Systemic Exposure to the Metabolites of Lesogaberan in Humans and Animals – a Case Study of Metabolites in Safety Testing

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ABBREVIATIONS: ADME, absorption, distribution, metabolism and excretion; AUC, area under the plasma concentration–time curve; \( C_t \), concentration at the time of the last plasma sample; DRM, drug-related material; FDA, US Food and Drug Administration; GERD, gastroesophageal reflux disease; HILIC, hydrophilic interaction liquid chromatography; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; \( k \), apparent terminal rate constant; LC-MS, liquid chromatography with mass spectrometry detection; LLOQ, lower limit of quantification; MIST, Metabolites in Safety Testing; PPI, proton pump inhibitor.
Abstract

During preclinical and early-phase clinical studies of drug candidates, exposure to metabolites should be monitored to determine whether safety conclusions drawn from studies in animals can be extrapolated to humans. Metabolites accounting for more than 10% of total exposure to drug-related material (DRM) in humans are of regulatory concern, and for any such metabolites adequate exposure should be demonstrated in animals before large-scale phase 3 clinical trials are conducted. We have previously identified six metabolites, M1–M6, of the gastroesophageal reflux inhibitor lesogaberan. Here, we measure exposure in humans, rats and beagle dogs to lesogaberan and these metabolites. Plasma samples were taken at various time points after lesogaberan dosing in two clinical and three preclinical studies. Concentrations of lesogaberan and its metabolites were measured, and exposures during a single dosing interval were calculated. The parent compound and metabolites M1, M2, M4 and M5 were together shown to comprise all significant exposure to DRM in humans. Only M4 and M5 were present at levels of regulatory concern (10.6% and 18.9% of total exposure to DRM, respectively, at steady state). Absolute exposure to M5 was greater in rats during toxicology studies than the highest absolute exposure observed in humans at steady state (117.0 µmol×h/l versus 52.2 µmol×h/l). In contrast, exposure to M4 in rats was less than 50% of the highest absolute exposure observed in humans. Further safety testing of this metabolite may therefore be required.
**Introduction**

In 2008, the US Food and Drug Administration (FDA) published guidance on monitoring exposure in humans and animals to the metabolites of novel drug candidates during preclinical and early-phase clinical safety studies (Metabolites in Safety Testing [MIST]) (FDA, 2008). According to this guidance, any metabolite for which the total exposure in humans accounts for more than 10% of exposure to the parent compound at steady state is of regulatory concern. For such metabolites, the absolute level of exposure in at least one animal species used in general toxicology studies should equal or exceed that observed in humans. If this requirement is not met for any metabolite of regulatory concern, further safety testing is required. Two strategies are recommended: the identification of and safety evaluation in an alternative animal species that produces the metabolite in sufficient quantities, or dosing of synthetic metabolite to a species already tested. Regulatory guidance does not distinguish between metabolite characteristics; stable, reactive and pharmacologically inert or active metabolites are treated the same.

The FDA MIST guidance has been superseded by guidance from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (ICH, 2009; ICH, 2012). The major difference between the FDA and ICH guidance lies in the definitions of metabolites of regulatory concern; according to the ICH, such metabolites are those for which exposure in humans accounts for more than 10% of the total exposure to drug-related material (DRM), not parent compound alone. The ICH guidance also considers adequate exposure in animals to metabolites of regulatory concern to be anything greater than 50% of the maximum absolute exposure observed in humans, unless the metabolite constitutes the majority of the total human exposure to DRM (ICH, 2012). In this case, exposure in animals should exceed the maximum absolute exposure observed in humans. The FDA
adopted the ICH guidance in 2010 (Yu et al., 2010), but has not officially changed or withdrawn its original guidance.

Although there is much published literature focusing on MIST strategies, perspectives and methodology, as well as reviews of the topic (Ma et al., 2010; Yu et al., 2010; Gao and Obach, 2011; Luffer-Atlas, 2012), there are very few published case studies demonstrating the application of regulatory guidance (Luffer-Atlas, 2008; Nedderman et al., 2011). Here, we report the results of MIST studies carried out during the preclinical and clinical development of the GABAB receptor agonist lesogaberan ([R]-[3-amino-2-fluoropropyl]phosphinic acid).

