CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: March 8, 2014

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 203-952 (Duopa; Levodopa-Carbidopa Intestinal Gel)

NDA 203-952 for Levodopa-Carbidopa Intestinal Gel (LCIG) in the treatment of "motor fluctuations in patients with advanced [redacted] (b) (4) Parkinson's disease [redacted] (b) (4) was originally submitted by AbbVie Inc. on November 16, 2012. A Refusal-to-File letter was issued on January 15, 2013 because of CMC, statistical, and clinical deficiencies. The NDA was resubmitted on June 21, 2013, and was filed (Agency letter, dated August 8, 2013). NDA 203-952 is a 505(b)(2) application, with Sinemet (NDA 17-555) as the Reference Listed Drug (RLD).

Clinical development of LCIG was conducted under IND 60663 (Nouvel Pharma; transferred to Solvay Pharmaceuticals). Nonclinical studies conducted to support clinical development consisted of local (GI) toxicity studies in minipig. The sponsor was also asked to address the high levels of hydrazine in LCIG and the proposed specification limits for two additional degradants, [redacted] (b) (4) and [redacted] (b) (4) (Meeting Minutes, dated February 18, 2011).

The following nonclinical studies were submitted in the NDA:

- local (GI) toxicity studies in minipig, including dose-ranging/MTD studies and pivotal 4-week studies.
- in vitro genotoxicity (Ames, chromosomal aberration) assays for [redacted] (b) (4) and [redacted] (b) (4)
- Four week oral toxicity studies of [redacted] (b) (4) and [redacted] (b) (4) in rat.

In addition, the sponsor provided safety assessments for [redacted] (b) (4), [redacted] (b) (4), hydrazine, and leachables in the LCIG product. These studies and documents were reviewed by Dr. LuAnn McKinney (*cf. Pharmacology/Toxicology NDA Review and Evaluation, LuAnn McKinney, DVM, DACVP, 2/21/2014*). Based on that review, Dr. McKinney concluded that the sponsor has adequate addressed the issues of local toxicity of LCIG and leachables in the LCIG product, but that none of the degradants have been adequately

Reference ID: 3467491

qualified at the proposed shelf-life limits. Although, Dr. McKinney also concluded that for [redacted] (b) (4) "levels in the drug product are not of concern" since [redacted] (b) (4) is a metabolite of carbidopa in humans.

Local Toxicity

In patients with Parkinson's disease, the RLD (Sinemet) is administered orally up to levodopa doses similar to those proposed for LCIG. However, there was concern that a daily 16-hr continuous intraduodenal (ID) infusion of LCIG might result in greater local toxicity to the gastrointestinal tract compared to the RLD. To address this concern, the sponsor conducted dose-ranging and 4-week local toxicity studies in male and female minipig. In general, the dosing regimen used in these studies approximated that proposed for humans, i.e., initial ID bolus followed by continuous ID infusion, although the infusion was for 24 hrs in minipig, compared to 16 hrs/day for humans. The results of the preliminary studies demonstrated the feasibility of the dosing regimen and tolerability of doses of carbidopa/levodopa up to 22.5/90 mg/kg/day.

In Study RD111302, female minipigs (3/group) were administered placebo gel or LCIG at final doses of 11.25/45 or 22.5/90 mg/kg/day (titration to final doses) for 4 weeks. In one HDF, displacement of the cannula (Day 6) resulted in premature sacrifice. Clinical signs ("highly exaggerated fear/startle and intense flight responses") were observed at both doses. Diazepam was administered IM to 1 MDF and all HDF from Day 7 on in order to control the clinical signs. No drug-related gross or microscopic findings were noted in the duodenum.

In Study RD111304, male minipigs (4/group) were administered placebo gel or LCIG for 4 weeks. Following a 2-day dose titration (both groups), the LD group was administered 11.25/45 mg/kg/day on Days 1-18 and 7.5/30 mg/kg/day on Days 19-28; the HD group received 22.5/90 mg/kg/day for the entire 28-day period. Because of clinical signs similar to those observed in females, diazepam was administered IM to all treated animals, beginning on Day 3 and throughout the remainder of the study at the HD; diazepam was discontinued at the LD on Day 22 following dose reduction. Upon microscopic examination of the duodenum, there was an increase in the incidence and severity of congestion and inflammatory cells in HD animals (slight vs minimal in C and LD groups). The sponsor attributed the dose-related findings to effects of LCIG on behavior rather than a direct tissue effect; Dr. McKinney concurred.

Therefore, the LCIG product did not induce notable local toxicity at doses fairly similar to those proposed for humans.

Impurities/Degradants

The shelf-life limits proposed for three carbidopa degradants, [redacted] (b) (4) [redacted] (b) (4) % relative to carbidopa monohydrate; [redacted] (b) (4) mg/day), [redacted] (b) (4) [redacted] (b) (4) % relative to carbidopa monohydrate; [redacted] (b) (4) mg/day), and hydrazine ([redacted] (b) (4) µg per g of LCIG; [redacted] (b) (4) mg/day) exceed the qualification thresholds ([redacted] (b) (4) respectively). In addition, hydrazine is a genotoxic carcinogen; therefore, the expected limit would be one resulting in ≤1.5 µg/day. The

Reference ID: 3467491

shelf-life limits for the RLD are not available to the sponsor; however, according to the review chemist, the proposed shelf-life limits for all three degradants are "at unprecedented levels" (*Chemistry Review, NDA 203952, Charles F. Jewell, 1/28/2014*).

Hydrazine

The sponsor provided the following information to support the proposed limit for hydrazine:

- published literature "for hydrazine toxicity, with identification of dose or plasma exposure margins versus hydrazine exposure for LCIG patients."
- plasma exposure to hydrazine following administration of isoniazid (approved for the treatment of tuberculosis) compared to that following LCIG.
- published literature on "long-term epidemiology and safety of isoniazid."

Based on all the data presented, the sponsor concluded that "As a known animal carcinogen and potential human carcinogen, the presence of the carbidopa degradation impurity hydrazine in LCIG represents a potential safety risk for patients." However, at the proposed shelf-life limit, "there is little human data to support hydrazine's carcinogenic potential and exposure to hydrazine during treatment with LCIG therefore appears to represent a small component of risk in the context of the overall benefit-risk for LCIG."

Nonclinical data: The sponsor acknowledged that hydrazine is genotoxic and that "The carcinogenicity of hydrazine has been well documented in the scientific literature and reflected in numerous hydrazine monographs." In a review of the data, IARC (IARC Monographs 71:991-1015, 1999) reported that hydrazine was mutagenic in bacteria and "…induced the formation of DNA adducts *in vitro* and of N7-methylguanine and O^6-methylguanine in liver of mice, rats and hamsters treated *in vivo*" and concluded that hydrazine is an animal carcinogen and "… *possibly carcinogenic to humans (Group 2B)*." However, the sponsor argued that hydrazine exerts its carcinogenic effects through an indirect genotoxic (or possibly non-genotoxic) mechanism, suggesting various modes of action, such as interaction with endogenous formaldehyde to form an intermediate (formaldehyde hydrazine) which is metabolized to a methylating agent (diazomethane) or a combination of increased DNA methylation accompanied by increased oxidative stress and sulfhydryl demand. Hydrazine may be a tumor promoter, although the sponsor noted that "The literature is not clear on this point…" The sponsor also hypothesized that hydrazine may exert its carcinogenic effects through an "indirect influence…on DNA alkylation (methylguanines)…" or through hypomethylation of DNA, which would "support a loss of genetic control and stability that can lead to inappropriate gene expression and impact on phenotypic expression." Although it was acknowledged that "…a specific mechanism has not been established…" and no studies were conducted to investigate a potential mechanism, the sponsor argued that hydrazine should be considered to be carcinogenic through a threshold-based mechanism.

The sponsor also argued that "While hydrazine has been demonstrated to be mutagenic in vitro, there is a lack of evidence for a similar effect in vivo…" or at least that "…hydrazine is genotoxic by the oral route but only at high dosages." As evidence for a

Reference ID: 3467491

lack of a genotoxic effect in vivo, the sponsor cited a published study (Douglas GR *et al. Carcinogenesis* 16:801-804, 1995) conducted in transgenic mice (Muta™mouse). According to the sponsor, Douglas *et al.* (1995) reported that "…no dose induced any lacZ mutations in lung, liver or bone marrow, indicating the absence of a measurable mutagenic action" following single oral (gavage) doses "up to a toxic dose (400 mg/kg)…" However, according to the authors, it is possible that "…repeated exposure is needed for induction and detection of mutagenicity in target tissues (since all the mutagenicity studies to date have involved acute exposures)." Becker *et al*. (Becker RA *et al. Carcinogenesis* 11:1181-1188, 1981) demonstrated DNA methylation (formation of 7-methylguanine) in livers of Fisher 344 rats administered hydrazine at oral doses of 3 mg/kg (4 daily doses) or 45-90 mg/kg (acute doses). Formation of 7-methylguanine was detected at all doses; however, at 3 mg/kg, 7-methylguanine was not detected following a single dose and was detected only at trace levels after the 3rd dose, but was "readily detected" after the 4th daily dose.

The sponsor cited three published carcinogenicity studies (Steinhoff D *et al. Exp Pathol* 39:1-9, 1990; Steinhoff D, Mohr U *Exp Pathol* 33:133-143, 1988; Bosan WS *et al. Carcinogenesis* 8(3):439-444, 1987) considered to have been "…closest in design to that of a conventional 2-year bioassay for carcinogenicity." At the dose (or highest dose) not associated with an increase in liver (rat, hamster) or lung (mouse) tumors in these studies, the sponsor calculated safety margins of 2, 1.3, and 10 in mouse, rat, and hamster, respectively, compared to that in humans at the maximum dose of hydrazine based on the proposed shelf-life limit ((b) (4) mg/day), on a mg/m^2 basis. The sponsor conducted a study to estimate plasma exposures achieved in the published studies in mouse (Steinhoff *et al.*, 1990) and rat (Steinhoff & Mohr, 1988). Based on these data, the sponsor estimated higher safety margins, i.e., (b) (4) and (b) (4) for mouse and rat, respectively. The sponsor used a mean plasma hydrazine AUC in humans of (b) (4) ng*hr/mL, although hydrazine was not detected in 10 of 11 subjects and a plasma AUC of (b) (4) ng*hr/mL was obtained in the one subject in which hydrazine was detected (*see* <u>*Clinical data*</u>). Using the higher plasma AUC, the safety margins would be (b) (4) and (b) (4) for mouse and rat, respectively. It is questionable how accurately the sponsor's data reflected exposures in the published studies; the mouse studies were conducted in different strains and while the rat studies were both conducted in Wistar rat, animals were obtained from different suppliers, and no plasma data were collected in the published study to help bridge between studies. However, even if one presumes a non-genotoxic, threshold-based mechanism for hydrazine-induced carcinogenicity and bases safety margins only on these three studies, there is no safety margin based on the most sensitive species (rat). In addition, as Dr. McKinney points out, there will be direct, prolonged local (GI) exposure to hydrazine in humans on LCIG therapy.

The genotoxic and carcinogenic potential of hydrazine (and 10 other "mutagenic carcinogens") has recently been extensively reviewed (Ellis P *et al. Regul Toxicol Pharmacol* 65:201-213, 2013). The authors (representatives of Kimberly-Clark and Pfizer Global Research and Development) stated that in reviewing genotoxicity data, where conflicts among studies existed, assessment conducted by various organizations (including IARC, NTP, US EPA, and WHO) were "relied upon." For carcinogenicity

Reference ID: 3467491

studies, the primary source was the Carcinogenic Potency Database (*cf.* Gold S, Zeiger E. Eds. *Handbook of carcinogenic Potency and Genotoxicity Databases.* CRC Press, Boca Raton, Fl, 1997); however, the authors also conducted a literature search for "more recent or supplementary information." The authors also reviewed the published literature on potential modes of action. For hydrazine, the authors concluded that "Overall, the weight of evidence suggests that hydrazine is a DNA-reactive carcinogen with a non-threshold mode of action" and estimated an acceptable daily intake (ADI) of 0.613 µg/day, based on the TD_{50} (0.613 mg/kg/day) in the most sensitive species (rat) and assuming a lifetime (70 year) exposure. This ADI was calculated based on a 50-kg human. If one instead calculates an ADI based on a (b) (4) kg human, the ADI would be (b) (4) µg/day. If one also assumes that LCIG will be primarily used in patients with advanced Parkinson's disease, and therefore an older population with a shorter duration of exposure (e.g., ~10 years), a higher ADI would be reasonable (e.g., (b) (4) µg/day). It is not possible, however, to justify the maximum daily dose of hydrazine from LCIG of (b) (4) mg/day, based on these data.

<u>Clinical data</u>: The sponsor argued that there are substantially higher plasma exposures to hydrazine in patients treated with isoniazid compared to those in patients on LCIG, but the extensive human experience with isoniazid has not identified a signal for carcinogenicity (the sponsor cited a number of publications, dated 1962-1990). According to isoniazid labeling, the maximum recommended dose is 900 mg (given 300 mg TID) 2-3 times per week, or ~260-386 mg/day averaged over a week. Two published studies of isoniazid were cited (Pea F *et al. Clin Pharmacokin* 17(2):145-154, 1999; Woo J *et al. J Med* 23(1):51-59, 1992). Pea *et al*. (1999) reported serum hydrazine levels in 26 patients (15 rapid acetylators, 11 slow acetylators) following a single oral isoniazid dose of 200 or 300 mg (based on body weight; ~4.68-4.75 mg/kg) (samples collected up to 12 hrs post dose). The data were provided only in a figure; the sponsor digitized the figure to estimate PK parameters. The sponsor estimated serum AUCs (presumed 0-12 hr) for hydrazine of (b) (4) and (b) (4) ng*hr/mL in rapid and slow acetylators, respectively. From the figure, it appears that C_{max} values were (b) (4) and (b) (4) ng/mL, respectively. Woo *et al.* (1992) reported a mean plasma hydrazine level of 3.1 ± 1.5 µg*hr/mL in 8 of 10 patients on isoniazid therapy (mean dose of 6.7 mg/kg [~400 mg], in 10 patients).The authors noted that plasma hydrazine levels were higher than previously reported (citing Beever IW *et al*. *Brit J Clin Pharmacol* 13:599P, 1982) because of the assay used, which measured free and hydrated forms. For example, Blair *et al*. (Blair IA *et al. Hum Exp Toxicol* 4:195-202, 1985) assessed plasma exposure to hydrazine following administration of isoniazid (300 mg QD) for 14 days to healthy male subjects. On Day 14, plasma levels at 1 hr post dose were 3.7 ± 1.1 and 9.9 ± 1.1 ng/mL in rapid and slow acetylators, respectively; pre-dose levels were 1.9 ± 0.6 and 7.1 ± 1.0 ng/mL, respectively. While the T_{max} of hydrazine was not reported, all serum/plasma values reported by Pea *et al*. (1999) and Woo *et al*. (1992) were higher than those reported by Blair *et al*. (1985).

The sponsor conducted a PK study (S187.3.001/S187.3.002; R&D/12/260) to determine plasma hydrazine levels in patients with advanced Parkinson's disease. (According to the sponsor's Hydrazine Risk Assessment [R&D/12/714], the data represent "Preliminary Results.") Patients received either LCIG (n = 11) or oral levodopa-carbidopa IR tablets

Reference ID: 3467491

(from Merck & Co and/or Mylan Pharmaceuticals; n = 5). Mean daily doses in patients receiving LCIG were 1284-1307 and 321-327 mg for levodopa and carbidopa, respectively; for those receiving the IR tablet, mean daily doses were 1325 and 331 mg, respectively. Hydrazine was detected in plasma only in 1 patient on LCIG and 2 patients on the IR tablet. In the patient on LCIG, the $AUC_{(0\text{-}16\ hrs)}$ was (b) (4) ng*hr/mL (plasma levels ranged from (b) (4) to (b) (4) ng/mL). In each patient on the IR tablet, hydrazine was detected at only one sampling time (b) (4) ng/mL at 4 hr, (b) (4) ng/mL at 8 hr; LLOQ = (b) (4) ng/mL). Although conclusions based on these data are limited by the small number of subjects and the absence of data on the actual amounts of hydrazine in the LCIG delivered, it is reasonable to conclude that plasma hydrazine exposure in patients on isoniazid are substantially higher than that anticipated with LCIG. Isoniazid is typically administered for up to 12 months, whereas LCIG will be administered chronically. The longer exposure in Parkinson's disease patients may limit the relevance of the isoniazid data.

Finally, the sponsor noted that an evaluation of foreign postmarketing data on patients treated with LCIG has not identified a carcinogenic risk, although "Currently, too few patients are enrolled in these data collection efforts to estimate cancer risk."

(b) (4) and (b) (4)

The sponsor provided the following information to support the proposed limits for (b) (4) and (b) (4)

- 4-week oral toxicity study in Sprague-Dawley rat.
- in vitro genotoxicity (Ames, chromosomal aberration assay in human peripheral blood lymphocytes) assays of (b) (4) and (b) (4)
- PK/TK data in rat, minipig, and human.

The sponsor conducted a 4-week toxicity study of (b) (4) and (b) (4) in rat and in vitro genotoxicity studies to directly qualify these degradants. Dr. McKinney considered the 28-day study adequate but of insufficient duration; 3-month bridging studies are typically required to qualify an impurity in a product intended for chronic use. (b) (4) and (b) (4) were negative in Ames assays but positive in in vitro chromosomal aberration assays in the absence ((b) (4), (b) (4)) and/or presence (b) (4) of metabolic activation.

To address concerns raised by the signal for genotoxicity, the sponsor conducted a PK study (Drug Metabolism Memo 01) to estimate the exposure of rats to (b) (4) and (b) (4) in a 2-year carcinogenicity study in Sprague-Dawley (S-D) rat briefly described by (b) (4) In the PK study, male Sprague-Dawley rats (n = 5) were administered carbidopa at doses of 1 and 100 mg/kg by oral gavage. Trace amounts of (b) (4) were detected in plasma (AUC at 100 mg/kg was (b) (4) ng*hr/mL); the highest dose of carbidopa tested in the 2-year study was 10 mg/kg. Therefore, the sponsor's data demonstrate that it is unlikely that exposure to (b) (4) was sufficient for the 2-year study to be considered an adequate assessment of the

Reference ID: 3467491

carcinogenic potential of (b) (4) The plasma AUC for (b) (4) was (b) (4) µg*hr/mL (C_{max} = (b) (4) µg/mL) at 100 mg/kg carbidopa. Therefore, there may have been some exposure to (b) (4) in the published 2-year carcinogenicity study (b) (4) although the extent of exposure is unlikely to have provided an adequate assessment of carcinogenic potential at the highest dose of carbidopa tested (10 mg/kg).

The sponsor stated that both (b) (4) and (b) (4) are metabolites of carbidopa in vivo, citing studies by (b) (4) and, for (b) (4) citing a study by (b) (4) (b) (4) reported the presence of (b) (4) in human urine, not plasma, and they stated that the presence of (b) (4) in human urine may have been the result of auto-oxidation during "manipulation of the urine samples." (b) (4) reported the presence of (b) (4) (b) (4)% of dose) in human urine following an oral dose (250 mg) of radiolabeled (b) (4) to 4 individuals; no plasma samples were collected. A brief literature search did not identify any additional publications documenting systemic exposure to (b) (4) following administration of (b) (4) detected (b) (4) (free and conjugated) in human urine ((b) (4)% of urinary radioactivity) following administration of radiolabeled (b) (4); plasma levels were not assessed.

