			BUP Maximum Human Dose (mg/kg)	[redacted] Level		Safety Margin[b]
			Highest Conc. in RBP-6000 (%)	Qualified Dose (mg/kg)	Human Equivalent Dose (mg/kg)[a]		Proposed Limit (%)	Maximum Dose at Limit (mg/kg)	
SD Tox	Rat	[redacted]							
	Dog								
MD Tox	Rat								
	Dog								
Genotox	Rat								
Male Fertility & Reproduction Tox	Rat								
Female Fertility & Reproduction	Rat								
Maternal & Developmental Tox	Rat								
Pre- & Post-natal Tox	Rat								
Pre-natal Tox	Rabbit								

BUP: Buprenorphine; MD: Multiple-dose; [redacted]; SD: Single-dose

[a] Human equivalent doses were calculated based on the FDA guidance *"Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"*

[b] Exposure ratio at maximum dose (at the specification limit) relative to human equivalent dose qualified in animal study

[c] Exit test result for RBP-6000 Lot 0042-001

From a local safety perspective, these data do not adequately characterize the potential local toxicity of [redacted] at the level proposed. However, the risk for local toxicity would not be expected to be any worse than for [redacted] based on the following considerations. [redacted]
and (Q)SAR analyses predicted comparable toxicity profiles for both compounds.

Figure 1. Structures (b) (4)

The degradant (b) (4) In addition, the intended SC route does not represent a uniquely sensitive tissue compared to the epidural, intranasal, or ocular routes, e.g., it is not enriched with neural tissue, and clinically, the same site will not be repeatedly injected. As noted above, the systemic safety of (b) (4) is covered. Given the risk benefit, the existing local tissue reactions, the likelihood that small elevations of local (b) (4) will not significantly alter the local tissue effects, the ability of these tissues to recover from minor insult, further nonclinical studies to characterize the impact of elevations of (b) (4) are not necessary. Therefore, based on all of these considerations, this reviewer considers the proposed specification for (b) (4) to be acceptable for this proposed drug product.

(b) (4)

According to the Applicant, the (b) (4) impurity (b) (4) is a degradation product observed in the drug product that appears to form most readily in forced degradation studies due to oxidative peroxide conditions. To justify the proposed specification of NMT (b) (4)%, the Applicant provided (Q)SAR data that predicted the degradant to be negative for mutagenicity in *E. Coli* and Salmonella strains. They also noted that concentrations of the (b) (4) impurity were present in nonclinical lots of RBP-6000 used in pivotal toxicology studies at up to (b) (4)% in repeat-dose chronic studies and up to (b) (4)% in reproductive and developmental toxicity studies as shown in the table below. From a systemic safety perspective, the human equivalent dose (HED) level of the degradant tested at the NOAEL of the dog chronic study provides adequate coverage for the potential level that could be exposed to humans based on the proposed specification as illustrated in the Applicant's table below.

Table 7. [redacted] Levels in Nonclinical Batches and Clinical Specifications

Toxicology Study	Species	BUP NOAEL (mg/kg)	[redacted] Level			BUP Maximum Human Dose (mg/kg)	[redacted] Level		Safety Margin[b]
			Highest Conc. in RBP-6000 (%)	Qualified Dose (mg/kg)	Human Equivalent Dose (mg/kg)[a]		Proposed Limit (%)	Maximum Dose at Limit (mg/kg)	
SD Tox	Rat	[redacted]							
	Dog								
MD Tox	Rat								
	Dog								
Genotox	Rat								
Male Fertility & Reproduction Tox	Rat								
Female Fertility & Reproduction	Rat								
Maternal & Developmental Tox	Rat								
Pre- & Post-natal Tox	Rat								
Pre-natal Tox	Rabbit								

BUP: Buprenorphine; MD: Multiple-dose; [redacted] SD: Single-dose

[a] Human equivalent doses were calculated based on the FDA guidance *"Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"*

[b] Exposure ratio at maximum dose (at the specification limit) relative to human equivalent dose qualified in animal study

[c] Exit test result for RBP-6000 Lot 0042-001

From a local safety perspective, the concentration tested in the chronic dog study at [redacted]% is only slightly lower than the proposed specification of NMT [redacted]%. [redacted]

Therefore, the proposed specification is considered acceptable.

Table 8. Comparison of Structures [redacted]

[redacted]

[redacted]

Elemental Impurities

The Applicant analyzed the finished drug product for elemental Impurities as per ICH Q3D (see Report FC-RPT-0280R). There are no elemental impurities that exceed the permitted daily exposures for a parenteral drug product.

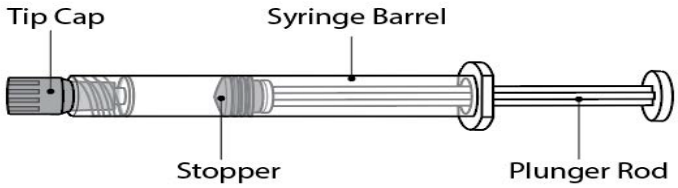
Container Closure System

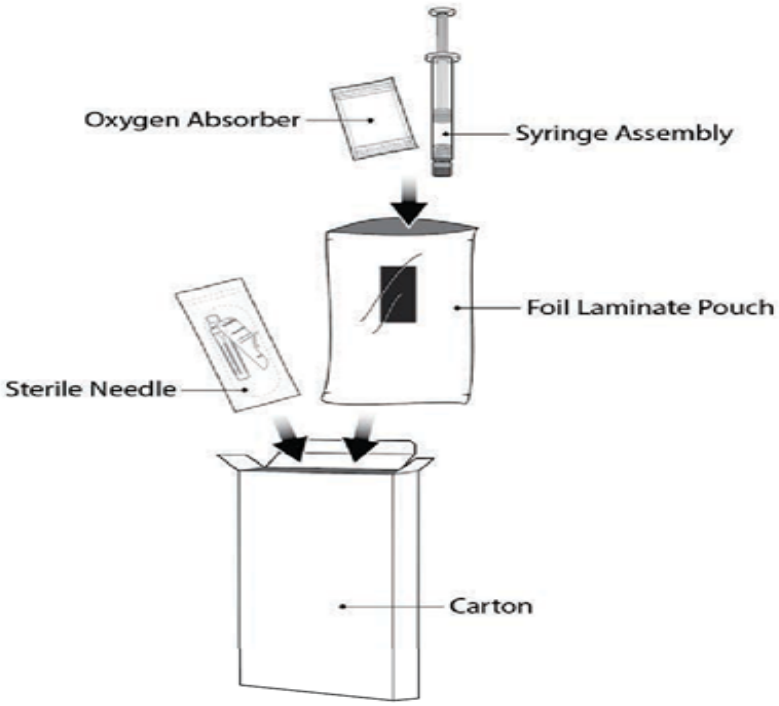
RBP-6000 is manufactured in both 100 mg and 300 mg dosage strengths, both of which are comprised of 18% buprenorphine/ATRIGEL® Delivery System formulation presented in a sterile pre-filled syringe assembly and a pre-packaged sterile needle for injection.

The RBP-6000 product is (b) (4)

(b) (4)

Figure 2. Drug Product Configuration





A description of the container closure system, including the device components, for the commercial RBP-6000 100 mg and 300 mg dosage strengths is provided in the table below.

Table 9. Details of Drug Product Container Closure System

Drug Product	Dosage Strength	Component Description	Sub-component Description and Use
RBP-6000	100 mg	1 mL Sterile Pre-filled Syringe Assembly (b) (4) (b) (4)	(b) (4)
			(b) (4)
		Safety Needle	(b) (4) Needle, 19 gauge 5/8 inch (b) (4)
RBP-6000	300 mg	2.25 mL Sterile Pre-filled Syringe Assembly (b) (4) (b) (4)	

Drug Product	Dosage Strength	Component Description	Sub-component Description and Use
		(b) (4)	
		Safety Needle	(b) (4) Needle, 19 gauge 5/8 inch (b) (4)

Extractables/Leachables Evaluation

To justify the safety of leachables arising from the primary or secondary container closure systems or from any upstream manufacturing processes, the Applicant performed an extensive series of extractables and leachables studies, which are listed in the table below:

Study Title	Report
Summary Extractables Report for RBP-6000 (1568 pages)	FC-RPT-0283R
Final Report for a Controlled Extraction Study on Syringe (b) (4) and Stoppers/Tip Caps in N-Methyl-2-Pyrrolidinone (106 pages – Attachment 1)	FC-RPT-0274R
Extractables Study of (b) (4) Syringe Components, Reckitt Benckiser Pharmaceuticals (135 pages – Attachment 2)	FC-RPT-0121R
Leachables and Extractables Assessment of (b) (4) Syringe for Storage/Delivery of RBP-6000 (20 pages – (b) (4) – Attachment 3)	FC-RPT-0288R
Assessment of Safety for Extractable Compounds of Syringe (b) (4) and Stoppers/Tip Caps in N-Methyl-2-Pyrrolidone (NMP) for RBP-6000 (210 pages – Attachment 4) – QSAR work	FC-RPT-0285R
Final Report for the Extractables Screening Study on ATRIGEL® Drug Product Vehicle (97 pages – Attachment 5)	FC-RPT-0279R

Assessment of Safety for Extractables for (b) (4) Syringes Filled with N-Methyl-2-Pyrrolidone or RBP-6000 (11 pages – Attachment 32/last one))	FC-RPT-0317R
Container Closure Integrity Testing for RBP-6000 Drug Product (13 pages)	FC-RPT-0296R.01
Leachables Testing of the RBP-6000 Product (230 pages)	**FC-RPT-0321R**

Extractables Testing

The series of extractions studies were conducted to evaluate the primary container closure syringe system filled with NMP, manufacturing process product-contact parts exposed to NMP, and secondary packaging components that were tested for volatile extractables. The Applicant justified the use of NMP since it is the relevant solvent in the drug product, comprising (b) (4)% of the formulation. Our CMC colleagues found this acceptable.

Appropriate analytical methodologies were used in these studies and included direct injection Gas Chromatography with Mass Spectrometric detection (GC-MS) for semi-volatile compounds, headspace GC-MS for volatile compounds, and Liquid Chromatography with Ultra-Violet and Mass Spectrometric detection (LC-UV-MS) for nonvolatile compounds. These techniques were primarily targeted towards organic extractable compounds and are suitable for the evaluation of extractables from the drug container closure system (b) (4). Elemental impurities were assessed using Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) or related techniques.

The Applicant proposed a toxicological threshold of concern of (b) (4)mcg/day with the following rationale based on intermittent dosing.

1. A lifetime 70 years (25,550 days divided by 28 days) of monthly doses (913 doses/lifetime), which is also the dosing interval for RBP-6000, is equivalent of 2.5 years of daily doses assuming all the extractable is absorbed in a single dose for the monthly product.

2. The ICH M7 Guidance, M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, May 2015, which is for drug impurities, can be applied to extractables/leachables as is warranted in this case.

3. A Threshold of Toxicological Concern (TTC) -based acceptable intake of 1.5 mcg/day is considered to be protective for a lifetime of daily exposure to a mutagenic impurity. Per ICH M7, the Acceptable Intake for an Individual Impurity relative to duration of exposure is as listed below.

Duration of treatment	≤1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [mcg/day]	120	20	10	1.5

The Applicant considers the duration of exposure at 2.5 years of daily exposure with 10 mcg/day allowed for impurity in drug with structural alert or positive Ames test but proposed to use a potential exposure level o (b) (4) mcg/day for these safety assessments based on advice provided by DAAAP with respect to the need to qualify the extractables/leachables for general toxicity as well as genotoxicity.

Reviewer's note:
The Applicant's proposed safety concern threshold of (b) (4) mcg/day (e.g., AET= (b) (4) mcg/mL based on 1.5 mL volume at MRHD of 300 mg) is appropriate from a general toxicity perspective. However, from a genetox perspective, 1.5 mcg/day is generally considered an appropriate qualification threshold for a chronically administered drug. It is acknowledged that the product is only administered intermittently at a monthly interval over a lifetime. If all of the leachable compounds are exposed only on the day of administration, then the Applicant's proposal to base the genetox on a shorter cumulative lifetime exposure may be acceptable. However, if the leachables are exposed slowly as the active ingredient is intended to be via the polymer depot, then lifetime exposure may actually exceed the estimated 2.5 years. For instance, if the leachable compounds are exposed over a 4-day period following each dose administration, then lifetime exposure would be 10 years, resulting in an appropriate genetox threshold of 1.5 mcg/day. However, since the total leachable exposure may occur over several days (<4 days per treatment), a higher total exposure may be acceptable. This may ultimately end up translating to a comparable threshold as proposed by the Applicant. Therefore, the reviewer considers the Applicant's proposed qualification threshold for leachables/extractables of (b) (4) mcg/day to be acceptable for this drug product. This Less Than Lifetime (LTL) exposure paradigm is consistent with the recommendations outlined in ICH M7, which states "In the case of intermittent dosing, the acceptable daily intake should be based on the total number of dosing days instead of the time interval over which the doses were administered and that number of dosing days should be related to the relevant duration category in Table 2. For example, a drug administered once per week for 2 years (i.e., 104 dosing days) would have an acceptable intake per dose of 20 mcg."

The primary container closure system, including the (b) (4) syringe, (b) (4) cap, and (b) (4) plunger were filled with NMP and stored at 75ºC for 23 days to represent a worst-case scenario. The methods were deemed adequate by the CMC review team.

Results of Extraction Studies

A summary of the results from the NMP extractables studies for organic compounds that were at or above the threshold (AET) of (b) (4) mcg/mL in the extract is presented in the table below. This AET corresponds to the requested safety concern threshold of (b) (4) mcg per injection for the largest volume to be administered (300 mg RBP-6000 dose) Note that the concentrations seen in the extractables studies are likely higher than what would be expected in the product formulation in leachables testing, due to the higher extraction temperatures used in the studies, compared to the commercial product storage temperature of 5°C. The last column shows the Applicant's Margin of Safety (MOS) calculated for each compound. This value is the ratio of the Permissible Daily Exposure (PDE) divided by the maximum amount delivered per dose.

Figure 3. Summary of NMP Extractables above (b) (4) mcg/day

Summary of NMP Extractables (b) (4) µg/mL

Retention Time (min)	Tentative Identification	Estimated Concentration (µg/mL)	Margin of Safety
(b) (4)			

(b) (4)

Note that the Applicant apparently decided arbitrarily that Margin of Safety (MOS) levels below (b) (4) were considered for leachables testing while most of the compounds that were well above this level were not included in leachables testing.

The Applicant did not elect to target every compound identified over the AET in the extraction studies in their leachable studies. They only analyzed the long-term stability samples for compounds that were detected in the extraction studies that did not have a margin of safety of at leas (b) (4) based on their PDE determinations. As such, the section below summarizes the extraction study screening process and reviews the toxicological risk assessments for those compounds present in the extraction studies over the qualification threshold of (b) (4) mcg/day but not evaluated in the leachable studies.

Our toxicological risk assessment based on the extractables data is summarized in the table below.

Table 10. Summary of Safety Evaluation of Extractable Compounds

Extractables Compound	Ret. Time (min)	Estimated Conc. (mcg/mL)	Total Exposure[a] (mcg)	Total Daily Exposure over month[b] (mcg/day)	PDE (mcg/day)	Safety Margin	Safety Justification
(b) (4)							Adequate. Below QT in leachables
							Adequate. Within PDE
							Adequate. Within PDE
							Adequate. Below QT in leachables
							Adequate. Below QT in leachables
GC/MS Headspace							
(b) (4)							Adequate. Within PDE
							Adequate. Within PDE

(b) (4)							Adequate. Within PDE
							Adequate. Within ICH Q3C PDE
							Adequate. Within PDE
							Adequate. Within PDE
							Adequate. Within PDE
							Adequate. Within PDE
							Adequate. Within PDE
							Adequate. Below QT in leachables
							Adequate. Below QT in leachables
							Adequate. Within IID
							Adequate. Below QT in leachables
LC/MS Electrospray Negative							
(b) (4)							Adequate. Within IID
							Adequate. Within IID
LC/MS Electrospray Positive							
(b) (4)							Adequate. Within PDE

a=Maximum exposure based on 1.47 dose volume at MRHD of 300 mg
b=Daily exposure calculated by dividing total leachable exposure by 28 days
QT=qualification threshold

The risk assessments for select extractables not monitored in leachables study are below. The reader is referred to the Leachable Testing Section for the risk assessments on all other compounds.

1. (b) (4)

(b) (4)

Neither the Applicant nor this reviewer was able to find information specifically about the toxicity of 1 (b) (4). The Applicant cited a published article by (b) (4))[1], which compiled toxicological information for over 540 extractables identified in polymers used in pharmaceutical applicants in order to assess risk using relevant tox endpoints. In this article, it was acknowledged that there was no published toxicity information for this compound, but the authors predicted it would have low oral toxicity based on results of Cramer classification and a Benigni/Bossa prediction of non-mutagenic using Toxtree, which is in silico software capable of making structure-based predictions for a number of toxicological endpoints that was developed by the Joint Research Centre's European Chemical's Bureau.

The Applicant also referred to data for a similar compound, (b) (4), for this risk assessment. Based on the comparable structure, as shown below, this reviewer considers this acceptable.

(b) (4)

(b) (4) is used as a (b) (4) in the chemical industry. (b) (4) is known to be moderately toxic by inhalation, and is also an eye and respiratory irritant. (b) (4) was negative for mutagenicity in the Ames test. The Applicant calculated a PDE of (b) (4) mg/day using LD_{50}= (b) (4) mg/kg from a single-dose rat IP study. However, repeat-dose toxicity information was found in a European Chemicals Agency (ECHA) safety assessment that included an assessment of a 28-week repeat-dose oral toxicity study[2]. The 28-day study was conducted using Sprague Dawley rats (N=12/sex/group) administered (b) (4) at doses of 1.5, 5, and 50 mg/kg/day in corn oil. The report concluded that the NOEL in males was (b) (4) mg/kg/day based on findings that included periportal hepatocyte hypertrophy at 50 mg/kg, atrophy of the seminiferous tubules and hyperplasia of Leydig cells at 50 mg/kg group, and a decrease in sperm

1 (b) (4)

2 (b) (4)

count in the lumen of the ductus epididymis at 50 mg/kg. The NOEL in females was (b) (4) mg/kg/day based on findings including periportal hepatocyte hypertrophy at 50 mg/kg.

For PDE calculations, the NOEL from male rats from the 28-day study was considered the most conservative.

PDE = _ (b) (4) mg

Based on the level of (b) (4) detected in extraction studies and the MRHD of RBP-6000, the potential exposure is (b) (4) mcg. The PDE= (b) (4) mg provides a margin of 21X.

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	10
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1

F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	1
F6 (if relying on LD50)	10	

2. (b) (4)

(b) (4)

(b) (4)

This reviewer identified an OECD Screening Information Dataset document (October, 1998)[3] that summarized toxicity information, including acute, repeat-dose, reprotox, and carcinogenesis study information. The report noted that the acute oral toxicity of (b) (4) appears to be low. This is consistent with the Applicant's LD_{50} info in the table below, which was from the (b) (4) citation[4]. The report also noted that no gross pathological or histopathological effects were reported at doses up to (b) (4) mg/kg in an OECD combined rat repeat-dose and reproductive/developmental toxicity screening test. This screen appears to generally consist of a 4-week dosing duration based on a Google search. In addition, (b) (4) was not mutagenic in the Ames with or without metabolic activation. None of these studies were provided or appear to be accessible by this reviewer, so an independent assessment of any data from studies with (b) (4) cannot be made. However, OECD is a regulatory body that is considered reliable for safety assessment purposes.

3 (b) (4).pdf

4 (b) (4)

Assay	Species	Route	Exposure	Effect	Source
Acute Toxicity	Rats	Oral	Single	[redacted] (b) (4)	
	Rats	Oral	Single		
	Guinea Pig	Dermal	Single		
	Rabbit	Oral	Single		
	Rat	Oral	Single		
	Guinea Pig	Dermal	Single		
	Mouse	Oral	Single		
	Rat	Oral	Single		
	Rat	**IV**	**Single**		
	Guinea Pig	SC	Single		
	Rabbit	Oral	Single		
	Rat	Inhalation	Single (6H)		
	Rabbit	Dermal	Single		
	Rabbit	Dermal	Single		
	Guinea Pig	Dermal	Single		

The Applicant's PDE of (b) (4) mg/kg was based on LD_{50}= (b) (4) mg/kg from a single-dose rat IV study. Although not ideal to base on LD_{50}, it appears this would be more conservative than using the (b) (4) mg/kg NOAEL value noted above for the safety assessment. An extra safety factor of 10 can be applied since this is not based on NOAEL/LOAEL information. Based on the factors used in the table below, this reviewer's calculation of the PDE is as follows:

PDE = _ (b) (4) mg

Based on the level of (b) (4) detected in extraction studies and the MRHD of RBP-6000, the potential exposure is (b) (4) mcg. The PDE= (b) (4) mg provides a margin of 66X.

Modifying Factors	Factor Recommendations	Factor Applied
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent)	10

	1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	10
F6 (if relying on LD50)	10	10

3. (b) (4)

(b) (4)

(b) (4) The European Chemicals Agency (ECHA) has performed a safety assessment on (b) (4) which included an evaluation of acute toxicity, 13-week repeat-dose oral toxicity, reproductive and developmental toxicity, and genetic toxicity studies. In an acute oral study in Wistar rats, there was no lethality at doses up to (b) (4) mg/kg. In a 13-week oral study in Sprague Dawley rats, the NOAEL was considered to be the highest dose tested at (b) (4) mg/kg. Pregnant female rats administered drug via inhalation at up to (b) (4) pm did not show adverse effects on either dams or fetuses. Moreover, (b) (4) was found to be negative in the Ames test[5].

5 (b) (4)

Based on this information, the 13-week NOAEL was used for PDE calculations as follows:

PDE = _ (b) (4) mg

Based on the level of (b) (4) detected in extraction studies and MRHD of RBP-6000, the potential exposure is (b) (4) mcg. The PDE= (b) (4) mg provides a margin of 80,000X.

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	5
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	1

F6 (if relying on LD50)	10	

4. (b) (4)

(b) (4)

(b) (4) is a (b) (4) listed in the ICH guidance for industry: Q3C document with a PDE of (b) (4) mg/day. Based on the level of (b) (4) detected in extraction studies and the MRHD of RBP-6000, the potential exposure is (b) (4) mcg. The PDE=(b) (4) mg/day provides a margin of 250X.

5. (b) (4)

(b) (4)

(b) (4) is a (b) (4) listed in the ICH guidance for industry: Q3C document with a PDE of (b) (4) mg/day. Based on the level of (b) (4) detected in extraction studies and the MRHD of RBP-6000, the potential exposure is (b) (4) mcg. The PDE= (b) (4) mg/day provides a margin of 6258X.

6. Unknown (b) (4)

The Applicant's risk assessment stated "The unknown extractable lacks proper identification. However, (b) (4) are generally not genotoxic. In this report, (b) (4) is another example of (b) (4) that can serve as surrogate compound. Any potential toxicity from these compounds would be expected to result from a similar mechanism. (b) (4) with an ICH Q3C PDE of (b) (4) mg/day or (b) (4) µg/day should be considered appropriate." This is supported by a published review (b) (4)

[6]. Based on this assessment, adding up all the unknown (b) (4) and assuming they are (b) (4) is likely a conservative approach.

7. (b) (4)

6 (b) (4)

(b) (4)

(b) (4) has not been tested for genetic toxicity and there are limited toxicity data available.

The Applicant provide a PDE assessment based on a rat 4-week repeat-dose toxicology study with a reported TD_{LO} of (b) (4) mg/kg and a PDE of (b) (4) mcg/day; however, the Applicant did not provide the reference for the study and therefore we cannot independently evaluate the conclusions or adequacy of the study report.

A search in Google identified a study by (b) (4) et al published in 2014 that tested either (b) (4) in a 13-week rat inhalation study[7]. Animals were dosed 6 hours per day, 5 days per week. There were no deaths or clinical signs or evidence of altered urinalysis, hematology, or clinical chemistry. Kidney and liver weights were increased in a dose dependent manner in males. There was a dose dependent increase in evidence of nephrotoxicity (cystic change in renal tubules, regenerative tubules, inflammatory cell infiltrates, and necrosis at all doses. The NOAEL in females was estimated to be the HD of (b) (4) ppm; the NOAEL in males was (b) (4) ppm. The full text was not available online. The exposure to (b) (4) ppm in these animals could be used to determine the theoretical dose using the method Derelanko[8].

The theoretical dose from an inhalation exposure:

Dose (mg/kg) = C x MV x T x D/BW

Where C = concentration in mg/L (ppm) = (b) (4) mg/L
MV = minute volume (L/min) = 54 mL/min = 0.054 L/min (Derelanko 3 mo rat)
T = time of exposure (min) = 360 min
D = deposition fraction into the respiratory tract = assume (b) (4) %)
BW = body weight = females ~232 grams per Derelanko = 0.232 kg

Dose (mg/kg) = (b) (4) mg/kg

7 (b) (4)

8 Derelanko MJ (2008) The Toxicologist's Pocket Handbook. New York: Informa Healthcare.

Modifying Factors	Factor Recommendations	Factor Applied
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	5
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	10 (no NOAEL, LOAEL unclear. Report not available.)
F6 (if relying on LD50)	10	

$$PDE = \frac{NOEL\ (mg/kg) \times Weight\ Adjustment\ (50\ kg)}{F1 \times F2 \times F3 \times F4 \times F5}$$

PDE = (b) (4) mcg/day

The levels detected are acceptable based on a PDE approach.

8. (b) (4)

(b) (4)

The Applicant's PDE assessment stated that there were no data on (b) (4) and therefore they proposed a PDE based on data from (b) (4)

(b) (4)

They stated that a 4-week rat oral toxicology study determined a TD_{LO} of (b) (4) mg/kg and estimated a PDE of (b) (4) mcg/day. However, they did not provide any reference for this study report and therefore we cannot independently confirm or evaluate this assessment.

We were able to identify a published 4-week inhalation toxicology study in 6-week old Sprague-Dawley rats with (b) (4) et al. treated Sprague Dawley rats with (b) (4) for 6 hours per day, 5 days per week for 4 weeks. They report that there were no changes in mortality, clinical signs, body weights, hematology, or gross or microscopic pathology. The study reports some statistically significant changes in food consumption, clinical chemistry, and organ weights were noted. But conclude that the NOAEL was above (b) (4) ppm, 6 h/day, 5 days per week. The exposure to (b) (4) ppm in these animals could be used to determine the theoretical dose using the method Derelanko.

The theoretical dose from an inhalation exposure:

Dose (mg/kg) = C x MV x T x D/BW

Where C = concentration in mg/L (ppm) = (b) (4) mg/L
MV = minute volume (L/min) = 54 mL/min = 0.054 L/min (Derelanko 3 mo rat)
T = time of exposure (min) = 360 min
D = deposition fraction into the respiratory tract = assume 0.1 (10%)
BW = body weight = females ~232 grams per Derelanko = 0.232 kg

Dose (mg/kg) = (b) (4) mg/kg

Modifying Factors	Factor Recommendations	Factor Applied
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	10
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	5
F6 (if relying on LD50)	10	

$$PDE = \frac{\text{NOEL (mg/kg)} \times \text{Weight Adjustment (50 kg)}}{F1 \times F2 \times F3 \times F4 \times F5}$$

PDE = (b) (4) mcg PDE

9. (b) (4)

(b) (4)

The Applicant's PDE assessment was based on a rat 4-week oral toxicology study with a reported TD_{LO} of (b) (4) mg/kg which they used to predict a PDE of (b) (4) mcg/day. However, they did not provide a reference for this citation and therefore we cannot independently evaluate the adequacy of the study or verify the assessment.

We identified data to determine that (b) (4) tested negative in the Ames assay[9]. We were able to identify a published used by the State of Michigan to establish a reference dose for methyl (b) (4) (b) (4) et al apparently dose male Fischer rats with either (b) (4) mg/kg undiluted (b) (4) by oral gavage 5 days per week for 4 weeks[11]. The dose of (b) (4) mg/kg produced a reduction in body weight and was deemed a NOAEL/LOAEL. The state of Michigan adjusted the NOAEL for the number of days of the week (b) (4) mg/kg) which may well be the reference used by the Applicant.

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	10

9 (b) (4)

10 (b) (4) .pdf

11 (b) (4)

F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	5
F6 (if relying on LD50)	10	

PDE = NOEL (mg/kg) x Weight Adjustment (50 kg)
F1 x F2 x F3 x F4 x F5

PDE = (b) (4) PDE

10. (b) (4)

(b) (4)

The Applicant proposed a PDE assessment based on a 4-week oral repeat-dose toxicology study and described a TD_{LO} of (b) (4) mg/kg and estimated a PDE of (b) (4) mcg/day; however, they did not identify the study or provide a reference and therefore we cannot independently evaluate the adequacy of the study.

We have identified study reports that indicate that (b) (4) tested negative in the Ames assay and has been tested in inhalation rat and mouse carcinogenicity studies by the National Toxicology Program[12]. In the NTP studies, F344

[12] (b) (4)

male rats were treated with (b) (4) ppm (b) (4) and female rats were treated with (b) (4) ppm (b) (4) 6 hours per day, 5 days per week for 105 weeks. The incidence of renal tubule adenomas and adenoma or carcinoma and of benign and malignant pheochromocytoma of the adrenal medulla were reported in (b) (4) ppm males. There was also significant nephropathy and adrenal pheochromocytoma in these animals. There were no tumors noted in female rats. In the 2-year mouse study, B6C3F1 mice were exposed to (b) (4) ppm (b) (4) for 6 hours per day, 5 days per week, for 105 weeks. There was an increased incidence of hepatocellular neoplasms in the (b) (4) ppm group which correlated with centrilobular hypertrophy, necrosis, syncytial alterations, and erythrophagocytosis of the liver in the (b) (4) ppm males. Uterine polyps were noted in female mice. The LD males could be used to estimate a NOEL for tumors in rats. The exposure to (b) (4) ppm in these animals could be used to determine the theoretical dose using the method Derelanko.

The theoretical dose from an inhalation exposure:

Dose (mg/kg) = C x MV x T x D/BW

Where C = concentration in mg/L (ppm) = (b) (4) mg/L
MV = minute volume (L/min) = 54 mL/min = 0.054 L/min (Derelanko 3 mo rat)
T = time of exposure (min) = 360 min
D = deposition fraction into the respiratory tract = assume 0.1 (10%)
BW = body weight = males ~220 grams per study report = 0.22 kg

Dose (mg/kg) = (b) (4) mg/kg

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study)	1

	10 (shorter than 3 months)	
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	10
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	5
F6 (if relying on LD_{50})	10	

PDE = NOEL (mg/kg) x Weight Adjustment (50 kg)
F1 x F2 x F3 x F4 x F5

PDE = (b) (4) mcg PDE

Although not all extractables were targeted by the Applicant in the leachable studies, several were targeted as followings:

1. (b) (4)
2.
3.
4.
5.
6.

The safety assessments for these compounds will be based on the results of the leachables studies.

==========

Screening Leachables Studies (described in FC-RPT-0321R)

In addition to the targeted leachables assessments discussed below, the Applicant conducted a broad screening study to look for (b) (4) that were observed in the extraction studies using a lower limit of (b) (4) mcg/mL.

Stability samples of RBP-6000 Lot 145 (samples stored at 5ºC for 27 months, (b) (4)).

Numerous compounds were detected, as summarized in the Applicant's tables below:

Figure 4. Summary of Results of Leachables Screening Study

Table 11: Direct-Injection GC/MS Results

Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
(b) (4)		

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(b) (4)

The Applicant's table below summarizes the results of the headspace analysis and the tentative identification of the compounds detected.

Table 12: Headspace GC/MS Results

Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
(b) (4)		

The Applicant evaluated the above lists of compounds and identified the most likely source of the compounds. As noted in the table above, most of the chemicals detected were believed to be related to the ingredients in the product (NMP solvent, PLGH polymer, or buprenorphine drug substance) or the syringe (b) (4). The remaining compounds, presumably from the container closure system, that are above the (b) (4) mcg/day SCT are listed in the table below.

Table 11. Summary of Non-Product Related Compounds from Screening Study Above (b) (4) mcg/day

Retention Time (min)	Tentative Identification	Estimated Concentration (mcg/mL)	MDD (mcg/day)	PDE (mcg)	Margin	Comment on Adequacy
(b) (4)						

(b) (4) was included in the targeted leachable studies and is discussed below. A toxicological risk assessment was provided for the (b) (4) (b) (4)

(b) (4)

As the compound has only tentatively been identified, the Applicant submitted risk assessment based on (b) (4)

(b) (4)

(b) (4) tested negative in the Ames assay[13]. The Applicant's PDE assessment is based on a 14-day rat oral toxicology study and a presumed TD_{LO} of (b) (4) mg/kg and estimated a PDE of (b) (4) mg/day. However, the reference was not provided and therefore, we could not independently evaluate the conclusions or adequacy of the study.

The Hazardous Substances Database (HSDB) identified an EPA report that included a 7-month oral gavage study in which rats were dosed with a 20% aqueous solution of (b) (4) resulting in doses of (b) (4) mg/kg[14]. The animals demonstrated a slight increase in body weight gain The high dose animals demonstrated slight reduction in acetylcholinesterase activity in blood. A conservative NOAEL using the MD was used for the PDE assessment.

F1 x F2 x F3 x F4 x F5

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent)	2

13 (b) (4)

14 (b) (4)

	1 (for repro studies entire organogenesis covered) 2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	1

PDE = (b) (4) mg/day

(b) (4)

(b) (4)

(b) (4) is listed in the IID as an (b) (4) It is also listed as being used in an (b) (4), although the maximum potency is not specified. (b) (4) The Applicant cites an EPA oral reference dose of (b) (4) mg/kg/day as justification for the safety of the (b) (4) mcg detected in the screening study. They added an additional safety factor of (b) (4) for the differential route of administration. We concur that the levels of (b) (4) pose no safety concerns.

During our filing review and in the 60-day filing letter, we noted that there were several tentatively identified compounds (e.g., (b) (4)) detected in this screening study and that long-term leachable data on three stability batches beyond 12-15 months was not yet submitted to the NDA. We also noted the impact of these

findings on the shelf-life was not clear, and if additional data were obtained to confirm the identity of these compounds or further quantitate the levels over the course of your stability, the Applicant should submit them to the NDA. We also noted that, as the NDA must be complete upon filing, these additional data may not be reviewed to support the application.

The NDA currently has leachables data from several batches on stability testing at up to 15 months under long-term conditions and at up to 14 months under accelerated conditions. Our CMC colleagues have informed us that this is adequate to support the proposed 18-month expiry. If these unknown compounds are not detected in these leachables data, then our concern for their safety should be minimal.

Definitive leachables study

All primary stability lots were evaluated for the following targeted leachables, using validated methods. Reporting thresholds are listed below:

Table 12. Targeted Leachables and Reporting Thresholds

Summary of Final Leachables and Test Methods

Method	Analyte	Reporting Limit (µg/g)	Range (µg/g)
	(b) (4)		

Results

The following tables show the levels of leachables detected in the primary stability lots for the 100 mg and 300 mg drug products that were put on stability under long-term (5°C) and accelerated (25°C) conditions for up to 15 months. Note that our CMC colleagues have noted that the duration of long-term data are adequate to support the proposed 18-month expiry.

Table 13. Results Leachables Primary Stability Lots 100 mg Drug Product

Sample ID	(b) (4)
Lot 192, 5°C, 3 Months	
Lot 192, 5°C, 13 Months	
Lot 192, 25°C, 12 Months	
Lot 193, 5°C, 3 Months	
Lot 193, 5°C, 13 Months	
Lot 193, 25°C, 12 Months	
Lot 194, 5°C, 3 Months	
Lot 194, 5°C, 13 Months	
Lot 194, 25°C, 12 Months	

(b) (4)

Table 14. Results Primary Stability Lots 300 mg Drug Product

Sample ID	(b) (4)
Lot 195, 5°C, 5 Months	
Lot 195, 5°C, 15 Months	
Lot 195, 25°C, 14 Months	
Lot 196, 5°C, 5 Months	
Lot 196, 5°C, 15 Months	
Lot 196, 25°C, 14 Months	
Lot 197, 5°C, 5 Months	
Lot 197, 5°C, 15 Months	
Lot 197, 25°C, 14 Months	

(b) (4)

Table 15. Summary Recent Lot Leachables 300 mg Drug Product

Sample ID	(b) (4)
Lot 208, 5°C, 0 Month	
Lot 208, 25°C, 1 Month	
Lot 209, 5°C, 0 Month	
Lot 209, 25°C, 1 Month	

(b) (4)

The summary table below shows the highest level of each targeted leachable compound that was detected across batches and time points from samples stored under long-term conditions (5°C) and whether they are adequately justified.

Table 16. Summary Risk Assessment Targeted Leachables

Compound	Highest Total Daily Exp. based on MRHD (mcg/day)	PDE (mcg/day)	Safety Margin	Justification
(b) (4)				Adequate. Within IID
				Adequate. Within IID
				Adequate. Within IID
				Adequate. Within PDE
				Adequate. Below QT
				Adequate. Below QT
				Adequate. Below QT

N/A=Not applicable since these compounds were below qualification threshold (QT) of (b) (4) mcg/day, are compounds used as excipients in FDA-approved drug products (within levels in the IID) or otherwise justified (see below).

Permissible Daily Exposures (PDEs) were determined for each relevant potential leachable for the most potentially extractable. However, note that since the (b) (4) were not detected above the reporting threshold of (b) (4) mcg/day, which is within the qualification thresholds for genotoxicity and general toxicity, a risk assessment for these compounds is not considered necessary.

Permissible Daily Exposure values were calculated per the ICH Q3C guidance (from Attachment 4) using the following formula.

$$PDE = \frac{\text{Effect Level x Weight Adjustment (50 kg)}}{F1 \times F2 \times F3 \times F4 \times F5}$$

Where:
F1=Extrapolation for species difference from human
F2= A factor of 10 to account for variability between individuals
F3= A variable factor to account for toxicity studies of short-term exposure
F4= A factor that may be applied in cases of severe toxicity
F5= A variable factor that may be applied if the NOEL was not established

1. (b) (4)

(b) (4)

(b) (4) was not detected in leachables study samples under long-term conditions (5°C); therefore, the maximum potential exposure to (b) (4) is based on the reporting threshold of (b) (4) mcg/day. The Applicant calculated a PDE= (b) (4) mg/day based on an LD_{50}= (b) (4) mg/kg identified in an acute mouse SC study using the following calculation. LD_{50} values are generally inappropriate to establish PDEs.

Importantly, (b) (4) is an excipient used in approved drug products and is listed in the FDA IID for numerous routes, (b) (4)

From a systemic perspective, the maximum potential daily exposure to (b) (4) of (b) (4) mcg through use of RBP-6000 is well within the IID max potency of (b) (4) mg for the oral route. From a local perspective, though (b) (4) is not specifically listed for the SC route, it is listed for the IM route at up to (b) (4)%, which exceeds the concentration of (b) (4)% from RBP-6000 based on (b) (4) mcg/1.5 mL MRHD. Therefore, the level of (b) (4) that was detected is considered justified.

2. (b) (4)

(b) (4)

(b) (4) was not detected in leachables study samples under long-term conditions (5°C); therefore, the maximum potential exposure to this compound is based on the reporting threshold of (b) (4) mcg/day. (b) (4) is an excipient used in approved drug products and is listed in the FDA IID (b) (4) Therefore, the level of (b) (4) acid that was detected is considered justified.

3. (b) (4)

(b) (4)

(b) (4) was not detected in leachables study samples under long-term conditions (5°C); therefore, the maximum potential exposure to this compound is

based on the reporting threshold of (b) (4) mcg/day. (b) (4) is an excipient in approved drug products and is listed in the FDA IID (b) (4) which exceeds the potential total exposure as a leachable. Therefore, the level of (b) (4) that was detected is considered justified.

4. (b) (4)

The Applicant notes that in their screening study they detected what they have tentatively identified as an (b) (4). The general structure is depicted below[15].

(b) (4)

The Applicant did not identify any data on (b) (4) as a class. They based their PDE assessment on a (b) (4) but provided no specific rationale for the selection of the molecule. (b) (4) of a similar structure and appears to be a reasonable member of the class. Both molecules do appear to contain (b) (4) The

15 (b) (4)
.

Applicant based their PDE on a rat oral LD_{50} of (b) (4) mg/kg of (b) (4) as follows.

- PDE = (b) (4)
- Note:
  - The Applicant chose 1 for F4 of 1 of 10 citing that no severe toxicity anticipated including genotoxicity and carcinogenicity was expected. (b) (4)

We do not believe use of an LD_{50} is ideal as the only endpoint examined is mortality.

We identified a teratology study testing a mixture of (b) (4) in rats and rabbits[16]. In the rabbit study, pregnant rabbits were dosed by oral gavage with (b) (4) mg/kg of the (b) (4) mixture from Gestation Day 2 to 16. There was no evidence of maternal toxicity. There were a slightly higher number of dead fetuses in the HD group, although these were dismissed by the authors as being from only 2 of the litters. The authors conclude the HD was the NOAEL. If we were to err on the side of caution, we could use the MD and adjusting for the percent of (b) (4), the NOAEL/LOAEL could be (b) (4) mg/kg (b) (4) % of mid-dose) as the NOAEL.

Modifying Factors	**Factor Recommendations**	**Factor Applied**
F1 (extrapolation between species)	5 (for rat to human) 12 (for mice to human) 2 (for dog to human) 2.5 (for rabbit to human) 3 (for monkey to human) 10 (for other animal to human)	2.5
F2 (variability between individuals)	10	10
F3 (tox studies of short duration)	1 (for year rodent or 7 year nonrodent) 1 (for repro studies entire organogenesis covered)	10

[16] (b) (4) .

	2 (6 month study in rodents) 5 (3 month rodent study) 10 (shorter than 3 months)	
F4 (severe toxicity)	1 if no severe toxicity concerns 1 for fetal toxicity with maternal toxicity 5 for fetal toxicity without maternal toxicity 5 for teratogenic effects with maternal toxicity 10 for teratogenic effect without maternal toxicity X for genotox or carci or neurotox?	1
F5 (if no NOAEL was established)	1 if NOAEL established Up to 10 if LOAEL only available pending severity of toxicity	1

PDE = [redacted] (b) (4) mcg/day

Finally, we note that [redacted] (b) (4) is an FDA approved intravenous drug [redacted] (b) (4) This compound is also a [redacted] (b) (4) Therefore, based on all three compounds, there appears to be adequate justification for the safety of the [redacted] (b) (4) potentially present in this drug product.3

Conclusions of the Extractable Leachable Assessments

Ideally, an Applicant will leverage the extractable data to inform the leachable studies and assay the long-term stability samples for any extractable that exceeds the SCT/AET. The Applicant, however, only validated methods for the definitive leachable assessment based on a toxicological risk assessment of the extraction studies with NMP, the solvent in the drug product. The results demonstrate low levels of leachables compounds in the RBP-6000 formulation, relative to the Permissible Daily Exposure (PDE) limits for these compounds, indicating human safety from leachables for this drug under proposed manufacturing and usage regarding leachables. For a discussion on the adequacy of the extractable leachable studies from a CMC perspective, the reader is referred to the CMC review.

The existing data support an expiry of 18 months.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication for RBP-6000 100 mg and 300 mg subcutaneous (SC) injection once monthly is treatment of moderate to severe opioid use disorder (OUD) in patients who have undergone induction to suppress opioid withdrawal signs and symptoms with a transmucosal buprenorphine-containing product. RBP-6000 should be used as part of a complete treatment plan to include counselling and psychosocial support. RBP-6000 is intended to be administered monthly only by subcutaneous injection in the abdominal region. The maximum recommended human dose is 300 mg monthly, but the dose may be decreased to 100 mg based upon tolerability.

2.7 Regulatory Background

IND 107607 (nonclinical related)
- preIND meeting April 27, 2010
- new IND September 17, 2010
- EOP2 meeting September 30, 2014
- preNDA December 15, 2016
- Fast Trace designation granted May 23, 2017

NDA 209819
- new NDA May 30, 2017

3 Studies Submitted

3.1 Studies Reviewed

Pharmacokinetics

INLS-R101-60-15: Quantitative Whole Body Autoradiography of Rats Following Subcutaneous Administration of ^{14}C-PLGH or ^{14}C-NMP (GLP)

RBRS-R010-60-11: Tissue Distribution of Radioactivity in Rats Following a Single Subcutaneous Dose of ^{14}C-ATRIGEL Using Quantitative Whole Body Autoradiography (non-GLP)

Acute toxicity

RBLS-R02-60-09: A Single Dose 4-Week Toxicokinetic and Toxicity Study of Buprenorphine/ATRIGEL (RBP-6000) Administered Subcutaneously to Sprague-Dawley Rats (GLP)

RBLS-C01-60-09: A 4-Week Toxicokinetic and Toxicity Study of Buprenorphine/ATRIGEL (RBP-6000) Administered Subcutaneously to Beagle Dogs (GLP)

Repeat-dose toxicity

RBLS-R04-60-09: A 6-Month Multi-Dose Toxicity and Toxicokinetic Study of Buprenorphine/ ATRIGEL (RBP-6000) Administered Subcutaneously to Sprague-Dawley Rats, with a 12-Week Recovery Period (GLP)

RBLS-C03-60-09: A 9-Month Multi-Dose Toxicity and Toxicokinetic Study of Buprenorphine/ATRIGEL® (RBP-6000) Administered Subcutaneously to Beagle Dogs, with a 12-Week Recovery Period (GLP)

Genotoxicity

RBLS-R08-60-10: Letter Report: RBP-6000: Toxicity Information for Micronucleus Test in Bone Marrow Cells of CD Rats: Single Subcutaneous Dose (GLP)

Reproductive and developmental toxicity

INLS-R103-60-16: A Combination Study for the Effects of RBP-6000 (Buprenorphine base in the ATRIGEL Delivery System), Administered by Subcutaneous Injection, on Male and Female Fertility and Embryo-Fetal Development, in the Sprague-Dawley Rat (GLP)

INLS-L105-60-16: RBP-6000: A Study for Effects on Embryo Fetal Developmental Toxicity in Rabbits with a Toxicokinetic Evaluation (GLP)

INLS-R104-60-16: A Pre and Post-natal Development Study Including Maternal Function of RBP-6000 (Buprenorphine in the ATRIGEL Delivery System) Administered by Subcutaneous Injection in the Sprague-Dawley Rat (GLP)

3.2 Studies Not Reviewed

NOTE: dose range finding studies were not formally reviewed but were essential to dose selection in definitive studies

Acute toxicity

RBRS-C045-60-09: A Non-GLP Maximum Tolerated Dose Study of an ATRIGEL Formulation Containing (b)(4)% Buprenorphine Base and an ATRIGEL Vehicle Formulation in Male Beagle Dogs

ATLS-156: A Single Dose One-Month Toxicokinetics and Toxicity Study in Rats with GHRP-1 in ATRIGEL Administered by Subcutaneous Injection (GLP)

– different title in nonclinical written to emphasize the Atrigel effect not GHRP-1

ATLS-169: A Single Dose One-Month Toxicity and Toxicokinetics Study in Rats with ATRIGEL/Octreotide Administered by Subcutaneous Injection (GLP)
– different title in nonclinical written to emphasize the Atrigel effect not drug

ATLS-170: A Single Dose One-Month Toxicity and Toxicokinetics Study in Rabbits with ATRIGEL/Octreotide Administered by Subcutaneous Injection (GLP)
– different title in nonclinical written to emphasize the Atrigel effect not drug

ATLS-157: A Single Dose One-Month Toxicokinetics and Toxicity Study in Dogs with GHRP-1 in ATRIGEL Administered by Subcutaneous Injection (GLP)
– different title in nonclinical written summary to emphasize the Atrigel effect not drug

Repeat Dose toxicity

ATLS-79: Systemic Toxicity Study in the Rat Following 4 and 12 Weeks of Subcutaneous Implantation ((b) (4) CDRH-type - nonGLP)

ATLS-192: A Multiple-Dose Six-Month Toxicity and Toxicokinetics with Reversibility in Rats with AL3937.01 Administered by Subcutaneous Injection (GLP)

ATLS-193: A Multiple-Dose Nine-Month Toxicity and Toxicokinetics with Reversibility in Rabbits with AL3937.01 Administered by Subcutaneous Injection (GLP)

Genotoxicity

789820: RBP-6000: Micronucleus Test in Bone Marrow Cells of CD Rats: Single Subcutaneous Dose and 24 h and 48 h Sampling with Toxicokinetic Blood Sampling (GLP)

Reproductive and Developmental toxicity

INLS-R102-60-15: Dose Range-Finding Study of Fertility and Embryo-Fetal Development of RBP-6000 (Buprenorphine/ATRIGEL) Administered by Subcutaneous Injection in Rats (GLP)

INRS-R148-60-16: A Dose Range-Finding Pre- and Post-natal Development Study of RBP-6000 (Buprenorphine in the ATRIGEL Delivery System) Administered by Subcutaneous Injection in the Sprague-Dawley Rat (non-GLP)

INRS-L146-60-15: A Dose Range-Finding Prenatal Developmental Toxicity and Toxicokinetic Study of RBP-6000 (Buprenorphine in the ATRIGEL Delivery System) Administered by Subcutaneous Injection in New Zealand White Rabbits (non-GLP)

3.3 Previous Reviews Referenced

IND 107607 (RBP-6000)

4 Pharmacology

No pharmacology studies were conducted for RBP-6000. Refer to approved buprenorphine drugs (NDA 20-732 for Subutex and NDA 18-401 for Buprenex) and buprenorphine-naloxone drugs (NDAs 20-733 and 22-410 for Suboxone), owned by the Applicant.

4.1 Primary Pharmacology

No new studies were completed or required. The reader is referred to the cross-referenced NDAs. As per the Applicant's other buprenorphine drug product NDAs, "Buprenorphine is a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor. Naloxone is a potent antagonist at mu opioid receptors and produces opioid withdrawal signs and symptoms in individuals physically dependent on full opioid agonists when administered parenterally."

4.2 Secondary Pharmacology

No new studies were completed or required. The reader is referred to the cross-referenced NDAs.

4.3 Safety Pharmacology

No new studies were completed or required. The reader is referred to the cross-referenced NDAs.

5 Pharmacokinetics/ADME/Toxicokinetics

Most specific Pharmacokinetics/ADME/Toxicokinetics studies were not reported except as reported in nonclinical toxicity studies that will be reported in Sections 6, 7, & 9 and the study/studies in section 5.1/ADME. Refer to approved buprenorphine drugs (NDA 20-732 for Subutex and NDA 18-401 for Buprenex) and buprenorphine-naloxone drugs (NDAs 20-733 and 22-410 for Suboxone), owned by the Applicant. Toxicokinetic data included in toxicity studies.

5.1 PK/ADME

Study Title: Quantitative Whole Body Autoradiography of Rats Following Subcutaneous Administration of ^{14}C-PLGH or ^{14}C-NMP (Study INLS-R101-60-15 - GLP)

The absorption and distribution of radioactivity were determined after administration of a single subcutaneous dose of ^{14}C-poly-lactide-co-glycolide (PLGH combined with a

target of 250 mg/kg (b) (4) buprenorphine base - Group 1) or ^{14}C-*N*-methyl-2-pyrrolidone (NMP combined with a target of 250 mg/kg (b) (4) buprenorphine base - Group 2) to male Sprague Dawley (SD) rats. At designated time points postdose following euthanasia, the animal carcasses were prepared and examined via quantitative whole body autoradiography (QWBA) analysis.

Key findings:

- After subcutaneous administration of ^{14}C-PLGH/buprenorphine or ^{14}C-NMP/buprenorphine to rats, radioactivity was widely distributed to most tissues (~50 tissues each for PLH and NMP) by the first collection time point (24 or 12 hours postdose, respectively). Most of the tissues reached maximum concentration (C_{max}) by 672 hours (28 days) for ^{14}C-PLGH/buprenorphine and by 12 hours for ^{14}C-NMP/buprenorphine. Radioactivity was still measurable in approximately half the tissues by 3360 hours (140 days) after ^{14}C-PLGH/buprenorphine dosing, suggesting that the radioactivity may have incorporated or bound to tissues or that radioactivity at the injection site is still absorbed and distributed to these tissues. After subcutaneous ^{14}C-+, radioactivity was cleared from or below the limit of quantitation for most of the tissues by 672 hours (28 days).

- Subcutaneous injection sites from two of the four animals administered ^{14}C-PLGH in RBP-6000 and sacrificed 3360 hours (140 days) postdose were processed for microscopic evaluation instead of QWBA. Four sections were trimmed from each dose site. No microscopic findings, including those consistent with the presence of any residual drug product including buprenorphine or PLGH, occurred at the subcutaneous injection sites.

==========

Study Title: A 35-Day Release Study of Three ATRIGEL® Formulations Containing 18% Buprenorphine Base Administered Subcutaneously in Rats with Two Injections per Rat (Study RBRS-R102-60-11 - nonGLP).

The purpose and primary objective of this study was to evaluate and compare the (b) (4) day release kinetics of three ATRIGEL formulation groups to two injection sites for 3 test articles (TAs). The second objective was to evaluate the statistical significance of any differences due to dorsal thoracic injection site or individual rat variability. The final objective was to evaluate local tissue reaction and implant characteristics.

Group I included TA 1 and 2 and Group II included TA 1 and TA 3

TA 1 - (b) (4) at 18% (b) (4) %: (b) (4) % buprenorphine, 50:50 PLGH (b) (4) and (b) (4) % NMP, respectively.
TA 2 - (b) (4) at 18% (b) (4) %: (b) (4) % buprenorphine, 50:50 PLGH (b) (4) (b) (4) % NMP, respectively
TA 3 - 18% (b) (4) %: (b) (4) % buprenorphine, 50:50 PLGH (b) (4) and (b) (4) % NMP, respectively.

On Day 0, each rat received two 100 mcL subcutaneous (SC) injections containing 18% buprenorphine in the dorsal thoracic (DT) region. Group I rats received one injection of Test Article (TA) 1 and one injection of TA 2, with alternating injection locations. Group II rats received one injection of TA 1 and one injection of TA 3, with alternating injection locations. On Days 1, 14, 21, and 35, thirteen rats per group were euthanized with CO_2 and the implants were retrieved for analysis of buprenorphine content using reverse phase high performance liquid chromatography (RP-HPLC). Macroscopic SC tissue reaction, relative to each formulation, was evaluated by gross examination of the implants and surrounding tissue.

Key findings:

- Injection site did not generally have a substantial effect on buprenorphine release at observation times during the 35 days (see Table). Buprenorphine release profiles for all three test articles were similar in shape and magnitude, regardless of implant injection site. Despite statistical differences in release observed on Days 1 and 21, it is difficult to discern any substantial differences between the three test articles. A high degree of animal-to-animal variability observed in this study also contributes to the complexity in finding any dissimilarity between test articles or injection sites. Macroscopic test site observations were unremarkable.

Table 17. Summary of Buprenorphine Release from Recovered Implants

Summary of Buprenorphine Release from Recovered Implants.

	Mean Buprenorphine Release % ± St. Dev.					
Time Point (Day)	TA 1		TA 2		TA 3	
1	Left Side	16.3 ± 10.6	Left	31.6 ± 7.2	Left	3.9 ± 8.6
	Right	19.8 ± 19.4	Right	19.2 ± 9.9	Right	7.5 ± 1.6
	Average	18.0 ± 15.1	**Average**	24.9 ± 10.6	**Average**	5.8 ± 6.0
14	Left	66.3 ± 14.7	Left	61.2 ± 25.1	Left	64.6 ± 22.9
	Right	57.1 ± 20.5	Right	53.8 ± 16.8	Right	58.0 ± 16.9
	Average	61.3 ± 18.3	**Average**	57.8 ± 21.2	**Average**	61.5 ± 19.8
21	Left	77.7 ± 15.0	Left	83.9 ± 5.4	Left	86.4 ± 7.6
	Right	76.1 ± 19.3	Right	57.6 ± 13.2	Right	86.2 ± 5.8
	Average	76.9 ± 16.7	**Average**	69.8 ± 16.9	**Average**	86.3 ± 6.4
35	Left	81.9 ± 16.3	Left	87.7 ± 9.3	Left	88.7 ± 7.0
	Right	82.9 ± 11.2	Right	84.4 ± 8.8	Right	76.4 ± 19.8
	Average	82.5 ± 13.6	**Average**	86.1 ± 8.8	**Average**	83.0 ± 15.1

5.2 Toxicokinetics

- included in toxicity studies

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: A Single-Dose 4-Week Toxicokinetic and Toxicity Study of Buprenorphine/ATRIGEL® (RBP-6000) Administered Subcutaneously to Sprague-Dawley Rats

Study no.:	RBLS-R02-60-09
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/Atrigel - (b) (4)% buprenorphine base in (b) (4)% 50/50 PLGH and (b) (4)% NMP), Lot No. 0042-01 - Control Article (Lot RB0042-21 & RB0042-01), 50:50 Poly(DL-lactide-co-glycolide) (PLGH) (b) (4), (b) (4), Lot (b) (4)) and N-methyl-2-pyrrolidone (NMP) (b) (4), Part (b) (4) Lot 05900219509, RB Part (b) (4)

Key Study Findings

- Ten rats/sex/group were injected once subcutaneously with 0 (Atrigel control Group 1), 10 (Group 2), 50 (Group 3), and 250 (Group 4) mg/kg RBP-6000 and sacrificed 4 weeks later. Toxicokinetic groups of 15/sex/dose were also included and evaluated with blood samples collected on Days -9, 1 (at 1, 2, 4, and 8 hours post dose), 2, 4, 8, 11, 15, 22, and 29.
- A full GLP protocol was conducted that included clinical signs, body weight, food consumption, clinical pathology, gross, macroscopic, and microscopic pathology, and toxicokinetics.
- Early test article-related effects observed included reduced food consumption and weight loss, and pica behavior (e.g., self-mutilation), a known effect of buprenorphine in rats. Animals from Group 2 (1 animal), 3 (6 animals), and Group 4 (7 animals) were euthanized for humane reasons due to the pica behavior. However, after preventative measures were in place to minimize the pica behavior

and the animals were stabilized after Week 2, there were no observed adverse effects in any of the RBP-6000-treated rats.

- For dermal dose-site scoring, erythema, and/or edema were the primary observations noted in the 250 mg/kg dose level in 7 of the 20 dosed rats and in 10 of 20 control rats. Erythema severity reached Grade 4 level (Draize severe). The erythema and/or edema were first noted on Day 3 with most resolving by Day 25 except for one high dose female which had the observation to the end of the study. Effects were attributed to Atrigel delivery system as effects occurred only in control and high dose groups which received 1.14 mL/kg versus 0.05 mL/kg (low dose) and 0.23 mL/kg (mid dose).
- No toxicologically relevant changes were observed in body weight/body weight gain, food consumption, clinical pathology, macroscopic pathology, and organ weights (changes were small, only affected males, and were without histologic correlates).
- The NOAEL for local safety is the mid-dose of 50 mg/kg (0.23 mL/kg) based on subcutaneous granulomas observed at the high-dose and vehicle control group. Note that the mid-dose level had a lower volume of the Atrigel vehicle, which likely resulted in the less toxicity than the vehicle control group.
- A NOAEL for systemic toxicity was proposed to be 250 mg/kg under the conditions of this study, but a low-observed-adverse-effect-level (LOAEL) of 10 mg/kg RBP-6000 may be more appropriate do to support needed as listed above.
- For toxicokinetics, C_{max} values (ng/mL) were 4.32, 10.0, & 26.4 in males and 2.53, 8.95, & 39.7 in females for the low-, mid- and high-dose groups, respectively. AUC_{0-24h} values (ng*h/mL) were 32.9, 76.6, & 228 in males and 13.0, 47.1, & 273 in females for the low, mid and high dose groups, respectively.

Methods

Doses:

Group	Number of Males	Number of Females	Dose Level[a] (mg/kg)	Dose Volume[a] (mL/kg)	Dose Concentration w/w%	Number of Males	Number of Females
						Terminal Necropsy Day 29/30	
1 (Control)	10	10	0 (Control)[d]	1.14	0	10	10
2[c]	10	10	10	0.05	20	10	10
3[c]	10	10	50	0.23	20	10	10
4[c]	10	10	250	1.14	20	10	10
5[b] (Control)	15	15	0 (Control)[d]	1.14	0	15	15
6[b]	15	15	10	0.05	20	15	15
7[b]	15	15	50	0.23	20	15	15
8[b]	15	15	250	1.14	20	15	15

[a] Doses were delivered within ± 20% of the desired dose.
[b] Animals in the toxicokinetic groups were used solely for the purpose of blood sample collections and were euthanized after the final collection and without necropsy on Day 29.
[c] On Day 4, Animal 2004 and Day 2 Animals 2510, 3002, 3003, 3006, 3007, 3503, 3510, 4004, 4008, 4010, 4502, 4503, 4505, and 4508 were euthanized for humane reasons due to severe behavioral issues (self-mutilation) which was a known buprenorphine effect in Sprague Dawley rats. Animals were added to the study to return the affected groups to the full complement. The euthanized animals were not included in the final evaluation of the study data;

[d] ATRIGEL Delivery System

Frequency of dosing: Single dose on Day 1
Route of administration: Subcutaneous
Dose volume: See table above
Formulation/Vehicle: Atrigel delivery system (NMP and PLGH)
Species/Strain: Sprague Dawley rats
Number/Sex/Group: 15/sex/group (toxicokinetic), 10/sex/group (main study)
Age: Approximately 13 weeks
Weight: 330.9 to 453.6 g for the males and 213.5 to 286.4 g for the females
Satellite groups: Toxicokinetic (TK) animals (no necropsy)
Unique study design: No. Doses of Atrigel in table.