Lesogaberan has been developed as a reflux inhibitor for the treatment of patients with gastroesophageal reflux disease (GERD) who have a partial response to proton pump inhibitor (PPI) therapy (Cioffi et al., 1999; Dent et al., 2005; Boeckxstaens et al., 2010a; Boeckxstaens et al., 2010b; El-Serag et al., 2010; Boeckxstaens et al., 2011). The parent compound was shown to be stable in human and animal hepatocytes in vitro, but to be extensively metabolised in humans in vivo (Niazi et al., 2011). Levels of metabolism in animals in vivo were considerably lower (data on file). This finding was communicated to the FDA, and further experiments were undertaken to identify lesogaberan metabolites and to develop methods for their quantification (Dunér et al., 2013; Ekdahl et al., 2013).

The metabolite profile of lesogaberan in rats was found to be similar to that in humans, despite lower overall levels of metabolism of the parent compound in rats (data on file). Six metabolites were identified in rat urine, the primary route of drug excretion (Ekdahl et al., 2013). These were designated M1–M6, and were shown by comparison with synthetic reference standards of their behaviour in liquid chromatography and mass spectrometry (LC-MS) to be: M1, ([2R]-3-acetamido-2-fluoropropyl)phosphinic acid; M2, 3-hydroxypropylphosphinic acid; M3, [2R]-2-
fluoro-3-hydroxyphosphonoylpropanoic acid; M4, ([2R]-2-fluoro-3-guanidinopropyl)phosphinic acid; M5, 3-hydroxyphosphonoylpropanoic acid; and M6, ([2R]-3-amino-2-fluoropropyl)phosphonic acid (Fig. 1) (Ekdahl et al., 2013). Qualified methods were subsequently developed to determine their plasma concentrations (Dunér et al., 2013). Here, we report the results of investigations conducted to determine exposure to lesogaberan and these six metabolites in humans, rats and beagle dogs.

Materials and Methods

Plasma samples and determination of metabolite concentrations

Animal and human plasma samples were obtained at various time points after lesogaberan dosing in five clinical and preclinical studies, as outlined in Table 1. All analyses were carried out in accordance with the methods and ethical standards outlined in the relevant study protocol. Metabolite concentrations in each plasma sample were measured using LC-MS, as described previously (Dunér et al., 2013). In the human studies, concentrations of the parent compound were measured by an accredited laboratory (PRA International-Bioanalytical Laboratory B.V., Assen, Netherlands) using LC-MS, and in the animal studies, as described previously (Fakt et al., 2003). In the human study in which 14C-labelled lesogaberan was administered, total radioactivity was measured by Covance Laboratories Ltd (Harrogate, UK) using liquid scintillation counting.

Pharmacokinetic calculations

The pharmacokinetic parameters of lesogaberan and its metabolites were calculated by non-compartmental analysis using WinNonlin Enterprise (Pharsight Corporation, Mountain View, CA). At steady state, the area under the plasma concentration–time curve for the parent
compound and each metabolite during a single dosing interval (AUC$_\tau$) was calculated using the linear trapezoidal method. For determination of AUC$_{0-\infty}$ in the human study in which a single 400 mg dose of lesogaberan was administered, AUC$_{0-48\,\text{h}}$ was calculated and extrapolated to infinity by adding $C_t/k$, where $C_t$ is the concentration at the time of the last plasma sample and $k$ is the apparent terminal rate constant, obtained by linear least-squares regression analysis of the logarithm of the last three plasma concentrations versus time. For the determination of 24-hour exposure in the human study in which twice-daily 400 mg doses were given, AUC$_\tau$ (12-hour exposure) was calculated and multiplied by 2.

**Results**

**Exposure to lesogaberan and its metabolites in humans.** Exposure to lesogaberan and its metabolites was measured in humans in three experiments carried out on plasma samples taken from two clinical studies (Table 1; Fig. 2, A and B). Two of these experiments assessed exposure after single lesogaberan doses (100 mg and 400 mg), and one assessed exposure during a 24-hour period at steady state during twice-daily 400 mg dosing. In the study assessing both single and repeated twice-daily 400 mg dosing, metabolites M3 and M6 were not present at levels above the lower limit of quantification, 0.1 µmol/l, at any of the time points analysed (Table 2). In this study, a single 400 mg lesogaberan dose was administered, after which plasma samples were taken for 48 hours. After a further 1-day washout period, a 5-day period of 400 mg twice-daily dosing was started, and plasma samples were obtained for 12 hours after the final dose. Because 24-hour exposure to either M3 or M6 at 0.1 µmol/l would represent a theoretical maximum AUC of 2.4 µmol×h/l (i.e. less than 10% of exposure to total DRM), these metabolites were concluded not to be of regulatory concern and were omitted from any further analysis (including in the 100 mg single-dose radiolabelled study).
In the study in which a single 100 mg dose was given, $^{14}$C-labelled lesogaberan was administered, allowing exposure to total radioactivity to be measured (Niazi et al., 2011). In this experiment, all radioactivity was accounted for by the parent compound and the four measured metabolites: the AUC$_{0-24\,\text{h}}$ values for lesogaberan, M1, M2, M4 and M5 summed to 105.4% of the AUC$_{0-24\,\text{h}}$ for total radioactivity (Table 2). Thus, it was concluded that no major metabolites remain to be identified and quantified.

