

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**206316Orig1Orig2s000**

**PHARMACOLOGY REVIEW(S)**

Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 206316

**Submission date:** 1/8/2014

**Drug:** Edoxaban Tosylate

**Applicant:** Daiichi Sankyo Inc.

**Indications:** 1. Reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation; 2. Treatment of deep vein thrombosis and pulmonary embolism;

(b) (4)

**Reviewing Division:** Division of Cardiovascular and Renal Products, Division of Hematology Products

**Discussion:** The primary and secondary pharmacology/toxicology reviewers found the information for edoxaban tosylate sufficient to support approval for the indications listed above.

Edoxaban is a factor Xa inhibitor. This is an existing Established Pharmacologic Class.

The carcinogenicity of edoxaban tosylate was assessed in two-year carcinogenicity studies in rats and mice. The executive carcinogenicity assessment committee found these studies to be acceptable and concurred that there were no drug-related neoplasms in either study.

A study in rats did not demonstrate any effect on fertility. Embryo fetal studies in rats and rabbits at substantial margins to human exposure showed no malformations in fetuses. Some embryofetal toxicity was observed although this occurred at doses that were maternally toxic and at exposures that were still substantially greater than human exposures.

**Conclusions:** I concur that the nonclinical information is adequate to support approval of edoxaban tosylate for the indications listed above. I have provided other comments on labeling to the review division separately.

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/s/  
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PAUL C BROWN  
11/07/2014

**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: 206316  
Supporting document/s: N-000 (SDN-001)  
Applicant's letter date: January 8, 2014  
CDER stamp date: January 8, 2014  
Product: SAVAYSA (edoxaban tosylate)  
Indication: 

- Treatment of deep vein thrombosis (DVT) (b) (4)
- Reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation

Applicant: Daiichi Sankyo Inc.  
Review Division: Division of Hematology and Oncology Toxicology (DHOT), for Division of Hematology Products (DHP)  
Reviewer: Shwu-Luan Lee, Ph.D.  
Supervisor/Team Leader: Haleh Saber, Ph.D.  
Division Director: John Leighton, Ph.D., DABT (DHOT)  
Ann Farrell, MD (DHP)  
Project Manager: Janet Higgins

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# 1 Executive Summary

## 1.1 Introduction

Edoxaban tosylate is an inhibitor of factor Xa, proposed for the following indications:

- Treatment of deep vein thrombosis (DVT) [REDACTED] (b) (4)
- Reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation.

The clinical data for DVT is being reviewed by the clinical team in the Division of Hematology Products (DHP), while the clinical data for atrial fibrillation is being reviewed by the Division of Cardiovascular and Renal Products (DCaRP). The nonclinical studies submitted to the NDA support both indications. This review addresses the primary and secondary pharmacology studies conducted with edoxaban in support of both indications. Results of safety pharmacology, ADME, and toxicology studies are being reviewed by Dr. Baichun Yang in DCaRP. Both reviews should be consulted to provide the complete understanding of the pharmacology and toxicology of edoxaban. For recommendation on approval, labeling, and impurity qualifications, see Dr. Yang's review.

## 1.2 Brief Discussion of Nonclinical Findings

Edoxaban (DU-176b) is an anti-coagulant exerting its pharmacodynamics effects mainly via inhibition of activated coagulation factor X (Factor Xa; FXa). Edoxaban also had inhibitory activity against thrombin. The  $K_i$  for FXa was  $\sim 0.6$  nM and for thrombin was 6  $\mu$ M, indicating less inhibition toward thrombin. Edoxaban demonstrated comparable FXa inhibition in human, rabbit, and cynomolgus plasma ( $K_i$  values  $\sim 0.5$ - $0.7$  nM), while less inhibition was observed in rat plasma. When two mutant forms of factor Xa were used in the assays, edoxaban exhibited comparable anticoagulation activity toward the wild-type or the mutants.

The three metabolites of edoxaban (D21-1402-0201, D21-2135-0101, D21-2393) also had anti-FXa activity and caused clotting time prolongation. Among these active metabolites, the human specific metabolite D21-2393 (10% of the total exposure in healthy human subjects) showed comparable anti-coagulant effects as edoxaban. In various animal models, oral administration of edoxaban resulted in dose-dependent anti-thrombotic activity, as manifested by reduced weight of thrombi, as well as prolongation of clotting time. Under the conditions tested, the antithrombotic effects, in terms of PT prolongation and inhibition of thrombosis, of edoxaban were comparable to enoxaparin (a low molecular weight heparin, which inhibits both FXa and thrombin) and warfarin (vitamin K antagonist).

Edoxaban inhibited platelet aggregation induced by thrombin, possibly via inhibition of thrombin, since edoxaban did not affect ADP, U46619 or collagen-induced platelet aggregation. In the *in vitro* studies, recombinant FVIIa, FEIBA (a plasma-derived activated prothrombin complex concentrate) or PPSB-HT (a prothrombin complex

concentrate) were used to determine the reversibility of edoxaban-induced anticoagulant activities. Under the conditions tested, reversibility of edoxaban-induced anticoagulation was demonstrated when these plasma factors were added to the mixture. Despite this reversibility, a conclusion cannot be made on the antidote effect of plasma factors in animals or in humans due to limitations of an in vitro study.

## 2 Drug Information

### 2.1 Drug

**CAS Registry Number:**

480449-71-6 (anhydrous free base)

**Generic Name**

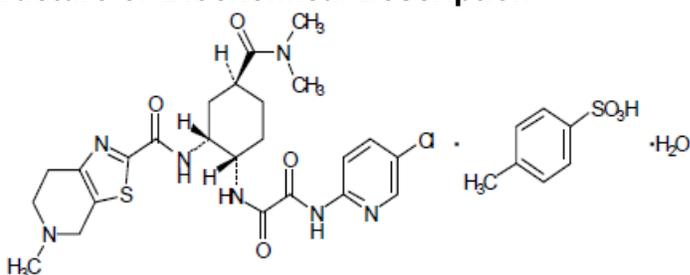
Edoxaban tosylate (p-toluenesulfonate) hydrate

**Code Name**

DU 176b (DU-176: free base of edoxaban), D11-4176b

**Chemical Name**

*N*-(5-Chloropyridin-2-yl)-*N'*-[(1*S*,2*R*,4*S*)-4-(*N,N*-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-*c*]pyridine-2-carboxamido)cyclohexyl] oxamide mono (4-methylbenzenesulfonate) monohydrate

**Molecular Formula/Molecular Weight** $C_{24}H_{30}ClN_7O_4S \cdot C_7H_8O_3S \cdot H_2O/738.27$ **Structure or Biochemical Description****Pharmacologic Class**

Factor Xa inhibitor.

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 63266 (venous thromboembolism); IND 77254 (pulmonary embolism)

### 3 Pharmacology Studies Submitted

#### 3.1 Pharmacology Studies Reviewed

| Study Number           | Study Title   |
|------------------------|---|
| Primary Pharmacology   |   |
| 20020850               | DU-176b: anticoagulant activity and its specificity for human factor Xa   |
| 20050516               | Effects of DU-176b on the activities of human factor IXa, factor Xa, and activated protein C  |
| AD07-H0067-R01         | DU-176b and (b) (4) pharmacogenomics  |
| GB11-H0026-R01         | Factor Xa inhibitory activities and anticoagulant effects of metabolites of DU-176b   |
| AD07-H0070-R01         | Factor Xa inhibitory activity and anticoagulant effect of D21-2393, a metabolite of DU-176b   |
| 20060456               | DU-176b: inhibitory effect of DU-176b on the activities of prothrombinase complex   |
| 20020847               | D11-4176b: Anti-thrombotic effect of D11-4176b in a rat A-V shunt model   |
| 20030369               | DU-176b: Anti-thrombotic effect of DU-176b in a rat venous stasis model   |
| 20020848               | D11-4176b: Anti-thrombotic effect of D11-4176b in a rat venous thrombosis model   |
| 20020849               | D11-4176b: Anti-thrombotic effect of D11-4176b in a rat tissue factor induced DIC model   |
| AD09-H0007-R01         | Antithrombotic effects of DU-176b and enoxaparin in a rat model of venous thrombosis  |
| BG11-H0012-R01         | Treatment of venous thrombosis with DU-176b, enoxaparin and fondaparinux in rats  |
| Secondary Pharmacology |   |
| 20060958               | DU-176b: Effects of DU-176b on human platelet aggregation   |
| 20030655               | Effects of anti-inhibitor coagulant complex, prothrombin complex concentrate and recombinant factor VIIa on prothrombin time (PT) prolonged by DU-176b                        |
| AD09-H0016-R01         | Effects of anti-inhibitor coagulant complex, prothrombin complex concentrate and recombinant factor VIIa on prothrombin time prolonged by DU-176                              |
| TMBM040                | Ex-vivo study to assess the potential of factor eight inhibitor bypass activity (FEIBA) or activated factor seven (rhFVIIa) to reverse the anticoagulant activity of edoxaban |
| AD09-H0008-R01         | Effects of DU-176b and enoxaparin on bleeding time in a rat tail bleeding model   |
| BG10-H0078-R01         | antithrombotic and bleeding prolongation effects of warfarin in rat models of venous thrombosis and tail bleeding   |
| BG12-H0020-R01         | Effects of tranexamic acid on prolonged bleeding time and prothrombin time induced by a supratherapeutic dose of DU-176b in rats  |
|                        |   |

### 3.2 Pharmacology Studies Not Reviewed

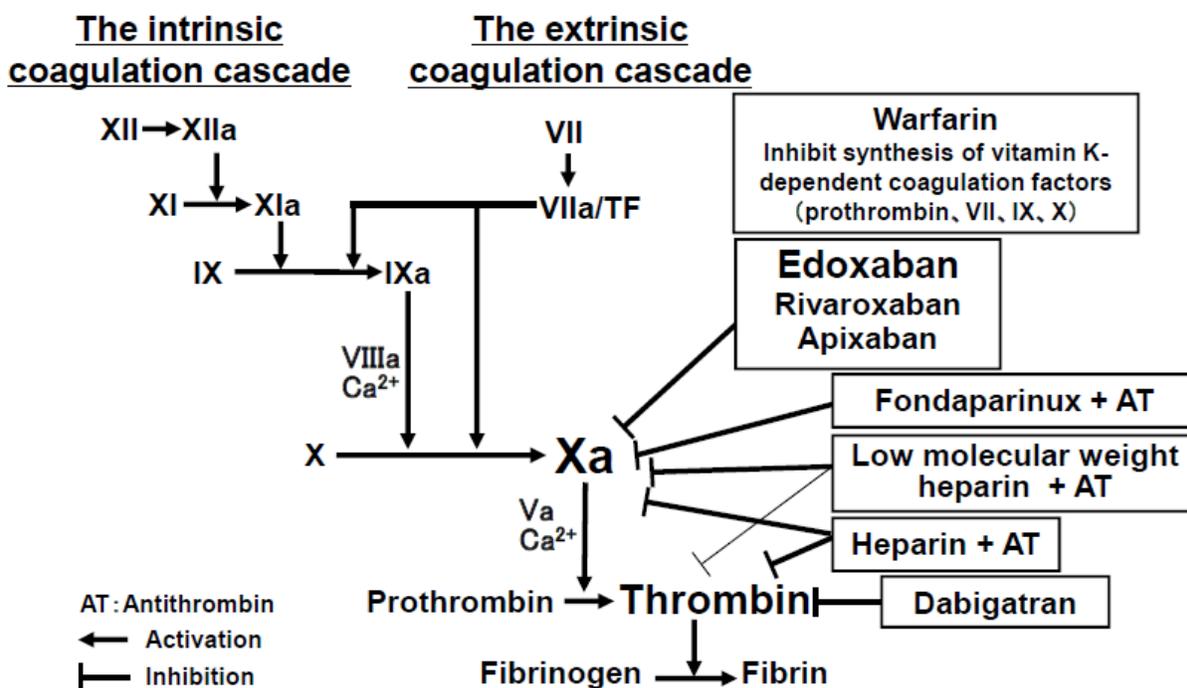
Study title: Antithrombotic and bleeding time prolongation effects of warfarin in rat models of venous thrombosis and tail bleeding (Study # BG10-H0078-R01, Module 4)

## 4 Pharmacology

### 4.1 Primary Pharmacology

Edoxaban exerts its anti-coagulant activity via inhibition of Factor Xa and prothrombinase complex in the blood coagulation cascade. Figure below (from the Applicant) depicts some of the factors and the sites of action of several anticoagulants.