Group	Number/Sex/Group	Buprenorphine Dose (mg/kg)	ATRIGEL Delivery System Exposure (mean mg) Males (M)/Females (F)	ATRIGEL Delivery System Exposure (mean mg/kg) M/F
1	10M/10F	0	446/316	1141.0/1267.5
2	10M/10F	10	17.6/11.2[a]	45.6/45.1
3	10M/10F	50	80.8/49.6[a]	211.7/198.8
4	10M/10F	250	414.4/262.4[a]	1056.6/1030.2

[a] ATRIGEL Delivery System is 80% of mean weight (mg) of total weight delivered

Deviation from study protocol: Nothing remarkable.

Observations and Results

Mortality

All animals (including the TK groups) were observed for clinical signs and mortality twice daily (a.m. and p.m.) beginning on the day of acclimation to housing and continuing through the days of necropsy. Observations were made by exception, with notations made for only those animals with findings.

On Day 4, Animal 2004 (Group 2) and Day 2 Animals 2510, 3002 (Group 3), 3003, 3006, 3007, 3503, 3510, 4004 (Group 4), 4008, 4010, 4502, 4503, 4505, and 4508 were euthanized for humane reasons due to severe behavioral issues such as self-mutilation from pica behavior which is reported to be a buprenorphine effect in Sprague Dawley rats. Animals were added to the study to return the affected groups to the full complement. The euthanized animals were not included in the final evaluation of the study data.

Clinical Signs

All animals (including the TK groups) were observed for signs of morbidity twice daily (a.m. and p.m.) beginning on the day of acclimation to housing and continuing through the days of necropsy. Observations were made by exception, with notations made for only those animals with findings. Detailed observations were conducted once daily (a.m.) on Day -7 to Days 30 and 29 (Sets A, B, and C, respectively).

Severe behavioral effects (self-mutilation due to pica behavior) were noted in the test article-dosed animals at all dose levels. Collars were placed on the rats to prevent them from chewing on their extremities. After approximately 48-hours post dose, these observations were absent or reduced. Many of the clinical observations were associated with this behavior such as abrasions, reddened areas, and swelling on or around forepaws. The incidence of these observations decreased after approximately 14 days post dose. All other noted observations were incidental findings, including, but not limited to, alopecia, broken/cracked teeth, and nasal discharge, and were of low incidence, not dose-dependent, noted in control animals, and/or are common findings for rats of this age.

Dermal Observations

Dermal observations were conducted predose on Day 1; once daily during Week 1 (Days 1 through 7); then Days 8, 11, 15, 18, 22, 25, and prior to necropsy on Day 29. Each animal in the main study groups (Groups 1 to 4) was removed from the cage as listed above and observed in detail according to the Macroscopic Dermal Grading System (Draize 2), which is based on the Draize method. A final dermal observation was made on the day of euthanasia prior to necropsy. The scores were recorded separately for each individual dose site.

For dermal dose-site scoring, erythema, and/or edema were the primary observations noted in the 250 mg/kg dose level in 7 of the 20 dosed rats and in 10 of 20 control rats. Erythema severity reached Grade 4 level (Draize severe). The erythema and/or edema were first noted on Day 3 with most resolving by Day 25 except for one high dose female which had the observation to the end of the study. In two high dose and 1 control animals, the observation was noted as severe. Dermal reactions were considered an effect of the ATRIGEL Delivery System injection. The control group received the greatest amount of the delivery system which was slightly more than the 250 mg/kg dosed animals. The 10 and 50 mg/kg dosed animals received a lower injection volume than the control and 250 mg/kg dose groups and had no observations of erythema and/or edema.

Body Weights

Main study and TK animals were weighed prestudy and prior to dosing on Day 1, then weekly thereafter. A final fasted body weight was obtained on the day of scheduled necropsy for calculation of organ-to-body weight ratios. Terminal body weights were not collected from animals found dead or euthanized moribund.

Body weights - While not of great magnitude, RBP-6000-related body weight decreases were noted in the 50 and 250 mg/kg dosed animals. The decreased body weights were most notable at Week 2 in male and female rats. At Week 2 for the 250 mg/kg dosed rats group 4), 9 of 10 males and 6 of 10 females lost weight compared to their Week 1 weights at -2% and -1%, respectively, for group means with nothing significant. For the same time period in the 50 mg/kg dosed rats (group 3), 3 of 10 males and 6 of 10 females had lost weight with group mean increasing for male and -2% for females. By Week 3, the 50 and 250 mg/kg dose level rats were gaining weight and by the end of the study were comparable to control rats. While body weight changes were noted, they were not considered toxicologically significant as Day 1 to Week 5 changes in body weight for males was +7%, +7%, +7%, and +5% for Atrigel controls and low, mid, and high dose RBP6000 groups, respectively. Day 1 to Week 5 changes in body weight for females was +11%, +7%, +4%, and +6% for Atrigel controls and low, mid, and high dose RBP6000 groups, respectively.

Body weight gain - This same toxicologically insignificant trend was also noted in the body weight gain data with a mean decease of body weight gain for the 250 mg/kg Group 4) dose level males and for the 50 and 250 mg/kg dose level females from Week 1 (Day 0 dosing) to Week 2. Although there was a decrease in body weight gain for the 10 mg/kg dose level males at the end of Week 2 and females at the end of Week 4, these changes were not statistically significant, within the reported variability observed in laboratory housed rats, and not considered toxicologically significant.

Food Consumption

Food consumption was measured for main study animals weekly. Food consumption for the TK animals was not assessed.

There was some test article-related reduction of food consumption during the first week after dosing apparently due to buprenorphine. In males, compared to Group 1 Atrigel controls (no saline control group), this was statistically significant for the males at both 50 (-16%) and 250 (-16%) mg/kg at Week 1 with a reduction of 9% in the low dose, 10 mg/kg male group. In females, compared to Group 1 Atrigel controls (no saline control group), there was no statistical significance but reduction was at 18%, 24% and 20% for low to high dose groups, respectively. From Week 2 to Week 3, food consumption increased in all RBP-6000 dosed groups, but was below control group values. From Week 3 to 4 through termination, food consumption in all test article dosed groups was comparable to the control rats. Over the course of Weeks -1 to 5, food consumption was reduced by 3%, 3%, and 9% in males and by 11%, 13%, and 10% in females, respectively, for the low-, mid-, and high-dose RBP-6000 groups compared to Atrigel controls.

When food consumption data is considered in light of body weight data, it is not considered toxicologically significant.

Ophthalmoscopy – Not evaluated

ECG – Not evaluated

Hematology

Blood was collected at terminal bleeding for main study animals. Parameters analyzed were as follows:

Hematology Parameters	
Red blood cell (RBC) count	Red cell distribution width (RDW)
Hemoglobin concentration	Reticulocyte count
Hematocrit	Platelet count
Mean corpuscular volume (MCV)	White blood cell (WBC) counts [a]
Mean corpuscular hemoglobin concentration (MCHC)	
Mean corpuscular hemoglobin (MCH)	

[a] Included total white blood cell, absolute polysegmented neutrophil, lymphocyte, monocyte, eosinophil, basophil, and other cell counts as appropriate.

There were no adverse test article-related changes in hematology parameters. White blood cell count, neutrophils, and lymphocytes increased in test article-dosed males (some statistically significant) which was reported likely related to abrasions (pica related) on paws, abrasions on tails, and broken teeth, and not considered RBP-6000 related. Other intergroup differences, some statistically significant, were dose-independent or within the range of variability for rodents thus were not considered

related to test article administration. The control group had no intergroup differences, and all values were considered within the range of variability for rodents.

Coagulation

Blood was collected at terminal bleeding for main study animals. Parameters analyzed were as follows:

Coagulation Parameters
Prothrombin time (PT)
Activated partial thromboplastin time (APTT)

There were no adverse changes in any coagulation parameters.

Clinical Chemistry

Blood was collected at terminal bleeding for main study animals. Parameters analyzed were as follows:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alkaline phosphatase (ALP)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Lactate dehydrogenase (LD)	Glucose
Total bilirubin	Cholesterol
Urea nitrogen (BUN)	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

There were no adverse changes in any serum chemistry parameters.

Urinalysis

Urine was collected overnight via urine collection cages during which time the animals were deprived of food prior to sacrifice. Parameters analyzed were as follows:

Urinalysis Parameters	
Color/Character	Ketones
pH	Bilirubin
Specific gravity	Occult blood
Protein	Microscopics
Glucose	Total Volume

There were no urinalysis parameter changes that were considered related to test article administration.

Gross Pathology

Main study animals from Groups 1-4 were necropsied on Day 29 or 30. All main study animals were subjected to a complete gross necropsy examination. The necropsy examination included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Gross findings related to the ATRIGEL Delivery System were present in most animals in all groups, including the vehicle controls, and consisted of dark or pale firm area/foci at the injection site. These correlated histologically with granulomas in the subcutaneous (SC) tissue.

Organ Weights

For all scheduled and unscheduled necropsies, the following organs (when present) were weighed before fixation. Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

Organs Weighed	
Adrenals	Ovaries
Brain	Pituitary
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Lungs	

Statistically significant (SS) decreases in liver to body weight ratio were observed in males dosed with 50 (-8%) and 250 mg/kg (-12%) of RBP-6000 compared to Atrigel control. At 10 mg/kg in males it was -7% but not statistically (NSS) significant. Absolute liver weights in males were decreased at 9%, 9%, and 15%, respectively, for the low-, mid-, and high-dose males at NSS levels. SS increases in adrenal weights (36% and 29% at 50 and 250 mg/kg, respectively, with NSS at 19% for low 10 mg/kg dose). SS adrenal to body weight ratio (100% and 100%) and adrenal to brain weight ratio (35% and 29%) were observed in males dosed with 50 and 250 mg/kg of RBP-6000, respectively. At the low dose of 10 mg/kg, these NSS increases were 100% and 24%, respectively.

The changes were considered test article-related, although the relationship may be indirect. The changes were small, only affected males, and were without histologic correlates. Adrenal weights are reported to increase in stressed animals. These animals had reduced food consumption during the dosing period, indicating they were under stress. There were no other alterations in organ weights or organ weight ratios in animals euthanized at terminal necropsy that could be attributed to the administration of RBP-6000.

Histopathology

Adequate Battery – yes

The following tissues and organs (or portions of), when present, were collected from any animal that died or was euthanized and preserved. For all animals necropsied, the tissues listed in the table were embedded in paraffin wax. For Groups 1 and 4, embedded tissues were sectioned, stained with hematoxylin and eosin, and examined.

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Glands (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Oviducts
Duodenum	Uterus
Jejunum	Cervix
Ileum	Vagina
Large Intestine	Endocrine
Cecum	Adrenals
Colon	Pituitary
Rectum	Thyroid/Parathyroids[a]
Pancreas	Harderian Gland (paired)
Liver	Skin/Musculoskeletal
Respiratory	Skin/Mammary Gland
Trachea	Bone (femur)
Lung with large bronchi	Bone (sternum)
Lymphoid/Hematopoietic	Skeletal Muscle (quadriceps femoris)
Bone Marrow (sternum)	Nervous/Special Sense
Thymus	Eyes with Optic Nerve
Spleen	Sciatic Nerve
Peyer's Patch	Brain
Lymph Nodes	Spinal Cord (cervical, thoracic, lumbar)
Mandibular	Other
Mesenteric	Animal Number (ear tag or tattoo and/or chip)
Inguinal	Gross Lesions
	Subcutaneous Injection Site[b]

[a] The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

[b] At least a 1 inch margin of skin surrounding the implant (at the injection site) was dissected. If multiple injections were administered, the skin and implant from the first injection were removed. If the implant was visible it remained intact and not disturbed by removal from the fatty tissue or fascia. The skin and the implant were removed in one piece and sectioned. If implant was not adhered to skin the implant was not collected, but the skin was collected.

Peer Review – not reported

Histological Findings –

Systemic effects: Histopathologically, there was an increased severity of vacuolar degeneration of pancreatic acinar cells in dosed animals. This change consisted of cytoplasmic vacuoles, which often contain cell debris, in some of the acinar cells. Minimal vacuolar degeneration was reported to be a common incidental finding in rats and was present in many controls in this study. However, there was a dose proportional increased severity (to mild or moderate) in animals dosed with 10, 50, and 250 mg/kg RBP-6000. It is possible that pancreatic vacuolar degeneration was indirectly related to test article administration and was related to test article-related lowered food consumption and weight loss. However, a direct relationship to RBP-6000 administration cannot be unequivocally ruled out. Pancreatic vacuolar degeneration was not considered adverse by the report because it is a common incidental finding in rats and the change consisted only in increased severity.

Incidence of Test Article-Related Histopathology Findings

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	50	250	0	10	50	250
Number of animals examined	10	10	10	10	10	10	10	10
Pancreas, Vacuolar Degeneration	9	10	10	10	6	10	10	9
Minimal	9	10	7	3	6	9	7	2
Mild	0	0	2	7	0	1	3	7
Moderate	0	0	1	0	0	0	0	0

Local effects: Dermal findings in all test groups were related to the Atrigel Delivery System and consisted of granulomas in the SC injection site of most animals. These were similar in appearance in control and dosed animals. There were no adverse local effects observed at the mid-dose level.

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	50 mg/kg	250 mg/kg	0 mg/kg	10 mg/kg	50 mg/kg	250 mg/kg
Number of Animals on Study :	10	10	10	10	10	10	10	10
Number of Animals Completed:	(10)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
INJECTION SITE, SUBCUTANEOUS;								
Examined	(10)	(0)	(0)	(10)	(10)	(0)	(0)	(10)
Within Normal Limits	1	0	0	0	1	0	0	0
Fibrosis; Subcutaneous; diffuse	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
Granuloma; Subcutaneous	8	0	0	10	8	0	0	9
Granuloma; Dermis	0	0	0	0	0	0	0	1
Inflammation, Granulomatous; Subcutaneous; focal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)

minimal	1	0	0	0	0	0	0	0
Ulcer	0	0	0	0	0	0	0	1
Infiltrate, Mononuclear Cell; Subcutaneous; diffuse	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
Infiltrate, Mononuclear Cell; Subcutaneous; multifocal	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0

All other histologic changes were considered incidental and unrelated to RBP-6000 administration because they were consistent with frequently observed incidental changes in Sprague Dawley rats at the Testing Facility, they occurred randomly within groups, or they were otherwise considered incidental.

Special Evaluation - none

Toxicokinetics

Blood samples were collected on Days -9, 1 (at 1, 2, 4, and 8 hours post dose), 2, 4, 8, 11, 15, 22, and 29. Animals were not fasted for sample collections. Three animals/sex/time point were rotated in Groups 5-8 and the same 3/sex were sampled on each occasion according to the following table.

No. of Rats	Time Points
3/sex/group	Day -9, Day 2, Day 22
3/sex/group	Day 1 1-hour pd, Day 4, Day 29
3/sex/group	Day 1 2-hour pd, Day 8
3/sex/group	Day 1 4-hour pd, Day 11
3/sex/group	Day 1 8-hour pd, Day 15

pd = post dose

Plasma concentrations of buprenorphine was generally maximal at 2 hours post dose, after which time plasma concentrations rapidly dropped until 24 hours post dose. After 24 hours post dose, there was a plateau phase. In the 10 mg/kg dose group, the terminal half-life of buprenorphine was estimated at 193 and 184 hours in males and females, respectively. Exposure tended to be lower in females than in males. However, sex-differences in exposure were within the inter-individual variability of the plasma concentration data. The increase in buprenorphine exposure was less than proportional to the increase in dose from 10 to 250 mg/kg (2.3- to 6.3-fold increase in exposure for a 25-fold increase in dose).

Males

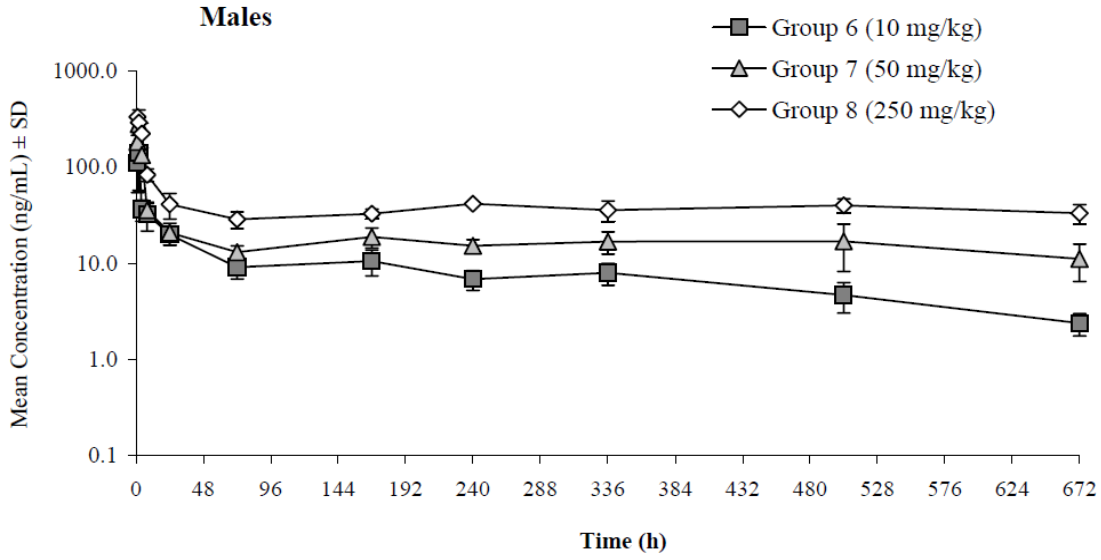
Group No.	Dose Level (mg/kg)	Cmax (ng/mL)	AUC(0-t) (ng•h/mL)	AUC(0-24) (ng•h/mL)	AUC(0-inf) (ng•h/mL)	AUC%extrap (tlast-inf)	Cmax/ Dose	AUC(0-t)/ Dose
6	10	141	5560	916	6224	10 7	14.1	556
7	50	282	11876	1535	a	43 3	5.63	238

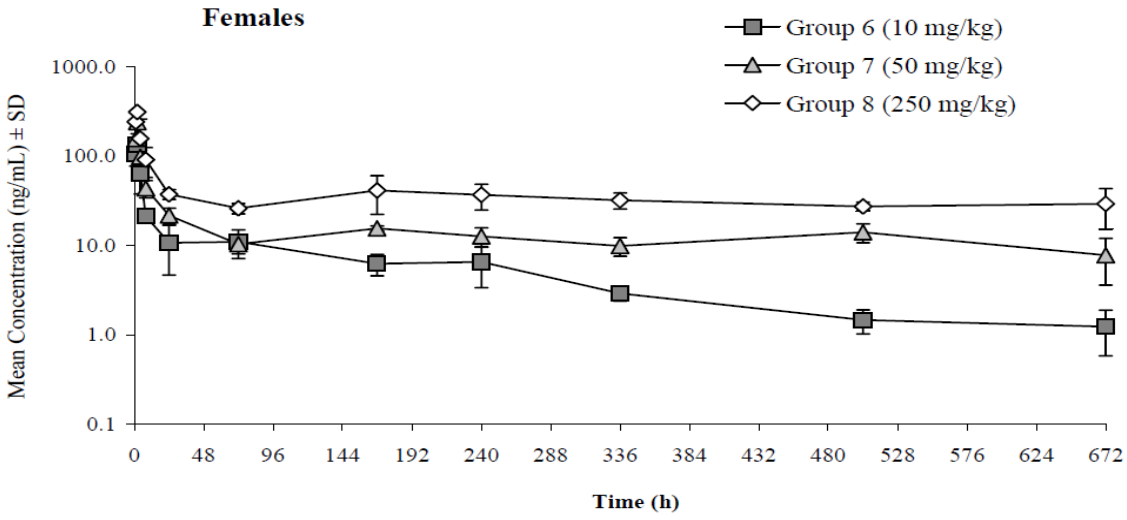
8	250	335	26256	2615	a	35 0	1.34	105

Females

Group No.	Dose Level (mg/kg)	Cmax (ng/mL)	AUC(0-t) (ng•h/mL)	AUC(0-24) (ng•h/mL)	AUC(0-inf) (ng•h/mL)	AUC%extrap (tlast-inf)	Cmax/ Dose	AUC(0-t)/ Dose
6	10	135	3675	803	4004	8 23	13.5	367
7	50	242	9422	1427	a	26 8	4.83	188
8	250	315	23141	2408	a	64 0	1.26	92.6

a Values are not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was <0.800.





Dosing Solution Analysis

The Applicant provided to the Testing Facility supporting documentation and/or assurances of GLP-required characterization and stability determination for the test article. A Certificate of Analysis, or equivalent document, was provided. According to the Sponsor, the concentration of the active ingredient in the test article was approximately (b) (4) % (w/w).

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Study title: A 4-Week Toxicokinetic and Toxicity Study of Buprenorphine/ATRIGEL® (RBP-6000) Administered Subcutaneously to Beagle Dogs

Study no.:	RBLS-C01-60-09
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - (b) (4)% buprenorphine base in (b) (4)% 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4)% N-methyl-2-pyrrolidone (NMP)), Lot 0042-01, Buprenorphine (b) (4)%, NMP (b) (4)%. - ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP), Lot 0042-21, NMP (b) (4)%.

Key Study Findings

- The objectives of this study were to evaluate any potential toxicity from the administration of RBP-6000 for 28 days following the subcutaneous injection(s) on Day 1 and determine the toxicokinetics of buprenorphine/ATRIGEL® (RBP-6000) when administered as a single dose to groups of 3 male and female Beagle dogs at doses of 0 (Atrigel control – 0.36 mL/kg), 20 (0.09 mL/kg), 40 (0.18 mL/kg) and 80 (0.36 mL/kg) mg/kg buprenorphine in Atrigel.
- All standard GLP protocol indices (e.g., clinical signs, body weights, clinical chemistry, gross and micropathology, and toxicokinetics - TK) were conducted in the dogs except for ECG and ophthalmology. All animals were sacrificed on Day 29.
- No treatment was associated with moribundity and/or early death in any animals and all animals survived to their scheduled terminal necropsy on Day 29 other than expected pharmacological effects due to buprenorphine. While some clinical signs

and changes were expected, due to the dehydration and exaggerated clinical findings (i.e., muscle tremors and ataxia) all dose levels were considered to cause treatment-related toxicity with the low dose of 20 mg/kg RBP-6000 being considered a reversible Lowest Adverse Effect Level (LOAEL) related to the buprenorphine as all RBP-6000 groups required some supportive care during the post-treatment phase.

- Expected effects of the injection of the ATRIGEL Delivery System (present in the test and control articles) included clinical signs at the injection site(s) of swelling, abrasion, reddening, and raised areas or masses. Dermal observations included edema, dermal irritation outside injection site(s), erythema, and erythema that extended beyond injection site(s).
- No other treatment-related effects were observed.
- The LOAEL for systemic effects was the low-dose due to exaggerated pharmacological clinical signs from buprenorphine that necessitated supportive care for all animals given RBP-6000.
- A NOAEL for local effects was not clearly determined. Animals from the RBP-6000 groups received approximately the same amount of drug per injection site (e.g., higher dose levels had more injections sites rather than more drug administered per site). That being said, only the high-dose RBP-6000 and vehicle control groups exhibited microscopic findings of granulomatous inflammation, ulcer, hemorrhage, and hyperplasia while the low- and mid-dose groups did not. As no necrosis or degeneration was observed at the injection sites, the 20 mg/kg concentration tested may be considered a LOAEL.
- At the LOAEL of 20 mg/kg, TK values were a C_{max} of 16 ng/mL in males and females and an $AUC_{0\text{-}24h}$ of 6214 ng*h/mL (males) and 5223 ng*h/mL (females).

Methods

Doses:

Group No.	Number of Males/Females	Dose Level[a] (mg/kg)	Dose Volume[a] (mL/kg)
1	3/3	0 (control)[b]	0.36
2	3/3	20	0.09
3	3/3	40	0.18
4	3/3	80	0.36

[a] Doses were delivered within ± 20% of the desired dose.
[b] ATRIGEL Delivery System

Frequency of dosing: Single dose
Route of administration: Subcutaneous
Dose volume: See Table above with all buprenorphine groups receiving the same concentration of buprenorphine (b) (4) % v/v)
Formulation/Vehicle: Atrigel Delivery system
Species/Strain: Beagle dog
Number/Sex/Group: 3
Age: 0.8 years of age (9.6 months)
Weight: 7.7 to 9.4 kg for the males and 7.2 to 9.2 kg for the females at the outset (Day -1) of the study
Satellite groups: No
Unique study design: No 24-hour necropsy, only Day 29 after single dose
Deviation from study protocol: Nothing remarkable

Study Design

Dosing - Subcutaneous dosing sites (one to six) were demarcated by a tattoo indicator on a shaved area of the animal's back (dorsal view). Consistent with the different dose volumes per group, Groups 1 and 4 animals received a total of three injections per animal (one injection administered at each of Sites 1, 2, and 3). Group 2 animals received one injection per animal administered at Site 1 and Group 3 animals received two injections per animal (one injection administered at each of Sites 1 and 2). Sites 4, 5, and 6 were apparently adjacent untreated controls.

Head

1	4	
2	5	Right
3	6	

Left

Tail

Observations and Results

Mortality

Animals were observed for signs of mortality twice daily (a.m. and p.m.) beginning 7 days prior to initiation of dosing, and continuing through the end of the in-life phase on Day 29.

The subcutaneous injection(s) of RBP-6000 to beagle dogs on Day 1 was not associated with early death in any animals. All animals survived to the Terminal Necropsy

Clinical Signs

Animals were observed for signs of morbidity twice daily (a.m. and p.m.) beginning 7 days prior to initiation of dosing, and continuing through the end of the in-life phase on Day 29.

The subcutaneous injection(s) of RBP-6000 to beagle dogs on Day 1 was not associated with moribundity in any animals. All animals survived to the terminal necropsy at Day 29. Not including anticipated mechanical effects of dosing and blood sampling and common background findings there were no unexpected clinical signs in the Atrigel control group. A small lump initially formed at each injection site due to implant formation by the test and control articles normally degraded over time (by Day 29).

Expected pharmacological effects of the test article (RBP-6000) due to buprenorphine included no feces (constipation), emesis, decreased activity, changes in food consumption (low or no food consumption), and decreased body weight. Watery feces were also observed. RBP-6000-related clinical signs were noted primarily between Days 1 through 7 for both sexes (see Table below), with resolution between Days 8 to 12 (data not shown). A definitive dose response relationship was not evident; however, when compared to the 20 and 40 mg/kg dose groups, the clinical signs were most frequent and severe for the 80 mg/kg dose group. Exaggerated pharmacological effects of the test article (RBP-6000) due to buprenorphine included muscle tremors and ataxia, and were reported for 2 of the 6 20 mg/kg animals and all 40 and 80 mg/kg animals on Day 2; and persisted through Day 4 for one 80 mg/kg male.

Due to the expected and exaggerated clinical signs from RBP-6000 treatment (decreased activity, dehydration, muscle tremors and ataxia, and low and/or no food consumption noted during Days 1 through 7), all RBP-6000-dosed animals received supportive care (80 mL Lactated Ringers administered subcutaneously) as follows:

- all 40 and 80 mg/kg animals on Day 3 (p.m.)
- all 20, 40, and 80 mg/kg animals on Day 4 (a.m. and p.m.)
- one 20 mg/kg female and two 80 mg/kg males on Day 5 (a.m. and p.m.)
- and one 80 mg/kg male on Day 6 (a.m.)

While some clinical signs and changes were expected, due to the dehydration and exaggerated clinical findings (i.e., muscle tremors and ataxia) all dose levels were considered to cause treatment-related toxicity with the low dose of 20 mg/kg RBP-6000 being considered a reversible Lowest Adverse Effect Level (LOAEL).

Clinical Signs, Food Consumption, and Clinical Evaluation/Treatment during Week 1

Dose Level (mg/kg)	Emesis	Decreased Activity	Decreased Skin Turgor[a, b]	SQ Fluids[b]	Dehydration[b]	Weight Loss[b]	Muscle Tremors/ Ataxia[b]	Low Food Consumption	No Food Consumed[a, b]
	Day 1								
20	0	3	0	0	0	0	0	0	1
40	0	1	0	0	0	0	0	0	0
80	0	6	0	0	0	0	0	0	0
	Day 2								
20	1	3	0	0	0	4	2	1	3
40	1	3	0	0	0	2	6	0	5
80	4	6	0	0	0	2	6	0	6
	Day 3								
20	1	2	1	0	0	1	0	0	4
40	0	3	0	6 (pm)	6	2	0	1	4
80	2	6	0	6 (pm)	6	5	1	3	3
	Day 4								
20	0	2	1	6 (am/pm)	6	0	0	0	0
40	0	0	0	6 (am/pm)	6	0	0	0	0
80	1	6	0	6 (am/pm)	6	0	1	0	0
	Day 5								
20	0	2	1	1 (am/pm)	1	0	0	0	0
40	0	0	0	0	0	0	0	0	0
80	0	2	0	2 (am/pm)	2	0	0	0	0
	Day 6								
20	0	2	1	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0
80	0	2	0	1 (am)	1	0	0	0	0
	Day 7								
20	0	3	0	0	1	0	0	5	0
40	0	0	1	0	0	0	0	2	0
80	0	2	0	0	0	0	0	0	0

[a] Per Summary and Individual Clinical Observations Tables
[b] Per written communications, veterinary requests, and clinical treatment records

Dermal Observations

Observations occurred Predose on Day 1; once daily during Week 1 (Days 1 through 7); then Days 8, 11, 15, 18, 22, 25, and prior to necropsy on Day 29 using a Draize 2 Scale method.

Expected effects from the injection of the Atrigel Delivery System (present in the test and control articles) included clinical signs at the injection site(s) (swelling, abrasion, reddening, and raised areas or masses). Dermal observations included edema, dermal irritation outside injection site(s), erythema, and erythema that extended beyond

injection site(s). Generally, the duration, frequency, and severity of the Atrigel Delivery System-related clinical signs and dermal observations correlated with increasing volume of the Atrigel Delivery System administered. The clinical signs and dermal observations were considered markedly frequent and severe for the 0 and 80 mg/kg groups that received three injections (largest volume).
Animals dosed at 20 mg/kg received one injection and had mildly frequent and severe reactions, while animals dosed at 40 mg/kg received two injections and had moderately frequent and severe reactions. The clinical signs and dermal observations were noted beginning on Day 1 (post dose) and the frequency and severity generally decreased beginning on Day 11. However, some of the clinical signs and dermal observations were still present through Day 29. Because the ATRIGEL Delivery System-related clinical signs and dermal observations were resolving after Day 11, they were not considered adverse by the report but were considered anticipated and adverse but reversible by this reviewer. On this basis, the low dose is the LOAEL with no apparent added effect due to presence of buprenorphine. This high dose and Atrigel-only effect were correlated with the ATRIGEL Delivery System-related histopathologic findings at the injection site(s) which included subcutaneous protein granulomatous inflammation with or without associated hemorrhage, observed in most animals from the control and high-dose groups, and subcutaneous fibroplasia, arterial adventitial hyperplasia, and ulceration in one 80 mg/kg female.

Edema was the most prominent and lasting dermal effect then dermal irritation outside the dosing site, erythema, and erythema outside the dosing site (see Table).

NDA 209819 – Local Irritation after Single Subcutaneous Dose in Dogs with RBP-6000 in Atrigel Delivery System for a 29-Day Study – Last Day Observed with 0.09 mL administered to separate dosing sites

Dose group[a] (mg/kg)	Dose volume (mL)	Sex	Edema (last day)	Irritation outside dose site (last day)	Erythema (last day)	Erythema outside dose site (last day)
0	0.36	M	29	8	3	0
		F	29	18	5	5
20	0.09	M	29	22	2	8
		F	29	22	1	8
40	0.18	M	29	22	NR[b]	NR
		F	29	29	NR	NR
80	0.36	M	29	29	NR	NR
		F	29	29	8	8

a - mg/kg buprenorphine in Atrigel
b – not observed to occur

Body Weights

Body weights were measured at least twice prior to the first dose (Weeks -2 and -1), once daily during Week 1 (Days 1 through 7), then on Days 8, 11, 15, 18, 22, 25, and

prior to necropsy on Day 29. For a single dose, 29-day observation period, body weight changes not total body weight is evaluated.

Body weight loss is an expected pharmacological effect of buprenorphine in the test article RBP-6000, primarily as a result of low and/or no food consumption. Buprenorphine-related body weight loss was noted for both sexes for all RBP-6000 dose levels beginning on Day 2, persisted through Day 29 (necropsy) and was consistent with changes in food consumption. A dose response relationship was not evident, with similar body weight loss for each sex across all three dose levels. On Day 29, intergroup differences in body weights attained statistical significance for females dosed at 20 mg/kg (Group 2). Although the body weights for the RBP-6000-dosed animals did not fully recover to prestudy levels by Day 29, the body weight loss stabilized by approximately Day 7, and was less pronounced through Day 29 (necropsy) (see Table). As such, the RBP-6000 (buprenorphine)-related body weight loss was not considered adverse for any of the dose levels by the report but the mid dose was considered a LOAEL by the reviewer.

Mean Absolute Body Weight Gain/Loss

	Days 1 to 7 (Day 1 Body Weight as Base)		Days 8 to 29 (Day 8 Body Weight as Base)		Days -8 to 29 (Day 1 Body Weight as Base)	
Dose Level	Males	Females	Males	Females	Males	Females
0 mg/kg	0.07 kg (0.64%) gain	0.07 kg (0.85%) gain	0.23 kg (2.74%) gain	0.17 kg (2.04%) gain	0.37 kg (4.20%) gain	0.20 kg (2.58%) gain
20 mg/kg	0.73 kg (8.90%) loss	0.77 kg (9.49%) loss	0.10 kg (1.51%) gain	0.30 kg (3.64%) loss	0.67 kg (7.97%) loss	1.17 kg (14.10%) loss
40 mg/kg	0.60 kg (6.79%) loss	0.60 kg (7.58%) loss	0.27 kg (3.29%) gain	0.00 kg (0.43%) gain	0.43 kg (4.81%) loss	0.67 kg (8.20%) loss
80 mg/kg	0.70 kg (8.24%) loss	0.67 kg (7.70%) loss	0.17 kg (2.16%) gain	0.17 kg (2.17%) loss	0.57 kg (6.67%) loss	0.80 kg (9.36%) loss

Food Consumption

Food consumption was measured by weighing the food bowl before and after the 4-hour feeding period at least twice prior to the day of dosing (Day 1), once daily during Week 1 (Days 1 through 7), then weekly thereafter starting on Day 14 through the day of necropsy.

Treatment-related changes in food consumption (low or no food consumption) was discussed in the Clinical signs section previously with associated decreased body

weight being discussed in the previous section to this one. The LOAEL is the low dose of 20 mg/kg RBP-6000.

Ophthalmoscopy – none

ECG – none

Clinical Pathology Evaluation

Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from all animals within one week prior to the initiation of dosing and on Day 29 prior to necropsy. Urine samples were obtained by drainage from special stainless-steel cage pans during Week -1 (prestudy) and on Day 29. The animals were fasted for at least 8 hours prior to blood collections for serum chemistry.

Hematology

Parameters Analyzed:

Hematology Parameters	
Red blood cell (RBC) count	Red cell distribution width (RDW)
Hemoglobin concentration	Reticulocyte count
Hematocrit	Platelet count
Mean corpuscular volume (MCV)	White blood cell (WBC) counts [a]
Mean corpuscular hemoglobin concentration (MCHC)	
Mean corpuscular hemoglobin (MCH)	

[a] Included total white blood cell, absolute polysegmented neutrophil, lymphocyte, monocyte, eosinophil, basophil, and other cell counts as appropriate.

There were no Atrigel- or RBP-6000-related changes in red blood cell parameters although reticulocytes were increased up to 3 fold from Day -4 to 29 in a non-dose responsive manner. In the white blood cell parameters, the eosinophil counts were increased on Day 29 compared to prestudy for RBP-6000 dosed animals at 20, 40, and 80 mg/kg and ranged from 1.90- to 3.85-fold and was larger than Atrigel control changes (see Table). Although the increased eosinophil counts were not considered to be adverse, the relationship to RBP-6000 is toxicological relevance is uncertain.

Fold Change for Mean Eosinophil Counts
(Day 29 Compared to Prestudy)

Dose Level	Males	Females
0 mg/kg	0.75	1.35
20 mg/kg	3.43	1.90
40 mg/kg	2.32	2.21
80 mg/kg	3.85	2.13

Coagulation

Parameters Analyzed:

Coagulation Parameters
Prothrombin time (PT)
Activated partial thromboplastin time (APTT)

There were no Atrigel- or RBP-6000-related changes in coagulation parameters.

Clinical Chemistry

Parameters Analyzed:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alkaline phosphatase (ALP)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Lactate dehydrogenase (LD)	Glucose
Total bilirubin	Cholesterol
Urea nitrogen (BUN)	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Bicarbonate

There were no Atrigel Delivery System- or RBP-6000-related changes in serum chemistry parameters.

Urinalysis

Parameters Analyzed:

Urinalysis Parameters	
Color/Character	Ketones
pH	Bilirubin
Specific gravity	Occult blood
Protein	Microscopics
Glucose	

There were no Atrigel Delivery System- or RBP-6000-related changes in urinalysis and urine chemistry parameters noted through Day 29.

Gross Pathology

All euthanized animals were subjected to a complete gross necropsy examination. The necropsy examination included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

The following tissues and organs were collected from all animals. Tissues were preserved in 10% neutral-buffered formalin (except for the eyes, which were preserved in Davidson's fixative for optimum fixation).

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Glands (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Ovaries
Stomach	Uterus
Small Intestine	Cervix
Duodenum	Vagina
Jejunum	Endocrine
Ileum	Adrenals
Large Intestine	Pituitary
Cecum	Thyroid/Parathyroids[a]
Colon	Skin/Musculoskeletal
Rectum	Skin/Mammary Gland
Pancreas	Bone (femoral head)
Liver	Bone (7th rib)
Gallbladder	Skeletal Muscle (psoas and diaphragm)
Respiratory	Nervous/Special Sense
Trachea	Eyes with Optic Nerve
Lung	Sciatic Nerve
Lymphoid/Hematopoietic	Brain

Bone Marrow (sternum)	Spinal Cord (thoracic)
Thymus	Other
Spleen	Animal Number Tattoo
Lymph Nodes	Gross Lesions
Axillary	Injection Sites[b]
Mesenteric	

[a] The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

[b] Scapular/interscapular area. At least a 1 inch margin of skin surrounding the implant (at the injection site) was dissected. When multiple injections were administered, the skin and implant from the first injection was removed. When the implant was visible, it remained intact and was not disturbed by removal from the fatty tissue or fascia. When the implant was adhered to the skin, the skin and implant were removed in one piece and the implant was sectioned with the skin. When the implant was not adhered to the skin, the implant and subcutaneous, fascia, and muscle tissue(s) were dissected such that the implant remained intact, and the implant was sectioned with the tissue(s). For animals that received multiple injections (Groups 1, 3, and 4), when the implant from the first injection site was not visible for collection, then the second injection site (Groups 1, 3, and 4) were examined for the presence of an implant and the implant was collected from the second injection site as detailed above (when present). When the implant from the second injection site was not visible, then the third injection site (Groups 1 and 4 only) was examined for the presence of an implant and the implant was collected from the third injection site as detailed above (when present). The injection site that the implant was collected from was documented and sectioned as detailed above.

Two bone marrow smears from the seventh rib of each animal were made, but were not examined microscopically because there were no suspected test article-related alterations observed in hematology parameters or in the standard histopathology sections of the bone marrow that would be further elucidated by examination of the smears. The bone marrow smears were retained and archived with the study.

There were no gross findings that were considered related to the administration of the test article other than those associated with the injection of the Atrigel vehicle.

Organ Weights

For all euthanized animals, the following organs were weighed before fixation with paired organs being weighed together. Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

The following organs were weighed:

Organs Weighed	
Adrenals	Ovaries
Brain	Pituitary
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Lungs	

Non-dose-proportional decreases in spleen and thymus weights and increases in thyroid weights were observed for organ weight, organ-to-body weight ratio, and organ-to-brain weight ratio (see Table). Statistical significance between treated and Atrigel controls is only observed in the mid-dose females for the spleen and likely due to the large standard deviations for the mean values (not shown) for the groups of 3 animals. Each organ will be discussed individually after the table.

NDA 209819 - Changes in Organ Weights, Organ-to-Body Weight Ratio, and Organ-to-Brain-Weight Ratio for Select Organs in Dogs after a Single Subcutaneous Dose of RBP-6000 Compared to the Atrigel Vehicle[a]

ORGAN and Dose Group[b]	Organ Weights (% Differences)		Organ/Body Weight Ratio (% Differences)		Organ/Brain Weight Ratio (% Differences)	
	Male	Female	Male	Female	Male	Female
Spleen						
Low dose	-27	-34	-16	-20	-25	-28
Mid dose	-31	-51[c]	-29	-43[c]	-28	-48[c]
High dose	-9	-33	+1	-28	-5	-28
Thymus						
Low dose	-51	-59	-42	-51	-50	-56
Mid dose	-31	-38	-26	-29	-27	-33
High dose	-52	-20	-45	-14	-50	-14
Thyroid						
Low dose	-19	+23	-8	+51	-16	+36
Mid dose	+25	-3	+13	+12	+34	+5
High dose	+18	+67	+28	+89	+25	+89

a – no other notable differences observed for other organs
b – 20, 40, & 80 mg RBP-600/kg, respectively
c – statistically signifi9cant at p>= 0.05

Non-dose-proportional decreased spleen weight, spleen to body weight ratio, and spleen to brain weight ratio were observed for dosed animals compared to Atrigel controls, with only the mid-dose females containing any statistical significance (p<= 0.05). Non-dose-proportional, test article-related decreased body weights were observed compared to controls. Moreover, the data suggested that decreased spleen weights and ratios in dosed females could not be completely accounted for by decreased body weight alone. There was no histologic correlation to decreased splenic weights and/or ratios in RBP-6000 dosed animals. Therefore, the spleen weight changes are not considered toxicologically significant.

Non-dose-proportional decreased thymic weights and ratios were observed for dosed animals compared to Atrigel controls with no statistical significance being identified. There were microscopic thymus findings of cortical hypocellularity in high-dose males and females and Atrigel vehicle control males, but the incidence does not correlate well with the organ weight changes. Therefore the toxicological relevance is unclear.

Non-dose-proportional increased thyroid weights and ratios were observed for dosed animals compared to Atrigel controls with not statistical significance being identified. These changes were not well correlated to histopathologic findings in animals examined. The increased variability of thyroid weights and ratios were considered additional evidence for a questionable association of these changes with the administration of the test article.

Histopathology

For all animals necropsied, the tissues listed in the table above were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The tissues from the control and high dose groups (Groups 1 and 4, respectively) were examined by a Veterinary Pathologist. Based on the histopathology evaluation for the control and high-dose groups (Groups 1 and 4, respectively) and per consultation with the Study Pathologist and the Sponsor, the low- and mid-dose groups (Groups 2 and 3, respectively) were not examined histologically, with the exception of the liver tissue for one Group 3 female based on gross observation.

Adequate Battery – yes

Peer Review – not reported/found

Histological Findings - There were no histopathologic findings that were considered related to the administration of the test article buprenorphine other than Atrigel Delivery System-related findings at the injection site(s) (see Table). These included subcutaneous protein granulomatous inflammation with or without associated hemorrhage, observed in most animals, and subcutaneous fibroplasia, arterial adventitial hyperplasia, and ulceration in one high-dose female animal. Protein granulomatous inflammation was in the subcutaneous tissue and was characterized by a well-defined, typically circumferential infiltrate of epithelioid and non-epithelioid macrophages and multinucleated giant cells, often with intracytoplasmic and free eosinophilic, large, globular protein deposits.

Compared to Atrigel controls, the character of this inflammation in test article-dosed animals were generally comparable with a possible increased incidence and severity in the high-dose group compared to the Atrigel controls. The nature of the inflammation was the same between groups and hence was considered related to administration of the delivery system with a possible buprenorphine influence in the high-dose group. This inflammation was graded as mild to marked and did not exhibit a biased severity between groups.

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations (with Severity) (Day 29)
Study: NNQ00003

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
INJECTION SITE(S);								
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	1	0	0	0	1	0	0	0
Fibroplasia; Subcutaneous; Dermis	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
moderate	0	0	0	0	0	0	0	1
Fibrosis; superficial; Dermis; focal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
Hemorrhage; Subcutaneous	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild	0	0	0	1	0	0	0	0
Hyperplasia; Artery; Adventitia; multifocal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
mild	0	0	0	0	0	0	0	1
Ulcer; left; focally extensive	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
moderate	0	0	0	0	0	0	0	1
Protein Granulomatous Inflammation; Subcutaneous	(1)	(0)	(0)	(3)	(2)	(0)	(0)	(3)
mild	0	0	0	1	0	0	0	0
moderate	1	0	0	1	1	0	0	2
marked	0	0	0	1	1	0	0	1

All other histopathologic findings, most notably the spleen, thymus, and thyroid which exhibited different weights and weight ratios than Atrigel controls, were considered incidental because they were consistent with spontaneous findings or normal physiologic processes in beagle dogs at the Testing Facility and/or the incidence and/or severity did not suggest an association with dosing with the test article (see Table).

Pathology - Intergroup Comparison of Gross/Histo Pathology Observations (Day 29)
Study: NNQ00003

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
SPLEEN;								
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	3	0	0	3	3	0	0	3

THYMUS;									
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	0	0	0	1	3	0	0	0
Hypocellularity; Lymphocyte; Cortex	3	0	0	2	0	0	0	3
Cystic Involution, Hassalls Corpuscles	1	0	0	0	0	0	0	0
THYROID;									
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	3	0	0	3	3	0	0	1
Cyst	0	0	0	0	0	0	0	1
Hyperplasia, C-Cell; focal	0	0	0	0	0	0	0	1

Special Evaluation

Toxicokinetics

Blood samples for toxicokinetics were collected on Day -4, post dose at 1, 2, 4, and 8 hours on Day 1 and Days 2, 3, 4, 5, 8, 11, 15, 18, 22, 25, and prior to necropsy on Day 29 at the listed time past the single dose on Day1 as noted in the Table.

Sampling Schedule

Group	Number of Animals/ Sex/ Group	Toxicokinetic Time-points (Hours Post Dose)															
		Day -4	Day 1				Day 2	Day 3	Day 4	Day 5	Day 8	Day 11	Day 15	Day 18	Day 22	Day 25	Day 29
		Pre-study	1	2	4	8	24	48	72	96	168	240	336	408	504	576	672
1 to 4	3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

X Sample collected

The toxicokinetics of buprenorphine and norbuprenorphine (norbuprenorphine not discussed in this review) were characterized after a single subcutaneous injection of Buprenorphine/ATRIGEL in beagle dogs, at dose levels of 20, 40, and 80 mg/kg. Buprenorphine profile was characterized by multiple peaks several days apart. Maximal concentrations of buprenorphine were observed either at the first or at second peak, with T_{max} ranging from 1 to 504 hours. Due to the fluctuating nature of the concentration vs. time profiles, a terminal phase could be characterized only for a few animals, with terminal half-lives ranging from 66.2 to 241 hours.

There were no sex-related trends in exposure. The increase in buprenorphine exposure was slightly greater than proportional to the increase in dose from 20 to 80 mg/kg in males, but it was less than proportional to the increase in dose in females. On sex-combined exposure parameters, the increase in exposure was proportional to the increase in dose from 20 to 80 mg/kg. The ratio of norbuprenorphine to buprenorphine exposure parameters was similar at all dose levels. Values are listed in the Table.

Buprenorphine Mean Exposure Parameters

			TK Parameters - Buprenorphine	
Gender	Group Number	Dose Level (mg/kg)	Mean Cmax ± SD (ng/mL)	Mean AUC(0-t) ± SD (ng*h/mL)
Males	2	20	15.5 ± 6.13	6214 ± 2967
	3	40	28.0 ± 10.30	11043 ± 4818
	4	80	97.2 ± 18.20	29708 ± 9153
Females	2	20	15.8 ± 7.80	5223 ± 2392
	3	40	55.7 ± 27.70	13486 ± 3549
	4	80	42.3 ± 10.30	17493 ± 7799
Sex-combined	2	20	15.6 ± 6.28	5718 ± 2470
	3	40	41.9 ± 24.10	12264 ± 4014
	4	80	69.7 ± 32.90	23600 ± 10129

Dosing Solution Analysis

The test article was provided by the Sponsor as a preformulated solution in syringes and was used as received. Stability analysis and verification of test article concentration were conducted by the Sponsor and reported as acceptable.

Based on the Certificates of Analysis (COA) included in the Test Article Report provided by the Sponsor, the absence of test article in the control article was confirmed, and the concentration of the test article was determined to be acceptable (99.94% purity) over the course of the study. Additionally, based on the analysis of samples stored at the Sponsor's facility under the same conditions as the Testing Facility and the Certificates of Stability (C of S), the test and control articles were determined to be stable over the course of the study.

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6.2 Repeat-Dose Toxicity

Study title: A 6-month Multi-dose Toxicity and Toxicokinetics Study of Buprenorphine/ATRIGEL® (RBP-6000) Administered Subcutaneously to Sprague-Dawley Rats, with a 12-week Recovery Period

Study no.:	RBLS-R04-60-09
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 17, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - (b) (4)% buprenorphine base in (b) (4)% 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4)% N-methyl-2-pyrrolidone (NMP)), Lot 0042-01, (b) (4)%. - ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP), Lot 0042-21.

Key Study Findings

- Male and female rats received six monthly subcutaneous doses to the same dosing area of the back at levels of 0 (Atrigel control), 10, 25, and 100 mg/kg buprenorphine in RBP-6000. Animals were sacrificed at 1, 2, and 3 months after the last dose.
- A full GLP study with all relevant indices was conducted including histology and toxicokinetics.
- There were no significant test article-related effects on body weight, food consumption, ophthalmology, hematology, serum chemistry, coagulation, or urinalyses parameters. Early mortalities occurred in all groups, but were not considered related to the administration of the test article.
- Dose related RBP-6000-related clinical observations noted in all dosed groups were overly aggressive behavior, urine stained fur, discolored urine (orange, red, and/or yellow), decreased activity, and broken/cracked teeth. All of these observations were also noted in the control animals with the exception of decreased activity. However, the incidence was low compared to the RBP-6000-dosed animals. Resolution occurred by the end of the recovery period
- Local effects: Dose site clinical observations (abrasion, reddened, and swollen) and dermal dose site scoring were similar in control and RBP-6000-dosed groups. The severity of abrasions was higher in the control animals. Dermal scoring noted edema and erythema in all animals on study, including the controls with up to Grade

3-4 but usually 0-2, Grades 0-2 in low-dose, 0-1 in mid-dose, up to Grade 4 in high-dose animals but usually 0-2. The dose response here is in relations to the amount of Atrigel. Eschar and test site staining was also noted in all groups with approximately equal incidence. The highest incidences of these observations were always the day after dosing and a few days post dose with the incidence decreasing over time. Microscopically, granulomas, which were characterized by macrophages, lymphocytes, plasma cells and multinucleated giant cells, were observed at injection sites in both vehicle- and RBP-6000-treated animals. At 30 days post last dose, the incidence and severity ranked from low to high as follows: LD>Veh>MD>HD, which suggests the findings were attributable to the vehicle and exacerbated by buprenorphine. By 90 days post last dose, the incidence and severity did not change much for the vehicle, MD, and HD groups, but there was complete recovery for the LD group. The LOAEL for local effects was the low-dose of 10 mg/kg/dose, which consisted of 0.045 mL/kg of the clinical product.

- The C_{max} of buprenorphine for the low- and mid-dose groups remained relatively similar between dosing occasions and between sexes, reflecting a good consistency of exposure at C_{max}, but the high-dose group was more variable. The C_{max} of buprenorphine was generally less than dose proportional. The $AUC_{(0-672)}$ of buprenorphine remained relatively similar between dosing occasions for the low- and mid-dose groups, but increased after repeated administration for the high-dose group, especially for the males. $AUC_{(0-672)}$ of buprenorphine was generally less than dose proportional at the early doses but became more than dose proportional for the later doses, which could result from a steady state being achieved.
- Systemic effects: Systemic target organs included the pancreas and lungs. Pancreatic acinar cell apoptosis and alveolar macrophage infiltrates in the lungs were seen with increased severity and incidence in 25 and 100 mg/kg RBP-6000-dosed rats compared to controls and 10 mg/kg RBP-6000-dosed groups. As these findings persisted at increased severity and incidence in 25 and 100 mg/kg RBP-6000-dosed rats compared to controls and 10 mg/kg RBP-6000-dosed groups, on Day 225 (90 days post dosing), 10 mg/kg could be at least described as a LOAEL and possible NOAEL according to this reviewer.
- At 10 mg/kg/day, the C_{max} ranged from 49.6-69.6 ng/mL and the $AUC_{(0-672)}$ for the month ranged from 3109-6926 ng*hour/mL.
- The final C_{max}s (68.0 ng/mL male and 142 ng/mL female) and $AUCs_{(0-24h)}$ (653 ng*h/mL male and 590 ng*h/mL female) were used in the Applicant's Safety Margins calculations whereas a mean of the six values yield C_{max}s of (62.9 ng/mL male and 73.2 ng/mL female) and $AUCs_{(0-24h)}$ (575 ng*h/mL male and 462 ng*h/mL female)

Methods

Doses:	0 (Atrigel control), 10, 25, and 100 mg/kg buprenorphine in RBP-6000. Doses selected based on information derived from previous studies (non-GLP MTD study, 4-week single dose study). There was no saline control group in this study.
Frequency of dosing:	Monthly for 6 months. The first day of dosing was designated Day 1 with subsequent doses given on Days 29, 57, 85, 113, and 141 (beginning of Month 6).
Route of administration:	Subcutaneous to the same demarcated site each month on the animal's shaved back
Dose volume:	See study design below
Formulation/Vehicle:	Atrigel - (b)(4)% PLGH (b)(4)% NMP in control and (b)(4)% PLGH: (b)(4)% NMP in drug product with (b)(4)% buprenorphine
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	28 per sex in main study and 9 per sex in toxicokinetic animals
Age:	13 weeks
Weight:	327.6 to 453.9 g for the males and 203.4 to 278.3 g for the females
Satellite groups:	Toxicokinetic groups 5-7 (surviving until scheduled euthanasia not necropsied) and main study recovery animals (necropsied at 1, 2, and 3 months after last dose).
Unique study design:	No.
Deviation from study protocol:	Nothing major reported.

Study Design

Group No.	No. of M/F	Dose Level[a] (mg/kg)	Dose Mass (mg/kg)[b]	Dose Volume[a] (mL/kg)	Buprenorphine Dose Concentration (w/w%)	Terminal Necropsy Day 169 M/F	Recovery Necropsy Day 197 M/F	Recovery Necropsy Day 225 M/F
1 (control)	28/28	0 (control)[d]	500	0.455	0	15/14	10/10	3/3
2	28/28	10	50	0.045	(b) (4)	14/14	10/10	3/2
3	28/28	25	125	0.114		13/14	10/10	3/3
4	28/28	100	500	0.455		14/14	9/10	3/3
5[c]	9/9	10	50	0.045		NA	NA	NA
6[c]	9/9	25	125	0.114		NA	NA	NA
7[c]	9/9	100	500	0.455		NA	NA	NA

M/F = Male/Female

[a] Doses were delivered within ± 20% of the desired dose mass.

[b] Dose volume was an approximation and only used after calculating target dose levels in w/w.

[c] Animals in the TK groups were used solely for the purpose of blood sample collections and were euthanized after the final collection without necropsy.

[d] ATRIGEL® vehicle.

Observations and Results

Mortality

All animals (including the TK groups) were observed for signs of mortality twice daily (a.m. and p.m.), beginning on the day of acclimation to housing and continuing through the day of necropsy.

No treatment related mortalities were observed in animals form all test groups that were euthanized early or found dead (see first table) as mortalities were dispersed across all groups, without regard to treatment (see second table).

Mortalities, Fate and Treatment Relationship

Animal/Sex/ Group	Fate	Study Day	Treatment Related	Explanation
1506/F/1	Euthanized	122	No	Due to broken teeth and declining health
2004/M/2	Euthanized	120	No[a]	Due to declining heath. Decreasing weight, urine staining, discolored urine, dose site abrasion, lethargy, and nasal discharge
2517/F/2	Found Dead	79	No	Found after clinical pathology sample collection and death considered to be procedure related
2528/F/2	Euthanized	196	No	Due to welfare issues related to severe foot lesions
3002/M/3	Euthanized	81	No	Due to broken teeth and declining health
3016/M/3	Found Dead	122	No[a]	Discolored urine, abrasion on hindpaw, and nasal discharge
3505/F/3	Found Dead	117	No[a]	Decreasing weight , eye discharge and nasal discharge
4022/M/4	Euthanized	83	No	Due to declining health and had prostatitis (see *Pathology Report*, Appendix 6)
4023/M/4	Euthanized	141	No	Due to welfare issues related to severe foot lesions
4526/F/4	Found Dead	19	No	Was noted as pale and lethargic prior to death. Death considered due to necrotizing vaginitis, pyonephritis, and septic pneumonia (see *Pathology Report*, Appendix 6)
5506/F/5	Euthanized	48	No	Due to fractured jaw
6501/F/6	Found Dead	198	No	Found 24 hours post TK blood collection; was noted with large hematoma in the jugular area and blood pooling in right axillary region. Death considered to be procedure related
6505/F/6	Found Dead	99	No	Died after TK blood collection while being treated by a veterinarian. Death considered to be procedure related
7005/M/7	Found Dead	7	No	Animal had weight loss, discolored urine, and rough hair coat after Day 1 TK blood collections. Death considered to be procedure related
7006/M/7	Euthanized	160	No	Due to declining health. Death considered to be procedure related
7503/F/7	Euthanized	85	No	Due to declining health. Death considered to be procedure related
7508/F/7	Found Dead	127	No	Found after TK blood collection. Death considered to be procedure related

[a] Histopathology evaluations were done on only lung, pancreas, and injection sites for the low and mid dose animals.

Summary of Mortalities

Dose Level mg/kg (Group)	No. per Sex/Group	Mortalities	
		Males	Females
0 (1)	28	0	1
10 (2 & 5)	37	1	3
25 (3 & 6)	37	2	3
100 (4 & 7)	37	4	3

Clinical Signs

All animals (including the TK groups) were observed for signs of morbidity twice daily (a.m. and p.m.), beginning on the day of acclimation to housing and continuing through the day of necropsy. Each animal in the Main Study groups (Groups 1 to 4) were observed cage side once daily for changes in general appearance and behavior.

RBP-6000-related clinical observations were overly aggressive behavior, urine stained fur, discolored urine (orange, red, and/or yellow), decreased activity, and broken/cracked teeth. All of these observations were also noted in the control animals with the exception of decreased activity. However, the incidence was low compared to the RBP-6000-dosed animals. Buprenorphine is known to cause pica behavior in Sprague-Dawley rats which increases the probability of the RBP-6000-dosed animals to chew on caging. The notable RBP-6000-related clinical observations were generally dose-related as listed in the table.

RBP-6000-related clinical observations (Main and Recovery Animals)

Clinical Observation	Dose Level (Male/Female No. Noted with Observation)			
	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg
Overly Aggressive	2/0	10/10	8/4	7/10
Urine Stained Fur	7/9	5/2	11/8	12/24
Discolored Urine	2/1	5/0	2/2	8/14
Decreased Activity	0/0	2/0	1/0	1/3
Broken/Cracked Teeth	1/1	1/3	2/2	3/4

All other noted observations were considered incidental findings that included, but were not limited to, alopecia, eye discharge, nasal discharge, abrasions other than at test site, and swollen neck/face (related to collars to prevent pica behavior) were of low incidence, not dose-dependent, noted in control animals, and/or are common findings for rats of this age.

Dermal Observations

Dermal observations were conducted for Groups 1-5 (Main Study animals only) prior to each monthly dose, then once daily for 7 days, and then once weekly prior to the next dose. A final dermal observation was made on the day of necropsy prior to euthanasia (scheduled and unscheduled). Each animal was removed from the cage according to the listed schedule and observed in detail according to the Macroscopic Dermal Grading

System which is based on the Draize 2 method with scoring ranges from 0 or 1 to 4 with 4 being most severe. Observations were made for erythema, edema, eschar, blanching, ulceration, necrosis, and superficial lightening. Observations were also made for desquamation, fissuring, eschar exfoliation, test sight staining, and erythema beyond the test site.

Dose site clinical observations (abrasion, reddened, and swollen) and dermal dose site scoring were similar in control and RBP-6000-dosed groups. The severity of abrasions was higher in the control animals. Dermal scoring noted edema and erythema in all animals on study, including the controls with up to Grade 3-4 but usually 0-2, Grades 0-2 in low-dose, 0-1 in mid-dose, up to Grade 4 in high-dose animals but usually 0-2. The dose response here is in relations to the amount of Atrigel. Eschar and test site staining was also noted in all groups with approximately equal incidence. The highest incidences of these observations were always the day after dosing and a few days post dose with the incidence decreasing over time. The dose site observations were considered related to the ATRIGEL Delivery System injection and not related to RBP-6000.

