## APPENDIX

### The Study Drug, Pexacerfont

*Pharmacological specificity.* The molecular formula of pexacerfont is $C_{18}H_{24}N_6O$, which corresponds to a molecular weight of 340.42. Nonclinical experience has shown it to be a potent and selective $CRF_1$ antagonist. It is specific for $CRF_1$ receptors and has greater than 1000-fold lower affinity for $CRF_2\alpha$ and $CRF_{2\beta}$ receptors and greater than 100-fold lower affinity for the CRF binding protein. Furthermore, in the rat, $CRF_1$ antagonists exhibit antidepressant-like behavior. In a number of studies, pexacerfont was active in reducing colonic motility and the frequency of diarrhea in stressed rats or rats given exogenous CRF (7).

*Pharmacokinetics.* The oral bioavailability of pexacerfont is 58% with a systemic clearance of 2 mL/min/kg in chimpanzees. $^{14}C$-labeled pexacerfont studies in humans indicate elimination of total radioactivity primarily in feces with substantial amounts present in urine. It is 96.1% protein bound in humans (7).

Pexacerfont is a weak inhibitor of CYP2D6 and is predominantly a CYP3A4 substrate. It does not produce any clinically meaningful changes in pharmacokinetics of compounds metabolized by CYP2D6, and potent CYP3A4 inhibitors produced no clinically meaningful changes in the pharmacokinetics of pexacerfont. Administration of pexacerfont to healthy volunteers showed that adverse events were generally similar to those of placebo. Additionally, in a multiple ascending dose study, it was not associated with an increased incidence of prolongations of QT interval or QTc (7).

*Safety.* Extensive safety studies have been completed in animals and support the safety of the doses chosen for this study. In human healthy volunteers, pexacerfont was well tolerated in

phase I single ascending- and multiple ascending-dose studies. Moreover, multiple doses of pexacerfont did not produce any consistent dose-related effects on cortisol or ACTH that would indicate a suppression of the ACTH or cortisol response. In a clinical study where healthy volunteers received pexacerfont up to 400 mg daily for 4-weeks, monitoring of serum cortisol measured throughout the day, salivary cortisol measured upon awakening and through 1 h after awakening, plasma cortisol after ACTH stimulation tests at near steady-state conditions, and both cortisol and ACTH after CRH stimulation tests at near steady-state conditions, did not find any dose-related or any clinically meaningful changes in either cortisol or ACTH levels after pexacerfont administration was compared to pre-dose conditions. Therefore, it was concluded that pexacerfont's activity is independent of effects on the HPA-axis since definite effects were observed in the rat IBS model but effects on cortisol were absent in both the rat and human.

**Method for Analysis of Gastrointestinal and Colonic Transit**

A variable region of interest program was used to quantitate the counts in the stomach and each of four colonic regions: ascending, transverse, descending, and combined sigmoid and rectum. These counts were corrected for isotope decay, tissue attenuation, and downscatter of $^{111}$In counts in the $^{99m}$Tc window (9,17).

*Gastric emptying* $t_{1/2}$ is a measure of the time for 50% of the radiolabeled meal (identifiable by radiolabeled tracer) to empty from the stomach. *Colonic filling at 6 hours, or* the proportion of the radiolabeled meal to have reached the colon at 6 hours, is an indirect measurement of small bowel transit time. *Ascending colon (AC) emptying* was summarized by the $t_{1/2}$ calculated by linear interpolation of values on the AC emptying curve. Overall *colonic transit* was summarized as the colonic geometric center (GC) at specified times. The GC is the weighted average of counts in the different colonic regions [ascending (AC), transverse (TC),

descending (DC), rectosigmoid (RS)] and stool, respectively 1 to 5. Thus, at any time, the proportion of counts in each colonic region is multiplied by its weighting factor as follows:

$$(\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \%\ stool \times 5)/100 = GC$$

Thus, a higher GC reflects a faster colonic transit. Colonic transit measurements reflect alterations of bowel function due to intestinal secretory or motility disorder; thus, in severe idiopathic constipation, GC at 24 hours is typically <1.7 (44) and, in carcinoid diarrhea, GC at 24 hours is $4.5 \pm 0.4$ [SEM (50)]. Similarly, ascending colon emptying $t_{1/2}$ is typically around 15 to 20 hours and is approximately halved by prokinetic agents (6,12,16,41) and this is positively correlated with stool consistency (12).

Colonic GC reflects the accelerated transit in D-IBS (11) and has been shown to be responsive to treatment with prokinetics such as prucalopride (6), tegaserod (41) and renzapride (12) in previous pharmacodynamic studies using the same methods in patients with constipation-predominant IBS (C-IBS) or functional constipation, and in documenting the retardation of transit caused by alosetron in D-IBS (49) and codeine in healthy controls (23).