In the PK study conducted by the sponsor (R&D/12/260), (b) (4) was not detected in plasma of 10 of 11 patients receiving LCIG or of all 5 receiving oral CD/LD tablets. In one patient receiving LCIG, (b) (4) was detected in only one plasma sample, at a level ((b) (4) ng/mL) near the LLOQ ((b) (4) ng/mL). Based on these data, the sponsor concluded that exposure to (b) (4) resulting from LCIG would be (b) (4)% of carbidopa levels, similar to that from the CD/LD tablet. (b) (4) was detected in plasma of 7/11 patients receiving LCIG and 4/5 patients receiving oral CD/LD tablets. In these patients, plasma levels were (b) (4) and (b) (4) ng/mL following LCIG and CD/LD tablets, respectively; plasma AUCs were 1 (b) (4) (mean = (b) (4)) and (b) (4) (mean = (b) (4) ng*hr/mL, respectively. The sponsor concluded plasma levels of (b) (4) following LCIG and CD/LD were (b) (4) and (b) (4)%, respectively, those of carbidopa. These data demonstrate that (b) (4) is likely to be a circulating metabolite in humans. However, T_{max} for (b) (4) in patients on LCIG was earlier than that for carbidopa, indicating that the higher circulating levels of (b) (4) following LCIG resulted from absorption of degradant (b) (4) Overall, the available data suggest that (b) (4) is a metabolite of carbidopa in vivo in humans following administration of carbidopa; but, they also suggest significant contribution to circulating levels from the presence of (b) (4) in LCIG as a degradant. The data do not support the sponsor's claim that (b) (4) is a metabolite of carbidopa in vivo in humans.

<u>Comments</u>: The sponsor has not adequately qualified the shelf-life limits proposed by the sponsor for carbidopa degradants, hydrazine, (b) (4), and (b) (4) Hydrazine is a recognized genotoxic (mutagenic, clastogenic) compound, as well as a multi-species carcinogen. The in vitro genotoxicity studies conducted by the sponsor demonstrated that

Reference ID: 3467491

both [redacted] (b) (4) and [redacted] (b) (4) while negative in the Ames assay, were clastogenic in mammalian cells. As genotoxic impurities, the expectation would be that the levels of each degradant in the drug product would be consistent with a daily dose of ≤1.5 µg/day for lifetime use or ≤10 µg/day for 1-10 years. (For hydrazine, these acceptable intakes (AI) are substantially higher than the AI estimated by Ellis *et al*., 2013.) However, none of these degradants can be lowered to even the higher level, based on the stability of carbidopa in LCIG under the proposed conditions of use.

The 4-week toxicity study of [redacted] (b) (4) in rat was adequate but of insufficient duration to assess the general toxicity of either degradant. For therapies intended for chronic use, the bridging study should be of 3 months' duration.

Based on review of the sponsor's information, Dr. McKinney has concluded that:

- Hydrazine has not been qualified at the proposed shelf life limit based on previous studies documenting hydrazine to be a genotoxic animal carcinogen.
- [redacted] (b) (4) has been adequately qualified at the proposed shelf life limit, based on the fact that it is a metabolite of carbidopa in vivo in humans.
- The clastogenicity of [redacted] (b) (4) and [redacted] (b) (4) are not of concern since the moiety responsible for the structural alert is shared by levodopa and carbidopa and the 2-year carcinogenicity study of levodopa-carbidopa (described in the Sinemet labeling) was negative for tumors; therefore, neither impurity needs to be controlled at a level providing a dose of ≤1.5 µg/day.
- [redacted] (b) (4) has not been qualified at the proposed shelf life limit because of the short duration of the repeat-dose toxicity study (4 weeks vs 3 months).

I concur with Dr. McKinney's conclusions regarding hydrazine and that the 4-week oral toxicity study was not of sufficient duration to qualify either [redacted] (b) (4) or [redacted] (b) (4) Although the sponsor's data confirm that [redacted] (b) (4) is a human metabolite of carbidopa in vivo, they also suggest that LCIG may result in higher exposure (~2-fold) to [redacted] (b) (4) than would be obtained from metabolism. The actual levels of [redacted] (b) (4) (or [redacted] (b) (4)) in the LCIG administered in the clinical study are unknown, suggesting that higher exposures could result. Therefore, it is unclear whether or not [redacted] (b) (4) should be considered qualified based on its being a metabolite. As Dr. McKinney notes, [redacted] (b) (4) does not appear to be a metabolite of carbidopa in vivo in humans or in rat.

It is not certain if one can conclude that there is no concern regarding the potential clastogenicity of [redacted] (b) (4) and [redacted] (b) (4) based on the negative 2-year carcinogenicity study of levodopa-carbidopa. It is unlikely that there was sufficient exposure to either degradant in that study. In addition, the highest dose of carbidopa tested (10 mg/kg/day) is substantially less than the maximum recommended clinical dose (200 mg/day) from Sinemet or from LCIG (500 mg/day), based on body surface area (mg/m^2). Dr. McKinney concluded, based on input from the chemistry team, that the carcinogenic potential of [redacted] (b) (4) and [redacted] (b) (4) associated with genotoxicity is not of concern since the structural alert ([redacted] (b) (4) is shared with carbidopa and levodopa, both of which were negative in the 2-year study. Whereas it is consistent with current guidance to dismiss

Reference ID: 3467491

concerns regarding the genotoxic potential of an impurity if the structural alert is shared with the parent compound and the parent compound is negative in genotoxicity assays, a positive signal for clastogenicity cannot be as easily dismissed, particularly based on data from an unrelated compound (levodopa). While levodopa and carbidopa were negative in the 2-year study, (b) (4) itself has clearly been shown to be carcinogenic in animals (b) (4) Therefore, one cannot necessarily generalize the negative results in a carcinogenicity study of one compound containing a specific structural alert to others containing the same structural alert.

The information provided by the sponsor does not fully qualify (b) (4) or (b) (4) at the propose shelf-life limits; however, it is clear that the primary concern regarding the carcinogenic potential of LCIG is the substantial amount of hydrazine formed under the proposed storage conditions. Although the 4-week toxicity study of (b) (4) and (b) (4) was of insufficient duration, no toxicities of concern were observed in that study and considering the amount of human experience to date and the inability of the sponsor to substantially reduce the levels of either (b) (4) or (b) (4), I don't believe that data from a 3-month study would have meaningful regulatory impact.

Leachables/Extractables

The sponsor reported the presence of a number of leachables/extractables (b) (4) Dr. McKinney has reviewed the information provided in support of the sponsor's proposed limits and finds it adequate to address any safety concerns. I concur.

Recommendations

There are concerns regarding the high shelf-life limits proposed for three degradants in LCIG, but the greatest concern is the potential for exposure of patients to substantial amounts of hydrazine, a known genotoxic animal carcinogen and possible human carcinogen. Considering the seriousness of the indication, if the LCIG product is considered to be of sufficient therapeutic benefit to warrant approval, I would suggest that the sponsor be asked to explore, if possible, strategies for lowering the amounts of degradants formed under the conditions of storage and use.

Reference ID: 3467491

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/s/

LOIS M FREED
03/08/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 203952
Supporting document/s:	eCTD
Applicant's letter date:	May 28, 2013
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Product:	Levodopa Carbidopa Intestinal Gel (LCIG)
Indication:	Parkinson's Disease
Applicant:	AbbVie, Inc.
Review Division:	Division of Neurology Products
Reviewer:	LuAnn McKinney, DVM, DACVP
Supervisor/Team Leader:	Lois M. Freed, PhD
Division Director:	Billy Dunn, MD
Project Manager:	Stacy Metz, PharmD

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Reference ID: 3459002

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 3
1.1 RECOMMENDATIONS 3
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 4
2 DRUG INFORMATION 4
2.1 (A) DRUG 5
2.1 (B) DRUG 5
2.2 RELEVANT INDS, NDAS, AND DMFS: 5
2.3 CLINICAL FORMULATION 5
2.4 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN: 6
2.5 REGULATORY BACKGROUND 6
3 STUDIES SUBMITTED 7
3.1 STUDIES REVIEWED 7
3.2 STUDIES NOT REVIEWED 8
3.3 PREVIOUS REVIEWS REFERENCED: NONE 8
4 PHARMACOLOGY 9
4.1 PRIMARY PHARMACOLOGY 9
4.2 SECONDARY PHARMACOLOGY 9
4.3 SAFETY PHARMACOLOGY 9
5 PHARMACOKINETICS/ADME/TOXICOKINETICS 9
5.1 PK/ADME 9
6 GENERAL TOXICOLOGY 9
7 IMPURITIES 38
7.1: PK/ADME 39
PHARMACOKINETIC STUDIES: 39
7.3 GENERAL TOXICOLOGY 47
7.4 GENETIC TOXICITY 50
8 LEACHABLES AND EXTRACTABLES 55
9 INTEGRATED SUMMARY AND SAFETY EVALUATION 58
LOCAL TOXICITY: 58
IMPURITIES 59
SUMMARY 65

Reference ID: 3459002

1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: No.

Levels of the degradant impurities hydrazine, (b) (4), and (b) (4) are not qualified and the drug product cannot be approved from a pharmacology/toxicology perspective.

Approval would be a matter of clinical judgment of the risk of exposure to the degradants and benefit to the patient population.

1.1.2 Additional Non Clinical Recommendations:

To support qualification of the degradants (b) (4) and (b) (4), further characterization of the genotoxic risk would be needed to support the proposed specifications.

1.1.3 Labeling

The labelling for the RLD does not list administered doses in nonclinical studies and, with the following exceptions, safety margins should be deleted from nonclinical sections 8.1 and 13.1.

Specific recommended changes from the sponsor's proposed labelling are:

NONCLINICAL TOXICOLOGY

Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Reference ID: 3459002

(b) (4)

1.2 Brief Discussion of Nonclinical Findings

This is a 505 (b)(2) application for a 4:1 combination of levodopa (LD) and carbidopa (CD) formulated as a cellulose gel to be administered by cannula into the jejunum. This NDA references the previous findings of safety and effectiveness for the Reference Listed Drug (RLD), Sinemet (NDA No. 017555), an oral tablet containing LD and CD in a ratio of 4:1. No new nonclinical studies were conducted to assess the pharmacology, PK/ADME, toxicity, genotoxicity, or effects on reproductive or developmental toxicology of levodopa and carbidopa alone or in combination. A required local irritation study was conducted in minipigs and the drug product was found to be non-irritating at the point of administration in the intestinal lumen.

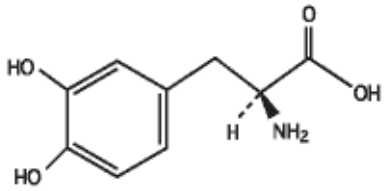
To qualify three carbidopa degradant impurities that exceed the allowable maximum exposure or the qualification threshold, the sponsor submitted TK and toxicity data and offered assessments of: hydrazine; (b) (4) and (b) (4) Hydrazine is a genotoxic animal carcinogen with an allowable exposure of 1.5 mcg/day and the data do not support the proposed daily dose (based on the maximum recommended human dose (MRHD) of 100 mL) of up to (b) (4) mg/day.

The sponsor proposes levels of the impurities (b) (4) and (b) (4) at (b) (4) w/w CD and (b) (4) w/w CD, respectively, yielding a total daily intake, based on the MRHD, of up to (b) (4) mg (b) (4) and up to (b) (4) mg (b) (4) Although not mutagenic, (b) (4) and (b) (4) are clastogenic and the acceptable daily intake of genotoxic impurities is 1.5 mcg. However, (b) (4) and (b) (4) share a structural alert for carcinogenicity with CD and LD, which have not been found to be carcinogenic when administered in combination in a 2-year bioassay. As such, it is unlikely that (b) (4) and (b) (4) would be carcinogenic based on this structural alert, and the levels need not be limited to those of genotoxic impurities. However, the levels of do exceed the qualification threshold for degradant impurities by (b) (4) fold (b) (4) and (b) (4) fold (b) (4) and a combined-administration toxicology study of these impurities was not adequate to support the proposed levels. (b) (4) was determined to be a minor metabolite of CD in clinical subjects and the levels in LCIG are not found to add significantly to exposure as a metabolite. (b) (4) was not detected in human plasma after CD administration, and thus is not proven to be a metabolite; the proposed level (b) (4) w/w CD) cannot be qualified from the submitted data.

2 Drug Information

2.1 (A) Drug

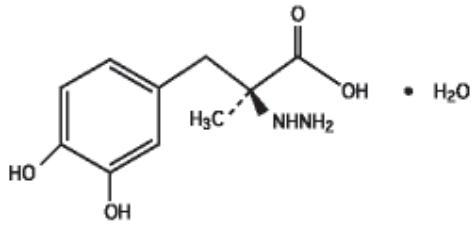
2.1.1 Generic Name: Levodopa
2.1.2 Chemical Name: L-Tyrosine, 3-Hydroxy or (-)-3-(3,4-dihydroxyphenyl)-L-alanine
2.1.3 Chemical formula/molecular weight: C9H11NO4/197.2
2.1.4 Structure:



2.1.5 Pharmacologic Class: aromatic amino acid

2.1 (B) Drug

2.1.6 Generic Name: Carbidopa
2.1.7 Chemical Name: Benzenepropanoic acid, (α-Hydrazino-3,4-dihydroxy-, α-methyl-, monohydrate)
2.1.8 Molecular Formula/Molecular Weight: C10H14N2O4•H2O/226
2.1.9 Structure:



2.1.10 Pharmacologic class: Aromatic Amino Acid Decarboxylation inhibitor

2.2 Relevant INDs, NDAs, and DMFs:

IND 60663

2.3 Clinical Formulation

2.3.1 Drug Formulation: LCIG is a suspension of levodopa-carbidopa monohydrate (4:1) in an aqueous carmellose sodium gel that is formulated at a target pH of (b) (4) Each mL of gel contains 20.0 mg levodopa, 5.0 mg carbidopa, and (b) (4) mg carmellose sodium in purified water (b) (4) The product is delivered from 100 mL cassettes, through a cannula, directly to the jejunum.

2.3.2 Comments on Novel Excipients: None

2.3.3 Comments on Impurities/Degradants of Concern:

The sponsor provided data and assessments to qualify three carbidopa degradant impurities in LCIG: hydrazine, [redacted] (b) (4), and [redacted] (b) (4) These are addressed in Section 7 and an extended discussion is found in the Integrated Summary and Evaluation.

Hydrazine (H_4N_2) is genotoxic, a known animal carcinogen, and is reasonably anticipated to be a human carcinogen. LCIG contains up to [redacted] (b) (4) mcg/mL of hydrazine. The sponsor's recommended maximum dose of [redacted] (b) (4) results in a maximum daily dose of [redacted] (b) (4) mg/day, exceeding the maximum daily dose of 1.5 mcg by over [redacted] (b) (4)-fold. A review of the literature and PK data from animals administered hydrazine, carbidopa, or LCIG and from clinical subjects administered LCIG or the RLD were submitted.

[redacted] (b) (4) and [redacted] (b) (4) are both carbidopa degradants and the sponsor considers them to be *in vivo* metabolites. Both were negative in the Ames test but positive in chromosomal aberration test. At the Sponsor's MRDD, the shelf life limit of the drug product allows levels resulting in a daily dose of [redacted] (b) (4) up to [redacted] (b) (4) mg/day and [redacted] (b) (4) up to [redacted] (b) (4) mg/day. To qualify these daily doses, the sponsor submitted PK data, results of genotoxicity tests, and a 4-week [redacted] (b) (4) toxicity study. Clinical (PK) data from subjects administered LCIG and subjects administered the RLD were also submitted.

2.4 Proposed Clinical Population and Dosing Regimen:

LCIG is proposed for the long-term treatment of patients with advanced [redacted] (b) (4) Parkinson's disease [redacted] (b) (4) The concentration of levodopa (LD) in LCIG is 20 mg/mL and of carbidopa is 5 mg/mL; individual 100 mL medication cassettes contain 100 g LCIG (2000 mg levodopa and 500 mg carbidopa). The product is delivered directly to the proximal small intestine (jejunum) via a Percutaneous Endoscopic Gastrostomy-Jejunal (PEG-J) tube and a portable programmable infusion pump. In contrast to the sponsor's maximum recommended daily dose (MRDD) of [redacted] (b) (4) LCIG, the clinical reviewer finds that the, based on LD, the MRDD should be 2000mg (100mL gel), with a daily bolus of 300 mg LD (15 mL gel) followed by continuous dose of LD for 16 hours up to a total of 100mL of LCIG in 24 hours.

2.5 Regulatory Background

IND 60663 was submitted on JUL 28, 2000 by [redacted] (b) (4) Solvay Pharmaceuticals, Inc. became the sponsor effective SEP 1, 2005. Fast Track Designation was granted on JAN 29, 2008; Abbott Products, Inc. (now AbbVie, Inc.) became the sponsor on MAR 25, 2010.

Reference ID: 3459002

NDA 203-952 was received NOV 16, 2012. A Refuse-to-File letter was issued on JAN 14, 2013; the application was resubmitted on 28 May, 2013, with a filing date of JUL 2, 2013.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacokinetics:

Memo 01 Hydrazine Pharmacokinetics following Oral Dosing in Swiss Webster Mice
Memo 02 Hydrazine Pharmacokinetics following Oral Dosing in Wistar Rats
Memo 01 Pharmacokinetics of Carbidopa, [redacted] (b) (4) and [redacted] (b) (4) following Intravenous and Oral Dosing in Rat

Toxicology:

RD 111277- Duodopa: Pilot Study for Gastric Evaluation of Continuous Duodenal Administration in Minipigs
RD 111278-Duodopa: maximum Tolerated Dose Study for Gastric Evaluation of Continuous Duodenal Administration in Minipigs
RD 111279- Duodopa: Maximum tolerated Dose Study by Continuous Duodenal Infusion Administration to Minipigs
RD 111302- Duodopa: Local Irritation Study of Continuous Duodenal Administration to Minipigs for 4 Weeks
RD 111303- Duodopa: Maximum Tolerated Dosage and Feasibility Study of Continuous Duodenal Infusion in Male Minipigs
RD 111304- Duodopa: Local Irritation Study of Continuous Duodenal Administration ot Male Minipigs for 4 Weeks
TA 11-113- Four-Week Oral Impurity Qualification Study of [redacted] (b) (4) and [redacted] (b) (4) (Degradants in Duodopa) in Sprague-Dawley Rats

Genetic Toxicology

TX 11-132- Bacterial Reverse Mutation Assay with [redacted] (b) (4)
TX 11-133- *In Vitro* Mammalian Chromosome Aberration Test with [redacted] (b) (4)
TX 11-134- Bacterial Reverse Mutation Assay with [redacted] (b) (4)
TX11-135- *In Vitro* Mammalian Chromosome Aberration Test with [redacted] (b) (4)

Assessments and analyses:

RD 07138- PIDE for [redacted] (b) (4) in Clarithromycin
RD 11170- Preclinical Safety Assessment of [redacted] (b) (4) Erythromycin Base/Clarithromycin Drug Substance
RD 11570- Safety Assessment of [redacted] (b) (4)
RD 11571- Safety Assessment of Leachables [redacted] (b) (4) in ABT-SLV-187
RD 11827- Determination of the Acceptable Daily Exposure (ADE) of [redacted] (b) (4)

Reference ID: 3459002

RD12714- Analysis of the Safety Risks Associated with Hydrazine as a Degradation Product in LCIG
RD 12997- Qualification of LCIG Impurities (b) (4) and (b) (4)

3.2 Studies Not Reviewed

P1074- Quantitation of Carbidopa, Levodopa, and (b) (4) in Gottingen Mini-Pig Plasma via HPLC with MS/MS Detection
RD111259- (b) (4) Validation of SLV187 degradation products (b) (4) and (b) (4) in Minipig Plasma with MS/MS Detection. Validation Report for a 96-Well Protein Precipitation Extraction HPLC Tandem Mass Spectrometric Method for the Determination of (b) (4) in Minipig Plasma

3.3 Previous Reviews Referenced: None

4 Pharmacology

4.1 Primary Pharmacology

Parkinson's disease is characterized by progressive degeneration of the dopaminergic nigrostriatal system and depletion of dopamine. Levodopa (LD), the precursor of dopamine, readily passes the blood-brain barrier and is the main therapeutic drug for symptoms of Parkinson's disease. Carbidopa (CD), a peripheral decarboxylase inhibitor which does not pass readily into the brain, prevents peripheral decarboxylation of LD and allows higher concentrations to reach the brain.