Dose Site Observations

Dose Site Ob	0 mg/kg Males/Females	10 mg/kg Males/Females	25 mg/kg Males/Females	100 mg/kg Males/Females
Abrasion	28/28	28/28	28/28	28/27
Reddened	21/21	11/18	9/15	10/22
Swollen	2/2	0/0	0/0	0/0
Mass	1/0	0/0	0/0	0/0
Abrasion Severity				
Slight	28/28	28/28	28/28	28/27
Moderate	7/7	1/1	0/1	1/4
Severe	2/3	0/0	0/0	0/0
Dermal Scoring				
Edema	all	all	all	all
Erythema	all	all	all	all

Body Weights

Body weights of Main Study and TK animals were noted twice prior to the first dose (Day 1) and weekly thereafter. For the Main Study animals, a final fasted body weight was noted on the day of scheduled necropsy for calculation of organ-to-body weight ratios.

Mean body weights were statistically significantly (SS) lower for 10, 25, and 100 mg/kg dosed males at Week 1 as compared with control animals. This related to an RBP-6000-related statistically significant lower mean body weight for the males at all RBP-

6000 dose levels throughout the dosing period (see table), which could be misleading if just compared on a control versus treated basis.

Summary of Mean Body Weights

Dose Level	Mean Weight (Male/Female in Grams)			
	Week 1	Week 12	Week 26	Week 32
0 mg/kg	413.7/242.8	558.9/286.2	669.9/317.7	676.8/331.8
10 mg/kg	387.5/247.3	514.8/287.6	587.9/318.8	648.4/307.0
25 mg/kg	390.5/245.1	524.0/283.5	594.8/324.1	637.0/318.7
100 mg/kg	385.2/244.3	515.5/286.8	601.5/317.5	573.8/328.8

However, considering within groups comparisons (e.g., Week 1 body weight for high-dose group to Week 32 body weight for high-dose group) identified Week 1 to 32 body weights increasing at 63%/37%, 67%/24%, 63%/30%, and 50%/35% for control, low-, mid-, and high-dose groups, respectively. High-dose males lost 5% of body weight from Week 26 to 32. Based on these observations, RBP-6000 dosing did not adversely affect body weights.

Mean body weight gains showed somewhat similar but differing results as listed in the table. While no RBP-6000 treatment effects was observed in females, there were some differences in males with statistically significant difference compared to control. The relative change of ~10% is notable but a lack of any dose-response and overlap of SDs (standard deviations), makes these effects of unknown toxicological significance.

Body weight gains over dosing period with RBP-6000 in rats dosed monthly for 6 months			
Gender	Dose group	Absolute weight gain week -1 to 24 (SD)	% weight gain week -1 to 24 (SD)
Male	Control[a]	245.66 g (51.80)	63.95 g (12.75)
	10 mg/kg	199.48 g (41.96)*	51.97 g (9.59)*
	25 mg/kg	192.89 g (38.95)*	49.94 g (8.60)*
	100 mg/kg	192.12 g (38.35)*	51.46 g (10.71)*
Female	Control[a]	81.29 g (19.12)	35.51 g (7.20)
	10 mg/kg	84.94 g (15.41)	36.64 g (6.38)
	25 mg/kg	81.91 g (18.31)	36.39 g (8.07)
	100 mg/kg	75.79 g (16.13)	33.15 g (6.94)

a – Atrigel only group
* - statistically significant at p <= 0.05 level compared to the control

These indices for body weights and body weight gains were also generally unremarkable for the recovery groups.

Food Consumption

Food consumption for Main study animals only was determined weekly beginning in Week -1.

Mean food consumption was in general comparable to controls in all RBP-6000-dosed groups. Although the mean food consumption was slightly lower at times for the 100 mg/kg-dosed males, it was not considered outside normal variability by the conducting laboratory.

Ophthalmoscopy

An ophthalmic exam was conducted on all Main Study animals during the pre-study period and on Groups 1 and 4 within a week of terminal necropsy.

There were no abnormal ophthalmic findings noted in the test article-dosed animals.

ECG – Not evaluated.

Clinical Pathology

Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from Main Study animals within one week prior to initiation of dosing, Week 12, prior to Day 84, and on Days 169, 197, and 225. Urine samples were obtained on ice by overnight cage collection from all available Main Study animals pre-study and prior to Terminal and Recovery necropsies.

Hematology – The following parameters were analyzed:

Hematology Parameters	
Red blood cell (RBC) count	Red cell distribution width (RDW)
Hemoglobin concentration	Reticulocyte count
Hematocrit	Platelet count
Mean corpuscular volume (MCV)	White blood cell (WBC) counts [a]
Mean corpuscular hemoglobin concentration (MCHC)	
Mean corpuscular hemoglobin (MCH)	

a Included total white blood cell, absolute polysegmented neutrophil, lymphocyte, monocyte, eosinophil, basophil, and other cell counts as appropriate.

There were no hematology changes that were considered related to test or control article administration. Intergroup differences, some statistically significant, were dose-independent or within the range of variability for rodents thus were not considered related to test article administration. The control group had no intergroup differences, and all values were considered within the range of variability for Sprague-Dawley rats over the time frame of the study.

Coagulation - The following parameters were determined:

Coagulation Parameters
Prothrombin time (PT)
Activated partial thromboplastin time (APTT)
Fibrinogen

There were no coagulation parameter changes that were considered related to test or control article administration. Intergroup differences, some statistically significant, were dose-independent or within the range of variability for rodents thus were not considered related to test article administration. The control group had no intergroup differences, and all values were considered within the range of variability for Sprague-Dawley rats over the time frame of the study.

Clinical Chemistry – The following parameters were analyzed:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alkaline phosphatase (ALP)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Lactate dehydrogenase (LD)	Glucose
Total bilirubin	Cholesterol
Urea nitrogen (BUN)	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

There were no serum chemistry changes that were considered related to test or control article administration.

Intergroup differences, some statistically significant, were dose-independent or within the range of variability for rodents thus were not considered related to test article administration. The control group had no intergroup differences, and all values were considered within the range of variability for Sprague-Dawley rats over the time frame of the study.

Notable was lactate dehydrogenase in males (see table). Note that Day 169, Day 197, and Day 225 were different animals (main study and two recovery groups). The toxicological significance of this is unknown as increases in this enzyme is the indicator of toxicity and as there was no histological correlate of toxicity.

Serum Chemistry - Males Summary

Study: STUDY NNQ00005
Compound: See Study Protocol
Sex: M
Analysis Framework: 1-Factor ANOVA
Primary Factor: Treatment Group Code

Parameter: Lactate Dehydrogenase - U/L

GROUP	STATISTIC	PRESTUDY	WEEK 12	DAY 169	DAY 197	DAY 225
1	Mean	1476.1	1332.3	1056.4	1236.1	1294.7
	SD	527.5	409.5	418.6	571.8	263.3
	N	28	28	15	10	3
	Statistical Sig					
2	Mean	1499.6	1529.0	968.1	1062.3	354.0
	SD	659.7	728.3	462.2	691.7	192.4
	N	27	28	14	10	3
	Statistical Sig					*
3	Mean	1666.7	1334.2	1296.5	1527.7	584.7
	SD	600.1	392.9	543.1	732.4	174.6
	N	24	28	13	10	3
	Statistical Sig					*
4	Mean	1491.7	1369.7	1159.2	741.0	649.0
	SD	611.1	591.2	614.6	389.8	108.0
	N	27	28	14	9	3
	Statistical Sig					*

* ANOVA with Dunnett's/Dunn's (p <= 0.05)
Groups with n<3 were excluded from statistical analysis

Urinalysis - The following parameters were analyzed:

Urinalysis Parameters	
Color/Character	Ketones
pH	Bilirubin
Specific gravity	Occult blood
Protein	Microscopics
Glucose	Total Volume

There were no urinalysis parameter changes that were considered related to test or control article administration. Intergroup differences, some statistically significant, were dose-independent or within the range of variability for Sprague-Dawley rats over the time frame of the study thus were not considered related to test article administration. The control group had no intergroup differences, and all values were considered within the range of variability for rodents.

Gross Pathology

All Main Study animals were subjected to a complete gross necropsy examination after sacrifice on Days 169, 197 (recovery group), 225 (recovery group). The necropsy examination included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

The following tissues and organs were collected for the main and recovery animals for all animals in groups 1-4 (main and recovery animals), not for TK groups:

Tissues Collected	
Cardiovascular Aorta Heart Digestive Salivary Glands (mandibular) Tongue Esophagus Stomach Small Intestine Duodenum Jejunum Ileum Large Intestine Cecum Colon Rectum Pancreas Liver Respiratory Trachea Lung with large bronchi Lymphoid/Hematopoietic Bone Marrow (sternum) Thymus Spleen Peyer's Patch Lymph Nodes Mandibular Mesenteric Inguinal	Urogenital Kidneys Urinary Bladder Testes Epididymides Prostate Seminal Vesicles Ovaries Oviducts Uterus Cervix Vagina Endocrine Adrenals Pituitary Thyroid/Parathyroids[a] Harderian Gland (paired) Skin/Musculoskeletal Skin/Mammary Gland Bone (femur) Bone (sternum) Skeletal Muscle (quadriceps femoris) Nervous/Special Sense Eyes with Optic Nerve Harderian gland Sciatic Nerve Brain Spinal Cord (cervical, thoracic, lumbar) Other Animal Number (ear tag or tattoo and/or chip) Gross Lesions SC Injection Site[b]

Two bone marrow smears were collected from the femoral shaft of each Main Study animal at necropsy.

Gross pathology/macroscopic pathology are reported for necropsies on Days 169 (main study - ~30 days post last dose), 197 (Recovery 1 – ~60 days post last dose), and 225 (Recovery 2 – ~90 days post last dose).

Day 169 - Treatment-associated gross observations included:

- Area foci dark (1/14 males and 2/15 females in Atrigel control group) and area foci pale (0/10 males and 0/10 females in low dose RBP-6000 group, 4/10 males and 1/10 females in mid-dose group, and 3/10 males and 7/10 females in high dose group) were observed at injection sites which correlated to microscopic findings of granulomas. This appears to primarily be a buprenorphine-related dose response effect with the Atrigel vehicle having some contribution.
- Pale areas in the lungs were observed in some rats (1/14 high dose females only) which correlated to alveolar macrophage infiltrates.

Day 197 - Treatment-associated gross observations included:

- Area foci dark (1/10 females in Atrigel control group and 1/10 males in high dose RBP-6000 group) and area foci pale (4/14 males and 3/15 females in low dose RBP-6000 group, 8/14 males and 9/14 females in mid-dose group, and 13/15 males and 14/14 females in high dose group) were observed at injection sites which correlated to microscopic findings of granulomas. This appears to be a buprenorphine-related dose response effect.
- Pale areas in the lungs were observed in some rats (1/10 high dose males only) which correlated to alveolar macrophage infiltrates.

Day 225 - Treatment-associated gross observations included:

- Area foci dark (1/3 females in Atrigel control group) and area foci pale (0/3 males and 0/2 females in low dose RBP-6000 group, 1/3 males and 0/3 females in mid-dose group, and 2/3 males and 2/3 females in high dose group) were observed at injection sites which correlated to microscopic findings of granulomas. This appears to be a buprenorphine-related dose response effect.
- Pale areas in the lungs were not observed.

In summary, there were local injection site findings observed in all dose groups including vehicle control, but exacerbated by buprenorphine that were largely resolved by the 90 days post the last dose. In addition, there were pale areas in lung at the high-dose, which also recovered by 90 days post last dose.

Organ Weights

The following organ weights were obtained at necropsy:

Organs Weighed	
Adrenals Brain Epididymides Heart Kidneys Liver Lungs	Ovaries Pituitary Spleen Testes Thymus Thyroid with parathyroids

Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

Organ weights, organ-to-body weights and organ-to-brain weights were reported for necropsies on Days 169, 197, and 225. The table contains select data followed by evaluation of each day's observations.

RBP-6000 Associated Organ Weight Findings for Rats on Necropsy Days 169, 197, and 225

		Day 169		Day 197		Day 225	
Parameters	Dose Groups[a]	Males	Females	Males	Females	Males	Females
Mean Body Weight	0 mg/kg	606.1200	294.5500	658.1400	303.9000	647.1333	306.4667
	10 mg/kg	562.6286	300.6143	576.4100*	310.4500	615.5333	288.5500
	25 mg/kg	554.2615*	291.7571	572.8500*	312.2300	612.6333	303.8333
	100 mg/kg	545.9357*	288.6571	594.2667*	301.8900	542.6667	311.6333
Mean Liver Weight	0 mg/kg	14.0191	6.8621	14.7259	6.8571	14.4703	7.5193
	10 mg/kg	11.4547*	7.2334	12.3408*	7.2446	13.2520	7.1115
	25 mg/kg	11.0398*	7.0211	12.0751*	7.9475	13.1173	7.3210
	100 mg/kg	10.2649*	6.8702	11.7593*	7.3659	10.9373	8.2770
Liver vs. Body Weight	0 mg/kg	0.023021	0.023270	0.022344	0.022545	0.022208	0.024533
	10 mg/kg	0.020352*	0.024008	0.021369	0.023349	0.021540	0.024650
	25 mg/kg	0.019873*	0.024012	0.021066	0.025413*	0.021283	0.024100
	100 mg/kg	0.018783*	0.023789	0.019828*	0.024459	0.020112	0.026733
Liver vs. Brain Weight	0 mg/kg	5.9491	3.3640	6.4451	3.3912	6.1775	3.498087
	10 mg/kg	4.9179*	3.3933	5.3591*	3.4047	5.5780	3.415706
	25 mg/kg	4.6773*	3.3296	5.1607*	3.8526	5.4386	3.551563
	100 mg/kg	4.3549 *	3.1975	5.0144*	3.5140	4.7925	4.005325
Lung vs. Body Weight	0 mg/kg	0.003134	0.004450	0.003056	0.004461	0.002671	0.005000
	10 mg/kg	0.003358	0.004629	0.003449*	0.004557	0.003520*	0.004950
	25 mg/kg	0.003357	0.004829	0.003437*	0.004514	0.003369	0.004933
	100 mg/kg	0.003363	0.004677	0.003317	0.004618	0.003451*	0.004900

[a] Drug dose, based on buprenorphine.
* Indicates a statistically significantly value.

Day 169

Notable observations in organ weights and ratios are included in the table for males.

- The actual toxicological significance of the liver changes are unknown but considered of minor consequence and variation with no histological correlate (see Histopathology section).
- The thymus and adrenal changes are considered real and explained by the report as due to stress with no histological correlate.
- There were no notable observations for females.

Organ weights, organ to body weight ratios and organ to brain weight ratios changes after 6 monthly doses with RBP-6000 (**Day 169 – 1 month after last dose**) compared to control (Atrigel) in male rats				
Organ	Dose (mg/kg)	Organ weight (g)	Organ/ body weight (%)	Organ/ brain weight (%)
Liver	10	-18%*	-11%	-17%*
	20	-21%*	-14%	-21%*
	100	-27%*	-19%*	-26%*
Thymus	10	-14%	-6%	-12%
	20	-32%*	-24%	-32%*
	100	-26%*	-18%	-26%*
Adrenal	10	+24%*	+33%*	+25%*
	20	+35%*	+46%*	+34%*
	100	+21%*	+33%*	+20%*

* - statistically significant at p <= 0.05 level

Day 197

Notable observations in organ weights and ratios are included in the table for males.

- The actual toxicological significance of the liver changes are unknown but considered of minor consequence and variation with no histological correlate (see Histopathology section).
- The adrenal changes are considered real and explained by the report as due to stress with no histological correlate.
- Testes/body weights were increased 21, 18, & 20% and brain/body weight 15, 17, and 13% for the low, mid, and high dose groups, respectively, at a SS level, but were not considered of any toxicological significance with no histological correlate.
- There were no notable observations for females.

Organ weights, organ to body weight ratios and organ to brain weight ratios changes after 6 monthly doses with RBP-6000 (**Day 197 – 2 months after last dose**) compared to control (Atrigel) in rats				
Organ	Dose (mg/kg)	Organ weight (g)	Organ/body weight (%)	Organ/brain weight (%)
Liver	10	-16%*	-4%	-17%*
	20	-18%*	-6%*	-20%*
	100	-20%*	-11%*	-23%*
Adrenal	10	+17%	+34%*	+17%
	20	+21%	+38%*	+18%
	100	+37%*	+51%*	+34%*

* - statistically significant at p <= 0.05 level

Day 225

Notable observations in organ weights and ratios are included in the table for males.

- The actual toxicological significance of the liver changes are unknown but considered of minor consequence and variation with no histological correlate. Of note is that the liver values are more closely aligned with control at this last sacrifice and listed as within normal limits by the report.
- A SS, RBP-associated increased lung to body weight ratios in male rats dosed at 10 and 100 mg/kg RBP-6000 (25 mg/kg is mid-dose). This alteration may be associated with alveolar macrophage infiltrates in the lungs, which was correlated histologically (see Histopathology section).
- Thymus values were again decreased as on the first observation Day 169 with no histological correlate.
- Spleen weights and ratios were decreased but not in a dose-responsive manner with no histological correlate.
- There were no notable observations for females.

Organ weights, organ to body weight ratios and organ to brain weight ratios changes after 6 monthly doses with RBP-6000 (**Day 225 – 3 months after last dose**) compared to control (Atrigel) in rats

Organ	Dose (mg/kg)	Organ weight (g)	Organ/body weight (%)	Organ/brain weight (%)
Liver	10	-8%	-3%	-10%
	20	-9%	-4%	-12%
	100	-24%	-9%	-22%
Thymus	10	-6%	-3%	-7%
	20	-12%	-9%	-15%
	100	-39%	-29%	-38%
Spleen	10	-17%	-13%	-18%
	20	-10%	-5%	-13%
	100	-26%	-13%	-26%
Lung	10	+24	+32*	+23
	20	+18	+26	+15
	100	+8	+29*	+10

* - statistically significant at p <= 0.05 level

Histopathology

The tissues from the control and high-dose groups (Groups 1 and 4, respectively) and gross lesions, were examined. Target tissues in the low- and mid-dose groups (Groups 2 and 3, respectively) of lung, pancreas, and injection sites were also examined.

Adequate Battery - yes

Peer Review – not reported/found

Histological Findings

Microscopic observations and severity for Days 169, 197, and 225 are summarized in the table below.

ATRIGEL Vehicle and RBP-6000 Associated Findings Combined for Male and Female Rats on Necropsy Days 169 197, and 225

Study Day			Day 169				Day197				Day 225			
Dose Level RBP-6000			0 mg/kg n = 29	10 mg/kg n = 28	25 mg/kg n = 27	100 mg/kg n = 28	0 mg/kg n = 20	10 mg/kg n = 20	25 mg/kg n = 20	100 mg/kg n = 19	0 mg/kg n = 6	10 mg/kg n = 5	25 mg/kg n = 6	100 mg/kg n = 6
Tissue	Finding	Severity												
Injection Site	Granulomas	Minimal	7/29	2/28	2/27	0/28	6/20	2/20	1/20	1/19	4/6	0/5	1/6	0/6
		Mild	2/29	4/28	3/27	1/28	2/20	1/20	3/20	1/19	0/6	0/5	0/6	1/6
		Moderate	1/29	0/28	10/27	25/28	0/20	0/20	2/20	16/19	0/6	0/5	1/6	4/6
		TOTAL	**10/29**	**6/28**	**15/27**	**26/28**	**8/20**	**3/20**	**6/20**	**18/19**	**4/6**	**0/5**	**2/6**	**5/6**
Lung	Alveolar Macrophage Infiltrates	Minimal	12/29	17/28	15/27	11/28	5/20	6/20	5/20	8/19	0/6	1/5	3/6	1/6
		Mild	1/29	5/28	10/27	13/28	3/20	10/20	12/20	5/19	2/6	2/5	3/6	4/6
		TOTAL	**13/29**	**22/28**	**25/27**	**24/28**	**8/20**	**16/20**	**17/20**	**13/19**	**2/6**	**3/5**	**6/6**	**5/6**
PancreasAcinar Cells	Increased Apoptosis	Minimal	6/29	12/28	8/27	9/28	3/20	1/20	10/20	8/19	1/6	1/5	2/6	3/6
		Mild	0/29	1/28	5/27	3/28	0/20	0/20	2/20	6/19	0/6	1/5	0/6	1/6
		TOTAL	**6/29**	**13/28**	**13/27**	**12/28**	**3/20**	**1/20**	**12/20**	**14/19**	**1/6**	**2/5**	**2/6**	**4/6**

[a] Severity grades for which there were no finding are omitted from the table.

Local effects
Injection site: Notable microscopic changes observed at injection sites included granulomas, which were characterized by macrophages, lymphocytes, plasma cells and multinucleated giant cells, in both vehicle- and RBP-6000-treated animals. At Day 169 (30 days post last dose), the incidence and severity ranked from low to high as follows: LD>Veh>MD>HD, which suggests the findings are attributable to the vehicle and exacerbated by buprenorphine. Incidence and severity were consistent for all groups at Day 197 (60 days post last dose) relative to Day 169. By Day 225 (90 days post last dose), the incidence and severity did not change much for the vehicle, MD, and HD groups, but there was complete recovery for the LD group.

Systemic effects
Lung: At Day 169, minimal to mild alveolar macrophage infiltrates were observed in nearly all RBP-6000 treated animals from LD to HD while approximately half of the vehicle control animals exhibited the finding mostly with minimal severity. Incidence and severity remained comparable for all groups between Days 169 and 179. By 90 days post dose, the incidence and severity did not change much for the MD and HD groups, but the LD group looked comparable to the vehicle control group.

Pancreas: Increase apoptosis of pancreatic acinar cells were observed in approximately half of the animals in all RBP-6000 groups while less than a quarter from the vehicle group exhibiting the finding, which suggests it may either be incidental or vehicle related and exacerbated by buprenorphine. The finding appeared to resolve over time with the LD and MD looking comparable to vehicle by 90 days post last dose though more than half of the HD group animals still exhibited the finding at this time.

Some additional detailed text describing the toxicities observed in these three target organs at each sacrifice time point are below.

Day 169 - Treatment-associated microscopic observations included:

- RBP-6000-associated findings at the injection sites
  - Minimal hair follicle atrophy at the injection sites occurred equally in control and RBP-6000-dosed rats, and was considered related to the ATRIGEL vehicle and RBP-6000 administration.
  - Minimal to moderate granulomas at the injection sites occurred in both control and RBP-6000-dosed rats, indicating a relationship with the administration of the ATRIGEL vehicle to inflammation; however, these findings were more prevalent and more severe in animals receiving 25 and 100 mg/kg RBP-6000.
  - Rats dosed at 10 mg/kg RBP-6000 had injection site granulomas similar in incidence and severity as controls.
  - Injection site granulomas were characterized by SC aggregates of macrophages, lymphocytes, plasma cells and multinucleated giant cells, frequently surrounding pockets or cysts of amorphous, pale basophilic material and/or eosinophilic, cellular material.
- RBP-6000-associated findings in the lungs
  - Minimal to mild alveolar macrophage infiltrates in the lungs were increased in incidence and severity in all RBP-6000 dosed groups compared to controls.
  - Alveolar macrophage infiltrates were characterized by macrophages with pale, basophilic, foamy cytoplasm occasionally associated with extracellular, amorphous, pale, basophilic material.
- RBP-6000-associated findings in the pancreases
  - There was a slightly increased severity of minimal to mild pancreatic acinar cell apoptosis in RBP-6000-dosed rats, compared to controls.

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg
Number of Animals on Study :	15	14	13	14	14	14	14	14
Number of Animals Completed:	(15)	(14)	(13)	(14)	(14)	(14)	(14)	(14)
LUNG;								
Examined	(15)	(14)	(13)	(14)	(14)	(14)	(14)	(14)
Within Normal Limits	9	5	1	4	7	1	1	0
Infiltrate, Macrophage; Alveolus	(6)	(9)	(12)	(10)	(7)	(13)	(13)	(14)
minimal	6	8	7	6	6	9	8	5
mild	0	1	5	4	1	4	5	9
Hypertrophy; Smooth muscle; Vascular	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
mild	0	1	0	0	0	0	0	0
PANCREAS;								
Examined	(15)	(14)	(13)	(14)	(14)	(14)	(14)	(14)
Within Normal Limits	5	4	1	5	9	4	9	9
Degeneration; Acinus; focally extensive	(1)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
mild	0	0	0	0	0	1	0	0
Degeneration; Islet	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	0	1	0	0	0	0	0	0
Infiltrate, Mononuclear Cell	(3)	(2)	(5)	(4)	(5)	(5)	(2)	(3)
minimal	3	2	5	4	5	5	2	3
Vacuolation, Cytoplasm; Acinar cell	(4)	(2)	(2)	(2)	(0)	(0)	(0)	(0)
minimal	4	2	2	2	0	0	0	0
Apoptosis Increased; Acinar cell	(6)	(8)	(10)	(8)	(0)	(5)	(3)	(4)
minimal	6	7	5	6	0	5	3	3
mild	0	1	5	2	0	0	0	1

Day 197 - Treatment-associated microscopic observations included:

- Findings in rats were similar to those described for Day 169 necropsies.
  - These findings consisted of granulomas at the injection sites and pancreatic acinar cell apoptosis that were more prevalent and severe in rats dosed with 25 and 100 mg/kg RBP-6000, but were comparable between control and 10 mg/kg RBP-6000-dosed rats.
  - Alveolar macrophage infiltrates in the lungs were increased in incidence and severity in all RBP-6000-dosed groups compared to controls.

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg
Number of Animals on Study :	15	14	13	14	14	14	14	14
Number of Animals Completed:	(15)	(14)	(13)	(14)	(14)	(14)	(14)	(14)
LUNG;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(10)
Within Normal Limits	8	4	1	3	4	0	2	3
Hemorrhage; multifocal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
Infiltrate, Macrophage; Alveolus	(2)	(6)	(9)	(6)	(6)	(10)	(8)	(7)
minimal	1	2	3	5	4	4	2	3
mild	1	4	6	1	2	6	6	4
PANCREAS;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(10)	(10)
Within Normal Limits	4	4	1	0	4	8	5	4
Degeneration; Islet	(1)	(2)	(2)	(1)	(0)	(0)	(0)	(0)
minimal	1	2	2	1	0	0	0	0
Infiltrate, Mononuclear Cell	(3)	(4)	(1)	(1)	(4)	(1)	(1)	(3)
minimal	3	4	1	1	4	1	1	3
Vacuolation, Cytoplasm; Acinar cell	(1)	(1)	(3)	(0)	(1)	(2)	(0)	(0)
minimal	1	1	3	0	1	2	0	0
Apoptosis Increased; Acinar cell	(1)	(1)	(8)	(9)	(2)	(0)	(4)	(5)
minimal	1	1	6	4	2	0	4	4
mild	0	0	2	5	0	0	0	1
Pigment; Islet; multifocal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild	0	0	0	1	0	0	0	0

<u>Day 225</u> - Treatment-associated microscopic observations included:

- Injection site granulomas, pancreatic acinar cell apoptosis, and alveolar macrophage infiltrates in the lungs persisted at increased severity and incidence in 25 and 100 mg/kg RBP-6000-dosed rats compared to controls and 10 mg/kg RBP-6000-dosed groups.

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg	0 mg/kg	10 mg/kg	25 mg/kg	100 mg/kg
Number of Animals on Study :	15	14	13	14	14	14	14	14
Number of Animals Completed:	(15)	(14)	(13)	(14)	(14)	(14)	(14)	(14)

LUNG;								
Examined	(3)	(3)	(3)	(3)	(3)	(2)	(3)	(3)
Within Normal Limits	3	1	0	1	1	1	0	0
Infiltrate, Macrophage; Alveolus	(0)	(2)	(3)	(2)	(2)	(1)	(3)	(3)
minimal	0	1	2	1	0	0	1	0
mild	0	1	1	1	2	1	2	3
PANCREAS;								
Examined	(3)	(3)	(3)	(3)	(3)	(2)	(3)	(3)
Within Normal Limits	0	0	0	0	1	2	1	2
Degeneration; Acinus; focally extensive	(0)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
mild	0	1	0	0	0	0	0	0
Degeneration; Islet	(1)	(1)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	0	1	0	0	0	0	0	0
mild	1	0	0	0	0	0	0	0
Infiltrate, Mononuclear Cell	(2)	(1)	(1)	(1)	(0)	(0)	(1)	(1)
minimal	2	1	1	1	0	0	1	1
Vacuolation, Cytoplasm; Acinar cell	(0)	(0)	(1)	(1)	(1)	(0)	(0)	(0)
minimal	0	0	1	1	1	0	0	0
Apoptosis Increased; Acinar cell	(0)	(2)	(1)	(3)	(1)	(0)	(1)	(1)
minimal	0	1	1	2	1	0	1	1
mild	0	1	0	1	0	0	0	0
Pigment; Islet; multifocal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0

Special Evaluation – none

Toxicokinetics

Three animals/sex/time point were rotated in Groups 5 through 7 and the same 3/sex were sampled on each occasion according to the following table.

No. of Rats	Time Points
3/sex/group	Day -3, Day 1 2-hr pd and 24-hr pd Day 29 1-hr pd and 8-hr pd Day 57 predose and 4-hr pd Day 71 Day 85 2-hr pd and 24-hr pd Day 113 1-hr pd and 8-hr pd Day 141 predose and 4-hr pd and Day 155 and Day 197
3/sex/group	Day 1 predose and 4-hr pd Day 15 Day 29 2-hr pd and 24-hr pd Day 57 1-hr pd and 8-hr pd Day 85 predose and 4-hr pd Day 99 Day 113 2-hr pd and 24-hr pd Day 141 1-hr pd and 8-hr pd and Day 169 and Day 211
3/sex/group	Day 1 1-hr pd and 8-hr pd Day 29 predose and 4-hr pd Day 43 Day 57 2-hr pd and 24-hr pd Day 85 1-hr pd and 8-hr pd Day 127 Day 141 2-hr pd and 24-hr pd and Day 183 and Day 225

hr = hour, pd = postdose

The following results for buprenorphine were observed in the table that contains C_{max} and $AUC_{(0-672)}$ for the six days of exposure (1/month – 672 hours) and the accumulation ratios at the different time points. The second table contains the $AUC_{(0-24h)}$ after the last dose on Day 141 which is used in safety calculations:

			C_{max} (ng/mL)						Ratio				
Gender	Group No.	Dose Level (mg/kg)	Day 1	Day 29	Day 57	Day 85	Day 113	Day 141	Day 29/ Day 1	Day 57/ Day 1	Day 85/ Day 1	Day 113/ Day 1	Day 141/ Day 1
Males	5	10	69.6	56.4	58.6	57.6	67.7	68.0	0.810	0.841	0.828	0.972	0.977
	6	25	242	153	150	86.0	68.0	130	0.632	0.619	0.356	0.281	0.537
	7	100	234	188	124	216	233	302	0.806	0.532	0.923	0.997	1.29
Females	5	10	67.3	56.6	66.0	49.6	57.4	142	0.841	0.980	0.737	0.853	2.11
	6	25	177	85.9	87.6	111	122	107	0.486	0.496	0.629	0.689	0.608
	7	100	462	440	194	221	370	1362	0.953	0.419	0.479	0.800	2.95

			$AUC_{(0-672)}$ (ng•h/mL)						Ratio				
Gender	Group No.	Dose Level (mg/kg)	Day 1	Day 29	Day 57	Day 85	Day 113	Day 141	Day 29/ Day 1	Day 57/ Day 1	Day 85/ Day 1	Day 113/ Day 1	Day 141/ Day 1
Males	5	10	5513	4687	6926	5268	5449	5657	0.850	1.26	0.955	0.988	1.03
	6	25	7212	14096	18381	12612	12851	15462	1.95	2.55	1.75	1.78	2.14
	7	100	16213	30544	41310	49351	49140	55274	1.88	2.55	3.04	3.03	3.41
Females	5	10	3109	3260	5046	4201	3626	4607	1.05	1.62	1.35	1.17	1.48
	6	25	6231	7315	11259	10627	13573	11359	1.17	1.81	1.71	2.18	1.82
	7	100	14663	24068	27310	25327	27815	41989	1.64	1.86	1.73	1.90	2.86

The final C_{max}s (68.0 ng/mL male and 142 ng/mL female) and $AUCs_{(0-24h)}$ (653 ng*h/mL male and 590 ng*h/mL female) were used in the Applicant's Safety Margins calculations

whereas a mean of the six values yield C_{max}s of (62.9 ng/mL male and 73.2 ng/mL female) and $AUCs_{(0\text{-}24h)}$ (575 ng*h/mL male and 462 ng*h/mL female)

Day 141

	Group	Dose Level	T_{max}	C_{max}		C_{trough}	$AUC_{(0\text{-}t)}$		$AUC_{(0\text{-}24)}$	$AUC_{(0\text{-}672)}$	$T_{½}$			
Gender	No.	(mg/kg)	(h)	(ng/mL)	SE	(ng/mL)	(ng•h/mL)	SE	(ng•h/mL)	(ng•h/mL)	(h)	C_{max}/D	C_{trough}/D	$AUC_{(0\text{-}672)}$/D
Males	5	10	1	68.0	25.5	2.55	6756	927	653	5657	262	6.80	0.255	566
	6	25	2	130	14.3	12.2	21714	1585	1127	15462	367	5.19	0.487	618
	7	100	2	302	11.0	73.7	101387	19290	3142	55274	656	3.02	0.737	553
Females	5	10	2	142	74.6	1.83	5319	696	590	4607	285	14.2	0.183	461
	6	25	2	107	16.0	4.80	14177	1830	978	11359	337	4.29	0.192	454
	7	100	2	1362	1039	59.2	69730	9972	4200	41989	617	13.6	0.592	420

The C_{max} of buprenorphine for the low- and mid-dose groups remained relatively similar between dosing occasions and between sexes, but the high-dose group was more variable. The C_{max} of buprenorphine was generally less than dose proportional.

The $AUC_{(0\text{-}672h)}$ of buprenorphine remained relatively similar between dosing occasions for the low group with the mid-dose group showing increases after the first dose (males) and second dose (females) that were comparable thereafter. For the high dose group, the $AUC_{(0\text{-}672)}$ increased after the first dose and generally continued increasing after repeated administration especially for the males. $AUC_{(0\text{-}672)}$ of buprenorphine was generally dose proportional for the low dose and greater than dose proportional for the mid-dose and high doe, more so for the high dose.

T_{max} for buprenorphine at usually 1-2 hours, suggesting rapid conversion to its metabolite (data tables not shown). Half-lives were apparently not reported.

The following C_{max}, $AUC_{(0\text{-}672)}$, and accumulation ratios for norbuprenorphine, the major metabolite of buprenorphine, are presented only as comparison for data completeness purposes but will not be discussed. Values for norbuprenorphine were generally at least 10-fold lower than buprenorphine for the considerably less biologically active norbuprenorphine.

Gender	Group No.	Dose Level (mg/kg)	C_{max} (ng/mL) Day 1	Day 29	Day 57	Day 85	Day 113	Day 141	Ratio Day 29/ Day 1	Day 57/ Day 1	Day 85/ Day 1	Day 113/ Day 1	Day 141/ Day 1
Males	5	10	2.53	1.77	3.19	2.06	3.38	3.23	0.698	1.26	0.813	1.33	1.28
	6	25	8.89	5.51	4.51	4.03	4.02	4.95	0.620	0.507	0.453	0.453	0.556
	7	100	17.9	10.2	5.90	14.4	33.6	23.1	0.571	0.330	0.804	1.88	1.29
Females	5	10	1.24	1.63	1.76	1.43	2.17	4.98	1.31	1.42	1.15	1.75	4.01
	6	25	4.40	2.67	3.07	3.60	8.21	3.38	0.606	0.698	0.818	1.87	0.768
	7	100	31.7	25.1	9.02	8.79	13.1	81.7	0.793	0.285	0.278	0.415	2.58

Gender	Group No.	Dose Level (mg/kg)	$AUC_{(0-672)}$ (ng•h/mL) Day 1	Day 29	Day 57	Day 85	Day 113	Day 141	Ratio Day 29/ Day 1	Day 57/ Day 1	Day 85/ Day 1	Day 113/ Day 1	Day 141/ Day 1
Males	5	10	133	64.0	105	133	146	115	0.480	0.789	1.00	1.10	0.864
	6	25	209	148	177	176	198	181	0.708	0.849	0.843	0.951	0.867
	7	100	499	556	513	701	868	778	1.11	1.03	1.40	1.74	1.56
Females	5	10	20.7	19.5	25.2	25.5	26.3	35.6	0.940	1.22	1.23	1.27	1.72
	6	25	63.1	56.3	71.9	49.5	65.1	93.7	0.892	1.14	0.785	1.03	1.48
	7	100	222	213	199	221	241	419	0.959	0.898	0.998	1.09	1.89

This TK assessment confirmed that 1) high and consistent (except for high dose males) exposures to parent drug, buprenorphine, and metabolite, norbuprenorphine, were achieved with SC injection of RBP-6000 in rats once every four weeks for six consecutive dosing periods and 2) significant exposure was maintained throughout the four-week dosing intervals following each dose.

Dosing Solution Analysis

According to the study report, appropriate documentation of the method of synthesis, fabrication, or derivation of the test and control articles are on file if this documentation is requested by regulatory agencies. The test and control (vehicle) articles were provided by the Sponsor as pre-filled syringes. The test and control articles were used as received for dose. Stability analysis and verification of test article concentration and control article were conducted by the Sponsor. Analysis of the test and control articles was performed by the Sponsor on samples stored at the Sponsor's facility under the same conditions as the Testing Facility. The stability exit testing began after the sixth and final dose in the test article and control groups.

Agreed upon by this reviewer, all product characteristics seemed to be within acceptable ranges upon the initial release of Lots RB0042-0 1 (test article) and RB0042-21 (control article). A comparison of the Certificates of Analysis and the Certificates of Stability for Lots RB0042-01 and RB0042-21 indicates that there were no substantial changes in the products over the course of 12 months for materials stored at 5 ± 2 ^{o}C. All characteristics were within generally acceptable ranges for the purposes of this study. The test article and control article are considered to have been stable for the duration of the study under the storage conditions per the study protocol at 2 to 8 ^{o}C.

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Study title: A 9-Month Multi-Dose Toxicity and Toxicokinetic Study of Buprenorphine/ATRIGEL® (RBP-6000) Administered Subcutaneously to Beagle Dogs, with a 12-Week Recovery Period

Study no.:	RBL-C03-60-09
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 17, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - (b) (4)% buprenorphine base in (b) (4)% 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4)% N-methyl-2-pyrrolidone (NMP)), Lot 0042-01, (b) (4)%. - ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP), Lot 0042-21.

Key Study Findings

- Male and female Beagle dogs received 0 (Atrigel control), 10, 20, or 40 mg/kg of RBP-6000 by subcutaneous (SC) injection monthly for nine (9) months and were necropsied approximately 30 (3/sex/dose), 60 (3/sex/dose), and 90 (2/sex/dose) days after the last dose.
- A complete GLP protocol was conducted as the animals were evaluated for clinical signs, dermal (injection site) observations, changes in food consumption and body weight, electrocardiograms (ECGs), ophthalmic examinations, serum chemistry, hematology, and coagulation, and urinalysis. Blood samples were collected for toxicokinetic analysis at various time points prior to and following each injection. Full necropsies were conducted on all animals, and tissues were collected, weighed, preserved, and processed. Groups 1 and 4 were examined microscopically.
- Clinical signs associated with the ATRIGEL® Delivery System occurred at the injection sites in all groups (test and control), and included edema/swelling, raised areas, masses, erythema/reddening, abrasion, eschar formation, ulceration, desquamation, and blanching. Edema, the most prominent and consistent effect, was observed in all animals in the control and RBP-6000 dose groups at most time points following each dose administration. Severity of edema reached Grade 4 (severe), but Atrigel control and RBP-6000 groups were similar in response.
- All animals survived to their respective scheduled necropsy time point on Study Days 253, 281, or 309. The once monthly subcutaneous injection of RBP-6000

(buprenorphine/ATRIGEL®) to beagle dogs for up to 9 months was not associated with any evidence of systemic toxicity other than anticipated pharmacological effects of buprenorphine. Therefore the NOAEL/LOAEL for systemic effects was the highest dose tested at 40 mg/kg.

- Notable local effects included microscopic findings of granuloma at the injection sites of animals from all study groups including vehicle control, indicating that the effects were primarily attributable to the Atrigel vehicle. Over time, granulomas at older injection sites tended to be less cellular, having a thinner fibrotic wall, degenerate central debris and characterized by collapse of the central core. These findings represented resolving inflammation at injection sites which had been used at earlier time points in the study and in all sites at the last study time point (Study Day 309). However, injection site inflammation persisted longer in RBP-6000-dosed animals relative to vehicle-treated animals, suggesting buprenorphine exacerbates the local effects. The dose of 40 mg/kg RBP-6000 was considered an acceptable LOAEL/NOAEL for local effects due to the general reversibility of the injection site changes.
- Combined (male and female) C_{max}s ranged from 10.3 to 34.9 ng/mL (low-dose), 17.2 to 49.8 ng/mL (mid dose), and 35.7 to 123 ng/mL (high dose) over the course of the study. Combined (male and female) AUC's over 672 hours (1 month) ranged from 4,033 to 7,102 ng*hour/mL (low dose), 7,134 to 13,192 ng*hour/mL (mid-dose), and 14,743 to 26,204 ng*hour/mL (high-dose) over the course of the study.

Methods

Doses:

Group No.	Number of Males/Females	Dose Level (mg BUP/kg)	Dose Mass (mg/kg)[a]	Dose Volume (mL/kg)[c]	Dose Formulation Concentration (w/w%)
1	8/8	0 (control)[b]	200	0.182	0
2	8/8	10	50	0.045	(b) (4)
3	8/8	20	100	0.091	
4	8/8	40	200	0.182	

[a] Doses were delivered within ± 20% of the desired dose mass.
[b] ATRIGEL® vehicle (ATRIGEL® Delivery System)
[c] Dose volume was an approximation and was only used after calculating target dose levels in w/w.

Doses selected based on information derived from previous studies (a non-GLP MTD, 4 week study).

Frequency of dosing: Once monthly for 9 months with the 1st injection on Day 1 of the study

Route of administration: Subcutaneous to the shaved back at differing dose sites for each month (2 sites for each monthly dose

Dose volume: See doses.

Formulation/Vehicle: Atrigel - (b) (4)% PLGH(b) (4)% NMP in control and (b) (4)% PLGH(b) (4)% NMP in drug product with(b) (4)% buprenorphine

Species/Strain: Beagle Dog

Number/Sex/Group: 8

Age: 10.7 to 12.3 months of age for the males and 12.4 to 13.8 months of age for the females

Weight: 8.2 to 10.3 kg for the males and 7.2 to 9.6 kg for the females

Satellite groups: 670 day and 90-day recovery groups; main study animals also for toxicokinetics

Unique study design: No

Deviation from study protocol: Nothing remarkable

Study Design

Group No.	No. of Males/Females	Dose Level (mg BUP/kg)[a]	No. Necropsied: Terminal 1 Day 253	Terminal 2 Day 281	Recovery Day 309
1	8/8	0 (control)[b]	3/3	3/3	2/2
2	8/8	10	3/3	3/3	2/2
3	8/8	20	3/3	3/3	2/2
4	8/8	40	3/3	3/3	2/2

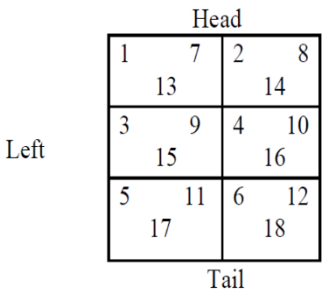
[a] Doses were delivered within ± 20% of the desired dose.
[b] ATRIGEL® vehicle (ATRIGEL® Delivery System)

Dosing is once monthly for 9 months on Days 1, 29, 57, 85, 113, 141, 169, 197, and

225. The Terminal 1 necropsy is Day 253 or 28 days (4 weeks) after the last injection with the next two necropsies are 8 weeks (Day 281) and 12 weeks (Day 309) after the last injection, respectively.

Dosing Scheme

Subcutaneous dosing sites (1 to 18) were demarcated by a tattoo indicator on a clipped area of the animal's back as indicated below (dorsal view). The dose was administered over 1 or 2 separate injection sites as necessary (dependent on dose volume). Using this dosing scheme, no injection site received repeated injections.



Right

Dose Site Order	
Dose Day	Dose Sites
1	1 and 2
29	3 and 4
57	5 and 6
85	7 and 8
113	9 and 10
141	11 and 12
169	13 and 14
197	15 and 16
225	17 and 18

Observations and Results

Mortality

Animals were observed for signs of mortality twice daily (a.m. and p.m.) beginning at least 7 days prior to initiation of dosing and continuing through the end of the in-life phase.

No mortality was reported.

Clinical Signs

Animals were observed for signs of morbidity twice daily (a.m. and p.m.) beginning at least 7 days prior to initiation of dosing and continuing through the end of the in-life phase.

Observed clinical signs were either associated with the expected pharmacological effects of buprenorphine (decreased activity and abnormal feces) covered in this section or the injection sites related to the Atrigel (swelling, abrasion, reddening, raised areas, and/or masses) covered in the Dermal Observations section following this section.

Decreased activity was observed in several animals of both sexes in the three RBP-6000-dose groups during the first few days following dosing on Day 1.

Observations of Decreased Activity Following Dose 1 (Days 1-28)				
Treatment	**Days Observed**	**Number of Animals Affected [n=8/sex/group]**	**Total Number of Observations**	**Range of Observations per Animal**
Group 1 (0 mg/kg)	----	----	----	----
Group 2 (10 mg/kg)	1-4	4	12	2 to 4
Group 3 (20 mg/kg)	1-3	8	16	1 to 3
Group 4 (40 mg/kg)	1-5	6	16	1-5
Total Observations of Decreased Activity (Days 1 – 253)				
Group 1 (0 mg/kg)	-	0	0	0
Group 2 (10 mg/kg)	1-230	7	22	1-7
Group 3 (20 mg/kg)	1-229	10	35	1-13
Group 4 (40 mg/kg)	1-230	8	21	1-5

---- = not applicable

Watery feces, abnormal feces color (red and/or black, orange), and mucus in feces were observed during Week 1 in a few animals in each of the three RBP-6000 dose groups.

Observations of Abnormal Feces				
Dose Group	Group 1 (0 mg/kg)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (40 mg/kg)
Watery Feces				
#Observations/#Affected	0/0	2/2	6/4	8/3
Days observed from - to	----	4-4	3-7	3-7
Abnormal Feces Color				
#Observations/#Affected	0/0	1/1	14/8	2/2
Days observed from - to	----	3-3	3-7	5-5
Mucus in Feces				
#Observations/#Affected	0/0	2/1	8/4	4/2
Days observed from - to	----	3-4	3-7	4-6
n=8/sex/group				

Dermal Observations

Doses 1-8: Prior to each monthly dose, then once daily for 7 days at approximately the same time as dosing, then once weekly at approximately the same time as dosing. Dose 9: Prior to dose, then once daily for 7 days at approximately the same time as dosing, then once weekly at approximately the same time as dosing through the day of necropsy. A final dermal observation was made on the day of necropsy prior to euthanasia. Each animal was removed from the cage according to the listed schedule and observed in detail according to the Macroscopic Dermal Grading System which is based on the Draize 2 method with scoring ranges from 0 or 1 to 4 with 4 being most

severe. Observations were made for erythema, edema, eschar, blanching, ulceration, necrosis, and superficial lightening. Observations were also made for desquamation, fissuring, eschar exfoliation, test sight staining, and erythema beyond the test site.

The most common dermal observation was edema with erythema and eschar being observed to a much lesser extent. Blanching, ulceration, and desquamation were also observed, but affected very few animals. Dermal irritation outside the test site was also observed and is not surprising considering the dosing locations and that the solvent dissipates from the injection site to form the implant. Dermal effects occurred in most animals in each of the 4 groups and is generally considered an effect of the he ATRIGEL® Delivery System with possible contribution by buprenorphine based on dose volume and buprenorphine concentration. Edema was observed in all animals in the Atrigel control and RBP-6000 dose groups at most time points following dose administration with some reversal in some animals before the next dose and certainly during the recovery periods within approximately 30-60 days after the last dose on Day 225 with the Atrigel control group exhibiting reversal in all animals at ~34 days and the other groups within 1-3 weeks later. Edema (swelling) ranged from very slight (Grade 1) to severe (Grade 4). There may have been a buprenorphine-related and/or dose volume dose-response in edema as the total number of edema observations recorded prior to necropsy on Day 253 for the 10 mg/kg group (low-dose) was less than the control group, while the 20 and 40 mg/kg groups (mid- and high-dose groups) were slightly greater in number but at only 10% difference. Isolated observations of edema occurred in a few animals in all 4 dose groups during the period between Days 253 and 281 (Terminal Necropsy 2) and in only one 20 mg/kg animal after that (Recovery). While inflammation was identified microscopically (see histopathology section), the edema/swelling at the injection sites may also have been associated with the small lump that forms at each injection site due to implant formation by the ATRIGEL®. Erythema (redness) which was typically very slight to well-defined (Grades 1 and 2) sometimes extended beyond the injection site and was observed most often during the period following the first 2 doses.

Body Weights

Body weights were measured twice prior to the first dose (Weeks -2 and -1), prior to dosing on Day 1, then weekly thereafter. Food was withheld before body weights were measured. A terminal body weight was obtained the day of necropsy for all animals. This body weight was used to calculate organ/body weight ratios.

Decreases in individual body weight occurred for both sexes in all RBP-6000 dose groups beginning at Week 1 following the first dose, which resulted in mean body weights for the three RBP-6000 dose groups that were statistically significantly (SS) lower than controls at various time points throughout the study (see Table). The effect on body weight was consistent with the observed reductions in food consumption and with the expected pharmacological effect of the buprenorphine contained in RBP-6000.

Study Weeks with Statistically Significant Changes in Body Weight		
Dose Group	Gender	Week
Group 2 (10 mg/kg)	Male	--------
	Female	24, 28-36
Group 3 (20 mg/kg)	Male	21, 23, 26, 28-30, 33, 35
	Female	3, 21-24, 28-36, 38-40
Group 4 (40 mg/kg)	Male	1, 21, 23, 26, 28-30, 33, 35, 40
	Female	3, 21, 22, 24, 28-36

Weeks 1-36, n= 8/sex/group (Terminal 1)
Weeks 37-40, n= 5/sex/group (Terminal 2)

RBP-6000 had an adverse effect on body weight that appeared to be buprenorphine-related. In brief, there was nearly no average weight gain for any RBP-6000 male or female group through the dosing period while the vehicle control groups steadily gained weight during this time. The effect appeared reversible as animals from the RBP-6000 groups gained weight during the recovery periods.

The RBP-6000-related decrease in body weight was initially observed on Day 7 following the first dose, when all but one RBP-6000-dosed males exhibited weight loss, which resulted in mean weight losses of approximately 6%, 6%, and 8% for the 10, 20, and 40 mg/kg-dosed males, respectively, relative to pre-study (Day -1). In the male control group, 4 of 8 male control animals had slight weight losses resulting in a group mean gain of less than 1% relative to pre-study. All RBP-6000-dosed females experienced weight loss following the first dose, resulting in mean losses of approximately 8%, 8%, and 9% for the 10, 20, and 40 mg/kg-dosed females, respectively, relative to pre-study (Day -1). Three (3) of 8 female control animals exhibited weight loss, resulting in a mean gain of less than 1% relative to pre-study.

The RBP-6000-effect on body weight was evident throughout the dosing phase of the study. Mean body weight for the 10 and 20 mg/kg-dosed males remained below pre-study levels for the first few weeks of study, followed by periodic increases to weights equal to or exceeding pre-study levels at several succeeding time points, while the 40 mg/kg-dosed males did not attain pre-study weight until the period following the first terminal necropsy (Week 36) and being most evident at the Week 44 terminal necropsy. All three RBP-6000-dosed female groups remained below the pre-study mean weight for the duration of the study through the Week 40 terminal necropsy (end of 1st recovery period) where the low- and mid-dose groups achieved Day -1 levels. The high-dose group (40 mg/kg) females remained below original body weights until the between the second necropsy and the final necropsy (Weeks 41 through 44). The table lists group mean body weight changes (%) relative to pre-study, measured at the end of the 4-week periods between each of the nine dose administrations.

Mean Percent Body Weight Gain by Dose Period Relative to Week -1 (Prestudy)											
Male	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36[a]	Week 40[b]	Week 44[c]
Group 1 (0 mg/kg)	+2.5 (3.9)	+3.9 (4.2)	+6.2 (5.6)	+4.8 (5.8)	+8.0 (7.3)	+7.2 (7.0)	+10.7 (7.7)	+10.9 (8.2)	+11.2 (9.7)	+1.1 (2.0)	+0.4 (2.1)
Group 2 (10 mg/kg)	-2.3 (4.4)	-1.8 (3.7)	-1.5 (3.7)	-1.9 (3.8)	+1.3 (2.7)	-0.8 (3.3)	-0.9 (3.5)	+0.5 (3.0)	+0.1 (4.5)	+1.2 (2.9)	+0.4 (2.2)
Group 3 (20 mg/kg)	-3.6 (5.4)	-1.5 (4.3)	-0.1 (5.9)	-1.9 (6.1)	-0.9 (6.6)	-2.3 (8.1)	-2.2 (7.4)	-0.4 (8.2)	-0.4 (8.7)	-0.1 (2.3)	+1.1 (2.9)
Group 4 (40 mg/kg)	-4.4 (3.4)	-2.0 (2.4)	-1.1 (4.4)	-3.9 (5.0)	-1.2 (6.2)	-2.9 (6.5)	-1.6 (6.1)	-0.9 (5.7)	-0.6 (5.7)	-1.1 (1.3)	+5.5 (1.4)
Female	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44
Group 1 (0 mg/kg)	+0.3 (3.4)	+1.5 (3.4)	+4.9 (5.2)	+4.2 (5.8)	+8.6 (7.0)	+8.6 (8.3)	+7.6 (7.7)	+7.6 (8.4)	+9.9 (8.4)	+1.1 (2.6)	+3.8 (0.9)
Group 2 (10 mg/kg)	-7.0 (3.6)	-6.7 (7.2)	-6.0 (9.6)	-6.3 (9.8)	-2.4 (7.5)	-5.0 (6.4)	-6.9 (5.8)	-5.8 (4.6)	-4.4 (6.1)	+2.1 (3.2)	-0.6 (0.9)
Group 3 (20 mg/kg)	-8.3 (5.6)	-6.4 (5.1)	-6.6 (6.7)	-6.7 (5.9)	-2.0 (7.6)	-4.5 (4.9)	-4.4 (5.2)	-2.9 (4.8)	-3.3 (6.9)	+0.4 (3.2)	+1.3 (0.0)
Group 4 (40 mg/kg)	-8.0 (4.3)	-3.6 (6.2)	-3.2 (5.5)	-5.0 (7.0)	-3.7 (7.1)	-5.4 (5.9)	-4.7 (5.5)	-4.0 (5.4)	-3.4 (6.7)	-0.9 (3.0)	+0.6 (0.80

Mean (SD) Weight Gain
[a] Week 36 Terminal Necropsy 1, n= 8/sex/group
[b] Week 40 Terminal Necropsy 2, n=5/sex/group (% change relative to Week 36)
[c] Week 44 Recovery Necropsy, n=2/sex/group (% change relative to Week 40)

The next table list how individual animals were affected relative to body weight changes (i.e., gain, loss, or no change) at the end of the 3 study phases. Buprenorphine appeared to affect body weight in a dose-responsive manner.

Text Table 7: Incidence of Body Weight Change at Selected Intervals

Male	Week 36[a]			Week 40[b]			Week 44[c]		
	Gain	Loss	No Change	Gain	Loss	No Change	Gain	Loss	No Change
Group 1 0 mg/kg	8	0	0	2	1	2	1	1	0
Group 2 10 mg/kg	3	4	1	4	1	0	1	1	0
Group 3 20 mg/kg	3	5	0	1	2	2	1	1	0
Group 4 40 mg/kg	4	4	0	0	3	2	2	0	0

Female	Week 36[a]			Week 40[b]			Week 44[c]		
	Gain	Loss	No Change	Gain	Loss	No Change	Gain	Loss	No Change
Group 1 0 mg/kg	7	1	0	2	1	2	2	0	0
Group 2 10 mg/kg	1	6	1	3	2	0	0	1	1
Group 3 20 mg/kg	3	5	0	2	3	0	2	0	0
Group 4 40 mg/kg	4	4	0	2	3	0	1	0	1

[a]Week 36 Terminal Necropsy 1, n= 8/sex/group, relative to Day -1
[b]Week 40 Terminal Necropsy 2, n=5/sex/group, relative to Week 36
[c] Week 44 Recovery Necropsy n=2/sex/group, relative to Week 40

Food Consumption

Food was measured by weighing the food bowl before and after the 4-hour feeding period. This was done twice prior to the first day of dosing (Day 1), then once weekly thereafter. Food consumption recorded prior to dosing and through the day of necropsy is presented in the study report.

RBP-6000-related decreases in food consumption occurred in all dose groups beginning on Day 2. This effect is consistent with the pharmacological effect of buprenorphine contained in RBP-6000. Individual animal and group mean quantitative food consumption was quite variable at all time points of the study including pre-study. The first dose of RBP-6000 resulted in the most notable decrease for the study with statistically significant reductions in quantitative food consumption on Day 2 (Week 1) for both sexes in all three RBP-6000 dose groups relative to the control group.

Quantitative Food Consumption Day -3 vs. Day 2[a]				
Dose Group	Gender	Day -3	Day 2	% Change
Group 1 (0 mg/kg)	Male	138.75 (78.08)	147.63 (89.94)	+6%
	Female	142.25 (71.07)	144.13 (76.12)	+1%
Group 2 (10 mg/kg)	Male	136.38 (71.54)	30.63* (49.51)	-78%
	Female	131.50 (75.05)	2.75* (5.34)	-98%
Group 3 (20 mg/kg)	Male	198.75 (47.66)	13.00* (31.50)	-93%
	Female	150.13 (79.87)	2.88* (5.46)	-98%
Group 4 (40 mg/kg)	Male	166.63 (57.80)	23.38* (24.84)	-86%
	Female	103.13 (72.18)	19.75* (34.01)	-81%

Mean (SD); N = 8/sex/group; * = Statistically significant

[a] Group mean grams consumed/day

Individual qualitative food consumption was also assessed daily. Qualitative assessment of reduced food consumption was also variable between individuals and groups at all time points, but tended to occur more often in the three RBP-6000 groups relative to controls but not in a dose-dependent manner.

Observations of Reduced Qualitative Food Consumption by Dose Period				
Dose Day Range	Group 1 (0 mg/kg)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (40 mg/kg)
1-28	6/4 (0)	66/15 (4/4)	82/15 (7/5)	87/14 (4/4)
29-56	25/10	38/11	36/11 (1/1)	43/13 (1/1)
57-84	7/5 (0)	31/10 (1/1)	20/8 (0)	22/9 (0)
85-112	8/6	21/12	20/9	19/8
113-140	32/9	45/10	45/13	51/13
141-168	21/8	52/13	35/13	48/11
169-196	37/11	61/14	45/13	56/12
197-224	47/12	61/15	51/13	55/11
225-252	37/11	42/13	39/14	46/14
253-280	18/5 (0)	41/9 (1/1)	29/10 (1/1)	43/9 (1/1)
281-309	20/3	26/7	24/6	17/6

No. Observations/No. Affected with reduced food consumption
(No. Observations/No. Affected) with no food consumption
Day 1-224 n= 8/sex/group ; Day 253-280 n= 5/sex/group; Day 281-309 n=2/sex/group

Ophthalmoscopy

Ophthalmic Examinations were performed on all main study animals during the pre-study period and Groups 1 and 4 on Day 225. No further examinations were required because no test article-related findings were observed at Day 225.

There were no ophthalmic findings associated with administration of RBP-6000.

ECG

Electrocardiograms were conducted pre-study and on Day 225 (Dose 9) at 2 hours (±15 minutes) post-dose, following the 2-hour post-dose TK sample collection. No further ECGs were required because no test article-related findings were observed at Day 225.

There were no abnormal electrocardiographic findings reported to be attributable to the administration of RBP-6000. While quantitative data were not provided, summary graphs were included and the ECG report was signed by a veterinary cardiologist.

Clinical Pathology

Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from all animals prior to the initiation of dosing (Day -6), prior to dosing on Days 85 and 169, and on Days 252, 280, and 308. Urine samples were

obtained by cage pan collection pre-study (Day -6) and from the bladder at each necropsy on Days 253, 281, and 309. The animals were fasted for at least 8 hours prior to blood collections for serum chemistry.

Hematology

The following parameters were analyzed:

Hematology Parameters	
Red blood cell (RBC) count	Red cell distribution width (RDW)
Hemoglobin concentration	Reticulocyte count
Hematocrit	Platelet count
Mean corpuscular volume (MCV)	White blood cell (WBC) counts[a]
Mean corpuscular hemoglobin concentration (MCHC)	
Mean corpuscular hemoglobin (MCH)	

[a] Included total white blood cell, absolute polysegmented neutrophil, lymphocyte, monocyte, eosinophil, basophil, and other cell counts as appropriate.

A blood smear was prepared from each hematology specimen.

There were no RBP-6000-related hematology changes. Any changes to hematology parameters were considered by the Applicant to be within the normal range of variability seen in laboratory-housed beagle dogs at this facility or were not biologically relevant.

Coagulation

The following parameters were analyzed:

Coagulation Parameters
Prothrombin time (PT)
Activated partial thromboplastin time (APTT)
Fibrinogen

There were no RBP-6000-related changes to prothrombin time (PT), activated partial thromboplastin time (APTT), or fibrinogen concentration.