Figure 1- The blood coagulation cascade and the sites of action of anticoagulants (Figure from the Applicant; Module 2)



Activated factor X (FXa) and prothrombin are involved in the formation of thrombin, which in turn catalyzes the conversion of fibrinogen to fibrin. Studies conducted to investigate the effects of edoxaban on Factor Xa (free or incorporated in the prothrombinase complex) and effects on two mutant forms of FXa are summarized below. In vitro and in vivo models used to characterize the anti-thrombotic effects of edoxaban are also summarized. These studies are from Module 4, Section 4.2.1.1.

**Study title: DU-176b: anticoagulant activity and its specificity for human factor Xa (Study # 20020850)**

The inhibitory effect of edoxaban on amidolytic activity of factor Xa was determined for various species based on the inhibitory constant (i.e.,  $K_i$  values). Additionally, the effect of edoxaban on amidolytic activity of other serine proteases, including thrombin, trypsin, chymotrypsin, plasmin, tissue plasminogen activator and human factor VIIa, was investigated. The effect of edoxaban on FXa-related clotting times, i.e., thrombin time (PT), activated thromboplastin time (APTT) and thrombin time (TT) of human plasma was determined.

**Methods:**

- Determination of  $K_i$  values for FXa from various species

The inhibitory effect of edoxaban (in the salt form edoxaban tosylate hydrate) on the amidolytic activity of human, monkey, rabbit and rat FXa (at 0.005 U/mL or 0.025 U/mL) was determined in a mixture of chromogenic substrate S-2222 (250 to 1000  $\mu$ M), FXa, and various concentrations of edoxaban (0.5 to 8 nM). The amidolysis was measured at absorption OD of 405 nm.

The slopes of the lines were calculated from the linear regression of the Lineweaver-Burk plot. The slopes were plotted against the three different concentrations of edoxaban (secondary plot). The  $K_i$  values of edoxaban for each enzyme (or FXa from different species) were determined from the linear regression of secondary plot. The  $K_i$  values over 100  $\mu$ M were expressed as ">100  $\mu$ M".

- $K_i$  value determination for other serine proteases:

The final concentration of enzymes, substrates and edoxaban (DU-176b) used in this study are shown as follows.

| Serine proteases | Concentration of enzyme | Substrate         | Concentrations of substrate | Concentrations of DU-176b |
|------------------|-------------------------|-------------------|-----------------------------|---------------------------|
| Thrombin         | 0.03 U/mL               | S-2238            | 25, 33, 50, 100 $\mu$ M     | 10, 20, 30 $\mu$ M        |
| Trypsin          | 0.3 U/mL                | S-2222            | 50, 67, 100, 200 $\mu$ M    | 100, 150, 200 $\mu$ M     |
| Chymotrypsin     | 0.005 U/mL              | S-2586            | 25, 33, 50, 100 $\mu$ M     | 100, 150, 200 $\mu$ M     |
| Plasmin          | 0.004 U/mL              | S-2251            | 250, 334, 500, 1000 $\mu$ M | 100, 150, 200 $\mu$ M     |
| rt-PA            | 750 U/mL                | S-2288            | 250, 334, 500, 1000 $\mu$ M | 100, 150, 200 $\mu$ M     |
| rhFVIIa / sTF    | 2 nM / 20 nM            | Spectrozyme fVIIa | 250, 334, 500, 1000 $\mu$ M | 100, 150, 200 $\mu$ M     |

- Anticoagulation activity of edoxaban:

The clotting times were determined following conventional methods, and the following was added to human plasma: thromboplastin C plus,  $\text{CaCl}_2$ , and thrombin for PT, APTT and TT determination, respectively. The prolongation ratios of the clotting time (PT, APTT and TT) to the mean value of the control clotting time were calculated, then the

CT2\* and their 95% confidence interval were determined with an ED<sub>50</sub> calculation software. To evaluate species specificity of edoxaban for the anticoagulant activity, PT was measured using human plasma, rat plasma, cynomolgus monkey plasma and rabbit plasma. The CT2 value was estimated from dose response curves. All assays were performed in triplicate.

**\*CT2: The concentrations required to double the clotting time**

#### Results:

- AntiFXa activity of edoxaban:  
Edoxaban exerted comparable inhibitory effects on FXa activity among different species, except for the rat plasma.

Table 1- AntiFXa activity of edoxaban in human, rat, rabbit and monkey plasmas

| Factor Xa (FXA): species | Ki (nM) |
|--------------------------|---------|
| Human                    | 0.561   |
| Rat                      | 6.98    |
| Rabbit                   | 0.457   |
| Cynomolgus monkey        | 0.715   |

- Effect of edoxaban on serine proteases:  
Except for thrombin, there was no inhibitory effect of edoxaban on the amidolysis activity of other serine proteases.

Table 2- Ki values of edoxaban on serine proteinases

| Serine protease | Ki (μM) |
|-----------------|---------|
| Thrombin        | 6       |
| Trypsin         | >100    |
| Chymotrypsin    | >100    |
| Plasmin         | >100    |
| Rt-PA           | >100    |
| rhFVIIa/sTF     | >100    |

- Anticoagulation activity of edoxaban:

Table 3- Clotting times of edoxaban in human, rat, rabbit and monkey plasma

#### Clotting times: human plasma

| Plasma clotting time | CT2 (μM) |
|----------------------|----------|
| PT                   | 0.256    |
| APTT                 | 0.508    |
| TT                   | 4.95     |

**\*CT2: The concentrations required to double the clotting time**

#### Effects on PT of plasma from various species

| Species           | PT: CT2 (μM) |
|-------------------|--------------|
| Rat               | 0.647        |
| Rabbit            | 0.149        |
| Cynomolgus monkey | 0.32         |

**Study title: DU-176b: Effects of DU-176b on the activities of human factor IXa, factor Xia, and activated protein C (Study # 20050516)**

**Key finding:**

Edoxaban had more inhibitory activity towards FXa compared to other factors tested in this study (FIXa, FXIa, and activated protein C).

**Methods:**

Edoxaban (DU-176b) at various concentrations or vehicle (5% DMSO) were mixed with the substrate (see table below). The reaction was started by the addition of the enzyme solution. The absorbance (O.D.) at 405 nm was monitored every 10 seconds at 30°C for 10 minutes, and the reaction velocities (O.D./min) were calculated from a slope of the absorbance curve.

**Concentration design:**

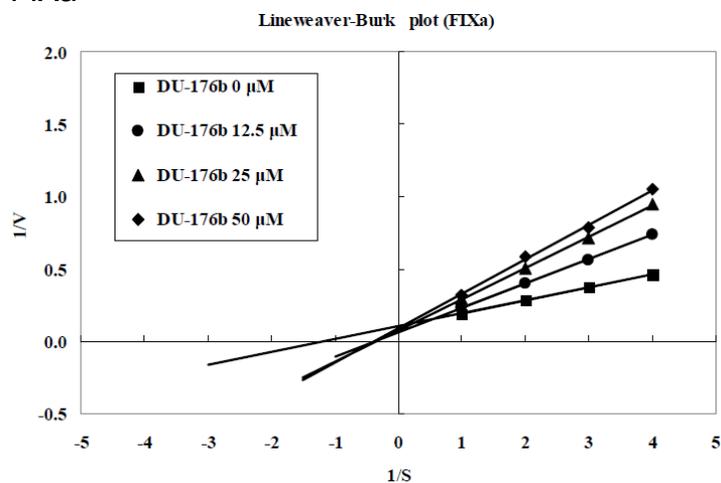
| Serine proteases | Concentrations of enzymes | Substrates       | Concentrations of substrates (mM) | Concentrations of DU-176b ( $\mu$ M) |
|------------------|---------------------------|------------------|-----------------------------------|--------------------------------------|
| FIXa $\beta$     | 6.25 U/mL                 | Spectrozyme fIXa | 0.25, 0.33, 0.50, 1.00            | 0, 12.5, 25, 50                      |
| FXIa             | 0.25 nM                   | S-2366           | 0.25, 0.33, 0.50, 1.00            | 0, 100, 150, 200                     |
| aPC              | 2.5 nM                    | S-2366           | 0.25, 0.33, 0.50, 1.00            | 0, 100, 150, 200                     |

**Results:**

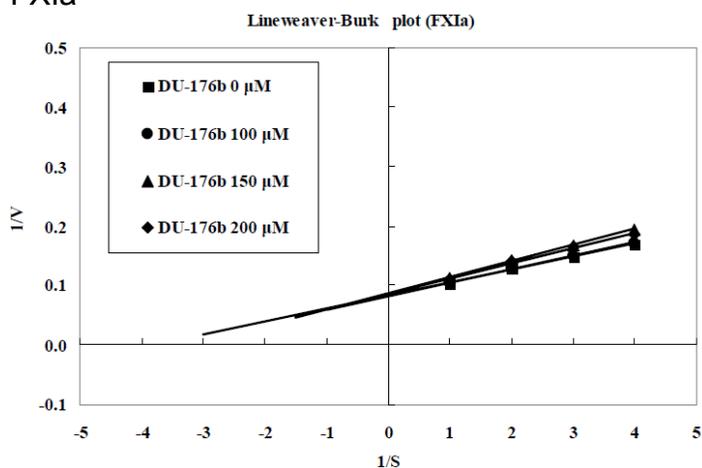
Lineweaver-Burk plots of the amidolytic activity of 6.25 U/mL FIXa, 0.25 nM FXIa, or 2.5 nM aPC against a chromogenic substrate in the absence and presence of edoxaban were depicted in the following figures (figures from the Applicant). Data are mean of triplicate measurements.