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted on LD or CD, alone or in combination.

4.3 Safety Pharmacology

No safety pharmacology studies were conducted on LD or CD, alone or in combination.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No new nonclinical studies were conducted to assess the PK/ADME or toxicokinetics (TK) of levodopa and carbidopa alone or in combination. TK data were collected in minipigs, rats, and mice to support levels of degradant impurities in the drug product.

6 General Toxicology

No new nonclinical studies were conducted to assess toxicity of LD and CD alone or in combination. Submitted studies of the LCIG were limited to feasibility and TK studies in minipigs and a subsequent pivotal local irritation study in minipigs. A general toxicity study to support qualification of CD degradants (b) (4) and (b) (4) is reviewed in Section 7.

6.1. Local irritation studies in the minipig.

In a series of feasibility studies and a pivotal 4-week study, LCIG was administered directly into the small intestine of Göttingen minipigs by a portable infusion pump through a surgically implanted percutaneous cannula. The product was delivered from 100 mL cassettes containing a fixed amount of LD (2000 mg) and CD (500 mg) and doses were expressed as mg/kg/day of CD/LD. A pre-determined bolus was administered each morning, followed by continuous perfusion for 24 hours; the dose

Reference ID: 3459002

was determined by the total volume of gel delivered to each minipig. Cassettes were replenished as necessary to maintain the assigned dose. Vehicle control animals were administered carmellose-sodium aqueous gel.

Postmortem examination was largely limited to the abdominal viscera and percutaneous catheterization sites. Histologic examination was limited to gross lesions and pre-determined sections of the duodenum, per protocol for the local irritation study.

6.2.1

Study title: Duodopa: Pilot Study for Gastric Evaluation of Continuous Duodenal Administration in Minipigs

Study no.:	RD 111277
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	FEB 17, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Duodopa®, Batch 5405, 96% (LD) & 95% (CD)

Key Study Findings:

- Animals evidenced inappetence and were "*subdued*" at 10/40 (CD/LD) mg/kg/day; they were unsteady and collapsed at 15/60 (CD/LD) mg/kg/day with slight tremors.
- Higher doses (20/80 CD/LD and 30/120 CD/LD mg/kg/day) were well tolerated and the sponsor suggested the tube had displaced into the stomach prior to administration of 20/80 CD/LD, rendering subsequent lack of findings not relevant.
- On radiologic exam, the cannula tips were found to have migrated from the jejunum to the stomach on Study Day 16 in both vehicle and dosed minipigs. Dosing was terminated; CM remained on saline infusion through Study Day 54.
- An MTD was not determined. Surgery was deemed feasible and cannula patency in the CM was maintained for one month.

Reference ID: 3459002

Methods

Doses:	Doses are expressed as mg of CD/LD. 16 days: Initial bolus (25% of daily dose) followed by continuous (24-hour) infusion of ascending doses of 10/40, 15/60, 20/80, 25/100, 30/120 mg/kg/day. 14 days: Continuous infusion at a stable dose of 30/120 mg/kg/day
Frequency of dosing:	24-hour continuous infusion by programmed infusion pump. Ascending Doses: 24 hours for 3 days, 3 days apart (see table below). Stable dose: every day for 14 days.
Route of administration:	Percutaneous Endoscopic Gastrostomy (PEG) tube with cannula extending into the duodenum.
Dose volume:	Varied with dose: The programmed rate of infusion from 100 mL cassettes determined the 24-hour dose.
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus,* Gottingen minipig
Number/Sex/Group:	1M-C, 2M-dosed
Age:	C: 12 weeks; Dose: 17 weeks.
Weight:	C: 11.2 kg; Dose: 13.8 and 13.6 kg
Satellite groups:	NA
Unique study design:	Surgical implantation of cannula and accommodation to pump-containing jackets prior to dosing by continuous infusion. CM administered vehicle gel for 30 days and saline for additional 21 days.
Deviation from study protocol:	Catheter displaced into stomach (estimated at ~ Study Day 9/10) in 2 dosed M and the study was terminated on Study Day 16.

Sponsor's Table, Dose groups:

Text Table 1. Schedule for Group 1

Day number	Animal number	Dose-carbidopa/levodopa (mg/kg/day)*	Total dose[a] (mg/kg/day)
1-3	92	10/40	12.5/50
4-6	94	15/60	18.5/60
7-9	92	20/80	25/100
10-13	94	25/100	31.3/125
14-16	92	30/120	37.5/150

* On the first day of each dose level, an initial bolus of 25% of the total daily dose was administered followed by the continuous infusion

[a] Total dose on first day of each dose level was the sum of the bolus dose and the 24 hour infusion dose

Reference ID: 3459002

Observations and Results
Mortality: None

Clinical Signs: Dose-related tremors, unsteadiness, and transient collapse were seen at 15CD/60LD mg/kg/day. Green material (suppurative) was seen at percutaneous catheter sites.

Body Weights: No drug related effects.

Food Consumption: No drug related effects.

Gross Pathology:
Intra-abdominal adhesions of cranial viscera (liver, gallbladder, spleen, and stomach) were seen in all animals. Thickening of the skin was observed at the percutaneous catheter site; green purulent material was noted at the site in one dosed animal. Duodenal mucosal congestion was seen in all animals.

Organ Weights: Heart and spleen weights were slightly higher in one dosed pig.

Histopathology: Tissues were collected, formalin-fixed, and retained. No microscopic examination was performed.

Stability and Homogeneity: Not done.

6.2.2

Study title: Duodopa: Maximum Tolerated Dose Study for Gastric Evaluation of Continuous Duodenal Administration in Minipigs.

Study no.:	RD 111278
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	SEP 23, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Duodopa®, 09H13G18, 97.4% (CD) & 97.5% (LD)

Key Study Findings

- Group 1: (Ascending doses) reduced from 2 F to 1. The catheter tip of one minipig with a Gastrostomy-duodenal catheter had "*retracted into stomach,*" and the animal was removed from study. Direct duodenal implantation of the cannula

proved successful in the remaining animals, and the procedure was adopted for subsequent studies.

- Group 2 (Stable dose): Marked repetitive behaviors were observed in 1 animal during constant dosing regimen at 30CD/120LD mg/kg/day.

- An MTD was not determined; however, 30CD/120LD mg/kg/day was deemed adequate for a 4-week study.

Methods

Doses:	Doses are expressed as mg of CD/LD. Group 1 – Initial bolus followed by continuous 24-hour infusion of ascending doses of: 10/40, 15/60, 20/80, 25/100, 30/120 mg/kg/day, each for two days, two days apart. Group 2 – 24-hour infusion at 30/120 mg/kg/day for 14 days.
Frequency of dosing:	24-hour continuous infusion by programmed pump. Initial daily bolus of 25% of total daily dose. Ascending Doses: 24-hour infusion for 2 days, every 2 days (see table below). Stable dose: every day for 14 days
Route of administration:	Intra-duodenal via PEG tube and jacketed portable infusion pump.
Dose volume:	100 mL cassettes containing 500 mg CD and 2000 mg LD per cassette. Daily doses were determined by programmed infusion rate of the pump.
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus*, Gottingen minipig
Number/Sex/Group:	2 F
Age:	Group 1: 6 months; Group 2: 8 months
Weight:	Group 1: 11.3 kg; Group 2: 13.8, 14.0 kg
Satellite groups:	NA
Unique study design:	Surgical implantation of catheter and accommodation to pump-containing jackets prior to dosing by continuous infusion.
Deviation from study protocol:	Group 1 reduced to 1 animal. Daily bolus reduced to 17% of dose.

Reference ID: 3459002

Study Design Ascending Dose (Group 1), Sponsor's table:

Group	Day	Treatment	Dose* (mg/kg/day)	Total dose[a] (mg/kg/day)	No. of animals assigned	Animal number assigned
1	1-2	Duodopa gel (carbidopa & levodopa)	10/40	12.5/50	2	1, 2
	3-4		15/60	18.5/60		
	5-6		20/80	25/100		
	7-8		25/100	31.3/125		
	9-10		30/120	35/140		

* On the first day of each dose level, an initial bolus of 25% of the total daily dose was administered followed by the continuous infusion

a Total dose on first day of each dose level was the sum of the bolus dose and the 24 hour infusion dose apart from Day 9 when, due to clinical signs the bolus was reduced to 17%

Observations and Results
Prior to dosing, animal number 1 of Group 1 was removed from the study and euthanized because the cannula tip had migrated into the stomach.

Clinical Signs:
Group 1: Animal was restless, "*unbalanced,*" vocalizing, agitated, and "*appeared uncomfortable*" at 30/120 CD/LD. The signs were most notable after the initial daily bolus and the bolus was reduced from 25% of the daily dose to 17%.
Group 2: On Study Day 1, one animal briefly collapsed on its rear legs and from Study Day 5 through Study Day 8 the animal was unsteady, agitated, and vocalizing, with repetitive licking movements and salivation. On Study Days 10-12, repetitive movements and salivation were observed in the same animal.

Mortality: None

Body Weights: There was a modest weight gain in Group 2.

Food Consumption: No dose effect.

Radiography: Study Days 4, 5, 11: The cannula tip remained in the duodenum in all minipigs.

Gross Pathology: Intra-abdominal adhesions of the cranial viscera were noted in all three minipigs.

Organ Weights: No drug-related effects.

Histopathology: Not done.

Stability and Homogeneity: Not reported.

Reference ID: 3459002

6.2.3

Study title: Duodopa: Maximum Tolerated Dose Study by Continuous Duodenal Infusion Administration to Minipigs

Study no.:	RD111279
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	AUG 5, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Duodopa®, 08H19G12, 97% CD & 96.5% LD

Key Study Findings

- LCIG was not tolerated at 30/120 mg/kg/day without 4 days of ascending-dose titration in advance of constant/stable dosing.
- MTD of LCIG in female minipigs was determined to be between 22.5/90 CD/LD and 30/120 CD/LD mg/kg/day. (An initial 10% bolus of the total dose was administered daily.)
- Adhesions and granulation tissue were seen at catheter insertions in the duodenum. No gross findings were noted at the catheter tips.
- After the second dose, T_{max} was approximately 1.5 hrs post-dose (CD) and 1 hr post-dose (LD) and varied from 1-12 hours for 3-O-Methyldopa (an LD metabolite).

Methods

Doses:	Doses are expressed as mg of CD/LD. Group 1: Initial 10% bolus followed by continuous 24-hour infusion of ascending doses to 90/360 mg/kg/day over 8 days. Group 2: Two doses (7.5/30 and 22.5/90 mg/kg) over 4 days of up-titration to the stable dose of 30/120 mg/kg/day for 7 days.
Frequency of dosing:	Group 1: 24 hours every 2 days; Group 2: Constant dose 24 hours for 7 days.

Reference ID: 3459002

Route of administration:	Intra-duodenal through PEG tube with jacketed portable infusion pump.
Dose volume:	100 mL cassettes contained 500 mg CD and 2000 mg LD. The 24-hour dose was determined by the programmed infusion rate of the pump. (See sponsor's tables below.)
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus*, Gottingen minipig
Number/Sex/Group:	2 F
Age:	Group 1: 37-40 weeks Group 2: 39-40 weeks
Weight:	Group 1: 16.3, 17.6 kg Group 2: 167.4, 16.9 kg
Satellite groups:	NA
Unique study design:	Surgical implantation of intra-duodenal cannula and accommodation to pump-containing jackets prior to dosing by continuous infusion. See dose escalation tables below.
Deviation from study protocol:	See table below: During Phase 2, after up-titration and 24 hours of infusion at 30/120 mg/kg/day, the drug was withdrawn and the animals were maintained drug-free for 7 days followed by infusion for 7 days at 22.5/90 mg/kg/day. One additional animal from Group 1, Phase 1 was added to Phase 2 at 30/120 mg/kg/day.

Sponsor's table: study design.

Table 5 Summary of Duodopa treatment groups

Group	Phase	Animal Number	Duodopa Dose (mg/kg/day)	Bolus dose
1	1	61	Escalating dose[a,b] (Day 1)	10% of total dose
1	1	62	Escalating dose[a] (Day 1-8)	10% of total dose
1	1	66	Escalating dose[a] (Day 1-6)	10% of total dose
2	2	63, 64	30/120[d]	10% of total dose
2	2	63, 64	22.5/90[c]	Titration phase – 10% of total dose Constant dose phase– 1.5/6 mg/kg
1	2	66	30/120[c]	Titration phase – 10% of total dose Constant dose phase – 1.5/6 mg/kg

[a] Escalating dose phase: Dose (carbidopa mg/kg/day with levodopa mg/kg/day): Day 1-2 15/60
Day 3-4 30/120
Day 5-6 60/240
Day 7-8 90/360

[b] On the morning of Day 2 at 15/60 mg/kg/day, the duodenal cannula of Animal 61 was found to have been pulled out. The animal was removed from the study and replaced by Animal 66

[c] Received Duodopa for 7 days, plus 4 days up-titration; 2 days at 7.5/30 and 15/60 mg/kg/day

[d] Dosing was stopped after 1 day of treatment (plus 2 days up-titration at 7.5/30 mg/kg/day)

Sponsor's table: Volumes of bolus and constant doses in Group 1

Table 6 Pump settings for dose escalation phase (Group 1)

	Day			
	1 to 2	3 to 4	5 to 6	7 to 8[a]
Initial bolus (mg/kg)	1.5/6	3.0/12	6.0/24	9.0/36
Initial bolus volume (mL/kg)	0.3	0.6	1.2	1.8
Infusion dose (mg/kg/day)	15/60	30/120	60/240	90/360
Infusion volume (mL/kg/day)	2.7	5.4	10.8	16.2
Infusion rate (mL/kg/h)	0.1125	0.225	0.450	0.675
Total dose (mg/kg/day)	15/60	30/120	60/240	90/360

[a] Only Animal 62 was dosed at this level and subsequently terminated on Day 8 on welfare grounds. Animal 66 completed the MTD phase through Day 6 and was maintained off dose until testing in Phase 2.

Sponsor's table: Volumes of bolus and constant doses in Group 2

Table 7 Pump settings for 7-day constant dose phase (Group 2)

	Day		
	T1a to T2a	T3a-T4a	1a to 7a
Initial bolus (mg/kg)	0.75/3	1.5/6	1.5/6
Initial bolus volume (mL/kg)	0.15	0.3	0.3
Infusion dose (mg/kg/day)	7.5/30	15/60	22.5/90
Infusion volume (mL/kg/day)	1.35	2.7	4.2
Infusion rate (mL/kg/h)	0.0563	0.1125	0.175
Total dose (mg/kg/day)	7.5/30	15/60	22.5/90

Following the MTD Phase 1 and approximately 5 weeks off dose, Animal 66 was treated in the constant dose Phase 2 at 30/120 mg/kg/day (compared to 22.5/90 mg/kg/day for the other two animals) after up-titration (Table 8 below).

Note: One animal from Group 1 was added to Group 2 at 30/120 mg/kg/day.

Excerpted from sponsor's table: Doses for Animal 66.

	1a to 7a
Initial bolus (mg/kg)	1.5/6
Initial bolus volume (mL/kg)	0.3
Infusion dose (mg/kg/day)	30/120
Infusion volume (mL/kg/day)	5.7
Infusion rate (mL/kg/h)	0.2375
Total dose (mg/kg/day)	30/120

Observations and Results

Mortality:

Limited to Group 1: On Study Day 2, the cannula was "*out of place*" in one animal, which was euthanized. On Study Day 9, a second animal was euthanized after the 4-hour TK sample because of bleeding hooves and snout, attributed to repetitive scraping and digging.

Clinical Signs: In all treated animals, there was a dose-related increase in agitation, vocalization, flight response, repetitive motions, third eyelid prolapse, irritability, abnormal posture, and "*tail unusually curled upwards.*"

Body Weights: Group 1: On the second day of 60/240 CD/LD, body weight losses of 0.1 and 0.3 kg were recorded.
Group 2: Body weight remained unchanged

Food Consumption: No differences.

Gross Pathology:

Euthanized animal (Day 9, Group 1): The duodenum was thickened and congested and there were numerous intra-abdominal adhesions.

Necropsy (per protocol) Group 2: Green purulent material was seen in the abdominal wall of 1/3 animals, intra-abdominal adhesions in 3/3, and congestion of duodenal mucosa in 1/3.

Organ Weights: There was no control group; weights were within normal limits for the species, with little variation between animals.

Histopathology

Adequate Battery: Yes. Examination limited to duodenum: gross findings and at pre-determined levels (per protocol).
Peer Review: No
Signed and dated path report: Yes

Histological Findings:
Euthanized animal, Group 1: Granulation tissue and intra-luminal neutrophilic debris were seen in sections from the duodenum.

Necropsy, per protocol, Group 2: Granulation tissue at the catheter insertion site in the duodenum was seen in 3/3 animals and intra-luminal neutrophils in the duodenum of 1/3 animals.

Toxicokinetics: Plasma levels of CD, LD, and 3-O-methyldopa (a metabolite of LD that was measured in clinical trials) were measured in exploratory TK studies.

Sponsor's Table: TK sampling times.

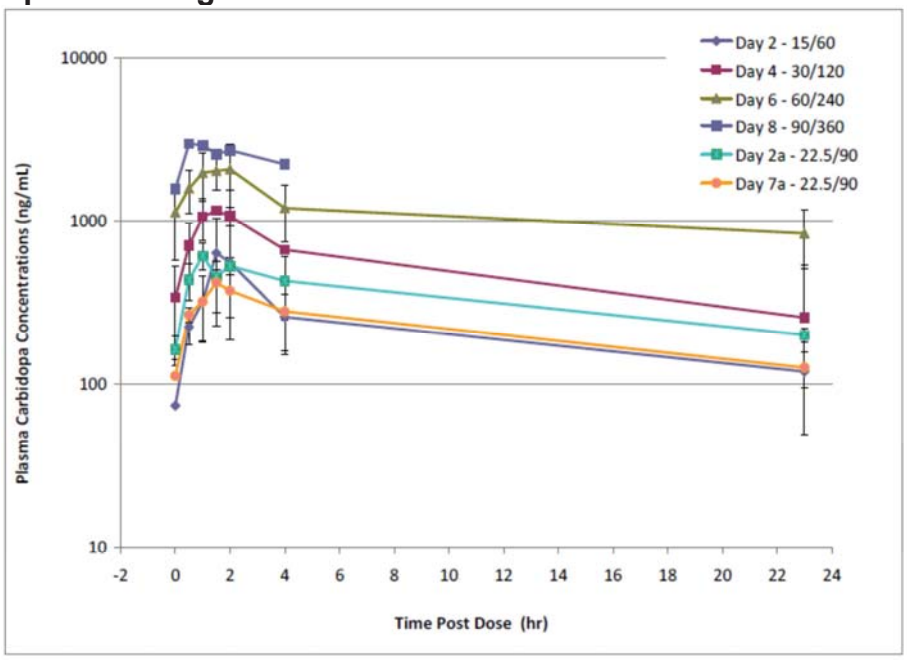
Occasion	Animals	Time of sampling (hours after dosing/hours GMT)
Phase 1: Day 2, 4, 6, 8*	62, 66	Before bolus dose, and 0.5, 1, 1.5, 2, 4, 23 hours after the end of the bolus dose at each dose level.
Phase 2: Day 2 and 7	63, 64, 66	Before bolus dose, and 0.5, 1, 1.5, 2, 4, 23 hours after the end of the bolus dose.

*Animal 62 only

Reference ID: 3459002

After the second dose, T_{max} was approximately 1.5 hrs post-dose (CD) and 1 hr post-dose (LD) and varied from 1 to 12 hours for 3-OMD. C_{max} increased dose-proportionally for CD, LD, and 3-OMD, with clear peaks related to bolus administration.