Clinical Chemistry

The following parameters were analyzed:

Serum Chemistry Parameters	
Alanine aminotransferase (ALT)	Total protein
Aspartate aminotransferase (AST)	Albumin
Alkaline phosphatase (ALP)	Globulin
Gamma-glutamyltransferase (GGT)	Albumin/globulin ratio
Lactate dehydrogenase (LD)	Glucose
Total bilirubin	Cholesterol
Urea nitrogen (BUN)	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Bicarbonate

There were no RBP-6000-related clinical chemistry changes. Any changes to clinical chemistry parameters were considered to be within the normal range of variability seen in laboratory-housed beagle dogs at this facility or were not biologically relevant.

Urinalysis

The following parameters were analyzed:

Urinalysis Parameters	
Color	Protein
Clarity	Glucose
Specific gravity	Ketones
Microscopic evaluation of urine sediment	Bilirubin
pH	Occult blood

For all animals, urine was collected from the bladder at necropsy and analyzed.

There were no RBP-6000-related urinalysis changes.

Gross Pathology

The animals were necropsied according to the following schedule:

Group No.	Day 253 (Terminal 1) No. of Males/Females	Day 281 (Terminal 2) No. of Males/Females	Day 309 (Recovery) No. of Males/Females
1	3/3	3/3	2/2
2	3/3	3/3	2/2
3	3/3	3/3	2/2
4	3/3	3/3	2/2

All animals were subjected to a complete gross necropsy examination. The necropsy examination included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

The following tissues and organs were collected from all animals. Tissues were preserved in 10% neutral-buffered formalin (except for the eyes, which were preserved in Davidson's fixative for optimum fixation).

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Glands (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Ovaries
Stomach	Uterus
Small Intestine	Cervix
Duodenum	Vagina
Jejunum	Endocrine
Ileum	Adrenals
Large Intestine	Pituitary
Cecum	Thyroid/Parathyroids[a]
Colon	Skin/Musculoskeletal
Rectum	Skin/Mammary Gland
Pancreas	Bone (femoral head)
Liver	Bone (7th rib)
Gallbladder	Skeletal Muscle (psoas and diaphragm)
Respiratory	Nervous/Special Sense
Trachea	Eyes with Optic Nerve
Lung	Sciatic Nerve
Lymphoid/Hematopoietic	Brain
Bone Marrow (sternum)	Spinal Cord (thoracic)
Thymus	Other
Spleen	Animal Number Tattoo
Lymph Nodes	Tarsal Gland
Axillary	Lacrimal Gland
Mesenteric	Gross Lesions
	Injection Site[b]

[a] The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.
[b] Scapular/interscapular area.

Two bone marrow smears from the seventh rib of each animal were made, but were not examined microscopically because there were no suspected test article-related alterations observed in hematology parameters or in the standard histopathology sections.

As noted previously, dosing was as follows:

Dose Site Order	
Dose Day	Dose Sites
1	1 and 2
29	3 and 4
57	5 and 6
85	7 and 8
113	9 and 10
141	11 and 12
169	13 and 14
197	15 and 16
225	17 and 18

Necropsy Days 253, 281, and 309 - RBP-6000-associated macroscopic findings were limited to injection sites and consisted of area/foci, pale, firm, or equivalent finding in animals examined on necropsy Days 253, 281, and 309 (see tables). The incidence of injection sites with findings per animal was higher in RBP-6000-dosed animals at Day 253 than Atrigel control. At Days 281 and 309, injection site findings were only observed in RBP-6000-dosed animals, but not in controls administered the ATRIGEL® Delivery System. No clear dose-related response was evident for injection site findings although higher incidences were usually associated with the high-dose. Injection site findings at earlier sites (Sites 1-16; Days 253, 281, and 309) were primarily observed in RBP-6000-dosed animals. Macroscopic injection site findings correlated with microscopic findings of subcutaneous granuloma, degenerate/necrotic cell debris, mononuclear cell infiltrate, fibroplasias, and/or hemorrhage (see histopathology section).

Incidence of Gross Injection Site Findings (Study Day 253)

			Males				Females			
		Group	1	2	3	4	1	2	3	4
		Dose (mg/kg/day)	0	10	20	40	0	10	20	40
		No. animals examined	3	3	3	3	3	3	3	3
Injection Site No.	Day Injected	Weeks since								
18	225	4	1	--	--	3	--	--	--	2
17	225	4	1	2	1	3	2	2	2	3
16	197	8	--	--	--	--	1	--	--	--
15	197	8	--	--	1	--	--	2	--	1
13	169	12	--	--	--	1	--	--	1	1
12	141	16	--	--	--	--	--	--	--	1
11	141	16	--	--	--	1	--	1	--	--
10	113	20	--	--	--	1	--	--	--	1
8	85	24	--	--	--	1	--	--	--	1
7	85	24	--	--	--	--	--	1	1	--
6	57	28	--	--	--	--	--	--	--	1
5	57	28	--	--	--	--	--	1	--	--
3	29	32	--	--	--	1	--	--	--	--
2	1	36	--	--	--	--	--	1	1	--
1	1	36	--	1	1	1	--	--	1	1
Total Injection Sites Affected			**2**	**3**	**3**	**12**	**3**	**8**	**6**	**12**

Early injection sites (sites 1 to 16) were only included in the table if a gross finding was observed.
Shaded rows represent injection sites that were used in all dose groups.
-- = No injection site findings observed.

Incidence of Gross Injection Site Findings (Study Day 281)

		Males				Females			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	20	40	0	10	20	40
No. animals examined		3	3	3	3	3	3	3	3
Injection Site Number	Weeks Since								
18	8	--	--	--	--	--	--	--	--
17	8	--	--	--	1	--	--	1	--
15	12	--	--	--	1	--	--	1	--
13	16	--	--	--	2	--	1	1	--
11	20	--	--	--	1	--	--	1	--
10	24	--	--	--	--	--	1	--	--
9	24	--	--	--	--	--	--	1	--
8	28	--	--	--	--	--	--	--	--
7	28	--	--	1	2	--	--	1	1
6	32	--	--	--	1	--	--	--	--
5	32	--	--	--	1	--	--	1	--
3	36	--	--	--	--	--	--	1	--
2	40	--	--	--	1	--	--	--	--
1	40	--	--	--	--	--	--	1	--
Site not specified		--	--	--	--	--	1	--	--
Total Injection Sites Affected		**0**	**0**	**1**	**10**	**0**	**3**	**9**	**1**

Early injection sites (sites 1 to 16) were only included in the table if a gross finding was observed.
Shaded rows represent injection sites that were used in all dose groups.
-- = No injection site findings observed.

Incidence of Gross Injection Site Findings (Study Day 309)

		Males				Females			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	20	40	0	10	20	40
No. animals examined		2	2	2	2	2	2	2	2
Injection Site Number	Weeks Since								
18	12	--	--	--	1	--	--	--	--
17	12	--	--	--	--	--	--	1	--
16	16	--	--	--	1	--	--	--	1
15	16	--	--	--	--	--	1	1	--
13	20	--	--	--	1	--	1	1	--
10	28	--	--	--	1	--	--	--	--
5	36	--	--	--	--	--	--	1	--
3	40	--	1	--	--	--	--	--	--
1	44	--	--	--	--	--	--	1	--
Total Injection Sites Affected		**0**	**1**	**0**	**4**	**0**	**2**	**5**	**1**

Early injection sites (sites 1 to 16) were only included in the table if a finding was observed.
Shaded rows represent injection sites that were used in all dose groups.
-- = No injection site finding observed.

Gross findings based upon time elapsed after injection show increased incidence of injection site findings in dosed animals as compared to controls for all injection sites for the course of the study. Females had a somewhat higher occurrence of injection site findings than males (46 versus 34) with occurrence in females distributed more evenly across doses, whereas high-dose males had a much higher number of injection site findings than mid- and low-dose males. A clear dose-incidence relationship is seen with sexes combined at numbers of 5, 16, 24, and 40 for the vehicle, LD, MD, and HD, respectively.

Incidence of Gross Injection Site Findings According to Time After Injection

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	20	40	0	10	20	40
No. animals examined	8	8	8	8	8	8	8	8
Weeks Since Injection								
4	2	2	1	6	2	2	2	5
8	0	0	1	1	1	2	1	1
12	0	0	0	3	0	0	3	1
16	0	0	0	4	0	3	2	2
20	0	0	0	3	0	1	2	1
24	0	0	0	1	0	2	2	1
28	0	0	1	3	0	1	1	2
32	0	0	0	3	0	0	1	0
36	0	1	1	1	0	1	4	1
40	0	1	0	1	0	0	1	0
44	0	0	0	0	0	0	1	0
Total Injection Sites with Gross Findings	**2**	**4**	**4**	**26**	**3**	**12**	**20**	**14**

Organ Weights

For all animals, the following organs (when present) were weighed before fixation. Paired organs were weighed together. The pituitary was weighed post fixation. Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

Organs Weighed	
Adrenals	Ovaries
Brain	Pituitary
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Lungs	

On Day 253 (1st necropsy), liver weights, liver/body weight ratios, and liver/brain weight ratios were no different among any of the groups. On Day 281 (2nd necropsy), liver weight were decreased 38%, 26%, and 94% for the low, mid, and high dose groups, respectively, compared to Atrigel control (statistical significance at p <= 0.05). Liver/body weight ratios were decreased 17%, 17%, and 23% for the low-, mid-, and high-dose groups, respectively, compared to Atrigel control (statistical significance at p <= 0.05 for high dose only). On Day 309 (3rd necropsy), all liver values were unremarkable. There was no associated histopathology with this observation, so its toxicological relevance is unknown.

No other alterations in organ weights or organ weight ratios in animals euthanized at Study Days 253, 281, or 309 were attributed to the administration of RBP-6000 or the ATRIGEL® Delivery System.

Histopathology

For all animals necropsied in the control and high-dose groups (Groups 1 and 4, respectively), the tissues listed in the table above and any gross lesions from the low- and mid-dose groups (Groups 2 and 3, respectively) were evaluated.

Adequate Battery – Yes

Peer Review – No

Histological Findings - No histological evidence of systemic toxicity was observed as RBP-6000-associated findings were limited to injection sites. Microscopic findings at Injection Sites 17 and 18 for animals examined on Day 253 and 281 were similar in incidence and severity among control and RBP-6000-dosed animals. However, RBP-6000-dosed animals had a higher incidence and severity of findings at Injection Sites 17 and 18 on Study Day 309 (see tables). Granulomas at older injection sites tended to be less cellular, having a thinner fibrotic wall, degenerate central debris and characterized

by collapse of the central core. These findings represented resolving inflammation at injection sites which had been used at earlier time points in the study and in all sites at the last study time point (Study Day 309). Although injection site findings were similar between control (which received ATRIGEL® Delivery System) and RBP-6000-dosed animals at relatively recent time points post injection, injection site inflammation persisted longer in RBP-6000-dosed animals. Cellular infiltration is observed in other organs but not at any comparable incidence or distribution across all dose groups. In summary, macroscopic and microscopic injection site findings appeared to be related to the ATRIGEL® Delivery System with injection site findings more persistent in RBP-6000-dosed animals.

Day 253

Pathology - Intergroup Comparison of Selected Histopathology Findings (with Severity) (Day 253)
Study: NNQ00004

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
INJECTION SITE(S);								
Examined	(0)	(1)	(2)	(2)	(1)	(2)	(3)	(2)
Within Normal Limits	0	0	0	0	0	0	0	0
Granuloma; Subcutaneous	(0)	(1)	(2)	(2)	(0)	(2)	(2)	(3)
mild	0	0	1	2	0	1	1	2
moderate	0	1	1	0	0	1	1	1
No Correlating Lesion	0	0	0	1	1	0	1	1
Degenerate/Necrotic Cell Debris; Subcutaneous; focal	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	0	0	1	0	0
INJECTION SITE 17;								
Examined	(3)	(2)	(1)	(3)	(3)	(2)	(2)	(3)
Within Normal Limits	0	0	0	0	0	0	0	0
Infiltrate, Mononuclear Cell; Subcutaneous	(2)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
mild	2	0	0	0	1	0	0	0
Fibroplasia; Subcutaneous	(2)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
mild	2	0	0	0	1	0	0	0
Granuloma; Subcutaneous	(1)	(2)	(1)	(2)	(2)	(2)	(2)	(2)
mild	0	2	0	0	0	0	0	0
moderate	1	0	1	2	2	2	2	2
Hemorrhage	(0)	(0)	(0)	(0)	(2)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
mild	0	0	0	0	1	0	0	0
Hemorrhage; multifocal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
No Correlating Lesion	0	0	0	1	0	0	0	1
INJECTION SITE 18;								
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	0	0	0	0	1	0	0	1
Infiltrate, Mononuclear Cell; Subcutaneous	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
Granuloma; Subcutaneous	(2)	(0)	(0)	(2)	(2)	(0)	(0)	(1)
moderate	2	0	0	2	2	0	0	1
Hemorrhage	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
Hemorrhage; multifocal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
Fibroplasia; Subcutaneous	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
No Correlating Lesion	0	0	0	1	0	0	0	1

Day 281

Pathology - Intergroup Comparison of Selected Histopathology Findings (with Severity) (Day 281)
Study: NNQ00004

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
Number of Animals on Study :	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
INJECTION SITE(S);								
Examined	(0)	(0)	(2)	(3)	(0)	(2)	(1)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Granuloma; Subcutaneous	(0)	(0)	(2)	(2)	(0)	(2)	(2)	(1)
minimal	0	0	0	0	0	1	0	0
mild	0	0	2	2	0	1	1	1
moderate	0	0	0	0	0	0	1	0
Infiltrate, Mononuclear Cell; Subcutaneous	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(0)
mild	0	0	0	1	0	0	0	0
moderate	0	0	0	1	0	0	0	0
Degenerate/Necrotic Cell Debris; Subcutaneous; focal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
mild	0	0	0	1	0	0	0	0
INJECTION SITE 17;								
Examined	(3)	(0)	(1)	(3)	(3)	(0)	(1)	(3)
Within Normal Limits	3	0	0	1	1	0	0	2
Infiltrate, Mononuclear Cell; Subcutaneous	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
Fibroplasia; Subcutaneous	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
Granuloma; Subcutaneous	(0)	(0)	(1)	(2)	(1)	(0)	(1)	(1)
mild	0	0	1	2	1	0	1	1
INJECTION SITE 18;								
Examined	(3)	(0)	(0)	(3)	(3)	(0)	(0)	(3)
Within Normal Limits	3	0	0	0	2	0	0	2
Infiltrate, Mononuclear Cell; Subcutaneous	(0)	(0)	(0)	(1)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
mild	0	0	0	1	0	0	0	0
Granuloma; Subcutaneous	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(1)
mild	0	0	0	2	0	0	0	1
Fibroplasia; Subcutaneous	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0

Day 309

Pathology - Intergroup Comparison of Selected Histopathology Observations (with Severity) (Day 309)
Study: NNQ00004

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	0 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg
Number of Animals on Study :	2	2	2	2	2	2	2	2
Number of Animals Completed:	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
INJECTION SITE(S);								
Examined	(0)	(1)	(0)	(1)	(0)	(1)	(1)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Granuloma; Subcutaneous	(0)	(1)	(0)	(2)	(0)	(1)	(1)	(1)
mild	0	0	0	1	0	0	0	1
moderate	0	1	0	1	0	1	1	0
Infiltrate, Mononuclear Cell; Subcutaneous	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
INJECTION SITE 17;								
Examined	(2)	(0)	(0)	(2)	(2)	(0)	(1)	(2)
Within Normal Limits	2	0	0	2	2	0	0	1
Infiltrate, Mononuclear Cell; Subcutaneous	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
Granuloma; Subcutaneous	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
moderate	0	0	0	0	0	0	1	0
INJECTION SITE 18;								
Examined	(2)	(0)	(0)	(2)	(2)	(0)	(0)	(2)
Within Normal Limits	2	0	0	1	1	0	0	1
Granuloma; Subcutaneous	(0)	(0)	(0)	(1)	(1)	(0)	(0)	(1)
minimal	0	0	0	0	1	0	0	0
mild	0	0	0	1	0	0	0	1

Special Evaluation – None

Toxicokinetics

On dosing days, up to 4 animals/sex/time point were sampled on each occasion at pre-dose and 1, 2, 4, 8, and 24 hours following each dose. All available samples were evaluated.

No. of Dogs	Time Points
4/sex/group[a]	Predose, 2-hours postdose, 8-hours postdose
4/sex/group[b]	1-hour post dose, 4-hours postdose, and 24-hours postdose

[a] The first 4 of 8 dogs/sex. [b] The second 4 of 8 dogs/sex.

On Days 15, 43, 71, 99, 127, 155, 183, 211, 239, and 252, up to 4 animals/sex/time point were sampled once at approximately the same time as dosing. All available samples were evaluated.

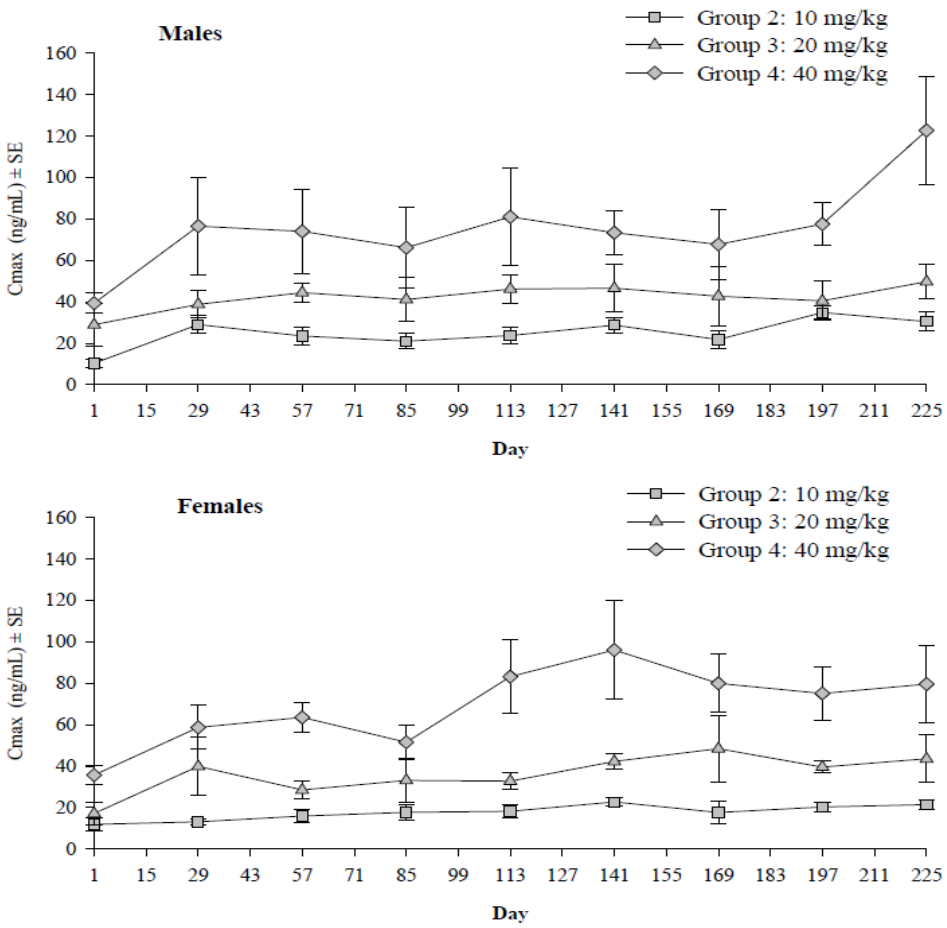
No. of Dogs	Time Points
4/sex/group[a]	Days 15, 71, 127, 183, and 239
4/sex/group[b]	Days 43, 99, 155, 211, and 252

[a] The first 4 of 8 dogs/sex. [b] The second 4 of 8 dogs/sex.

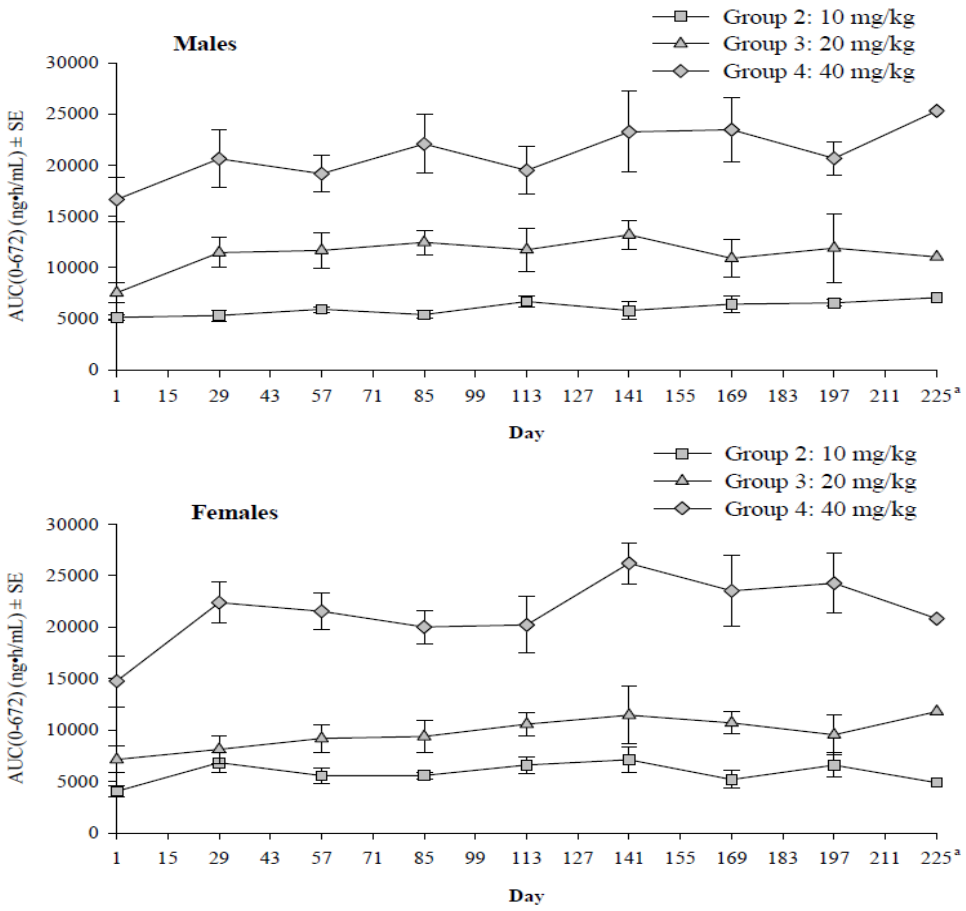
On Days 267 and 280, up to 5 animals/sex/time point were sampled at approximately the same time as dosing. On Days 295 and 308, up to 2 animals/sex/time point were sampled at approximately the same time as dosing. All available samples were evaluated.

The toxicokinetics of buprenorphine was characterized in Beagle dog plasma following once monthly subcutaneous administration at dose levels of 10, 20, and 40 mg/kg. Buprenorphine concentrations tended to be sustained between 8 hours and 4 weeks post-dose.

C_{max} of Buprenorphine versus Study Day



Overall exposure (AUC) of Buprenorphine versus Study Day



a AUC(0-672) was generated by extrapolation to 672 h and therefore does not have a standard error.

The estimated $t_{1/2}$ of the recovery phase was between 260 and 561 hours for buprenorphine. Exposure of buprenorphine as defined by C_{max} and $AUC_{(0-672)}$, increased with increasing dose level and was generally proportional to dose within the range tested (10 to 40 mg/kg of RBP-6000). No notable accumulation was observed for the $AUC_{(0-672)}$ of buprenorphine, but modest accumulation was observed for C_{max} (accumulation ratios between 1.10 and 3.37) at all dose levels. There was generally no notable difference in the exposure between males and females.

Combined (male and female) C_{max}s ranged from 10.3 to 34.9 ng/mL (low-dose), 17.2 to 49.8 ng/mL (mid-dose), and 35.7 to 123 ng/mL (high-dose) over the course of the study. Combined (male and female) AUC's over 672 hours (1 month) ranged from 4033 to 7,102 ng*hour/mL (low-dose), 7,134 to 13192 ng*hour/mL (mid-dose), and 14,743 to 26,204 ng*hour/mL (high-dose) over the course of the study.

Accumulation Ratios of Buprenorphine in Beagle Dog Plasma Following Subcutaneous Injection of Buprenorphine/ATRIGEL® (RBP-6000)

			C_{max} (ng/mL)									Ratio							
	Group	Dose Level	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day 29/	Day 57/	Day 85/	Day 113/	Day 141/	Day 169/	Day 197/	Day 225/
Gender	No.	(mg/kg)	1	29	57	85	113	141	169	197	225	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
Males	2	10	10.3	29.1	23.5	21.0	23.8	28.8	21.8	34.9	30.5	2.81	2.27	2.03	2.30	2.78	2.11	3.37	2.95
	3	20	29.0	38.8	44.4	41.1	46.2	46.6	42.7	40.5	49.8	1.34	1.53	1.42	1.59	1.61	1.48	1.40	1.72
	4	40	39.3	76.5	74.0	66.2	81.0	73.4	67.7	77.5	123	1.95	1.88	1.68	2.06	1.87	1.72	1.97	3.12
Females	2	10	11.9	13.2	16.0	17.8	18.3	22.8	17.6	20.4	21.5	1.10	1.34	1.49	1.54	1.91	1.48	1.71	1.80
	3	20	17.2	39.9	28.5	33.2	32.8	42.3	48.4	39.6	43.5	2.32	1.66	1.93	1.91	2.46	2.82	2.30	2.53
	4	40	35.7	58.7	63.5	51.6	83.2	96.1	80.0	75.1	79.6	1.65	1.78	1.44	2.33	2.69	2.24	2.10	2.23

			$AUC_{(0-672)}$ (ng•h/mL)									Ratio							
	Group	Dose Level	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day 29/	Day 57/	Day 85/	Day 113/	Day 141/	Day 169/	Day 197/	Day 225/
Gender	No.	(mg/kg)	1	29	57	85	113	141	169	197	225	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
Males	2	10	5124	5307	5919	5401	6677	5799	6416	6537	7064	1.04	1.16	1.05	1.30	1.13	1.25	1.28	1.38
	3	20	7529	11452	11675	12443	11736	13192	10901	11897	11036	1.52	1.55	1.65	1.56	1.75	1.45	1.58	1.47
	4	40	16646	20609	19157	22066	19474	23233	23446	20656	25285	1.24	1.15	1.33	1.17	1.40	1.41	1.24	1.52
Females	2	10	4033	6833	5561	5573	6599	7112	5186	6585	4876	1.69	1.38	1.38	1.64	1.76	1.29	1.63	1.21
	3	20	7134	8112	9176	9362	10569	11439	10687	9535	11788	1.14	1.29	1.31	1.48	1.60	1.50	1.34	1.65
	4	40	14743	22372	21531	20019	20219	26204	23524	24262	20819	1.52	1.46	1.36	1.37	1.78	1.60	1.65	1.41

This toxicokinetic assessment confirmed that 1) consistent and high exposures to buprenorphine was achieved with subcutaneous injection of RBP-6000 in dogs once every 4 weeks for 9 consecutive dosing periods; 2) significant exposure was maintained throughout the 4-week dosing intervals following each dose; 3) control group animals remained drug naive throughout the study.

Dosing Solution Analysis

As per the study report, appropriate documentation of the method of synthesis, fabrication, or derivation of the test and control articles are on file and are available to the appropriate regulatory agencies should it be requested. The test and control (vehicle) articles were provided by the Sponsor as pre-filled syringes. The test and control articles were used as received for dose. Stability analysis and verification of test article concentration and control article were conducted by the Sponsor. Analysis of the test and control articles was performed by the Sponsor on samples stored at the Sponsor's facility under the same conditions as the Testing Facility. The stability exit testing began after the sixth and final dose in the test article and control groups.

Agreed upon by this reviewer, all product characteristics seemed to be within acceptable ranges upon the initial release of Lots RB0042-0 1 (test article) and RB0042-21 (control article). A comparison of the Certificates of Analysis and the Certificates of Stability for Lots RB0042-01 and RB0042-21 indicates that there were no

substantial changes in the products over the course of 12 months for materials stored at 5 ± 2°C. All characteristics were within generally acceptable ranges for the purposes of this study. The test article and control article are considered to have been stable for the duration of the study under the storage conditions per the study protocol at 2 to 8°C.

The test and control articles conformed with product characteristics and specifications when supplied for this study and remained within these parameters under the prescribed storage conditions through the duration of the study. Buprenorphine in the test formulation was 98% and 97% target concentration at the initial and end of study assays, respectively.

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7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

No specific studies were conducted for RBP-6000. Refer to approved buprenorphine drugs (NDA 20732 for Subutex and NDA 18401 for Buprenex) and buprenorphine-naloxone drugs (NDAs 20733 and 22410 for Suboxone), owned by the Applicant.

7.2 *In Vitro* Assays in Mammalian Cells

No specific studies were conducted for RBP-6000. Refer to approved buprenorphine drugs (NDA 20732 for Subutex and NDA 18401 for Buprenex) and buprenorphine-naloxone drugs (NDAs 20733 and 22410 for Suboxone), owned by the Applicant.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: RBP-6000: Micronucleus Test in Bone Marrow Cells of CD Rats: Single Subcutaneous Dose and 24 h and 48 h Sampling with Toxicokinetic Blood Sampling

Study no:	RBLS-R08-60-10 (789280)
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 10, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - (b) (4) % buprenorphine base in (b) (4) % 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4) % N-methyl-2-pyrrolidone (NMP), Lot 0042-01, buprenorphine (b) (4) %, NMP (b) (4) %

- ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP), Lot 0042-01, (b) (4)% NMP
- Positive control cyclophosphamide, Lot 079K1569

Key Study Findings

- The objective of this study was to assess the genotoxic effects of the RBP-6000 and Atrigel at equivalent amounts of N-methyl-2-pyrrolidone (NMP) *in vivo*, as determined by its potential to induce micronuclei (micronucleated polychromatic erythrocytes - MN-PCE) in the bone marrow of CD rats after 0 hour subcutaneous dosing at the maximum tolerated dose with a 24 hour and 48 hour sampling regimen.
- Dose levels of 125, 250, and 500 mg/kg buprenorphine as RBP-6000 were injected subcutaneously in three rats per sex as well as 2000 mg/kg Atrigel Delivery system (the buprenorphine vehicle) which is an equivalent dose of NMP as 500 mg/kg buprenorphine at RBP-6000. Cyclophosphamide was the positive control.
- At both 24 and 48 hours, no increase in percentage of MN-PCE were observed for any RBP-6000 groups or the Atrigel group compared to historical control levels. The cyclophosphamide group exhibited significant increases in MN-PCEs at 0.99% compared to historical control values with a mean of 0.04% with a maximum frequency of 0.13% MN-PCEs.
- Exposure to buprenorphine was indicated by detected levels in the blood.
- Based on this study, buprenorphine in Atrigel at the maximum tolerated dose (MTD) and NMP in RBP-6000 and Atrigel at the same dose levels were not genotoxic.
- **Note** – Atrigel Delivery system (b) (4)% PLGH: (b) (4)% NMP) and RBP-6000 (b) (4)% buprenorphine: (b) (4)% PLGH: (b) (4)% NMP) at (b) (4) has same amount of NMP as (b) (4)

Methods

Doses in definitive study:

Dose Group	Target Test Dose	Treatment and Number of Rats		
		Dosing (h)	24 h Sample	48 h Sample
Vehicle Control	2000 mg ATRIGEL Delivery System [a]/kg	0	5M + 5F	5M + 5F
Low Dose	125 mg Buprenorphine/kg (625 mg RBP-6000/kg)	0	5M	
Mid Dose	250 mg Buprenorphine/kg (1250 mg RBP-6000/kg)	0	5M	
High Dose a	500 mg Buprenorphine/kg (2500 mg RBP-6000/kg)	0	5M + 5F	10M + 10F
Positive Control	50 mg Cyclophosphamide/kg	0	5M	

[a] Vehicle treated animals received a dose of ATRIGEL Delivery System with NMP level equivalent to the High Dose treated animals.

- 2000 mg/kg Atrigel (b) (4)

- 500 mg/kg buprenorphine in RBP-6000 (b) (4)

Frequency of dosing: Once

Route of administration: Subcutaneous

Dose volume: 5 mL/kg

Formulation/Vehicle: Atrigel

Species/Strain: (Crl:CD) Sprague Dawley rats

Number/Sex/Group: 5-10 (see Table above)

Satellite groups: Satellite groups of rats were dosed at 0 h and blood samples taken for drug plasma level analysis as follows:

Dose Group	Target Test Dose (equiv. buprenorphine mg/kg)	Treatment and Number of Animals			
		Dose (h)	Sample (h)	Males	Females
Vehicle Control[a]	0	0 h	1	181-183	184-186
			2		
			4		
High Dose	500		1	187-189	190-192
			2		
			4		

[a] Vehicle treated animals received a dose of Atrigel with NMP level equivalent to the High Dose treated animals.

Basis of dose selection: Previous MTD study (RBLS-R08-60-10, 787959) and toxicity study in this report

Negative control: 2000 mg/kg Atrigel

Positive control: 50 mg Cyclophosphamide

Study Validity

The following 3 acceptance criteria were satisfied:

General - The assay was considered acceptable as the prepared slides showed uniform staining properties and sufficient number of PCE cells present to allow accurate micronucleus determination.

Negative Controls - The assay was considered acceptable as the MN-PCE frequencies for the vehicle control dosed rats were within the expected historical range. The ranges are defined in accordance with (b) (4) experience of the bone marrow micronucleus test using CD rats.

Positive Controls - The assay was considered acceptable as an adequate positive control response for at least 2 animals and the dose group as a whole was observed.

Evaluation Criteria

Negative Response - The test would be judged negative if no biologically relevant increases in the numbers of micronucleated polychromatic erythrocytes (MN-PCE) were observed, relative to the concurrent and established historical control frequencies for MN-PCE induction.

Positive Response - The test was judged positive if an increase in the number of MN-PCE was obtained for one or more of the test item treated dose groups. That is, an increase greater than 10% over the expected historical control ranges for a group of animals. The increase observed should be biologically relevant and statistically significant relative to concurrent and historical control frequencies for MN-PCE and/or MN-NCE induction.

Positive Response - The test was judged positive if an increase in the number of MN-PCE was obtained for one or more of the test item treated dose groups. That is, an increase greater than 10% over the expected historical control ranges for a group of animals. The increase observed should be biologically relevant and statistically significant relative to concurrent and historical control frequencies for MN-PCE and/or MN-NCE induction.

Inconclusive Response - The test would be considered inconclusive if the levels of MN-PCE within any one dose group were increased above the established historical control frequencies for MN-PCE induction, but not high enough to meet the criteria for a positive response. That is an increase up to 10% over the maximum negative control frequency for a group of animals.

Results

Toxicity Study - In the preliminary toxicity study, groups of male and female CD rats received subcutaneous doses of RBP-6000 ranging from 500 to 1000 mg/kg/day. Groups of rats were also treated with ATRIGEL Delivery System at the equivalent level of NMP to RBP-6000 at 500 or 1000 mg buprenorphine/kg dose group. Clinical signs included lump at dosing site, subdued behavior, hunched posture, irregular respiration,

staggering, partly-closed eyes, rolling gait, dark staining at the injection site, pale extremities. Clinical signs and mortality, which occurred in the 100 mg/kg Atrigel group, are listed in the table. Based on this data, the MTD was determined to be 500 mg/kg buprenorphine as RBP-6000. For NMP, this is equivalent to 2,000 mg/kg Atrigel regarding the dose of NMP.

Toxicity Study
Clinical Signs and Deaths

Test No.	Dose Level (mg buprenorphine /kg)	Animal Nos.	Clinical Signs Observed (in one or more animals per group) (Days 1 = Dosing Day)		No. of Deaths
1 ATRIGEL Delivery System	(NMP equivalent to 1000 mg of RBP-6000)	1-3M 7-9F	Day 1	Lump at dosing site, Subdued behaviour, Hunched (1-3M, 7-9F), Irregular breathing, Staggering, Eyes part-closed (7-9F), Rolling gait (3M, 7-9F), Dark staining on dose-site, Pale extremities (9F) Stained savings (1-3M) HK (7-9F)	3
			Day 2	Hunched, Staggering (1-3M), Lump at dosing site (1+3M)	0
			Days 3-4	Hunched, Staggering (1-3M), Lump at dosing site (1+3M), Swollen abdomen (2M)	0
1 RBP-6000	1000	4-6M 10-12F	Day 1	Lump at dosing site, Hunched, Subdued behaviour, Staggering (4-6M, 10-12F), Irregular respiration (10-11F), Rolling gait (6M)	0
			Day 2	Lump at dosing site, Hunched, Subdued behaviour, Staggering (4-6M, 10-12F), Piloerection (10M), Staining on face (10-12F), Swollen abdomen (4M, 10-12F)	0
			Days 3-4	Lump at dosing site, Hunched, Staggering (4-6M, 10-12F), swollen abdomen (4-6M, 12F)	0
2 ATRIGEL Delivery System	(NMP equivalent to 500 mg of RBP-6000)	13-15M 16-18F	Day 1	Lump at dosing site (13-15M, 16-18F), Subdued Behaviour (13-15M, 17F)	0
			Day 2	Lump at dosing site (13-15M, 16-18F), Subdued Behaviour (13-15M, 17F), Rolling gait (13-14M, 17F)	0
			Days 3-4	Lump at dosing site (13-15M, 16-18F), Rolling gait (13-15M)	0
2 RBP-6000	500	19-21M 22-24F	Day 1	Lump at dosing site (19-21M, 22-24F), Subdued Behaviour (19-21M)	0
			Day 2	Lump at dosing site (19-21M, 22-24F), Subdued Behaviour (19-21M)	0
			Days 3-4	Lump at dosing site (19-21M, 22-24F), Subdued Behaviour (23F)	0

HK = Humanely killed

Group No.	Target Dose Level (mg/kg)	No. of Deaths
1 (Vehicle)[a]	1000	3
1 (RBP-6000)		0
2 (Vehicle)[a]	500	0
2 (RBP-6000)		0

[a] Vehicle control animals received a dose of Atrigel equivalent to that in the dose of RBP-6000

Test Conduct - In the micronucleus test, 3 groups of rats were dosed subcutaneously at 0 hour with RBP-6000 at target doses of 125, 250, and 500 mg buprenorphine/kg as the low-, mid-, and high-dose groups, respectively. Concurrent vehicle and positive control groups of rats were similarly dosed subcutaneously at 0 hour. Sampling for all groups was at 24 and 48 hours.

Micronucleus Test - Clinical Signs and Deaths

Treatment	Animal Nos.	Clinical Signs Observed (in one or more animals per group) (Day 1 = Dosing Day)		No. of Deaths
ATRIGEL Delivery System (2000 mg/kg, or NMP equivalent to 500 mg buprenorphine/kg of RBP-6000)	101-110M 111-120F	Day 1	Lump at dosing site (101-110M; 111-120F), Subdued behaviour (101-110M; 115-117F), Rolling gait (108M).	0
		Day 2	Lump at dosing site (101-110M; 111-120F), Subdued behaviour and Rolling gait (119F).	0
		Day 3	Lump at dosing site (106-110M; 116-120F), Rolling gait (119F).	0
RBP-6000 (125 mg buprenorphine/kg)	121-125M	Day 1	Lump at dosing site (121-125M).	0
		Day 2	Lump at dosing site (121-125M).	0
RBP-6000 (250 mg buprenorphine/kg)	126-130M	Day 1	Lump at dosing site (126-130M), Rolling gait (126M).	0
		Day 2	Lump at dosing site (126-130M).	0
RBP-6000 (500 mg buprenorphine/kg)	131-145M 146-160F	Day 1	Lump at dosing site (131-145M; 146-160F), Subdued behaviour (131-145M; 146, 148 - 151, 153-160F) Rolling gait (131-133, 135, 138-142, 144-145M; 146, 148 - 151, 153-157, 160F).	0
		Day 2	Lump at dosing site (131-145M; 151-160F), Subdued behaviour (134, 135M, 146-160F), Rolling gait (131, 137, 138, 142, 145M; 151-154, 157, 160F), Hunched, Brown staining on muzzle (160F)	0
		Day 3	Lump at dosing site (136-140, 142-145M; 151-160), Subdued behaviour (137, 138, 141, 142, 145M; 153, 154, 156-160F), Rolling gait (138, 141, 145M; 151, 154, 157, 158, 160F), Piloerection, Hunched (160F).	0
Cyclophosphamide (50 mg/kg)	161-165M	Day 1	Lump at dosing site (161-165M).	0
		Day 2	Lump at dosing site (161-165M).	0

Note – Atrigel Delivery system (b) (4)% PLGH: (b) (4)% NMP) and RBP-6000 (b) (4)% buprenorphine: (b) (4)% PLGH: (b) (4)% NMP)

(b) (4)

As noted in the table, no sex only or sex combined group other than the positive control groups exceeded a percentage of micronucleated bone marrow polychromatic erythrocytes (MN-PCE) of 0.06% at 24 or 48 hours. The positive control group % MN-PCE was 0.99%. Historical control (information contained in Appendix) was a mean of 0.04% with a maximum frequency of 0.13%. As noted previously, a positive response is an increase greater than 10% over the expected historical control ranges for a group of animals, which did not occur here except for the positive control groups.

RBP-6000 is not considered genotoxic and neither is NMP in Atrigel at the MTD for the drug product.

Summary of Assessment Data

Treatment	Sample Time (h)	Sex	No. of Rats Scored	Erythrocytes				
				Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE
				No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	Mean ± S.D.
2000 mg ATRIGEL Delivery System/kg	24	M	5	2	10005	3	0.03	0.60 ± 0.07
		F	5	3	10001	2	0.02	0.55 ± 0.09
		M/F	10	5	20006	5	0.02	0.57 ± 0.08
	48	M	5	3	10002	6	0.06	0.61 ± 0.05
		F	5	2	10001	4	0.04	0.54 ± 0.07
		M/F	10	5	20003	10	0.05	0.58 ± 0.07
125 mg buprenorphine/kg	24	M	5	1	10003	2	0.02	0.59 ± 0.08
250 mg buprenorphine/kg	24	M	5	2	10006	2	0.02	0.53 ± 0.10
500 mg buprenorphine/kg	24	M	5	2	10002	2	0.02	0.53 ± 0.05
		F	5	2	10004	1	0.01	0.62 ± 0.08
		M/F	10	4	20006	3	0.01	0.57 ± 0.08
	48	M	5	6	10002	4	0.04	0.49 ± 0.10
		F	5	4	10002	4	0.04	0.51 ± 0.08
		M/F	10	10	20004	8	0.04	0.50 ± 0.08
50 mg Cyclophosphamide/kg	24	M	5	50α	10001	99	0.99Φ	0.33 ± 0.04

PCE = Polychromatic erythrocytes
MN-PCE = Micronucleated PCE
NCE = Normochromatic erythrocytes
MN-NCE = Micronucleated NCE
Φ = Positive response in PCE
α = Evident response in NCE

Toxicokinetics - The plasma analysis showed animals were exposed to buprenorphine as levels of buprenorphine were between 104 and 346 ng/mL in the satellite animals treated at the high dose level. No apparent toxicokinetic analysis (e.g., C_{max}, AUC) were apparently reported.

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7.4 Other Genetic Toxicity Studies

N/A

8 Carcinogenicity

No carcinogenicity studies were conducted for RBP-6000. Refer to approved buprenorphine drugs (NDA 20732 for Subutex and NDA 18401 for Buprenex) and buprenorphine-naloxone drugs (NDAs 20733 and 22410 for Suboxone), owned by the Applicant. As part of the pre-IND meeting of April 27, 2010 (IND 107607) for this NDA 209819, no additional carcinogenicity studies would be anticipated pending absence of data of carcinogenic concern from planned 6- and 9- month toxicity studies in rats and dogs, respectively, that differ from the known carcinogenic potential of buprenorphine. This lack of a need for additional carcinogenicity studies was confirmed in the nonclinical review by this reviewer in DARRTS (June 18, 2013) of those chronic rat and dog studies.

As NMP is technically novel for a chronic, non-oncology indication, the Applicant provided a literature-based safety justification for NMP. NMP has been reported to be negative in rats and positive in mice (hepatocellular adenoma which correlated with hepatocellular hypertrophy). Refer to the appendix on NMP for further information.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

(Embryo-Fetal Combination)

Study title: A Combination Study for the Effects of RBP-6000 (Buprenorphine Base in the ATRIGEL® Delivery System), Administered by Subcutaneous Injection, on Male and Female Fertility and Embryo-Fetal Development, in the Sprague-Dawley Rat

Study no.:	INLS-R103-60-16
Study report location:	eCTD in Global Submit Review
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 24, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - 18% buprenorphine base in (b) (4)% 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4)% N-methyl-2-pyrrolidone (NMP)

- 100 mg, Lot 158, (b) (4)% of buprenorphine content
- ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP)
  - 100 mg placebo (Lot 202), (b) (4)% NMP
  - 300 mg placebo (Lot 203), (b) (4)% NMP
- 0.9% Sodium chloride, Lots 52-071-JT, 53-027-JT, 54-017-JT, 56-025-JT

Key Study Findings

- A comprehensive GLP study protocol was employed to assess male and female fertility and embryo-fetal development. Twenty-three (23) male and female rats were dosed subcutaneously (SC) with saline, 300, 600, & 900 mg/kg buprenorphine as RBP-6000 (ATRIGEL® Delivery System vehicle) and three (3) equivalent dose volumes of Atrigel to the buprenorphine dose groups. Females were dosed on Day 1 and Gestation Day 7, which were approximately 22 to 38 days apart. Males were dosed on Days 1, 28, & 56, then mated with non-treated females. Treated females were sacrificed on GD 20 and untreated females were sacrificed on GD 13.
- Following two dose cycles, female survival, macroscopic findings, and organ weights were unaffected by any of the ATRIGEL® control or RBP-6000 dose levels. The majority of clinical signs were related to the injection site(s). Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals in the Atrigel control (low, mid, and high) and RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine). Clinical signs indicative of maternal toxicity were observed during the gestation period and consisted of lower body weight, body weight gain, and food consumption. These findings were observed in the high-Atrigel control group and the 900 mg/kg buprenorphine administered group indicating maternal toxicity was not confined to exposure to buprenorphine but also to high exposure levels of Atrigel.
- Following three dose cycles (one every 28 days), male toxicity was observed in all of the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) and included morbundity/mortality at 900 mg/kg buprenorphine and lower body weight, body weight gain, and food consumption at 300, 600, and 900 mg/kg buprenorphine. Treatment-related reductions in testes and epididymis weights (absolute and relative to body weight) were noted in the high-Atrigel control group and at 900 mg/kg buprenorphine.
- Male fertility and reproduction indices were lower as evidenced by abnormal sperm parameters (low motility, low mean number of sperm, and higher percentage of abnormal sperm in mid- and high-ATRIGEL® control groups and at 600 and 900 mg/kg buprenorphine.

- In untreated females (mated to treated males), higher mean post-implantation loss, higher mean number of resorptions, lower mean number of viable fetuses/litter size, lower mean gravid uterine weight, lower fetal body weights were observed in the high-Atrigel control group and at 900 mg/kg buprenorphine. However, no similar effects were observed in the low and mid-ATRIGEL® control groups and at 300 and 600 mg/kg buprenorphine.
- The results of this study indicate that toxicity can be induced by high doses of both the ATRIGEL Delivery System as well as RBP-6000, and it therefore are not likely be solely due to buprenorphine exposure. Because the ATRIGEL Delivery System is comprised of only NMP and PLGH, and the latter has a benign and extensively characterized safety profile, it is presumed that much of the toxicity observed is due to exposure to NMP. However, buprenorphine treatment does appear to have an increase in head malformations over and above that of Atrigel
- Based on these results, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 300 mg/kg buprenorphine for RBP-6000 and the low-Atrigel control group. The NOAEL for female fertility and reproductive parameters was 600 mg/kg buprenorphine for RBP-6000 the mid-ATRIGEL® control group. The NOAEL for induced fetal malformations was 300 mg/kg buprenorphine for RBP-6000 and the low-Atrigel control group. A NOAEL for male general toxicity could not be determined for RBP-6000 as effects were observed in all dose groups, however the NOAEL for the ATRIGEL® Delivery System control groups was the mid-ATRIGEL® control group. The NOAEL for male reproductive and fertility parameters was 300 mg/kg buprenorphine for RBP-6000 and the mid-ATRIGEL® control group, however the NOAEL for sperm parameters (percent abnormal sperm) was the low-ATRIGEL® control group. In all, the overall NOAEL for fertility and embryo-fetal toxicity is 300 mg/kg buprenorphine for RBP-6000 (1.5 mL/kg) and the low-Atrigel control group (1.23 mL/kg).
- Toxicokinetic values in males for the low dose RBP-6000 group were C_{max}s of 310 ng/mL (Day 1) and 475 ng/mL (Day 56), $AUCs_{0\text{-}24h}$ of 3,420 ng*h/mL (Day 1) and 5,690 ng*h/mL (Day 56), and $AUCs_{0\text{-}168h}$ of 9,150 ng*h/mL and 23,000 ng*h/mL (Day 56). Toxicokinetic values in females for the low dose RBP-6000 group were C_{max}s of 266 ng/mL (Day 1) and 217 ng/mL (GD 7)), $AUCs_{0\text{-}24h}$ of 2,660 ng*h/mL (Day 1) and 2,720 ng*h/mL (GD 7), and $AUCs_{0\text{-}168h}$ of 7,050 ng*h/mL and 11,300 ng*h/mL (GD 7).

Methods

Doses:	0 (saline), 0 (Atrigel), 300, 600, and 900 mg buprenorphine in Atrigel. The dose levels were selected based on data from previously conducted toxicological studies with RBP-6000 in rats including dose range finding study INLS-R102-60-15.
Frequency of dosing:	Dosing of the treated females occurred on Day 1 and Gestation Day (GD) 7 (approximately 22 to 38 days apart) and dosing of the treated males occurred on Days 1, 28, and 56. All rats wore Elizabethan head collars for 72 hours following each dose administration.
Dose volume:	See study design
Route of administration:	Subcutaneous
Formulation/Vehicle:	Atrigel
Species/Strain:	CD® [Crl:CD®(SD)] rats
Number/Sex/Group:	23
Satellite groups:	Toxicokinetics
Study design:	See below
Deviation from study protocol:	Nothing significant

Study Design

Study Design							
					Number of Animals		
Group Number	Dose Level of Buprenorphine Base (mg/kg)	Total Dose Volume (mL/kg)[a, e]	Dose Volume per Injection (mL/kg)[a, e]	Total Number of Injection Sites	Treated Males[b, c]	Treated Females[c]	Untreated Females
1	0 (Saline)	4.5	1.5	3	-	23	-
2	0 (Control 1)[d]	1.23	1.23	1	-	23	-
3	0 (Control 2)[d]	2.46	1.23	2	-	23	-
4	0 (Control 3)[d]	3.69	1.23	3	-	23	-
5	300	1.5	1.5	1	-	23	-
6	600	3.0	1.5	2	-	23	-
7	900	4.5	1.5	3	-	23	-
8	0 (Saline)	4.5	1.5	3	23	-	23
9	0 (Control 1)[d]	1.23	1.23	1	23	-	23
10	0 (Control 2)[d]	2.46	1.23	2	23	-	23
11	0 (Control 3)[d]	3.69	1.23	3	23	-	23
12	300	1.5	1.5	1	23	-	23
13	600	3.0	1.5	2	23	-	23
14	900	4.5	1.5	3	23	-	23

[a] For treated groups, the dose volume was calculated using 18% (w/w) buprenorphine and a density of 1.15 g/mL. For control groups, the dose volume was calculated using a density of 1.15 g/mL.
[b] The male animals remained naïve for the first mating period with the treated females and continued on study and were dosed.
[c] A subset of 3 animals/group were used for TK blood sample collection.
[d] The ATRIGEL® control groups received an approximately equivalent amount of ATRIGEL® as delivered to the treated groups (1.23 mL/kg/injection).
[e] Doses were delivered within ±20% of the desired dose.
- = Not applicable

All rats received 1, 2, or 3 injections of approximately the same volume size to reach their appropriate dose level.

The nominal dose levels of buprenorphine base tested and the equivalent amounts of RBP-6000 and the content of the three Atrigel dose groups are as follows:

Dose Levels of Buprenorphine Base Relative to RBP-6000	
Buprenorphine base (mg/kg)	RBP-6000 (mg/kg)
300	(b) (4)
600	
900	

Dose Levels of ATRIGEL® Delivery System and its Components		
ATRIGEL® Delivery System (mg/kg)	NMP (mg/kg)	PLGH (mg/kg)
		(b) (4)

NMP - N-methyl-2-pyrrolidone; PLGH - polylactide-co-glycolide

Dosing

Injection sites were rotated to minimize irritation. Prior to administration (within 3 days), the hair was clipped from the dorsal surface of each animal in order to mark, number and assess the placement sites for the SC dose according to the following:

Rat Dosing Diagram

Head	
A	D
E	B
C	F

Observations and Results

TREATED FEMALES

Mortality

All animals were observed for mortality twice daily.

All treated females in the ATRIGEL® control (low, mid, and high) and RBP-6000 groups (300, 600 and 900 mg/kg buprenorphine) survived to the scheduled necropsy

Clinical Signs – Treated Females

All animals were observed for morbidity and injury twice daily. Additionally, collar checks were performed every 12 hours from collar application until removal. Once daily for 3 days after each dose administration (75 minutes ±15 minutes post-dose on dosing days) and once weekly thereafter, each animal was removed from the cage and given a detailed clinical examination. Animals were also given a detailed clinical examination prior to necropsy. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration.

General - Swelling of the face, salivation, lacrimation, and material around the eyes and/or nose (black and/or red) in a few female animals (up to 7) and was observed at all dose levels during the treatment period (premating/mating and gestation periods). These findings generally occurred following the dose and application of the collar and were considered in the report to be related to improperly fitted collars.

Detailed – Reduced activity during the premating/mating period was observed at a higher incidence at 600 and 900 mg/kg buprenorphine (18-20 animals) and was considered a known pharmacologic effect of buprenorphine.

The majority of clinical signs in the study were related to the injection site(s). Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals in the ATRIGEL® control (low, mid, and high) and RBP-6000

administered groups (300, 600 and 900 mg/kg buprenorphine). This observation was not considered adverse according to the report because this was an expected finding associated with the subcutaneous delivery of the formulation and formation of the drug depot. Additional clinical signs related to the injection site(s), seen in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (300, 600 and 900 mg/kg buprenorphine), were considered likely related to the unique properties, such as the formation of palpable drug depot and delivery system associated with the subcutaneous dose route of administration. The most prevalent of these findings was scabbing at the injection site(s) which occurred in all Atrigel control (low, mid, and high) and RBP-6000 administered groups at a much higher incidence than the saline control as there was little to no local effect from saline injection. Other findings at the injection site(s) were observed on occasion in one of more of these groups, including skin discoloration, sparse hair, and abrasion(s).

Increased incidence of reduced activity during the treatment period the premating/mating period was observed in treated females at a higher incidence at 600 and 900 mg/kg buprenorphine (18-20 animals) and is considered to be a known pharmacologic effect of buprenorphine. A few other clinical findings in the Atrigel control (low, mid, and high) and RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) were observed infrequently and were considered incidental and unrelated to treatment.

Body Weight – Treated Females

Body weights of treated females were recorded at the initiation of dose administration on Day 1 and twice weekly at 3- and 4-day intervals prior to and during cohabitation. Mated treated females with a confirmed GD 0 were weighed on GD 0, 4, 7, 10, 13, and 20. After cohabitation, treated females with no evidence of mating were weighed at 3- and 4-day intervals until euthanasia. Body weight changes were calculated for treated females between each weighing interval and over the entire premating period and on GD 0-4, 4-7, 7-10, 10-13, 13-20, 0-7, 7-20, and 0-20. Adjusted body weight (GD 20 body weight minus gravid uterine weight) and adjusted body weight change (GD 0 to 20) were also calculated.

Premating – Premating mean body weights in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) were generally comparable to the saline control group.

Summary of Premating Body Weight Values - FEMALE

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Body Weight Values g													
	1	219.8	11.57	23	216.1	11.93	23	220.0	10.73	23	220.3	12.39	23
	4	217.9	14.33	23	213.3	11.46	23	210.8	15.86	23	202.3[b]	12.45	23
	8	233.3	12.03	23	227.7	12.85	23	233.1	15.61	23	232.0	14.01	23
	11	239.7	14.29	23	231.8	13.44	23	241.5	16.17	23	244.4	17.20	23
	15	249.3	16.65	23	239.5	13.62	23	249.3	15.16	23	249.6	18.70	23

Summary of Premating Body Weight Values - FEMALE

Endpoint	Study Interval (Day)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Body Weight Values g										
	1	218.6	11.10	23	221.0	12.41	23	218.4	15.34	23
	4	222.6	11.49	23	218.4	12.16	23	212.3	14.60	23
	8	236.3	10.25	23	235.3	12.08	23	230.0	14.77	23
	11	242.6	11.98	23	241.0	13.32	23	235.8	14.63	23
	15	247.0	11.75	23	247.9	15.12	23	247.2	18.39	23

Mean premating body weight gains exhibited some ups and downs over the different time periods with usually next interval recovery and comparable Day 1-15 gains for all groups. Possibly notable is the high-ATRIGEL® control group (highest volume of Atrigel) where mean body weight on Day 4 was statistically lower (-7%) and correlated with a statistically lower mean body weight gain from Day 1-4 in comparison to the saline control (-18.0 g vs. -2.0 g).

Summary of Premating Body Weight Change Values - FEMALE

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Body Weight Change Values g													
	1-4	-2.0	4.91	23	-2.9	4.92	23	-9.2[a]	8.78	23	-18.0[b]	10.48	23
	4-8	15.5	7.48	23	14.4	6.07	23	22.3[a]	6.76	23	29.7[b]	7.18	23
	8-11	6.3	6.99	23	4.2	7.04	23	8.3	5.59	23	12.4[a]	6.83	23
	11-15	9.7	6.08	23	7.7	4.46	23	7.9	6.01	23	5.2	7.59	23
	1-15	29.5	7.74	23	23.4	11.37	23	29.3	11.02	23	29.3	12.78	23

Summary of Premating Body Weight Change Values - FEMALE

Endpoint	Study Interval (Day)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Body Weight Change Values g										
	1-4	3.9[a,f]	7.05	23	-2.5	7.77	23	-6.2[j]	6.94	23
	4-8	13.7	6.62	23	16.9[g]	5.09	23	17.7[j]	6.66	23
	8-11	6.3	4.78	23	5.7	5.21	23	5.8[j]	5.02	23
	11-15	4.4[a]	4.66	23	6.9	5.55	23	11.4[j]	6.71	23
	1-15	28.4	8.66	23	26.9	10.41	23	28.8	9.26	23

Gestation - Statistically lower mean gestation body weights and body weight gain were observed in the high-ATRIGEL® control group and at 900 mg/kg buprenorphine in comparison to the saline control group and were considered treatment-related and adverse. Mean gestation body weights in the high-ATRIGEL® control group and at 900 mg/kg buprenorphine were statistically lower on GD 10 (-10% and -8%, respectively), GD 13 (-6% and -9%, respectively), and GD 20 (-13% and -18%, respectively) in comparison to the saline control group. These differences in mean gestation body weight in the high-ATRIGEL® control group correlated with statistically lower mean body weight gain from GD 7-10 (-28.6 g vs. +0.1 g), GD 13-20 (+46.2 g vs. +80.2 g), GD 7-20 (+52.2 g vs. +105.9 g), and GD 0-20 (+88.7 g vs. +139.2 g) in comparison to the saline control group. Likewise, at 900 mg/kg buprenorphine the lower mean gestation body weights correlated with statistically lower mean gestation body weight gain from GD 7-10 (-12.6 g vs. +0.1 g), GD 13-20 (+37.0 g vs. +80.2 g), GD 7-20 (+46.7 g vs. +105.9 g), and GD 0-20 (+75.0 g vs. +139.2 g) in comparison to the saline control group. These two (2) groups have a similar dose volume of Atrigel at 3 times their respective low-dose group and 1/3 more than their respective mid-dose group.

Mean gestation body weight and body weight gain at low- and mid-ATRIGEL® control and at 300 and 600 mg/kg buprenorphine were generally comparable to the saline control group and unaffected by treatment. Mean gestation body weight and body weight gain in the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) were generally comparable to their respective ATRIGEL® control group (low, mid, or high) which were comparable to the saline control for the low- and mid-dose groups.