Figure 2- Kinetic analysis of the inhibitory effect of edoxaban

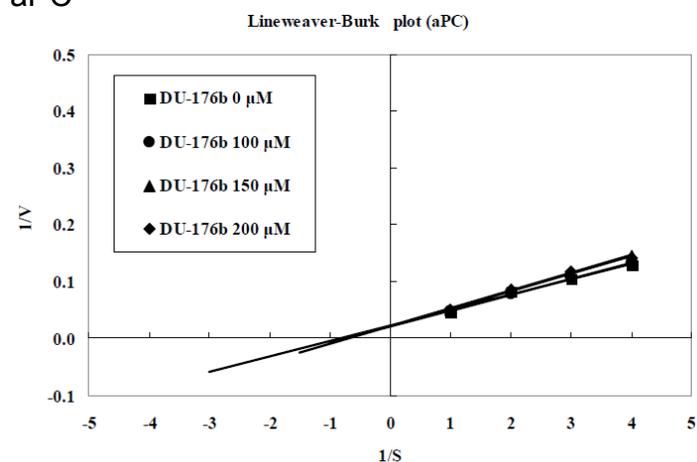
FIXa



## FXIa



## aPC



The  $K_i$  (inhibition constant) values derived from the kinetic studies were:

- 41.7  $\mu\text{M}$  for FIXa
- >100  $\mu\text{M}$  for FXIa
- > 100  $\mu\text{M}$  for and aPC.

The  $K_i$  value of edoxaban for FXa was 0.561 nM (see Study # 20020850, above). Thus, the  $K_i$  values for FIXa, FXIa, and aPC were more than 70,000-times higher than that for FXa.

**Study title:** DU-176b and (b) (4) pharmacogenomics (Study # AD07-H0067-R01, Module 4)

*Reviewer's note:* the part of the study report regarding (b) (4)

**Key finding:**

Edoxaban exhibited comparable anticoagulant activity toward wild type and two mutants of FXa.

Methods

In this study, the effect of two different mutations (amino acid substitutions in the Factor X (FX) gene): 1) Ala112Thr (A112T) or 2) Gly152Arg (G152R), on the FXa inhibitory properties of DU-176b was determined. The mutant FXs were prepared by site-directed mutagenesis in the pCMV4-ss-pro-II-hFX plasmid. The plasmids were then transformed into competent cells for larger scale plasmid production. Each FX plasmid, i.e., pCMV-Script-ss-pro-II-hFX-WT, pCMV-Script-ss-pro-II-hFX-A112T or pCMV-Script-ss-pro-II-hFX-G152R, was transfected into human embryonic kidney (HEK) 293 cells to obtain recombinant FXs. Both wild type and mutant factor X were activated by RVVX, the factor X activating enzyme isolated from Russell's viper venom.

The anticlotting activities of edoxaban (DU-176b, Lot# FC208) were examined by measuring the plasma clotting time, prothrombin time (PT) and activated partial thromboplastin time (aPTT) using recombinant FXs, with or without the mutations A112T or G152R, mixed with FX-deficient plasma. Also, the inhibitory effects of edoxaban on FXas with or without a mutation were assessed by measuring the amidolytic activity of recombinant FXas in the presence of edoxaban and calculating the inhibition constants (Ki value). The methods of clotting time, CT2, and Ki value determination are described as above. The recombinant wild type or mutant factor Xs were added to human factor X-deficient plasma and spiked with edoxaban (0.01  $\mu$ M to 1.0  $\mu$ M, n = 3 each). PT and APTT were measured and the respective CT2 values were calculated.

Results:

Table 4- Inhibitory effects on factor Xa and anticoagulant activities of edoxaban in wild type and mutant FXas

| FactorXa  | Ki (nM) | PTCT2 ( $\mu$ M) | APTTCT2 ( $\mu$ M) |
|-----------|---------|------------------|--------------------|
| Wild type | 0.89    | 0.12             | 0.5                |
| Ala112Thr | 0.85    | 0.12             | 0.45               |
| Gly152Arg | 1.1     | 0.11             | 0.46               |

**Study title: Factor Xa inhibitory activities and anticoagulant effects of metabolites of DU-176b (Study # GB11-H0026-R1, Module 4)**

**Study title: Factor Xa inhibitory activity and anticoagulant effect of D21-2393, a metabolite of DU-176b (Study # AD07-H0070-R01, Module 4)**

**Key findings:**

The results indicated that D21-1402-0201, D21-2135-0101, D21-2393 were active metabolites of edoxaban (DU-176b), but D21-3231-0101 was an inactive metabolite.

Methods:

The factor Xa inhibitory effects and anticoagulant activities of D21-2393, a human specific metabolite, and other metabolites, D21-1402-0201, D21-2135-0101, and D21-

3231-0101, of edoxaban, were studied *in vitro* and compared to those of the parent compound. The measurement of antiamidolytic activity of factor Xa and anticoagulant activity (clotting times and CT2) are described above, under Study # 20020850.

Results:

Anti-factor Xa activity

Parent drug edoxaban (DU-176b) and its metabolites (including D21-1402-0201, D21-2135-0101, and D21-2393) inhibited human and rat FXa activity. The 50% inhibition concentration (IC<sub>50</sub>) values are summarized below. (Tables from the Applicant, Pharmacology written summary, Module 2)

Table 5- Inhibitory effects of metabolites (D21-1402-0201, D21-2135-0101, D21-3231-0101, and D21-2393), and edoxaban on factor Xa

| Compounds                 | Human                            |                       | Rat                              |                       |
|---------------------------|----------------------------------|-----------------------|----------------------------------|-----------------------|
|                           | Concentration (nM) <sup>a)</sup> | IC <sub>50</sub> (nM) | Concentration (nM) <sup>a)</sup> | IC <sub>50</sub> (nM) |
| D21-1402-0201             | 2.5 to 20 <sup>b)</sup>          | 6.9                   | 10 to 80 <sup>b)</sup>           | 28                    |
| D21-2135-0101             | 0.625 to 5 <sup>b)</sup>         | 2.7                   | 5 to 40 <sup>b)</sup>            | 14                    |
| D21-3231-0101             | 1000 <sup>b)</sup>               | > 1000                | 1000 <sup>b)</sup>               | > 1000                |
| D21-2393                  | 0.625 to 5 <sup>b)</sup>         | 1.8                   | 5 to 40 <sup>b)</sup>            | 10                    |
| Edoxaban tosilate hydrate | 0.625 to 5 <sup>b)</sup>         | 3.0                   | 5 to 40 <sup>b)</sup>            | 13                    |

a: n = 2 or 3, b: 0.2% dimethyl sulfoxide (DMSO) for control

The Ki value was determined for metabolite D21-2393 in human and rat plasma; see Table below.

Table 6- Inhibitory Effects of D21-2393 and edoxaban on factor Xa

| Species | Ki (nM)  |   |
|---------|----------|---|
|         | D21-2393 | Edoxaban tosilate hydrate <sup>a)</sup> |
| Human   | 0.767    | 0.561                                   |
| Rat     | 6.88     | 6.98                                    |

Anticoagulant activity:

Tables are from the Applicant (Pharmacology written summary, Module 2, Section 2.6.2.2.4.2).

Table 7- Anticoagulant activities of metabolites (D21-1402-0201, D21-2135-0101, D21-3231-0101, and D21-2393) and edoxaban

| Compounds                 | Human   |                         | Rat   |                         |
|---------------------------|---|-------------------------|---|-------------------------|
|                           | Concentration ( $\mu\text{M}$ ) <sup>a)</sup> | PTCT2 ( $\mu\text{M}$ ) | Concentration ( $\mu\text{M}$ ) <sup>a)</sup> | PTCT2 ( $\mu\text{M}$ ) |
| D21-1402-0201             | 0.125 to 1 <sup>b)</sup>                      | 0.66                    | 0.25 to 2 <sup>b)</sup>                       | 1.6                     |
| D21-2135-0101             | 0.0625 to 0.5 <sup>b)</sup>                   | 0.26                    | 0.125 to 1 <sup>b)</sup>                      | 0.67                    |
| D21-3231-0101             | 5 <sup>b)</sup>                               | > 5                     | 5 <sup>b)</sup>                               | > 5                     |
| D21-2393                  | 0.0625 to 0.5 <sup>b)</sup>                   | 0.28                    | 0.125 to 1 <sup>b)</sup>                      | 0.80                    |
| Edoxaban tosilate hydrate | 0.0625 to 0.5 <sup>b)</sup>                   | 0.23                    | 0.125 to 1 <sup>b)</sup>                      | 0.57                    |

A similar result was obtained in Study AD07-H0070-R01, when D 21-2393 was tested:

| Species | PTCT2 ( $\mu\text{M}$ ) |   | APTTCT2 ( $\mu\text{M}$ ) |   |
|---------|-------------------------|---|---------------------------|---|
|         | D21-2393                | Edoxaban tosilate hydrate <sup>a)</sup> | D21-2393                  | Edoxaban tosilate hydrate <sup>a)</sup> |
| Human   | 0.258                   | 0.256                                   | 0.811                     | 0.508                                   |
| Rat     | 0.898                   | 0.647                                   | 3.63                      | ND                                      |

**Study title: DU-176b: inhibitory effect of DU-176b on the activities of prothrombinase complex (Study # 20060456, Module 4)**

**Key finding:**

Edoxaban acted as an inhibitor of both free FXa and FXa in a prothrombinase complex. When a chromogenic substrate was used the  $K_i$  value was 0.9 nM and when prothrombin substrate was used in the assay  $K_i$  was 3 nM. The  $K_i$  for FXa was previously determined to be 0.561 nM.

Prothrombinase was reconstituted with human factor Xa (FXa), human factor Va, phosphatidylcholine/phosphatidylserine (PC/PS) liposome, and  $\text{CaCl}_2$  to form the prothrombinase complex. The competitive inhibition of edoxaban on the substrate S-2222 cleavage was investigated in the incubation of prothrombinase complex, the substrate and edoxaban.

**Methods:**

The objective of this study was to demonstrate that edoxaban exerted inhibitory effects not only on free FXa but also FXa incorporated in the prothrombinase complex. In brief, edoxaban (DU-176b) at different concentrations or control (5% DMSO) was mixed with the enzyme solution (containing FXa, FVa, PC/PS and  $\text{CaCl}_2$ ). The substrate at various concentrations, either S-2238 or prothrombin, was then added and the mixture was incubated. When prothrombin was used as the substrate, the incubation

was stopped by the addition of EDTA, and then S-2238 was added to the mixture. The thrombin activity was measured by monitoring the absorbance (O.D.) at 405 nm.

Concentration design: diagram from the study report

| Prothrombinase complex                      | Concentrations of prothrombinase complex constituents                         | Substrates  | Concentrations of substrates ( $\mu\text{M}$ ) | Concentrations of DU-176b (nM) |
|---|---|-------------|--|--------------------------------|
| In the case of S-2222 as the substrate      | FXa 0.4 nM<br>FVa 10 nM<br>PC/PS 25 $\mu\text{M}$<br>CaCl <sub>2</sub> 2.5 mM | S-2222      | 250, 333,<br>500, 1000                         | 0*, 0.4, 0.8, 1.2              |
| In the case of prothrombin as the substrate | FXa 0.2 pM<br>FVa 10 nM<br>PC/PS 25 $\mu\text{M}$<br>CaCl <sub>2</sub> 2.5 mM | Prothrombin | 0.0078, 0.0156,<br>0.0625, 0.25                | 0*, 2, 4, 6                    |

\*: 5% DMSO solution

**In this report, concentrations of DU-176b (edoxaban) were expressed as the amount of anhydrous freebase (conversion factor: 0.7424).**

The reaction velocities (O.D./min) were calculated from a slope of the absorbance curve. Thrombin concentration in the reaction mixture was determined from a standard curve prepared from O.D./min by known concentrations of thrombin. The rate of thrombin generation (nM/min) during incubation for 3 min was calculated based on the thrombin concentration.