At steady state, after 5 days of twice daily 400 mg dosing, exposure to M1 accounted for 7.1% of exposure to total DRM, exposure to M2 accounted for 1.7%, exposure to M4 accounted for 10.6% and exposure to M5 accounted for 18.9% (Table 2). Similar results were seen in the single-dose experiments. Thus, only M4 and M5 were shown to be metabolites of regulatory concern, according to the ICH guidance (ICH, 2009; ICH, 2012).

**Exposure to lesogaberan and its metabolites in animals.** Exposure to parent compound and metabolites was measured in rats and beagle dogs during three preclinical studies (Table 1; Table 3; Fig. 2, C–E). All calculations were for exposure during a single 24-hour dosing interval at steady state after repeated once-daily lesogaberan administration and, in all studies, no adverse effects were observed at the highest dose tested. Exposure to M2 was not measured in rats in the 52-week study, as this metabolite was not of regulatory concern in humans. Exposure to M4 in rats could only be measured in the 7-day study, owing to lack of availability of plasma samples in the 52-week study at the time that M4 was identified and the quantification methodology developed. Exposure to M4 was not measured in beagle dogs, as it was expected from the results of early dog absorption, distribution, metabolism and excretion (ADME) studies to be present only at very low levels (data on file).
Of the two metabolites of regulatory concern, the highest absolute AUC observed in humans for M5, 52.2 µmol×h/l (AUCₜ after twice-daily 400 mg dosing), was exceeded in rats after 7 days of once-daily 176 mg/kg dosing (117.0 µmol×h/l). Thus, extrapolation to humans of safety conclusions from preclinical studies performed in rats can be considered valid with respect to this metabolite. In contrast, the highest AUC for M4 in animals, which was only measured in the 7-day study in rats, did not exceed 50% of the highest AUC observed in humans (i.e. was below the level considered adequate in the ICH MIST guidance for a metabolite that does not constitute the majority of exposure to total DRM in humans) (ICH, 2012). Further toxicology testing may therefore be necessary for this metabolite. However, the development program for lesogaberan has been halted, as phase 2b study results did not meet criteria for progression into phase 3 clinical trials (Shaheen et al., 2012).

Discussion

Although the current literature on MIST includes numerous methodology, perspective and strategic papers, and literature reviews (Ma et al., 2010; Yu et al., 2010; Gao and Obach, 2011; Luffer-Atlas, 2012), there are very few case studies of the application of regulatory guidance (Luffer-Atlas, 2008; Nedderman et al., 2011). Here, we have presented the results of MIST experiments carried out during the clinical development of lesogaberan, which therefore represent an important addition to available reports. The experiments were initially undertaken after communication to the FDA of results showing that, despite the high in vitro stability of lesogaberan in hepatocytes from various species, it is highly metabolised in humans in vivo. In the human phase 1 ADME study, in which ¹⁴C-labelled lesogaberan was administered orally and intravenously to healthy individuals, 84% of the dose was excreted in urine (based on recovery of radioactivity after both oral and intravenous dosing), but renal clearance of unchanged parent
compound accounted for only approximately 22% of total clearance (Niazi et al., 2011). Thus, the majority of the parent compound was metabolised. After oral dosing, total plasma radioactivity levels were clearly higher than the concentration of the parent compound at time points later than 1 hour after dosing, indicating circulating metabolites. In contrast, parent compound accounted for approximately 65% of total DRM excreted in urine and faeces in rats, and approximately 74% in dogs (data on file). Experiments were therefore conducted to identify the metabolites of lesogaberan in humans, and to develop methods to quantify them in plasma.