Summary of Gestation Body Weight Values

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Body Weight Values													
g													
	0	261.6	20.91	21	244.7	17.97	22	261.8	26.66	19	258.0	18.78	21
	4	284.8	21.50	21	271.6	17.11	22	283.6	22.60	20	282.3	22.06	21
	7	294.9	21.69	21	282.3	15.92	22	294.3	19.60	20	294.5	22.81	21
	10	295.0	21.53	21	278.8	20.85	22	286.4	19.70	20	285.9[b]	19.72	21
	13	320.6	25.36	21	306.2	19.44	22	316.4	22.78	20	300.5	25.37	21
	20	400.8	46.10	21	383.2	38.52	22	401.9	28.65	20	346.7[b]	41.40	21

Summary of Gestation Body Weight Values

Endpoint	Study Interval (Day)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Body Weight Values										
g										
	0	256.2	17.50	20	259.5	25.19	22	255.1	23.06	21
	4	278.7	16.31	20	282.4	23.19	22	275.7	22.42	21
	7	289.4	14.66	20	289.3	22.95	22	283.4	21.02	21
	10	286.0	16.01	20	280.2	23.60	22	270.8[b]	20.06	21
	13	314.4	16.87	20	312.5	24.24	22	293.2[b]	21.27	21
	20	389.9	37.01	20	393.0	33.97	22	330.1[b]	41.76	21

Summary of Gestation Body Weight Change Values

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Body Weight Change Values													
g													
	0-4	23.2	6.59	21	26.9	8.60	22	21.8	9.13	19	24.3	7.61	21
	4-7	10.1	3.87	21	10.7	5.28	22	10.8	7.55	20	12.1	6.95	21
	7-10	0.1	8.50	21	-3.5	13.03	22	-8.0[a]	9.37	20	-28.6[b]	11.64	21
	10-13	25.6	9.49	21	27.4	10.14	22	30.1	9.47	20	34.7[a]	8.92	21
	13-20	80.2	26.39	21	77.0	26.20	22	85.5	11.73	20	46.2[b]	27.43	21
	0-7	33.3	8.38	21	37.6	8.85	22	32.2	12.16	19	36.4	8.44	21
	7-20	105.9	29.08	21	100.9	30.15	22	107.6	15.39	20	52.2[b]	32.77	21
	0-20	139.2	33.43	21	138.5	30.71	22	139.7	15.22	19	88.7[b]	31.88	21

Summary of Gestation Body Weight Change Values

Endpoint	Study Interval (Day)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Body Weight Change Values g										
	0-4	22.5	9.51	20	22.9	7.24	22	20.6	8.56	21
	4-7	10.7	6.10	20	7.0	6.28	22	7.8	5.65	21
	7-10	-3.4	10.19	20	-9.1	13.67	22	-12.6[bJ]	9.55	21
	10-13	28.4	8.96	20	32.3	12.05	22	22.4[J]	9.83	21
	13-20	75.5	26.06	20	80.5	16.60	22	37.0[b]	27.16	21
	0-7	33.2	10.62	20	29.8	8.09	22	28.3[J]	7.64	21
	7-20	100.5	28.43	20	103.7	18.28	22	46.7[b]	35.16	21
	0-20	133.7	25.71	20	133.5	17.52	22	75.0[b]	35.57	21

Food and Water Consumption – Treated Females

All animals were observed for the availability of food and water twice daily. Food consumption was recorded weekly for all animals prior to pairing for mating. During the pairing period, food consumption was not recorded for any animals. Any males that completed mating were placed back on measured food consumption weekly until euthanasia. Food consumption was recorded on the corresponding gestation body weight days for mated females (treated and untreated).

Female - Mean premating food consumption in the Atrigel control groups (low, mid, and high) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) were generally comparable to the saline control group and the respective Atrigel control groups. At 900 mg/kg buprenorphine, mean food consumption was statistically lower during Week 1 (-8%) and Week 2 (-10%) in comparison to the saline control group and the high-Atrigel control group, respectively. While likely treatment related, these differences in mean food consumption were slight in magnitude and were not considered of toxicological concern.

Summary of Premating Food Consumption Values - FEMALE

Endpoint	Study Interval (Week)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Food Consumption g/animal/day													
	1	23.27	2.047	23	21.94	2.101	23	23.24	3.070	23	21.24	2.791	23
	2	20.80	2.586	23	19.83	1.957	23	21.99	2.185	23	22.73	2.278	23

Summary of Premating Food Consumption Values - FEMALE

Endpoint	Study Interval (Week)	300 mg/kg Mean	300 mg/kg SD	300 mg/kg N	600 mg/kg Mean	600 mg/kg SD	600 mg/kg N	900 mg/kg Mean	900 mg/kg SD	900 mg/kg N
Food Consumption g/animal/day										
	1	23.43	2.619	23	22.11	2.207	23	21.32[3]	2.259	23
	2	20.71	1.828	23	20.86	2.146	23	20.53[]	2.025	23

Gestation – Treated Females

Mean gestation food consumption in the low- and mid-ATRIGEL® control groups and at 300 and 600 mg/kg buprenorphine were comparable to the saline control group. At 900 mg/kg buprenorphine, mean gestation food consumption was statistically lower during GD 0-4 (-10%), GD 4-7 (-10%), GD 13-20 (-11%), GD 0-7 (-10%), GD 7-20 (-19%), and GD 0-20 (-16%) in comparison to the saline control group. Similarly, when the 900 mg/kg buprenorphine administered group was compared to the high-ATRIGEL® control group, statistically lower mean food consumption was observed during GD 0-4 (-14%), GD 4-7 (-13%), and GD 0-7 (-13%). These differences in mean food consumption at 900 mg/kg buprenorphine when compared to the saline and the high-ATRIGEL® control groups were considered treatment-related and adverse.

A trend in lower mean gestation food consumption was also noted in the high-ATRIGEL® control group when compared to the saline control group beginning at approximately GD 7. Mean gestation food consumption in the high-ATRIGEL® control group was 45%, 12%, 11%, 22% (statistically significant), and 13% lower during GD 7-10, 10-13, 13-20, 7-20, and 0-20, respectively, in comparison to the saline control group. These differences in mean gestation food consumption were considered treatment-related and adverse.

Summary of Gestation Food Consumption Values

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Food Consumption g/animal/day													
	0-4	28.6	2.76	21	28.1	2.05	22	28.5	2.39	19	29.8	2.96	21
	4-7	30.4	3.43	21	29.8	2.58	22	28.7	6.88	20	31.6	2.75	21
	7-10	37.1	29.43	21	27.4	5.95	22	26.0	4.29	18	20.3	5.36	21
	10-13	28.9	2.92	20	27.9	2.00	22	29.4	3.54	20	25.3	10.03	21
	13-20	30.1	3.32	21	29.3	3.28	22	30.5	2.27	20	26.7	7.63	21
	0-7	29.4	2.74	21	28.8	2.12	22	28.6	3.83	19	30.6	2.72	21
	7-20	31.9	9.33	21	28.5	2.64	22	29.3	2.00	20	24.9[a]	6.79	21
	0-20	30.9	5.81	21	28.6	2.18	22	29.0	2.30	19	26.9	4.55	21

Summary of Gestation Food Consumption Values

Endpoint	Study Interval (Day)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Food Consumption g/animal/day										
	0-4	28.3	2.81	20	28.4	3.06	22	25.7[b,j]	2.61	21
	4-7	29.1	2.78	20	28.6	3.64	22	27.5[a,j]	2.82	21
	7-10	26.8	4.89	20	26.1	4.44	22	23.2	4.10	21
	10-13	27.5	2.50	20	27.4	2.54	22	26.3	3.58	21
	13-20	27.9	2.59	20	27.9[h]	2.43	22	26.7[a]	3.72	21
	0-7	27.5	2.62	20	27.4	3.13	22	26.5[a,j]	2.48	21
	7-20	27.5	1.89	20	27.4[g]	2.12	22	25.8[a]	3.40	21
	0-20	27.5	2.02	20	27.4	2.30	22	26.0[a]	2.96	21

Mean gestation food consumption in the untreated females was generally comparable across the groups.

Estrous Cycle Determination – Treated Females

At the initiation of dose administration and until evidence of copulation was observed or the cohabitation period ended, treated females were examined daily by vaginal lavage to establish estrous cyclicity. Untreated females were examined daily during the mating period until evidence of copulations was observed (no cyclicity reported).

Mean estrous cycle length (days) was statistically longer (7.9 days versus 4.6 days) with a correlating statistically reduced mean number of cycles (1.4 cycles versus 2.3 cycles) in the high-ATRIGEL® control group and outside recent historical control data

(reference contained in report) in comparison to the saline control group and was considered treatment-related and adverse.

Estrous cyclicity (mean number of days and mean number of cycles) was unaffected by treatment in the ATRIGEL® control groups (low and mid) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) when compared to the saline control group.

Summary of Premating Estrous Cycling

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Mean Cycle Length (Days)	4.6	0.76	21	4.5	0.79	23	5.8	3.05	20	7.9[b]	3.62	21
No. of Cycles (Count)	2.3	0.64	21	2.5	0.67	23	2.2	0.77	20	1.4[b]	0.75	21
Animals with No Cycles			2			0			3			2

Summary of Premating Estrous Cycling

	300 mg/kg			600 mg/kg			900 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Mean Cycle Length (Days)	4.5	0.98	23	4.7	0.52	23	4.6[h]	0.71	22
No. of Cycles (Count)	2.6	0.66	23	2.3	0.56	23	2.4[h]	0.66	22
Animals with No Cycles			0			0			1

Breeding Procedures and Reproduction/Fertility Results – Treated Females

On Day 15 for the treated females (dosed on Day 1), each female was housed in the cage of a naïve male from the corresponding treatment group. The day on which positive evidence of copulation was observed was considered GD 0 (dosing on GD 7). After evidence of mating was observed, the female was returned to an individual cage for the remainder of the study. If mating was not confirmed during the 14-day mating period, the original male remained on study and the treated female was paired with a second male that successfully mated with its female for an additional 7 days. The maximum pairing period was 21 days, at the end of which any females with no confirmed evidence of mating were returned to individual cages until scheduled euthanasia.

There were no adverse effects on female mating, fertility and fecundity indices by RBP-6000 or the Atrigel vehicle at the doses tested. Female mating, fertility, and fecundity indices ranged from 91.3% to 100% in the Atrigel control groups (low, mid, and high) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) and

were comparable to the saline control group (95.7% to 100%) and each of the respective Atrigel control groups. In addition, the mean copulatory interval (days to mating) was comparable and within statistical margins across all groups (Atrigel control groups and RBP-6000 administered groups) and ranged from 2.0 to 4.4 in comparison to the saline control group at 3.8 days.

Summary of Reproductive and Fertility Parameters

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Females on Study		23	23	23	23
No. Females Paired		23	23	23	23
No. Females Mated		23	23	23	23
No. Pregnant		22	23	22	22
Female Mating Index (%)		100.0	100.0	100.0	100.0
Female Fertility Index (%)		95.7	100.0	95.7	95.7
Female Fecundity Index (%)		95.7	100.0	95.7	95.7
Females with Confirmed Mating Day		22	22	21	22
Copulatory Interval (Days)					
	Mean	3.8	2.0	2.5	2.3
	SD	3.37	1.05	1.60	1.08
	N	22	22	21	22

Summary of Reproductive and Fertility Parameters

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
No. Females on Study		23	23	23
No. Females Paired		23	23	23
No. Females Mated		23	23	23
No. Pregnant		21	22	22
Female Mating Index (%)		100.0	100.0	100.0
Female Fertility Index (%)		91.3	95.7	95.7
Female Fecundity Index (%)		91.3	95.7	95.7
Females with Confirmed Mating Day		22	23	22
Copulatory Interval (Days)				
	Mean	2.6	4.4	3.4
	SD	2.42	4.61	3.70
	N	22	23	22

Disposition of Females with No Evidence of Mating and Males

Females - All females with no confirmed mating date that appeared to be non-pregnant on the basis of body weight and shape were euthanized 13 days after the last scheduled pairing day and examined as described in Postmortem Study Evaluations. Females that appeared to be pregnant on the basis of body weight and shape were euthanized as identified to prevent delivery in the cage and loss of the litter. Uterine and ovarian examinations were conducted as described and reported in Postmortem Study Evaluations section.

Males - Upon completion of most of the GD 13 uterine examinations after the second cohabitation with the untreated females, all surviving treated males were observed externally, euthanized, and subjected to a necropsy and sperm analysis as described and reported in Postmortem Study Evaluations section.

Toxicokinetics

Blood samples were collected from the last three surviving treated animals per group for determination of the plasma concentrations of buprenorphine and norbuprenorphine. Samples were collected from treated females pre-dose and at 2, 12, 24, 72, 168, and 336 (Day 1 only) hours post-dose on Day 1 and GD 7, and prior to euthanasia on GD 20. Samples were collected from treated males pre-dose and at 2, 12, 24, 72, 168, 336, and 504 hours post-dose on Days 1 and 56, pre-dose on Day 28, and prior to euthanasia on Day 106. The animals were not fasted prior to blood collection.

Treated females

Exposure, as assessed by buprenorphine mean C_{max}, $AUC_{0\text{-}24h}$ (1 day), and $AUC_{0\text{-}168h}$ (7 days) values for females, increased with the increase in dose level from 300 to 900 mg/kg (see table). The increases in mean C_{max} and $AUC_{0\text{-}24h}$ for Days 1 versus 7 were generally dose proportional (AR). The increases in mean $AUC_{0\text{-}168h}$ values were greater than dose proportional suggesting possible accumulation of buprenorphine.

Summary of Mean Buprenorphine C_{max}, $AUC_{0\text{-}24}$, and $AUC_{0\text{-}168}$ in Female Rat Plasma

Interval	Dose Group	Dose Level (mg/kg)	C_{max} (ng/mL)	$AUC_{0\text{-}24}$ (ng·hr/mL)	$AUC_{0\text{-}168}$ (ng·hr/mL)	AR		
						C_{max}	$AUC_{0\text{-}24}$	$AUC_{0\text{-}168}$
Day 1	5	300	266	2660	7050	NA	NA	NA
	6	600	394	3960	10600	NA	NA	NA
	7	900	526	6940	15700	NA	NA	NA
GD 7	5	300	217	2720	11300	0.847	1.09	1.69
	6	600	377	4430	17000	0.971	1.12	1.61
	7	900	587	6710	22600	1.11	0.956	1.42

AR – Accumulation ratio; NA – Not applicable

Treated Males

Exposure, as assessed by buprenorphine mean C_{max}, AUC_{0-24h} (1 day), and AUC_{0-168h} (7 days), increased with the increase in dose level from 300 to 900 mg/kg. The increases in mean C_{max} was generally dose proportional (AR). The AUC_{0-24h}, and AUC_{0-168} values were greater than dose proportional (AR), more so for AUC_{0-168h}. Accumulation of buprenorphine was observed after three doses regarding the AUCs but not C_{max}.

Summary of Mean Buprenorphine C_{max}, AUC_{0-24}, and AUC_{0-168} in Male Rat Plasma

Interval	Dose Group	Dose Level (mg/kg)	C_{max} (ng/mL)	AUC_{0-24} (ng·hr/mL)	AUC_{0-168} (ng·hr/mL)	AR		
						C_{max}	AUC_{0-24}	AUC_{0-168}
Day 1	12	300	310	3420	9150	NA	NA	NA
	13	600	634	7310	16500	NA	NA	NA
	14	900	948	11200	28500	NA	NA	NA
Day 56	12	300	475	5690	23000	1.54	1.84	2.42
	13	600	571	10100	37600	1.01	1.53	2.46
	14	900	845	14200	57200	0.852	1.66	2.32

AR – Accumulation ratio; NA – Not applicable

Dosing Solution Analysis

Fresh control articles, 0.9% Sodium Chloride for Injection, USP and Atrigel placebo, were dispensed for use on study prior to each dose and were stored refrigerated at 2 to 8°C. The test article, RBP-6000 (18% buprenorphine base in ATRIGEL® Delivery System), was used as received from the Applicant. The test article was administered neat (undiluted). Formulations were dispensed prior to each dose and were stored refrigerated at 2 to 8°C.

Post Mortem Study Evaluations/Necropsy – Treated Females

Macroscopic

Necropsy examinations were performed under procedures approved by a veterinary pathologist on all animals. Collection of gross lesions from treated animals necessitated collection of sufficient corresponding tissues from control animals for comparison purposes.

Maternal necropsy findings appeared to correlate with the clinical observations related to the injection site(s). These findings in the ATRIGEL® control groups (low, mid, and high) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) included abrasions/scabs, skin discoloration, and foreign material (test material

implant). The few other macroscopic findings observed were infrequent and considered unrelated to treatment.

Organ Weights

Protocol-specified tissues and organs from all animals were removed, examined, weighed, and fixed for possible histopathological examination. For males, body, testes, epididymides, seminal vesicles with coagulating glands, and prostate weights for males were recorded and organ/body weight ratios were calculated. The left and right testes and epididymides were weighed separately, and a combined weight was analyzed. The right testis and epididymis were utilized for sperm analysis, while the left testis and epididymis were fixed for possible histopathological examination. For females, body, gravid uterus, and ovary (combined) weights (treated and untreated) were recorded.

Organs to be Weighed and Tissues to be Preserved: All Parental Animals

Tissue	Organ Weight Taken	Collected and Preserved
Epididymis, left (fixed in Bouin's fixative)	X	X
Epididymis, right	X	
Ovaries	X	X
Prostate	X	X
Seminal vesicles with coagulating glands	X	X
Testis, left (fixed in Bouin's fixative)	X	X
Testis, right	X	
Uterus (both horns) with cervix	X	X
Vagina		X
Gross lesions		X
Identified target organs[a]		X
[a]Potential target organs (and potential target organ gross lesions) will be designated by the Study Director, Pathologist, and/or Sponsor based on experimental findings.		

Mean terminal body weight and mean uterus w/cervix weight were statistically lower in the high-ATRIGEL® control group (-14% and -62%, respectively) and at 900 mg/kg buprenorphine (-18% and -68%, respectively) in comparison to the saline control and considered treatment related and toxicologically relevant (see Tables). Mean terminal body weight and mean uterus w/cervix weight in the low- and mid- ATRIGEL® control groups and at 300 and 600 mg/kg buprenorphine were comparable to the saline control group and unaffected by treatment. Mean ovary weights were generally comparable across the groups and unaffected by treatment. A slight yet statistically significant decrease (-13%) in mean ovary weight was observed at 900 mg/kg buprenorphine in comparison to the saline control group and may have been attributed to the lower body weights observed in this group according to the report.

Summary of Organ Weight Values - FEMALE
Terminal

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight												
g	396	46	21	378	39	22	397	29	20	340[b]	43	20
Ovaries												
g	0.136	0.020	21	0.133	0.018	22	0.139	0.017	20	0.121	0.018	21
Uterus w/ cervix												
g	87.467	28.912	21	81.665	21.479	22	88.215	12.720	20	33.235[b]	29.725	21

Summary of Organ Weight Values - FEMALE
Terminal

	300 mg/kg			600 mg/kg			900 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight									
g	388	36	20	390	34	22	325[b]	42	21
Ovaries									
g	0.142	0.021	20	0.144	0.025	22	0.119[a]	0.012	21
Uterus w/ cervix									
g	83.169	26.953	20	84.799	19.014	22	28.270[b]	25.483	21

N - Number of measures used to calculate mean
SD - Standard Deviation
a - Significantly different from 0 mg/kg (Saline); ($p<0.05$)
b - Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Uterine and Ovarian Examinations – Treated Females

Dams - On GD 20, each treated female was euthanized. The skin was reflected from a ventral midline incision to examine mammary tissue and locate any subcutaneous masses. The uterus was excised, and the gravid uterine weight was recorded. The location of viable and nonviable fetuses, early and late resorptions for each uterine horn, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Uteri from females that appeared nongravid were opened examined for detection of implantation sites. The foci, if detected, were considered early resorptions, and data from this female were included in mean calculations. If no foci were detected, the female was considered to be nonpregnant.

Fetuses - Each implant was categorized according to the following criteria. Viable fetuses responded to touch. Nonviable fetuses did not respond to touch and had no signs of autolysis. Late resorptions were characterized by recognizable fetal form, but undergoing autolysis. Early resorptions were characterized as implantation sites that had no recognizable fetal characteristics. The fetuses were removed by making a dorsal incision longitudinally along both uterine horns. The embryonic membrane of each fetus was gently removed, and each fetus was pulled away from the placenta, fully extending the umbilical cord. The placentae were examined grossly.

There were a number of adverse effects observed in the uterine examination that were mostly observed in the high-dose Atrigel group and high-dose RBP-6000 group. In summary, these included higher incidence of females with all resorptions and lower number of females with viable fetuses at Day 20, a lower number of viable fetuses per animal, lower litter size per animal, and higher post-implantation loss.

The pregnancy index was 100% in the low-ATRIGEL® control group, 95.7% in the mid- and high-Atrigel control groups and at 600 and 900 mg/kg buprenorphine, 91.3% at 300 mg/kg buprenorphine and comparable to the saline control group at 95.7% (see Table below). There was one non-pregnant animal in the saline control, mid- and high-Atrigel control, 600 mg/kg, and 900 mg/kg buprenorphine groups and two non-pregnant in the 300 mg/kg buprenorphine group. There were two females in the mid-Atrigel control group and one female in saline control group, low-Atrigel control group, high-ATRIGEL® control group, 300 mg/kg, and 900 mg/kg buprenorphine groups with undetected matings (pregnant with no confirmed mating date). The saline control group and the low-Atrigel control group had one female each with all resorbed fetuses.

A higher incidence of females with all resorbed fetuses was observed in the high-Atrigel control group (5 females) and at 900 mg/kg buprenorphine (7 females with all resorptions. These pregnancy outcomes in the high-Atrigel control group and at 900 mg/kg buprenorphine were considered treatment-related and adverse. The number of females for evaluation (viable fetuses on Day 20 gestation), were comparable for all groups except the high-Atrigel control group and the 900 mg/kg buprenorphine group which were reduced.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Females on Study	23	23	23	23
No. Not Pregnant	1	0	1	1
No. Pregnant	22	23	22	22
Pregnancy Index Percent	95.7	100.0	95.7	95.7
No. Females with All Resorptions	1	1	0	5
No. Females Pregnant by Stain	0	0	0	0
No. Females with Viable Fetuses Day 20 Gestation	20	21	20	16
No. Pregnant Females with No Confirmed Mating Date	1	1	2	1

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	300 mg/kg	600 mg/kg	900 mg/kg
No. Females on Study	23	23	23
No. Not Pregnant	2	1	1
No. Pregnant	21	22	22
Pregnancy Index Percent	91.3	95.7	95.7
No. Females with All Resorptions	0	0	5
No. Females Pregnant by Stain	0	0	2
No. Females with Viable Fetuses Day 20 Gestation	20	22	14
No. Pregnant Females with No Confirmed Mating Date	1	0	1

Mean number of corpora lutea, mean number of implantation sites, and preimplantation loss in the high-Atrigel control group and at 900 mg/kg buprenorphine were unaffected and comparable to the saline control group. Uterine parameters (mean number of corpora lutea, mean number of implantation sites, preimplantation loss, mean number of viable fetuses/litter size, and fetal sex ratios) in the low and mid-Atrigel control groups and at 300 and 600 mg/kg buprenorphine were unaffected and comparable to the saline control group. All uterine parameters in the RBP-6000 administered groups were comparable to their respective ATRIGEL® control group. The number viable fetuses on Day 20 gestation, were comparable for all groups except the high-Atrigel control group and the 900 mg/kg buprenorphine group which were reduced by ~2/3 and considered Atrigel-related as the values in those two (2) groups were comparable.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Corpora Lutea					
No. per Animal	Mean	18.6	18.0	18.6	18.1
	SD	2.61	2.37	2.48	2.84
	N	20	21	20	16
Implantation Sites					
No. per Animal	Mean	15.6	14.8	17.1	16.8
	SD	4.81	3.46	2.68	2.44
	N	21	22	20	21
Preimplantation Loss					
% per Animal	Mean	12.31	13.19	8.15	7.01
	SD	17.384	13.726	11.029	9.274
	N	20	21	20	16
Viable Fetuses					
No. per Animal	Mean	14.5	13.4	15.2	5.4[b]
	SD	5.33	3.67	2.18	5.54
	N	21	22	20	21
Fetal Sex Ratio					
% Males per Animal	Mean	48.7	49.0	49.6	50.6
	SD	15.32	11.40	11.69	25.12
	N	20	21	20	16

b – Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
Corpora Lutea				
No. per Animal	Mean	19.2	18.7	17.5
	SD	3.54	2.85	3.13
	N	20	22	14
Implantation Sites				
No. per Animal	Mean	14.5	15.5	14.3
	SD	4.73	3.57	5.04
	N	20	22	21
Preimplantation Loss				
% per Animal	Mean	24.49	16.73	18.21
	SD	25.398	18.627	25.375
	N	20	22	14
Viable Fetuses				
No. per Animal	Mean	13.7	13.9	4.5[b]
	SD	4.75	3.64	4.29
	N	20	22	21
Fetal Sex Ratio				
% Males per Animal	Mean	48.1	55.9	43.5
	SD	21.93	13.41	28.51
	N	20	22	14

Mean post-implantation loss was statistically higher (67.65% and 66.35%) in the high-Atrigel control group and at 900 mg/kg buprenorphine, respectively, and correlated with statistically higher mean number of resorptions (early plus late) of 11.4 per litter in the high-Atrigel control group and 9.8 per litter at 900 mg/kg buprenorphine. As a result, mean number of viable fetuses/litter size was statistically lower (5.4 per litter and 4.5 per

litter) in the high-ATRIGEL® control group and at 900 mg/kg buprenorphine, respectively. The adverse effects on these uterine parameters in the high-Atrigel control group and at 900 mg/kg buprenorphine were considered treatment-related.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Postimplantation Loss					
% per Animal	Mean	11.24	13.05	10.41	67.65[b]
	SD	21.359	22.099	8.940	32.411
	N	21	22	20	21
Nonviable Fetuses					
No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.21	0.00	0.00
	N	21	22	20	21
Litter Size					
No. per Animal	Mean	14.5	13.4	15.2	5.4[b]
	SD	5.33	3.69	2.18	5.54
	N	21	22	20	21
Resorptions: Early + Late					
No. per Animal	Mean	1.0	1.4	1.9	11.4[b]
	SD	0.97	1.74	1.74	5.77
	N	21	22	20	21

b – Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
Postimplantation Loss				
% per Animal	Mean	4.95	10.17	66.35[b]
	SD	8.829	9.787	31.657
	N	20	22	21
Nonviable Fetuses				
No. per Animal	Mean	0.0	0.0	0.0
	SD	0.00	0.00	0.00
	N	20	22	21
Litter Size				
No. per Animal	Mean	13.7	13.9	4.5[b]
	SD	4.75	3.64	4.29
	N	20	22	21
Resorptions: Early + Late				
No. per Animal	Mean	0.8	1.5	9.8[b]
	SD	1.29	1.53	6.20
	N	20	22	21

The increased resorptions were clearly early resorptions in the high-Atrigel control group and at 900 mg/kg buprenorphine,

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Resorptions: Early					
No. per Animal	Mean	1.0	1.4	1.9	11.1[b]
	SD	0.89	1.74	1.74	5.78
	N	21	22	20	21
Resorptions: Late					
No. per Animal	Mean	0.0	0.0	0.0	0.2
	SD	0.22	0.00	0.00	0.44
	N	21	22	20	21

b – Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
Resorptions: Early				
No. per Animal	Mean	0.8	1.5	9.5[b]
	SD	1.29	1.47	6.28
	N	20	22	21
Resorptions: Late				
No. per Animal	Mean	0.0	0.0	0.3
	SD	0.00	0.21	0.56
	N	20	22	21

Mean gravid uterine weight and mean final body weight were statistically significantly lower in the high-Atrigel control group (-54% and -12%, respectively) and at 900 mg/kg buprenorphine (-54% and -13%, respectively) in comparison to the saline control group. These lower weights correlated with the higher mean number of resorptions reported previously and the lower mean fetal body weights, which will be reported later, observed in the high-Atrigel control group and at 900 mg/kg buprenorphine. Mean adjusted final body weight (body weight minus gravid uterine weight) and mean adjusted weight change (GD 0-20) in the high-Atrigel control group and at 900 mg/kg buprenorphine were comparable to the saline control group. Mean gravid uterine weight, mean final body weight, mean adjusted final body weight, and mean adjusted body weight change (GD 0-20) in the low and mid-Atrigel control groups and at 300 and 600 mg/kg buprenorphine were comparable to the saline control group and unaffected by treatment. Mean gravid uterine weight, mean final body weight, mean adjusted final body weight, and mean adjusted body weight change (GD 0-20) in the RBP-6000 administered groups were comparable to their respective Atrigel control group suggesting that any adverse effects were primarily Atrigel-related.

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, g	91.6	22.34	20	85.5	11.81	21	88.2	12.72	20	42.2[b]	28.58	16
Final Body Weight, g	407.5	35.31	20	390.0	21.99	21	401.9	28.65	20	357.7[b]	38.41	16
Adjusted Final Body Weight, g	315.9	22.52	20	304.5	19.95	21	313.7	22.61	20	315.5	27.16	16
Adjusted Weight Change from Day 0, g	52.3	11.35	20	58.7	10.33	21	52.2	11.99	19	56.9	12.79	16

b – Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values

	300 mg/kg			600 mg/kg			900 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, g	83.2	26.95	20	84.8	19.01	22	42.0[b]	19.68	14
Final Body Weight, g	389.9	37.01	20	393.0	33.97	22	354.7[b]	22.29	14
Adjusted Final Body Weight, g	306.7	17.24	20	308.2	24.11	22	312.7	25.30	14
Adjusted Weight Change from Day 0, g	50.5	14.64	20	48.7	8.44	22	54.0	9.85	14

Fetal Examinations – Treated Females

Each fetus was individually weighed, sexed, tagged, and examined for external malformations and variations. Fetuses were then euthanized and approximately one-half of the fetuses in each litter were fixed for soft tissue defects using the Wilson razor-blade sectioning technique or fixed by a method similar to that described by Dawson for subsequent skeletal examination. Fetal findings were classified as malformations or developmental variations.

Fetal Sex Ratio

There were no vehicle- or RBP-6000-related effects on fetal sex ratio. As reported previously and contained in previous tables, mean fetal sex ratio (% males per litter) ranged from 43.5% to 55.9% in the Atrigel control groups (low, mid, and high) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) and were comparable to 48.7% in the saline control group and the respective Atrigel control group.

Fetal Body Weights

There were vehicle- and RBP-6000-related effects on fetal body weights. Mean fetal body weights (male, female, and sexes combined) were statistically significantly lower in the high-Atrigel control group and at 900 mg/kg buprenorphine in comparison to the saline control group. Mean fetal body weights (sexes combined) were 16% and 13% statistically significantly lower in the high-Atrigel control group and at 900 mg/kg buprenorphine, respectively, and these differences were considered treatment related and likely related to the larger volume dose of Atrigel. Mean fetal body weights (male, female, and sexes combined) in the low and mid-Atrigel control groups and at 300 and 600 mg/kg buprenorphine were comparable to the saline control group and unaffected by treatment.

Summary of Fetal Body Weight Values, g

Endpoint			0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Fetal Weight						
	Males	Mean	4.28 (4.45)	4.30 (4.35)	4.08 (4.20)	3.70 (3.36)[b]
		SD	0.566	0.443	0.439	0.235
		N	19	21	20	14
	Females	Mean	4.01 (4.09)	4.00 (4.04)	3.78 (3.87)	3.32 (3.12)[b]
		SD	0.587	0.373	0.395	0.454
		N	20	21	20	15
	Males + Females	Mean	4.15 (4.27)	4.14 (4.21)	3.94 (4.06)	3.50 (3.21)[b]
		SD	0.581	0.396	0.418	0.411
		N	20	21	20	16

b – Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Summary of Fetal Body Weight Values, g

Endpoint			300 mg/kg	600 mg/kg	900 mg/kg
Fetal Weight					
	Males	Mean	4.38 (4.45)	4.19 (4.24)	3.78 (3.42)[b]
		SD	0.768	0.436	0.596
		N	19	22	12
	Females	Mean	4.02 (4.07)	3.92 (3.96)	3.57 (3.35)[b]
		SD	0.548	0.471	0.654
		N	19	22	13
	Males + Females	Mean	4.28 (4.33)	4.08 (4.14)	3.60 (3.29)[b]
		SD	0.805	0.455	0.660
		N	20	22	14

External Fetal Examinations

A statistically higher litter incidence of **total** external malformations was observed in the high, 900 mg/kg buprenorphine group (64.3% of litters observed with a malformation) in comparison to 5% of litters in the saline control group. While not statistically significant, a dose-responsive increase in the incidence of total malformations was observed in low Atrigel control group (4.8%), the mid-Atrigel control group (20.0%), and high-Atrigel

control group (31.3%). A dose-response effect was also observed for the buprenorphine groups at 5.0, 18.2, and 64.3%, respectively. There appears that buprenorphine may be and additive component in the high-dose buprenorphine group to the Atrigel alone.

Summary of External Malformations and Developmental Variations

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Litters Evaluated	20	21	20	16
No. Fetuses Evaluated	305	294	303	114
Total Malformations				
No. Litters(%)	1 (5.0)	1 (4.8)	4 (20.0)	5 (31.3)
No. Fetuses(%)[1]	1 (0.3)	1 (0.3)	6 (2.0)	8 (7.0)
Total Variations				
No. Litters(%)	0 (0.0)	0 (0.0)	1 (5.0)	1 (6.3)
No. Fetuses(%)[1]	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.9)

Summary of External Malformations and Developmental Variations

Observation	300 mg/kg	600 mg/kg	900 mg/kg
No. Litters Evaluated	20	22	14
No. Fetuses Evaluated	274	306	94
Total Malformations			
No. Litters(%)	1 (5.0)	4 (18.2)	9 (64.3)[b]
No. Fetuses(%)[1]	1 (0.4)	5 (1.6)	13 (13.8)
Total Variations			
No. Litters(%)	0 (0.0)	0 (0.0)	3 (21.4)
No. Fetuses(%)[1]	0 (0.0)	0 (0.0)	3 (3.2)

1 - Not statistically analyzed
b - Significantly different from 0 mg/kg (Saline); ($p<0.01$)

It was reported that the individual external malformations observed were either seen at a higher incidence or have not been observed in historical control data (contained in report). Malformations of the head were predominately observed at 900 mg/kg buprenorphine, however a higher litter incidence for a few of the same malformations was observed in the mid-and high-Atrigel control groups and at 600 mg/kg buprenorphine. Entire edema in 1 fetus in 2 litters of high dose Atrigel and high dose buprenorphine is of unknown toxicological significance. Some of these external malformations were dose-responsive and were considered treatment related and toxicologically relevant (Table) as they were not observed in saline controls. No significant differences in incidence of external malformations or variations were

observed when comparing the RBP-6000 administered groups to their respective Atrigel control group which have the same Atrigel vehicle.

Summary of External Malformations (% of Litters Affected)							
Malformation Type	**Saline Control**	**Low-ATRIGEL® Control**	**Mid-ATRIGEL® Control**	**High-ATRIGEL® Control**	**300 mg/kg BUP**	**600 mg/kg BUP**	**900 mg/kg BUP**
Multiple craniofacial abnormality	0.0	0.0	0.0	0.0	0.0	0.0	21.4
Eyes, anophthalmia	0.0	0.0	0.0	12.5	5.0	9.1	21.4
Eyes, microphthalmia	0.0	0.0	5.0	6.3	0.0	4.5	14.3
Open eye	0.0	0.0	0.0	12.5	0.0	0.0	7.1
Jaw(s), agnathia	0.0	0.0	10.0	25.0	5.0	9.1	35.7
Jaw(s), micrognathia	0.0	0.0	0.0	6.3	0.0	4.5	21.4
Mouth, absent	0.0	0.0	0.0	12.5	5.0	4.5	21.4
Mouth, smaller than normal	0.0	0.0	10.0	12.5	0.0	9.1	14.3
Nares, absent	0.0	0.0	0.0	0.0	0.0	0.0	7.1
Nares, misshapen	0.0	0.0	0.0	18.8	5.0	4.5	14.3
Nares, proboscis	0.0	0.0	0.0	0.0	0.0	0.0	21.4
Papillae, absent	0.0	0.0	10.0	25.0	5.0	13.6	28.6
Pinna(e), malpositioned	0.0	0.0	10.0	25.0	5.0	9.1	35.7
Tongue, absent	0.0	0.0	5.0	18.8	5.0	4.5	28.6
BUP – buprenorphine							

Observations not included in the table were external malformations observed in the low-Atrigel control group and at 300 mg/kg buprenorphine that were either in a single fetus or were not dose-responsive and considered unrelated to treatment. The few external variations observed in the Atrigel control groups and/or the RBP-6000 administered groups were seen at a low incidence, comparable to the saline control group, or generally within historical control data (contained in report) and were considered spontaneous and unrelated to treatment.

Fetal Visceral Examinations

Overall, there appears to be dose-responsive increases in total malformations and total variations for both Atrigel and buprenorphine dose groups with the mid-doses being the LOAELS.

Summary of Visceral Malformations and Developmental Variations

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Litters Evaluated	20	21	20	15
No. Fetuses Evaluated	156	145	152	58
Total Malformations				
No. Litters(%)	1 (5.0)	0 (0.0)	2 (10.0)	5 (33.3)
No. Fetuses(%)[1]	1 (0.6)	0 (0.0)	2 (1.3)	6 (10.3)
Total Variations				
No. Litters(%)	0 (0.0)	2 (9.5)	5 (25.0)	5 (33.3)
No. Fetuses(%)[1]	0 (0.0)	2 (1.4)	10 (6.6)	6 (10.3)

1 – not statistically analyzed

Summary of Visceral Malformations and Developmental Variations

Observation	300 mg/kg	600 mg/kg	900 mg/kg
No. Litters Evaluated	19	22	13
No. Fetuses Evaluated	135	154	46
Total Malformations			
No. Litters(%)	0 (0.0)	1 (4.5)	5 (38.5)
No. Fetuses(%)[1]	0 (0.0)	1 (0.6)	9 (19.6)
Total Variations			
No. Litters(%)	2 (10.5)	2 (9.1)	3 (23.1)
No. Fetuses(%)[1]	2 (1.5)	2 (1.3)	4 (8.7)

Specifically, at 900 mg/kg buprenorphine, a higher litter incidence of the visceral malformation complete situs inversus of the thoracic cavity (38.5%) was observed in comparison to the saline control group (0.0%). While not statistically significant, a higher litter incidence of the visceral malformations complete situs inversus of the abdomen (30.8%), persistent truncus arteriosus (15.4%), and discontinuous interventricular septum (15.4%) were observed at 900 mg/kg buprenorphine in comparison to the saline control group (0.0%). Similarly, in the high-Atrigel control group a higher litter incidence of complete situs inversus of the thoracic cavity (13.3%) and complete situs inversus of the abdomen (13.3%) was observed in comparison to the saline control group (0.0%). These visceral malformations in the high-Atrigel control group and at 900 mg/kg buprenorphine were observed at a higher litter incidence in comparison to the saline control group and were either seen at a higher incidence or have not been observed in historical control data (contained in report) and were considered treatment related and toxicologically relevant.

Summary of Individual Fetal Visceral Observations

Observation	Classification	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Abdomen, Situs inversus complete	M				
No. Litters(%)		0 (0.0)	0 (0.0)	1 (5.0)	2 (13.3)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	1 (0.7)	3 (5.2)

Thoracic cavity, Situs inversus complete	M				
No. Litters(%)		0 (0.0)	0 (0.0)	1 (5.0)	2 (13.3)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	1 (0.7)	3 (5.2)
Interventricular septum, Discontinuous	M				
No. Litters(%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (1.7)
Heart - entire, Persistent truncus arteriosus	M				
No. Litters(%)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

No. - Number
M - Malformation
P - Pathological
V - Variation

1 - Not statistically analyzed

Observation	Classification	300 mg/kg	600 mg/kg	900 mg/kg
Abdomen, Situs inversus complete	M			
No. Litters(%)		0 (0.0)	0 (0.0)	4 (30.8)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	7 (15.2)
Thoracic cavity, Situs inversus complete	M			
No. Litters(%)		0 (0.0)	0 (0.0)	5 (38.5)[a]
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	8 (17.4)
Interventricular septum, Discontinuous	M			
No. Litters(%)		0 (0.0)	0 (0.0)	2 (15.4)
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	2 (4.3)
Heart - entire, Persistent truncus arteriosus	M			
No. Litters(%)		0 (0.0)	0 (0.0)	2 (15.4)(
No. Fetuses(%)[1]		0 (0.0)	0 (0.0)	2 (4.3)

A few other visceral malformations were observed in either the Atrigel control groups (low and mid) or the RBP-6000 administered groups (300 and 600 mg/kg buprenorphine), but were seen at a low incidence or within historical control data and considered spontaneous and unrelated to treatment. A few dissimilar visceral variations were observed in either the Atrigel control groups or the RBP-6000 administered groups, but were seen at a low incidence, were not dose responsive, or were observed at a similar incidence to historical control data and considered spontaneous and unrelated to treatment.

No significant differences in incidence of visceral malformations or variations were observed when comparing the RBP-6000 administered groups to their respective Atrigel control group.

Fetal Skeletal Examinations

A statistically significant (SS) higher litter incidence of total skeletal malformations was observed in the high-Atrigel (57.1%) control group and the 900 mg/kg buprenorphine (84.6%) group. Dose response effects were observed as the mid-Atrigel (25%) control groups and 600 mg/kg buprenorphine (36.4%) group incidences were greater than the saline control value of 10%. The higher incidence of these skeletal malformations in the mid- and high-Atrigel control groups and the 600 and 900 mg/kg buprenorphine treated groups was considered treatment related and toxicologically relevant.

No significant differences in incidence of skeletal malformations or variations were observed when comparing the RBP-6000 administered groups to their respective Atrigel control group although the incidence was 11.4% and ~27.6% higher in the mid- and high-dose buprenorphine compare their Atrigel counterparts suggesting a possible contribution of buprenorphine to the increased incidence.

Summary of Skeletal Malformations and Developmental Variations

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Litters Evaluated	20	21	20	14
No. Fetuses Evaluated	149	149	151	56
Total Malformations				
No. Litters(%)	2 (10.0)	0 (0.0)	5 (25.0)	8 (57.1)[a]
No. Fetuses(%)[1]	2 (1.3)	0 (0.0)	6 (4.0)	12 (21.4)
Total Variations				
No. Litters(%)	17 (85.0)	16 (76.2)	20 (100.0)	13 (92.9)
No. Fetuses(%)[1]	58 (38.9)	56 (37.6)	82 (54.3)	37 (66.1)

No. - Number
a - Significantly different from 0 mg/kg (Saline); ($p<0.05$)
1 - Not statistically analyzed

Summary of Skeletal Malformations and Developmental Variations

Observation	300 mg/kg	600 mg/kg	900 mg/kg
No. Litters Evaluated	20	22	13
No. Fetuses Evaluated	139	152	48
Total Malformations			
No. Litters(%)	1 (5.0)	8 (36.4)	11 (84.6)[b]
No. Fetuses(%)[1]	1 (0.7)	11 (7.2)	14 (29.2)
Total Variations			
No. Litters(%)	18 (90.0)	20 (90.9)	12 (92.3)
No. Fetuses(%)[1]	75 (54.0)	92 (60.5)	35 (72.9)

No. - Number
b - Significantly different from 0 mg/kg (Saline); ($p<0.01$)
1 - Not statistically analyzed

The higher incidence of total skeletal malformations was consistent with several individual skeletal malformations (predominately skull malformations) observed in these groups and were either seen at a higher incidence, occasionally SS higher or have not been observed in historical control data (contained in report). No skeletal malformations were observed in the low-Atrigel control group.

A SS higher litter incidence of rudimentary ribs (skeletal variation – not shown in a table) was observed in the high-Atrigel control group (71.4%), 600 mg/kg buprenorphine (68.2%), and 900 mg/kg buprenorphine (76.9%) in comparison to the saline control group (25%) and was slightly outside the historical control data of 60% (contained in report for 601 fetuses, 379 litters, 21% of fetuses, and 60% of litters). While considered treatment related, the magnitude of change was slight in comparison to historical control data, there was no impact on total variations observed, and this skeletal variation (rudimentary ribs) was considered not toxicologically relevant. The few other skeletal variations observed in the Atrigel control groups and/or RBP-6000 administered groups were seen at a low incidence, comparable to the saline control or generally within historical control data and were no considered toxicologically relevant.

Summary of Skeletal Malformations (% of Litters Affected)							
Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	300 mg/kg BUP	600 mg/kg BUP	900 mg/kg BUP
Neural arches fused (cervical vertebra)	0.0	0.0	5.0	21.4	0.0	9.1	46.2[a]
Alisphenoid misshapen	0.0	0.0	5.0	7.1	5.0	9.1	23.1
Basisphenoid misshapen	0.0	0.0	5.0	28.6	5.0	13.6	38.5[a]
Frontal bone misshapen	0.0	0.0	5.0	7.1	5.0	0.0	23.1
Mandible absent	0.0	0.0	5.0	21.4	5.0	9.1	38.5[a]
Maxilla misshapen	0.0	0.0	0.0	7.1	5.0	4.5	30.8
Nasal bone fused	0.0	0.0	0.0	14.3	5.0	4.5	23.1
Nasal bone misshapen	0.0	0.0	0.0	0.0	0.0	0.0	15.4
Palatine misshapen	0.0	0.0	10.0	28.6	5.0	13.6	38.5[a]
Premaxilla misshapen	0.0	0.0	0.0	7.1	5.0	0.0	30.8
Pterygoid process misshapen	0.0	0.0	5.0	35.7	5.0	13.6	30.8
Squamosal misshapen	0.0	0.0	5.0	35.7[a]	5.0	9.1	38.5[a]
Tympanic ring fused	0.0	0.0	0.0	14.3	5.0	4.5	15.4
Tympanic ring malpositioned	0.0	0.0	5.0	7.1	0.0	9.1	23.1
Tympanic ring misshapen	0.0	0.0	0.0	21.4	5.0	4.5	23.1

[a]Statistically significant when compared to the saline control group
BUP – buprenorphine

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In-life Examinations – Treated Males and Untreated Females

[refer to duplicate methods in the previous sections for those same sections that follow as appropriate]

Mortality

All treated males in the ATRIGEL® control (low, mid, and high) survived to the scheduled necropsy. All untreated females survived to scheduled necropsy.

In treated males, treatment-related mortality/moribundity was observed at 900 mg/kg buprenorphine. A total of 7 of 23 treated males were either euthanized due to

moribundity (2) or were found dead (5) during the study between Days 40 and 60. Several of these animals exhibited one or more of the following clinical signs: reduced activity, dehydration, hunched posture, cold to the touch, thin body condition, red material around the eyes, nose, and/or mouth, salivation, difficult breathing, red urine, tremors, and vocalization. At necropsy, a definitive cause of moribundity/mortality could not be determined, however, five of the seven animals (4 found dead and 1 euthanized) were noted with macroscopic findings of black foci on the stomach. Other animals were noted with either a small thymus (2 found dead and 1 euthanized) or red foci on the thymus (2 found dead). One animal (euthanized) had bilateral dilation and enlargement of the kidneys, tan foci on the kidneys, red discolored spleen and thymus, swollen ureters and bladder (red fluid in the bladder and red/black foci on the bladder). Depletion of body fat was also noted in two animals (1 found dead and 1 euthanized) and correlated with the lower food consumption observed in this group.

Two animals, one low dose and one high dose buprenorphine were euthanized on Day 81 and 52, respectively, either due to complications at the dosing site (purulent discharge or loss of the drug depot respectively. The mortality of these animals was not considered treatment-related. One mid-dose animal was euthanized due to morbundity on Day 33. Clinical observations for this animal included difficult breathing, red material around the eyes and nose, hypersensitive to touch, hunched posture, cold to touch, and thin body condition. Macroscopic findings at necropsy included body fat depletion and swollen urinary bladder with calculi present. Furthermore, two low dose buprenorphine animals were found dead on Days 11 and 18, respectively. Macroscopic findings for one of the animals included enlarged kidneys and swollen bladder and calculi present in the ureters, kidneys and bladder. Macroscopic findings for the other animal included red foci on the stomach, swollen ureters, and red fluid in the bladder and ureters.

The moribundity/mortality observed at 300 and 600 mg/kg buprenorphine did not appear dose-responsive and the macroscopic findings may suggest possible urinary tract obstruction or hemorrhage of the urinary tract and were therefore not considered treatment-related by the report.

Detailed clinical signs

General - Swelling of the face, salivation, lacrimation, and material around the eyes and/or nose (black and/or red) in treated males (up to 22 animals) was observed at all dose levels during the treatment period for males). These findings generally occurred following the dose and application of the collar and were considered in the report to be related to improperly fitted collars.

Detailed – Reduced activity during the treatment period was observed at a higher incidence at 600 and 900 mg/kg buprenorphine (6 animals) and was considered a known pharmacologic effect of buprenorphine.

The majority of clinical signs in the study were related to the injection site(s). Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (300, 600 and 900 mg/kg buprenorphine). This observation was not considered adverse according to the report because this was an expected finding associated with the subcutaneous delivery of the formulation and formation of the drug depot. Additional clinical signs related to the injection site(s), seen in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (300, 600 and 900 mg/kg buprenorphine), were considered likely related to the unique properties, such as the formation of palpable drug depot and delivery system associated with the subcutaneous dose route of administration. The most prevalent of these findings was scabbing at the injection site(s) which occurred in all Atrigel control (low, mid, and high) and RBP-6000 administered groups at a much higher incidence than the saline control as there was little to no local effect from saline injection. Other findings at the injection site(s) were observed on occasion in one of more of these groups, including skin discoloration, sparse hair and abrasion(s).

Increased incidence of reduced activity during the treatment period the premating/mating period was observed 900 mg/kg buprenorphine treated males (6 animals) and is considered to be a known pharmacologic effect of buprenorphine. A few other clinical findings in the Atrigel control (low, mid, and high) and RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) were observed infrequently and were considered incidental and unrelated to treatment.

The clinical findings observed in the untreated females were similar across the groups and are considered common for this species of rat used in this study.

Body Weight

Untreated females were weighed weekly prior to and during cohabitation. Mated untreated females with a confirmed GD 0 were weighed on GD 0, 4, 7, 10, and 13. After cohabitation, untreated females with no evidence of mating were weighed twice weekly at 3- and 4-day intervals until euthanasia. Body weight changes were calculated for the untreated females between each weighing interval and on GD 0-4, 4-7, 7-10, 10-13, 0-7, 7-13, and 0-13.

Premating and gestation – Mean premating body weights and gestation body weight and body weight change in the untreated females were comparable across the groups.

Males (premating and post mating)

Atrigel groups

Male premating mean body weights and body weight change in the Atrigel control groups (low, mid, and high) were generally comparable to the saline control group. Reduced mean body weights and/or body weight loss was observed in the saline

control and all Atrigel control groups (low, mid, and high) following each test article administration (Days 1, 28, and 56).

Body weight changes were statistically lower in the Atrigel control groups (mid and high) during Days 1-4, 28-31, 56-59, and 1-70 (high Atrigel control group only) and this was similar with statistically significantly (SS) lower (≤ 8%) mean body weights on Days 4, 8, 31, and 59. These differences in mean body weight and body weight change in the mid- and high Atrigel control groups were considered treatment-related. But, due to the slight magnitude of change and occurrences only after administration of the dose, these differences were not considered toxicologically relevant.

Mean body weights and body weight change in the low Atrigel control group was comparable to the saline control group and unaffected by treatment. During the pairing and post-mating period male mean body weights and body weight change in the Atrigel control groups (low, mid, and high) were comparable to the saline control group.

Buprenorphine groups (see Tables)

Mean body weights in males were statistically significantly (SS) lower throughout the premating, pairing, and post-mating period in comparison to the saline control group in the low dose (5-7%), mid-dose (5-8%), and high dose (6-12%), around the time of test article administration. Similarly, reduced body weight change was observed at dosing time. All were considered treatment-related although. The overall body weight change during the premating period (Days 1-70) was SS lower in comparison to the saline control and the low-Atrigel control group, respectively for the low dose (-44% and -41%), mid-dose (-48% and -43%), and high dose (-67% and -57%) groups. These differences in mean body weights and body weight change correlated with lower mean food consumption and were considered treatment-related and toxicologically relevant.

Mean male body weight change during the pairing and post-mating period at 300, 600, and 900 mg/kg buprenorphine was comparable to the saline control group and with each respective Atrigel control group.

Mean premating body weights and gestation body weight and body weight change in the untreated females were comparable across the groups.

Food Consumption

Atrigel groups (see Tables)

Male premating and post-mating mean food consumption in the Atrigel control groups (low, mid, and high) were comparable to the saline control group (see Tables). Select lower food consumption values, while likely related to treatment, were slight in magnitude of change, sporadic in nature, and not considered toxicologically relevant as they were in the 10-20% range Weeks 1, 4 and 8 with a compensatory higher mean

food consumption in Week 2 for the high Atrigel group (see tables). Mean food consumption was statistically significantly lower (-12% and -23%) in the mid- and high Atrigel control groups, respectively, during Week 1. In addition, in the high-Atrigel control group, mean food consumption was statistically lower (-9% and -17%) during Weeks 4 and 8, respectively. These lower food consumption values, while likely related to treatment, were slight in magnitude of change, sporadic in nature and not considered adverse. A compensatory statistically higher mean food consumption value was observed during Week 2 in the high-Atrigel control group.

Buprenorphine groups (see Tables)

300 mg/kg Buprenorphine - Mean food consumption was statistically significantly (SS) lower for the 300 mg/kg buprenorphine group (pre-mating - Weeks 1-3, 5, 6, and 9; post-mating - Week 14), ranging from 11% to 18% lower in comparison to saline control values. When compared to the low-Atrigel control group, mean food consumption was statistically lower during Week 5 (-13%), 6 (-17%), and 14 (-13%). The lower mean food consumption at 300 mg/kg buprenorphine correlated with lower mean body weights and body weight change and were considered treatment-related and toxicologically relevant.

600 mg/kg Buprenorphine - Mean food consumption was SS lower for the 600 mg/kg buprenorphine group (pre-mating - Weeks 1-3) ranging from 9% to 24% lower in comparison to saline control values. When compared to the mid-ATRIGEL® control group, mean food consumption was statistically lower during Weeks 1 (-14%) and 2 (-10%). The lower mean food consumption at 600 mg/kg buprenorphine correlated with lower mean body weights and body weight change and were considered treatment-related and toxicologically relevant.

900 mg/kg Buprenorphine - Mean food consumption was SS lower for the 900 mg/kg buprenorphine group (pre-mating period - Weeks 1-8) ranging from 13% to 31% lower in comparison to saline control values. When compared to the high-ATRIGEL® control group, mean food consumption was statistically lower during Weeks 2 (-21%), 3 (-12%), 5 (-26%), 6 (-25%), and 7 (-23%). The decreases in mean food consumption at 900 mg/kg buprenorphine correlated with lower mean body weights and body weight change and were considered treatment-related and toxicologically relevant.

Mean gestational food consumption in the untreated females were comparable across the groups.

Summary of Food Consumption Values - MALE

Endpoint	Study Interval (Week)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Food Consumption g/animal/day													
Premating	1	30.25	2.713	23	27.80	3.938	23	26.52[a]	4.374	23	23.29[b]	2.834	23
	2	31.50	2.249	23	30.45	4.863	23	31.57	2.813	23	33.48[a]	2.206	23
	3	32.22	1.856	23	29.78	5.709	23	30.07	4.500	23	31.86	2.589	23
	4	30.50	2.118	23	30.05	2.506	23	29.12	3.259	23	27.79[b]	2.157	23
	5	32.93	4.253	23	33.15	2.628	23	32.53	4.904	23	35.68	2.548	23
	6	32.00	5.875	23	32.55	2.741	23	31.02	5.153	23	31.42	3.528	23
	7	33.28	2.646	23	33.42	2.735	23	33.72	4.039	22	33.44	3.487	23
	8	26.93	1.962	23	27.32	3.146	23	25.11	2.888	23	22.27[b]	3.631	23
	9	33.81	2.983	23	33.14	4.934	23	34.19	3.080	23	34.48	5.599	23
Postmating	14	31.89	2.160	23	32.37	2.361	23	31.78	3.976	23	30.49	2.412	23

Summary of Food Consumption Values - MALE

Endpoint	Study Interval (Week)	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Food Consumption g/animal/day										
Premating	1	24.69[b]	4.258	23	22.93[b,e]	3.668	23	20.94[b]	4.577	23
	2	26.91[b]	3.621	22	28.35[b,f]	2.754	23	26.61[b,h]	3.230	23
	3	28.59[b]	2.313	21	29.23[b]	2.587	23	27.99[b,h]	3.730	21
	4	29.82	2.825	21	29.60	2.617	23	26.10[b]	3.901	23
	5	28.90[b,d]	2.111	21	31.42	2.996	22	26.34[a,h]	9.731	22
	6	26.95[a,d]	5.196	21	30.97	5.374	22	23.44[a,g]	10.081	21
	7	31.67	2.196	21	32.01	3.264	22	25.74[b,g]	9.032	19
	8	27.40	3.360	21	26.66	3.707	22	18.86[b]	6.914	14
	9	31.05[a]	2.803	21	31.99	4.520	22	26.64	11.144	15
Postmating	14	28.09[a,c]	5.162	20	30.21	3.041	22	30.56	3.633	14

N - Number of measures used to calculate mean
SD - Standard Deviation

[a]Significantly different from 0 mg/kg (Saline); (p<0.05)
[b]Significantly different from 0 mg/kg (Saline); (p<0.01)
[c]Significantly different from 0 mg/kg (Control 1); (p<0.05)
[d]Significantly different from 0 mg/kg (Control 1); (p<0.01)
[e]Significantly different from 0 mg/kg (Control 2); (p<0.05)
[f]Significantly different from 0 mg/kg (Control 2); (p<0.01)
[g]Significantly different from 0 mg/kg (Control 3); (p<0.05)
[h]Significantly different from 0 mg/kg (Control 3); (p<0.01)

Estrous Cyclicity – Untreated females

Estrous cyclicity in the untreated females was comparable across the groups.

Reproductive and Fertility Indices – Treated males and untreated females

No buprenorphine-related effects were seen in male reproductive and fertility indices in the low- and mid-Atrigel control groups and at 300 and 600 mg/kg buprenorphine. The male mating, fertility, and fecundity indices in these groups ranged from 90.5% to 100% and were comparable to the saline control values (91.3% to 100%) (see Tables).

In the high-Atrigel control group and at 900 mg/kg buprenorphine, while the mating index (males with evidence of mating) was 100% and comparable to the saline controls at 100%, the male fertility and fecundity indices were 65.2% and 35.7% in the high-Atrigel control group and at 900 mg/kg buprenorphine, respectively. The saline control was at 91.3% and outside recent historical control data for this laboratory (76% to 100%; contained in report). These lower fertility and fecundity indices at the high-Atrigel control group and at 900 mg/kg buprenorphine were considered treatment-related and toxicologically relevant.

The mean copulatory interval (days to mating) ranged from 2.6 to 4.2 days in the Atrigel control groups (low, mid, and high) and at 300 and 600 mg/kg buprenorphine and was comparable to the saline controls at 2.3 days and within the recent historical control range of 2.2 to 4.5 days. The mean copulatory interval for the 600 mg/kg buprenorphine group was 4.8 days and while not statistically significant was outside the historical control range and considered to be uncharacteristic and a delay of time to mating. This difference in the copulatory interval was considered treatment-related and toxicologically relevant.

Summary of Reproductive and Fertility Parameters

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Males on Study		23	23	23	23
No. Males Paired		23	23	23	23
No. Males Mated		23	23	23	23
No. Males Impregnating a Female		21	22	22	15
Male Mating Index (%)		100.0	100.0	100.0	100.0
Male Fertility Index (%)		91.3	95.7	95.7	65.2
Male Fecundity Index (%)		91.3	95.7	95.7	65.2
Females with Confirmed Mating Day		23	23	23	23
Copulatory Interval (Days)					
	Mean	2.3	3.1	2.9	2.6
	SD	1.47	3.33	3.16	2.76
	N	23	23	23	23

Summary of Reproductive and Fertility Parameters

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
No. Males on Study		21	22	14
No. Males Paired		21	22	14
No. Males Mated		20	22	14
No. Males Impregnating a Female		19	22	5
Male Mating Index (%)		95.2	100.0	100.0
Male Fertility Index (%)		90.5	100.0	35.7[b]
Male Fecundity Index (%)		95.0	100.0	35.7[b]
Females with Confirmed Mating Day		22	23	22
Copulatory Interval (Days)				
	Mean	4.2	3.0	4.8
	SD	3.53	2.86	5.49
	N	22	23	22

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

[b]Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Sperm Analysis – Treated males only (see Tables)

A section of the right or left vas deferens was utilized for automated evaluation of sperm motility (viability). The right cauda epididymis was weighed and used for manual (visual) assessment of sperm concentration. Slides were prepared for assessment of sperm morphology from the motility preparations. The right testis was frozen (at approximately -10 to -30°C), to be used if necessary, to prepare samples for possible analysis of spermatid head count. Sperm evaluations were performed for all males in each group euthanized at study termination.

High dose groups - All sperm parameters for the high-Atrigel control group were statistically significant (SS) affected in comparison to the saline control group. Sperm motility (53.9% vs. 96.7% in the saline control group), total sperm count per cauda epididymis, and sperm concentration per gram cauda epididymis were lower (53% vs.34%). In addition, abnormal sperm were observed in the high-Atrigel control group at 55.67% in comparison to the saline control group at 3.45%. As with the high dose Atrigel group, the 900 mg/kg buprenorphine group had all sperm parameters SS different in comparison to the saline control group with low sperm motility (43.6% vs. 96.7% in the saline control group), lower total sperm count per cauda epididymis, and lower sperm concentration per gram cauda epididymis (64% and 49%), respectively. In addition, at 900 mg/kg buprenorphine, 62.91% abnormal sperm was observed in comparison to the saline control group at 3.45%. These differences in sperm parameters in the high-Atrigel control group and at 900 mg/kg buprenorphine were considered treatment-related and toxicologically relevant.

Mid-dose groups - In the mid-Atrigel control group and at 600 mg/kg buprenorphine (mid-dose), percent abnormal sperm was SS higher at 9.33% and 11.61%, respectively, compared to saline control of 3.45%. This SS increase in percent abnormal sperm in the mid-Atrigel control group and 600 mg/kg buprenorphine were outside the historical control data range for this laboratory (2.4% to 8.2%; contained in report). These differences were considered treatment related and toxicologically relevant. Other sperm parameters (percent motility, total sperm count per cauda epididymis, sperm concentration per gram cauda epididymis) in the mid-Atrigel control group and at 600 mg/kg buprenorphine were comparable to the saline control group and/or the respective Atrigel control group.