Sponsor's Figure:



Sponsor's Tables:

Table 9 Toxicokinetic Parameters for Carbidopa [a]

Phase	Dose [b]	Day in Phase	C_{max} (ng/mL)	C_{max}/D (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC (ng•hr/mL)	AUC/D (ng•hr/mL)/(mg/kg/day)
1	15	2	636 ± 409	42.4 ± 27	1.5 ± 0	5130 ± 2420	342 ± 161
2 [c]	22.5	2a	626 ± 95.5	27.8 ± 4.2	2.5 ± 2	7850 ± 1990	349 ± 88
2 [c]	22.5	7a	419 ± 146	18.6 ± 6.5	1.5 ± 0	5110 ± 1930	227 ± 86
1	30	4	1340 ± 91.9	44.5 ± 3.1	1.5 ± 1	12300 ± 2690	410 ± 88
1	60	6	2180 ± 700	36.3 ± 11.7	1.8 ± 0	26200 ± 9900	437 ± 164
1	90 [d]	8	2960	32.9	0.5	nc	nc

a. Means ± SD are shown. b. Doses are for carbidopa in mg/kg/day. c. Because of a technical limitation to the LIMS analysis, results for Phase 2 are averages for Animals 63 and 64 only (i.e., not including Animal 66).
d . Data for one animal
nc : not calculated.

Table 10 Toxicokinetic Parameters for Levodopa [a]

Phase	Dose [b]	Day in Phase	C_{max} (ng/mL)	C_{max}/D (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC (ng•hr/mL)	AUC/D (ng•hr/mL)/(mg/kg/day)
1	60	2	13300 ± 2260	222 ± 37.5	11.8 ± 15.9	237000 ± 15600	3960 ± 262
2 [c]	90	2a	26900 ± 7140	298 ± 79.2	1.3 ± 1.1	437000 ± 69300	4860 ± 771
2 [c]	90	7a	23100 ± 849	257 ± 9.2	0.8 ± 0.4	411000 ± 19800	4570 ± 219
1	120	4	44800 ± 11800	373 ± 99	1 ± 0.7	660000 ± 303000	5500 ± 2520
1	240	6	108000 ± 15300	451 ± 64.3	0.8 ± 0.4	1630000 ± 382000	6790 ± 1580
1	360 [d]	8	180000	500	1.5	nc	nc

a. Means ± SD are shown. b. Doses are for levodopa in mg/kg/day. c. Because of a technical limitation to the LIMS analysis, results for Phase 2 are averages for Animals 63 and 64 only (i.e., not including Animal 66).
d . Data for one animal
nc : not calculated.

Table 11 Toxicokinetic Parameters for 3-OMD [a]

Phase	Dose [b]	Day in Phase	C_{max} (ng/mL)	C_{max}/D (ng/mL)/(mg/kg/day)	T_{max} (hr)	AUC (ng•hr/mL)	AUC/D (ng•hr/mL)/(mg/kg/day)
1	60	2	27700 ± 10400	461 ± 173	23 ± 0	486000 ± 95500	8100 ± 1590
2 [c]	90	2a	56300 ± 10800	625 ± 120	1.3 ± 1.1	1150000 ± 184000	12800 ± 1980
2 [c]	90	7a	72500 ± 1200	805 ± 12.7	2 ± 2.8	1470000 ± 7070	16300 ± 141
1	120	4	94600 ± 64300	790 ± 538	12.3 ± 15.2	1970000 ± 1430000	16400 ± 11900
1	240	6	161000 ± 98100	669 ± 409	1.3 ± 0.4	3170000 ± 1930000	13200 ± 8080
1	360 [d]	8	224000	622	1.5	nc	nc

a. Means ± SD are shown. b. Doses are for levodopa in mg/kg/day. c. Because of a technical limitation to the LIMS analysis, results for Phase 2 are averages for Animals 63 and 64 only (i.e., not including Animal 66).
d . Data for one animal
nc : not calculated.

Stability and Homogeneity: Not done

6.2.4

Study title: Duodopa®: Local Irritation Study of Continuous Duodenal Administration to Minipigs for 4 Weeks

Study no.: RD111302
Study report location: eCTD 4.3.2.2
Conducting laboratory and location: (b) (4)
Date of study initiation: MAY 6, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Duodopa®, #09I21G13, 98%CD, 98% LD

Key Study Findings:

- Adverse clinical signs of flight response and repetitive behaviors seen at both dose levels.

- After diazepam (IM) was co-administered in all HDF and one MDF, clinical signs were controlled and the doses deemed tolerable.

- No changes were observed in the duodenal mucosa; gross and microscopic lesions were attributed to surgical attachment of the cannula.

- The T_{max} of CD was ~1-2 hours. Dose-related increases in C_{max} and $AUC_{23\ hr}$ of CD were slightly less than dose-proportional, but there was no accumulation over the 28-day study. The concentration and AUC of (b) (4) increased proportionately to CD dose. The T_{max} of (b) (4) was also 1-2 hours post-dose and levels were measurable through post-dose hour 4.

- The C_{max} and AUC_{23} of LD were greater than proportional on Study Day 2 but closer to dose-proportional by Study Day 28; there was no accumulation over the 28 days. The T_{max} of LD was 1-1 ½ hrs. The T_{max} of the levodopa metabolite 3-0-Methyldopa was later than that of LD. Concentration and exposures increased with LD dose; however, there was some accumulation at the highest LD dose level.

Methods

Doses:	Doses are expressed as mg of CD/LD. 0 (Saline),0 (Vehicle), CD/LD up-titrated (see below) to 11.25/45, 22.5/90 mg/kg/day
Frequency of dosing:	Initial daily bolus, followed by continuous 24-hour infusion.
Route of administration:	Intra-duodenal through gastrostomy tube with jacketed portable infusion pump
Dose volume:	Bolus: 0.15 mL/kg, titrate to 0.225 (Low Dose) to 0.45 (HD) mL/kg; Continuous infusion 1.35, titrated to 2.025 (Low Dose) or 4.05 (HD) mL/kg/day.
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus*; Gottingen minipig
Number/Sex/Group:	3F
Age:	24-32 weeks
Weight:	12.9-16.6 kg
Satellite groups:	NA
Unique study design:	Surgical implantation of intra-duodenal cannula and accommodation to pump-containing jackets prior to dosing by continuous infusion.
Deviation from study protocol:	Study dates and animal ages shifted due to later animal delivery dates; animals shifted between groups (see table below). Pathology peer review conducted at HLS. No effect on data.

Sponsor's Table: study design

Group	Treatment	Dose# (mg/kg/day)	Number of animals Female	Animal numbers Female
1	Control (saline)	0	3	1, 2, 3
2	Control (placebo)	0	3	4, 5, 6
3	Duodopa	11.25/45	3	7, 9, 11*, 13+
4	Duodopa	22.5/90	3	8**, 10, 12

Total dose of each dose level was the sum of the bolus dose and the 24 hour infusion dose.

* On the morning of Day 2 (prior to dosing), Animal 11 of Group 4 was found to be intolerant to treatment. An assessment was made of the clinical signs displayed by this animal (intense and sustained neurological signs including highly exaggerated startle/fear/flight responses to the extent that the animal was running into the pen sides and was thereby at significant risk of injuring itself) and as a result dosing was suspended on Day 2 for welfare reasons. Following discussion with the Sponsor, Animal 11 was moved into Group 3 and dosing was continued on Day 3.

** Animal 8 from Group 3 was moved into Group 4 as requested by the Sponsor. Animal 8 was dosed at the Group 3 dose level from the titration phase (Days 1a and 2a) and also on Days 1, 2 and 3. Animal 8 was moved and dosed as a Group 4 animal from Day 4.

\+ Animal 13 was added to Group 3 of this study to replace Animal 7 which was removed from the study due to a displaced cannula on Day 6. Animal 13 was dosed according to Group 3 levels (two days of titration followed by 30 days of continuous infusion).

Observations and Results

Mortality: On Study Day 6, the cannula was found to be displaced in 1 HDF; the animal was subsequently euthanized.

Clinical Signs: Dose-related signs of an exaggerated startle response (to include self-injury), vocalization, and repetitive behavior resulting in injury to hooves and snout, and dark colored urine were observed.

Body Weights: There were no differences in mean body weight gain between groups.

Food Consumption: There were no differences between groups.

Gross Pathology: Thickening and swelling at the site of the cannula insertion through the abdominal wall, intra-abdominal adhesions, and swelling at duodenal cannula insertion site were noted in all groups.

Organ Weights: Not done

Histopathology
Adequate Battery, per protocol: Harvested tissues limited to esophagus, stomach, duodenum, jejunum, cecum and colon, and any gross lesions.

Signed Pathology Report: yes

Peer Review: Yes (by Sponsor)

Reference ID: 3459002

Histological Findings:
In all groups, granulation tissue, suture granulomas, and necrotic debris in and on the tunica muscularis and serosa were observed at the cannula insertion into the duodenum. No changes were noted in the duodenal mucosa.

There were no histologic evaluations of the gross lesions noted at the cannula site in the skin and abdominal wall.

Toxicokinetics: LD, CD, and the metabolites 3-OMD, [redacted] (b) (4), and [redacted] (b) (4) were assessed on Study Days 2 and 28. CD, LD, and 3-OMD were measured up to 23 hours after initiation of infusion. [redacted] (b) (4) and [redacted] (b) (4) were measured between 1 and 4 hours after initiation of infusion.

The C_{max} of LD was dose-proportional and $AUC_{23\ hr}$ greater than dose proportional; no accumulation was apparent. C_{max} of CD was 36% less and the $AUC_{23\ hr}$ was 24% less than dose-proportional. C_{max} and $AUC_{23\ hr}$ of 3-O-MD were up to 1.5 times greater than dose-proportional and there was accumulation at the HD. The C_{max} and $AUC_{23\ hr}$ of [redacted] (b) (4) increased in a dose-proportional manner. [redacted] (b) (4) was below the limit of quantification [redacted] (b) (4) ng/mL) in all samples.

Sponsor's tables: Cmax and AUC values for LD, CD, 3-OMD, and [redacted] (b) (4)

Carbidopa/ levodopa Dose level (mg/kg/day)	Levodopa C_{max} (ng/mL) Day 2	Levodopa C_{max} (ng/mL) Day 28	Levodopa AUC_{23} (ng.h/mL) Day 2	Levodopa AUC_{23} (ng.h/mL) Day 28
11.25/45	10000 (-)	12200 (-)	165000 (-)	179000 (-)
22.5/90	22900 (1500)	20500 (2600)	438000 (32000)	371000 (28000)

(T_{max} 0.5-1.5 hr)

Carbidopa/ levodopa Dose level (mg/kg/day)	Carbidopa C_{max} (ng/mL) Day 2	Carbidopa C_{max} (ng/mL) Day 28	Carbidopa AUC_{23} (ng.h/mL) Day 2	Carbidopa AUC_{23} (ng.h/mL) Day 28
11.25/45	273 (-)	217 (-)	2100 (-)	1780 (-)
22.5/90	407 (126)	235 (99)	3320 (600)	2600 (770)

(T_{max} 1-2 hr)

.

Levodopa Dose level (mg/kg/day)	3-O-methyldopa C_{max} (ng/mL) Day 2	3-O-methyldopa C_{max} (ng/mL) Day 28	3-O-methyldopa AUC_{23} (ng.h/mL) Day 2	3-O-methyldopa AUC_{23} (ng.h/mL) Day 28
45	13600 (-)	26600 (-)	306000 (-)	563000 (-)
90	41300 (6200)	50800 (3300)	873000 (121000)	1070000 (60000)

(T_{max} 4-23 hr)

Note: 11.25/45 CD/LD mg/kg/day

22.5/90 CD/LD mg/kg/day

Carbidopa/ levodopa Dose level (mg/kg/day)	(b) (4) C_{max} (ng/mL) Day 2	Day 28	AUC_{1-4} (ng.h/mL) Day 2	Day 28
11.25/45	(b) (4)			
22.5/90				

(T_{max} 1-2 hr)

Stability and Homogeneity: Conformed to standard (per certificate of analysis).

6.2.5

Study title: Duodopa: Maximum Tolerated Dosage and Feasibility Study of Continuous Duodenal Infusion in Male Minipigs

Study no.: RD 111303
Study report location: eCTD 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: JUL 1, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Duodopa®
Sinemet®

Key Study Findings

- Marked dose-related clinical signs were attributed to CD/LD and inflammation at the surgical insertions sites recorded, but no gross or histopathology examinations were made, per protocol.
- MTD was 22.5CD/90LD after a 2-day titration period.
- TK was determined for CD, LD, 3-O-MD, (b) (4) and (b) (4) after intra-duodenal LCIG and CD, oral CD/LD, and IV CD.

Methods

Doses:	Doses are expressed as mg of CD/LD. Phase 1: Infusion Ascending 7.5/30 to 30/120 mg/kg/day (repeated in second week) Phase 2: Infusion Ascending 7.5/30 to 30/120 mg/kg/day (Stable doses in second week at 22.5/90 mg/kg/day for 7 days) Phase 3: -CD initital daily bolus infusion: 1.5 mg/kg (Study Day 1) and 3.0 mg/kg CD (Study Day 3). (CD powder in 50mM citrate buffer, pH 3.0) - PO Sinemet CR tablets: 25/100 mg/kg on Study Day 9 and 75/300 mg/kg on Study Day 11. - IV CD 1.5 mg/kg on Study Day 15 and 3.0 mg/kg on Study Day 17 (pH 3.0, cyclodextrin+ 5% dextrose in water) in a ratio of 50 vehicle:10CD:40LD)
Frequency of dosing:	See above
Route of administration:	Intra-duodenal continuous infusion of LCIG or CD through gastrostomy tube with jacketed portable infusion pump; PO (Sinemet® CR); IV Carbidopa
Dose volume:	100 mL cassettes contained 500 mg CD/2000 mg LD. The daily bolus and 24-hour dose was determined by the programmed infusion rate of the pump.
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus*, Gottingen Minipig
Number/Sex/Group:	Phase 1: 3M Phase 2: 4M Phase 3: 3M
Age:	29-30 weeks
Weight:	14.4-15.5 kg
Satellite groups:	None
Unique study design:	Surgical implantation of intra-duodenal cannula and accommodation to pump-containing jackets prior to dosing by continuous infusion. Phase 3 (PO and IV administration of parent drugs and PO of the RLD) in a TK study of parent drugs and 4 metabolites.
Deviation from study protocol:	Minor, with no effect on data.

Reference ID: 3459002

Study Design: Sponsor's Table:

Phase (Group)	Day	Treatment	Route	No. of animals	Animal numbers	Total Dose* (mg/kg/day)
1 (1)	1 to 2	Duodopa gel	Duodenal infusion	3	14, 16 ,17	7.5/30
	3 to 4	Duodopa gel	Duodenal infusion	3	14, 16 ,17	15/60
	5	Duodopa gel	Duodenal infusion	3	14, 16 ,17	30/120
	6 to 7	-	-	-	-	-
	8 (Titration 1)	Duodopa gel	Duodenal infusion	3	14, 16 ,17	7.5/30
	9 (Titration 2)	Duodopa gel	Duodenal infusion	3	14, 16 ,17	15/60
	10 to 11	Duodopa gel	Duodenal infusion	3	14, 16 ,17	22.5/90
2 (1)	1a (Titration 1)	Duodopa gel	Duodenal infusion	3	15, 16 ,17	7.5/30
				1	14	7.5/30
	2a (Titration 2)	Duodopa gel	Duodenal infusion	3	15, 16 ,17	15/60
				1	14	7.5/30
	1-7 (Constant dose)†	Duodopa gel	Duodenal infusion	3	15, 16 ,17	22.5/90
				1	14	15/30
3 (1)	1	Carbidopa	Bolus (duodenum)	3	15, 16 ,17	1.5
	3	Carbidopa	Bolus (duodenum)	3	15, 16 ,17	3.0
	9	Sinemet tablets#	Oral	3	15, 16 ,17	25/100
	11	Sinemet tablets#	Oral	3	15, 16 ,17	75/300
	15	Carbidopa	Intravenous	3	15, 16 ,17	1.5
	17	Carbidopa	Intravenous	3	15, 16 ,17	3.0

Sinemet® CR tablets: continuous release formulation of levodopa (LD) and carbidopa (CD) at 25 mg CD and 100 mg LD per tablet
* All doses in Phase 3 were given as a single dose
† Except Animal 14 which was killed on Day 2 of the constant dose phase

In Phases 1 and 2 a bolus of 10% of total daily dose was administered daily over 5 minutes followed by continuous infusion at the selected dose level for the remainder of the 24 hours, and again for a second day at that dose.

Observations and Results

Mortality: During Phase 1 on the second day of constant dosing (Study Day 11), one animal was euthanized due a kinked cannula that was attributed to massive adhesions involving the liver and duodenum.

Clinical Signs: Hyperactivity, "*flight response*", salivation, repeated behaviors, vocalizing, circling, and bleeding hooves (from hyperactivity) were seen at all doses and sexual excitement and aggression were seen at the HD.

On Study Day 11 of Phase 1, the animal that was subsequently euthanized was observed to be *"extremely agitated hyperalert* [sic], *and continually spinning and darting around the pen."* The animal was also observed to *"launch itself in a vertical supine position with enormous force against the gate immediately after the blood draw."*

Body Weights: Phase 1: There was uniform body weight gain within the groups.
Phase 2: 2/3 animals gained weight, but 1/3 had lost 3% body weight by the end of the stable dose period.
Phase 3: 2/3 animals gained weight, but 1/3 lost 2% by the end of all treatments.

Food Consumption: There was no observed treatment effect on food consumption.

Gross Pathology: Limited to the animal euthanized in Phase 1.
Massive adhesions involving the liver and duodenum around the cannula exit site and multiple serosal and intra-abdominal adhesions were noted. Suture granulomas and marked thickening of the tunica muscularis were noted in the duodenum.

Histopathology: Limited to animal euthanized in Phase 1.
Adequate Battery: Harvested tissues were limited to esophagus, stomach, duodenum, jejunum, cecum and colon, and any gross lesions. Histologic examination was limited to duodenum.

Peer Review: *"A reviewing pathologist undertook a peer review of the microscopic findings."* (sponsor)

Signed Pathology Report: Yes

Histological Findings: Limited to the animal euthanized in Phase 1.

Duodenum - Administration Site	Granulation Tissue: moderate Neutrophilic Debris: moderate Suture Granuloma: minimal Serosa - Adhesions: slight Mucosa - Epithelial Congestion: minimal
Duodenum - Dark depression	Mucosa - Epithelial Congestion: slight
Cannula Liver Adhesions	Granulation Tissue: marked Serosa - Adhesions: moderate

Toxicokinetics:
Carbidopa, Levodopa, 3-O-Methyldopa, (b) (4), (b) (4) (by precipitate-HPLC-MS/MS) and Hydrazine (by SALLE-HPLC-MS/MS) were measured in plasma collected from animals in each phase.

TK parameters were not calculated for hydrazine; plasma levels were reported in the appended Study RD111271.

Summary data for Carbidopa, Levodopa, 3-O-Methyldopa and (b) (4) are shown in the sponsor's tables below. (b) (4) was above the LLOQ ((b) (4) ng/mL) only in Phase 3, at 5 minutes after 1.5 mg/kg CD (IV) and 5, 20, 45 and 90 minutes after 3 mg/kg (IV) CD; values ranged from (b) (4) to (b) (4) ng/mL.

- Phase 1: Samples taken on the second day of each dose up-titration of LCIG.

