

**2.6.6.5 Carcinogenicity**

N/A

**2.6.6.6 Reproductive and developmental toxicology**

N/A

**2.6.6.7 Local tolerance**

The first local toxicity study that the sponsor performed, MB 06-14381.15, was considered adequate to support the initial clinical trials, but was not considered a definitive study due to notable deficiencies. The sponsor was informed that an adequate local toxicity would need to be performed; that study should specifically address the following deficiencies (excerpted from the May Proceed Letter, dated 9/22/06, next page):

- a. **the lack of data on administered dose. It was impossible to determine which, if any, animals received drug at the specified dose. The only data available for verifying dosing (i.e., changes in weight of the spray pump vial) suggest a relatively large interanimal variability in the administered dose.**
- b. **only one dose level was tested. At least 2 dose levels of zolpidem LS should be tested and compared to appropriate control groups, in order to identify a maximum tolerated and a no-effect dose. (Alternatively, the high dose may be justified on the basis of maximum feasible dose.) The clinical formulation should be used and the dosing frequency should be at least equal to, and preferably exceed, the proposed human dosing frequency.**
- c. **the oral mucosa was examined only at 24 hours postdose. It is recommended that examinations be conducted prior to and at a reasonable interval following dosing.**

As performed, the study demonstrated that a zolpidem lingual spray formulation similar to that intended for market performed as a mild irritant, and severe local reactions did not occur. There was one instance (one animal with abraded mucosa, on one day) that **demonstrated “sloughing in several areas”** of the oral mucosa at 24-hours postdose; otherwise, incidences of irritation were scored as **“discoloration and slight sloughing” and/or “slight redness, sloughing and dryness.”** No animals were identified with frank ulceration of the oral mucosa or cracking and bleeding of the junction of the lips. The results of histopathological evaluation were varied, but demonstrated sporadic evidence of mild irritation (inflammatory cell infiltration and/or hyperplasia) of the trachea and/or buccal mucosa; neither a saline nor water control was used, so some of the slight changes observed could be attributed to test article and/or placebo. Additionally, although the nominal 10 mg dose should exceed the local clinical exposure (based on surface area considerations), it is notable that only a single dose level was tested. This study was previously reviewed in detail (IND #71,290 N000 P/T safety review).

With regard to the lingual sprays used in these studies, the sponsor provided Table 2.6.7-4 (below) detailing the impurity levels the drug product lots used in the local toxicity assays. The definitive local toxicity study (Study 12230.02.01) was conducted with the proposed commercial drug product, but slightly different than that used in the definitive clinical PK studies. Please **see the sponsor’s table, next page.** The sponsor also provided

a table demonstrating that the proposed commercial product conforms to the Inactive Ingredient Guide (see below).

Table 2.6.7-4. Toxicology: Drug Substance
[Redacted]

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Component	Unit Composition (% w/w), Formula Number					
	009-00 <sup>a</sup>	023-00 <sup>b</sup>	026-00 <sup>c</sup>	027-00 <sup>d</sup>	027-01 <sup>e</sup>	027-02 <sup>f</sup>
[Redacted]						
[Redacted]						
Citric Acid, Monohydrate <i>USP</i>						
Hydrochloric Acid <sup>g</sup>						
[Redacted]						
Propylene Glycol, <i>USP</i>						
Benzoic Acid, <i>USP</i>						
Neotame						
Artificial Cherry Flavor						
Purified Water, <i>USP</i>						

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**Table 1.11.2-1. Composition of Placebo and Corresponding Formulations of ZolpiMist**

Component	Unit Composition (% w/w), Formula Number			
	030-00 <sup>a</sup>	030-01 <sup>b</sup>	027-01 <sup>c</sup>	027-02 <sup>d</sup>
Zolpidem Tartrate, Ph. Eur.				
Citric Acid Monohydrate, USP				
Hydrochloric Acid <sup>e</sup>				
Propylene Glycol, USP				
Benzoic Acid, USP				
Neotame				
Artificial Cherry Flavor				
Purified Water, USP				

a  
b  
c  
d  
e

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**Table 3.2.P.2.1-1. Conformance to Inactive Ingredient Guide for Approved Drug Products**