Low dose groups - Sperm parameters (percent motility, total sperm count per cauda epididymis, sperm concentration per gram cauda epididymis, and percent abnormal) in the low-Atrigel control group and at 300 mg/kg buprenorphine were comparable to the saline control group and/or the respective Atrigel control group.

Summary of Sperm Evaluation

Endpoint	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Sperm % Motility	96.7	3.09	22	93.5	7.52	23	94.7	6.28	23	53.9[b]	46.19	22
Total Sperm Count per Cauda Epididymis x 10^8	3.054	0.822	23	3.124	0.607	23	2.649	0.414	23	1.441[b]	0.704	23
Sperm Concentration per gram Cauda Epididymis x 10^8	9.177	2.395	23	9.782	1.721	23	9.267	1.113	23	6.030[b]	2.388	23
% Abnormal	3.45	2.849	22	4.30	3.305	23	9.33[a]	9.402	23	55.67[b]	39.363	21

Summary of Sperm Evaluation

Endpoint	300 mg/kg Mean	SD	N	600 mg/kg Mean	SD	N	900 mg/kg Mean	SD	N
Sperm % Motility	91.7	9.56	19	89.7	15.93	22	43.6[b]	44.08	12
Total Sperm Count per Cauda Epididymis x 10^8	3.041	0.844	20	2.778	0.557	22	1.094[b]	0.793	15
Sperm Concentration per gram Cauda Epididymis x 10^8	9.149	2.302	20	9.127	1.357	22	4.716[b]	2.914	15
% Abnormal	4.76	8.047	19	11.61	21.127	22	62.91[b]	31.024	11

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b]Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Uterine and Ovarian Examinations – Untreated Females (see Tables)

On GD 13, each untreated female was euthanized, and the uterus and ovaries were examined for the number of normally developing embryos, resorptions, and the total number of implantations. The number of corpora lutea on each ovary was also recorded. Uteri from females that appeared non-gravid were opened fixed for detection of implantation sites. If no foci were seen, the female was considered non-pregnant.

The number of untreated females (mated with treated males) with viable embryos available for evaluation on GD 13 was 21, 21, 21, 15, 21, 23 and 8 in the saline control, low-Atrigel control, mid-Atrigel control, high-Atrigel control, 300, 600, and 900 mg/kg buprenorphine, respectively. There were 2, 1, 2, 8, 5, 1, and 18 non-pregnant females in these same groups, respectively.

The pregnancy outcome (8 and 18 non-pregnant females) in the high-Atrigel control group and at 900 mg/kg buprenorphine, respectively, could be correlated with the abnormal sperm evaluations (low motility, low mean number of sperm, and higher percentage of abnormal sperm) and was considered treatment-related and

toxicologically relevant. Also related to the abnormal sperm evaluations and considered treatment related and toxicologically relevant were the mean number of implantation sites (11.9 per animal vs. 15.4 per animal in saline controls), mean number of viable embryos (11.3 per animal vs. 14.7 per animal in saline controls), and preimplantation loss (28.82% vs. 6.98% in saline controls) in the high-Atrigel control group (untreated females). These values were not statistically lower, but were reported outside recent historical control data and considered related to the abnormal sperm.

Also related to the abnormal sperm evaluations and considered treatment related and toxicologically relevant in the 900 mg/kg buprenorphine (untreated females) group were mean number of implantation sites (7.8 per animal vs. 15.4 per animal in saline controls), mean number of viable embryos (7.0 per animal vs. 14.7 per animal in saline controls), and preimplantation loss (49.04%). These indices were SS lower and outside recent historical control data for the testing laboratory.

Untreated females in the high-Atrigel control group and at 900 mg/kg buprenorphine groups had corpora lutea, mean number of resorptions, and post-implantation loss that were comparable to the saline control group and/or the respective Atrigel control group. In the untreated females, ovarian and uterine parameters (corpora lutea count, number of implantation sites, viable embryos, and resorptions, and pre-and post-implantation loss) in the low and mid-Atrigel control groups and respective buprenorphine groups (low and mid) were comparable to both the saline control group and/or the respective Atrigel control group.

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Females on Study	26	23	25	23
No. Not Pregnant	2	1	2	8
No. Pregnant	24	22	23	15
No. Females Pregnant by Stain	0	0	0	0
No. Females with Viable Embryos Day 13 Gestation	21	21	21	15
No. Pregnant Females with No Confirmed Mating Date	3	1	2	0

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint	300 mg/kg	600 mg/kg	900 mg/kg
No. Females on Study	27	25	27
No. Not Pregnant	5	1	18
No. Pregnant	22	24	9
No. Females Pregnant by Stain	1	0	0
No. Females with Viable Embryos Day 13 Gestation	21	23	8
No. Pregnant Females with No Confirmed Mating Date	1	1	1

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Corpora Lutea					
No. per Animal	Mean	16.7	17.2	17.2	16.1
	SD	2.39	2.66	2.64	3.73
	N	21	21	21	15
Implantation Sites					
No. per Animal	Mean	15.4	15.9	16.0	11.9
	SD	1.88	2.26	1.94	5.76
	N	21	21	21	15
Preimplantation Loss					
% per Animal	Mean	6.98	6.97	6.69	28.82
	SD	9.490	9.184	8.336	33.232
	N	21	21	21	15
Viable Embryos					
No. per Animal	Mean	14.7	14.7	15.0	11.3
	SD	2.31	2.52	2.41	5.63
	N	21	21	21	15
Postimplantation Loss					
% per Animal	Mean	4.94	7.73	6.23	4.00
	SD	6.173	8.559	7.244	5.703
	N	21	21	21	15

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
Corpora Lutea				
No. per Animal	Mean	17.7	16.8	13.4
	SD	2.76	2.62	4.10
	N	21	24	8
Implantation Sites				
No. per Animal	Mean	14.3	14.0	7.8[a]
	SD	3.91	4.80	6.58
	N	22	24	8
Preimplantation Loss				
% per Animal	Mean	14.74	17.85	49.04[b]
	SD	15.116	24.763	34.438
	N	21	24	8
Viable Embryos				
No. per Animal	Mean	13.4	13.4	7.0[a]
	SD	4.28	4.75	6.68
	N	22	24	8
Postimplantation Loss				
% per Animal	Mean	11.06	7.06	9.99
	SD	21.288	11.782	16.042
	N	22	24	8

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Resorptions: Early + Late					
No. per Animal	Mean	0.7	1.2	1.0	0.5
	SD	0.90	1.45	1.02	0.74
	N	21	21	21	15

Summary of Maternal and Developmental Observations at Uterine Examination - Untreated

Endpoint		300 mg/kg	600 mg/kg	900 mg/kg
Resorptions: Early + Late				
No. per Animal	Mean	1.0	0.7	0.8
	SD	0.90	0.70	1.16
	N	22	24	8

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

[a]Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b]Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Postmortem Study Evaluations – Treated Males and Untreated Females

Macroscopic

A treatment-related higher incidence of small testes (left and right; up to 6 animals affected) and small epididymides (left, right, and right cauda; up to 10 animals affected) was observed in the high-Atrigel control group and at 900 mg/kg buprenorphine group in comparison to the saline control group. These macroscopic findings correlated with reduced organ weights and reduced sperm observed in these groups and was considered toxicologically relevant. In addition, a higher incidence of small thymus (up to 9 animals affected) was observed in the high-Atrigel control group and at 900 mg/kg buprenorphine in comparison to the saline control group and was considered treatment-related. These findings were considered related to NMP toxicity by the report (DEI Malek et al. 1997 – to be reviewed in the safety assessment of NMP in this NDA review).

Macroscopic findings at the injection site(s) tended to correlate with the clinical observations. Findings related to the injection site(s) in the Atrigel control groups (low, mid, and high) and the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) included abrasions/scabs, skin discoloration, and foreign material (test material implant). The few other macroscopic findings observed were seen infrequently and considered unrelated to treatment.

Macroscopic findings in the untreated females were infrequent and comparable across the groups.

Organ Weights (see Tables)

Treatment-related organ weight reductions were present in the testes and epididymides in the high-Atrigel control group and at 900 mg/kg buprenorphine in comparison to the saline control group. In the high-Atrigel control group, statistically significant (SS) decreases in testis and epididymis weight (absolute and relative to body weight) of 16% and 24%, respectively, was observed in comparison to the saline control group. Similarly, at 900 mg/kg buprenorphine, a statistically significant lower absolute testis weight (-22%), absolute epididymis weight (-26%), and epididymis weight relative to body weight (-19%) was observed in comparison to the saline control group. These differences in testis and epididymis weights (absolute and relative to body weight) were reported to correlate with reduced sperm in these groups and were considered toxicologically relevant.

Mean terminal body weights at 300, 600, and 900 mg/kg buprenorphine were SS lower (9%, 7%, and 8%, respectively) in comparison to the saline controls. At 300 and 900 mg/kg buprenorphine, mean terminal body weights were also SS lower (9% and 8%, respectively) in comparison to their respective Atrigel control group. These differences in mean terminal body weights correlated with the lower body weights observed in the RBP-6000 administered groups (300, 600, and 900 mg/kg buprenorphine) throughout the treatment and posttreatment period. A non-dose responsive statistically significant

increase in testes weight relative to body weight was observed at 600 mg/kg buprenorphine, but was considered incidental and unrelated to treatment.

Mean organ weights in the untreated females (ovaries and uterus with cervix at GD 13) were comparable across the groups.

Summary of Organ Weight Values - MALE
Terminal

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight												
g	570	36	23	569	32	23	557	37	23	567	46	23
Epididymides												
g	1.422	0.160	23	1.396	0.112	23	1.318	0.089	23	1.077[b]	0.196	23
Epididymides/BWt												
%	0.2511	0.0344	23	0.2459	0.0240	23	0.2374	0.0206	23	0.1905[b]	0.0340	23
Prostate gl												
g	1.328	0.186	23	1.312	0.207	23	1.415	0.242	23	1.372	0.223	23
Prostate gl/BWt												
%	0.2339	0.0355	23	0.2312	0.0384	23	0.2542	0.0423	23	0.2424	0.0391	23
Sem. ves. w/ coag. gl												
g	2.761	0.362	23	2.682	0.359	23	2.753	0.365	23	2.654	0.297	23
Sem. ves. w/ coag. gl/BWt												
%	0.4852	0.0612	23	0.4723	0.0667	23	0.4946	0.0609	23	0.4693	0.0511	23
Testes												
g	3.483	0.407	23	3.496	0.232	23	3.415	0.213	23	2.927[b]	0.455	23
Testes/BWt												
%	0.6148	0.0848	23	0.6158	0.0522	23	0.6153	0.0525	23	0.5188[b]	0.0872	23

Summary of Organ Weight Values - MALE
Terminal

	300 mg/kg			600 mg/kg			900 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight									
g	518[b,d]	33	20	531[b]	34	22	522[b,g]	42	15
Epididymides									
g	1.399	0.196	20	1.349	0.117	22	1.055[b]	0.139	15
Epididymides/BWt									
%	0.2712	0.0415	20	0.2549	0.0245	22	0.2036[b]	0.0316	15
Prostate gl									
g	1.355	0.222	20	1.297	0.220	22	1.211	0.201	15
Prostate gl/BWt									
%	0.2620	0.0436	20	0.2456	0.0470	22	0.2324	0.0366	15
Sem. ves. w/ coag. gl									
g	2.759	0.428	20	2.861	0.467	22	2.653	0.278	15
Sem. ves. w/ coag. gl/BWt									
%	0.5333	0.0793	20	0.5391	0.0822	22	0.5125	0.0723	15
Testes									
g	3.457	0.679	20	3.579	0.340	22	2.711[b]	0.603	15
Testes/BWt									
%	0.6716	0.1413	20	0.6761[e]	0.0687	22	0.5260	0.1363	15

N - Number of measures used to calculate mean
SD - Standard Deviation

[b]Significantly different from 0 mg/kg (Saline); ($p<0.01$)
[d]Significantly different from 0 mg/kg (Control 1); ($p<0.01$)
[g]Significantly different from 0 mg/kg (Control 3); ($p<0.05$)

Summary of Organ Weight Values - Untreated - FEMALE
Terminal

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight												
g	303	23	21	310	22	21	313	18	21	311	22	15
Ovaries												
g	0.116	0.015	21	0.119	0.019	21	0.118	0.018	21	0.114	0.017	15
Uterus w/ cervix												
g	8.800	1.216	21	9.709	1.689	21	9.482	1.576	21	7.318	3.287	15

Summary of Organ Weight Values - Untreated - FEMALE
Terminal

	300 mg/kg			600 mg/kg			900 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight									
g	313	27	21	310	25	23	315	12	8
Ovaries									
g	0.112	0.013	21	0.111	0.018	23	0.103	0.019	8
Uterus w/ cervix									
g	8.611	1.694	21	8.441	2.635	23	4.761	3.910	8

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9.2 Embryonic Fetal Development

Study title: RBP-6000: A Study for Effects on Embryo Fetal Development in Rabbits with Toxicokinetic Evaluation

Study no.:	INLS-L105-60-16
Study report location:	eCTD 4.2.3.5.2; SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 5, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/Atrigel - 18% buprenorphine base in (b) (4)% 50/50 PLGH and (b) (4)% NMP), 100 mg, Lot 206. 300 mg, Lot 205, Purity NR - RBP-6000 Control Article: 100 mg placebo, Lot 206. 300 mg placebo, Lot 205 - 50:50 Poly(DL-lactide-co-glycolide) (PLGH) and N-methyl-2-pyrrolidone (NMP), Purity NR - 0.9% Sodium Chloride USP, Lot 52-071-JT, Purity NR

Key Study Findings

- This study was conducted in 23 time-mated female New Zealand White rabbits/ group to determine the potential developmental toxicity, including the teratogenic potential, of 78, 155, and 390 mg/kg RBP-6000 (18% buprenorphine base in the ATRIGEL® Delivery System) and respective equivalent amounts of ATRIGEL® (Atrigel control groups) administered by a single subcutaneous injection on Gestation Day 7 (GD 7). A 0.9% saline control group was included. All animals were sacrificed for full examination on GD 29 in this complete GLP protocol study.
- Maternal toxicity was observed at the high-Atrigel control group and at the mid (155 mg/kg) and high (390 mg/kg) RBP-6000 groups. Toxicity included abortions (high-Atrigel control group and high buprenorphine groups), decreased body weight gain and reduced food consumption during the gestation period. Maternal survival and macroscopic findings were unaffected at all ATRIGEL® control and RBP-6000 dose levels.
- The majority of clinical signs in the study were related to the local toxicity of injection site(s) plus sporadic occurrences of scabbed areas, abrasions, and discolored skin. Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals (low, mid, and high) in the ATRIGEL® control and buprenorphine groups. These observations were not considered adverse because this was an expected finding associated with the delivery of the formulation

and formation of the drug depot. Decreased activity was also observed in the RBP-6000-administered groups and considered to be a known buprenorphine pharmacologic effect.

- Developmental toxicity including total litter resorption, increase in mean post-implantation loss, increased mean number of resorptions and decreased mean number of viable fetuses were observed in the high Atrigel and RBP-6000 groups and not considered buprenorphine related.
- Decreased mean fetal body weights were observed in the high Atrigel and RBP-6000 groups. The differences in fetal body weight appear to correlate with reduced maternal food consumption observed in these groups. An increased litter incidence of external malformations, visceral and skeletal malformations and variations were observed in the high-Atrigel and RBP-6000 groups. In addition, at the mid-dose RBP-6000, animals had an increased litter incidence of skeletal malformations. These effects on fetal development were considered test article related and toxicologically relevant.
- A few skeletal malformations were either seen in the Atrigel control groups (low [total skeletal malformations statistically higher or the low RBP-6000 group, but were seen at a low incidence, comparable to the saline control, not dose-responsive or within historical control data and considered spontaneous and unrelated to treatment.
- The NOAEL for embryofetal development effects is the low-dose of RBP-6000 and the mid-dose may be an acceptable LOAEL. However, note that the observed effects appear to be attributable to the Atrigel vehicle as the adverse changes observed in the RBP-6000 groups were never greater than the corresponding Atrigel control groups.
- The NOAEL for maternal toxicity is the low-dose of RBP-6000 which consisted of 78 mg/kg buprenorphine (also containing (b) (4) mg/kg of NMP)
- The NOAEL for developmental effects for the ATRIGEL® control groups is the mid-dose, which contained (b) (4) mg/kg of NMP.
- At the low buprenorphine dose, the C_{max} was 26.1 ng/mL and the AUC_{0-336h} (14 days – major organogenesis period) was 3,010 ng*h/mL.

Summary of Mean Buprenorphine C_{max} and AUC_{0-336} in Plasma of Pregnant Rabbits

Dose Group	Dose Level of Buprenorphine Base (mg/kg)	C_{max} (ng/mL)	AUC_{0-336} (ng·hr/mL)
5	78	26.1	3010
6	155	49.7	7880
7	390	171	18700

Methods

Doses:

Group Assignments		
Group Number	Dose Level of Buprenorphine Base (mg/kg)	Number of Time-mated Females
1	0 (Saline)	23
2	0 (Control 1)[a]	23
3	0 (Control 2)[a]	23
4	0 (Control 3)[a]	23
5	78	23
6	155	23
7	390	23

[a]The ATRIGEL® control groups received the equivalent amount of ATRIGEL® as delivered to the RBP-6000-administered groups (maximum of 1.6 mL/kg).

The dose levels were selected based on data from previously conducted toxicological studies with this test article (buprenorphine base in ATRIGEL® Delivery System or RBP-6000) in rabbits including DRF study INRS-L146-60-15.

Frequency of dosing:	Single dose on Day 7 of gestation
Dose volume:	See Study Design
Route of administration:	Subcutaneous
Formulation/Vehicle:	Atrigel Delivery System
Species/Strain:	New Zealand White Hra:(NZW)SPF rabbits
Number/Sex/Group:	23 time-mated females/group
Satellite groups:	No – same animals used for toxicokinetics
Study design:	See study design below – Respective Atrigel control groups received equal volume of Atrigel as RBP-6000 group
Deviation from study protocol:	Nothing significant

Study Design

Study Design					
Group Number	Dose Level of Buprenorphine Base (mg/kg)	Total Dose Volume (mL/kg) [a, c]	Dose Volume per Injection (mL/kg) [a, c]	Total Number of Injection Sites	Number of Time-mated Females
1	0 (Saline)	1.9	0.38	5	23
2	0 (Control 1)[b]	0.31	0.31	1	23
3	0 (Control 2)[b]	0.62	0.31	2	23
4	0 (Control 3)[b]	1.6	0.31	5	23
5	78	0.38	0.38	1	23
6	155	0.76	0.38	2	23
7	390	1.9	0.38	5	23

[a] Dose volume was calculated using 18% (w/w) buprenorphine base and a density of 1.15 g/mL.
[b] The ATRIGEL® control groups received the equivalent amount of ATRIGEL® as delivered to the RBP-6000-administered groups (maximum of 1.6 mL/kg).
[c] Doses were delivered within ±20% of the desired dose.

Dosing was to scapular region on the back. Number of injections is related to dose volume (i.e., 1.6 mL is ~5x volume of 0.31 mL).

Rabbit Dosing Diagram

Head	
A	B
C	D
E	

Dose Levels of ATRIGEL® Delivery System and its Components			
Group Description	ATRIGEL® Delivery System (mg/kg)	NMP (mg/kg)	PLGH (mg/kg)
Low-ATRIGEL (Control 1) Mid-ATRIGEL (Control 2) High ATRIGEL (Control 3)	[redacted] (b) (4)		
NMP - N-methyl-2-pyrrolidone; PLGH - polylactide-co-glycolide			

Dose Levels of Buprenorphine Base Relative to RBP-6000	
Buprenorphine base (mg/kg)	RBP-6000 (mg/kg)
78 155 390	[redacted] (b) (4)

Observations and Results

Mortality

All animals were observed for mortality twice daily.

None of the treated dams died prematurely during the study. Three pregnant dams from the Atrigel high-dose group appeared to have abortions on GDs 22, 19, and 19 and three from the RBP-6000 high-dose (390 mg/kg buprenorphine) group aborted on GDs 23, 24, and 21. These abortions were considered treatment-related and toxicologically relevant. All other animals survived to the scheduled necropsy on GD 29.

Clinical Signs

All animals were observed for morbidity and injury twice daily. Daily from GD 7 through 29 (75 minutes ±15 minutes on Day 7), each animal was removed from the cage and given a detailed clinical examination. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration and any atypical behavior, such as tremors, convulsions, and reactivity to handling.

The majority of clinical signs in the study were related to the injection site(s). Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals in the Atrigel control (low, mid, and high) and RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine) (see Tables). This observation was not considered adverse because this was an expected finding associated with the SC delivery of the formulation and formation of the drug depot. Additional clinical signs related to the injection site(s), seen in the ATRIGEL® control (low, mid, and high) and RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine), were considered likely related to the unique properties, such as the formation of palpable drug depot from the delivery system associated with the SC dose route of administration. The most prevalent of these findings was scabbing at the injection site(s) which occurred in all Atrigel control (low, mid, and high) and RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine), but was observed at a higher incidence in the high-Atrigel control group (18 animals) and in the 390 mg/kg buprenorphine group (16 animals) in comparison to 6 animals in the saline control group. Other findings at the injection site(s) observed in all groups to some extent included skin discoloration and abrasion(s). These injection site(s) findings were considered not adverse. Reduced activity was observed at a higher incidence in the RBP-6000-administered groups (78 [6 animals], 155 [3 animals], and 390 mg/kg buprenorphine [2 animals]) and was considered a known pharmacologic effect of buprenorphine.

A limited incidence of a clinical sign, including feces few/absent, was likely related to treatment (pharmacologic action), because of the onset and duration (GD 8-13 predominately) and coincided with reduced food consumption. These findings were

seen at a higher frequency in the high-Atrigel control group and the RBP-6000 groups (78, 155, and 390 mg/kg buprenorphine).

Findings of red material in the cage pan were associated with females that either aborted or had total litter resorption at uterine examination in the high-Atrigel control group and the 390 mg/kg buprenorphine group.

Summary of Gestation Detailed Clinical Observations*
Days 7 to 29

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Number of Animals Observed	23	23	23	23
Excretion				
Feces abnormal shape/size, Small	0/0	0/0	0/0	0/0
Feces abnormal shape/size, Small, clumped	0/0	0/0	0/0	0/0
Feces discolored, Black	0/0	0/0	0/0	0/0
Feces few/absent	4/3	1/1	1/1	17/7
Feces soft	5/2	12/3	5/2	1/1
Material in pan/bedding, Red	1/1	0/0	0/0	43/10
External Appearance				
Swelling, Injection Site	4/2	456/23	463/23	465/23
Swelling, Injection Site 2	0/0	0/0	447/23	442/23
Swelling, Injection Site 3	0/0	0/0	0/0	448/23
Swelling, Injection Site 4	1/1	0/0	0/0	458/23
Swelling, Injection Site 5	0/0	0/0	0/0	465/23
Thin	0/0	0/0	0/0	0/0
Scabbed area, Injection Site	19/4	52/5	35/4	69/10
Scabbed area, Injection Site 2	6/2	0/0	33/5	38/6
Scabbed area, Injection Site 3	0/0	0/0	0/0	93/8
Scabbed area, Injection Site 4	3/1	0/0	0/0	71/7
Scabbed area, Injection Site 5	2/1	0/0	0/0	121/9

+ Number of times observed/Total number of animals affected

Summary of Gestation Detailed Clinical Observations*
Days 7 to 29

Observation	78 mg/kg	155 mg/kg	390 mg/kg
Number of Animals Observed	23	23	23
Excretion			
Feces abnormal shape/size, Small	0/0	2/1	0/0
Feces abnormal shape/size, Small, clumped	0/0	1/1	0/0
Feces discolored, Black	1/1	0/0	0/0
Feces few/absent	55/13	83/20	69/18
Feces soft	10/2	21/5	28/4
Material in pan/bedding, Red	0/0	0/0	56/10

External Appearance			
Swelling, Injection Site	492/23	520/23	507/23
Swelling, Injection Site 2	0/0	496/23	490/23
Swelling, Injection Site 3	0/0	4/1	482/23
Swelling, Injection Site 4	0/0	0/0	479/23
Swelling, Injection Site 5	0/0	0/0	481/22
Thin	3/1	1/1	0/0
Scabbed area, Injection Site	61/5	28/4	8/2
Scabbed area, Injection Site 2	0/0	27/2	28/3
Scabbed area, Injection Site 3	0/0	0/0	72/7
Scabbed area, Injection Site 4	0/0	0/0	77/6
Scabbed area, Injection Site 5	0/0	0/0	100/10

+ Number of times observed/Total number of animals affected

Other – Skin discoloration (white, yellow, red, purple, black, brown) at injection site only in high-dose Atrigel and RBP-6000 groups.

Body Weight

Body weights for all animals were measured and recorded on GD 0, 7, 10, 13, 16, 19, 21, 25, and 29. Individual body weight change was calculated for the following GD intervals: 0-7, 7-10, 10-13, 13-16, 16-19, 19-21, 21-25, 25-29, 7-29, and 0-29. Adjusted body weight (GD 29 body weight minus gravid uterine weight) and adjusted body weight change (GD 0 to 29) were also calculated.

Mean gestation body weights in the high-Atrigel control group were statistically significantly (SS) lower on GD 19 and 21 (-5% and -6%, respectively) (see Tables). These slight body weight decreases were considered transient in nature, slight in magnitude, and not of significant toxicological relevance. Mean body weights were unremarkable in all of the other groups and for RBP-6000-administered groups when compared to the corresponding values in the saline control group or their respective Atrigel control groups.

Notable mean SS body weight loss occurred in the high-Atrigel control group (-0.105 kg) and in the RBP-6000-administered groups (-0.099, -0.096, and -0.141 kg for the 78, 155, and 390 mg/kg buprenorphine groups, respectively) from GD 7-10 (GD 7 1st day of dosing), compared to a slight gain in the saline control group (+0.003). These body weight losses were considered primarily related to administration of the vehicle formulation (Atrigel Delivery System) although there was some dose-responsiveness between the mid- and high-dose RBP-6000 groups. As the overall body weight changes (GD 7-29 and 0-29) at the low-dose RBP-6000 (78 mg/kg buprenorphine) was comparable to saline control, this dose was considered unaffected by treatment as were the low- and mid-dose Atrigel groups.

The high-Atrigel control group, as well as in the mid- and high-dose RBP-6000-administered groups (155 and 390 mg/kg buprenorphine) lower mean gestation body

weight change of -41%, -43%, and -60%, respectively, was observed from GD 7-29 and in the 390 mg/kg buprenorphine group mean gestation body weight change was -40% lower from GD 0-29 in comparison to the saline control group. These differences in mean gestation body weight change at the high-Atrigel control group and the 155 and 390 mg/kg buprenorphine groups were considered treatment-related and toxicologically relevant. The SS differences in body weight gain correlate with reduced maternal food consumption observed in these groups.

Mean gestation body weight change in the RBP-6000 groups (78, 155, and 390 mg/kg buprenorphine) were generally comparable to their respective Atrigel control groups (low, mid, and high). The exception to this was during GD 7-10 at 78 and 155 mg/kg buprenorphine in which SS lower mean gestation body weight gains were observed in comparison to their respective Atrigel control groups (-0.099 kg vs. -0.006 kg and -0.096 kg vs. 0.019 kg, respectively).

Food Consumption

All animals were observed for the availability of food and water twice daily. Food consumption was recorded daily and reported on the corresponding body weight days. Daily food consumption values are presented individually but not statistically analyzed.

Generally, mean gestation food consumption in the Atrigel control groups (low, mid, and high) were comparable to the saline control group except on GD 7-10 (day 7 1st day of dosing) when the mean food consumption was statistically significantly (SS) lower (-54%) in the high Atrigel control group in comparison to the saline control group (see Tables). The lower food consumption value correlated with lower gestation body weights and was considered treatment-related and toxicologically relevant. The SS higher mean food consumption value (+59%) during Gestation Days 25-29 in the high-Atrigel control group which was considered to be a compensatory effect.

On GD 7-10 (GD 7 1st day of dosing), mean gestation food consumption was SS lower at 78, 155, and 390 mg/kg buprenorphine at -78%, -83%, and -89% in comparison to the saline control group, respectively, and -76%, -83%, and -76% in comparison to their respective Atrigel control groups Similarly, mean gestation food consumption at 78, 155, and 390 mg/kg buprenorphine during GD 10-13 was -51%, -56%, and -44% in comparison to the saline control group, respectively, and -47%, -55%, and -40% lower in comparison to their respective Atrigel control groups. While not dose responsive, these changes were considered buprenorphine related as the Atrigel groups were not similarly affected.

Mean gestation food consumption did return to normal values or slightly higher in all RBP-6000-administered groups for the remainder of the gestation period. However, at the two lower buprenorphine dose groups (78 and 155 mg/kg buprenorphine), mean food consumption was statistically lower during GD 7-29 (-21% and -28%, respectively) and during GD 0-29 (-16% and -22%, respectively) in comparison to the saline control group.

The lower food consumption values at 155 and 390 mg/kg buprenorphine had a significant impact on mean gestation body weight gain early in gestation (GD 7-10) and later in gestation (GD 7-29 and/or GD 0-29) and were considered treatment-related and toxicologically relevant. Mean food consumption was statistically lower in the 78 and 155 mg/kg buprenorphine groups during GD 7-29 (-16% and -26%, respectively) and 20% lower at 155 mg/kg buprenorphine during GD 0-29 in comparison to their respective Atrigel control groups. Mean gestation food consumption at 390 mg/kg buprenorphine was comparable to the high-Atrigel control group during GD 7-29 and 0-29.

At 78 mg/kg buprenorphine the lower food consumption values during early gestation (GD 7-10) and late gestation (GD 7-29 and GD 0-29) had little impact on mean gestation body weights or body weight gain and was therefore considered treatment-related.

Summary of Gestation Food Consumption Values

Endpoint	Study Interval (Day)	0 mg/kg (Saline) Mean	SD	N	0 mg/kg (Control 1) Mean	SD	N	0 mg/kg (Control 2) Mean	SD	N	0 mg/kg (Control 3) Mean	SD	N
Food Consumption g/animal/day													
	0-7	122.96	21.783	23	125.60	20.177	23	118.31	23.524	22	123.15	17.433	23
	7-10	137.20	28.070	23	126.28	27.831	23	132.48	27.988	22	62.57[b]	29.873	23
	10-13	129.72	37.265	23	121.16	27.326	23	126.17	26.279	22	120.94	28.622	23
	13-16	123.59	40.215	23	122.70	29.416	23	116.42	31.666	22	133.19	26.873	23
	16-19	146.59	25.929	23	140.20	35.884	23	140.38	23.646	22	121.53	45.706	23
	19-21	144.15	24.910	23	134.04	35.800	23	135.48	21.993	22	122.12	37.361	21
	21-25	118.46	30.747	23	106.83	42.835	23	110.70	24.732	22	136.11	21.801	20
	25-29	86.09	36.655	23	81.84	34.751	23	92.27	26.935	22	136.58[b]	19.919	20
	7-29	123.49	22.367	23	116.08	25.933	23	119.51	19.620	22	120.43	18.993	21
	0-29	123.35	20.700	23	118.38	21.845	23	119.22	18.420	22	120.75	16.413	21

Summary of Gestation Food Consumption Values

Endpoint	Study Interval (Day)	78 mg/kg Mean	SD	N	155 mg/kg Mean	SD	N	390 mg/kg Mean	SD	N
Food Consumption g/animal/day										
	0-7	124.70	20.786	23	117.83	21.573	22	125.79	19.346	23
	7-10	30.84[b,d]	27.689	23	23.18[b,f]	19.383	22	14.78[b,h]	15.403	23
	10-13	64.00[b,d]	32.664	23	56.83[b,f]	33.972	22	72.88[b,h]	37.011	23
	13-16	101.33	35.102	23	93.97	37.823	22	116.13	30.769	23
	16-19	136.86	24.135	23	123.26	36.480	22	133.30	31.844	23
	19-21	136.93	20.884	23	120.27	35.837	22	135.43	22.835	22
	21-25	121.87	24.407	23	113.99	23.546	22	140.75[a]	19.619	21
	25-29	97.64	24.258	23	90.85	26.580	22	132.71[b]	16.572	20
	7-29	97.63[b,c]	16.264	23	88.71[b,f]	23.047	22	107.35	17.835	22
	0-29	104.18[b]	13.406	23	95.74[b,f]	21.398	22	111.31	16.182	23

N - Number of measures used to calculate mean
SD - Standard Deviation

[a] Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)
[c] Significantly different from 0 mg/kg (Control 1); ($p<0.05$)
[d] Significantly different from 0 mg/kg (Control 1); ($p<0.01$)
[f] Significantly different from 0 mg/kg (Control 2); ($p<0.01$)
[h] Significantly different from 0 mg/kg (Control 3); ($p<0.01$)

Toxicokinetics

Blood samples were collected from the last three animals per group via the jugular vein for determination of the plasma concentrations of buprenorphine and norbuprenorphine. Samples were collected at 1, 2, 4, 6, 8, 12, 24, 48, 72, 168, 240, and 336 hours post-dose on GD 7 and once on GD 29. The animals were **not** fasted prior to blood collection.

All concentration values of buprenorphine in the control groups were below the lower limit of quantitation (< 0.200 ng/mL). Exposure, as assessed by buprenorphine mean C_{max} and $AUC_{0\text{-}336h}$ (14 days) values, increased with the increase in RBP-6000 dose level from 78 to 390 mg/kg. The increases in mean C_{max} and $AUC_{0\text{-}336h}$ values were generally dose proportional. Norbuprenorphine levels were not listed by this reviewer as values ~15-20 fold (C_{max}) and ~75-100 (AUC) lower than buprenorphine with much less biological activity.

Summary of Mean Buprenorphine C_{max} and $AUC_{0\text{-}336}$ in Plasma of Pregnant Rabbits

Dose Group	Dose Level of Buprenorphine Base (mg/kg)	C_{max} (ng/mL)	$AUC_{0\text{-}336}$ (ng·hr/mL)
5	78	26.1	3010
6	155	49.7	7880
7	390	171	18700

Dosing Solution Analysis

Documentation of the strength, purity, composition, stability, and other pertinent information for the lot of control article (0.9% Sodium Chloride for Injection, USP) used on study was limited to that information listed on the label and accompanying documentation of this commercially available product.

The Sponsor provided documentation of the strength (for test article only), purity, composition, stability, and other pertinent information for each batch of control (ATRIGEL® Delivery System) and test article used on study.

Necropsy

Females showing signs of premature delivery earlier than 24 hours of scheduled euthanasia were subjected to a necropsy and uterine examinations. In the event that a female delivered, the aborted material (embryos, fetuses, resorptions, placentae) were randomly assigned a uterine position for identification purposes.

On GD 29, each surviving female was euthanized and immediately subjected to a laparohysterectomy. The uterus was excised, and the gravid uterine weight was recorded. Beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses, early and late resorptions for each uterine horn, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded. Uteri from females that appeared nongravid were stained for detection of implantation sites and if no foci were detected, the female was considered to be nonpregnant.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Maternal Necropsy

A complete necropsy was performed on all surviving does with emphasis on structural abnormalities or pathologic changes that may have influenced the pregnancy. The presence of lesions or other abnormal conditions in the doe were noted and described.

The pregnancy index was 100% in the saline control group, low- and high-Atrigel control groups, 78 and 390 mg/kg buprenorphine groups; it was 95.7% in the mid-Atrigel control group and 155 mg/kg buprenorphine group.

Three females aborted and ten females had total litter resorption (100% post-implantation loss) in the high-Atrigel control group. Three females aborted and eight females had total litter resorption (100% postimplantation loss) in the high-dose RBP-6000 group (390 mg/kg buprenorphine) (see Tables). These events were considered treatment-related and toxicologically relevant. The higher rate of abortions correlates with reduced maternal food consumption observed in these groups noted previously. As a result, the numbers of pregnant females with viable fetuses for evaluation at uterine examination were 23, 23, 22, 10, 23, 22, and 12 in the saline control group, the low-, mid- and high-Atrigel control groups, and the 78, 155, and 390 mg/kg buprenorphine groups, respectively.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)

No. Abortions	0	0	0	3
No. Females with All Resorptions	0	0	0	10
No. Females with Viable Fetuses Day 29 Gestation	23	23	22	10

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	78 mg/kg	155 mg/kg	390 mg/kg
No. Abortions	0	0	3
No. Females with All Resorptions	0	0	8
No. Females with Viable Fetuses Day 29 Gestation	23	22	12

Mean post-implantation loss was statistically significantly (SS) increased (75.7% and 77.3%) in the high-dose Atrigel control group and the high-dose RBP-6000 group (390 mg/kg buprenorphine), respectively (see Tables). This increased loss correlated with SS increased mean number of resorptions of 6.6 per litter (early and early plus late) in the high-Atrigel control group and 7.0 per litter (early and early plus late) at 390 mg/kg buprenorphine. As a result, mean number of viable fetuses/litter size was SS reduced (2.2 per litter) in the high-Atrigel control group and at 390 mg/kg buprenorphine. The adverse effects on these uterine parameters in the high-Atrigel control group and at 390 mg/kg buprenorphine were considered treatment-related and toxicologically relevant.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Postimplantation Loss					
% per Animal	Mean	8.22	6.98	4.58	75.68[p]
	SD	12.127	7.786	9.157	28.817
	N	23	23	22	20
Nonviable Fetuses					
No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	N	23	23	22	20
Litter Size					
No. per Animal	Mean	8.2	8.4	8.3	2.2[p]
	SD	2.52	2.46	2.53	2.48
	N	23	23	22	20
Resorptions: Early + Late					
No. per Animal	Mean	0.7	0.6	0.5	6.6[p]
	SD	1.02	0.59	0.91	2.52
	N	23	23	22	20
Resorptions: Early					
No. per Animal	Mean	0.5	0.4	0.4	6.6[p]
	SD	0.79	0.51	0.90	2.52
	N	23	23	22	20

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		78 mg/kg	155 mg/kg	390 mg/kg
Postimplantation Loss				
% per Animal	Mean	6.62	8.42	77.28[b]
	SD	7.298	16.654	30.964
	N	23	22	20
Nonviable Fetuses				
No. per Animal	Mean	0.0	0.0	0.1
	SD	0.00	0.00	0.22
	N	23	22	20
Litter Size				
No. per Animal	Mean	8.3	7.6	2.3[b]
	SD	2.03	2.22	3.08
	N	23	22	20
Resorptions: Early + Late				
No. per Animal	Mean	0.6	0.8	7.0[b]
	SD	0.66	1.41	2.99
	N	23	22	20
Resorptions: Early				
No. per Animal	Mean	0.6	0.7	7.0[b]
	SD	0.66	1.42	3.02
	N	23	22	20

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Mean number of corpora lutea, mean number of implantation sites, and preimplantation loss in all Atrigel control groups and all buprenorphine were unaffected and comparable to the saline control group. All uterine parameters in the RBP-6000-administered groups were comparable to their respective Atrigel control group.

The mean number of viable fetuses/animal was statistically significantly (SS) reduced in the high-dose Atrigel and RBP-6000 groups by 73% (2.2 versus 8.2) (see Tables).

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Viable Fetuses					
No. per Animal	Mean	8.2	8.4	8.3	2.2[b]
	SD	2.52	2.46	2.53	2.48
	N	23	23	22	20

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	78 mg/kg	155 mg/kg	390 mg/kg

Viable Fetuses				
No. per Animal	Mean	8.3	7.6	2.2[b]
	SD	2.03	2.22	3.04
	N	23	22	20

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

[a] Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Mean gravid uterine weight was statistically significantly (SS) lower in the high-Atrigel control group and at 390 mg/kg buprenorphine (-46% and -51%, respectively) in comparison to the saline control group (see Tables). These lower weights correlated with the increase in mean number of resorptions and the lower mean fetal body weights observed in these groups. Mean gravid uterine weight was unaffected in the low- and mid- ATRIGEL® control groups and at 78 and 155 mg/kg buprenorphine. Mean final body weight, mean adjusted final body weight, and mean adjusted weight change (GD 0-29) were unaffected in all groups compared to saline control and RBP-6000-administered groups were comparable to their respective Atrigel control group.

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values

	0 mg/kg (Saline)			0 mg/kg (Control 1)			0 mg/kg (Control 2)			0 mg/kg (Control 3)		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, kg	0.505	0.1294	23	0.502	0.1179	23	0.491	0.1198	22	0.275[b]	0.0729	10

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values

	78 mg/kg			155 mg/kg			390 mg/kg		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, kg	0.507	0.1176	23	0.453	0.1033	22	0.249[b]	0.1503	12

N - Number of measures used to calculate mean
SD - Standard Deviation

[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Offspring (Malformations, Variations, etc.)

Fetal Examinations

Each fetus was individually examined for external malformations and variations. Following the external examination, each fetus was euthanized, weighed, and subjected to a fresh fetal soft tissue dissection. Any visceral abnormalities were recorded. The sex of each fetus was documented. After dissection and examination of internal organs was complete, each fetus was eviscerated and skinned and processed for subsequent skeletal examination. Fetal findings were classified as malformations or developmental variations under procedures approved by a developmental toxicologist.

Fetal Sex Ratio

Fetal sex ratios (% males) was increased by 37% in the high RBP-6000 group but without statistical significance. However, it was considered unusually high in comparison to the saline control, respective Atrigel control group, and historical control data for the conducting laboratory (contained in report). Considering the low number of viable fetuses in this group, the toxicologically relevance cannot be determined.

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Fetal Sex Ratio					
% Males per Animal	Mean	55.5	52.1	51.7	56.5
	SD	15.90	14.21	19.89	25.39
	N	23	23	22	10

Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		78 mg/kg	155 mg/kg	390 mg/kg
Fetal Sex Ratio				
% Males per Animal	Mean	48.5	52.4	77.4
	SD	18.47	18.69	25.45
	N	23	22	12

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

[a] Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Fetal Body Weights

Mean fetal body weights (male, female, and sexes combined) were statistically significantly (SS) lower in the high-Atrigel control group and the 390 mg/kg buprenorphine group in comparison to the saline control group (see Tables). Mean fetal body weights (sexes combined) were SS lower at 11% and 4% in the high-Atrigel control group and at 390 mg/kg buprenorphine, respectively. The significantly lower fetal body weights correlate with reduced maternal food consumption observed in these groups. The report considered these differences treatment related and toxicologically relevant but this reviewer considers them of unknown toxicological relevance.

Mean fetal body weights (male, female, and sexes combined) in the low and mid-Atrigel control groups and at 78 and 155 mg/kg buprenorphine were comparable to the saline control group. The 78 and 155 mg/kg buprenorphine groups were comparable to their respective Atrigel control group.

Summary of Fetal Body Weight Values, g

Endpoint			0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Fetal Weight						
	Males	Mean	43.39 (44.28)	43.63 (44.81)	43.22 (44.14)	39.18 (35.08)[b]
		SD	5.418	4.832	5.272	4.458
		N	23	23	21	9
	Females	Mean	43.48 (43.93)	41.79 (42.44)	42.67 (43.45)	38.04 (34.26)[b]
		SD	5.069	4.823	4.362	6.030
		N	22	23	20	9
	Males + Females	Mean	43.50 (44.42)	42.90 (44.09)	43.33 (44.28)	38.93 (35.10)[b]
		SD	4.996	4.635	4.508	4.531
		N	23	23	21	10

Summary of Fetal Body Weight Values, g

Endpoint			78 mg/kg	155 mg/kg	390 mg/kg
Fetal Weight					
	Males	Mean	44.36 (45.68)	43.44 (43.50)	41.87 (36.85)[b]
		SD	5.605	5.725	4.131
		N	22	22	12
	Females	Mean	43.24 (43.67)	42.95 (42.91)	41.85 (39.25)
		SD	5.417	5.319	6.468
		N	23	21	6
	Males + Females	Mean	43.94 (44.91)	43.18 (43.32)	42.01 (37.37)[b]
		SD	5.271	5.089	4.409
		N	23	22	12

N - Number of measures used to calculate mean
SD - Standard Deviation
( ) - Least Square Mean
[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Fetal External Examinations

A statistically increased litter incidence of total external malformations was observed at 390 mg/kg buprenorphine (33.3% of litters observed with a malformation) and in the high- Atrigel control group (80.0% of litters observed with a malformation) in comparison to 0% of litters in the saline control group. The individual external malformations observed were either seen at an increased incidence or have not been observed in historical control data (contained in report). These external malformations were considered treatment related and toxicologically relevant.

External malformations observed in the low and mid-Atrigel control groups and in the 78 and 155 mg/kg buprenorphine groups were seen either in a single fetus or were not dose-responsive and considered unrelated to treatment. The few external variations observed in the Atrigel control groups (low, mid, and high) and/or the RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine) were seen at a low incidence, comparable to the saline control group, or generally within historical control data and were considered spontaneous and unrelated to treatment.

No significant differences in the litter incidence of external malformations or variations were observed when comparing the RBP-6000-administered groups to their respective Atrigel control group.

Summary of External Malformations (% of Litters Affected)								
Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	78 mg/kg BUP	155 mg/kg BUP	390 mg/kg BUP	HC
Abdomen, gastroschisis	0.0	0.0	0.0	30.0[b]	0.0	0.0	0.0	NS
Genital tubercle, misshapen	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Umbilical omphalocele	0.0	0.0	0.0	30.0[b]	4.3	0.0	33.3[b]	5.6
Hindlimbs malrotated	0.0	0.0	0.0	30.0[b]	0.0	0.0	0.0	5.0
Tail absent	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Tail short	0.0	0.0	0.0	50.0[a]	4.3	0.0	8.3	5.0

[a]Statistically significant when compared to the saline control group
[b]Excessively higher than or not seen in recent historical control data
BUP – buprenorphine; HC – Historical Control; NS – Not seen in Historical Control

Fetal Visceral Examinations

A statistically significant (SS) increased litter incidence of total visceral malformations was observed at 390 mg/kg buprenorphine (66.7% of litters observed with a malformation) and in the high-Atrigel control group (90.0% of litters observed with a malformation) in comparison to 4.3% of litters in the saline control group (see Table). The individual visceral malformations observed were either seen at an increased incidence or have not been observed in historical control data (contained in report). These visceral malformations were considered treatment related and toxicologically relevant.

Similarly, a SS increased litter incidence of total visceral variations was observed at 390 mg/kg buprenorphine (83.3% of litters observed with a variation) and in the high-Atrigel control group (80.0% of litters observed with a variation) in comparison to 26.1% of litters in the saline control group (no Table). These included a smaller than normal ductus arteriosus at a SS higher litter incidence (40%) in the high-Atrigel control group and while not statistically significant, at 390 mg/kg buprenorphine was observed at a litter incidence of 16.7% and has not been observed in recent historical control data. The visceral variation, azygous lobe absent (right lung) was also SS higher in the high-Atrigel control group and the 390 mg/kg buprenorphine group at incidences of 60% and

58.3%, respectively). These visceral variations were considered treatment related and toxicologically relevant by the report.

The few other visceral malformations and variations observed in the Atrigel control groups (low, mid, and high) and/or the RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine) were seen at a low incidence, comparable to the saline control group, or generally within historical control data and were considered spontaneous and unrelated to treatment.

No significant differences in the litter incidence of visceral malformations or variations were observed when comparing the RBP-6000-administered groups to their respective Atrigel control group.

Summary of Visceral Malformations (% of Litters Affected)

Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	78 mg/kg BUP	155 mg/kg BUP	390 mg/kg BUP	HC
Colon, malpositioned	0.0	0.0	0.0	40.0[a,b]	0.0	0.0	8.3[b]	NS
Kidney, fused	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Kidney, malrotated	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	5.6
Spleen, smaller than normal	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Urinary bladder, misshapen	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Aortic arch, dilated	0.0	8.7	4.5	60.0[a,b]	4.3	0.0	41.7[a,b]	13.0
Interventricular septum, discontinuous	0.0	8.7	4.5	90.0[a,b]	4.3	0.0	41.7[a,b]	13.0
Pulmonary trunk, Smaller than normal	0.0	8.7	0.0	40.0[a,b]	0.0	0.0	16.7[b]	NS

[a]Statistically significant when compared to the saline control group
[b]Excessively higher than or not seen in recent historical control data
BUP – buprenorphine; HC – Historical Control; NS – Not seen in Historical Control

Fetal Skeletal Examination

A statistically significant (SS) increase in litter incidence of total skeletal malformations was observed at 155 and 390 mg/kg buprenorphine (31.8% and 91.7% of litters observed with a malformation, respectively) and in the high-Atrigel control group (100% of litters observed with a malformation) in comparison to 4.3% of litters in the saline

control group (see Tables for effects in litter and fetuses). While not always SS, all other treatment group litters (Atrigel and buprenorphine had increase in incidence of total skeletal malformations per litter compared to saline control. For skeletal variations, from 91.3-100% of litters were affected across all groups including saline control but the percent of fetuses affected was higher in the high dose Atrigel (97.7%) and high dose buprenorphine (90.9%) groups compare to the other groups which were comparative at a range of 49.2-55.7% including the saline control. In addition, at 155 mg/kg buprenorphine, an increased litter incidence of skeletal malformations was observed. The majority of skeletal malformations observed at 155 mg/kg buprenorphine were also observed in the high-Atrigel control and at 390 mg/kg buprenorphine group and were either not seen or were observed at a higher litter incidence that recent historical control data. These effects on fetal development were considered test article-related and toxicologically relevant.

Overall, skeletal malformations (% of litters) were: saline control (4.3%); low (34.8%), mid (22.7%), and high (100%) dose Atrigel; and low (21.7%), mid (31.8%0) and high (91.7%) dose buprenorphine. These skeletal malformations were considered seen at a low incidence, comparable to the saline control, not dose-responsive or within historical control data and considered spontaneous and unrelated to treatment.

Summary of Skeletal Malformations and Developmental Variations

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
No. Litters Evaluated	23	23	22	10
No. Fetuses Evaluated	189	194	183	44
Total Malformations				
No. Litters(%)	1 (4.3)	8 (34.8)[a]	5 (22.7)	10 (100.0)[b]
No. Fetuses(%)[1]	1 (0.5)	12 (6.2)	6 (3.3)	33 (75.0)
Total Variations				
No. Litters(%)	22 (95.7)	23 (100.0)	22 (100.0)	10 (100.0)
No. Fetuses(%)[1]	95 (50.3)	108 (55.7)	90 (49.2)	43 (97.7)

Summary of Skeletal Malformations and Developmental Variations

Observation	78 mg/kg	155 mg/kg	390 mg/kg
No. Litters Evaluated	23	22	12
No. Fetuses Evaluated	190	167	44
Total Malformations			
No. Litters(%)	5 (21.7)	7 (31.8)[a]	11 (91.7)[b]
No. Fetuses(%)[1]	6 (3.2)	12 (7.2)	21 (47.7)
Total Variations			
No. Litters(%)	21 (91.3)	22 (100.0)	11 (91.7)
No. Fetuses(%)[1]	100 (52.6)	91 (54.5)	40 (90.9)

No.- Number

[1] Not statistically analyzed
[a] Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[b] Significantly different from 0 mg/kg (Saline); ($p<0.01$)

Individual fetal skeletal malformations based on the percent of litters affected are contained in the following tables. Other than some increases in the high dose buprenorphine groups but never greater than the high dosed Atrigel group, no buprenorphine-specific effects were observed with the NOAEL being the low dose burpehorphine groups and the LOAEL being the mid-dose buprenorphine group.

Summary of Skeletal Malformations (% of Litters Affected)								
Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	78 mg/kg BUP	155 mg/kg BUP	390 mg/kg BUP	HC
Caudal Vertebra(e), all absent	0.0	0.0	0.0	20.0	0.0	0.0	0.0	NS
Caudal Vertebra(e), one or more absent	0.0	0.0	0.0	60.0[a]	4.3	0.0	0.0	NS
Caudal Vertebra(e), one or more fused	0.0	0.0	0.0	40.0[a]	0.0	0.0	16.7[b]	5.0
Cervical Vertebra(e), centra fused	0.0	4.3	4.5	20.0[b]	4.3	0.0	0.0	5.3
Cervical Vertebra(e), neural arches absent	0.0	0.0	0.0	50.0[a]	0.0	0.0	16.7[b]	NS
Cervical Vertebra(e), neural arches fused	0.0	0.0	4.5	20.0[b]	0.0	0.0	8.3	5.0
Cervical Vertebra(e), neural arches misaligned	0.0	0.0	4.5	30.0[b]	0.0	0.0	0.0	5.3
Cervical Vertebra(e), neural arches misshapen	0.0	4.3	4.5	70.0[a]	4.3	0.0	58.3[a]	8.7
Lumbar Vertebra(e), neural arches absent	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	5.3
Lumbar Vertebra(e), neural arches misshapen	0.0	0.0	0.0	20.0[b]	0.0	4.5	0.0	NS

Summary of Skeletal Malformations (% of Litters Affected)								
Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	78 mg/kg BUP	155 mg/kg BUP	390 mg/kg BUP	HC
Ribs, costal cartilage fused	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	10.5
Ribs, absent	0.0	4.3	0.0	50.0[a]	0.0	0.0	16.7[b]	9.1
Ribs, branched	0.0	4.3	0.0	20.0[b]	0.0	4.5	0.0	5.0
Ribs, fused	0.0	4.3	4.5	90.0[a]	0.0	9.1	50.0[a]	14.3
Sacral Vertebra(e), centra fused	0.0	0.0	0.0	20.0[b]	0.0	4.5	8.3	NS
Sacral Vertebra(e), neural arches absent	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Sacral Vertebra(e), neural arches fused	0.0	0.0	0.0	20.0[b]	0.0	4.5	0.0	NS
Sacral Vertebra(e), neural arches misaligned	0.0	0.0	0.0	20.0[b]	0.0	4.5	0.0	NS
Sacral Vertebra(e), neural arches misshapen	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Skull, exoccipital bone misshapen	0.0	0.0	0.0	20.0[b]	0.0	0.0	8.3	NS
Sternum, Sternebra(e) fused	4.3	17.4	9.1	30.0[b]	8.7	18.2	8.3	26.1
Thoracic Vertebra(e), centra absent	0.0	4.3	0.0	40.0[a]	0.0	4.5	0.0	4.5
Thoracic Vertebra(e), centra fused	0.0	4.3	0.0	30.0[b]	4.3	0.0	8.3	5.9
Thoracic Vertebra(e), neural arches absent	0.0	4.3	0.0	60.0[a]	0.0	0.0	16.7[b]	9.1
Thoracic Vertebra(e), neural arches fused	0.0	8.7	0.0	70.0[a]	0.0	9.1	16.7[b]	9.5
Thoracic Vertebra(e), neural arches misaligned	0.0	8.7	0.0	50.0[a]	0.0	0.0	25.0[b]	5.3
Thoracic Vertebra(e), neural arches misshapen	0.0	13.0	4.5	60.0[a]	0.0	9.1	16.7[b]	9.5

[a]Statistically significant when compared to the saline control group
[b]Excessively higher than or not seen in recent historical control data
BUP – buprenorphine; HC – Historical Control; NS – Not seen in Historical Control

Individual skeletal variations were observed on a per litter basis (see Table) in the high-Atrigel control group and the 390 mg/kg buprenorphine group that were either statistically significant in comparison to the saline control group, seen at an increased

litter incidence to historical control data, or have not been observed in historical control data and are summarized in the following Table. The increased incidence of these skeletal variations in the high-Atrigel control group and the 390 mg/kg buprenorphine-administered group were considered treatment related and toxicologically relevant.

Summary of Skeletal Variations (% of Litters Affected)

Malformation Type	Saline Control	Low-ATRIGEL® Control	Mid-ATRIGEL® Control	High-ATRIGEL® Control	78 mg/kg BUP	155 mg/kg BUP	390 mg/kg BUP	HC
Cervical vertebra(e), centra additional ossification center	4.3	4.3	22.7	80.0[a,b]	13.0	36.4[a,b]	83.3[a,b]	14.3
Cervical vertebra(e), hemicentric centra	0.0	0.0	0.0	50.0[a,b]	0.0	0.0	25.0[b]	5.3
Cervical vertebra(e), neural arches additional ossification center	13.0	8.7	36.4	70.0[a,b]	4.3	4.5	8.3	19.0
Ribs, costal cartilage misaligned	0.0	0.0	0.0	30.0[b]	0.0	0.0	0.0	9.1
Sacral vertebra(e), centra absent	0.0	0.0	0.0	30.0[b]	0.0	0.0	0.0	NS
Sacral vertebra(e), centra hemicentric	0.0	0.0	0.0	20.0[b]	0.0	0.0	0.0	NS
Skull, interparietal bone bipartite	4.3	4.3	13.6	30.0[b]	0.0	9.1	41.7[b]	11.1
Skull, parietal bone, additional ossification center	0.0	4.3	0.0	40.0[a,b]	8.7	4.5	8.3	22.7
Sternum, sternebra(e) unossified	39.1	56.5	72.7	90.0[b]	21.7	50.0	58.3	71.4
Thoracic vertebra(e), centra hemicentric	0.0	4.3	0.0	70.0[a,b]	0.0	4.5	25.0[b]	11.1

[a]Statistically significant when compared to the saline control group
[b]Excessively higher than or not seen in recent historical control data
BUP – buprenorphine; HC – Historical Control; NS – Not seen in Historical Control

Maternal Macroscopic Observations

Maternal necropsy findings tended to correlate with the clinical observations resulting in descriptions related to the injection site(s). Findings related to the injection site(s) in the Atrigel control groups (low, mid, and high) and the RBP-6000-administered groups (78, 155, and 390 mg/kg buprenorphine) included abrasions/scabs, skin discoloration, and foreign material (test material implant). The few other macroscopic findings observed were seen infrequently and considered unrelated to treatment.

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9.3 Prenatal and Postnatal Development

Study title: A Pre- and Post- Natal Development Study including Maternal function of RBP-6000 (Buprenorphine in the Atrigel® Delivery System) Administered by Subcutaneous injection in the Sprague-Dawley Rat

Study no.:	INLS-R104-60-16
Study report location:	eCTD 4.2.3.5.3; SDN 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 27, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	- RBP-6000 (Buprenorphine/ATRIGEL - 18% buprenorphine base in (b) (4)% 50/50 Poly(DL-lactide-co-glycolide) (PLGH) and (b) (4)% N-methyl-2-pyrrolidone (NMP)) - 100 mg, Lot 207, (b) (4)% of buprenorphine content - (b) (4)% NMP - ATRIGEL® vehicle (b) (4)% 50/50 PLGH and (b) (4)% NMP) - 100 mg placebo, Lot 157, (b) (4)% NMP - 0.9% Sodium chloride, Lots 52-071-JT, 53-027-JT, 54-017-JT, 56-025-JT

Key Study Findings

- Twenty-five (25) timed pregnant female Sprague Dawley rats/group were tested to evaluate the possible adverse effects of 0 (saline), 50, 150, and 300 mg/kg of buprenorphine in RBP-6000 (18% buprenorphine base in the ATRIGEL® Delivery System) on the pregnant/lactating female and on the development of the conceptus and the offspring following exposure of the female during implantation (Gestation Day 7) during weaning (Lactation Day 7). Developmental evaluation of the offspring included evaluations of behavior and fertility. In addition, the developmental toxicity profile (including the above-mentioned endpoints) of the Atrigel Delivery System was also evaluated at equivalent amounts of Atrigel as delivered to the RBP-6000-administered groups.
- For this complete GLP study, all appropriate indices of a pre/post-natal study were evaluated except no toxicokinetic (TK) information was obtained. TK information for

the female rat was obtained at 300 mg/kg buprenorphine in RBP-6000 in a previous study on Day 1 and Gestation Day 7, approximately 22-38 days apart.

- No adverse effects from administration of RBP-6000 (50, 150, and 300 mg/kg buprenorphine) or the Atrigel Delivery System control (low, mid, and high) in P females were observed on survival, clinical observations, gestation and lactation body weights, body weight change, food consumption, parturition, weaning of the F1 offspring, or macroscopic observations.
- No effects were observed on F1 survival, clinical observations, sexual maturation, behavioral assessments (passive avoidance and motor activity), reproductive parameters (mating, fertility, and fecundity indices), macroscopic findings or GD 13 Cesarean section data.
- A transient reduction in male F1 body weights at 150 and 300 mg/kg buprenorphine (8% to 13% lower than saline controls and 8% to 16% lower than each respective Atrigel control group) was observed throughout the lactation and early post-weaning periods (PND 21 through PND 28), but body weights returned to saline control levels during the F1 growth phase. F1 body weights in the 50 mg/kg buprenorphine group and the Atrigel control groups (low, mid, and high) were unaffected. No other adverse effects were observed.
- On the basis of the transient F1 male body weight effects, the NOAEL/LOAEL is 300 mg/kg.

Methods

Doses:

Group Assignments		
Group Number	Dose Level of Buprenorphine Base (mg/kg)	Number of Time-mated Females
1	0 (Saline)	25
2	0 (Control 1)[a]	25
3	0 (Control 2)[a]	25
4	0 (Control 3)[a]	25
5	50	25
6	150	25
7	300	25

[a] The ATRIGEL® control groups received an approximately equivalent amount of ATRIGEL® as delivered to the treated groups (0.21, 0.62, or 1.23 mL/kg/injection).

The dose levels were selected based on available data from previous studies including dose range finding study INRS-R148-60-16.