Sponsor's Tables:

Carbidopa/Levodopa Dose level (mg/kg/day)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_t (ng.h/mL)
7.5/30 (Day 2)	243 (256)	1990 (1460)
15/60 (Day 4)	599 (337)	5580 (3210)
22.5/90 (Day 11)	609 (203)	9720 (-[a])

[a] n=2

Reference ID: 3459002

Carbidopa/Levodopa Dose level (mg/kg/day)	Levodopa C_{max} (ng/mL)	Levodopa AUC_t (ng.h/mL)
7.5/30 (Day 2)	6230 (2090)	97500 (16300)
15/60 (Day 4)	15200 (1800)	247000 (36000)
22.5/90 (Day 11)	26400 (3000)	482000 (-[a])

[a] n=2

Carbidopa/Levodopa Dose level (mg/kg/day)	(b) (4) C_{max} (ng/mL)	(b) (4) AUC_{1-4} (ng.h/mL)
7.5/30 (Day 2)	(b) (4)	
15/60 (Day 4)		
22.5/90 (Day 11)		

[a] n=1, AUC not reported as less than 3 quantifiable plasma concentrations

- Phase 2: After dose up-titration, samples were taken on 2nd and 7th day of constant infusion of LCIG.

Sponsor's Tables:

Carbidopa/Levodopa Dose level (mg/kg/day)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_t (ng.h/mL)
22.5/90 (Day 2)	583 (46)	6930 (2130)
22.5/90 (Day 7)	480 (18)	5240 (1580)

Carbidopa/Levodopa Dose level (mg/kg/day)	Levodopa C_{max} (ng/mL)	Levodopa AUC_t (ng.h/mL)
22.5/90 (Day 2)	24600 (2300)	455000 (80000)
22.5/90 (Day 7)	26600 (4400)	410000 (93000)

Carbidopa/Levodopa Dose level (mg/kg/day)	(b) (4) C_{max} (ng/mL)	(b) (4) AUC_{1-4} (ng.h/mL)
22.5/90 (Day 2)	(b) (4)	
22.5/90 (Day 7)		

- Phase 3: Carbidopa duodenal bolus administration days 1 and 3. Bioavailability ~ 5%.

Sponsor's Tables:

Carbidopa Dose level (mg/kg)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_t (ng.h/mL)
1.5	194 (114)	258 (124)
3	269 (193)	426 (306)

(b) (4)

Carbidopa Dose level (mg/kg)	C_{max} (ng/mL)	AUC_t (ng.h/mL)
1.5	(b) (4)	
3		

[a] n=1

- Phase 3: Sinemet CR PO

Sponsor's Tables:

Carbidopa bioavailability ~2% (75 mg PO) and 3.3% (25 mg PO); $t_{1/2}$ = 1-2 hr.

Carbidopa/Levodopa Dose level (mg)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_t (ng.h/mL)
25/100	78.1 (76.7)	161 (160)
75/300	88.3 (76.5)	299 (304)

Levodopa: T_{max} = 3hr; $t_{1/2}$=1.5 and 2.7 hr)

Carbidopa/Levodopa Dose level (mg)	Levodopa C_{max} (ng/mL)	Levodopa AUC_t (ng.h/mL)
25/100	2300 (1040)	9930 (3920)
75/300	4540 (1900)	32800 (9200)

(b) (4) Tmax = 0.75-3hr

(b) (4)

Carbidopa/Levodopa Dose level (mg)	C_{max} (ng/mL)	AUC_t (ng.h/mL)
25/100	(b) (4)	
75/300		

[a] Calculated from less than three quantifiable plasma concentrations, not reported
[b] Calculated from n=1 animal with three quantifiable plasma concentrations

- Phase 3: CD IV (as a 5 minute bolus).

Sponsor's Tables:

CD: T_{max} = 5 minutes post-dose; $t_{1/2}$ = 1.6-1.8 hr.

Carbidopa Dose level (mg/kg)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_t (ng.h/mL)
1.5	6880 (1260)	4590 (1070)
3	11300 (4500)	8140 (1820)

(b) (4) T_{max} = 5 minutes post-bolus; $t_{1/2}$ = 0.2 hr

Carbidopa Dose level (mg/kg)	C_{max} (ng/mL)	AUC_t (ng.h/mL)
1.5	(b) (4)	
3		

(b) (4) T_{max} = 5 minutes post-bolus; $t_{1/2}$ not calculable.

Carbidopa Dose level (mg/kg)	C_{max} (ng/mL)	AUC_t (ng.h/mL)
1.5	(b) (4)	
3		

[a] Less than three quantifiable plasma concentrations, omitted from mean
[b] Calculated from n=1 animal with three quantifiable plasma concentrations

Hydrazine levels were presented in Annex 3, from study report RD111261 in Department R46W of Abbott Laboratories, USA. The Principal Investigator was (b) (4)

Plasma concentrations of hydrazine were reported; however, TK parameters were not calculated.

Sponsor's Tables: Hydrazine plasma concentrations

Table 1 **Phase 1 - Plasma concentration values of Hydrazine following continuous duodenal administration of Duodopa® to male minipigs as presented in report R&D/11/1261**

Day 2 - 7.5/30 mg/kg/day Carbidopa/Levodopa

Time (hours)	Concentration (ng/mL) * Minipig identification number 14	16	17
1			(b) (4)
2			
4			

Day 4 - 15/60 mg/kg/day Carbidopa/Levodopa

Time (hours)	Concentration (ng/mL) * Minipig identification number 14	16	17
1			(b) (4)
2			
4			

Day 11 – 22.5/90 mg/kg/day Carbidopa/Levodopa

Time (hours)	Concentration (ng/mL) * Minipig identification number 14	16	17
1			(b) (4)
2			
4			

* The method used to determine plasma hydrazine concentrations was not fully validated. At the request of the sponsor the values were taken from the listings of Abbott report R&D/11/1261, presented in this table, and are considered exploratory: no analyses or pharmacokinetic evaluation was performed.

All samples taken relative to the end of the bolus dose.

a. No sample available

Table 2 **Phase 2 – Plasma concentration values of Hydrazine on Days 2 and 7 of continuous duodenal administration of Duodopa® to male minipigs at a dose level of 22.5/90 mg/kg/day as presented in report R&D/11/1261**

Day 2

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
1			(b) (4)
2			
4			

Day 7

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
1			(b) (4)
2			
4			

All samples taken relative to the end of the bolus dose.

a. No sample available

Table 3 **Phase 3 – Plasma concentrations of Hydrazine following duodenal bolus administration of carbidopa to male minipigs as presented in report R&D/11/1261**

Day 1 – 1.5 mg/kg Carbidopa administered by intraduodenal bolus

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.5			(b) (4)
1			
1.5			
2			
4			
8			

Day 3 – 3 mg/kg Carbidopa administered by intraduodenal bolus

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.5			(b) (4)
1			
1.5			
2			
4			
8			

*All samples taken relative to the end of the intravenous slow bolus dose

Table 4 **Phase 3 – Plasma concentrations of Hydrazine following oral administration of Sinemet CR to male minipigs as presented in report R&D/11/1261**

Day 9 – 1 Sinemet® tablet – 25/100 mg Carbidopa/Levodopa

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.75			(b) (4)
1.5			
3			
5			
10			
24			

Day 11 – 3 Sinemet® tablets –75/300 mg Carbidopa/Levodopa total dose

Time (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.75			(b) (4)
1.5			
3			
5			
10			
24			

Table 5 **Phase 3 – Plasma concentrations of Hydrazine following intravenous bolus administration of carbidopa to male minipigs as presented in report R&D/11/1261**

Day 15 – 1.5 mg/kg Carbidopa administered by intravenous bolus

Actual time (hours)	Time* (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.167	0.0833			(b) (4)
0.417	0.3333			
0.833	0.75			
1.583	1.5			
3.083	3			
8.083	8			

Day 17 – 3 mg/kg Carbidopa administered by intravenous bolus

Actual time (hours)	Time* (hours)	Concentration (ng/mL) Minipig identification number 15	16	17
0.167	0.0833			(b) (4)
0.417	0.3333			
0.833	0.75			
1.583	1.5			
3.083	3			
8.083	8			

*All samples taken relative to the end of the intravenous slow bolus dose

6.2.6

Study title: Duodopa®: Local Irritation Study of Continuous Duodenal Administration to Male Minipigs for 4 Weeks

Study no.:	RD 111304
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	07 SEP 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Duodopa®,

Key Study Findings

- Intramuscular diazepam was administered to HD animals to control clinical signs. IM diazepam was administered to MD animals but was stopped after a reduction in the dose and subsequent reduction in the signs.

- Minimal-to-slight congestion of the lamina propria [sub-mucosa] were noted "*in the region of, or slightly distal from, the cannula tip,*" corresponding to reddened duodenal mucosa in 1/4 CM, 1/4 MDM, and 3/4 HDM.

- Microscopic evidence of epithelial ulceration, in the vicinity of the cannula tip, was seen in 1 CM and 1 HDM. Minimal-to-slight numbers of inflammatory cells (neutrophils and mononuclear cells) were noted in the lamina propria of the same CM and 3 HDM.

- The T_{max} of CD was ~1.5-2 hours (range 0.5 to 2 hours). Dose-related increases in C_{max} and $AUC_{23\ hr}$ of CD were approximately dose-proportional and there was no accumulation over the 28-day study.

- The T_{max} of LD was ~ 0.5 (range 0.5 to 4.0 hrs). The C_{max} and $AUC_{23\ hr}$ of LD were roughly dose-proportional and there was no accumulation over the 28 days.

Methods

Doses:	Doses are expressed as mg of CD/LD. See table below
Frequency of dosing:	Continuous infusion up-titration for 2 days followed by stable dose for 28 days. Daily bolus (5% dose), followed by 24-hr infusion
Route of administration:	Intra-duodenal through gastrostomy tube with

	jacketed portable infusion pump
Dose volume:	Titration: 4.05, 2.025, 4.05 mL/kg/day Constant: 4.05, 2.1375, 4.275 mL/kg/day
Formulation/Vehicle:	Aqueous carmellose sodium gel
Species/Strain:	*Sus domesticus*, Gottingen Minipig
Number/Sex/Group:	4 M
Age:	23-26 weeks
Weight:	11.9-14.0 kg
Satellite groups:	None
Unique study design:	Surgical implantation of intra-duodenal cannula and accommodation to pump-containing jackets prior to dosing by continuous infusion.
Deviation from study protocol:	Minor- the deviations did not impact the outcome. (Low Dose was reduced on Study Day19; the High Dose was not.)

Sponsor's Table

Group	Treatment	Dose# (mg/kg/day)	Number of animals Male	Animal numbers Male
1	Control (placebo)	0	4	72-75
2	Duodopa	11.25/45* 7.5/30+	4	76-79
3	Duodopa	22.5/90	4	80-83

Total dose of each dose level was the sum of the bolus dose and the 24 hour infusion dose.
* Total dose of Group 2 during Days 1 to 18.
+ Total dose of Group 2 during Days 19 to 28.

Sponsor's Tables and Comments, pump settings for continuous infusion

Pump settings for Days 3 to 28 (up to Day 18 for Group 2)

Group Compound	1 Control (Placebo)	2 Duodopa (½ high dose)	3 Duodopa (high)
Infusion period (hours/day)	24	24	24
Initial bolus (mg/kg)*	0	0.5625/2.25	1.125/4.5
Initial bolus volume (mL/kg)	0.45	0.1125	0.225
Infusion volume (mL/kg/day)	4.05	2.1375	4.275
Infusion rate (mL/kg/h)	0.16875	0.0890625	0.178125
Dose concentration (mg/mL)	0	5/20	5/20
Total dose (mg/kg/day)[a]	0	11.25/45	22.5/90

* On each day an initial bolus was given over 5 minutes. This was followed by the subsequent infusion dose to make up 24 hours of dose administration. The bolus volume was calculated as 5% of the 24 hour infusion dose.
[a] Total dose at each dose level was the sum of the bolus dose (either 10% or 5% of the total daily dose) given over 5 min in the morning plus the remaining 90% or 95% of the total dose given over the remainder of the 24 hours.

The dose level in Group 2 was reduced at the Sponsor's request from Day 19 until Day 28; the bolus dose remained at 5% of the total daily dose.

Pump settings for Days 19 to 28 (Group 2 only)

Group Compound	2 Duodopa (⅓ high dose)
Infusion period (hours/day)	24
Initial bolus (mg/kg)*	0.375/1.5
Initial bolus volume (mL/kg)	0.075
Infusion volume (mL/kg/day)	1.425
Infusion rate (mL/kg/h)	0.059375
Dose concentration (mg/mL)	5/20
Total dose (mg/kg/day)[a]	7.5/30

Observations and Results:
On Study Day 2, both LD and HD groups were administered diazepam for relief of clinical signs in order to facilitate dosing and handling. The LD was reduced to 7.5/30 mg/kg/day from Study Day 19; from Study Days 20-22 diazepam was discontinued in all Group 2 animals.

Mortality: On Study Day 23, one HDM had a blocked or broken cannula that could not be repaired and the animal was euthanized on Study Day 24. Gross examination and tissue collection were performed per protocol.

Clinical Signs: A dose-related marked flight response, startle reflex, vocalizing, hyper-activity, repetitive movements, and aggression were somewhat responsive to low doses of IM diazepam (0.25 mg/kg and adjusted as needed).

Body Weights: All groups gained body weight; however, treated groups had slightly lower body-weight gain than controls.

Sponsor's Table:

Bodyweight - group mean values (kg)

Group	:	1	2		3
Compound	:	Control (Placebo)	Duodopa		Duodopa
Days	:	1-28	1-18	19-28	1-28
Total dose (mg/kg/day)	:	0	11.25/45	7.5/30	22.5/90

Group /Sex		Day -14	Day -7	Day 1a	Day 1	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 24	Day 28
1M	Mean	11.6	11.9	12.9	13.0	13.1	13.2	13.4	13.7	13.7	14.1	13.8	14.4
	SD	0.51	0.66	0.83	0.84	0.87	0.95	1.18	1.19	1.31	1.17	1.21	0.93
	N	4	4	4	4	4	4	4	4	4	4	4	4
2M	Mean	11.3	12.0	12.7	12.8	12.8	12.9	13.1	13.2	13.4	13.5	13.3	13.8
	SD	0.73	0.56	0.58	0.60	0.54	0.82	0.99	0.83	0.87	0.88	0.80	1.02
	N	4	4	4	4	4	4	4	4	4	4	4	4
3M	Mean	11.6	12.2	12.7	13.0	12.8	12.8	12.8	13.1	13.7	13.9	13.5	13.8
	SD	0.60	0.69	0.64	0.68	0.67	0.82	0.50	0.41	0.56	0.69	0.42	0.88
	N	4	4	4	4	4	4	4	4	4	4	4	3

a Day 1 of titration phase

Reference ID: 3459002

Food Consumption:
There was no drug effect on food consumption. (One HDM had not been administered CD/LD for 24 hours and failed to consume the daily total food ration after it was dosed with diazepam.)

Gross Pathology:
There were extensive intra-abdominal adhesions of the liver, abdominal wall, spleen, and small intestines observed in all groups. Reddened mucosa was observed in the duodenum of 1/4 CM, 1/4 MDM, and 3/4 HDM.

Summary of findings in the duodenum for animals killed after 4 weeks of treatment and the decedent animal

Group/sex Dose (mg/kg/day)	1M 0	2M 11.25/45 to 7.5/30	3M 22.5/90
Red area(s)	1	1	3
Number of animals examined	4	4	4

Histopathology: limited
Adequate Battery: Harvested tissues limited to esophagus, stomach, duodenum, jejunum, cecum and colon, and any gross lesions.

Histologic examination limited to duodenum (four sections (per protocol) at 3 cm intervals distal to the cannula tip) and any grossly observable lesions.

Pathology Report: Yes

Peer Review: No

Histological Findings:
No histologic changes were observed in duodenal sections examined per protocol (at 3 cm intervals distal to the catheter tip).
In sites noted grossly as "*red,*" minimal-to-slight congestion of the lamina propria [sub-mucosa] were noted "*in the region of, or slightly distal from, the cannula tip.*"
Slight epithelial ulcerations and inflammatory cells (neutrophils and mononuclear cells) in the lamina propria were observed in the areas corresponding to reddened mucosa seen in 1/3 CM and 3/3 HDM.

The pathologist attributed all mucosal changes to the presence of the cannula. The apparent dose-response was attributed to physical irritation and trauma by the catheter tip in the presence of increased physical activity in the HD animals.

Reference ID: 3459002

Sponsor's Table:

Summary of findings in the duodenum for animals killed after 4 weeks of treatment and the decedent animal

Group/sex Dose (mg/kg/day)	1M 0	2M 11.25/45 to 7.5/30	3M 22.5/90
Congestion			
Minimal	1	1	0
Slight	0	0	4
Total	0	1	4
Increased inflammatory cells			
Minimal	1	0	0
Slight	0	0	3
Total	1	0	3
Number of tissues examined	4	4	4

Granulation tissue, seen histologically in the duodenum, was attributed to surgical placement of the cannulas.

Toxicokinetics:

The C_{max} and $AUC_{23\ hr}$ of carbidopa increased in a dose-proportional manner. The T_{max} was ~1.5 or 2 hours post-dose (range 0.5-2 hrs).

The C_{max} and $AUC_{23\ hr}$ of levodopa increased in a dose-proportional manner. The T_{max} was ~ 0.5 hours (range 0.5-4 hrs).

Sponsor's Tables: C_{max} and AUC_{23} of CD and LD. (Standard Deviation)

Carbidopa/levodopa dose level (mg/kg/day)	Carbidopa C_{max} (ng/mL)	Carbidopa AUC_{23} (ng.h/mL)
11.25/45	463 (342)	3220 (2090)
22.5/90	836 (255)	6400 (1860)

Carbidopa/levodopa dose level (mg/kg/day)	Levodopa C_{max} (ng/mL)	Levodopa AUC_{23} (ng.h/mL)
11.25/45	9900 (2850)	157000 (34000)
22.5/90	23800 (2700)	377000 (76000)

7 Impurities

The sponsor submitted PK, general and genotoxicity studies, and reviews of the literature to support qualification of the carbidopa degradants, (b) (4) (b) (4) and

hydrazine. Clinical PK data from subjects administered LCIG and subjects administered the RLD were also submitted.

7.1: PK/ADME

Pharmacokinetic studies:

7.1.1
A-15067: Drug Metabolism Memo 02- Hydrazine Pharmacokinetics following Oral Dosing in Wistar Rats. (Non-GLP, unaudited report)

In this memo the sponsor compared PK data after administration of hydrazine by oral gavage to administration in drinking water to M and F Wistar rats. The dose levels replicate those used in 2 published carcinogenicity studies cited in document "RD12714 – Analysis of the Safety Risks Associated with Hydrazine as a Degradation Product in LCIG."

Preclinical Safety, Abbott Laboratories, Abbott Park, IL 60064
NonGLP/Unaudited.

Oral Gavage: 5/sex Wistar rats, 300-500 g, were administered 0.0, 1, or 5 mg/kg hydrazine (anhydrous free base) in sterile water for injection. Blood samples were taken at 0.5, 1, 1.5, 2, 4, 7, and 12 hours after dosing.

Drinking water: Male rats received 0.2, 1, or 5 mg/kg in daily intake of 25 mL water for 21 days. Steady-state PK samples were taken on Study Day 19 (0 and 8 hours) and Study Day 20 (4 and 12 hours) relative to the room dark cycle.

Frozen plasma samples from both groups were thawed, salt-assisted liquid/liquid extracted (SALLE), and the acetone azine was analyzed by HPLC-MS/MS.