### Results:

- Synthetic chromogenic substrate S-2222:

Edoxaban exerted a dose-dependent and competitive inhibition of S-2222 cleavage by the prothrombinase complex, with a  $K_i$  value of 0.903 nM (data not shown). Based on the result obtained in Study # #20020850, edoxaban competitively inhibited free FXa with a  $K_i$  value of 0.561 nM (see above). Thus, edoxaban appeared to be a competitive inhibitor of S-2222 cleavage by both free FXa and prothrombinase complex with nearly comparable  $K_i$  values.

- Physiological substrate prothrombin:

Edoxaban also exerted an inhibitory effect on prothrombinase complex using prothrombin as the substrate, with a  $K_i$  value of 2.98 nM (data not shown). The  $K_i$  value of edoxaban for the inhibition of prothrombinase activity in prothrombin cleavage is approximately 5 fold higher than that for S-2222 cleavage by FXa (2.98 nM versus 0.561 nM, respectively).

### **Animal models** (Studies all from Module 4, Section 4.2.1.1)

Rat arterio-venous (A-V) shunt model, described below.

### **Key finding** (from studies 20020847, 20030369, 20020848, 20020849):

Edoxaban had anticoagulant activity in animals as demonstrated by reduction in thrombus weight and anti-FXa activity.

**Study title: D11-4176b: Anti-thrombotic effect of D11-4176b in a rat A-V shunt model (Study # 20020847)**

Male Wistar rats were treated via oral gavage with edoxaban (D11-4176b) (0.5, 2.5 and 12.5 mg/kg in 0.5% methyl cellulose solution, 0.5% MC) or the control vehicle (0.5% MC). The shunt was constructed between the left carotid artery and the right jugular vein 30 minutes following the treatment of edoxaban. The thrombi formed in the shunt were measured quantitatively (as the protein content in the thrombus) and the levels of edoxaban in the plasma were also determined (HPLC-mass). The anti-FXa activity of edoxaban, expressed as the anti-amidolytic activity of the drug, was measured using S-2222 as a substrate. When the amidolytic activity of FXa was inhibited, less S-2222 was in the assay system and hence a reduced absorption (at 405 nm, expressed as mOD/min) in the collected plasma samples (according to the method of Bradford; Biochem. 72: 248-254, 1976). Results were expressed as amidolytic activity of human FXa. In addition, prothrombin time (PT) was measured.

Rat venous stasis model**Study title: DU-176b: Anti-thrombotic effect of DU-176b in a rat venous stasis model (Study # 20030369)**

The anti-thrombotic effect of oral edoxaban (DU-176b) was also evaluated in a venous stasis model in rats. The treatment and doses of edoxaban are described above. Venous stasis was performed 30 minutes following edoxaban treatment, by ligating tightly the inferior vena cava and bigger branches with a cotton thread below the left renal vein, and after ten minutes the inferior vena cava was ligated 1.5 cm below the first ligature. Thrombi were removed 60 minutes after ligation and the thrombus weight was measured. Anti-FXa activity, plasma concentration of edoxaban and PT were also determined (see above).

Rat thrombosis model**Study title: D11-4176b: Anti-thrombotic effect of D11-4176b in a rat venous thrombosis model (Study # 20020848)**

Treatments with edoxaban and vehicle control are described above. The doses of edoxaban were 0.1, 0.5 and 2.5 mg/kg. Thrombosis formation was induced 30 minutes following edoxaban treatment, by inserting a platinum wire sharpened at one tip by 2 cm into the abdominal vein below the renal vessel and the wire was left for 60 minutes. One mL of 1% glutaraldehyde in 10 mM phosphate buffered saline (PBS: pH 7.3) was injected into the lower vena cava to fix the thrombus in situ followed by injection of 1 mL of 3.13 % sodium citrate to block thrombus formation afterward. The wire was dissected free immediately and placed in glutaraldehyde in 10 mM PBS until weighing. The weight of the thrombus with wire was noted. Each wire was wiped free of thrombus and re-weighted to obtain the thrombus weight

Rat tissue factor induced disseminated intravascular coagulation (DIC) model**Study title: D11-4176b: Anti-thrombotic effect of D11-4176b in a rat tissue factor induced DIC model (Study # 20020849)**

Treatments with edoxaban and vehicle control are described above. The doses of edoxaban were 0.1, 0.5 and 2.5 mg/kg. Tissue factor (Thromboplastin C Plus) (Dade Behring, Liederbach, Germany) (0.8 U/mL, in saline) was infused into the rat femoral vein for 1 minute at a dose of 3.1 mL/kg. Two groups of male rats were designated as the control: sham control (administered with 0.5% MC and IV infusion with saline only), and vehicle control (treated with 0.5% MC and received tissue factor infusion).

The following assessment endpoints were determined: Platelet number, concentrations of TAT\*, fibrinogen and D11-4176 in rat plasma, S-2222 amidolytic activity in rat plasma.

\*TAT: thrombin-antithrombin complex; the rat plasma TAT levels were determined by an enzyme immunoassay via Enzygnost® TAT micro.

### Results

Intravenous tissue factor treatment significantly reduced the platelet number from  $64.6 \times 10^4$  (mean  $\pm$  standard error) to  $39.8 \times 10^4$  platelets/ $\mu$ L. Tissue factor also significantly increased the concentration of TAT in rat plasma to 290.5 ng/mL compared to that of the sham group (3.4 ng/mL). In addition, the concentration of fibrinogen in rat plasma was reduced significantly by tissue factor from 202.2 to 124.4 mg/dL.

### Summary of results of four anti-thrombosis studies:

|                                 | Anti-thrombotic effect*  | Anti-FXa effect (amidolytic activity)**  | PT prolongation                          | Edoxaban plasma concentration   |
|---------------------------------|--|--|--|---|
| A-V shunt model                 | Protein content in thrombus↓ : 59% and 47% of the control at 2.5 and 12.5 mg/kg  | ↓ Absorption from 7.2 (control) to 1.0 and 0.5 mOD/min at doses of 2.5 and 12.5 mg/kg  | 18 sec (control) to 21 sec at 12.5 mg/kg | 21, 172 and 396 ng/mL at 0.5, 2.5 and 12.5 mg/kg<br>Not linear, rise at 2.5 mg/kg     |
| Venous stasis                   | Thrombus weight↓: 43%, 16% and 13% of the control at 0.5, 2.5 and 12.5 mg/kg   | ↓ Absorption from 13.8 (control) to 0.8 and 0.3 mOD/min at doses of 2.5 and 12.5 mg/kg | 18 sec (control) to 24 sec at 12.5 mg/kg | 21, 194 and 449 ng/mL at 0.5, 2.5 and 12.5 mg/kg<br>Not linear, rise at 2.5 mg/kg     |
| Thrombosis model                | Thrombus weight↓: 30% of the control at 2.5 mg/kg  | ↓ Absorption from 7.6 (control) to 6.4 and 2.2 mOD/min at doses of 0.5 and 2.5 mg/kg   | Not remarkable                           | At 2.5 mg/kg: 188 ng/mL and 106 ng/mL at 30 min and 90 min after dosing, respectively |
| Tissue factor induced DIC model | <ul style="list-style-type: none"> <li>• Restoration of platelet counts reduced by tissue factor.at <math>\geq</math> 0.1 mg/kg.</li> <li>• Decreases in TAT levels at <math>\geq</math> 0.1 mg/kg.</li> <li>• Increases in fibrinogen reduced by tissue factor.at <math>\geq</math> 0.1 mg/kg.</li> </ul> | ↓ Absorption from 8.7 (control) to 6.1 and 1.5 mOD/min at doses of 0.5 and 2.5 mg/kg   | Not conducted                            | <5, 16 and 163 ng/mL at 0.1, 0.5 and 2.5 mg/kg<br>Greater than unity.                 |

\*Dose-dependent and statistically significant

\*\* Expressed as the reduction in the absorption (mOD/min) at 405 nm. (See "model" under Study #20020847)

Comparisons of various antithrombotic agents in the venous thrombosis model in rats:

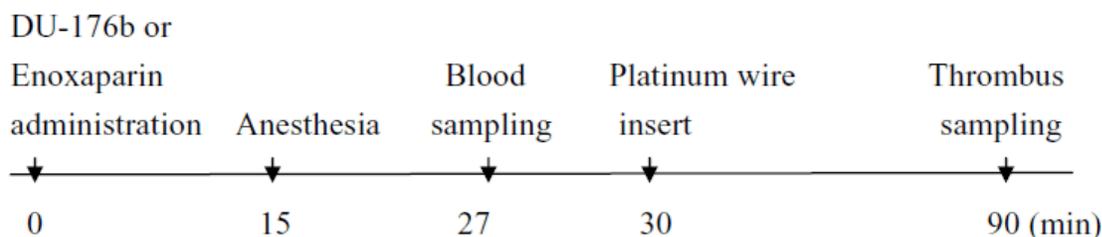
The antithrombotic effects of edoxaban were compared with other anticoagulants in a rat venous thrombosis model. Comparators were enoxaparin and fondaparinux (Arixtra), drugs approved for the treatment of acute deep vein thrombosis and pulmonary embolism. The following studies are summarized and discussed together.

**Study title: Antithrombotic effects of DU-176b and enoxaparin in a rat model of venous thrombosis (Study # AD09-H0007-R01, Module 4).****Study title: Treatment of venous thrombosis with DU-176b, enoxaparin and fondaparinux in rats (Study # BG11-H0012-R01)**Methods

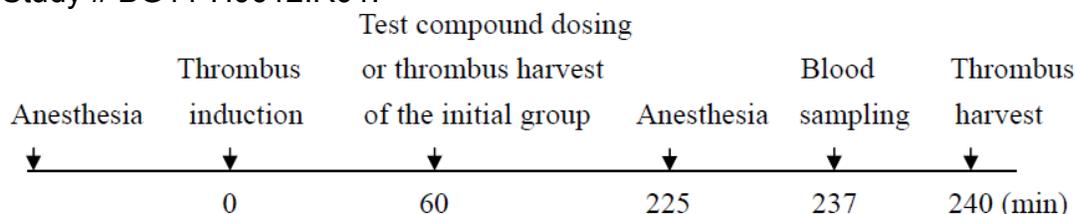
In the rat venous thrombosis model, venous thrombosis in the inferior vena cava was induced by partial stenosis (method, see above) with or without topical application of 10% ferric chloride solution. After 1-h thrombus maturation, the thrombus wet weights of the treated and control groups were measured.