Frequency of dosing: Twice (GD 7 and LD 7)

Dose volume: See study design (Atrigel controls received same volume of Atrigel as received by respective RBP-6000 group

Route of administration: Subcutaneous to the shaved back. Neck collars were used for approximately 48-hours post dose to prevent access to the dosing site.

Formulation/Vehicle: Atrigel

Species/Strain: CD® [Crl:CD® Sprague Dawley rats

Number/Sex/Group: 25 time-mated females

Satellite groups: No. No toxicokinetic evaluation.

Study design: See below

Deviation from study protocol: Nothing remarkable.

Study Design

Dosing Regimen				
Group Number	Buprenorphine Dose Level (mg/kg)	Total Dose Volume (mL/kg)	Buprenorphine Base Dose Concentration (% w/w)	Number of Injection Sites per Dosing Day
1	0 (Saline)	1.5	0	1
2	0 (Control 1)[a]	0.21	0	1
3	0 (Control 2)[a]	0.62	0	1
4	0 (Control 3)[a]	1.23	0	1
5	50	0.25	18	1
6	150	0.75	18	1
7	300	1.5	18	1

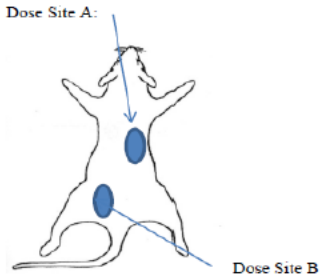
[a] The ATRIGEL® control groups received an approximately equivalent amount of ATRIGEL® as delivered to the treated groups (0.21, 0.62, or 1.23 mL/kg/injection).

Dose Levels of Buprenorphine Base Relative to RBP-6000	
Buprenorphine base (mg/kg)	RBP-6000 (mg/kg)
50	(b) (4)
150	
300	

Dose Levels of ATRIGEL® Delivery System and its Components			
Group Description	ATRIGEL® Delivery System (mg/kg)	NMP (mg/kg)	PLGH (mg/kg)
Low-ATRIGEL (Control 1) Mid-ATRIGEL (Control 2) High ATRIGEL (Control 3)	(b) (4)		

NMP - *N*-methyl-2-pyrrolidone; PLGH – poly(lactide-co-glycolide)

Dosing – On the back to Site A on GD 7 and to Site B on LD 7.



Methods

Mortality

All animals were observed for mortality twice daily.

Clinical Signs

All animals were observed for morbidity and injury twice daily. Additionally, collar checks were performed every 12 hours from collar application until removal. Once daily for 3 days after each dose administration (75 minutes ±15 minutes postdose on dosing days) and once weekly thereafter, each animal was removed from the cage and given a detailed clinical examination. Animals were also given a detailed clinical examination prior to necropsy. On occasion, clinical observations were recorded at unscheduled intervals. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration.

F1 animals that continued on study were given a similar detailed examination weekly. These examinations began after all animals were selected.

Food and Water Consumption

All animals were observed for the availability of food and water twice daily. Individual food consumption for the P females was recorded on the corresponding body weight days and calculated for the same intervals.

Food consumption was not recorded for the F1 animals.

Body Weights and Body Weight Changes

Individual body weights were recorded for all P (parental) females on GD 7, 10, 13, 18, and 20, and on LD 0, 4, 7, 10, 14, 17, and 21. Body weight change was calculated for the following intervals: GD 7-10, 10-13, 13-18, 18-20, and 7-20, and LD 0-4, 4-7, 7-10, 10-14, 14-17, 17-21, and 0-21.

Body weight measurements for the F1 animals began after all the animals were selected. Individual body weights were recorded weekly for the F1 males until termination. The F1 females were weighed weekly until positive evidence of copulation was observed. During gestation, F1 female body weights were recorded on GD 0, 7, 10, and 13, and body weight change was calculated for the following GD intervals: 0-7, 7-10, 10-13, and 0-13.

P Parturition and F1 Litter Observations

Toward the end of the gestation period, P females were examined twice daily for signs of parturition. The mated females were allowed to give birth (F1). The duration of

gestation was calculated, and any difficulties occurring at parturition were recorded. The day on which all pups were delivered was designated as LD 0. The litters were examined as soon as possible after delivery. Litter size, number of stillborn and liveborn pups, number of males and females, individual body weights, and gross abnormalities of the pups were recorded for each litter.

On LD 0, all live pups from each litter were tattooed to maintain individual identity, and eight pups of equal sex distribution (four males and four females), when possible, were randomly selected. These eight pups from each litter were evaluated for various behavioral and developmental indices. Litters were housed with the dams for 3 weeks after birth (LD 0 to 21). The dams and litters were observed daily for survival and behavioral alterations in nesting and nursing, and the presence of dead pups was recorded.

Pups were individually weighed and examined externally on LD 0, 4, 7, 14, and 21.

F1 Behavioral and Developmental Indices

Static Righting Reflex - On LD 2, each pup was tested for a complete righting response within a 15-second time period. Pups that did not exhibit the response initially were retested daily until the response was observed.

Pinna Detachment - On LD 2, each pup was observed for unfolding of the pinna. Pups that did not have both pinnae detached on LD 2 were observed daily until detachment was complete.

Cliff Aversion - On LD 11, prior to eye opening, each pup was tested for cliff aversion. Pups not perceiving depth by moving away from the edge on LD 11 were retested daily until a response was observed.

Eye Opening - On LD 13, each pup was observed for eye opening. The development was considered complete when both eyes were fully open. Pups not achieving this landmark on LD 13 were observed daily until both eyes were opened.

Air Drop Righting Reflex - On LD 16, each pup was tested for air drop righting reflex. A righting reflex was recorded as present for those pups that were able to turn over in the air and land upright on all four legs when dropped from a height of approximately 30 cm. Any pup that did not respond on LD 16 was tested on a daily basis until the response was observed.

Neuropharmacological Evaluation - On LD 21, after weaning was complete, each pup was given a neuropharmacological evaluation

Auditory Response - Each pup was evaluated at PND 22 for auditory response. Each pup was observed for movement of the ears in response to a sound emitted from a Galton whistle at a distance of 25 cm. Three trials were performed.

Vaginal Opening - Beginning on PND 28, F1 female pups selected to continue on study for behavioral and reproductive assessment were examined for the presence of vaginal opening. Pups that did not demonstrate vaginal opening were examined daily until this landmark was observed. A body weight was measured and recorded on the day each animal achieved this landmark.

Preputial Separation - Beginning on PND 35, F1 male pups selected to continue on study for behavioral and reproductive assessment were examined for preputial separation. Pups that did not show complete retraction of the penile prepuce were examined daily until this landmark was observed. A body weight was measured and recorded on the day each animal achieved this landmark.

Measurement of Motor Activity - Motor activity was evaluated on pups selected to continue on study. The activity of each pup was assessed at 37 days of age using a Hamilton Kinder Motor Monitor System equipped with an electronic analyzer-recorder. The test period was 20 minutes (four 5 minute intervals per animal). Movement was recorded by 16 photocell sensors. A sensor check was conducted daily during the testing period. Basic movements, fine movements, rearing counts, and total distance were recorded.

Step-through Passive Avoidance Test - Learning and memory were evaluated on pups selected to continue on study using the step-through passive avoidance test. Testing initiated between 70 and 85 days of age for each selected rat. The test was conducted in a fully automated, computerized system consisting of light and dark components separated by a mechanical door. In each trial, animals moving to the dark compartment were shocked. Animals were considered to have learned the appropriate response (i.e., not to leave the light compartment) if they did not pass into the dark compartment for two consecutive 3 minute trials. Animals were evaluated for a maximum of five trials on the day of testing.

Selection of F1 Rats - The pups were weighed individually and observed on PND 28. A minimum of one male and one female pup from each litter in each group was randomly selected to continue on study for assessment of sexual maturation, and behavioral and reproductive performance. A maximum of 25 males and 25 females per group were selected. If there were less than 25 litters in a group, additional pups were chosen from randomly selected litters. At PND 28, the remaining offspring from each litter were euthanized and subjected to a necropsy.

F1 Breeding Procedures (Reproductive/Fertility Assessment) - When the selected F1 animals were at least 80 days of age and the step-through passive avoidance testing was complete, males and females of the same treatment group were placed together in the cage of the male at a ratio of 1:1 for mating. Care was taken to avoid pairing siblings. Daily inspection for a copulatory plug in situ or vaginal lavage for sperm was performed by 10:00 a.m. daily on the females during the cohabitation period. The day on which positive evidence of copulation was observed was considered GD 0. After

evidence of mating was observed, the female was returned to an individual cage for the remainder of the study.

The maximum pairing period was 20 days, at the end of which any females with no confirmed evidence of mating were returned to individual cages until scheduled euthanasia. All females with no confirmed mating date that appeared to be nonpregnant on the basis of body weight and shape were euthanized 13 days after the last scheduled pairing day and examined. Females with unconfirmed mating dates that appeared to be pregnant on the basis of body weight and shape were euthanized as identified to prevent delivery in the cage and loss of the litter as described in the Uterine and Ovarian Examinations Section. These data are reported individually, but not statistically analyzed.

Termination of F1 Males - After the cohabitation period, the F1 males were individually housed until completion of the uterine examinations. After determination that there was no evidence of impaired fertility, the males were euthanized and subjected to a necropsy with emphasis placed on congenital anomalies.

Necropsy and Histopathology

After weighing on LD 4, each litter was reduced to the eight randomly selected pups from LD 0. The culled pups were euthanized and examined for external abnormalities. Pups with abnormalities were fixed and saved for possible future examination, and the remaining carcasses were discarded. Any intact, dead pups or pups euthanized *in extremis* were necropsied to the fullest possible extent, examined for anomalies, and pups with abnormalities were fixed and saved for possible future examination. In addition, the hearts of these pups were dissected. Pups found dead on LD 0 had their lungs removed and placed in tap water to determine if they floated (i.e., liveborn) or sank (i.e., stillborn). In the event stillborn status could not be determined, the pup was considered liveborn for reporting purposes.

Any females with total litter loss were euthanized and subjected to a necropsy and the number of uterine implantation scars was recorded. On LD 21, the P females were euthanized and subjected to a necropsy and the number of uterine implantation scars was recorded.

Necropsy examinations were performed on all animals (P and selected F1) found dead, euthanized *in extremis*, or at scheduled termination, and F1 pups not selected to continue on study. Gross lesions from all animals were saved in 10% neutral buffered formalin. Collection of gross lesions from treated animals necessitated collection of sufficient corresponding tissues from control animals for comparison purposes. The carcasses were then discarded.

Observations and Results

F_0 Dams

Survival: All females in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (50, 150 and 300 mg/kg buprenorphine) survived to the scheduled necropsy.

Clinical signs: General - Swelling of the face, lacrimation, and material around the eyes and/or nose (black and/or red) in a few animals (up to 8) was observed during the gestation and lactation periods in either the saline control, Atrigel, and/or the RBP-6000 administered groups. These findings generally occurred following the dose and application of the collar and were considered related to improperly fitted collars.

Detailed - The majority of clinical signs in the study were related to the injection site(s). Swelling at the injection site(s) was the most prevalent observation during the study period and was seen in all animals in the ATRIGEL® control (low, mid, and high) and RBP-6000 administered groups (50, 150 and 300 mg/kg buprenorphine). This observation was not considered adverse by the report because this was an expected finding associated with the subcutaneous delivery of the formulation and formation of the drug depot. Other findings at the injection site(s) included scabbing, which occurred at a higher incidence than the treated groups versus the saline control (up to 22 animals vs. 8), skin discoloration and abrasion(s).

Body weight: Gestation Body Weight and Body Weight Change – Mean gestation body weight and body weight change in the RBP-6000 administered groups (50, 150, and 300 mg/kg buprenorphine) and Atrigel control (low, mid, and high) groups were generally comparable to the saline control group (see Tables). Mean gestation body weight and body weight change in the RBP-6000 administered groups (50, 150, and 300 mg/kg buprenorphine) were generally comparable to their respective ATRIGEL® control group (low, mid, or high). There were some differences which were not dose responsive, sporadic, and not of notable difference.

Lactation Body Weights and Lactation Body Weight Changes – Mean lactation body weights and body weight changes for the buprenorphine groups were generally comparable to the saline control group (see Tables). The differences were slight in magnitude (<10%), not dose-responsive, and while potentially treatment-related, as they were decreases, are not considered adverse. Mean lactation body weight and body weight change in the Atrigel control (low, mid, and high) groups were also comparable to the saline control group and unaffected by treatment. All changes were considered incidental and not toxicologically relevant.

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Food consumption: Gestation - Mean gestation food consumption in the RBP-6000 administered groups (50, 150, and 300 mg/kg buprenorphine) were generally comparable to the saline control group. A statistically lower (-8% and -9%) mean food consumption was observed from GD 13 to GD 18 at 50 and 300 mg/kg buprenorphine, respectively, and 11% lower from GD 7 to GD 20 at 300 mg/kg buprenorphine in comparison to the saline control group. These differences in mean food consumption were not dose-responsive, did not impact mean gestation body weight adversely, and were not considered toxicologically meaningful. Mean gestation food consumption in the ATRIGEL® control groups (low, mid, or high) were comparable to the saline control group and unaffected by treatment.

Mean gestation food consumption in the 150 mg/kg buprenorphine was statistically lower (-10%, -18%, and -13%) from GD 10 to GD 13, GD 13 to GD 18, and GD 7 to GD 20, respectively, in comparison to the mid-Atrigel control group. At 300 mg/kg buprenorphine, mean food consumption was statistically lower (-42%, -12%, and -14%) from GD 7 to GD 10, GD 13 to GD 18, and GD 7 to GD 20, respectively, in comparison to the high-Atrigel control group. These differences at 150 and 300 mg/kg buprenorphine in comparison to each respective Atrigel control group were considered related to buprenorphine administration.

Lactation - Mean lactation food consumption in the RBP-6000 administered groups (50, 150, and 300 mg/kg buprenorphine) were generally comparable to the saline control group. The exception to this was at 150 mg/kg buprenorphine, in which mean food consumption values from LD 17 to LD 21 and LD 0 to LD 21 were statistically lower (-12% and -9%, respectively), in comparison to the saline control group. These differences were slight in magnitude, did not impact lactation body weights adversely, were not dose-responsive, and therefore not considered treatment-related or toxicologically relevant. Mean lactation food consumption in the Atrigel control groups (low, mid, or high) were comparable to the saline control group and unaffected by treatment.

Mean lactation food consumption at 150 mg/kg buprenorphine was statistically lower during all intervals of the lactation period (LD 0 to LD 4, LD 4 to LD 7, LD 7 to LD 10, LD 10 to LD 14, LD 14 to LD 17, LD 17 to LD 21, and LD 0 to LD 21) and ranged from 11% to 18% lower in comparison to the mid-Atrigel control group. At 300 mg/kg buprenorphine, mean food consumption was statistically lower (-12%, and -9%) from LD 17 to LD 21 and LD 0 to LD 21, respectively, in comparison to the high-Atrigel control group. These differences in mean food consumption at 150 and 300 mg/kg buprenorphine in comparison to each respective Atrigel control group were not dose-

responsive and considered unrelated to treatment. Mean lactation food consumption at 50 mg/kg buprenorphine was comparable to the low-Atrigel control group.

Necropsy observation: Macroscopic – Maternal necropsy findings tended to correlate with the clinical observations resulting in descriptions related to the injection site(s). Findings related to the injection site(s) in the Atrigel control groups (low, mid, and high) and the RBP-6000 administered groups (50, 150, and 300 mg/kg buprenorphine) included abrasions/scabs, skin discoloration, and foreign material (test material implant). The few other macroscopic findings observed were seen infrequently and considered unrelated to treatment.

Toxico-kinetics: Not evaluated

Dosing Solution Analysis: Fresh control articles, 0.9% Sodium Chloride for Injection, USP and ATRIGEL® placebo, were dispensed for use on study prior to each dose and were stored refrigerated at 2 to 8°C. The test article, RBP-6000 (18% buprenorphine base in ATRIGEL® Delivery System), was used as received from the Sponsor. The test article was administered neat (undiluted). Formulations were dispensed prior to each dose and were stored refrigerated at 2 to 8°C.

Other: None

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F1 Pup Survival: Mean litter sizes were unaffected by treatment as there were no treatment effects in the RBP-6000 groups (50, 150, and 300 mg/kg buprenorphine), the Atrigel control groups, and saline controls on LD 4 post-cull and for the remainder of lactation to weaning and on PND 28. The buprenorphine groups were also comparable to each respective Atrigel group.

While the pup lactation index was unaffected by treatment, the pup viability index (mean percent per litter) was decreased in the saline control and buprenorphine groups with large standard deviations compared to the Atrigel control groups (see Tables). The toxicological relevance is unknown as the decrease was not dose related and only the mid-dose RBP-6000 groups showed a statistical difference compared to saline control.

Summary of P Natural Delivery and Litter Data

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Pup Survival Indices					
Viability Index	Mean %/Litter	93.98	98.41	97.54	99.07
	SD	20.141	3.260	4.167	2.622
	N	25	25	25	25

Endpoint		50 mg/kg	150 mg/kg	300 mg/kg
Pup Survival Indices				
Viability Index	Mean %/Litter	89.75	77.86[a,f]	89.89
	SD	21.114	24.905	20.838
	N	25	24	25

N- Number of measures used to calculate mean
SD- Standard Deviation
No.- Number

[a]Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[f]Significantly different from 0 mg/kg (Control 2); ($p<0.01$)

F1 Clinical signs:

No effect of RBP-6000 or Atrigel control was observed from the pre-weaning (PND 0 to PND 21) and post-weaning (PND 28) for F1 pup detailed clinical examinations. The few findings observed among the F1 pups occurred at low incidence and were considered unrelated to treatment, one such being increased activity in 14 preweaning high-dose RBP-6000 pups but no others in the other groups. The overall number of observations are contained in the Tables for pre- and post-weaning.

Summary of F_1 Preweaning Pup Detailed Clinical Observations[+] - MALE AND FEMALE

Days 0 to 21

Observation	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Number of Animals Alive at Start of Interval	312	314	308	316
Number With No Abnormalities Detected	276	296	290	282

Observation	50 mg/kg	150 mg/kg	300 mg/kg
Number of Animals Alive at Start of Interval	259	262	300
Number With No Abnormalities Detected	229	237	268

F1 Pup Body Weights: At birth, mean pup body weights were statistically lower in males, females, and sexes combined at 150 mg/kg buprenorphine and in females at 300 mg/kg buprenorphine in comparison to each respective Atrigel control group. Beginning on PND 4 (pre-culling) through weaning (PND 21) and PND 28, statistically lower mean pup body weights (male, females, and sexes combined) were observed at 150 and 300 mg/kg buprenorphine in comparison to saline controls and each respective Atrigel control group (see Table – data only for sexes combined). These differences at 150 and 300 mg/kg buprenorphine were generally statistically significant relative to saline controls and each respective Atrigel control group in both sexes and were considered consistent with a delay in development and related to buprenorphine administration in the P females. Mean F1 pup body weights in the 50 mg/kg buprenorphine group and in the Atrigel control groups (low, mid, and high) were comparable to the saline control and unaffected by maternal treatment. At 50 mg/kg, mean F1 pup body weights were generally comparable to the low Atrigel control group.

Summary of Least Square Mean F_1 Pup Body Weights (g)
(% Difference from Saline Control/%Difference from ATRIGEL Control Group)[a]

Day	Saline Control	ATRIGEL low	ATRIGEL mid	ATRIGEL high	50 mg/kg	150 mg/kg	300 mg/kg
PND 0	6.83	7.27[b] (+6%)	7.12	7.14	7.03	6.49[d] (NA/-9%)	6.80
PND 4 (precull)	11.35	12.02	11.95	11.69	11.13[e] (NA/-7%)	10.05[c, d] (-11%/-16%)	10.40[c, d] (-8%/-11%)
PND 4 (postcull)	11.36	11.92	11.89	11.59	11.47	10.33[b, d] (-9%/-13%)	10.41[b, d] (-8%/-10%)
PND 7	18.98	19.95	19.92	19.59	19.00	17.11[c, d] (-10%/-14%)	17.02[c, d] (-10%/-13%)
PND 14	36.45	37.21	37.62	36.78	35.53	32.83[c, d] (-10%/-13%)	31.77[c, d] (-13%/-14%)
PND 21	57.08	59.10	59.63	58.22	56.81	51.60[c, d] (-10%/-13%)	51.35[c, d] (-10%/-12%)
PND 28	96.48	99.58	99.34	96.64	95.98	87.58[c, d] (-9%/-12%)	86.70[c, d] (-10%/-10%)

[a] Percent differences shown for statistically significant intervals only.
[b] Significantly different from Saline control (p value <0.05).
[c] Significantly different from Saline control (p value <0.01).
[d] Significantly different from ATRIGEL® control (p value <0.01).
[e] Significantly different from ATRIGEL® control (p value <0.05).

Pup Macroscopic Examination: No effect was observed from the macroscopic examination of stillborn pups, LD 4 culled pups (examined externally), pups found dead during lactation and PND 28 unselected pups in the RBP-6000 groups (50, 150, and 300 mg/kg buprenorphine) or the ATRIGEL® control groups (low, mid, and high). The few findings observed among pups occurred at low incidence or had a presence in control pups and were

considered unrelated to the treatment.

F1 Behavioral, Sensory, and Developmental Indices

Lactation Period: No effect from administration of Atrigel control (low, mid, and high) and RBP-6000 (50, 150, and 300 mg/kg buprenorphine) was observed from the F1 pup preweaning assessments for physical development, sensory response and reflex performance. A few statistical differences (lower or higher) were observed in the RBP-6000 administered groups in comparison to the saline control in the cliff aversion, eye opening, and air drop righting reflex evaluations, but were slight in magnitude, generally within or near historical control ranges (contained in report) and therefore not considered treatment related or adverse. Auditory response of the F1 pups was 100% in all groups.

F1 Sexual Maturation: No effect from administration of Atrigel and RBP-6000 was observed on F1 sexual maturation endpoints. The mean age for vaginal opening in the Atrigel control groups and the RBP-6000 groups ranged from 31.3 to 32.1 days and was comparable to the saline controls at 31.8 days. Mean body weights, in the RBP-6000 groups, when attaining vaginal opening were statistically lower (-8%, -7%, and -9%, respectively) at 50, 150, and 300 mg/kg buprenorphine in comparison to each respective Atrigel control group and was statistically lower (-9%) at 300 mg/kg buprenorphine in comparison to the saline control group. While these differences were consistent with the lower body weights observed in the RBP-6000 F1 pups throughout the lactation period and there was no impact on attainment of vaginal opening, these differences were not considered toxicologically relevant.

The mean age for preputial separation in the Atrigel control groups and the RBP-6000 groups ranged from 40.4 to 44.0 days and was comparable to the saline controls at 41.5 days. Mean body weights at attainment of preputial separation in the Atrigel control groups and the RBP-6000 groups were comparable to the saline controls and each respective Atrigel control group and unaffected by treatment.

Summary of F_1 Sexual Maturation

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Vaginal Opening (Days)	Mean	31.8	32.1	31.5	31.5
	SD	1.46	1.44	1.48	1.19
	No. of Pups Passing	25	25	25	25
Body Weight on Day Passed Vaginal Opening, g	Mean	115.9	125.0	116.0	115.3
	SD	15.73	13.92	10.14	12.50
	No. of Pups	25	25	25	25
Preputial Separation (Days)	Mean	41.5	40.4	40.7	42.2
	SD	3.10	1.61	1.34	2.68
	No. of Pups Passing	25	25	25	25
Body Weight on Day Passed Preputial Separation, g	Mean	233.9	228.7	231.8	240.0
	SD	35.00	18.76	26.85	30.85
	No. of Pups	25	25	25	25

Summary of F_1 Sexual Maturation

Endpoint		50 mg/kg	150 mg/kg	300 mg/kg
Vaginal Opening (Days)	Mean	31.5	31.6	31.3
	SD	1.56	1.08	1.25
	No. of Pups Passing	25	25	25
Body Weight on Day Passed Vaginal Opening, g	Mean	115.2[c]	108.2[e]	105.2[a,g]
	SD	10.30	7.91	9.54
	No. of Pups	25	25	25
Preputial Separation (Days)	Mean	41.2	44.0	41.9
	SD	1.96	7.74	2.11
	No. of Pups Passing	25	25	25
Body Weight on Day Passed Preputial Separation, g	Mean	232.2	241.6	226.3
	SD	18.67	70.11	25.21
	No. of Pups	25	25	25

SD - Standard Deviation
No. - Number

[a]Significantly different from 0 mg/kg (Saline); ($p<0.05$)
[c]Significantly different from 0 mg/kg (Control 1); ($p<0.05$)
[e]Significantly different from 0 mg/kg (Control 2); ($p<0.05$)
[g]Significantly different from 0 mg/kg (Control 3); ($p<0.05$)

F1 Motor Activity:

F1 Motor Activity and Learning and Memory Assessments – Postweaning

F1 Learning and Memory Evaluations:

No effect was observed on motor activity (e.g., basic movement, fine movement, rearing, total distance) in all RBP-6000 groups or all Atrigel control groups when compared to the saline control group. No effect was observed in the RBP-6000 groups when compared to each respective Atrigel control group.

No effect was observed on learning and memory from the passive

F1 Mortality:

avoidance testing in all RBP-6000 groups or all Atrigel control groups (low, mid, and high). In both male and female F1 animals, the incidence of passive animals (i.e., number of animals successfully passing the test) and the incidence of animals passing the test after the minimum of three trials were comparable to saline controls and each respective Atrigel control group.

F1 In-life Examinations

F1 Detailed Clinical Observations:

All F1 pups selected to continue on study in all RBP-6000 groups and all Atrigel control groups survived to scheduled termination except for 1 low-dose RBP-6000 male (sacrificed *in extremis* with cervical mass on Day 38) and one mid-dose Atrigel male (found dead Day 49, no clinical findings, red foci on thymus). No treatment-related findings were observed and because there was no mortality observed in the higher RBP-6000 groups and the high Atrigel control group, this mortality/morbundity was not considered treatment related.

F1 Body Weights and Body Weight Changes:

No apparent clinical effects were observed from the detailed clinical evaluations of the F1 pups in all the RBP-6000 groups or all the Atrigel control groups during the premating, mating, and gestation (females only) periods. Most findings observed in F1 animals occurred at low incidence or with comparable frequency as saline controls and each respective Atrigel control group. Apparent dose-response was observed in the RBP-6000 groups for sparse hair in males in the face and limbs which were also increased in the mid-and high-dose Atrigel groups. Clinical observations were considered incidental and unrelated to treatment.

No adverse effects on the growth of the F1 pups over the premating period (after PND 28) and over the pairing and postmating period were observed in all RBP-6000 groups or all Atrigel control groups. Mean F1 body weights for both males and females were generally comparable to the saline controls and each respective Atrigel control group throughout the study.

For F1 premating males, there were a few intervals when mean body weights in the 150 or 300 mg/kg buprenorphine groups were statistically significantly (SS) lower compared to mean saline control values. Premating male body weights were SS reduced at 150 mg/kg buprenorphine (-10% and -8% during premating Weeks 1 and 2, respectively), and at 300 mg/kg buprenorphine (-6% and -7%, during premating Weeks 7 and 8, and -7% on postmating Week 12). In addition, at 150 mg/kg buprenorphine, mean body weights were SS lower (-13%, -11%, and -8%) during Week 1, 2, and 3, respectively, in comparison to the mid-Atrigel control group. As these differences

occurred sporadically, the amount of change was slight, and were not clearly dose-responsive, they were considered incidental and unrelated to treatment. For F1 premating females, body weights were generally comparable to the saline controls and each respective Atrigel control group.

For F1 females, mean gestation body weight and body weight change in all RBP-6000 groups or all Atrigel control groups were generally comparable to the saline control group and RBP-6000 groups were comparable to each respective Atrigel control group. Mean body weights were up to 5% lower in the high buprenorphine group and up to 3% lower in the high-dose Atrigel groups compared to saline control and not considered toxicologically relevant. A SS higher mean body weight change (+55%) was observed during GD 10 to GD 13 (55%) and GD 0 to GD 13 (28%) in the low-dose, 50 mg/kg buprenorphine group in comparison to the low-Atrigel control group. Based on the direction of change (higher), these differences were considered incidental and not toxicologically meaningful. No differences were observed in other F1 female groups during gestation.

F1 Reproductive Performance:

No effect was observed on reproductive performance of the F1 animals in all RBP-6000 groups or all Atrigel control groups. Male and female mating indices in the RBP-6000 groups and the Atrigel control groups ranged from 96% to 100% and were comparable to the saline control group (100%).

Male and female fecundity indices (ratio of pregnant to number mated) in the RBP-6000 groups and the Atrigel control groups ranged from 84% to 100% and were comparable to the saline control group (92%) (see Tables). Fertility indices (ratio of pregnant to number paired) in the RBP-6000 groups and the Atrigel control groups ranged from 84% to 100% and were comparable to the saline control (92%). In addition, mating, fertility and fecundity indices were within recent historical control data (92% to 100% [mating index] and 84% to 100% [fertility and fecundity indices]) in both the RBP-6000 groups and the Atrigel control groups.

Summary of F_1 Reproductive and Fertility Parameters

Endpoint	0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)

Female Mating Index (%)	100.0	100.0	100.0	96.0
Female Fertility Index (%)	92.0	84.0	100.0	84.0
Female Fecundity Index (%)	92.0	84.0	100.0	87.5
Male Mating Index (%)	100.0	100.0	100.0	96.0

Summary of F_1 Reproductive and Fertility Parameters

Endpoint	50 mg/kg	150 mg/kg	300 mg/kg
Female Mating Index (%)	100.0	100.0	100.0
Female Fertility Index (%)	100.0	92.0	92.0
Female Fecundity Index (%)	100.0	92.0	92.0
Male Mating Index (%)	100.0	100.0	100.0

The mean Copulatory Interval (i.e., mean number-of-days to mating) in the RBP-6000 groups ranged from 2.9 to 4.2 days and in the Atrigel control groups ranged from 2.9 to 4.1 days and were comparable to the 3.0 days in the saline controls (see Tables).

Summary of F_1 Reproductive and Fertility Parameters

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Copulatory Interval (Days)					
	Mean	3.0	3.2	2.9	4.1
	SD	2.15	2.51	1.74	5.17
	N	25	25	21	24

Summary of F_1 Reproductive and Fertility Parameters

Endpoint		50 mg/kg	150 mg/kg	300 mg/kg
Copulatory Interval (Days)				
	Mean	3.0	2.9	4.2
	SD	2.27	3.02	4.60
	N	24	25	25

F1 Uterine Examination:

N - Number of measures used to calculate mean
SD - Standard Deviation

No effect was observed on GD 13 uterine and ovarian parameters in the F1 animals in all RBP-6000 groups or all Atrigel control groups (see Tables). The mean number of corpora lutea, uterine implantation sites, viable embryos and resorption sites per animal and mean pre- and post-implantation loss indices were comparable to the saline controls and each respective Atrigel control group.

Summary of F_1 Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg (Saline)	0 mg/kg (Control 1)	0 mg/kg (Control 2)	0 mg/kg (Control 3)
Corpora Lutea					
No. per Animal	Mean	17.6	17.0	17.3	16.5
	SD	2.76	2.73	3.08	4.21
	N	23	21	21	21
Implantation Sites					
No. per Animal	Mean	16.0	15.3	14.6	14.7
	SD	2.61	4.03	4.15	4.92
	N	23	21	21	21
Preimplantation Loss					
% per Animal	Mean	8.44	11.53	16.37	13.03
	SD	11.465	20.816	22.215	17.128
	N	23	21	21	21
Viable Embryos					
No. per Animal	Mean	15.3	14.4	13.9	13.4
	SD	2.67	3.92	4.02	5.54
	N	23	21	21	21
Postimplantation Loss					
% per Animal	Mean	4.60	10.15	4.49	12.41
	SD	6.225	21.310	6.256	18.605
	N	23	21	21	21
Resorptions: Early + Late					
No. per Animal	Mean	0.7	1.0	0.7	1.2
	SD	0.96	0.92	1.01	1.34
	N	23	21	21	21

F1 Macroscopic Examinations:

Summary of F_1 Maternal and Developmental Observations at Uterine Examination

Endpoint		50 mg/kg	150 mg/kg	300 mg/kg
Corpora Lutea				
No. per Animal	Mean	17.4	17.9	17.5
	SD	1.81	2.44	2.21
	N	24	23	23
Implantation Sites				
No. per Animal	Mean	16.3	16.3	16.3
	SD	1.16	2.05	1.77
	N	24	23	23
Preimplantation Loss				
% per Animal	Mean	5.76	8.27	6.36
	SD	6.439	10.656	8.383
	N	24	23	23
Viable Embryos				
No. per Animal	Mean	15.3	15.4	15.8
	SD	1.43	2.10	2.08
	N	24	23	23
Postimplantation Loss				
% per Animal	Mean	5.89	5.35	3.11
	SD	5.527	4.948	3.829
	N	24	23	23
Resorptions: Early + Late				
No. per Animal	Mean	1.0	0.9	0.5
	SD	0.91	0.81	0.59
	N	24	23	23

N - Number of measures used to calculate mean
SD - Standard Deviation
No. - Number

No effect was observed from the macroscopic examinations of the F1 animals in the RBP-6000 groups or all Atrigel control groups at scheduled termination of the reproductive phase (see Tables for overall summary). The few findings observed among the F1 animals occurred at low incidence or with similar frequency as saline controls and were considered unrelated to treatment.

Summary of F_1 Macroscopic Observations - MALE

Terminal

Tissue / Observation	Severity	0 mg/kg (Saline) DOS	0 mg/kg (Saline) SNC	0 mg/kg (Control 1) DOS	0 mg/kg (Control 1) SNC	0 mg/kg (Control 2) DOS	0 mg/kg (Control 2) SNC	0 mg/kg (Control 3) DOS	0 mg/kg (Control 3) SNC
Number of Animals Examined		0	25	0	25	1	24	0	25
all tissues									
within normal limits		0	22	0	23	0	20	0	23

Tissue / Observation	Severity	50 mg/kg DOS	50 mg/kg SNC	150 mg/kg DOS	150 mg/kg SNC	300 mg/kg DOS	300 mg/kg SNC
Number of Animals Examined		1	24	0	25	0	25
all tissues							
within normal limits		0	23	0	23	0	23

DOS - Died or euthanized on study
SNC - Scheduled necropsy

Summary of F_1 Macroscopic Observations - FEMALE

Terminal

Tissue / Observation	Severity	0 mg/kg (Saline) DOS	0 mg/kg (Saline) SNC	0 mg/kg (Control 1) DOS	0 mg/kg (Control 1) SNC	0 mg/kg (Control 2) DOS	0 mg/kg (Control 2) SNC	0 mg/kg (Control 3) DOS	0 mg/kg (Control 3) SNC
Number of Animals Examined		0	25	0	25	0	25	0	25
all tissues									
within normal limits		0	25	0	24	0	23	0	24

Tissue / Observation	Severity	50 mg/kg DOS	50 mg/kg SNC	150 mg/kg DOS	150 mg/kg SNC	300 mg/kg DOS	300 mg/kg SNC
Number of Animals Examined		0	25	0	25	0	25
all tissues							
within normal limits		0	25	0	22	0	22

DOS - Died or euthanized on study
SNC - Scheduled necropsy

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Sublocade is a once a month SC depot injection of buprenorphine intended to assist in the treatment of opioid use disorder. The Applicant is leveraging, in part, their previous data generated in support of Buprenex, Subutex, and Suboxone. However, given the novel route of administration and the use of a novel excipient, many of the pivotal studies needed to be repeated with the drug product formulation and augmented by data in the literature for the novel excipient, NMP.

Adequate data were provided to support the safety of the drug substance and drug product specifications. Although the DP specification for the drug product degradant (b) (4), were higher than tested in the local tissue toxicity studies, (b) (4) the injection site does not contain uniquely sensitive tissues. The proposed specifications are adequate.

Extensive extractable leachable studies on the container closure system were provided. Although adequate details were missing from the toxicology risk assessments for potential leachables, the review team was able to adequately justify the safety of the container closure system for this drug product, as this product is hoped to add to the current armamentarium for the treatment of opioid use disorder, which has risen to crisis level in the United States.

The Drug Product does contain a novel excipient, NMP, which was tested in part via the toxicology studies with the drug product formulation. However, as the NMP in the formulation dissipates into the surrounding tissues and is eliminated in the first few days after treatment with Sublocade, the safety assessment required augmentation with data from the literature, specifically for the reproductive and developmental effects of NMP. The existing literature, although a worst-case scenario as the NMP was dosed every day, provides a better assessment of the potential risk of NMP to the developing fetus because it is not known when a patient could be treated with Sublocade prior to conception and during pregnancy. As such, NMP exposure margins suggest greater risk than buprenorphine alone and should be included in the drug product labeling.

Potential human toxicity issues with Sublocade identified in nonclinical animal studies deal with local, injection site effects and potential systemic effects identified in single and repeat dose studies, an in vivo genotoxicity assay, and a battery of reproductive toxicity studies.

In terms of local tissue effects, the SC injection of a prolonged release polylactide co-glycolide (PLG) polymer-based drug product formulation is expected to result in an acute and chronic local tissue reaction primarily characterized by the formation of a local granulomatous tissue response. This response has both an acute and chronic component, the former being driven by the entire drug product including NMP and the latter being driven by the PLG and buprenorphine. The fate of the injected polymer can be predicted by the experiences with PLG-based depot injections. The polymer slowly

degrades to lactic acid and glycolic acid, which are then shunted through the Kreb cycle and used by the body. Because the breakdown products are ultimately used by the body, the radiolabeled distribution studies do not clearly characterize the half-life of the polymer. The injection site is rotated, and therefore, the local tissue effects are not likely to overlap between injections. The risk for potential local tissue reactions in humans, however, cannot be eliminated. The reader is referred to the clinical safety data for further details.

During the course of the review, the Division specifically noted that the existing repeat-dose toxicology studies demonstrated two findings that required justification for their potential clinical relevance, specifically, pancreatic acinar cell degeneration/apoptosis and alveolar macrophage infiltrates in the repeat-dose toxicology studies. The Applicant considered these findings to be secondary to the local inflammatory response and stress. They did note that pancreatic acinar cells did have an apparent treatment-related increase in acinar cell apoptosis and degeneration; however, they noted that this could be related to the decreased food consumption and weight loss noted in treated animals and therefore part of the an opioid-mediated stress response. As these findings are not expected to occur either from PLG depot injections and were not reported in the published studies with NMP, the most likely explanation is that they were due to the prolonged exposure to buprenorphine. Following discussions at the EOP2 meeting on these topics, pancreatic function was specifically monitored in the Phase 3 clinical studies and no adverse effects were noted (see clinical review). In addition, the lung infiltrates observed with RBP-6000 and Atrigel vehicle appeared to be incidental since additional studies that included a saline arm showed either comparable incidence in the vehicle arm as saline, or the findings were not observed in either groups.

Reproductive and developmental toxicity studies for buprenorphine have been completed via a variety of routes of administration leading to lengthy summary of adverse effects in the drug product labeling. Many of these findings are difficult to interpret clinically given the different routes of administration, none of which were the SL route as indicated for Subutex and Suboxone. The studies completed with Sublocade, however, are more relevant as the Sublocade drug product produces a consistent level of buprenorphine and avoids the repeated C_{max} values of an immediate-release formulation, which can confound study clinical relevance of the study results. The results of the studies with RBP-6000 suggest a relatively large safety margin with respect to buprenorphine. However, the studies with Sublocade cannot be used to characterize the risk of NMP, which shows very different kinetics than the buprenorphine and potentially greater risk to the developing fetus and subsequent offspring. Hence we recommend that the data with NMP be included in the labeling, as these data are probably more relevant to the risk of Sublocade than buprenorphine alone.

Based on the proposed Sublocade doses of 100 mg and 300 mg buprenorphine monthly by subcutaneous injection, nonclinical testing identified dose ratios/safety margins based on the nonclinical studies blood level-based nonclinical TK versus clinical PK values. In addition, Human Equivalent Dose (HED) safety margins were

calculated for the Atrigel Delivery System, N-methyl-2-pyrrolidone (NMP) and Poly(lactide-co-glycolide) (PLGH).

Safety Margins - Exposure Multiples are generally considered acceptable at >= 1. Note that NOAEL/LOAEL for acute rat study was a conservative interpretation of pancreatic effects that could be considered background, but were not in this case because of dose responsive incidence and severity. However the occurrence could have be spurious and occurred for unknown reasons/conditions.

Acute and Chronic Toxicity (TK-PK based)

Safety Margins for RBP-6000 Based on Maximum Buprenorphine and 24-hour Exposure (AUC_{0-24h})							
Animals						**Exposure Multiple Based on Human Exposure from Study RB-US-12-0005**	
Study	**Species**	**BUP NOAEL/ LOAEL (mg/kg)**	**Sex**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng·h/mL)**	**Based on SD C_{max} of 4.817 ng/mL[a]**	**Based on SD AUC_{0-24h} of 85.09 ng·h/mL[a]**
Single-dose Toxicology	Rat	10 10	M	4.32 2.53	32.9 13.0	0.9 0.5	0.4 0.15
	Dog	20 20	M F	16 16	214 [c] 180 [c]	3.3 3.3	2.5[c] 2.1 [c]
Study	**Species**	**BUP NOAEL (mg/kg)**	**Sex**	**C_{max} (ng/mL)**	**AUC_{0-24h} (ng·h/mL)**	**Based on MD C_{max} of 9.637 ng/mL[d]**	**Based on MD AUC_{0-24h} of 178.109 ng·h/mL[d]**
Repeat-dose Toxicology	Rat	10 10	M	68 142	653 590	7 15	4 3
	Dog	40 40	M F	123 80	1150 1071	13 8	6 6

BUP: Buprenorphine; C_{max}: Maximum plasma buprenorphine concentration; DART: Developmental and reproductive toxicity; F: Female; M: Male; MD: Multiple-dose; NOAEL: No observed adverse effect level; SD:Single-dose

[a] Overall PK parameter observed after 1st injection of RBP-6000 (single 300 mg buprenorphine dose) in multicenter, multiple-dose study of RBP-6000 in treatment-seeking opioid-dependent subjects, Study RB-US-12-0005. Patients were inducted and stabilized over a 13-day period on doses of Subutex at 8 to 24 mg prior to receiving their 1st injection.

[b] Average C_{max} calculated from 3M and 3F satellite animals (average of maximum from sampling at 1, 2 and 4 hours post--dose)

[c] Nonclinical AUC_{0-24h} were calculated for this study so safety margins could be converted from AUC_{0-last} (dog) or AUC_{0-336h}.

[d] Overall PK parameter observed after 4th injection (Day 85) of RBP-6000 (300 mg buprenorphine) in multicenter, multiple-dose study of RBP-6000 in treatment-seeking opioid-dependent subjects, Study RB-US-12-0005.

Animals						Exposure Multiple Based on Human Exposure from Study RB-US-12-0005	Exposure Multiple Based on Human Exposure from Study RB-US-13-0001	
Study	**Species**	**BUP NOAEL (mg/kg)**	**Sex**	**AUC_{0-672h} (ng·h/mL) for First Dose**	**AUC_{0-672h} (ng·h/mL) for Last Dose**	**Based on SD AUC_{0-672h} of 1268.012 ng·h/mL[a]**	**Based on MD AUC_{0-672h} of 3230.873 ng·h/mL[b]**	**Based on MD AUC_{0-672h} of 4370.848 ng·h/mL[c]**
Repeat-dose Toxicology	Rat	10	M	5513	5657	4.3	1.8	1.3
		10		3109	4607	2.5	1.4	1.1
	Dog	40	M	16646	25285	13	7.8	5.8
		40	F	14743	20819	12	6.4	4.8

BUP: Buprenorphine; DART: Developmental and reproductive toxicity; F: Female; M: Male; MD: Multiple-dose; ND: Not determined; NOAEL: No observed adverse effect level; SD: Single-dose

a Overall AUC_{0-672h} observed after 1st injection of RBP-6000 (single 300 mg buprenorphine dose) in multi-center, multiple-dose study of RBP-6000 in treatment-seeking opioid-dependent subjects, Study RB-US-12-0005. Patients were inducted and stabilized over a 13-day period on doses of Subutex at 8 to 24 mg prior to receiving their 1st injection.

b Overall PK parameter observed after 4th injection (Day 85) of RBP-6000 (300 mg buprenorphine) in multi-center, multiple-dose study of RBP- 6000 in treatment-seeking opioid-dependent subjects, Study RB-US-12-0005.

c Overall AUC_{0-672h} observed after 6th injection (Day 141) of RBP-6000 (300 mg buprenorphine) in Phase 3 multiple-dose study of RBP-6000 in treatment-seeking opioid-dependent subjects, Study RB-US-13-0001.

Reproductive Toxicity (TK-PK based)

Animal Doses of RBP-6000 (mg/kg)	Animal $AUC_{0\text{-}24h}$ (ng.h/mL)	Animal $AUC_{0\text{-}336h}$ (ng.h/mL)	MD Human $AUC_{0\text{-}24h}$ (ng.h/mL) of 178.1[a]	SD Human $AUC_{0\text{-}672h}$ (ng.h/mL) of 1268 [b]	Human Exposure Margins [c]
Fertility and Early Embryonic Development – Rat					
Females					
300	2720				15.3
600	4430				24.9
900	6710				37.7
Males					
300	5690				32
600	10100				56.7
900	14200				79.7
Embryofetal Development – Rat					
300	2720				15.3
600	4430				24.9
900	6710				37.7
Embryofetal Development – Rabbit [d]					
78	N/A	3010			2.4
155	N/A	7880			6.2
390	N/A	18700			14.7

a. Human $AUC_{0\text{-}24h}$ observed after 4th injection (Day 85) of RBP-6000 (300 mg buprenorphine) in multicenter, multiple-dose Study RB-US-12-0005
b. Human $AUC_{0\text{-}672h}$ observed after 1st injection of RBP-6000 (single 300 mg buprenorphine dose) in multicenter, multiple-dose Study RB-US-12-0005
c. Exposure margins were calculated using $AUC_{0\text{-}24h}$ values when available. In addition, AUC values from single-dose nonclinical studies were compared to single-dose human AUC, and AUC values from repeat-dose nonclinical studies were compared to multi-dose human AUC.
d. $AUC_{0\text{-}336h}$ is presented because $AUC_{0\text{-}24h}$ was not calculated for this study; Exposure margins were calculated using human $AUC_{0\text{-}672h}$ since $AUC_{0\text{-}336h}$ were not evaluated, and therefore represent more conservative margins.

Atrigel Toxicity (HED-based)

Safety Margins for ATRIGEL Delivery System and Individual Components based on Human Equivalent doses (HED)a							
Study	Species	Highest Dose Qualified in Animal Studies (mg/kg)			Safety Factor Based on HED		
		ATRIGEL Delivery System	NMP	PLGH	ATRIGEL Delivery System	NMP	PLGH
Single-dose Toxicology	Rat	1205	723	482	8.5	8.3	8.7
	Dog	196	118	78	4.6	4.6	4.7
Repeat-dose	Rat	1290	774	516	9.1	8.9	9.3

Toxicology	Dog	98	59	39	2.3	2.3	2.4
Fertility and embryo-fetal toxicity[b]	Rat (M)	1415[c]	863	552	10.0	10.0	10.0
	Rat (F)	2829[c]	1726	1103	20.0	20.0	20.0
Pre- and post-natal development	Rat (P)	1415	863	552	10.0	10.0	10.0
	Rat (F1)	1415	863	552	10.0	10.0	10.0
Embryo-fetal development	Rabbit (F)	713	435	278	10.0	10.0	10.0

F: Female; M: Male; NMP: *N*-methyl-2-pyrrolidone; PLGH: Poly(lactide-co-glycolide) with carboxylic acid endgroup

[a] Safety factor is calculated by dividing the human equivalent dose from toxicity studies by the human dose; human equivalent dose calculations based on FDA Guidance "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." Human dose of ATRIGEL Delivery System in RBP-6000 is 1366 mg or 22.8 mg/kg. Human dose of NMP and PLGH in RBP-6000 is estimated to be 833 mg (13.9 mg/kg) and 533 mg (8.9 mg/kg), respectively.

[b] NOAEL is presented for each species tested.

[c] NOAEL for male reproductive and fertility indices was 2829 mg ATRIGEL/kg body weight and represent 20-fold safety margins for both NMP and PLGH; however, the NOAEL of 1415 mg/kg was due to adverse effects on sperm function (percent abnormal sperm).

12 Appendix/Attachments

Appendix 1: NMP Toxicological Risk Assessment (completed by Drs. Elizabeth Bolan and R. Daniel Mellon)

Review of Toxicity Data for NMP in Indivior's Sublocade (RBP-6000)

Sublocade, code name RBP-6000, is a sterile, non-aqueous solution for subcutaneous injection indicated for the treatment of opioid use disorder. It contains 100 mg or 300 mg of buprenorphine base (BUP) in the ATRIGEL delivery system. This delivery system is designed to deliver buprenorphine over a minimum of 28 days after subcutaneous injection. The ATRIGEL delivery system is composed of a 50:50 poly (DL-lactide-coglycolide) polymer (PLGH), (b) (4) and *N*-methyl-2-pyrrolidone (NMP) as the solvent during delivery. (b) (4) .

The formulation of Sublocade is depicted in the table below.

Table 18. Composition of RBP-6000 (Sublocade) Drug Product

Component	Nominal (% w/w)	100 mg Syringe (mg)	300 mg Syringe[a] (mg)
Buprenorphine	18	100	300
50:50 Poly(DL-lactide-co-glycolide)	(b) (4)	178	533
N-methyl-2-pyrrolidone		278	833
			(b) (4)
Approximate Delivered Volume (mL[b])	-	0.5	1.5

[a] Delivered mass does not equal the sum of the components due to rounding
[b] Approximate volume based on the density of buprenorphine-ATRIGEL

Regulatory History

As discussed during development, the use of NMP in this drug product formulation is technically novel. According to the Inactive Ingredients Database (IID), NMP is currently use as an excipient in two FDA approved drug products, as noted by the Applicant in their table below, reproduced from the submission.

Table 19. FDA Inactive Ingredient Database Listing of N-methyl-2-pyrrolidone

Inactive Ingredient	Route; Dosage Form	CAS Number	UNII	Maximum Potency
N-methyl-2-pyrrolidone	Periodontal; Drug Delivery System	872-50-4	JR9CE63FPM	(b) (4)
N-methyl-2-pyrrolidone	Subcutaneous; Injection	872-50-4	JR9CE63FPM	25.85%

Each SC injection of the 300 mg BUP strength of the RBP-6000 drug product contains 833 mg of NMP (b) (4)). Sublocade is intended to be administered once every 28 days with no limitation on the duration of treatment (i.e., chronic/lifetime). Although NMP can be found in previously approved products (one for acute periodontal use only, the second for an advanced cancer indication), the safety has not been characterized for this population (chronic use, not advanced cancer indication). Therefore, the use in Sublocade for a chronic non-oncology indication is novel.

At the preIND meeting held in 2010, the Agency and the Sponsor began the discussion of the nonclinical developmental requirements for this program. At that time, the Division agreed that no new carcinogenicity studies would be required for the drug product if there were no findings of concern in the 6-month and 9-month rat and dog studies. As the question was primarily focused on the buprenorphine in the drug product, the Applicant specifically asked about NMP at this time. According to the official meeting minutes, the Division noted that "if the clinical exposure to the excipient was at a level below the highest approved level, this would be reassuring. The Sponsor noted that it appeared that exposure to NMP and PLGH would be at levels below those experienced from previously-approved products." The Sponsor then asked if literature could be used to address the Division's concerns. The Division replied that literature would not likely be adequate, but may be supportive.

In the EOP2 meeting held in 2014, the Division noted that although no new studies with buprenorphine were needed, the Sponsor must still address the safety of the excipients, including NMP and we were specifically concerned about the adequacy of the reproductive and developmental effects of the compound. The Division specifically indicated that the Sponsor must "Provide a toxicological risk assessment for the novel use of the excipient NMP in your formulation. In the risk assessment, address what is known regarding the impact of the compound on general toxicity, genetic toxicity, reproductive and developmental toxicity, and carcinogenicity, as per the FDA guidance document …"

At the preNDA meeting held in 2016, the Division again discussed NMP, specifically stating:

> Your proposed reproductive and developmental study program testing the final drug product formulation does not appear to be adequate to characterize the safety of NMP in ATRIGEL because, as designed, NMP exposures would not be adequate over the course of the standard dosing interval for these studies. If you choose to conduct new reproductive and developmental toxicology studies with the ATRIGEL vehicle and/or with its individual components, we recommend that the studies be designed such that adequate systemic exposures to the individual components are achieved throughout the critical exposure periods as appropriate for the specific study protocols. For example, embryofetal development studies with NMP should employ daily dosing by an appropriate route (e.g., subcutaneous) throughout the period of organogenesis. High dose selection must be based on appropriate justification (e.g., maternal toxicity, maximum feasible dose, multiples of clinical exposure) per the ICH guidance for industry: *S5A Detection of Toxicity to Reproduction for Medicinal Products*, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074950.pdf.

The Division noted that the reproductive and developmental studies conducted with RBP-6000 would not result in adequate exposure to NMP over the critical periods of vulnerability intended to be studies by these assays. The Division also noted that a literature-based safety justification would permit filing of an NDA; however, the adequacy of the literature would be determined during the NDA review.

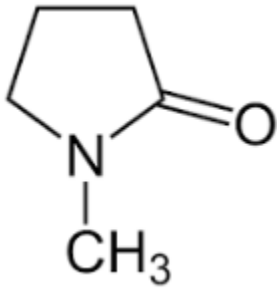
The Applicant has conducted a toxicologic evaluation of NMP which includes study reports testing the drug product formulation and published literature. A full battery of reproductive and developmental toxicology studies with saline, RBP-6000 and ATRIGEL alone (PLGH and NMP) has been conducted. However, as noted above, the dosing regimens are not adequate to characterize the potential for reproductive and developmental toxicity in the studies. Although these studies are adequate to address the safety of the buprenorphine and (b) (4) PLGH polymer, (b) (4) , the systemic exposure of NMP over the course of the reproduction studies is inadequate.

N-methyl-2-pyrrolidone (NMP)

N-methyl-2-pyrrolidone (CAS No. 872-50-4) is a water-miscible, polar aprotic organic solvent used in the manufacture of paint removers, pesticides, herbicides, fungicides, pigments, cosmetics and pharmaceuticals. In rats, it has shown to be absorbed rapidly

via inhalation, oral and dermal administration (World Health Organization, 2001). The structure is depicted below.

Figure 5. Structure of NMP (C_5H_9NO)



NMP is a commonly used solvent in pharmaceutical manufacturing. In fact, ICH Q3C(R6) specifically discusses NMP as a residual solvent in Part III of the document. The Expert Working Group (EWG) revisited the PDE for NMP in 2000 based on new data (chronic oral carcinogenicity study conducted by the NMP Producers Group (unpublished data) and a reproductive and developmental toxicology study published in 1994 (Hass et al., 1994). As noted in the ICH Q3C document, Hass et al. exposed pregnant Wistar rats to 150 ppm NMP for 6 hours a day from Gestation Day 7-20. The rats were allowed to deliver and offspring were evaluated. There was no evidence of maternal toxicity or physical abnormalities noted. However, there were reduced weight of the offspring, pre-weaning development was impaired, and the pups performed worst at higher cognitive function in solving a difficult task compared to control animals. A NOAEL was not established in this study. The EWG elected to keep NMP in Class 2 residual solvent and set an PDE of not more than (NMT) 5.3 mg/day, as follows:

Figure 6. PDE Calculation for NMP as per ICH Q3C(R6)

$$150\ \text{ppm} = \frac{150 \times 99.13}{24.45} = 608.16\ \text{mg/m}^3 = 0.608\ \text{mg/L}$$

$$\text{For continuous dosing} = \frac{0.608 \times 6}{24} = 0.152\ \text{mg/L}$$

$$\text{Daily dose} = \frac{0.152 \times 290}{0.33} = 133.58\ \text{mg/kg}$$

$$\text{PDE} = \frac{133.58 \times 50}{5 \times 10 \times 1 \times 5 \times 5} = \mathbf{5.3\ mg/day}$$

$$\text{Limit} = \frac{5.3 \times 1000}{10} = \mathbf{530\ ppm}$$

This PDE remains in the most current ICH Q3C document (Revision 6, Step 4, dated October 20, 2016).

ADME

NMP is readily absorbed across human skin, the gastrointestinal and respiratory tracts. It is rapidly distributed in the body to most organs including male sex organs. Wells and Digenis reported that after IV administration of 45 mg/kg of radiolabeled NMP to male Sprague Dawley rats, the radiolabel had a plasma half-life of 7-10 hours. Approximately 70% of the label was excreted in urine within 12 hours and 80% by 24 hours. At 6 hours, the highest radioactivity was present in the liver and intestines (Wells and Digenis, 1988).

Sitarek and Kilanowicz also studied the tissue distribution of radiolabeled IP NMP in female and male rats and noted that the highest levels at 4 hours were in the adrenals, kidneys, seminal vesicles, testes, muscles, liver, brain and lungs, as depicted in the tables below (Sitarek and Kilanowicz, 2006).

Table 1. Radioactivity concentration in different male rat tissues after administration of ^{14}C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/male)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.11 ± 0.11[a]	0.89 ± 0.01	0.05 ± 0.01	0.04 ± 0.001	0.04 ± 0.001
Kidneys	1.40 ± 0.15	1.08 ± 0.02	0.11 ± 0.02	0.04 ± 0.001	0.04 ± 0.001
Spleen	0.69 ± 0.10	0.67 ± 0.10	0.05 ± 0.01	0.03 ± 0.002	0.03 ± 0.0007
Lungs	1.04 ± 0.13	0.86 ± 0.14	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.002
Brain	1.05 ± 0.11	0.91 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.0007
Adrenals	1.42 ± 0.10	1.03 ± 0.10	0.07 ± 0.01	0.06 ± 0.001	0.05 ± 0.0007
Sciatic nerve	0.96 ± 0.04	1.04 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.007
Muscles	1.32 ± 0.19	1.44 ± 0.01	0.03 ± 0.001	0.07 ± 0.004	0.02 ± 0.002
Fat	0.83 ± 0.30	0.67 ± 0.10	0.04 ± 0.01	0.06 ± 0.02	0.02 ± 0.001
Epididymis	0.89 ± 0.08	0.82 ± 0.02	0.05 ± 0.02	0.04 ± 0.005	0.03 ± 0.003
Testicles	1.28 ± 0.14	1.08 ± 0.009	0.05 ± 0.02	0.03 ± 0.005	0.03 ± 0.001
Seminal vesicles	1.33 ± 0.14	0.99 ± 0.08	0.09 ± 0.07	0.04 ± 0.003	0.02 ± 0.002
Whole blood	0.87 ± 0.04	0.70 ± 0.05	0.18 ± 0.003	0.17 ± 0.01	0.18 ± 0.01

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 male rats at each time.
To assess ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12%, and muscles as 40% of whole b.w. [17].

Table 2. Concentration of radioactivity in different female rat tissues after administration of ^{14}C-N-methyl-2-pyrrolidone in a single i.p. dose of 250 mg/kg b.w. (350 kBq/female)

Tissue	4 h	8 h	24 h	48 h	72 h
Liver	1.45 ± 0.16[a]	0.88 ± 0.03	0.06 ± 0.01	0.04 ± 0.005	0.04 ± 0.001
Kidneys	1.70 ± 0.14	1.16 ± 0.22	0.09 ± 0.01	0.05 ± 0.002	0.06 ± 0.007
Spleen	0.87 ± 0.10	0.47 ± 0.05	0.05 ± 0.009	0.04 ± 0.002	0.03 ± 0.004
Lungs	1.70 ± 0.16	1.23 ± 0.06	0.06 ± 0.01	0.05 ± 0.001	0.05 ± 0.002
Brain	1.59 ± 0.19	1.14 ± 0.19	0.02 ± 0.002	0.02 ± 0.002	0.03 ± 0.0007
Adrenals	1.48 ± 0.18	0.98 ± 0.10	0.06 ± 0.005	0.06 ± 0.008	0.05 ± 0.003
Sciatic nerve	1.57 ± 0.16	1.52 ± 0.27	0.05 ± 0.007	0.05 ± 0.003	0.05 ± 0.0007
Muscles	2.02 ± 0.17	1.48 ± 0.12	0.04 ± 0.001	0.07 ± 0.004	0.04 ± 0.007
Fat	0.43 ± 0.08	0.82 ± 0.26	0.06 ± 0.006	0.06 ± 0.004	0.03 ± 0.006
Ovaries	1.66 ± 0.20	0.93 ± 0.16	0.05 ± 0.01	0.05 ± 0.008	0.04 ± 0.005
Uterus	1.19 ± 0.09	0.94 ± 0.03	0.04 ± 0.01	0.04 ± 0.005	0.04 ± 0.007

i.p. – intraperitoneally; b.w. – body weight; a – mean radioactivity (kBq/g tissue) ± SD from 4 female rats at each time.
To assess ^{14}C distribution, blood was accepted as 7 ml/100 g b.w. [16], fat tissue as 12% and muscles as 40% of whole b. w. [17].

The Applicant also completed their own radiolabeled PK/distribution study with ^{3}H-NMP delivered via the Atrigel delivery system (Study INLS-R101-60-15). As noted in the table below reproduced from the report, radiolabeled material peaked in blood and plasma at about 8 hours post injection. Using the blood values in the table below, 97% of the radiolabel is accounted for in blood following SC injection of RBP-6000.