Results: Sponsor's table:

Table 3. Hydrazine Pharmacokinetics following Oral Dosing in Wistar Rats (Oral Gavage vs. Drinking Water Dosing)

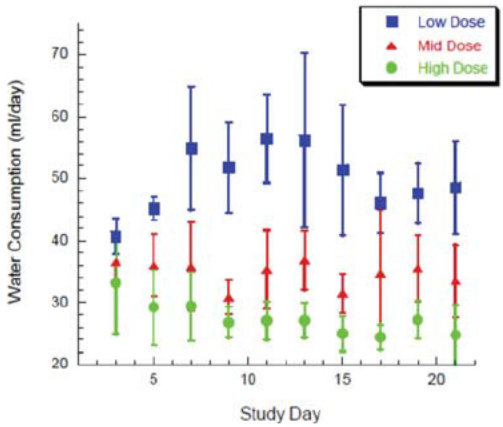
Dose	Route	C_{max}	C_{max}/D	T_{max}	$t_{1/2}$	AUC	AUC/D
							(b) (4)
0.2	Gavage						
0.40 (0.02)	Water						
1.0	Gavage						
1.39 (0.08)	Water						
5.0	Gavage						
5.48 (0.32)	Water						

Data provided as Mean (SEM)
Dose (mg/kg (gavage) or mg/kg/day (water)); C_{max} (ng/ml); C_{max}/D (ng/ml per mg/kg); T_{max} (hr); $t_{1/2}$ (hr);
AUC (ng•hr/ml); AUC/D (ng•hr/ml per mg/kg)
n.f. – unable to estimate plasma elimination half-life
n.a. – not applicable

Oral Gavage: Hydrazine was rapidly absorbed, with a T_{max} of [redacted] (b) (4) hour and an elimination half-life o [redacted] (b) (4) hours. The C_{max} and AUC were slightly greater than dose-proportional and there were no consistent differences between sexes.

Drinking water: Over time, there was a dose-related reduction in water consumption in MD and HD animals.

Sponsor's figure:



Following oral administration in the drinking water, the C_{max} was recorded eight hours after the initiation of the dark cycle and plasma levels were compared to those from oral gavage.

Sponsor's table:

Table 3. Hydrazine Pharmacokinetics following Oral Dosing in Wistar Rats (Oral Gavage vs. Drinking Water Dosing)

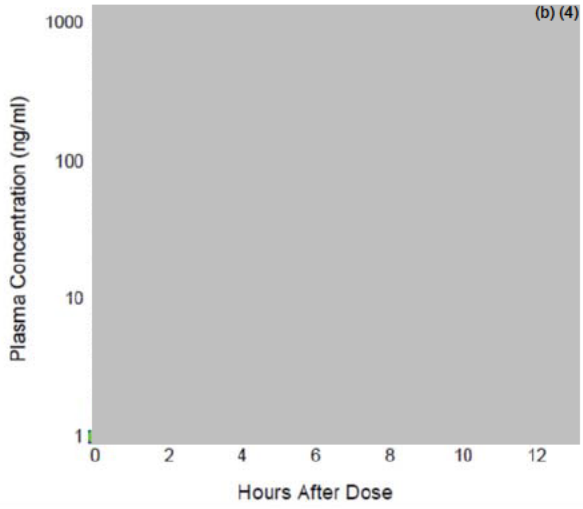
Dose	Route	C_{max}	C_{max}/D	T_{max}	$t_{1/2}$	AUC	AUC/D
0.2	Gavage	[redacted]					(b) (4)
0.40 (0.02)	Water						
1.0	Gavage						
1.39 (0.08)	Water						
5.0	Gavage						
5.48 (0.32)	Water						

Data provided as Mean (SEM)
Dose (mg/kg (gavage) or mg/kg/day (water)); C_{max} (ng/ml); C_{max}/D (ng/ml per mg/kg); T_{max} (hr); $t_{1/2}$ (hr);
AUC (ng•hr/ml); AUC/D (ng•hr/ml per mg/kg)
n.f. – unable to estimate plasma elimination half-life
n.a. – not applicable

AUC values were ~3-8-fold higher from continuous dosing in drinking water than those from dosing by gavage.

Sponsor's figure:

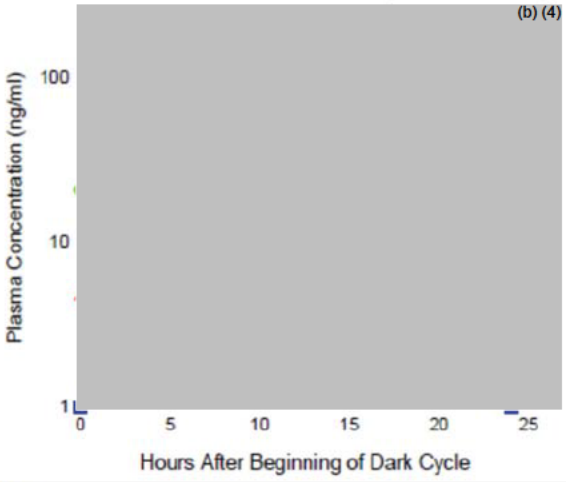
Figure 1. **Hydrazine Plasma Concentrations following Oral (Gavage) Dosing in Wistar Rats**



Mean (±SEM, n=5 rats per gender per timepoint)

Sponsor's Figure:

Figure 4. **Hydrazine Plasma Concentrations following Oral Dosing via Drinking Water in Male Wistar Rats (Day 19)**



Mean (±SEM, n=5)

7.1.2

A-156057 Drug Metabolism Memo 01: Hydrazine Pharmacokinetics following Oral Dosing in Swiss Webster Mice.

In this memo, the sponsor compared PK data for administration of hydrazine by oral gavage to administration in drinking water to M and F Swiss Webster mice. The dose levels replicate those used in published carcinogenicity studies cited in document "RD12714 – Analysis of the Safety Risks Associated with Hydrazine as a Degradation Product in LCIG."

Preclinical Safety, Abbot Laboratories, Abbott Park, IL 60064
NonGLP/ Unaudited.

Oral Gavage: M and F mice were administered single 0.4, 2, or 10 mg/kg doses of hydrazine in water for injection. 5/sex were euthanized and plasma samples were taken at each time period of 0.5, 1, 1.5, 2, 4, 7, and 12 hours post-dose.

Drinking water: M mice were administered ~0.4, 2, or 10 mg/kg/day hydrazine in the drinking water. On Study Day 19, samples were drawn from five mice at each time period of 0, 4, 8, and 12 hours after the beginning of the animal room dark cycle.

Results:
Oral Gavage: There were no appreciable differences between sexes. Hydrazine was detected in circulation at 0.5 hr and the half-life was short ((b) (4) hr). C_{max} was dose proportional in all three dose groups; AUC values were dose-proportional at MD and HD and slightly less than dose-proportional at LD.

Drinking Water: Following oral administration in the drinking water, the C_{max} was recorded four hours after the initiation of the dark cycle and values were roughly similar to oral gavage and, unlike the oral gavage, AUC values were greater than proportional to the administered dose in all dose groups. (Note: A dose-related reduction in water consumption was attributed by the sponsor to the adverse taste of hydrazine.)

Sponsor's figure:

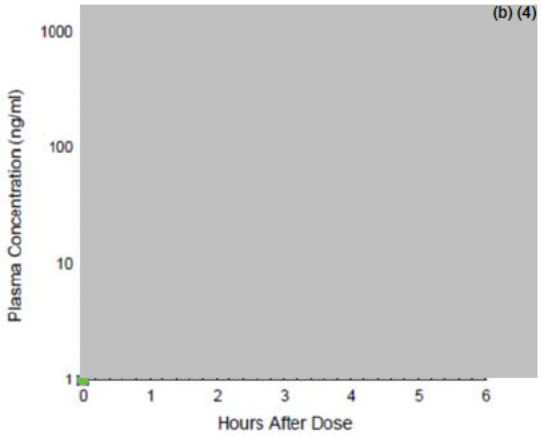
Table 3. Hydrazine Pharmacokinetics following Oral Dosing in Swiss Webster Mice (Oral Gavage vs. Drinking Water Dosing)

Dose	Route	C_{max}	C_{max}/D	T_{max}	$t_{1/2}$	AUC	AUC/D
0.4	Gavage						(b) (4)
0.65 (0.02)	Water						
2.0	Gavage						
2.4 (0.04)	Water						
10.0	Gavage						
7.25 (0.14)	Water						

Data provided as Mean (SEM)
Dose (mg/kg (gavage) or mg/kg/day (water)); C_{max} (ng/ml); C_{max}/D (ng/ml per mg/kg); T_{max} (hr); $t_{1/2}$ (hr);
AUC (ng•hr/ml); AUC/D (ng•hr/ml per mg/kg)
n.f. – unable to estimate plasma elimination half-life
n.a. – not applicable

Sponsor's figure:

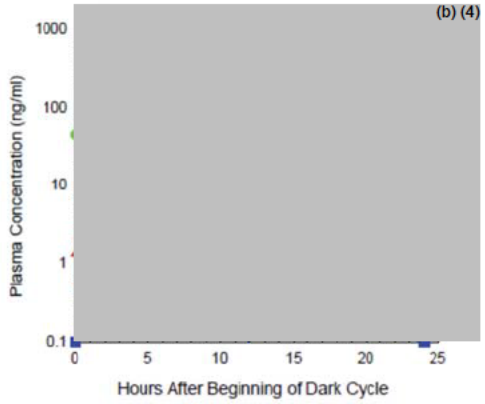
Figure 1. Hydrazine Plasma Concentrations following Oral (Gavage) Dosing in Swiss Webster Mice



Mean (±SEM, n=5 mice per gender per timepoint)

Sponsor's figure:

Figure 4. Hydrazine Plasma Concentrations following Oral Dosing via Drinking Water in Male Swiss Webster Mice (Day 19)



Mean (±SEM, n=5 mice per timepoint)

7.1.3

LCIG Drug Metabolism Memo 01: Pharmacokinetics of Carbidopa, (b) (4) and (b) (4) following Intravenous and Oral Dosing in Rat.

Preclinical Safety, Abbot Laboratories
Abbott Park, IL 60064
(Non-GLP, unaudited)

In this memo, the sponsor administered carbidopa, (b) (4), or (b) (4) in 10% DMSO in D5W, and compared PK data for the degradants after intravenous and oral gavage administrations to M Sprague-Dawley rats

See the table below for the study design.

Sponsor's Table: Doses are given in mg/kg.

Protocol	Compound	Lot #	Rte	Dose	Formulation	n
V11-1090	(b) (4)	1824749-0	IV	1	Solution (1 mL/kg)	3
V11-1090		1824749-0	PO	1	Solution (1 mL/kg)	3
V11-1091		1824755-0	IV	1	Solution (1 mL/kg)	3
V11-1091		1824755-0	PO	1	Solution (1 mL/kg)	3
V11-2320		1858842-0	PO	3	Solution in buffer (1 mL/kg)	3
V11-2320		1858842-0	PO	10	Solution in buffer (1 mL/kg)	3
V11-1092	Carbidopa	1420924-0	IV	1	Solution (1 mL/kg)	3
V11-1092	Carbidopa	1420924-0	PO	1	Solution (1 mL/kg)	3
V11-2318	Carbidopa	C0040408/2	PO	100	Suspension in buffer (1 ml/kg)	3

Compound – compound administered with the dose in mg/kg; Rte – dosing route; IV – intravenous; PO – oral;

Sequential tail-vein samples were taken at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 hours post-dose. IV-dosed animals were also sampled at 0.1 hr. post dose.

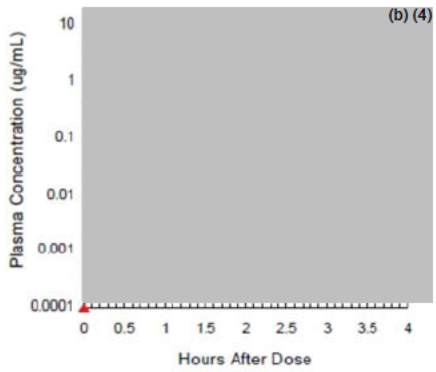
Results:
(b) (4) and (b) (4) were rapidly cleared after intravenous dosing in rats, with apparent elimination half-life of (b) (4) minutes. After oral administration, (b) (4) reached peak concentrations at (b) (4) minutes, with (b) (4)% bioavailability (C_{max}= (b) (4) mcg/mL). Oral bioavailability of (b) (4) was (b) (4)% (C_{max} = (b) (4) mcg/mL after 1 mg/kg PO) and the T_{max} was (b) (4) minutes. At 3 and 10 mg/kg PO, the AUC∞ and the C_{max} of (b) (4) were relatively dose-proportional.

Carbidopa was cleared more slowly after IV administration (half-life = 0.5 -1 hours). Carbidopa was slowly absorbed after oral dosing, peak plasma concentrations were at 0.5 to 1.5 hours, and the C_{max} was 0.051 mcg/mL. After 100 mg/kg PO, the C_{max} was less than dose-proportional and the T_{max} averaged 2.3 hrs (1.5-5.6 hrs).

After administration of carbidopa, plasma levels of (b) (4) paralleled carbidopa, albeit (b) (4) fold lower. Only trace amounts of (b) (4) were detected after oral dosing with carbidopa (C_{max} values of (b) (4) mcg/mL); the AUC∞ was (b) (4)-fold lower than carbidopa).

Sponsor's Figure:

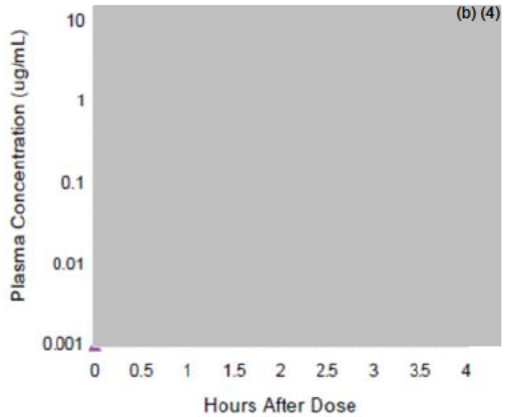
Figure 1. (b) (4) Plasma Concentrations following a 1 mg/kg IV or Oral Dose in Rat



Mean (±SEM, n=3); data from V11-1090

Sponsor's Figure:

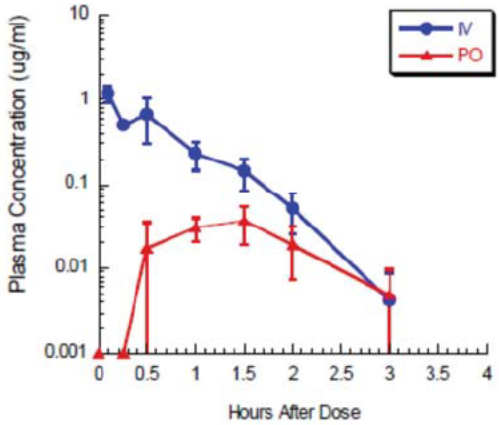
Figure 2. (b) (4) **Plasma Concentrations following a 1 mg/kg IV or 1, 3,. 10 mg/kg Oral Dose in Rat**



Mean (±SEM, n=3); data from V11-1091 and V11-2320

Sponsor's Figure:

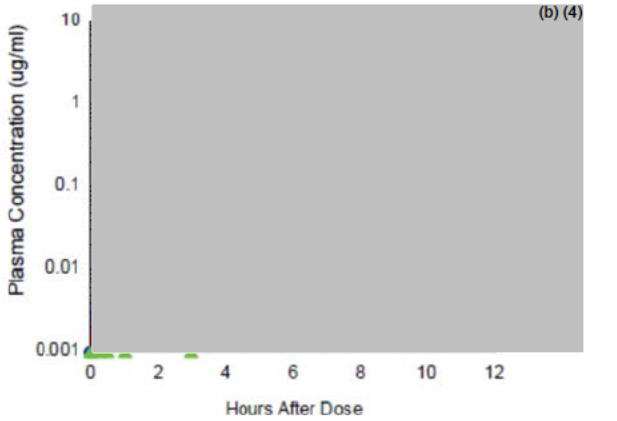
Figure 3. **Carbidopa Plasma Concentrations following a 1 mg/kg IV or Oral Dose in Rat**



Mean (±SEM, n=3); data from V11-1092

Sponsor's Figure: LOQ ~1-2 ng/mL per 0.2 mL plasma

Figure 4. Plasma Concentrations of Carbidopa, (b) (4) and (b) (4) following a 100 mg/kg Oral Dose of Carbidopa in Rat



Mean (±SEM, n=3); data from V11-2318

Table: PK after IV and Oral doses of carbidopa, (b) (4) and (b) (4) in rat.

Mg/kg	1 IV			1 PO	1 PO	3 PO	10 PO	1 PO	100 PO
Administered compound	(b) (4)	(b) (4)	CD	(b) (4)	(b) (4)	(b) (4)	(b) (4)	CD	CD
$t_{1/2}$ (hr)	(b) (4)		0.47	(b) (4)				n.f.	2.5
T_{max} (hr)			NA					1.2	2.3
Cmax mcg/mL			NA					.051	0.81
BA (%)			NA					7.5	3.9
AUC (ug*hr/mL)			0.94					0.71	3.72

n.f.: Sponsor "*unable to estimate*".

Table: PK of CD and metabolites after 100 mg/kg CD (PO) to rat.

100 mg/kg CD PO	CD	(b) (4)	(b) (4)
$t_{1/2}$ (hr)	2.5	(b) (4)	
Cmax (mcg/mL)	.81		
T_{max} (hr)	2.3		
AUC_t (mcg*h/mL)	3.72		
CD/Metabolite (AUC)	NA		
Metab/CD (AUC)	NA		

n.f.: Sponsor "*unable to estimate*".

7.3 General Toxicology

7.3.1

Study title: Four-Week Oral Impurity Qualification Study of (b) (4) and (b) (4) (Degradants in Duodopa) in Sprague-Dawley Rats.

Study no.:	TA11-113
Study report location:	eCTD: 4.2.3.7.6
Conducting laboratory and location:	Global Pharmaceutical Research and Development, Preclinical Safety Division, Toxicology, Abbott Laboratories, Abbott Park, IL
Date of study initiation:	AUG 16, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) ot No 1860195, 94.3%
	(b) (4) lot No 1860196, 97.5%

Key Study Findings:

- NOEL: None. Mild decreases in total white cell count and absolute lymphocyte counts were seen in both dose groups. A trend to increased thyroid weights compared to concurrent controls was seen in both sexes of both dose groups.
- Drug-related changes were limited to slight decreases in lymphocytes and total white blood cell count as compared to controls.
- The NOAEL was at the HD (15/10 mg/kg/day (b) (4)).

Methods

Doses:	(b) (4) 0/0, 6/4, 15/10 mg/kg/day
Frequency of dosing:	Dose divided twice daily for 28 days
Route of administration:	PO
Dose volume:	1 mL/kg
Formulation/Vehicle:	25mM Citrate buffered deionized water (pH 3.0)
Species/Strain:	Rat/Sprague-Dawley [Crl: CD® (SD)]
Number/Sex/Group:	10
Age:	9 weeks
Weight:	198-342 grams
Satellite groups:	No
Unique study design:	No baseline and no TK samples.
Deviation from study protocol:	"Samples for serological testing were not collected near the beginning of the study." The lack of serology for infectious agents had no impact on the study results.

Sponsor's Table, Dose groups:

Test Group	Dosage (b) (4) mg/kg/day (*mg/kg/dose*)[a]	Concentration of (b) (4) (mg/mL)[b]	Dosage (b) (4) mg/kg/day (*mg/kg/dose*)[c]	Concentration of (b) (4) (mg/mL)[b]	Main Study Male	Main Study Female
Group 1 (vehicle control)	0[d]	(b) (4)	0[d]	(b) (4)	10	10
Group 2 (low)	6 (*3*)		4 (*2*)		10	10
Group 3 (high)	15 (*7.5*)		10 (*5*)		10	10

a. (b) (4) (Lot 1860195). Expressed as free active moiety. Assigned chemical potency = 943 mg/g of test item. Correction factor for free active moiety = 1.060.

b. Expressed as mg free active moiety/mL.

c. (b) (4) (Lot 1860196). Expressed as free active moiety. Assigned chemical potency = 975 mg/g of test item. Correction factor for free active moiety = 1.026.

d. Vehicle: 25 mM Citrate buffer pH 3.0.

e. The dose volume was 1 mL/kg.