Experiment diagrams:

## Study # AD09-H0007.R01:



## Study # BG11-H0012.R01:



## Study result summary of the two studies: the data represent group means (n=8)

| Agents  | Thrombus weight (µg)                     | PT prolongation (seconds)   | APTT prolongation (second) |
|---|--|-----------------------------|----------------------------|
| Study # AD09-H0007-R01                                |  |                             |                            |
| Edoxaban: 0, 1, 3, and 10 mg/kg (oral)                | 128, 87* (32%), 86* (33%), and 49* (61%) | 18.4, 17.8, 18.8 and 23.1*  | 19.0, 18.2, 19.2 and 18.3  |
| Enoxaparin: 0, 100, 300 and 1000 IU/kg (subcutaneous) | 167, 139 (19%), 112 (33%), and 46* (73%) | 18.6, 18.5, 19.3, and 20.3* | 19.1, 21, 24.9* and 44.8*  |
| Study # BG11-H0012-R01                                |  |                             |                            |
| Vehicle: 0.5w/v% methyl cellulose 400 solution        |  |                             |                            |

|   |  |                            |                           |
|---|--|----------------------------|---------------------------|
| Edoxaban: 0, 1, 3, and 10 mg/kg (oral)                  | 13.7, 10.5 (24%), 7 (49%), and 7 (49%)   | 18.9, 18.9, 19.6, and 24.4 | Not conducted             |
| Enoxaparin: 0, 300, 1000, and 2000 IU/kg (subcutaneous) | 12, 7* (42%), 4.3* (64%) and 6* (50%)    | Not conducted              | 22.2, 29.9, 81*, and 122* |
| Fondaparinux: 0, 0.3, 1 and 3 mg/kg (subcutaneous)      | 13.2, 6.6 (50%), 5.6 (58%) and 3.6 (73%) | 19.8, 20.2, 20.4 and 20.4  | 24.9, 25.3, 25.1 and 25.1 |

\*Statistically significant changes; data in the parenthesis: % inhibition (from the control)

Vehicle: 0.5w/v% methyl cellulose 400 solution

Edoxaban (DU176b): Lot # MH414A

### **Conclusion:**

- Study # AD09-H0007-R01

Orally administered edoxaban in rats, up to 10 mg/kg, induced a dose-dependent prevention of venous thrombus formation (as shown by reduction in protein content of thrombus) and prolonged prothrombin time, but did not affect the APTT values. On the other hand, low molecular weight heparin, enoxaparin (up to 1000 IU/kg, administered via intravenous injection), exerted dose-dependent anti-thrombotic activity as well as clotting time prolongation (both PT and APTT). Edoxaban prolonged clotting times, indicating inhibition of fibrin formation and anticoagulant activity. The different effects in clotting times (especially APTT) observed in edoxaban and enoxaparin may be due to different anti-thrombosis mechanisms: edoxaban as a direct FXa inhibitor, while enoxaparin acts via antithrombin-dependent inhibition of FXa and thrombin.

- Study # BG11-H0012-R01

The three antithrombotic agents, edoxaban, enoxaparin and fondaparinux exhibited dose-dependent prevention in venous thrombosis in rats. Unlike the effect of edoxaban and enoxaparin on PT or APTT prolongation, fondaparinux, an antithrombin-dependent FXa inhibitor, did not have effects on clotting times under the conditions of the study.

## **4.2 Secondary Pharmacology**

### **In vitro studies**

#### **Studies conducted to show Effects on platelet aggregation**

**Study title: DU-176b: Effects of DU-176b on human platelet aggregation (Study title # 20060958, Module 4)**

#### **Key finding:**

Edoxaban (DU-176b) inhibited platelet aggregation only when aggregation was induced by thrombin. No significant inhibitory effect was noted when platelet aggregation was induced by collagen, U46619 (a synthetic thromboxane A<sub>2</sub> receptor agonist), or ADP.

#### **Methods:**

This study was conducted to investigate whether edoxaban has inhibitory activity through the blood coagulation pathway (i.e. by inhibiting thrombin-induced aggregation) or whether it can also inhibit other mechanisms of platelet aggregation (i.e. by inhibiting collagen-, U46619-, and ADP-induced platelet aggregation).

Human blood samples were collected from healthy volunteers. Several test samples were prepared from the whole blood: platelet rich plasma (PRP), platelet poor plasma (PPP), washed platelet suspension (WP). The IC<sub>50</sub> values were obtained for collagen-,

U46619-, and ADP-induced platelet aggregation (in platelet rich plasma). IC<sub>50</sub> values were also obtained for thrombin-induced platelet aggregation (in washed platelet suspension).

- Test article: Edoxaban (DU-176b, Lot #FC208), 0.01, 1 and 100 μM (when collagen, U46619 or ADP were used for platelet aggregation); 0.1, 1 and 10 μM (when thrombin was used for platelet aggregation)
- Positive control: Abciximab (an anti-glycoprotein IIb/IIIa antibody), 0.2 and 20 μg/mL
- Negative/vehicle control: DMSO

Note: collagen, U46619 (synthetic thromboxane A<sub>2</sub> receptor agonist), ADP cause platelet aggregation through calcium flux and platelet shape changes, while thrombin is involved in the prothrombinase-FXa complex formation.

#### Procedure:

Edoxaban, abciximab or vehicle was incubated with the plasma; then the platelet aggregation agonist was added to the mixture and the extent of aggregation was measured. The light transmission of platelet rich plasma or washed platelet suspension increased with the formation of platelet aggregates in the plasma samples. The extent of aggregation was estimated by the percentage of maximum increase in light transmission, with the PPP representing 100% transmittance. The concentration of the agonist was set such that >60% platelet aggregation will be induced.

#### Results:

- Collagen, U46619 and ADP induced platelet aggregation in PRP:  
DU-176b, at concentrations up to 100 μM, did not inhibit platelet aggregation induced by collagen, U46619, or ADP. Thus the IC<sub>50</sub> under the study condition was greater than 100 μM.
- Thrombin induced platelet aggregation in WP suspension:  
DU-176b inhibited thrombin-induced WP aggregation with mean IC<sub>50</sub> value of 2.90 μM (95% CI 1.99-3.81 μM).

### **Studies conducted to evaluate effects of hemostatic agents to reverse the anti-coagulation activity of edoxaban**

#### Background:

The following agents were used to address reversibility of edoxaban-induced anticoagulation:

- Recombinant human factor VIIa (rFVIIa) (NovoSeven, NJ):  
By enhancing thrombin generation, rFVIIa helps to form stable fibrin plugs, so that rFVIIa maintains hemostasis in the absence of factor VIII or factor IX.
- FEIBA:  
FEIBA is a plasma-derived, activated prothrombin complex concentrate (aPCC). The major components of FEIBA include FVII, IX, X, and activated FVII and FX and prothrombin. It has been used to control bleeding in patients with factor VIII or IX

inhibitors. It is postulated that when FEIBA is introduced to a rabbit model of bleeding, it would trigger the FXa binding to the surface of activated platelets at the site of bleeding. Prothrombin and FXa would convert transfused and endogenous prothrombin directly into thrombin, bypassing the need for FVIII and FIX (Wilde, Pathophysiol Hemost Thromb 32 (Suppl 1): 9-12, 2002).

- PPSB-HT

PPSB-HT is a prothrombin complex concentrate (PCC). PPSB-HT contains non-activated vitamin K-dependent coagulation factors and heparin, and is used to treat patients with hemophilia B.

#### Experiment procedures for Study # 20030655 and AD09-H0016-R01

Edoxaban (at final concentrations of 0, 150, and 300 or 450 ng/mL in the reaction mixture) and recombinant factor VIIa (for concentrations in the reaction mixture, see tables below) were mixed in pooled human plasma obtained from adult healthy volunteers. The prothrombin time (PT) was measured after addition of Thromboplastin C Plus (a thromboplastin agent, catalyzing the conversion of prothrombin to thrombin) or HemosIL PT-fibrinogen HS PLUS at 37 °C.

Edoxaban and rFVIIa, FEIBA or PPSB-HT were incubated together before the addition of thromboplastin agent.

**Study title: DU-176b: Effect of recombinant factor VIIa on prothrombin time prolonged by DU-176b (Study # 20030655, Module 4).**

#### **Key finding:**

Under the conditions tested, Factor VIIa reversed the anticoagulation activity of edoxaban, such as edoxaban-induced PT prolongation. No conclusion can be made on the antidote effect of Factor VIIa due to limitations of an in vitro study.

The table is from the Applicant (Module 2, Section 2.6.2.3.3, Pharmacology written summary, Table 3.4) (data verified by the reviewer).

Table 8- Reversal effect of recombinant factor VIIa on PT prolonged by edoxaban

| Edoxaban tosilate hydrate (ng/mL) | rFactor VIIa (ng/mL) | PT (s)                   |
|-----------------------------------|----------------------|--------------------------|
| 0                                 | 0                    | 13.9 ± 0.3               |
| 0                                 | 5                    | 12.9 ± 0.3 <sup>a)</sup> |
| 0                                 | 50                   | 10.4 ± 0.2 <sup>a)</sup> |
| 0                                 | 500                  | 8.6 ± 0.3 <sup>a)</sup>  |
| 150                               | 0                    | 27.0 ± 1.5 <sup>b)</sup> |
| 150                               | 5                    | 24.2 ± 1.4 <sup>c)</sup> |
| 150                               | 50                   | 18.2 ± 1.3 <sup>c)</sup> |
| 150                               | 500                  | 14.8 ± 0.8 <sup>c)</sup> |
| Edoxaban tosilate hydrate (ng/mL) | rFactor VIIa (ng/mL) | PT (s)                   |
| 0                                 | 0                    | 13.7 ± 0.2               |
| 0                                 | 5                    | 13.0 ± 0.2 <sup>a)</sup> |
| 0                                 | 50                   | 11.0 ± 0.3 <sup>a)</sup> |
| 0                                 | 500                  | 8.4 ± 0.2 <sup>a)</sup>  |
| 450                               | 0                    | 46.0 ± 1.5 <sup>b)</sup> |
| 450                               | 5                    | 43.2 ± 2.4 <sup>c)</sup> |
| 450                               | 50                   | 33.2 ± 1.7 <sup>c)</sup> |
| 450                               | 500                  | 24.4 ± 1.1 <sup>c)</sup> |

rFactor VIIa: recombinant activated coagulation factor VII, PT: prothrombin time

Data represent mean ± standard deviation, n = 6/group

a:  $P < 0.05$  compared to the control group (Dunnett's multiple comparison test)

b:  $P < 0.05$  compared to the control group (Aspin-Welch t test)

c:  $P < 0.05$  compared to the edoxaban alone group (Dunnett's multiple comparison test)

**Study title: Effects of anti-inhibitor coagulant complex, prothrombin complex concentrate and recombinant factor VIIa on prothrombin time prolonged by DU-176 (Study #AD09-H0016-R01, Module 4)**

### Key findings:

The anticoagulant activities of edoxaban, such as PT prolongation, were reversed by FEIBATM, PPSB-HT and recombinant factor VIIa.