Mean concentrations of radioactivity in blood and plasma at specified times after a single subcutaneous administration of ^{14}C-NMP/buprenorphine in RBP-6000 to male Sprague Dawley rats (Group 2, 250 mg buprenorphine/kg)

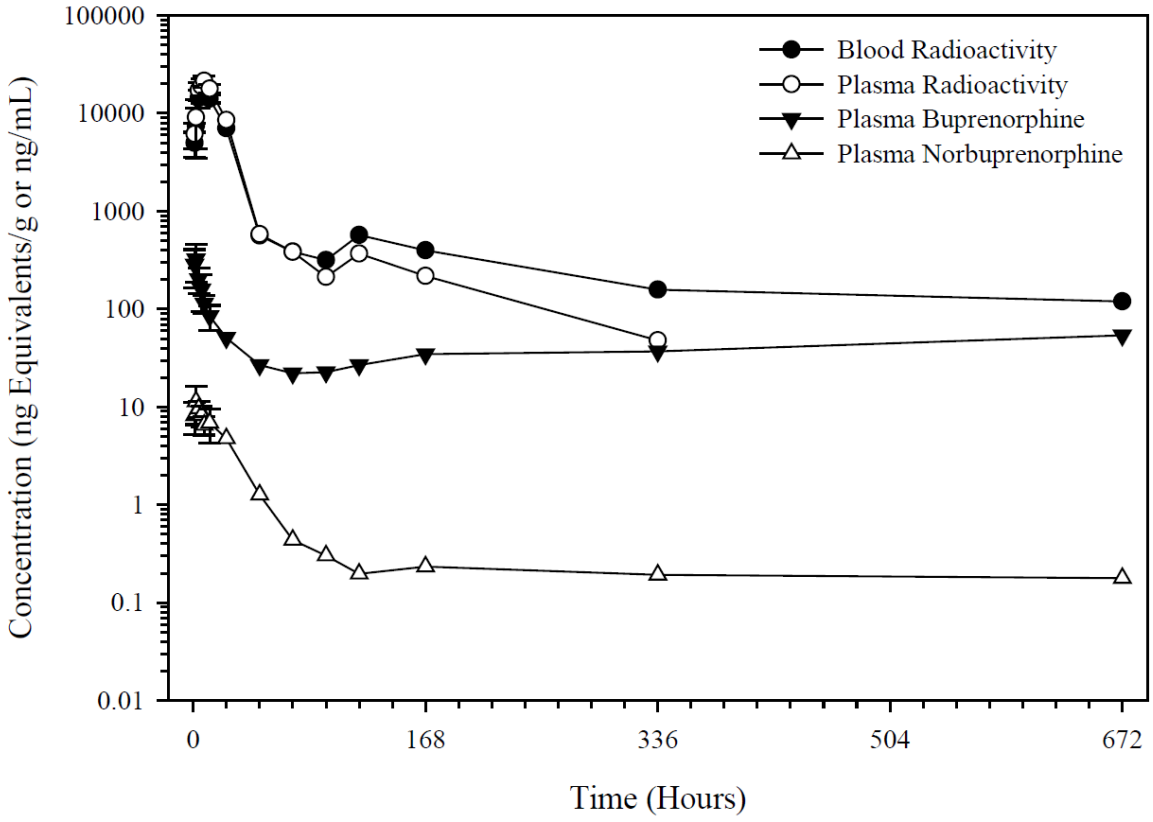
Sample	Time Point	No. of Animals	Concentration (ng Equivalents ^{14}C-NMP/g) Mean	SD
Blood	1 h	10	4980	1430
Blood	2 h	10	7450	3940
Blood	4 h	10	14300	2900
Blood	6 h	10	15600	3050
Blood	8 h	10	18100	2340
Blood	12 h	10	14100	1290
Blood[a]	12 h	2	13800	N.A.
Blood[a]	24 h	2	7020	N.A.
Blood[a]	48 h	2	564	N.A.
Blood[a]	72 h	2	386	N.A.
Blood[a]	96 h	2	317	N.A.
Blood[a]	120 h	2	572	N.A.
Blood[a]	168 h	2	399	N.A.
Blood[a]	336 h	2	158	N.A.
Blood[a]	672 h	2	120	N.A.
Plasma	1 h	10	6150	1780
Plasma	2 h	10	9100	4770
Plasma	4 h	10	17200	3530
Plasma	6 h	10	18900	3780
Plasma	8 h	10	21400	2730
Plasma	12 h	10	17800	1860
Plasma[a]	12 h	2	16900	N.A.
Plasma[a]	24 h	2	8540	N.A.
Plasma[a]	48 h	2	582	N.A.
Plasma[a]	72 h	2	384	N.A.
Plasma[a]	96 h	2	213	N.A.
Plasma[a]	120 h	2	369	N.A.
Plasma[a]	168 h	2	218	N.A.
Plasma[a]	336 h	2	47.9	N.A.
Plasma[a]	672 h	2	0.00	N.A.

h Hours.
N.A. Not applicable.
SD Standard deviation.
a Sample collected at the time of sacrifice.
Note: The lower limits of quantitation for blood and plasma were 16.7 and 36.2 ng equivalents ^{14}C-NMP/g, respectively.

The Quantitative Whole Body Autoradiography (QWBA) studies in rat show that the NMP delivered via the Atrigel system is also largely eliminated systemically within three days after a SC injection.

Thus, in contrast to buprenorphine and the PLGH vehicle, the NMP is released as a bolus from the injection site. This is graphically represented in the figure below from the report, comparing the levels of radiolabeled NMP to the levels of buprenorphine and norbuprenorphine via this product.

Concentrations of radioactivity in blood and plasma and buprenorphine and norbuprenorphine in plasma at specified times after a single subcutaneous administration of ^{14}C-NMP/buprenorphine in RBP-6000 to male Sprague Dawley rats (Group 2, 250 mg buprenorphine/kg)



Note: Plasma radioactivity was below the limit of quantitation after 336 hours.

Further, the QWBA studies also demonstrates that after SC injection, NMP via Atrigel distributes to many different tissues. The tissue distribution data are depicted in the Applicant's tables below:

Table 12
Average (n = 2) concentrations of radioactivity in blood and tissues determined by quantitative whole-body autoradiography at specified times after a single subcutaneous administration of ^{14}C-NMP/buprenorphine in RBP-6000 to male Sprague Dawley rats (Group 2, 250 mg buprenorphine/kg)

	Average Concentration (ng Equivalents ^{14}C-NMP/g)								
	Sacrifice Time								
Tissue	12 h	24 h	48 h	72 h	96 h	120 h	168 h	336 h	672 h
Adrenal gland(s)	8570	4860	1130	1110	892	1380	635	205	BLQ
Arterial wall	12800	9390	1650	1950	1360	1490	1730	739	451
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	10600	6680	576	996	439	591	535	153	BLQ
Bone	9110	1940	BLQ	209	BLQ	154	122	BLQ	ND
Bone marrow	5260	5310	2180	1630	904	1930	1050	144	BLQ
Brain cerebellum	6360	4380	306	429	407	351	287	137	BLQ
Brain cerebrum	5880	4440	319	386	300	356	268	131	BLQ
Brain medulla	5850	3950	261	356	390	298	254	117	BLQ
Brain olfactory lobe	5860	4470	455	515	428	532	469	340	165
Bulbo-urethral gland	7740	5560	695	627	448	977	740	241	115
Cecum	7630	11600	6290	1840	931	4280	2170	328	BLQ
Diaphragm	9150	6070	622	523	365	1020	611	159	130
Dose site	1740000[a]	614000	349000	702000	335000	357000	273000	53000	8750
Epididymis	6970	4750	394	433	279	479	244	206	BLQ
Esophagus	9840	8080	867	1070	429	1070	628	185	BLQ
Exorbital lacrimal gland	8170	5600	611	862	545	1160	1020	216	BLQ
Eye lens	11800	6780	499	339	189	188	218	BLQ	ND
Eye uveal tract	8100	4650	302	343	ND	ND	ND	ND	ND
Eye(s)	8080	4950	385	287	185	222	182	ND	ND
Fat (abdominal)	2880	737	132	159	116	211	173	BLQ	BLQ
Fat (brown)	5490	3700	595	500[a]	572[a]	1260	1470	ND	ND
Harderian gland	5430	3310	884	743	567	873	641	209	BLQ

BLQ Below the limit of quantitation (<101 ng equivalents ^{14}C-NMP/g).
h Hours.
ND Not detectable (sample shape not discernible from background or surrounding tissue).
a Sample(s) were above the upper limit of quantitation (>1060000 ng equivalents ^{14}C-NMP/g); therefore, this value is extrapolated.
b Average is the result of one value.

Table 12 (continued)
Average (n = 2) concentrations of radioactivity in blood and tissues determined by quantitative whole-body autoradiography at specified times after a single subcutaneous administration of ^{14}C-NMP/buprenorphine in RBP-6000 to male Sprague Dawley rats (Group 2, 250 mg buprenorphine/kg)

	Average Concentration (ng Equivalents ^{14}C-NMP/g)								
	Sacrifice Time								
Tissue	12 h	24 h	48 h	72 h	96 h	120 h	168 h	336 h	672 h
Intra-orbital lacrimal gland	7000	4900	603[b]	959	544	1650[b]	944	238	BLQ
Kidney cortex	11000	7140	1440	1220	875	1680	1130	270	112
Kidney medulla	12000	7950	2150	1420	993	1920	1310	285	115
Kidney(s)	11400	7360	1680	1290	933	1780	1230	278	113
Large intestine	6890	6270	2810	2330	561	1150	1260	305	157
Liver	9300	6100	1590	1360	910	1480	1020	245	BLQ
Lung(s)	8550	5300	792	741	668	1000	965	256	BLQ
Lymph node(s)	8090	5660	990	914	644	1170	765	245	BLQ
Muscle	8590	5250	351	315	227	355	350	130	BLQ
Myocardium	9300	5740	657	1240	482	881	685	282	158
Nasal turbinates	12800	6130	2880	2530	2120	2050	2320	1230	573
Pancreas	8540	6100	830	860	425	863	721	185	BLQ
Pituitary gland	7480	6310	767	849	706	887	775	226	ND
Preputial gland	18000[b]	8820	1690	876	1050	891	762	171	BLQ
Prostate gland	8990	6590	735	693	465	702	536	150	BLQ
Salivary gland(s)	8340	5780	761	1010	785	1570	1100	317	124
Seminal vesicle(s)	10400	5050	565	590	391	561	458	272	BLQ
Skin (nonpigmented)	7840	5060	790	591	480	588	575	179	BLQ
Small intestine	8680	6960	2700	1550	723	1670	1130	201	ND
Spinal cord	4190	3930	306	329	309	312	240	108	BLQ
Spleen	9140	5740	1240	1320	734	1670	1080	216	BLQ
Stomach	9000	6690	950	1070	633	1230	998	277	BLQ
Testis(es)	7510	5130	528	467	329	545	395	147	BLQ

BLQ Below the limit of quantitation (<101 ng equivalents ^{14}C-NMP/g).
h Hours.
ND Not detectable (sample shape not discernible from background or surrounding tissue).
b Average is the result of one value.

Table 12 (continued)
Average (n = 2) concentrations of radioactivity in blood and tissues determined by quantitative whole-body autoradiography at specified times after a single subcutaneous administration of ^{14}C-NMP/buprenorphine in RBP-6000 to male Sprague Dawley rats (Group 2, 250 mg buprenorphine/kg)

	Average Concentration (ng Equivalents ^{14}C-NMP/g)								
	Sacrifice Time								
Tissue	12 h	24 h	48 h	72 h	96 h	120 h	168 h	336 h	672 h
Thymus	8280	5530	753	1270	901	994	1060	179	BLQ
Thyroid	10500	7850	757	1060	771	1110	839	210	BLQ
Urinary bladder	ND	ND	13100	3160	1120	332	2030	444	223
Urine	290000	494000	21200	21300	5000	10300	2410	194	BLQ

BLQ Below the limit of quantitation (<101 ng equivalents ^{14}C-NMP/g).
h Hours.
ND Not detectable (sample shape not discernible from background or surrounding tissue).

Evaluation of the data in the table above suggest that NMP distributes widely in the body after SC injection via RBP-6000. Although the highest level of radioactivity was obviously the injection site, other tissues with high levels of radioactivity included the preputial gland, urinary bladder, nasal turbinates, arterial wall, kidney (total, cortex and medulla), eye lens, cecum, and thyroid.

ADME Conclusions

The above data support the Applicants statement that, in contrast to buprenorphine and PLGH, the NMP rapidly disperses from the injection site and distributes to tissues. A

majority of the total 833 mg amount of NMP in the dosage form appears to be eliminated from the body within the first several days after injection with peak levels in urine occurring by ~24 hours. This has significant implications for the toxicological risk assessment, particularly the studies evaluating the reproductive and developmental effects of the drug product, as noted by the Division in developmental discussions with the Applicant. The studies with RBP-6000, which was dosed every 28 days in these studies, will result in most of the exposure to NMP only within the first few days after the injection. The Applicant states that in the rat and rabbit embryofetal studies conducted with RBP-6000 this ~three-day exposure covers a critical period during organogenesis. They argue that the embryofetal findings in their studies are comparable to those observed in the literature reports (i.e., decreased body weight gain, increased resorption of fetuses, decreased fetal body weight, increased fetal skeletal abnormalities, testicular degeneration in males and thymic atrophy in females; see reviews below). Although perhaps qualitatively similar, the NOAEL for these studies may differ depending on the day the drug is administered with respect to the ongoing development of the fetus. Therefore, we do not believe that the studies with RBP-6000 alone fully characterize the effects of NMP over the full critical periods of development these studies are intended to characterize. Therefore, the literature for NMP dosed daily will be necessary to support this drug product application and appropriately inform labeling.

For the purposes of this review, the worst-case MDD of NMP will be 833 mg/day, as injected via this product. Although one could distribute this dose over the entire 28-day period, the PK data do not support this rationale if the toxicological effects are due to C_{max} or the critical periods of exposure are not covered. A dose of 833 mg/60 kg person = 513.7 mg/m^2 on a body surface area basis. This daily dose is greater than any of the other two FDA approved drug products making this use novel in terms of dose, duration, and risk-benefit. Further, (b) (4), this dose is also 157 times higher than the ICH Q3C(R6) PDE of 5.3 mg/day.

TOXICOLOGICAL RISK ASSESSMENT

The Applicant has provided a review of the literature in support the safety of NMP. Although there are multiple toxicology studies in the literature with NMP, none of the studies use the subcutaneous route of administration. Bridging studies with appropriate TK have not been provided. Therefore exposure margins will be based on body surface area comparisons.

General Toxicology

General toxicology studies with RBP-6000 were conducted in support of this NDA. These studies provide an adequate safety assessment of the NMP in the drug product formulation. The reader is referred to the body of this review for further details.

In addition, the Applicant summarized acute and repeat-dose general toxicology data from the published literature. This appendix will focus only on the published subchronic

and toxicology studies submitted (Becci et al., 1983, Lee et al., 1987, Malek et al., 1997, Malley et al., 1999). The primary literature references cited by the Applicant and the reported NOAELs are depicted in the Applicant's table below, which also assumes 833 mg of NMP per day:

Species	Duration	Route	NOAEL (mg/kg)	Safety Margin[a]	Source
B6C3F1 mice	28 days	Oral / feed	720 (males) 920 (females)	4x (males) 5x (females)	Malek *et al* 1997
B6C3F1 mice	90 days	Oral / feed	277	2x	Malley *et al* 1999
Crl:CD BR rats	28 days	Oral / feed	429 (male) 1548 (female)	5x 18x	Malek *et al* 1997
Crl:CD BR rats	90 days	Oral / feed	169 (male) 217 (female)	2x 3x	Malley *et al* 1999
Beagle dogs	90 days	Oral / feed	250	10x	Becci *et al* 1983

[a]Safety Multiples are based on NOAELs in mg/kg, corresponding to a multiple of the highest proposed dose strength of RBP-6000 in humans (which contains 833 mg of NMP or 13.9 mg/kg in a 60 kg subject)

Subchronic Toxicity

As noted in the table above, there are 90-day oral rat, mouse, and dog toxicology studies. Based on the NOAEL reported, the NOAELs predict safety margins of between 2 to 18x the maximum daily dose of NMP via RBP-6000.

In the 90-day rat study, rats were administered 3000, 7500, or 18000 ppm NMP via the diet. Based on food consumption, male doses were 169, 433, and 1057 mg/kg and female doses were 217, 565, and 1344 mg/kg at the low-, mid-, and high-dose groups (LD, MD, and HD), respectively (Malley et al., 1999). The LD was considered the NOAEL for both sexes. Exposure margins at the NOAEL were 2x and 2.5x in males and females respectively, based on body surface area comparisons. There were no effects of NMP on mortality. The HD group demonstrated reduced body weights (~25% decrease compared to controls). The MD and HD male animals demonstrated increased foot splay values, low arousal, and palpebral closure in the neurological assessments, consistent with a general sedative effect of the compound. HD animals demonstrated increased relative liver, kidney and lung weights and decreased relative brain and testes weights. Microscopically, the high-dose group female demonstrated hepatocellular hypertrophy which was considered an adaptive response. An increase in hemosiderin deposits in spleen was also noted in both HD males and females. There was no adverse histopathology in the CNS. The NOAEL was the low dose. These doses provide a safety margin of 2x and 2.5x for males and females for the MDD via RBP-6000 based on body surface area.

In the 90-day mouse study, mice were fed diets containing 1000, 2500, or 7500 ppm NMP which resulted in mean daily doses of 277, 619, and 1931 mg/kg for both sexes based on food consumption data (Malley et al., 1999). There was no effect on mortality. There was a dose related increase in liver weights. Evidence of centrilobular hepatocellular hypertrophy was found in the HD animals, which was attributed to an adaptive response. The LD was considered the NOAEL/LOAEL based on liver weight increases. This dose provides a safety margin of 1.6x for the MDD via RBP-6000 based on body surface area.

In the 90-day dog study, beagles were administered NMP via the diet resulting in daily doses of 25, 79, and 250 mg/kg (Becci et al., 1983). There was no impact on survival. HD animals demonstrated reduced body weight gain compared to controls (↓53% males, ↓37% females). Decreased cholesterol, serum albumin, and total protein levels in MD and HD male dogs was noted; however, there were no histological correlates to explain these findings. Platelet counts increased with dose at Week 8, but levels were subsequently decreasing by Week 12. Based on these unexplained changes, the HD was deemed the NOAEL which provides a 10x safety margin for the proposed MDD of NMP via RBP-6000.

Chronic Toxicity

In a 2-year inhalation toxicology study in male rats, Lee et al report that 0.04 or 0.4 mg/L for 6 hours a day NMP, 5 days a week did not result in any clear treatment related histopathology (Lee et al., 1987). This concentration in air, assuming 290 L/day (12 L/h) and 0.5 kg body weight, as per the Applicant, would result in 58 mg/kg dose (0.7x the MDD).

Malley et al. treated rats with oral 1600, 5000, or 15000 NMP via the diet for 2-years resulting in estimated daily intakes of 66.4, 207, and 678 mg/kg in males and 87.8, 283, and 939 mg/kg in females (Malley et al., 2001). NMP reduced body weights in HD males and females by 25 and 35% relative to controls which corresponded to lower food consumption and food efficiency. HD males demonstrated reduced survival which was associated with an increased incidence of chronic progressive nephropathy/uremia. Decreased survival in MD males was associated with an increase in pituitary and skin tumors which were not dose dependent and therefore, not likely treatment related. As noted in the table below, NMP increased the incidence of chronic progressive nephropathy, increased pigment containing macrophages in the spleen, produced hepatocellular fat accumulation, and generated testicular, bone and kidney damage in males. In females, the only findings were increased pigmented macrophages in the spleen and lymphoid depletion. The NOAEL was 5000 ppm (207 and 283 mg/kg in males and females, respectively) which suggests a safety margin of 2.4x and 3.2x for males and females, respectively, for the MDD of NMP via Sublocade.

Figure 7. Non-neoplastic Lesions in 2-year Oral Rat Toxicology Study (Malley et al., 2001)

Lesion Concentration (ppm)	0	1600	5000	15 000
Male rats				
Severe chronic progressive nephropathy	7/62	10/62	12/62	25/62#
Moderate accumulation of pigment containing macrophage, spleen	18/62	14/62	16/62	28/62#
Hepatic centrilobular fatty change	4/62	8/62	4/62	19/62#
Thrombus in renal vein of kidney	0/62	4/62	4/62	11/62#
Polyarteritis, cecum	0/62	1/31	0/49	5/62*
Lymphoid depletion, mesenteric lymph node	10/62	10/30	9/48	28/60*
Hypertrophy/cystic degeneration, adrenal cortex	18/62	10/34	22/52	28/62*
Polyarteritis, testes	10/62	16/62	11/62	31/62#
Bilateral degeneration/atrophy, seminiferous tubules	15/62	17/62	15/62	35/62#
Bilateral oligispermia/germ cell debris in epididymides	11/62	14/62	12/62	35/62#
Fibrous osteodystrophy, femur/knee	3/62	5/32	5/49	13/62*
Fibrous osteodystrophy, sternum	2/60	5/32	5/49	11/62*
Polyarteritis, mesenteric lymph node	0/60	0/30	0/48	5/62*
Female rats				
Moderate accumulation of pigment containing macrophage, spleen	38/62	35/62	36/62	51/62#
Lymphoid depletion, mesenteric lymph node	15/58	18/47	11/41	29/60*

#Statistically significant by Cochran Armitage test for trend, $p < 0.05$.
*Statistically significant by Fisher's exact test, $p < 0.05$.

Malley et al. also treated mice for 18 months with oral NMP via the diet (600, 1200, or 7200 ppm) resulting in daily doses of 89, 173, or 1089 mg/kg in males and 115, 221, or 1399 mg/kg in females based on food consumption (Malley et al., 2001). There were no clear effects of NMP on body weight or food consumption; however, the NMP appeared to stick to the food hopper, so the sporadic changes in BW are difficult to interpret. There was no increased mortality in males or females. NMP significantly increased liver weights, likely the result of the increased incidence of liver tumors and hepatocellular hypertrophy in this study (see carcinogenicity section below). In males, absolute kidney weights were decreased in all dose groups although there were no histopathological correlates to explain this finding and the effects were not clearly dose dependent. Absolute testes weight was increased in the HD males; relative weights were increased in all treatment groups without histopathological correlates. The same can be said of the increased relative adrenal weight in HD males and lower ovarian weights in MD and HD females. In females, absolute brain weight increases in all treatment group without histopathological changes noted.

Table 20. Organ Weights 18-Month Oral Mouse Study (Malley et al., 2001)

Dosage (ppm)	0	600	1200	7200
Male mouse				
Absolute liver weight (mg)	1437.3 (407.8)	1308.7 (150.2)	1455.8 (388.3)	1776.9** (467.9)
Relative liver weight (% of body weight)	3.8 (1.0)	3.6 (0.3)	4.0* (1.5)	5.0** (1.1)
Absolute kidney weight (mg)	684.4 (70.1)	633.4** (54.5)	633.6** (68.2)	635.8** (58.3)
Relative kidney weight (% of body weight)	1.8 (0.2)	1.7 ** (0.2)	1.7** (0.2)	1.8 (0.2)
Absolute testes weight (mg)	228.8 (15.4)	235.0 (16.5)	233.8 (14.8)	238.2** (18.4)
Relative testes weight (% of body weight)	0.62 (0.06)	0.65* (0.07)	0.65* (0.08)	0.67** (0.08)
Absolute adrenal weight (mg)	5.4 (1.2)	5.5 (1.4)	5.2 (1.4)	5.6 (0.9)
Relative adrenal weight (% of body weight)	0.014 (0.003)	0.015 (0.004)	0.014 (0.004)	0.016* (0.003)
Female mouse				
Absolute liver weight (mg)	1273.6 (181.2)	1241.3 (159.9)	1293.8 (189.4)	1423.1 (691.8)
Relative liver weight (% of body weight)	3.9 (0.6)	4.0 (0.4)	4.0 (0.6)	4.6** (1.9)
Absolute brain weight (mg)	498.2 (24.5)	515.8** (24.2)	518.2** (26.6)	508.8* (24.5)
Relative brain weight (% of body weight)	1.6 (0.3)	1.7 (0.3)	1.6 (0.3)	1.7 (0.2)
Absolute ovarian weight (mg)	36.7 (44.4)	64.5 (263.8)	22.9* (28.2)	26.6* (25.9)
Relative ovarian weight (% of body weight)	0.111 (0.103)	0.203 (0.824)	0.074 (0.108)	0.085 (0.078)

*Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.05$.
**Statistically significant difference by one-way analysis of variance and Dunnett's tests, $p < 0.01$.

Table 21. Summary of Test Substance-related Non-neoplastic Lesions in 18-month Oral Mouse Study (Malley et al., 2001)

	Concentrations (ppm)			
Lesion	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

As per the authors, the NOAEL was reported to be LD in males and the MD in females, the former apparently driven primarily by the liver effects in males at 1200 ppm. These NOAELs suggest exposure margins of 0.5x and 1.3x for the MDD of NMP via Sublocade.

Unlike the chronic rat repeat-dose toxicology study with RBP-6000, there was no mention in this study of pancreatic acinar cell apoptosis or alveolar macrophage infiltrates in the lung in any of these studies.

REPRODUCTION AND DEVELOPMENTAL TOXICITY

As noted above, the Applicant's study testing RBP-6000 to characterize the potential for toxicity on fertility endpoints in males and females was considered inadequate for NMP because the dosing interval employed did not provide systemic exposure of NMP throughout the critical time periods. In their literature review, the Applicant identified studies in male and female rats, as follows:

Table 22. Published Two-generational Male Reproductive Toxicology Studies

Species	Duration	Route	NOAEL (mg/kg)	Safety Margin[a]	Source
Rats	100 days (males) 143 days (females)	Whole body inhalation	30[b]	0.35x	Solomon *et al* 1995
Rats	10 weeks	Oral gavage	300 (male fertility) 100 (prenatal)	3.5x 1.2x	Sitarek and Stetkiewicz 2008

[a]Safety Multiples are based on NOAELs in mg/kg, corresponding to a multiple of the highest proposed dose strength of RBP-6000 in humans (which contains 833 mg of NMP or 13.9 mg/kg in a 60-kg subject)

[b]Dose was converted from ppm to mg/kg/day as previously described[2]

Table 23. Published Developmental Toxicity Studies in Rats

Type of Rat Toxicity Study	Dosing Route	Doses[a]	Toxicity		NOAEL[a]/Safety Multiple[b]	Reference
			Foetal	Maternal		
Developmental, whole body inhalation, GD 6-20, 6 hours/day	Inhalation	0 ppm (0 mg/kg)	None	None	Maternal toxicity = 30 ppm (18 mg/kg) **Safety margin: 0.20x** Developmental toxicity = 60 ppm (35 mg/kg) **Safety margin: 0.41x**	Saillenfait et al. 2003
		30 ppm (18 mg/kg)	None	None		
		60 ppm (35 mg/kg)	None	Reduced weight gain		
		120 ppm (71 mg/kg)	Reduced foetal weight	Reduced weight gain, food consumption		
Developmental: GD 6-20	Oral	0 mg/kg	None	None	Maternal toxicity = 250 mg/kg **Safety margin: 3x** Developmental toxicity = 125 mg/kg **Safety margin: 1.5x**	Saillenfait et al. 2002
		125 mg/kg	None	None		
		250 mg/kg	Decreased foetal weight	None		
		500 mg/kg	Malformation, decreased foetal weight	Reduced weight, food consumption		
		750 mg/kg	Malformation, decreased foetal weight	Reduced weight, food consumption		
Developmental, whole body inhalation, GD 7-20, 6 hours/day	Inhalation	0 ppm	None	None	Maternal toxicity = 150 ppm (88 mg/kg) **Safety margin: 1.0x** Developmental toxicity LOAEL = 150 ppm (88 mg/kg) **Safety margin: ND**	Hass et al. 1994
		150 ppm (88 mg/kg)	Decreased body weight and neuro-behavioural effects	None		
Developmental, whole body inhalation, GD 6-15, 6 hours/day	Inhalation	0 mg/L	None	None	Maternal toxicity = 0.1 mg/L (15 mg/kg) **Safety margin: 0.17x** Developmental toxicity = 0.36 mg/L (52 mg/kg) **Safety margin: 0.61x**	Lee et al. 1987
		0.1 mg/L (15 mg/kg)	None	None		
		0.36 mg/L (52 mg/kg)	None	Lethargy and irregular respiration in first 3 days of exposure		
Developmental, dermal, GD 6-15	Dermal	0 mg/kg	None	None	Maternal toxicity = 237 mg/kg **Safety margin: 2.8x** Developmental toxicity = 237 mg/kg **Safety margin: 2.8x**	Becci et al. 1982
		75 mg/kg	None	None		
		237 mg/kg	None	None		
		750 mg/kg	Increased resorption and delayed ossification, decreased foetal weight	Decreased body weight gain		

LOAEL = lowest-observed-adverse-effect level; NOAEL = no observed adverse effect level
GD = gestational day; ND = Not determined
[a] Doses in mg/L or ppm were converted to mg/kg and the safety margin was calculated[1]
[b] Safety Multiples are based on the human equivalent dose at the NOAEL in mg/kg, corresponding to a multiple of the highest proposed dose strength of RBP-6000 in humans (which contains 833 mg of NMP or 13.9 mg/kg in a 60-kg subject).

Male and Female Fertility and Early Embryonic Development

As per ICH S5(R2), fertility and early embryonic development studies are intended to test for toxic effects of a compound resulting from treatment prior to mating (males and females) through mating and implantation. For females, the studies should characterize effects on the estrus cycle, tubal transport, implantation, and development of preimplantation states of the embryo as well as mating behavior and fertility

assessments. The acceptable dosing regimen for females is to dose them at least 2 weeks prior to mating through implantation in females (for rats implantation is generally by Gestation Day 6-7 of pregnancy). For males, the studies should evaluate the impact of a drug on functional effects (e.g., libido, epididymal sperm maturation) that may not be detected via histopathological examination of the male reproductive tissues. Although spermatogenesis in the rat lasts 63 days, acceptable dosing regiments require that the rats be tested for at least 28 days prior to mating.

Male Fertility

Solomon et al. evaluated male fertility as part of a two-generation study in rat. Males were exposed to whole body inhalation of 10, 51, or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to end of mating period (100 days total) (Solomon et al., 1995). No effects on male fertility were observed but limited endpoints were assessed. Male mating and fertility indices were assessed and both were based only on reproductive performance (i.e., number of rats copulating or litters sired). Testes were weighed and reproductive organs were examined for gross lesions. No evaluation of spermatogenesis or histopathology of reproductive organs was performed. The NOEL for male reproductive performance is the highest dose tested, 68 mg/kg, which is 0.8-fold the human dose of 833 mg based on BSA. As this study does not test doses high enough to inform labeling, this publication has limited utility for the RBP-6000 safety assessment.

Sitarek and Stetkiewicz administered NMP to male rats orally at doses of 100, 300 and 1000 mg/kg for 10 weeks prior to mating (Sitarek and Stetkiewicz, 2008). Males were mated with untreated females then necropsied and epididymis and testis were fixed for histopathological evaluation. The individual stages of spermatogenesis were assessed. Viability and development of offspring were observed up to 28 days. The doses of 100 and 300 mg/kg did not significantly alter fertility or spermatogenesis. However, significantly lower viability of the offspring in the first four days of life was seen at 300 mg/kg and above. Infertility and extensive damage to the seminiferous epithelium and the seminal tubule of the testis were observed at 1000 mg/kg (11.6x the MDD). The NOEL for male fertility is 300 mg/kg (3.5-fold the human dose of 833 mg based on BSA) although the NOEL for paternal effects of prenatal development of the progeny is 100 mg/kg which is approximately equal to the human dose of 833 mg based on BSA. This study appears to contain all the primary endpoints of a male fertility study. Exposure data are not available, and oral dosing may result in reduced bioavailability compared to SC administration. The daily exposures to NMP in this study are far more than what would occur via RBP-6000, and therefore this study likely overestimates the potential toxicity of the drug product to males. The male fertility data for RBP-6000, which dosed male rats once a month for three months prior to mating, is likely more predictive of the potential toxicity of the drug product on male reproductive capacity. As noted in the primary review, the NOAEL for male fertility in that study suggested a safety margin of 10x for NMP under the conditions of the study. Although the findings in the Sitarek and Stetkiewicz study are still concerning, particularly the apparent male-mediated adverse

impact on subsequent offspring, as these endpoints are not tested in standard fertility or addressed via pre- and postnatal development studies due to lack of male dosing in the study designs. In terms of labeling, given the small to non-existent safety margin, labeling (Section 13.1 and 8.1) could state:

> Section 13.1
> Adverse effects on testes and male fertility were noted in published study in which rats were treated for 10 weeks with daily oral doses of NMP, an excipient in Sublocade at greater than 11.6 times the MDD and resulted in male-mediated adverse effects on offspring (decreased pup weight and survival) at daily doses 3.5 times the MDD of NMP delivered by Sublocade. No adverse effects were noted at oral doses equivalent to the dose of NMP delivered by Sublocade.
>
> Section 8.1
> Male-mediated adverse effects on offspring (decreased pup weight and survival) were noted in a published study in which male rats were treated orally for 10 weeks with NMP, an excipient in Sublocade at daily doses 3.5 times the MDD of NMP delivered by Sublocade. No adverse effects were noted at oral doses equivalent to the dose of NMP delivered by Sublocade.

Female Fertility and Early Embryonic Development

Solomon et al. evaluated female fertility as part of their two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Treated males were mated with treated females. Female mating and fertility indices were assessed and both were based only on reproductive performance (i.e., number of rats copulating or bearing litters). The uterus was weighed and reproductive organs were examined for gross lesions. Live and dead fetuses, resorptions, corpora lutea, and fetal weights were evaluated. The female fertility parameters that were evaluated appeared to be adequate in this study and no NMP-related effects were observed. However, the highest dose of NMP tested does not provide coverage for the proposed human dose via Sublocade. The NOEL for female fertility is the highest does tested, 68 mg/kg, which is 0.8-fold the human dose of 833 mg based on BSA. As this study did not test doses high enough to inform labeling, the results of this publication have limited utility for the RBP-6000 safety assessment.

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and though gestation and lactation (Sitarek et al., 2012). The fertility index was significantly lower at 450 and 1000 mg/kg (5.2 and 11.6 times the MDD). No standard early embryonic endpoints were examined

(only fertility index = percentage of pregnant females in mating female group). However, examination of the HD females that did not get pregnant revealed evidence of early resorptions indicating intrauterine mortality of the embryos. The NOEL for fertility in this study is 150 mg/kg and is 1.8-fold the human dose of 833 mg based on BSA. The authors note that the 150 mg/kg dose "slightly toxic" (body weight changes) to the dams. It should be noted that although there was no effect on female fertility parameters at 150 mg/kg, pup survival was decreased at this dose. Both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all treated groups, as noted in the table below from the publication.

Table 24. Effect of NMP on Reproductive Performance in Female Rats and their Progeny (Sitarek et al., 2012)

Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5[a]	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82[b]
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1[b]
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4[b]	68.2[b]
Viability	94.0	86.4[b]	71.6[b]	0
Lactation	96.1	78.2[b]	43.4[b]	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.
[a]Mean ± SD.
[b]Significantly different ($p < 0.05$) from control value.
F, female; M, male; GD, gestation day.
Index of fertility—percentage of pregnant females in mating females group.
Index of viability—percentage of pups born alive that survived to 4 days.
Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

The NOEL for female fertility is 150 mg/kg which is 1.8-fold the human dose of 833 mg based on BSA. No NOAEL for pup effects can be reported (<1.8x the MDD). Given the limited endpoints, this study is technically not an adequate full assessment of early embryonic development. Given the lack of exposure data, the lack of adequate early embryonic development endpoints in these studies, and the likelihood that oral bioavailability is not 100%, a definitive SC EFD study should be completed.

In terms of labeling, given the small to non-existent safety margin for an impact on offspring, labeling (Section 8.1) could state:

> Section 8.1
> Adverse effects on offspring (decreased pup survival) was noted in a published study in which female rats were treated orally for 2 weeks prior to mating and

through gestation and lactation with NMP, an excipient in Sublocade, at doses 1.8 times the MDD of NMP delivered by Sublocade. A NOAEL was not demonstrated.

Taken together, the literature reports provide an adequate assessment of the effects on male fertility for NMP and limited date on female fertility and early embryonic development. However, adverse effects in the offspring of both treated males and females are observed at doses of NMP that have no effect on fertility index. The key results of these studies are summarized below.

Table 25. Summary Table of Effects of NMP on Male and Female Fertility

Effect Reported	***Species, Route***	***Treatment Period***	***Dose***	***Estimated Exposure Margin Based on BSA***	***Citation***	***Comment on Adequacy***
Female Fertility						
Fertility decreased at 450 and 1000 mg/kg	Rat, oral	F: 5 days/wk 2 wks prior to mating, during mating, gestation and lactation	NOEL F fert: 150 mg/kg	1.8 X	(Sitarek et al., 2012)	No early embryonic development endpoints, only a fertility index determined.
7% decrease in fetal weight in F1 fetal offspring	Rat, inhalation	6 h/day, 7 d/wk M: 100 days total (from age 34 days to end of mating period F: 134 days total (from age 34 d days to weaning with interruption between GD 20- Day 4 post-partum)	206 mg/m^3 (30 mg/kg)	0.35 X	(Solomon et al., 1995)	Inadequate doses to provide adequate coverage for NMP via Sublocade.
4-11 % transient			>41 mg/m^3			

decrease pup weight						
Male Fertility						
Male infertility and damage to seminiferous epithelium and tubules (at 1000 mg/kg)	Rat, oral	Treated from age 7 weeks 5 days/wk for 10 weeks and for 1 wk during mating, mated with untreated F	NOEL for M fertility: 300 mg/kg	3.5 X	(Sitarek and Stetkiewicz, 2008)	Endpoints are acceptable to assess male fertility
Survival rate of pups lower than controls at 300 mg/kg treated M untreated F			NOEL for effects on offspring of treated M: 100 mg/kg	1.2 X		Endpoint not examined in typical studies, but suggests male mediated adverse effects on offspring.

Embryofetal Development (EFD)

Embryofetal Development in Rat

The Applicant's study testing RBP-6000 to characterize the potential for embryofetal toxicity potentially attributable to NMP is considered inadequate because of the dosing interval employed does not provide adequate systemic levels of NMP throughout the relevant time period during gestation (organogenesis). As per ICH S5(R2), embryo-fetal development studies are intended to detect adverse effects on the pregnant female and development of the embryo and fetus consequent to test article exposure of the female implantation to closure of the hard palate. The studies should characterize the toxicity profile of the test article compared to nonpregnant females as well as assess embryofetal death, altered growth and structural changes (including soft tissue and skeletal examination). The acceptable dosing regimen is to dose pregnant females from implantation to the closure of the hard palate (GD 6 to 18 for mice and rats; GD 6-20 for rabbits) and then sacrifice females one day prior to parturition (GD 18 mice; GD 20-21 rats; GD 29 rabbits). Fetuses should be examined for soft tissue and skeletal changes. At terminal examination, the following endpoints are recorded: macroscopic examination of all adults (histological evaluation), corpora lutea number, number of live and dead implantations, individual fetal body weight, fetal abnormalities, and gross evaluation of placenta.

The Applicant provided a literature review to address the safety of NMP on embryofetal development. Reports from the literature in rat are available for dermal, oral and inhalation routes of administration. It has been shown that NMP causes embryo- and fetotoxicity as well as malformations. NMP also causes maternal toxicity. Based on body surface area comparisons, the data in the literature are inconsistent with regard to the dose at which various effects are observed. These inconsistencies are most likely due to differential exposure via the routes of administration tested and the lack of toxicokinetic data needed to more accurately compare exposure.

Becci et al. examined the teratogenic potential of NMP in rats following dermal administration of 75, 237, or 750 mg/kg NMP from GD 6-15 (0.9, 2.8, or 8.7x the MDD) (Becci et al., 1982). Maternal toxicity was noted at 750 mg/kg as decreased body weight gain. Reduced number of viable fetuses, increased resorptions, and skeletal abnormalities (missing sternabrea extra or fused ribs, incomplete ossification of vertebra, incomplete closing of skull, fused atlas) were noted at 750 mg/kg group. There was no evidence of teratogenicity or maternal toxicity noted in the 75 or 237 mg/kg groups. The NOAEL for EFD provides a safety margin of 2.8x.

Lee et al. treated pregnant rats via inhalation with 0.1 or 0.36 mg/L (15 or 52 mg/kg) for 6 hours per day from GD 6-15 (Lee et al., 1987). Evidence of maternal toxicity was limited to sporadic lethargy and irregular respiration. Evaluation of the fetuses at GD 15 revealed no evidence of teratogenicity of other adverse fetal effects. The HD was the NOAEL for EFD and predicts an exposure margin of 0.6x.

Hass et al. treated rats 0 or 165 ppm NMP via whole body inhalation on GD 4-20 (Hass et al., 1995). Increased pre-implantation loss, delayed ossification of the skeleton and decreased fetal body weight was observed in the absence of maternal toxicity. No NOAEL can be established in this study. The dose of 165 ppm (97 mg/kg) is approximately equal to the human dose of 833 mg based on BSA. As this study did dose slightly earlier than a standard EFD study, implantation sites were assessed which could be used to inform embryonic development endpoints.

Solomon et al. evaluated embryofetal development as part of a two-generation study in rat (Solomon et al., 1995). Females were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to weaning with interruption between GD 20 to Day 4 post-partum (134 days total). Males were exposed to whole body inhalation of 10, 51 or 116 ppm NMP (corresponding to 6, 30, or 68 mg/kg) for 6 h/day 7 day/week from age 34 days to end of mating period (100 days total). Treated males were mated with treated females. Gestation and viability indices were similar between control and exposed groups. No malformations or variations were attributed to exposure of NMP, although the doses used were relatively low and the highest dose provides an exposure margin of less than one for the proposed human dose. The only finding was reduction of body weight of F1 offspring when both parents were exposed to 116 ppm NMP. Therefore, the NOEL for embryofetal development is the mid dose, 51 ppm (30 mg/kg), which is 0.4x the human dose of 833 mg based on BSA.

Saillenfait et al. orally administered 125, 250, 500, or 750 mg/kg NMP to pregnant rats from GD 6-20 (Saillenfait et al., 2002). Maternal toxicity (decreased body weight gain and food consumption) was evident at 500 and 750 mg/kg. NMP increased resorptions at these same doses. Fetal malformations (imperforate anus, absence of tail, anasarca, malformations of the great vessels and cervical arches) were noted at 500 and 750 as well and one fetus in the 250 mg/kg group showed similar evidence of teratogenicity. Decreased fetal weights were evident at 250 mg/kg and above. The maternal NOAEL was 250 mg/kg (3x the MDD); however the developmental NOAEL was 125 mg/kg (1.5x the human dose of 833 mg based on BSA. Given the presumed lack of 100% oral bioavailability, these margins may be lower following SC administration.

Saillenfait et al. exposed pregnant rats to NMP via whole body inhalation at 0, 30, 60 and 120 ppm (18, 35, or 71 mg/kg) 6 h/day from GD 6-20 (Saillenfait et al., 2003). Maternal toxicity was evident as transient body weight gain decreases at 60 and 120 ppm. Reduced fetal body weights were observed at 120 ppm. No effects were noted on the number of corpora lutea, implantation sites, dead or live fetuses. No NMP-related visceral or skeletal variations or malformations were observed. The NOEL for EFD effects is 60 ppm (35 mg/kg) is 0.4-fold the human dose of 833 mg based on BSA. Transient maternal toxicity was observed at this dose as a 17% decrease in body weight GD 6-13.

Table 26. NOAEL and Adverse Effects in Published Rat EFD Studies

Study	Route	NOAEL for EFD	Exposure Margin (BSA)	Dose for AEs	Adverse Fetal Effects	Exposure Margin	Comment on Adequacy
(Becci et al., 1982)	Dermal	237 mg/kg	2.8	750 mg/kg	Fetal lethality Increased resorptions Skeletal malformations	8.7x	No exposure data No maternal toxicity
(Lee et al., 1987)	Inhalation	52 mg/kg	0.6		None	0.6x	No maternal toxicity No exposure data
(Solomon et al., 1995)	Inhalation	30 mg/kg	0.4		None	0.4x	In adequate dosing to provide coverage for clinical
(Hass et al., 1995)	Inhalation	<97 mg/kg	<1	97 mg/kg	Preimplantation loss Delayed ossification Decreased fetal body weight	1x	No exposure data No NOAEL
(Saillenfait et al., 2002)	Oral	125 mg/kg	1.5	250 mg/kg	Increased resorptions Fetal malformations	3x	No maternal toxicity. No exposure data

							SM could be lower with SC
(Saillenfait et al., 2003)	Inhalation	35 mg/kg	0.4		None	0.4x	Maternal toxicity noted at this dose, unclear significance

Collectively, the potential embryofetal effects in rat with NMP has been tested in rats via oral, dermal, and inhalation routes of administration. No studies with the SC route have been completed and exposure data are not available. The existing data suggest adverse effects on fetal development at exposures that 1 to 9 times the MDD of NMP via Sublocade. As these adverse effects appear to result in exposure margins that are smaller than the exposure margins for buprenorphine-related adverse effects, the findings should be included in the product labeling. Recommended language for the label follows:

> Preimplantation losses, delayed ossification and reduced fetal body weights were reported in published studies following treatment of pregnant rats during organogenesis with NMP, an excipient in Sublocade, via inhalation at approximately equivalent doses of NMP delivered by Sublocade. Fetal malformations and resorptions have also been reported following oral administration of 3 times the MDD of NMP delivered by Sublocade at the MDD based on a body surface area comparison.

It is not clear how the inhalation and oral routes of administration compare the proposed SC route of administration. As oral delivery is not likely 100% bioavailable and malformations may have a smaller safety margin that predicted by these data, a definitive SC EFD study in the rat should be completed as a Postmarketing Requirement (PMR). Given the indication and potential public health benefit of this drug product, the study can be completed as a Postmarketing Requirement (PMR) with the above labeling.

Embryofetal Development in Rabbit

(b) (4)

(b) (4)

An embryofetal development study in the rabbit is recommended as a PMR.

In terms of the impact on labeling, suggesting language is as follows:

> Post-implantation loss and increased cardiovascular and skull malformations were demonstrated in pregnant rabbits administered oral NMP, an excipient in Sublocade, at doses 3.2 times the human MDD of NMP via Sublocade in the absence of maternal toxicity. No adverse effects were reported at an oral dose equivalent to the MDD via Sublocade based on a body surface area comparison.

Embryofetal Development in the Mouse

Although not specifically identified or discussed by the Applicant, Becci et al cite a report from Schmidt in 1976[18] that apparently tested IP NMP in the mouse model (Becci et al., 1982). According to Becci:

> Schmitt (1976) found that N-methylpyrrolidone caused dose-dependent embryotoxic and teratogenic effects in AJ JENA and C57BL mice when given in single or repeated i.p. doses on various days of gestation. The most pronounced embryotoxic effect of N-methylpyrrolidone was noted after a single i.p. administration of 165 mg/kg was given on the 7th day postconception. Twenty-three percent of all implanted fetuses died. The same dose level of N-methylpyrrolidone given on the 9th day caused the highest rate of fetal malformations, 18.6%.

A dose of 165 mg/kg in the mouse is approximately equivalent to the MDD in humans via Sublocade.

The Schmidt data were also summarized by the WHO as follows (World Health Organization, 2001):

> NMP doses of 14–166 mg/kg body weight singly or repeatedly intraperitoneally administered to mice during various phases of pregnancy caused increased post-implantation loss and a reduced body weight of the fetuses. Morphological defects such as exencephaly, open eyelids, microphthalmia, cleft palate, oligodactyly, shortened or kinked tails, fusions and curvature of neck and chest vertebrae, and fusion of sternebrae and ribs were observed. The LOAEL for repeated doses was 74 mg/kg body weight administered on days 7–11 of

[18] Schmidt, R. (1976). Tierexperimentelle Untersuch ungen zur embryotoxischen und teratogenen Wirkung von N-Methyl-Pyrrolidon (NMP). Biol. Rundsch. 14:38-41.

gestation. No information on maternal toxicity is given in this study; thus, evaluation of the results is difficult (Schmidt, 1976).

The LOAEL dose of 74 mg/kg in the mouse is 0.4x the MDD in humans via Sublocade.

Peri- and Post-Natal Development (PPND)

The Applicant's study with RBP-6000 to characterize the potential for peri- and postnatal development toxicity of NMP is considered inadequate because the dosing interval employed does not provide adequate systemic exposure of NMP throughout the study. As per ICH S5, PPND studies are intended to characterize the effects of a drug on the pregnant/lactating female and on development of the conceptus and the offspring after exposure of the female from implantation through weaning. Endpoints in the maternal animals should include clinical signs and mortalities, food intake and body weight changes, duration of pregnancy, and partition. Endpoints in the offspring include macroscopic examinations at necropsy, abnormalities, number of live/dead offspring, body weight at birth, pre- and post-weaning survival and growth, maturation and fertility of the offspring, physical development, sensory function and reflexes, and behavior. Specific functional testing methods is not outlined by ICH S5; however, studies that assess sensory function, motor activity, and learning and memory are recommended.

Hass et al. treated pregnant rats via whole body inhalation to 0 or 150 ppm (88 mg/kg = 1 x the MDD of NMP via Sublocade) NMP for 6 h/day on GD 7-20 (Hass et al., 1994). The authors state that this dose level was chosen so as not to cause maternal toxicity or decrease viability of the offspring. Although the study did not include exposures during lactation, the offspring were allowed to be born and were evaluated via functional assessments, similar to a PPND study. Exposed offspring showed decreases in body weight and had delayed physical development. Performance in tasks including the reversal procedure (retesting with a new platform location) in the Morris Water Maze and operant delayed spatial alteration (Skinner boxes) was impaired in exposed offspring. No differences between NMP-exposed animals and control animals were seen in motor function (rotarod), activity level (open field) and learning tasks of low complexity. No NOAEL can be established for PPD parameters and the exposure at the dose employed (88 mg/kg) is approximately equal to exposure of 833 mg in human based on BSA).

Sitarek et al. treated female rats with oral NMP 150, 450, or 1000 mg/kg for 5 days/week two weeks before mating, during mating, and through gestation and lactation (Sitarek et al., 2012). The authors considered the 150 mg/kg dose "slightly toxic" (body weight changes) to the dams. The incidence of stillbirths was increased at 1000 mg/kg. At 150 mg/kg there was no effect on fertility but pup survival was decreased. As previously noted in the fertility section of this appendix, both the index of viability (% of pups born alive that survived to 4 days) and index of lactation (% of pups alive at 4 days that survived to 21 days) were significantly lower than control for all groups, as illustrated in the table below again reproduced from the publication.

Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5[a]	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82[b]
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1[b]
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4[b]	68.2[b]
Viability	94.0	86.4[b]	71.6[b]	0
Lactation	96.1	78.2[b]	43.4[b]	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.
[a]Mean ± SD.
[b]Significantly different ($p < 0.05$) from control value.
F, female; M, male; GD, gestation day.
Index of fertility—percentage of pregnant females in mating females group.
Index of viability—percentage of pups born alive that survived to 4 days.
Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

In terms of typical PPND endpoints the offspring were only evaluated for general appearance, litter weight, mean pup weight, and mortality from birth to PND 21. As such, there are limited PPND endpoints in this report. No NOAEL was established for this study for these PPD endpoints. The low dose of 150 mg/kg is 1.8-fold the human dose of 833 mg based on BSA.

In terms of labeling, based on these two published studies, the following can be included in Section 8.1:

> Decreased pup survival was noted following oral treatment of pregnant rats prior to and during gestation and lactation with NMP, an excipient in Sublocade at doses 1.8 times the MDD. Developmental delays and impaired cognitive function were reported in pups born to pregnant rats treated with NMP via inhalation during gestation at doses equivalent to the MDD of NMP via Sublocade based on a body surface area comparison.

The reports reviewed in the literature do not provide adequate exposure margins or complete methodology to accurately assess effects of NMP on PPD endpoints. Therefore a definitive PPND study the rat should be completed. Given the indication and potential public health benefit of this drug product, the study can be completed as a Postmarketing Requirement (PMR) with the above labeling.

Overall Reproduction and Developmental Conclusions

The published literature on NMP clearly suggest the potential for adverse effects on development. As these studies exposed the animals to NMP during the full critical

periods of development, they provide a more comprehensive assessment of the potential adverse effects of NMP exposure via RBP-6000 than the studies completed by the Applicant with RBP-6000. However, they are limited as they employ different routes of administration which likely have different bioavailabilities. Therefore, as the safety margins for the RBP-6000 studies suggest reduced risk and the toxicities noted appear to be largely driven by the vehicle rather than buprenorphine alone, the adverse findings and smaller exposure margins for NMP from the published studies should be included in labeling. Finally, as the exposure data do not exist for these other routes of administration and SC could result in increased exposures compared to oral studies, definitive studies for NMP should be completed via the SC route as post marketing requirements.

GENETIC TOXICOLOGY

The Applicant provided a literature review of published genetic toxicology studies, as summarized their table below.

Test Method	Test Organisms/Species	Results	References
Salmonella/microsome assay	TA97, TA98, TA100, TA2638[a] UTH8413, UTH8414	-	Wells *et al.* 1988
	TA102, TA104[a]	±*	Wells *et al.* 1988
	TA98, TA104[b]	-	Wells *et al.* 1988
Micronucleus test	NMRI mice	-	Engelhardt and Fleig 1993
Bone marrow chromosome analysis	Chinese hamsters	-	Engelhardt and Fleig 1993
Aneuploidy induction	*Saccharomyces cerevisiae* Strain D61.M	+	Mayer and Goin 1988
	S. Cerevisiae strain D61.M	+	Mayer and Goin 1988
	S. Cerevisiae strain D61.M	+	Zimmermann *et al.* 1988

Source: Engelhardt and Fleig 1993
* The authors indicated that the increase was less than 2-fold over background without a clear linear dose-response relationship. No increases were observed in 6 other strains of *Salmonella typhimurlum.*
[a] Standard plate incorporation assay
[b] Preincubation assay

NMP was tested in the Ames Assay using strains TA1535, TA1537, TA1538, TA98, TA100, TA102, and TA104 in the presence and absence of metabolic activation (Mortelmans et al., 1986, Wells et al., 1988). In strains TA102 and TA104, small increases in revertants above control were observed. However, the increases were less than two-fold above control values and therefore do not meet the criteria for a positive response per the OECD protocol. NMP was negative in the Ames Assay in all other strains tested.

NMP was found to be negative in the mouse micronucleus assay and Chinese hamster bone marrow assay (Engelhardt and Fleig, 1993).

NMP was found to be induce aneuploidy in yeast (Mayer et al., 1988, Zimmermann et al., 1988). As discussed in Engelhardt and Fleig (1993), a cold shock treatment condition that destroys the spindles and has questionable relevance in intact mammals was used in these studies. Further work by Basler showed that various organic solvents that were known to be potent inducers of aneuploidy in yeast did not increase the frequency of micronucleated polychromatic erythrocytes in bone marrow cells of hamsters (Basler, 1986). Further, the weight of evidence suggests that the genotoxic potential for NMP is minimal.

CARCINOGENICITY

Two studies in rats and one in mice evaluating the carcinogenicity of NMP were identified in the literature.

Lee and colleagues exposed rats to 0, 0.04 or 0.4 mg/L NMP for 6 h/day 5 days/week via whole body inhalation for two years (Lee et al., 1987). No treatment-related tumors were identified in either males or females. The NOEL for carcinogenic effects of NMP is the high dose, 0.4 mg/L (58 mg/kg/day) yields and exposure margin of 0.7-fold the human dose of 833 mg based on BSA.

Malley et al. assessed the carcinogenic effect of NMP in rats and mice (Malley et al., 2001). The administration of NMP was in the diet and food consumption was measured. Although not a GLP study, an adequate assessment of organs and tissues were conducted and it appears that methods are acceptable. In rats, NMP was administered in the diet at 0, 1600, 5000, or 15000 ppm for approximately two years. No carcinogenic effects of NMP were observed at any dose. The mean daily consumption at 15000 ppm, the NOEL for oncogenic effects was 678 mg/kg in males and 939 mg/kg in females. Therefore, the exposure margins at the NOEL for oncogenic effects is 8-fold and 11-fold in males and females, respectively, as compared to the human dose of 833 mg based on BSA.

In mice, NMP was administered in the diet at 0, 600, 1200, or 7500 ppm for approximately 18 months. Increases in hepatocellular adenoma and carcinoma were

observed in both males and females at the highest dose tested. In females, the hepatocellular carcinoma was within the historical control range. The mean daily consumption of NMP at 1200 ppm, the NOEL for oncogenic effects was 173 mg/kg in males and 221 mg/kg in females (Malley et al., 2001). Therefore, the exposure margins at the NOEL for oncogenic effects is 1-fold and 1.3-fold in males and females, respectively. The dose at which a statistically significant increase in hepatocellular adenomas and carcinomas were noted (7200 ppm) was estimated to result in daily exposures of 1089 mg/kg/day in male mice and 1399 mg/kg/day in female mice. Based on a body surface area basis, these doses were ~6 and 8 times the maximum human daily dose via the drug product.

Table 27. Summary of Neoplastic Lesions in Male and Female Mice

	Concentration (ppm)			
Lesion	0	600	1200	7200
Male mice				
Hepatocellular adenoma	5/50	2/50	4/50	12/50*
Hepatocellular carcinoma	4/50	1/50	3/50	13/50*
Pulmonary adenoma	1/50	2/50	3/50	5/50
Pulmonary adenocarcinoma	1/50	4/50	6/50	2/50
Total animals with benign tumors	15/50	5/50	8/50	18/50
Total animals with malignant tumors	9/50	7/50	11/50	14/50
Female mice				
Hepatocellular adenoma	2/50	2/50	1/50	7/50*
Hepatocellular carcinoma	0/50	0/50	0/50	3/50*
Total animals with benign tumors	10/50	6/50	5/50	12/50
Total animals with malignant tumors	12/50	5/50	9/50	10/50

*Statistically significant differences at $p < 0.05$ by the Cochran Armitage test for trend.

Table 28. Summary of NMP-related Non-neoplastic Lesions in Male and Female Mice

	Concentrations (ppm)			
Lesion	0	600	1200	7200
Male mice				
Hepatocellular clear cell foci	0/50	1/50	1/50	6/50*
Hepatocellular basophilic foci	4/50	2/50	3/50	5/50
Hepatocellular eosinophilic foci	1/50	2/50	2/50	19/50*
Hepatocellular centrilobular hypertrophy	0/50	0/50	3/50	43/50*
Cellular alterations in liver	5/50	5/50	6/50	25/50*
Female mice				
Hepatocellular clear cell foci	0/50	0/50	0/50	1/50
Hepatocellular basophilic foci	2/50	2/50	1/50	10/50*
Hepatocellular eosinophilic foci	1/50	0/50	0/50	6/50*
Hepatocellular centrilobular hypertrophy	0/50	1/50	0/50	0/50
Cellular alterations in liver	3/50	2/50	1/50	17/50*

*Statistically significant by Cochran Armitage test for trend, $p < 0.05$.

According to Malley et al., Van Esch and Kroes included an NMP solvent control arm in a toxicology studies in Swiss mice (Van Esch and Kroes, 1972). Mice presumably received 25 mg (~25 g body weight = 1000 mg/kg or 3000 mg/m^2) NMP a total of 9 times over the 18-month assay (Day 14, Month 1.5, 3, 4.5, 6, 10, 14, and 17). No increased tumors were noted; however, a saline/water control arm was also not included in this study. One incidence of hepatocellular carcinoma was noted in the control group and one in one of the test arms. Although of limited utility given the study limitations, the dose tested was approximately 6 times the MDD and the dosing interval was punctuated, similar to that which will occur via the proposed dosing regimen.

The Applicant did not provide a mode of action (MOA) assessment to address the hepatic tumors in the mice. Although rodent liver tumors are frequently not believed to be relevant to humans, a MOA assessment is generally completed to put these findings into perspective (Holsapple et al., 2006). For example, a MOA assessment for phenobarbital-induced liver tumors in mice and rats has been undertaken and the data suggest that the increased cellular proliferation, decreased apoptosis, formation of altered hepatic foci, and ultimate liver tumors in rodents did not appear to translate to human risk (Elcombe et al., 2014). This conclusion was primarily driven by data that demonstrated that, unlike rodent hepatocytes, human hepatocytes did not proliferation in vitro in response to phenobarbital. That coupled with a lack of an epidemiological signal for increased tumors following extensive human exposure to phenobarbital support the conclusion that the risk of liver tumors in humans appear to be low for phenobarbital.

Like phenobarbital, repeat-dose toxicology studies with NMP demonstrate increased hepatocellular hypertrophy, suggesting that these liver tumors in mice may not correlate with increased human risk. However, as a similar MOA assessment has not be undertaken for NMP, we recommend that the product labeling include the following:

> NMP, an excipient in Sublocade, produced an increase in hepatocellular adenomas and carcinomas in male and female mice at 6 and 8 times the maximum daily dose (MDD) of NMP via Sublocade. No tumors were noted at 1 and 1.3 times the MDD. The clinical significance of these findings is unclear. In 2-year inhalation and dietary studies in rats, NMP did not result in evidence of carcinogenicity.

Further, as this risk may not be relevant and could impact prescription practices, we recommend that a post-marketing requirement be instituted to conduct a MOA for NMP induced liver tumors, which could support removal of the above labeling statement.

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/s/

RICHARD D MELLON on behalf of GARY P BOND
11/20/2017

ELIZABETH BOLAN
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JAY H CHANG
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RICHARD D MELLON
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I concur.