Observations and Results

Mortality: None

Clinical Signs: (Twice weekly) No differences between groups.

Body Weights: (Twice weekly) No differences between groups.

Food Consumption: (Once weekly) No differences between groups.

Ophthalmoscopy: (Baseline and "near the end of dosing period.") There was no individual animal ophthalmologic report and no abnormalities were reported in the summary tables.

Hematology: (Study Day 29) Total white blood cell counts were decreased in LDM (19%) and HDM (17%) and lymphocytes were decreased in LDM (22%) and HDM (22%) compared to mean values in CM.

Coagulation: (Study Day 29) No differences between groups.

Clinical Chemistry: (Study Day 29). ALT and AST were increased in 1 LDF (3x highest control value of AST and ALT) and 1 HDF (3x ALT and 2x AST highest control value). No baseline samples were taken.

Urinalysis: (Study Day 24) Ketones were detected in the urine from all treated rats and 5 CM and 3 CF. Levels in MD rats were similar to controls (25 mg/dL); levels in 1 MDM, 4 HDM, and 2HDF were 100 mg/dL, indicating a dose-response.

Note: According to the Sinemet ® label, Carbidopa/Levodopa "*may cause a false-positive reaction for urinary ketone bodies when a test tape is used for determination of ketonuria.*" The UA methodology was not provided in the study report, but the apparent increase in urine ketones may be an artifact.

Gross Pathology: There were no differences between groups.

Organ Weights: There was a trend to increased thyroid weights in all dosed M and F.

Histopathology

Adequate Battery: Yes
Pathology report: Yes
Peer Review: No

Histological Findings: All examined tissues were within limits of normal for the species.

Stability: The drug concentration ranged from 90-100% in retained samples.

Homogeneity: The relative SD of drug concentration ranged from 2.1-4.5%

Toxicokinetics: None.

Reference ID: 3459002

7.4 Genetic Toxicity

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

Study title: Bacterial Reverse Mutation Assay with (b) (4)
Study no.: TX11-132
Study report location: eCTD 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Date of study initiation: 29 AUG 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) Lot # 1860195, 94.3% purity

Key Study Findings:

There was no evidence of mutagenicity in any of the strains with or without S-9.

Methods

Strains: TA98, TA100, TA1515, TA1537, WP2*uvr*A.
Concentrations in definitive study: 15, 50, 150, 500, 1500, and 5000 mcg/plate
Basis of concentration selection: Initial toxicity-mutation assay
Negative control: Vehicle
Positive control: 2-nitrofluorene, sodium azide, 9-aminoacridine, methylmethanesulfonate
Formulation/Vehicle: 25 mM citrate buffer, pH 3, deionized distilled water.
Incubation & sampling time: 42-72 hours.

Study Validity: Study is valid.

Results

- By pre-incubation assay, no increase in revertant colonies were seen with or without Aroclor 1254-induced rat liver S9.
- Cytotoxicity was seen at 5000 mcg/plate, and there was no precipitation at any concentration.

7.4.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test with [redacted] (b) (4)

Study no.:	TX11-133
Study report location:	eCTD 4.2.3.7.7
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	31 AUG 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] (b) (4) #1860195, 94.3% pure

Key Study Findings

- Positive induction of structural chromosome aberrations in both non-activated and Aroclor 1254-induced rat liver S9-activated human PBL.
- No induction of numerical chromosome aberrations in both non-activated and S9-activated human PBL.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	Rat Liver S9: 20uL/mL of medium. Without S9: 143, 285, 460, 570, 660, 750, 810 mcg/mL
Basis of concentration selection:	With S9: OECD guideline Without S9: cytotoxicity
Negative control:	Vehicle
Positive control:	Mitomycin C
Formulation/Vehicle:	25 mM citrate buffer, pH 3, deionized distilled water
Incubation & sampling time:	Without S9: 4 or 20 hours With S9: 4 hours exposure +16 hours untreated

Study Validity: Study is valid.

Cytotoxicity:
In the preliminary assay:
At nine dose levels from 0.166 to 1660 mcg/mL, [redacted] (b) (4) did not precipitate at any concentration. Cytotoxicity was noted at ≥498 mcg/mL at 4 hours and ≥166 mcg/mL at 20 hours without S9, although there was no cytotoxicity with S9.

In the final assay:
After 4 hours, at 570 mcg/mL in the absence of S-9, there was a 53% reduction in mitotic index compared to vehicle control.

After 4 hours, at 1660 mcg/mL with S9, there was a 49% reduction in mitotic index compared to vehicle control.
After 20 hours, at 162 mcg/mL in the absence of S-9, there was a 52% reduction in the mitotic index compared to vehicle control.

Results:

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Untreated	-S9	4	15.5	200	200	0.005	±0.071	0.0	0.5
25mM citrate buffer, pH 3	-S9	4	16.2	200	200	0.005	±0.071	0.0	0.5
(b) (4)									
143	-S9	4	12.0	200	200	0.000	±0.000	0.0	0.0
285	-S9	4	11.2	200	200	0.000	±0.000	0.0	0.0
570	-S9	4	7.6	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	8.1	200	100	0.220	±0.462	0.0	20.0**
Untreated	+S9	4	11.9	200	200	0.000	±0.000	0.0	0.0
25mM citrate buffer, pH 3	+S9	4	10.8	200	200	0.005	±0.071	0.0	0.5
(b) (4)									
285	+S9	4	8.6	200	200	0.005	±0.071	0.0	0.5
810	+S9	4	7.6	200	200	0.005	±0.071	0.0	0.5
1660	+S9	4	5.5	200	200	0.000	±0.000	0.0	0.0
CP, 5	+S9	4	4.7	200	100	0.160	±0.395	0.0	15.0**
Untreated	-S9	20	14.9	200	200	0.000	±0.000	0.0	0.0
25mM citrate buffer, pH 3	-S9	20	14.9	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
36	-S9	20	13.4	200	200	0.005	±0.071	0.0	0.5
72	-S9	20	13.3	200	200	0.025	±0.157	0.0	2.5*
162	-S9	20	7.2	200	200	0.045	±0.208	0.0	4.5**
MMC, 0.3	-S9	20	6.8	200	100	0.220	±0.543	0.0	17.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.

<u>Without S9 @ 4 hr:</u>

There was no increase in structural or numerical aberrations compared to vehicle.

<u>Without S9 @ 20 hr:</u>

Structural aberrations were increased at 72 and 162 mcg/mL.
There was no increase in numerical aberrations.

<u>With S9 @ 4 hr:</u>

No increase in structural or numerical aberrations (up to 166 mcg/mL).

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

Study title: Bacterial Reverse Mutation Assay with (b) (4)
Study no.: TX11-134
Study report location: eCTD 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Date of study initiation: SEP 07, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: (b) (4) Lot # 1860196, 97.5% purity

Key Study Findings:

No evidence of mutagenicity in any of the strains with or without Aroclor 1254-induced rat liver S-9.

Methods

Strains: TA98, TA100, TA1535, TA1537, WP2*uvr*A.
Concentrations in definitive study: 15, 50, 150, 500, 1500, and 5000 mcg/plate
Basis of concentration selection: Initial toxicity-mutation assay
Negative control: Vehicle
Positive control: 2-nitrofluorene, sodium azide, 9-aminoacridine, methylmethanesulfonate
Formulation/Vehicle: 25 mM citrate buffer, pH 3, deionized distilled water.
Incubation & sampling time: 42-72 hours.

Study Validity: Study is valid

Results: By the preincubation assay, no increase in revertant colonies were seen with or without S9, no cytotoxicity was seen at 5000 mcg/plate, and there was no precipitation at any concentration.

Neither precipitate nor background lawn toxicity was observed; however, a reduction in revertant count was observed with S9 at 5000 µg per plate with tester strain TA1535.

7.4.4 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test with [redacted] (b) (4)
Study no.: TX11-135
Study report location: eCTD 4.2.3.7.7
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: AUG 31, 2011
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [redacted] (b) (4) Lot # 1860196, 97.5% pure

Key Study Findings :

- Induction of structural chromosome aberrations in both non-activated and Aroclor 1254-induced rat liver S9 S9-activated human PBL.
- No induction of numerical chromosome aberrations in either non-activated or S9-activated human PBL.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	4Hr: 500, 750, 100, 1500, 1960 mcg/mL; 20 hr: 250, 500, 550, 630, 700, 850, 1000 mcg/mL
Basis of concentration selection:	20 Hr: 50% mitotic inhibition; 4 Hr: OECD recommended 10 mM.
Negative control:	Vehicle
Positive control:	Mitomycin C
Formulation/Vehicle:	25 mM citrate buffer, pH 3, deionized distilled water.
Incubation & sampling time:	4 hrs and 20 hrs (-S9), 4 hrs (+ S9).

Study Validity: Study is valid

Cytotoxicity:

In the preliminary assay:

[redacted] (b) (4) did not precipitate at any concentration. Cytotoxicity was noted at 1960 mcg/mL at 20 hours without S9, although there was no cytotoxicity at 4 hours with or without S9.

In the final assay:

After 4 hours, at 1960 mcg/mL in the absence of S9, there was a 28% reduction in mitotic index compared to vehicle control.

After 4 hours, at 1960 mcg/mL with S9, there was a 14% reduction in mitotic index compared to vehicle control.

After 20 hours, at 550 mcg/mL, there was a 56% reduction in the mitotic index compared to vehicle control.

Results:

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Untreated	-S9	4	14.8	200	200	0.000	±0.000	0.0	0.0
25 mM citrate buffer, pH 3	-S9	4	14.6	200	200	0.000	±0.000	0.0	0.0
(b) (4)									
1000	-S9	4	13.2	200	200	0.030	±0.171	0.0	3.0*
1500	-S9	4	11.2	200	200	0.040	±0.196	0.0	4.0**
1960	-S9	4	10.5	200	200	0.065	±0.247	0.5	6.5**
MMC, 0.6	-S9	4	7.8	200	100	0.270	±0.529	0.0	24.0**
Untreated	+S9	4	12.7	200	200	0.000	±0.000	0.0	0.0
25 mM citrate buffer, pH 3	+S9	4	13.7	200	200	0.000	±0.000	0.5	0.0
(b) (4)									
1000	+S9	4	13.4	200	200	0.005	±0.071	0.0	0.5
1500	+S9	4	11.8	200	200	0.025	±0.157	0.0	2.5*
1960	+S9	4	11.8	200	200	0.020	±0.140	0.0	2.0
CP, 5	+S9	4	6.3	200	100	0.160	±0.395	0.0	15.0**
Untreated	-S9	20	15.5	200	200	0.000	±0.000	0.0	0.0
25 mM citrate buffer, pH 3	-S9	20	15.6	200	200	0.005	±0.071	0.0	0.5
(b) (4)									
250	-S9	20	13.7	200	200	0.000	±0.000	0.0	0.0
500	-S9	20	7.1	200	200	0.015	±0.122	0.0	1.5
550	-S9	20	6.9	200	200	0.035	±0.184	0.0	3.5*
MMC, 0.3	-S9	20	7.4	200	100	0.380	±0.814	0.0	27.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using the Fisher's Exact test.

Without S9 @ 20 hr:

Structural aberrations were increased from 550 mcg/mL to ??.
There were no increased numerical aberrations at any concentration.

Without S9 @ 4 hr:

Structural aberrations were increased at 1000, 1500, and 1960 mcg/mL.
Numerical aberrations were not increased at any concentration.

With S9 @ 4 hour:

Structural aberrations were statistically increased at 1500 mcg/mL.
Numerical aberrations not increased at any concentration.

8 Leachables and Extractables

8.1 R&D/11/570: Safety Assessment of (b) (4)
The sponsor finds that: *"The U.S. EPA and the European Food Safety Authority have established a reference dose or tolerable daily intake at (b) (4) µg/kg/day, or (b) (4) mg/ (b) (4) kg …in pharmaceutical products for adults and children over six years of age."*

According to the EPA's IRIS chronic health hazard assessments for non-carcinogenic effects of [redacted] (b) (4) the LOAEL for reduced mean body weight in rats was [redacted] (b) (4) mg/kg/day in chronic oral bioassay in rats and mice by the National Toxicology Program [redacted] (b) (4) With an uncertainly value of 1000, the reference dose for chronic oral exposure is [redacted] (b) (4) mg/kg/day [redacted] (b) (4) mg/day). On a mg/kg basis, the allowed human equivalent (HED) chronic exposure would be [redacted] (b) (4) mg/day.

8.2 R&D/1071138: an Abbot, Inc. inter-office memo entitled: <u>PIDE for</u> [redacted] (b) (4) <u>in clarithromycin</u>

The sponsor finds that: *"There is presently no ICH-derived PDE for* [redacted] (b) (4) *"The US EPA has established an oral reference dose of* [redacted] (b) (4) *mg/day. As* [redacted] (b) (4) *can often be a potential residual solvent in drug manufacture, it can be classified by its toxicity profile like a* [redacted] (b) (4) *with a PDE of* [redacted] (b) (4) *mg/day."*

This sponsor's calculated [redacted] (b) (4) Permissible Daily Exposure was determined for exposure in an antibiotic, not a chronically administered drug, and no references were submitted with the memo.

According to the EPA's IRIS chronic health hazard assessments for non-carcinogenic effects of [redacted] (b) (4) the NOAEL for body weight depression in rats was [redacted] (b) (4) mg/kg/day in a chronic oral study in rats and mice [redacted] (b) (4) The LOAEL for decreased body weight gain was the HD of [redacted] (b) (4) mg/kg/day. With an uncertainty factor of 100, the reference dose for chronic oral exposure, based on the NOAEL of [redacted] (b) (4) mg/kg/day is [redacted] (b) (4) mg/kg/day, or a daily intake of [redacted] (b) (4) mg.

8.3 R&D 11/170: Preclinical Safety Assessment of [redacted] (b) (4) **Erythromycin Base/Clarithromycin Drug Substance**

The sponsor cites a NSF oral risk assessment document that finds the NOAEL in subchronic studies to be [redacted] (b) (4) mg/kg/day, and finds that: *"*[redacted] (b) (4) *(R&D/11/170) has a PDE of* [redacted] (b) (4) *mg/day. Based on a reduction of body weight in rats in a chronic study at* [redacted] (b) (4) *mg/kg/day, a permissible daily exposure of* [redacted] (b) (4) *mg/day is calculated for humans"* for the daily intake in an antibiotic.

In a 13-week oral toxicity study in rats and mice, [redacted] (b) (4) peroxisome proliferation was seen at [redacted] (b) (4) mg/kg/day and the NOEL for target organ effects was [redacted] (b) (4) mg/kg/day in rats; the NOEL in mice was [redacted] (b) (4) mg/kg/day.

The PDE is calculated using an appropriate Uncertainty factor (500x) from the lowest NOAEL dose of the most sensitive species from a chronic study: [redacted] (b) (4) mg/day [redacted] (b) (4) kg adult is accepted.

8.4: Safety Assessment of Leachables (b) (4) (b) (4) and (b) (4) in ABT-SLV187

At 200 mg/day of LCIG, at levels calculated at 3 standard deviations above the average amounts in 6 analyzed batches, the sponsor anticipates a maximal daily intake of the three leachables as reported in the table below:

Name	Maximal Daily Exposure (mg/day)	PDE (60 kg adult) (mg/day)	Margin
(b) (4)			

With concurrence of the Chemistry reviewer, the anticipated levels of these leachables are reasonably safe.

8.5 Determination of the Acceptable Daily Exposure (ADE) of (b) (4)

Under eCTD 3.2.P, the sponsor explains that (b) (4) is in certain types of tubing that might be used in administration of LCIG, with a maximal exposure to (b) (4) estimated to be less than (b) (4) mcg/day.

> *(b) (4) is excluded as a potential leachable for LCIG based on its absence from the aqueous extracts. However, it was assessed as a special case because it was confirmed in the organic extract of the (b) (4) tube and connector, and the connector of both (b) (4) tubes, all (b) (4) components."*

(b) (4) has caused tumors in rats and mice; however, the mechanism through which (b) (4) induces carcinogenesis in rodents (peroxisome proliferation) is likely not relevant to human exposure. At (b) (4) mg/kg, reproductive effects in male rats are the most sensitive endpoints to calculate an acceptable daily exposure (ADE). The sponsor calculated an uncertainty factor of 60 (Inter-individual variability of 10 times an interspecies factor of 6), and the subsequent ADE for a (b) (4) kg human would be (b) (4) mg/kg/day

Name	Maximal Daily Exposure	ADE (60 kg adult)	Margin
(b) (4)	(b) (4)		100-fold

This reviewer agrees that the Acceptable Daily Exposure for (b) (4) is (b) (4) mg for a 60 kg individual (or, as calculated by the sponsor, (b) (4) mcg/day for a 50 kg individual). At a possible daily exposure of (b) (4) mcg, the margin of safety is 100-fold and, therefore, (b) (4) offers no toxicologic risk.

9 Integrated Summary and Safety Evaluation

Parkinson's disease is characterized by progressive degeneration of the dopaminergic nigrostriatal system and depletion of dopamine. Levodopa (L-dopa), the precursor of dopamine, readily passes the blood-brain barrier and is the mainstay of medical therapy. Carbidopa (CD), a peripheral decarboxylase inhibitor, which does not pass readily into the brain, prevents peripheral decarboxylation of L-dopa and allows higher concentrations to reach the brain.

This is a 505(b)(2) application for Levodopa/Carbidopa Intestinal Gel (LCIG) with Sinemet® (levodopa/carbidopa tablets) as the reference listed drug. LCIG, packaged in 100 mL cassettes, is delivered directly to the proximal small intestine using a portable infusion pump that delivers the gel through a percutaneous endoscopic gastrostomy tube with extension to the jejunum (PEG-J).

Submitted studies were limited to a series of feasibility and toxicokinetic evaluations leading to a 4-week local-irritation study of continuous duodenal infusion of LCIG in the minipig and PK and toxicity studies to support qualification of carbidopa degradants. Assessments of the safety of leachables and extractables from the delivery system were also submitted.

Local Toxicity:

The sponsor submitted a GLP-compliant 28-day GI irritation study (RD111304) of LCIG to address a change in formulation and route of administration from that of the reference listed drug. The study was adequately conducted and meets the requirements for the application. Two treatment groups and one vehicle group of 4 M minipigs per group, with surgically implanted percutaneous duodenal catheters, were up-titrated with LCIG for two days. They were maintained at constant intra-duodenal (CD/LD) doses of 0/0 mg/kg for 28 days, 11.25/45 mg/kg for 18 days and 7.5/30 mg/kg for 10 days (Low Dose), or 22.5/90 mg/kg for 28 days (High Dose). IM diazepam was administered to both dosed groups to ameliorate severe clinical signs of hyperactivity, “flight response,” salivation, vocalizing, circling, and repetitive behaviors. The Low Dose was reduced to the initial titration dose on Study Day 19 and diazepam was discontinued in that group.

One HDM was euthanized on Study Day 24 because of a blocked cannula and was examined grossly and histologically per protocol. Despite the adverse clinical signs, food consumption, body weight, and body weight gain did not differ among the three groups. The toxicokinetic data showed systemic exposure to CD and LD: both the C_{max} and $AUC_{23\ hr}$ of CD and LD increased in a dose-proportional manner, further evidence that the duodenum was exposed to the LCIG and that LD and CD crossed the duodenal mucosa.