Ingredient	ZolpiMist	IIG Database Levels
	Quantity (% w/v or mg/unit dose)	Highest Quantity Approved for an Oral Dosage Form With a Similar Route of Administration
Artificial Cherry Flavor		
Benzoic Acid		
Citric Acid, Monohydrate		
Hydrochloric Acid		
Neotame		
Propylene Glycol		

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Study title: 28-Day Oral Irritation Study in Sprague Dawley Rats

Key study findings:

- Note: the spray formulations have very low pH
- Evidence of mild irritancy potential for oral mucosae & skin, possibly for respiratory mucosa (mortality of one vehicle treated animal)
- Suggestion of a very slight delay in wound healing (~1 day difference)

Study no.: 12230.02.01  
 Volume #, and page #: Electronic document, 314 pgs  
 Conducting laboratory and location:  

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Date of study initiation: 6/19/07  
 GLP compliance: Yes, pg 8  
 QA reports: yes (X) no ( ) pg 9, except Introduction & References  
 Drug, lot #, and % purity: Formula 027-02

100 bottles of Zolpidem Tartrate Lingual Spray, 5 mg/100µl (zolpidem oral spray)  
 Lot No. 07C02 20070330M  
 Lot No. H59/L-1; expiration date unknown; retest date September 26, 2007), from

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Formulation/vehicle: Formula 030-01, "Based on Formula 027"  
 33 bottles of Zolpidem Tartrate Lingual Spray Placebo (Lot No. AA0391; expiration date unknown; retest date October 23, 2007) from NovaDel Pharma Inc. (Flemington, NJ)  
 Negative control: Sterile water

Methods

Species: Male Sprague Dawley rats  
~~CD® (SD)IGSBR~~  
 ~9 weeks of age, 295.0-385.2 g  
 Certified Rodent Diet #5002  
 or Certified Rodent Diet #2016  
 and tap water *ad libitum*

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Doses: 0 (water), 0 (vehicle), 10 mg and 20 mg

**Study design:**

Dose concentration and homogeneity analysis was not performed for this study. Documentation of delivered dose was achieved by weighing the dosing container prior to and after each dose administration and by determination of plasma drug levels.

The experimental design was described in the sponsor's table:

Dose Group	Test Substance	Dose	Total No. of Animals per Group	No. of Animals Euthanized				
				Day 2	Day 5	Day 15	Day 29	Day 43 <sup>a</sup>
1	Water	0	23	3	5	5	5	5
2	Vehicle	0	20	0	5	5	5	5
3	Test Article	Low (2 sprays/day)	20	0	5	5	5	5
4	Test Article	High (4 sprays/day)	20	0	5	5	5	5

<sup>a</sup> Recovery Group

**Mucosal Abrasion:** Prior to dosing D1, rats were anesthetized and a steel file \_\_\_\_\_ was used to lightly abrade the left buccal mucosa down to the level of the submucosa.

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**Dose Procedure:** Following mucosal abrasion, rats were exposed to water, vehicle, or the drug formulation once per day by oral spray on D1-28 or up to the day prior to the animal's scheduled euthanasia. Animals were anesthetized for the dosing procedure. Groups 1 and 2 received four oral sprays of sterile water or vehicle, respectively. Group 3 received two oral sprays of the drug, and Group 4 received four oral sprays of the drug. For Groups 1, 2, and 4, animals received two sprays initially and were dosed with the final two sprays after all animals in the group were dosed with the first two sprays. The elapsed time between the first two sprays and the last two sprays was approximately the same length each day. In order to document the amount of test substance delivered, all dosing containers were weighed prior to and after each dose administration. In addition, animals in groups receiving four sprays had container weights collected after the administration of the first two sprays and prior to administration of the last two sprays. For dosing procedure documentation, four animals in the vehicle control group were video taped (digital) during dosing to assess whether there was any significant loss due to **backsplash out of the animal's mouth during** the dosing procedure; this was a qualitative assessment only.

**Clinical Observations:** All rats were observed twice daily throughout the study periods for signs of moribundity and mortality; detailed observations were performed once daily.