FDA and ICH guidance recommends that any metabolite for which systemic exposure in humans at steady state accounts for 10% or more of total exposure to DRM is of regulatory concern (FDA, 2008; ICH, 2009; ICH, 2012). For such metabolites, the absolute level of exposure observed in at least one animal species used in general toxicology studies should be 50% or more of the maximum exposure observed in humans, in order to conclude that the contribution of the metabolite to the toxicity of the drug has been established (ICH, 2012). An exception to this is in cases in which the metabolite constitutes the majority of human total DRM exposure; for any such metabolite, exposure in animals should be shown to exceed the maximum level observed in humans. For any metabolites of regulatory concern that do not meet these requirements, further toxicology studies are recommended. Any potential concerns regarding metabolite toxicity should be resolved before beginning large-scale phase 3 clinical trials (ICH, 2009).

The high in vitro stability of lesogaberan presented initial challenges for metabolite identification, exacerbated by the highly polar and zwitterionic nature of the parent compound and its metabolites, which resulted in poor LC retention and MS response. Consequently, the elegant in vivo non-radiolabelled cross-species systemic metabolite exposure comparison that
has been proposed in the literature could not be applied (Ma et al., 2010; Gao and Obach, 2011).

Our standard approach to the early assessment of metabolite exposures under steady-state conditions would compare human plasma samples obtained after dosing to the highest level expected to be used in the clinic, with samples obtained from animal species at the highest ‘no observed adverse effect’ level. For lesogaberan, however, because of the very low metabolic turnover \textit{in vitro} in combination with the poor MS response of formed metabolites, the metabolite profile could not be assessed using samples from our early studies. It was therefore not possible to follow this relatively simple protocol for early exposure comparisons.

These difficulties were overcome by the use of hydrophilic interaction liquid chromatography (HILIC) to separate metabolites excreted in rat urine after administration of a high dose of lesogaberan (rats had previously been shown to have a similar lesogaberan metabolite profile to humans, despite overall lower levels of metabolism of the parent compound), coupled with detection using linear trap quadrupole orbitrap MS (Ekdahl et al., 2013). In HILIC, a mixture of an organic solvent and water is used in the mobile phase, together with a hydrophilic silica or modified silica stationary phase; this offers better retention, separation, sensitivity and efficacy than traditional reversed-phase LC in the separation of small and highly polar compounds (Ikegami et al., 2008; Chirita et al., 2010; Hsieh, 2010; Jian et al., 2011). HILIC offers the further benefit of being favorable for electrospray MS owing to the high organic content of the eluent.

Six unique compounds (M1–M6; Fig. 1) in addition to the parent compound were detected in rat urine, and their identities were confirmed by comparison of their LC-MS properties with those of synthesised reference compounds (Ekdahl et al., 2013), followed by unambiguous structural elucidation by nuclear magnetic resonance spectroscopy (data on file). Lesogaberan was found
to be metabolised via oxidative pathways, including by deamination and subsequent oxidation to the corresponding carboxylic acid, oxidation to the phosphonic acid, and conjugation to an N-acetylated species. The routes to formation of the more surprising defluorinated and guanidino metabolites have previously been discussed (Ekdahl et al., 2013). Bioanalytic methods were subsequently developed and qualified for the determination of metabolite concentration in human and animal plasma samples (Dunér et al., 2013). This is in accordance with the “tiered approach” recommended by the European Bioanalytical Forum (Timmerman et al., 2010): preliminary screening to detect metabolites, followed by the development and use of qualified and/or validated bioanalytic methods to determine absolute parent compound and metabolite exposures.