No gross or histologic changes were seen in sections taken per protocol at 3 cm intervals distal to the catheter tip. Outside of catheter-placement-associated changes, gross changes in the duodenum were limited to areas of reddened mucosa in 1/4 CM,

Reference ID: 3459002

1/4 MDM, and 3/4 HDM "*in the region of, or slightly distal from, the cannula tip.*" Epithelial erosions and minimal-to-slight numbers of inflammatory cells (neutrophils and mononuclear cells) in the lamina propria were noted in the same CM and 3 HDM. No evidence of increased proliferation was observed. While not adverse, erosion and congestion noted in CM indicate that the catheter is an irritant and the dose-related increase in incidence, although not in severity, is interpreted as exacerbation of the mechanical irritation. The sponsor argues that the dose-related increases in clinical hyperactivity resulted in increased mechanical irritation by the catheter. Alternatively, the LCIG may be a mild irritant; however, the evidence (a marked dose-related increase in physical activity and no increase in lesion severity) supports the sponsor's position and this reviewer concurs that these changes are attributable to the effect of LCIG on animal behavior.

The sponsor submitted an additional GLP 28-day study in F minipigs. Small group size (3/group), early euthanasia (1 HDF on Study Day 6) and inter-animal variability necessitated shifting animals between groups and the study was not considered pivotal. The clinical signs were as described above; however, there was no reported evidence of mucosal irritation, reddening, or inflammation.

Also submitted were a series of non-GLP feasibility, dose-ranging, pilot PK, and TK studies in minipigs. After a series of attempts to replicate the clinical procedure (gastrostomy-duodenal catheterizations), direct percutaneous catheterization of the duodenum proved to be reliable, with the least adverse effects. There were consistent findings related to surgical placement of the cannulas: skin infections at the percutaneous site, adhesions of the cranial abdominal viscera, and suture granulomas where the catheter entered the duodenum and intraluminal neutrophils were seen to varying degrees in both C and dosed minipigs. Dose levels were limited by the marked clinical response to LCIG, ranging from hyperactivity and repetitive movements in M and F to sexual excitement and aggression in M, at all doses.

This gel formulation of levodopa-carbidopa combination was found to be non-irritating at the point of administration in the intestinal lumen. The API is active in the minipig and caused marked and adverse behavioral activity. The TK evidence that systemic absorption was adequate and the pathology data show that the compound itself caused no adverse anatomic changes. Adverse post-mortem findings were limited to complications at the sites of catheter implantation and to irritation caused by the physical presence of the catheter.

Impurities

The shelf-life limits of three specified impurity degradants of carbidopa in LCIG are proposed at levels above the qualification threshold: (b) (4) (b) (4) and hydrazine. The sponsor offered data and rationale to qualify (b) (4) at NMT (b) (4) w/w Carbidopa), (b) (4) at NMT (b) (4) w/w Carbidopa, and Hydrazine at NMT (b) (4) mcg/g LCIG. The daily

exposure from the MRHD of 100 mL LCIG would be (b) (4) mg (b) (4) (b) (4) mg (b) (4) and (b) (4) mcg ((b) (4) mg) hydrazine.

(b) (4)

The sponsor submitted data and a rationale to qualify (b) (4) levels ((b) (4) w/w CD) in LCIG that yield a daily dose, at the MRHD of 100 mL LCIG, of (b) (4) mg (b) (4) ((b) (4) mg/kg or (b) (4) mg/m^2). The sponsor argued that the impurity is qualified on the basis of the results of genotoxicity assays, findings in a 28 day rat oral toxicity study, and the levels of (b) (4) in human plasma after oral administration of CD products.

To assess the genotoxic potential of (b) (4) the sponsor submitted two *in vitro* studies: (b) (4) was negative in the Ames test; however, it was positive for structural chromosomal aberrations in human peripheral blood lymphocytes with and without S-9 activation. Both studies were adequately conducted and the results are considered valid and, under the conditions of these studies, (b) (4) was clastogenic but not mutagenic.

According to the sponsor and confirmed by the chemistry reviewer (pers. comm. DEC 27, 2013), (b) (4) shares an (b) (4) structural alert for carcinogenic potential with LD and CD. The parent compound is mutagenic and clastogenic but has not been found to be carcinogenic: "*In a two-year bioassay of SINEMET®, no evidence of carcinogenicity was found in rats receiving doses of approximately two times the maximum daily human dose of carbidopa*" alone or in combination with levodopa (*Sinemet labeling*). (b) (4) reported that 10 mg/kg/day CD in combination with 20, 50, or 100 mg/kg/day LD for two years "showed no pathologic changes due to treatment." From the data submitted in Memo A-156057, the bioavailability of CD is quite low and, at 10 mg/kg/day, the doses in the 2-year bioassay may not have been adequate. However, LD shares the same structural alert, and was administered at adequate doses (up to 100 mg/kg/day) and was also not found to be carcinogenic. As such (b) (4) which shares the same carcinogenic alert, should not be carcinogenic through the clastogenic mechanism, and daily intake need not be limited to that for genotoxic impurities of 1.5 mcg/day.

(b) (4) is a known metabolite of carbidopa, (b) (4) and is detected in urine of humans, monkeys, dogs, and rats after oral administration of C^{14} labelled CD (b) (4) The sponsor detected (b) (4) in rat plasma ((b) (4) ng/mL) after oral administration of 100 mg/kg CD. (b) (4) was also detected in minipig plasma after oral administration of CD, intraduodenal administration of CD, and after intraduodenal administration of LCIG.

In Study R&D 12/260, the sponsor reported low levels of (b) (4) in human plasma after intra-jejunal administration of 300-400 mg CD in LCIG ((b) (4) ng/mL (b) (4)) and 300-500 mg CD by oral administration of LD/CD ((b) (4) ng/mL (b) (4)) to PD patients. The sponsor interpreted the data from 7 patients administered LCIG to show the average AUC of (b) (4) to be less than (b) (4)% that of the average AUC of CD after

administration of LCIG containing (b) (4)% (b) (4) After oral administration of the CD/LD to 4 subjects, the sponsor found the averaged AUC of (b) (4) was approximately (b) (4)% that of the average AUC of carbidopa. The sponsor "*concluded that plasma concentrations of …* (b) (4) *…are less than* (b) (4)*% of carbidopa plasma concentrations and do not meaningfully add to plasma concentrations derived as carbidopa metabolites.*"

According to the Clinical Pharmacology reviewers, the data after oral administration of CD/LD show that (b) (4) is a metabolite of carbidopa: the median T_{max} of (b) (4) is greater than that of CD (6 hr vs., 1.5 hr). This indicates that (b) (4) as a metabolite, is formed *in vivo* after oral administration of CD.

However, after LCIG administration the median T_{max} of (b) (4) is less than that of CD (1 hr vs. 2 hr), indicating that the plasma (b) (4) was a degradant impurity in the LCIG, formed and absorbed along with the parent CD. The reviewers also found that because of the limited number of subjects and inter-patient variability of plasma levels, the exposures should not be averaged; further, the range after LCIG ((b) (4) ng/mL) is greater than that from the RLD (b) (4) ng/mL). As such, (b) (4) is a minor metabolite of LCIG and the amounts as a degradant in LCIG did not significantly increase the plasma levels resulting from metabolism.

(b) (4) is genotoxic and the carcinogenic risk is not concerning. (b) (4) is considered to be a minor metabolite after CD administration to humans and the proposed levels are not found to add significantly to exposure to (b) (4) resulting from metabolism.

(b) (4)

The sponsor offered data and a rationale to qualify (b) (4) levels ((b) (4) w/w CD) in LCIG that yield a daily dose, at the MRHD of 100 mL LCIG, of (b) (4) mg (b) (4) ((b) (4) mg/kg or (b) (4) mg/m^2). As with (b) (4) the sponsor argues that (b) (4) is qualified on the basis of the results of genotoxicity assays, findings in a 28 day rat oral toxicity study, and the levels of (b) (4) in human plasma after oral administration of CD products.

In submitted genotoxicity studies, (b) (4) was also found to be clastogenic but not mutagenic. Like (b) (4) (b) (4) shares the same (b) (4) structural alert for carcinogenic potential with levodopa and the parent compound, carbidopa. As such (b) (4) should not be carcinogenic through the clastogenic mechanism, and daily intake need not be limited to that for genotoxic impurities (1.5 mcg/day).

(b) (4) is a putative metabolite of CD. The sponsor detected (b) (4) in rat plasma just above the LLOQ (b) (4) ng/mL) after oral administration of 100 mg/kg CD. (b) (4) was also detected at up to (b) (4) ng/mL in minipig plasma after intravenous administration of 3 mg/kg CD (T_{max}= (b) (4) minutes). Based on the data from 7 patients administered LCIG and 4 administered oral LD/CD the sponsor concluded *"that plasma concentrations of …* (b) (4) *…do not meaningfully add to plasma concentrations derived as carbidopa metabolites.*" However, in Study R&D 12/260, the sponsor reported no (b) (4) in human

plasma (LLOQ (b) (4) ng/mL) after intra-jejunal administration of 300-400 mg CD in LCIG or 300-500 mg CD by oral administration of LD/CD to PD patients. The literature reports that the presence of (b) (4) was considered to be an artefact of sample handling when detected at low levels in human urine after administration of C^{14} labelled CD. (b) (4)

Thus, (b) (4) is not established to be a human metabolite of LCIG. , and exposure would come from its presence as a degradant impurity in the drug product.

A 4-week oral toxicity study (TA11-113) was conducted in SD rats (10/sex/group). (b) (4) was co-administered with (b) (4) at doses of 6/4 and 15/10 mg/kg/day (b) (4) Drug-related changes, compared to concurrent controls, were limited to a mild (22%) reduction in circulating lymphocytes in both groups and a similar reduction in white blood cell counts. No other toxic effects were reported, and the HD (15/10 mg/kg/day (b) (4) or (b) (4) mg/m^2) was the NOAEL. On a mg/m^2 basis, the safety margin to the proposed level was (b) (4) fold.

The mild drug-related systemic changes show that some drug was absorbed and, were the study of sufficient length, the study could be considered supportive. Although the results of this study suggest minimal effects of co-administered (b) (4) and (b) (4) the submitted 28-day study is not of sufficient length to allow qualification of the impurities.

Like (b) (4) (b) (4) is genotoxic but shares a structural alert with CD, LD and (b) (4) The lack of carcinogenicity in the two year study of combined doses of CD/LD mitigates concern that (b) (4) would be carcinogenic based on the structural alert. (b) (4) is not proven to be a human metabolite and exposure would be as a degradant impurity in LCIG. Based on a MRHD of 500 mg CD, the proposed level of (b) (4) ((b) (4) mg/day) exceeds the qualification threshold (b) (4) mg/day) by (b) (4) fold. The 28-day study indicates the compound may be of low toxicologic risk; however, the study is of insufficient length, and the proposed levels that cannot be qualified from the submitted data.

Hydrazine:

Hydrazine (H_4N_2) is genotoxic, a known animal carcinogen, and is possibly carcinogenic to humans (Group 2B). (IARC Monographs; Vol 71, *Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide*, World Health Organization, 1999) and (The Carcinogenic Potency Data Base, http://toxnet.nlm.nih.gov/cpdb/chempages/HYDRAZINE.html). Hydrazine is formed in LCIG (b) (4). LCIG is a suspension in an aqueous gel, formulated at a target pH of (b) (4) and warmed to room temperature and the sponsor anticipates a maximal end-of-shelf-life content of (b) (4) mcg of hydrazine in a 100 mL LCIG cassette. Levels of carcinogenic impurities in approved drugs should not exceed 1.5 mcg per day and the sponsor presents a rationale to qualify hydrazine at (b) (4) mcg/mL in each 100 mL cassette, resulting in a daily exposure of up to (b) (4) mcg/day.

A review of the literature and PK data were submitted for animals administered hydrazine, carbidopa, or LCIG and clinical subjects administered LCIG or the RLD. The sponsor submitted data from mice, rats, and PD patients to establish that the circulating level of hydrazine in humans is considerably lower than that found to be carcinogenic in mice and rats.

In two memos, the sponsor submitted non-GLP, unaudited studies that compare the PK data of hydrazine after either oral gavage or administration in drinking water to Wistar rats (M and F) and Swiss Webster mice (M and F) at dose levels similar to those reported in 2 published carcinogenicity studies in rats (Steinhoff and Mohr, 1988) and mice (Steinhoff et al, 1990). Both routes of administration resulted in rapid absorption and metabolism of hydrazine; similar plasma concentrations were achieved in M and F and the kinetics were dose-proportional. As in the published studies, doses in the M rats and mice were subject to reduced water intake attributed to the taste and odor of hydrazine.

In the published rat study, there was an 11% increase in liver tumors at 2.5 mg/kg (HDM) and 2.86 mg/kg (HDF). The HD in this referenced study fell between MD (1.39 mg/kg) and HD (5.48 mg/kg) used in the submitted PK study: the C_{max} ranged from 29 ng/mL (MD) to 116 ng/mL (HD) and the AUC from 100.4 ng*hr/mL (MD) to 1412 ng*hr/mL. The pharmacokinetics were dose-proportional and it can be assumed that the exposures in the referenced study fell approximately midway between those of the MD and HD of the submitted study with a subsequent C_{max} ~ 45ng/mL and an of AUC ~650 ng*hr/mL.

In the referenced mouse study, there were 40% more benign lung tumors in the HD than in Controls. The HD in the submitted study (7.3 mg/kg) approximates the daily doses in the referenced study (8.3 mg/kg in HDM and 10 mg/kg in HDF); the resulting C_{max} and AUC were 157ng/mL and 1560 ng*hr/mL, respectively.

After administration of LCIG to 11 PD patients (R&D 12/260), measurable hydrazine levels were detected within the first 12 hours in 5 of 7 samples from one patient receiving 150 mL LCIG): range (b) (4) ng/mL and the $AUC_{0\text{-}16\ hr}$ was calculated at (b) (4) ng*hr/mL. When patients were administered ~ 1500/350 mg/day oral CD/LD, hydrazine was detected at single, different, time-points in 2 patients ((b) (4) ng/mL at 4 hours in one patient and (b) (4) ng/mL at 8 hours in the other).

The sponsor states that *"plasma hydrazine concentrations measured in mice and rats at doses associated with tumors in the respective carcinogenicity studies are many fold higher than the plasma hydrazine concentrations measured in human subjects given LCIG."* Data from a single patient, however, cannot support that conclusion. The sponsor indicates (Study R&D 12/260) that the sampling interval after CD/LD administration may have been too long. That hydrazine was not detected in 10/11 patients administered LCIG may also reflect methodology rather than metabolism.

Reference ID: 3459002

The sponsor found that mean plasma hydrazine levels in minipigs administered approximately twice the MRHD of LCIG are approximately 10-fold less than after oral CD/LD and offers that as evidence that human exposure would also be low. However, the levels were seen sporadically in 2 of three animals and, as in human pharmacokinetic data (R&D 12/260), the data points cannot be averaged. Hydrazine kinetics were not computed and, given a reported marked difference in CD kinetics in those same minipigs, the parallels cannot be drawn.

From a review of the literature, the sponsor finds hydrazine to be a "weak" carcinogen (Steinhoff and Mohr, 1988) with an unknown mechanism of action for tumorigenicity which, because it is seen mostly at "cytotoxic" doses, is threshold-dependent.

Alternately, the mechanism may involve hypomethylation of DNA rather than direct cytotoxicity, and thus "*may lead to an increase in potential for aberrant gene expression*" rather than direct interaction with DNA. Thus, "*higher rates of aberrant…gene expression would be expected in animals receiving the highest doses of hydrazine*" (Fitzgerald and Shank, 1996), supporting a threshold-dose effect (Williams, 2008).

Based on a threshold mechanism, the sponsor proposes the "*dose threshold for tumor induction*" to be at the MD in the referenced rat study, of (b) (4) mg/kg/day (b) (4) mg/m^2/day). Exposures from a similar dose level in the submitted rat PK study (b) (4) mg/kg) were a C_{max} of (b) (4) ng/mL and an AUC of (b) (4) ng*hr/mL. However, rather than being *"many fold higher,"* as the sponsor contends, plasma levels in the individual patient administered LCIG (b) (4) ng/mL and a calculated $AUC_{0\text{-}16\ hr}$ at (b) (4) ng*hr/mL) exceed the approximated "tumor threshold" plasma levels in the rat. The single data points from 2 patients given the RLD approximate the "tumor threshold" (b) (4) ng/mL at 4 hrs. in one patient and (b) (4) ng/mL at 8 hrs. in the other). As stated, data from solitary patients are limited; however, the reported levels do not offer a margin above the "threshold for tumor induction in the rat."

In further interspecies comparisons, plasma hydrazine levels from intraduodenal administration of LCIG to minipigs seem to approximate those of the patient administered LCIG through the J-PEG, and those levels approximate the highest no-tumor dose seen in the rat. If the approximated plasma levels were to be compared, there would be only a modest margin to the doses that caused tumors in rats.

The sponsor points out that human exposure to hydrazine has been seen at the industrial level and from use of the anti-tuberculosis medication isoniazid and that there is no evidence of tumors associated with either industrial accidents, occupational exposure, or in tubercular subjects administered isoniazid for months "*to years.*"

Rather than the prolonged administration of LCIG, isoniazid is usually administered for 6-9 months; however, there are published PK data of hydrazine exposure from isoniazid administration, and retrospective follow-up epidemiologic data suggest that isoniazid administration is not associated with an increased incidence of tumors. In two

Reference ID: 3459002

publications (Pea et al., 1999) and (Woo et al., 1992), therapeutic isoniazid doses of 5-7 mg/kg were found to result in a hydrazine AUC of ~3,000 ng*hr/mL (in subjects that did not rapidly acetylate hydrazine). These levels are (b) (4) times higher than that seen from LCIG in humans and minipigs and are higher than the proposed "threshold" for tumorigenicity.

As a final rationale based on reported human exposure to hydrazine, the sponsor discussed risk to the patients administered LCIG subsequent to regulatory approval in Europe. The sponsor reports 27 cases of neoplasms reported from an estimated 8,648 patient-years of exposure to LCIG (reporting rate, 325 per 100,000 person-years) "*since first launch*." There is no pattern of systemic tumor incidence or type, and a solitary "intestinal tumor" was reported. The sponsor finds *"…there is little human data to support hydrazine's carcinogenic potential and exposure to hydrazine during treatment with LCIG therefore appears to represent a small component of risk in the context of the overall benefit-risk for LCIG*."

While it is true that hydrazine may be a "weak" carcinogen, and there is no solid epidemiologic data to support carcinogenicity in humans, hydrazine is a clastogenic, mutagenic animal carcinogen. That isoniazid, yielding hydrazine exposures greater than those from LCIG, is not clearly associated with tumors in humans offers some comfort.

Although only one intestinal tumor has been reported among those patients administered LCIG, the gel and degradant hydrazine are administered directly to the mucosa of the small intestine – an organ of high cell turnover. As such, there remains concern for both the clastogenic and carcinogenic properties of hydrazine. Further, if hydrazine is an epigenetic carcinogen and hypomethylation of DNA is part of the mechanism of tumorigenicity, hydrazine may allow an increase in DNA adducts and a subsequent increased risk of tumors. Although the risk of carcinogenesis may be modest, this reviewer cannot support the levels of hydrazine proposed by the sponsor.

Summary

1) The sponsor submitted numerous feasibility and exploratory studies of intra-intestinal administration of LCIG to minipigs. Although there is considerable trauma associated with intra-duodenal cannulation, LCIG is not found to be a local irritant.

2) The reported leachables and extractables present no toxicologic concerns.

3) Three carbidopa degradant impurities are present in LCIG at levels that exceed thresholds of qualification. The sponsor submitted data and assessments in support of qualification of levels of (b) (4) and (b) (4) to include genotoxicity and general toxicity studies. A literature review and assessment of the safety of the levels of hydrazine were submitted and reviewed.

Reference ID: 3459002

- (b) (4) is a metabolite of CD in humans and, as such, levels in the drug product are not of concern.
- The submitted toxicology study does not support qualification of the proposed levels of the degradant impurity (b) (4) and levels exceed the qualification threshold.
- The levels of hydrazine exceed the allowable daily exposure and are not acceptable on a pharmacologic/toxicologic basis.

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/s/

LUANN MCKINNEY
02/21/2014

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02/21/2014