**Mucosal Observations:** The oral cavity was observed prior to abrasion on D1, prior to dosing on D1-D28, ~2 hours after dosing on D1-D28, and once daily thereafter. The evaluation included: the color, edema, erythema, sloughing, bleeding, or ulceration of the oral tissues (including the teeth), as well as the presence of dryness, roughness, cracking, or bleeding of the lips.

**Body Weights:** Each animal was weighed during W-1, D1, D2, D5, D8, D15, D22, D29, D36 and D43.

**Plasma Drug Level Determinations:** Blood samples were collected from the five rats/group on D5, D15, D29, and D43 prior to sacrifice. Samples were also taken from 5 rats in each recovery group at ~1 hr after dosing on D1, D15 and D28. Samples were collected from the retro-orbital plexus.

**Macroscopic Pathology:** All rats were necropsied. Rats were sacrificed by CO<sub>2</sub> asphyxiation, and samples of all tissues/organs were saved in 10% neutral buffered formalin for possible histopathological evaluation. The following tissues were collected, on the respective days:

- D2 (from 3 water controls): buccal mucosa at the abrasion site to evaluate adequacy and uniformity of the abrasion technique
- D5, D15, D29 and D43: oral cavity and adjacent structures, including the labial junctions, buccal mucosa (including the area that was abraded), gingival tissues, tongue, palate (hard and soft), parotid salivary gland, submandibular lymph nodes, nasopharynx and nasal passages, larynx, trachea, bronchi, esophagus, stomach, brain and any tissues that appear abnormal during gross examination.

**Histology:** All tissues listed were processed to slides. The fixed tissues were cut into sections (~5 µm), mounted on glass slides, and stained with hematoxylin and eosin.

**Microscopic Observations:** With the exception of the brain sections, all slides from D2, D5, D15, and D29 were submitted to a veterinary pathologist for evaluation and diagnosis. Findings were diagnosed and categorized using standardized nomenclature. A four-step grading system was used to rank the severity of microscopic lesions for comparison among groups. Pathology examination for D43 slides was performed on target tissues and drug-related gross lesions identified during the D29 examination.

### **Results:**

Water control animals M1-11 may not have received full sprays during dosing on Day 1. The dosing procedure was videotaped for vehicle control animals; most animals were dosed without apparent significant loss of dose. The amount of test substance (i.e., water, vehicle, or zolpidem oral spray) delivered was variable, but animals appeared to be **exposed daily. The sponsor's calculated mean** average mass of test substance delivered to Groups 1, 2, 3, and 4 was 0.365, 0.411, 0.206, and 0.412 g/animal/day, respectively; these values were within ±10% of nominal. The sponsor recorded the presence of one or more small droplets of liquid observed around the mouths of animals after dosing, although it was not possible to determine whether this liquid consisted of test substance formulation or saliva. The incidence of droplets of fluid observed around the mouths of **the animals was presented in the sponsor's table**, below. Because of the nature of the dosing procedure, it is possible that a portion of the dose volume from some animals may have been lost due to backsplash or dripping; the highest incidences of droplet loss were observed in the HD group.

## Incidence of Droplets of Fluid Around Mouth

Day	Incidence of Droplets of Fluid Around Mouth <sup>a</sup>			
	Group 1	Group 2	Group 3	Group 4
1	3/48	0/40	0/20	0/40
2	0/40	3/38	0/20	0/40
3	0/40	1/38	2/40	6/40
4	0/40	4/38	0/20	0/40
5	0/30	4/28	4/15	9/30
6	0/30	7/28	2/15	7/30
7	3/30	18/28	10/15	23/30
8	1/30	5/28	4/15	2/30
9	0/30	7/28	3/15	9/30
10	0/30	6/28	3/15	4/30
11	0/30	11/28	1/15	1/30
12	1/30	0/28	2/15	10/30
13	0/30	3/28	3/15	3/30
14	4/30	7/28	3/15	2/30
15	0/20	5/20	3/10	2/20
16	2/20	6/20	5/10	8/20
17	2/20	6/20	0/10	1/20
18	1/20	3/20	2/10	1/20
19	6/20	1/20	3/10	3/20
20	0/20	7/20	1/10	4/20
21	6/20	9/20	0/10	7/20
22	1/20	0/20	2/10	1/20
23	2/20	3/20	2/10	1/20
24	0/20	5/20	2/10	1/20
25	0/20	0/20	0/10	0/20
26	0/20	0/20	0/10	0/20
27	0/20	2/20	0/20	0/20
28	1/20	5/20	1/10	2/20