### Methods

In brief, prothrombin time (PT) was measured in the presence or absence of edoxaban (DU-176b, 150 and 300 ng/mL) in combination with FEIBA (0.15, 0.5 and 1.5 U/mL), prothrombin complex concentrate (PPSB-HT, 0.15, 0.5 and 1.5 U/mL), or rFVIIa (100, 300 and 1000 ng/mL). Saline was as a negative control.

### Results:

Edoxaban alone prolonged PT, while FEIBA, PPSB-HT or rFVIIa alone shortened PT. PT prolongation induced by edoxaban was shortened by FEIBA, PPSB-HT or rFVIIa in a concentration-dependent manner (data not shown). Based on the study results, the anticoagulant effect of edoxaban, such as PT prolongation, could be reversed in vitro by FEIBA, PPSB-HT and rFVIIa.

**Study title: Ex-vivo study to assess the potential of factor eight inhibitor bypass activity (FEIBA; FEIBATM) or activated factor seven (rhFVIIa) to reverse the anticoagulant activity of edoxaban (Study # TMBM040, Module 4)**

**Key findings:**

Under the conditions tested, the biologic activities of edoxaban, such as PT prolongation, were reversed by FEIBA and recombinant factor VIIa (rFVIIa). However, certain activities of edoxaban, like inhibition of intrinsic factor X activity, were not reversed by these agents. No conclusion can be made on the effect of plasma factors in vivo.

Methods

Pooled human plasma was incubated with edoxaban (0 [diluent], 500 and 1000 ng/mL)\* for 1 hour, then FEIBA (0.75, or 1.5 U/mL) or rFVIIa (0.8, or 1.8 µg/mL) was spiked in the same samples for further incubation. Aliquots were removed and centrifuged to separate the plasma layer at 0.25, 0.5, 1, 2, and 4 h during this second incubation. Samples were tested for the following biomarkers: PT, APTT, anti-factor Xa activity (FXa activity was measured by a chromogenic method, expressed in terms of LMWH activity; method described under primary pharmacology studies), thrombin generation activity (TGA)\*\* parameters (lag time [TGA-lag], time to peak [TGA-TTP], peak thrombin generation [TGA-Peak], velocity index [TGA-VI], and endogenous thrombin potential [TGA-ETP]), intrinsic factor X (IFX) activity, anti-thrombin activity, and D-dimer\*\*\* (immune-turbidometric method).

\* According to the Applicant: 500 and 1000 ng/mL were supratherapeutic concentrations of edoxaban. Diluent: deionized water.

\*\* Thrombin generation assay (TGA): the following is excerpted from the study Appendix 1.

- TGA is based on monitoring the fluorescence generated by thrombin cleavage of a fluorogenic substrate over time upon activation of the coagulation cascade. From the changes in fluorescence over time, the concentration of thrombin in the sample can be calculated using the respective thrombin calibration curve. The rate of increase in thrombin concentration over time then allows to calculate generation of thrombin in the sample per minute and to plot this value over time for the whole coagulation process. This then results in the visualization of the different phases of clot formation.
- The endogenous thrombin potential (ETP), defined as the amount of thrombin which can be generated after the in vitro activation of coagulation with tissue factor (as a trigger) and phospholipids (as a platelet substitute) (Hemker *et al.*, Thrombosis and Haemostasis, 70: 617-624, 1993).

\*\*\* D dimer

D dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two cross-linked D fragments of the fibrin protein. Polystyrene particles covalently coated with a monoclonal antibody are aggregated when mixed with samples containing D-dimer. The aggregation is then detected turbidometrically via the increase in turbidity.

Increased levels of D-dimer indicate tendency of thrombosis. D-dimer testing is of clinical use when there is a suspicion of deep venous thrombosis (DVT), pulmonary embolism (PE) or disseminated intravascular coagulation (DIC).<sup>1</sup>

### Results:

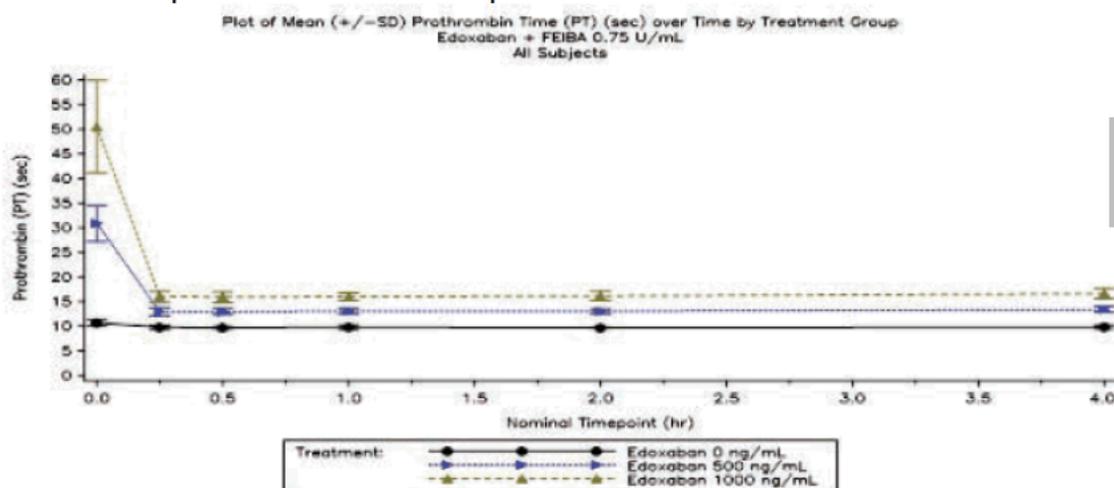
- Biomarkers responding to edoxaban (500 and 1000 ng/mL) treatment that were reversed by hemostatic agents:

Edoxaban prolonged PT and APTT, increased anti-factor Xa activity, and inhibited intrinsic factor X activity and thrombin generation relative to vehicle-treated samples. Such effects were mostly concentration-dependent. FEIBA and rFVIIa, reversed the effect of edoxaban on PT, APTT, and anti-factor Xa activity from 0.25 h to 4 hours. The following figures depict the results (figures from the Applicant, Pharmacology written summary, Module 2, Section 2.6.2.3.5). However, the effect of edoxaban on thrombin generation parameters (such as peak thrombin generation and endogenous thrombin potential) were only partially reversed by FEIBA and rFVIIa, with a gradual onset that plateaued by 4 hours (data not shown).

Figure 3- Reversal of edoxaban induced PT and APTT prolongation and anti-Factor Xa activity by FEIBA and rFVIIa

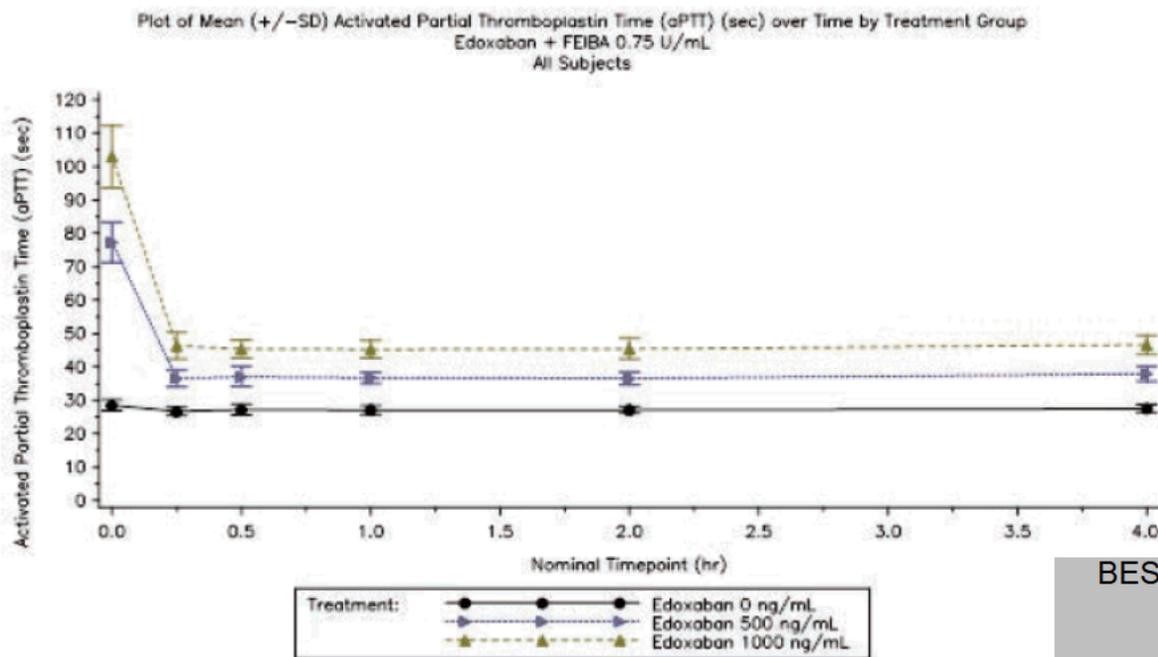
#### Reversal by FEIBA (0.75 U/mL)

PT prolongation: Pooled human plasma was incubated with edoxaban for 1 hour, then FEIBA was spiked in the same samples for further incubation.



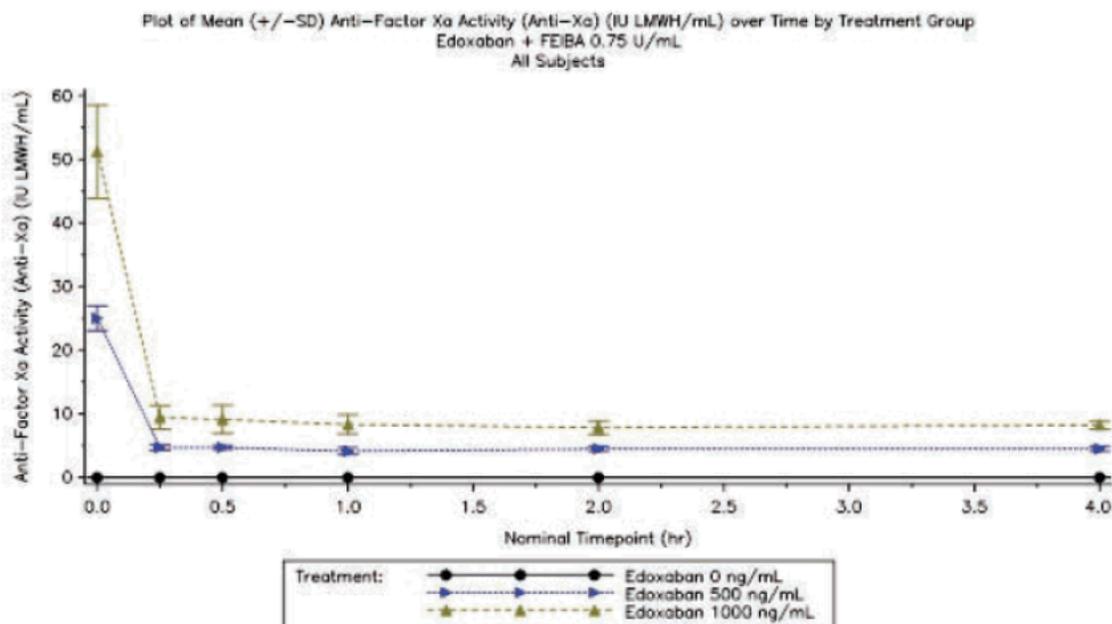
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APTT prolongation: Pooled human plasma was incubated with edoxaban for 1 hour, then FEIBA was spiked in the same samples for further incubation.