In the experiments described in the current report, we have analysed samples taken from human ADME (single 100 mg dose) and dose escalation (single and repeat 400 mg dosing) studies, and animal studies carried out in rats and beagle dogs. For beagle dogs, plasma samples were remainder from a 12-month toxicology study, while in rats, the 7-days study was carried out in order to mimic longer term safety studies in rats, from which no sample was left to analyse and for which the maximum dose was 176 mg/kg. The 52-week rat samples were remainder from a carcinogenesis study, which confirmed the results from the 7-day study. In the human dose escalation study, use of plasma samples for exploratory metabolite work was specified in the protocol. We have shown that, in addition to parent compound, four of the six metabolites (M1, M2, M4 and M5) identified in rats account for all significant exposure to DRM in humans. Of these, only two (M4 and M5) are present at levels of regulatory concern. M5 was particularly prevalent, representing 18.9% of total exposure to DRM at steady state, while metabolite M4 was closer to the 10% threshold, at 10.6% of total exposure to DRM at steady state. Overall exposure to each metabolite was similar in humans after single doses and at steady state.
For M5, absolute exposures observed in rats at the highest doses tested in both the 7-day and 52-week studies exceeded the greatest absolute exposure seen in humans. Adequate exposure to this metabolite could be achieved in animals despite overall lower levels of metabolism than in humans, as high lesogaberan doses could be administered without apparent safety concerns. The contribution of M5 to the toxicology of lesogaberan can therefore be considered to have been established. This was not the case, however, for M4, the concentration of which was only measured in the 7-day rat study. For this metabolite further safety testing may be required (although it should be noted that 400 mg twice daily is a very high lesogaberan dose in humans, and was considerably higher than the highest dose administered during the phase 2b study [240 mg twice daily] (Shaheen et al., 2012)). Possibilities for such experiments include testing an alternative animal species that generates M4 in sufficient quantities, or dosing animals directly with synthetic M4. However, because the phase 2b study results did not meet the criteria for progression into phase 3 clinical trials, the development program for lesogaberan has been halted (Shaheen et al., 2012). Further toxicology experiments are therefore likely to be delayed until the future developmental process for this compound becomes clear.

In conclusion, we have described how MIST regulatory guidance was followed during the development of the drug candidate lesogaberan. Despite challenges such as the high polarity, low molecular weight and low MS response of lesogaberan and its metabolites, robust quantification was achieved that allowed comparisons of metabolite exposure in humans and animals to be made with confidence. Our data indicate that further studies would be necessary to ensure adequate exposure of at least one animal species to metabolite M4 before large-scale phase 3 trials could be conducted. If the clinical development program for lesogaberan is continued, we are well-equipped to address the safety assessment of this remaining metabolite.
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Authorship Contributions

Participated in research design: Holmberg, Ekdahl and Weidolf.

Conducted experiments: None of the authors.

Contributed new reagents or analytic tools: Ekdahl.

Performed data analysis: Holmberg.

Wrote or contributed to the writing of the manuscript: Holmberg, Ekdahl and Weidolf.
References


ICH (2012) M3(R2) Implementation Working Group, M3(R2) guideline: guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals questions & answers (R2). Available from: [http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Q_As/M3_R2_Q_A_R2_Step4.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Q_As/M3_R2_Q_A_R2_Step4.pdf) (accessed 07 November 2012)


Footnotes

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Figures and Tables

**Fig. 1.** Chemical structures of lesogaberan and metabolites M1–M6. Lesogaberan, [2R]-3-amino-2-fluoropropylphosphinic acid; M1, [2R]-3-acetamido-2-fluoropropylphosphinic acid; M2, 3-hydroxypropylphosphinic acid; M3, [2R]-2-fluoro-3-hydroxyphosphonoylpropanoic acid; M4, ([2R]-2-fluoro-3-guanidinopropyl)phosphinic acid; M5, 3-hydroxyphosphonoylpropanoic acid; M6, ([2R]-3-amino-2-fluoropropyl)phosphonic acid.

**Fig. 2.** Lesogaberan and metabolite concentrations plotted against time after the final lesogaberan dose in clinical and preclinical studies. (A) Humans, single 100 mg dose (14C-labelled); (B) humans, 400 mg twice daily for 5 days; (C) beagle dogs, 28 mg/kg once daily for 52 weeks; (D) male rats, 176 mg/kg once daily for 7 days; (E) male and female rats, 90 mg/kg once daily for 52 weeks. The lower graphs in each panel are at an increased magnification to show the concentrations of metabolites present at low levels.
TABLE 1. Summary of lesogaberan dosing regimens and plasma samples analysed (a subset of all samples obtained) in the clinical and preclinical studies