One vehicle control rat was found dead after dosing on D1; although the sponsor identified this animal as being from Group 1 (water; all 15 animals recorded as alive on day 1), the clinical observations and pathology report indicate that the animal was from group 2 (vehicle; only 14 animals alive on day 1). The sponsor attributed the death to respiratory distress due to aspiration of the oral spray. Clinical observations included dyspnea and cyanosis of both ears; dark lung and liver were noted at necropsy, which corresponded with histologic findings of mild hemorrhage and minimal cellular infiltration of the lung and minimal cytoplasmic vacuolization within the centrilobular zone of the liver. Other histologic findings in addition to mild acute inflammation of left buccal mucosa (abrasion site) included minimal acute inflammation of the periodontal gingiva, larynx, and labial junction, and mild lymphoid necrosis of the submandibular lymph node.

Mean body weights were higher in the vehicle, LD and HD groups on D1. Animals in the vehicle, LD and HD treated groups tended to have increased mean body weight gains compared to the water controls. Mean body weight gains over D1-D29 (notably, with animals dropping from the average as scheduled) appeared increased in the vehicle, LD and HD treated groups (+13-39%, compared to water controls).

The primary clinical signs observed were alopecia (Groups 1, 2, 3, and 4), sore and/or ulcer on different sites of the body (Groups 2, 3, and 4), and scab (Group 4). The incidence of alopecia was highest in the HD group (in up to 8 rats, versus a maximum of 4 LD or vehicle). These observations may have been related to the low pH of the sprayed vehicle and test article (pH 1.1 and 2.3, respectively), and it was suggested that grooming might have spread the vehicle or test article to the different areas of the body (forefeet, forelimbs, hindlimbs, abdomen and/or chest) where the findings were noted. Sores,

ulcers and/or scabs were noted in a few vehicle and treated animals; the incidence was highest in the HD group (affecting up to 2 vehicle, 1 LD and 4 HD animals on a given day). These findings might also represent irritation due to direct contact or spreading of the sprayed vehicle or drug. Discharge from the eye was reported in 1 vehicle control animal (D11-29) and discharge from the nose was reported in 3 HD animals on D14. The zolpidem oral spray was reported to cause hypoactivity in the 16/20 animals in the HD group on D1, at ~40-50 minutes postdose. Hypoactivity was not reported on subsequent days; however, clinical observations for the HD group were recorded prior to treatment on D2 and ~2-3 hrs postdose on D3, D4 and D6-D28. Notably, hypoactivity was not observed on D5, even though observations were recorded at 1-1.5 hr postdose.

Mucosal observations indicated some potential for irritation, and possibly very slightly delayed healing. Due to the abrasion procedure, low to moderate erythema at the abrasion site in all groups from D1-D5; rough lips (Groups 2, 3, and 4) were also observed between D1-D5. The incidences suggested that the presence of erythema might have been slightly more protracted (by about a day) in the LD and HD groups. Furthermore, erythema at the abrasion site was also observed in 1/15 LD and 3/15 HD on D8-D9, 1/15 HD on D13, and 1-3/10 LD on D19-22. Edema was noted at the abrasion site in 1/15 HD animals on D7-D8. Other mucosal observations included tongue ulceration and bleeding in the vehicle control animals on D1-D3. No lesions were reported on the right (unabraded) buccal mucosa.

There were few macroscopic findings in the study. At the D15 sacrifice, alopecia of the skin was noted in 1/4 vehicle animals and 2/5 LD animals; additionally, a focus in the lung was observed in 1 vehicle treated animal. On D29, 1/5 vehicle animals showed alopecia at multiple sites (4), 1/5 LD animals showed alopecia on one forelimb/forefoot and 1-3/5 HD animals showed alopecia at 1-2 sites; additionally, 1 HD animal showed a lesion on the tail. On D43, alopecia was observed in 1 LD animal (2 sites) and 1 HD animal on a single site; a stomach plaque was observed in 1 HD animal.