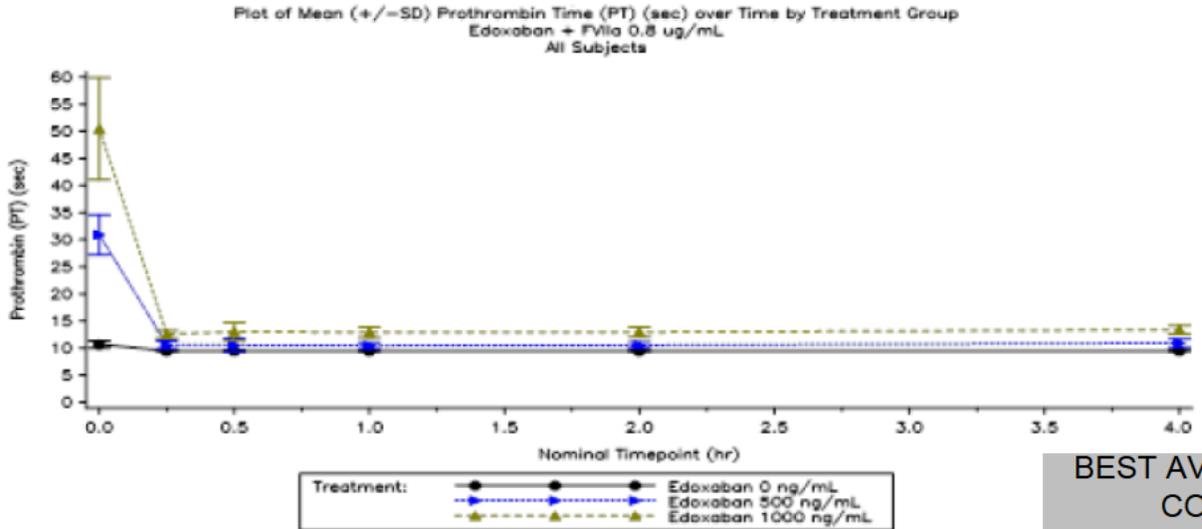
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Anti-Factor Xa activity: Pooled human plasma was incubated with edoxaban for 1 hour, then FEIBA was spiked in the same samples for further incubation.



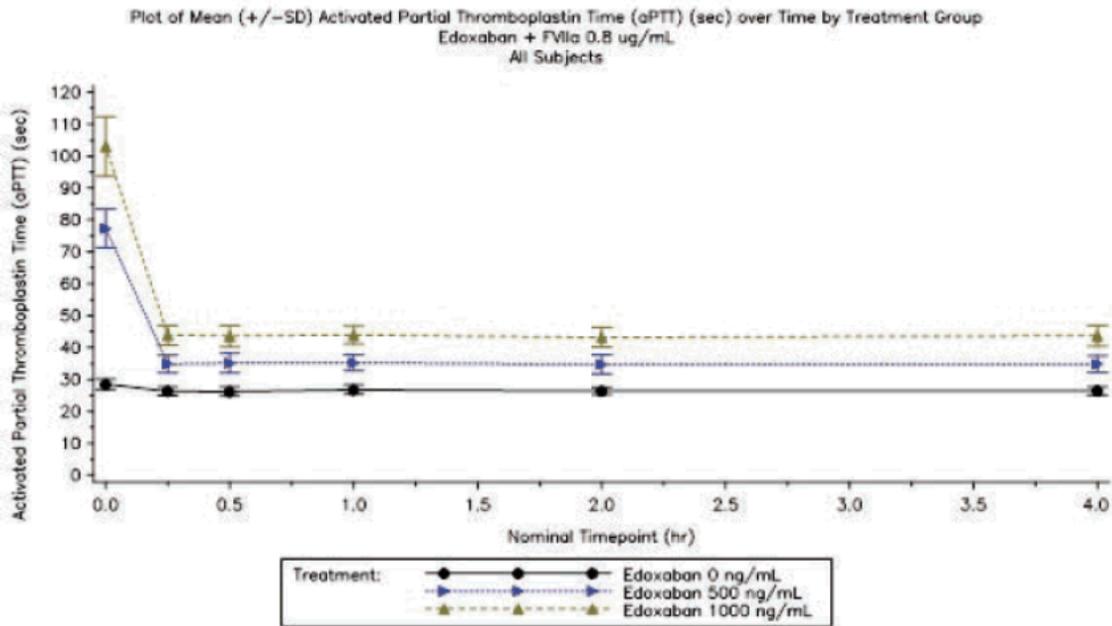
Reversal by rFVIIa (0.8 µg/mL)

PT prolongation: Pooled human plasma was incubated with edoxaban for 1 hour, then rFVIIa was spiked in the same samples for further incubation.

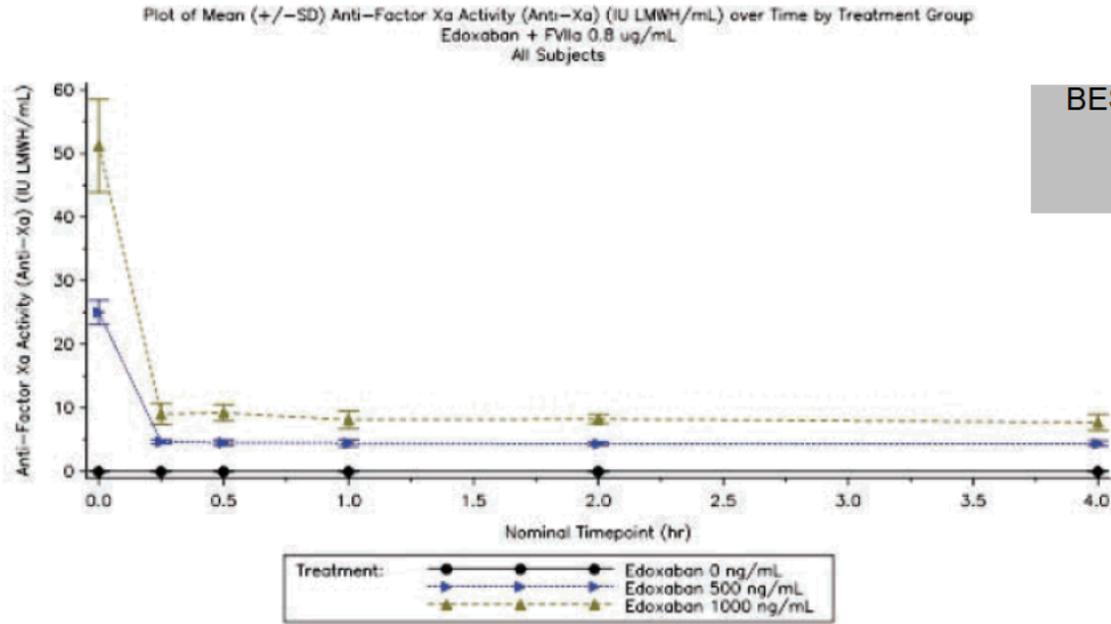


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APTT prolongation: Pooled human plasma was incubated with edoxaban for 1 hour, then rFVIIa was spiked in the same samples for further incubation.



Anti-Factor Xa activity: Pooled human plasma was incubated with edoxaban for 1 hour, then rFVIIa was spiked in the same samples for further incubation.



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- Biomarkers responding to edoxaban (500 and 1000 ng/mL) treatment that were **not** reversed by hemostatic agents:

Edoxaban at 500 and 1000 ng/mL inhibited Factor X activity. Only a relatively small degree of reversal by FEIBA and rFVIIa was observed for inhibition of Factor X activity (< 30%). See figures below, figures from the Applicant. Edoxaban treatment alone did not change levels of D-dimer. There were also no apparent changes in D-dimer levels following treatment with either FEIBA or rFVIIa (data not shown).

Figure 4- Partial reversal of edoxaban inhibition of factor X by FEIBA and rFVIIa

Reversal by FEIBATM (0.75 U/mL): Pooled human plasma was incubated with edoxaban for 1 hour, then FEIBA was spiked in the same samples for further incubation. Factor X activity was expressed as % of control (i.e., absorption of diluent at 405 nm).

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Reversal by FVIIa (0.8  $\mu\text{g}/\text{mL}$ ): Pooled human plasma was incubated with edoxaban for 1 hour, then rfactor VIIa was spiked in the same samples for further incubation.

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Conclusion:

Under the conditions tested, FEIBA (0.75 U/mL) and rFVIIa (0.8  $\mu\text{g}/\text{mL}$ ) showed reversal of edoxaban-induced anticoagulant activity within 4 hours after adding FEIBA or rFVIIa to the mixture of human plasma and edoxaban, based on PT, APTT, and anti-factor Xa activity measured.

**Summary of reversal anticoagulant activity studies:**

Under the conditions tested, reversibility of edoxaban-induced anticoagulation was demonstrated when plasma factors were added to the mixture. Despite this reversibility, a conclusion cannot be made on the antidote effect of plasma factors due to limitations of an in vitro study.

- In the in vitro studies, recombinant FVIIa, FEIBA (a plasma-derived activated prothrombin complex concentrate) or PPSB-HT (a prothrombin complex concentrate) incubated together with edoxaban or added following pre-treatment of the plasma samples with edoxaban, exerted inhibitory effects towards anticoagulant activity of edoxaban. Reversal of edoxaban-induced anticoagulation activity was demonstrated by shortening PT prolongation or APTT prolongation and reversal of anti-FXa activity. However, certain anticoagulant activities of edoxaban, such as anti- intrinsic FX activity, were only partially reversed by these agents.

**In vivo studies****Studies on bleeding effects**

The following two studies are discussed together, because of similar study conditions and objectives.

**Study title: Effects of DU-176b and enoxaparin on bleeding time in a rat tail bleeding model (Study # AD09-H0008-R01, Module 4)**

**Study title: antithrombotic and bleeding prolongation effects of warfarin in rat models of venous thrombosis and tail bleeding (Study # BG10-H0078-R01, Module 4)**

**Key findings:**

- Edoxaban, enoxaparin and warfarin inhibited venous thrombus formation (thrombosis) in the tail bleeding model in rats. The effect was dose-dependent.
- Under the conditions tested, there was a higher ratio of BT2 (estimated dose required to double bleeding time) to ED<sub>50</sub> (therapeutic index) for edoxaban than that for enoxaparin.

The anti-coagulation of edoxaban, enoxaparin (a low molecular weight heparin, which inhibits both FXa and thrombin) and warfarin (vitamin K antagonist) was assessed by the determination of the tail template bleeding time in male Wistar rats.