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Species</th>
<th>Lesogaberan Dose(s)</th>
<th>Dosing Prior to Plasma Samples</th>
<th>Time of obtaining Plasma Samples after Dosing (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C00017</td>
<td>Humans</td>
<td>100 mg (14C-labelled)</td>
<td>Single dose</td>
<td>1, 2, 3, 4, 6, 8, 12, 18, 24</td>
</tr>
<tr>
<td>C00030</td>
<td>Humans</td>
<td>400 mg</td>
<td>Single dose</td>
<td>2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 36, 48</td>
</tr>
<tr>
<td>C00030</td>
<td>Humans</td>
<td>400 mg</td>
<td>Twice daily for 5 days</td>
<td>0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12</td>
</tr>
<tr>
<td>VKS0499</td>
<td>Beagle dogs</td>
<td>28 mg/kg</td>
<td>Once daily for 52 weeks</td>
<td>0.5, 1, 2, 4, 8, 24</td>
</tr>
<tr>
<td>VKS0664</td>
<td>Rats (male)</td>
<td>35, 90, 176 mg/kg</td>
<td>Once daily for 7 days</td>
<td>0.5, 1, 3, 5, 8, 12, 18, 24</td>
</tr>
<tr>
<td>VKS0507</td>
<td>Rats (male/female)</td>
<td>10, 30, 90 mg/kg</td>
<td>Once daily for 52 weeks</td>
<td>0.5, 1, 3, 8, 24</td>
</tr>
</tbody>
</table>
TABLE 2. Mean AUC values for lesogaberan and its metabolites in human studies

<table>
<thead>
<tr>
<th>AUC value measured</th>
<th>Lesogaberan Dose</th>
<th>Dosing Regimen</th>
<th>Radioactivity AUC, µmol×h/l (%) Parent; % Total DRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0–24 h&lt;/sub&gt;</td>
<td>100 mg Single dose</td>
<td>28.100</td>
<td>17.7 (100.0; 59.7)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–&lt;/sub&gt;∞</td>
<td>400 mg Single dose</td>
<td>–</td>
<td>87.9 (100.0; 52.8)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;τ x 2&lt;/sub&gt;</td>
<td>400 mg Twice daily for 5 days</td>
<td>–</td>
<td>171 (100.0; 61.8)</td>
</tr>
</tbody>
</table>

–, not measured; AUC, area under curve; DRM, drug-related material (expressed as the sum of the AUCs for parent compound and measured metabolites).
### TABLE 3. Mean AUC\(_\tau\) values for lesogaberan and its metabolites in animal studies

<table>
<thead>
<tr>
<th>Species</th>
<th>Lesogaberan</th>
<th>Dosing Regimen</th>
<th>(\text{AUC}_\tau, \text{µmol}\times\text{h/l})</th>
<th>Lesogaberan</th>
<th>M1</th>
<th>M2</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beagle dogs</td>
<td>28 mg/kg</td>
<td>Once daily for 52 weeks</td>
<td>712</td>
<td>3.25</td>
<td>3.85</td>
<td>–</td>
<td>9.11</td>
<td></td>
</tr>
<tr>
<td>Rats (male)</td>
<td>35 mg/kg</td>
<td>Once daily for 7 days</td>
<td>322</td>
<td>3.24</td>
<td>1.54</td>
<td>1.31</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90 mg/kg</td>
<td></td>
<td>784</td>
<td>7.59</td>
<td>3.41</td>
<td>2.76</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>176 mg/kg</td>
<td></td>
<td>1280</td>
<td>12.2</td>
<td>5.75</td>
<td>4.71</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Rats (male)</td>
<td>10 mg/kg</td>
<td>Once daily for 52 weeks</td>
<td>107</td>
<td>0.790</td>
<td>–</td>
<td>–</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td></td>
<td>253</td>
<td>1.90</td>
<td>–</td>
<td>–</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90 mg/kg</td>
<td></td>
<td>632</td>
<td>6.59</td>
<td>–</td>
<td>–</td>
<td>77.0</td>
<td></td>
</tr>
<tr>
<td>Rats (female)</td>
<td>10 mg/kg</td>
<td>Once daily for 52 weeks</td>
<td>90.5</td>
<td>0.809</td>
<td>–</td>
<td>–</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td></td>
<td>277</td>
<td>5.06</td>
<td>–</td>
<td>–</td>
<td>43.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90 mg/kg</td>
<td></td>
<td>648</td>
<td>10.4</td>
<td>–</td>
<td>–</td>
<td>87.3</td>
<td></td>
</tr>
</tbody>
</table>

–, not measured; AUC, area under curve.
Lesogaberan (AZD3355)

M1: C$_4$H$_{10}$NO$_2$PF

M2: C$_5$H$_{11}$NO$_3$PF

M3: C$_3$H$_6$O$_4$PF

M4: C$_4$H$_{11}$O$_2$N$_3$PF

M5: C$_3$H$_7$O$_4$PF

M6: C$_3$H$_9$O$_3$N$_2$PF
FIGURE 2

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