Generally, histological assessment showed signs of mild irritancy and inflammatory reactions; for details of D5, D15, D29 and D43 sacrifices, see the excerpts from the **sponsor's summary table K2, below**. Microscopic observations performed for the 3 water control animals on D2 indicated that the abrasion procedure was mostly successful; two of 3 water control animals demonstrated mild ulcer and acute inflammation of the left buccal mucosa. Histology from D5 sacrifice animals demonstrated variable signs of inflammatory responses at the abrasion site and related tissues. Signs of minimal-mild inflammation and/or damage were generally observed in the left buccal mucosa, labial junction, tongue, larynx and submandibular lymph node. Microscopic findings on D15 demonstrated few remaining left buccal mucosa findings, but occasional minimal-mild lymphoid necrosis of the submandibular lymph node remained. Additionally, mild chronic inflammation of the lung was observed in 1 vehicle control animal and minimal-mild inflammation and/or epidermal hyperplasia of forelimb skin was observed in 1 LD animal. On Day 29, histologic evaluations demonstrated that 2 LD animals showed subacute inflammation of the trachea, and inflammation, epidermal hyperplasia, hyperkeratosis and fibrosis of varying skin sites were occasionally observed in vehicle,

LD and HD animals. At the recovery assessment, inflammation was observed in the buccal mucosae of 1LD and 2HD animals; although the findings are not consistent with D29 findings, these results suggest some variability of irritancy and possibly of the abrasion procedure. Minimal-mild epidermal hyperplasia, hyperkeratosis and parakeratosis of forelimb skin were observed 1LD and 1HD animal.

Table K2

28-Day Oral Irritation Study in Sprague Dawley Rats

Microscopic Observations: Day 5 Euthanasia and Early Death

Dose	0 4 sprays/day (Water)	0 4 sprays/day (Vehicle)	Low 2 sprays/day (Zolpidem oral spray)	High 4 sprays/day (Zolpidem oral spray)
Animal Number	1 1 1 1 M M M M 0 0 0 0 4 5 6 7 8	2 2 2 2 2 2 M M M M M M 2 2 2 2 2 3 4 5 6 7 8 3	3 3 3 3 3 M M M M M 4 4 4 4 4 4 5 6 7 8	4 4 4 4 4 M M M M M 6 6 6 6 6 4 5 6 7 8
Day of Death/Euthanasia	0 0 0 0 5 5 5 5 I	0 0 0 0 0 0 5 5 5 5 5 1 I	0 0 0 0 0 5 5 5 5 5 I	0 0 0 0 0 5 5 5 5 5
Tissue				
-lesion				
Buccal mucosa, left				
-fibrosis	0 0 2 0 2	2/5	0 0 0 0 0 0	0/6
-inflammation, acute	0 1 0 0 0	1/5	0 0 0 0 0 2	1/6
-inflammation, chronic	0 0 0 0 1	1/5	0 0 0 0 0 0	0/6
-ulcer	0 0 0 0 2	1/5	0 0 0 0 0 0	0/6
-regeneration, skeletal muscle	0 0 0 0 0	0/5	0 0 0 0 0 0	0/6
Gingiva, periodontal				
-inflammation, acute	1 1 2 1 1	5/5	1 1 1 1 1 1	6/6
Submandibular lymph node				
-necrosis, lymphoid	0 1 0 0 0	1/5	0 1 1 0 0 2	3/6
Larynx				
-inflammation, acute	0 0 0 0 0	0/5	0 0 0 0 0 1	1/6
Labial junction				
-inflammation, acute	0 0 N N 0	0/3	0 0 0 0 0 1	1/6
Tongue				
-fibrosis	0 0 0 0 0	0/5	0 0 0 0 0 0	0/6

No microscopic lesions were observed in the following tissues: right buccal mucosa, hard palate, soft palate, parotid salivary gland, nasal passages, trachea, bronchus, esophagus, and stomach.

- 0 = Lesion not observed
- 1 = Lesion of minimal severity
- 2 = Lesion of mild severity
- 3 = Lesion of moderate severity
- 4 = Lesion of marked severity
- N = Tissue insufficient
- M = Tissue missing
- \* = Nonprotocol-specified tissue
- I = Incidence: Number of animals with lesion/number of animals examined

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