**Test compounds:**

- Edoxaban (DU-176b, free base; Lot # AE303): 3, 10 and 20 mg/kg  
Vehicle: 0.5w/v% methyl cellulose 400 solution (0.5% MC, Lot No. PEE2201)
- Enoxaparin sodium (Lot # 12003J, content 2000 IU anti-Xa activity in a syringe of 0.2 mL): 800, 1600 and 3200 IU/kg  
Vehicle: saline
- Warfarin (lot # CDQ5092): doses see below  
Vehicle: 0.5w/v% methyl cellulose 400 solution (0.5% MC, Lot No. STR3766)

Treatment:

The solutions (0.30 and 1.0 mg/mL) and suspension (2.0 mg/mL) of DU-176b and vehicle (0.5% MC) were orally administered to rats (n=8/group) at a volume of 10 mL/kg 30 min before the induction of bleeding. The solutions of enoxaparin (160, 320 and 640 IU/mL) and vehicle (saline) were subcutaneously administered to rats (n=8/group) at a volume of 5 mL/kg 30 min before the induction of bleeding (incisions with a blade on the tail). Warfarin was orally administered on Day -4 as loading doses (LD) and Day -3, Day -2, and Day -1 as maintenance doses (MD). The doses of warfarin (LD + MD) were 0.30 mg/kg + 0.10 mg/kg, 0.45 mg/kg + 0.15 mg/kg and 0.60 mg/kg + 0.20 mg/kg (n=8/group)

Twenty-seven min after the dosing, blood was collected from the jugular vein for the measurement of prothrombin time (PT) and activated partial thromboplastin time (APTT).

In Study # BG10-H0078-R1, the antithrombotic effects were also assessed for warfarin. Venous thrombosis was induced by the insertion of a platinum wire into the inferior vena cava of rats.

Study endpoint:

Bleeding time was defined as the interval from the tail incision to the last detectable blood stain.

Results:

The rat model of tail template bleeding

- Edoxaban: oral doses; the data in the table indicate the mean of the group (n=8)

|          | Bleeding time (min) | PT (sec)   | APTT (sec) |
|----------|---------------------|------------|------------|
| Control  | 3.2 (1)*            | 18.3       | 18.7       |
| 3 mg/kg  | 4.4 (1.4)           | 20.7 (sig) | 15.7       |
| 10 mg/kg | 5.4 (1.7)           | 23.6 (sig) | 17.2       |
| 20 mg/kg | 5.3 (1.7)           | 24 (sig)   | 17.5       |

**\*fold of the control; sig: statistically significant**

Thus, estimated dose required to double bleeding time (BT2) was >20 mg/kg.

- Enoxaparin: subcutaneous doses, the data in the table indicate the mean of the group (n=8)

|            | Bleeding time (min) | PT (sec)   | APTT (sec)  |
|------------|---------------------|------------|-------------|
| Control    | 3.3 (1)*            | 18.5       | 18.9        |
| 800 IU/kg  | 5.1 (1.6)           | 19.6       | 33.9        |
| 1600 IU/kg | 5.1 (1.5)           | 20.8 (sig) | 66.5 (sig)  |
| 3200 IU/kg | 21.6 (6.5) (sig)    | 21.6 (sig) | 109.5 (sig) |

**\*fold increase compared to the control; sig: statistically significant**

Thus, estimated dose required to double bleeding time (BT2) was 1700 IU/kg.

- Warfarin: oral doses, the data in the table indicate the mean of the group (n=8)

|         | Bleeding time (min) | PT (sec) | APTT (sec) |
|---------|---------------------|----------|------------|
| Control | 4.1 (1)*            | 19.7     | 22.7       |

|            |                  |            |            |
|------------|------------------|------------|------------|
| 0.1 mg/kg  | 6.7 (1.6)        | 27         | 35.3       |
| 0.15 mg/kg | 6.3 (1.5)        | 36.1 (sig) | 42.5 (sig) |
| 0.2 mg/kg  | 17.3 (4.2) (sig) | 62.8 (sig) | 65.5 (sig) |

\*fold increase compared to the control; sig: statistically significant

Thus, estimated dose required to double bleeding time (BT2) was 0.16 mg/kg.

In addition, the result of warfarin in the rat model of venous thrombosis (Study # BG10-H0078-R01) is summarized as the following.

Warfarin: oral doses, the data in the table indicate the mean of the group (n=8)

|            | Protein content of thrombus ( $\mu$ g) | PT (sec)   | APTT (sec) | Inhibition (%) of thrombus formation |
|------------|--|------------|------------|--------------------------------------|
| Control    | 186                                    | 19.7       | 21.9       | 0.1                                  |
| 0.1 mg/kg  | 123                                    | 25.9       | 34.5       | 33.7                                 |
| 0.15 mg/kg | 42 (sig)                               | 40.3 (sig) | 44.7 (sig) | 77.5                                 |
| 0.2 mg/kg  | 14 (sig)                               | 58 (sig)   | 57.3 (sig) | 92.5                                 |

#### Conclusion:

- All the three agents, i.e., edoxaban, enoxaparin and warfarin, inhibited venous thrombus formation (thrombosis) in the tail bleeding model in rats. The inhibition was dose-dependent. The doses required for 50% inhibition of thrombus formation ( $ED_{50}$ ) of edoxaban, enoxaparin and warfarin were 1.9 mg/kg (oral), 500 IU/kg (SC), and 0.12 mg/kg, respectively.
- The ratios of BT2 to  $ED_{50}$  (the therapeutic indexes) were > 10.5 for edoxaban, 3.4 for enoxaparin and 1.3 for warfarin.
- In terms of clotting time prolongation, while enoxaparin and warfarin prolonged both PT and APTT, edoxaban only prolonged PT but not APTT, under the condition of the study. Differential effects on clotting time were also noted for enoxaparin and warfarin: warfarin prolonged both PT and APTT and enoxaparin prolonged APTT more than PT.

Based on the higher ratio of BT2 to  $ED_{50}$  (therapeutic index) for edoxaban when compared with enoxaparin and warfarin, the Applicant indicated that bleeding risk of edoxaban is lower than warfarin and enoxaparin (Pharmacology written summary, Section 2.6.2.3.2). However, the assessment of edoxaban and warfarin was carried out in separate studies; it may not be justified to draw such a conclusion.

#### **Assessment of the effect of tranexamic acid (Cyklokapron) on edoxaban induced PT and APTT prolongation**

**Study title: Effects of tranexamic acid on prolonged bleeding time and prothrombin time induced by a supratherapeutic dose of DU-176b in rats (Study # BG12-H0020-R01, Module 4).**

#### **Key finding:**

The anti-fibrinolytic agent, tranexamic acid, did not reverse the bleeding time and prothrombin time prolonged by edoxaban.



Table 9- The effects of tranexamic acid on edoxaban-induced prolongation of bleeding time and prothrombin time

| Group                     | Dose (mg/kg) | Bleeding time (min)          | PT (s)                         |
|---------------------------|--------------|------------------------------|--------------------------------|
| 5% glucose + saline       | 0 + 0        | 3.2 ± 1.1                    | 18.6 ± 0.8                     |
| Edoxaban tosilate hydrate | 3.0          |                              |                                |
| +                         | +            | 10.2 ± 6.5 <sup>**</sup>     | 39.4 ± 3.2 <sup>***</sup>      |
| saline                    | 0            |                              |                                |
| Edoxaban tosilate hydrate | 3.0          |                              |                                |
| +                         | +            | 8.3 ± 7.8 <sup>**</sup> , a) | 38.3 ± 2.6 <sup>***</sup> , a) |
| Tranexamic acid           | 100          |                              |                                |

PT: prothrombin time

Data represent mean ± standard deviation, n = 10 rats/group; \*\* and \*\*\*: statistically significant changes.

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/s/  
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SHWU LUAN LEE  
08/19/2014

HALEH SABER  
08/19/2014

**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: 206316  
Supporting document/s: 1, 21, 22, 25, 26, 27  
Applicant's letter date: 1/8/2014  
CDER stamp date: 1/8/2014  
Product: Savaysa™ (edoxaban tosylate) tablets  
Indication: 1. Reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation;  
2. Treatment of deep vein thrombosis and pulmonary embolism;  
 (b) (4)  
Applicant: Daiichi Sankyo Inc.  
Review Divisions: Division of Cardiovascular and Renal Products  
Reviewers: Baichun Yang, PhD, DATB  
Supervisors: Thomas Papoian, PhD, DATB  
Division Directors: Norman L Stockbridge, MD, PhD  
Project Managers: Alison Blaus, RAC

**Disclaimer**

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## 1 Executive Summary

### 1.1 Introduction (and Clinical Rationale)

Activated coagulation factor X (factor Xa) is the serine protease located in the final common pathway of the coagulation cascade which catalyzes the conversion of prothrombin to thrombin. Factor Xa inhibition reduces thrombin generation, prolongs clotting time, and reduces the risk of thrombus formation. Edoxaban tosylate hydrate (DU-176b) is a highly selective and direct inhibitor of factor Xa, and has been developed as an oral anticoagulant agent for indications including: 1) Reduction in the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation; 2) Treatment of deep vein thrombosis and pulmonary embolism; (b) (4)

### 1.2 Brief Discussion of Nonclinical Findings

The nonclinical profile of DU-176b and its main human specific metabolite D21-2393 were investigated in a series of pharmacological, pharmacokinetic, and toxicological studies. Findings from pivotal toxicological studies included (I) increased polyploidy in chromosomal aberration tests; (II) hemorrhage in mice, rats, rabbits, and monkeys; (III) more post-implantation loss, less live fetuses, lower fetal weight, increased gall bladder and skeletal variations, and delayed avoidance response in a learning test in F1 females, which were associated with maternal hemorrhagic toxicity; and (IV) higher mortality in male rats at the high dose in a 2-year carcinogenicity study that was associated with higher incidence and greater severity of centrilobular hepatocellular degeneration/ necrosis.

Numerical chromosome aberrations (polyploidy) observed in DU-176b or D21-2393-treated Chinese Hamster Lung (CHL) cells and human peripheral lymphocytes were the only positive finding among a battery tests for genotoxicity. The increased polyploidy in chromosomal aberration test using human peripheral lymphocytes was associated with cell toxicity (mitotic index reduction up to 83%), which lowered the weight for genotoxic potential. Meanwhile, DU-176b did not show any genotoxic potential in in vitro micronucleus test using human peripheral lymphocytes, in vivo bone marrow micronucleus tests in rats, and liver micronucleus test in rats, indicating that DU-176b has little potential to induce aneuploidy. Although aneuploidy is known to be correlated with cancer and birth defects (1), negative results in several micronucleus tests and in an unscheduled DNA synthesis test in rat liver demonstrated no damage to chromosomes or DNA in these in vitro and in vivo studies. Based upon a weight of evidence approach, DU-176b is not considered to pose a genotoxic risk.

In animal toxicity studies, including repeated-dose oral toxicity studies, 2-year carcinogenicity studies, and reproductive and developmental studies, hemorrhagic findings and anemia were noted in monkeys at DU-176b doses of  $\geq 15$  mg/kg/day, in mice at 500 mg/kg/day, in rats at  $\geq 200$  mg/kg/day, and in rabbits at  $\geq 30$  mg/kg/day, leading to deteriorated animal condition or animal deaths. These findings are thought to

be the exaggerated anticoagulant effect of DU-176b (its principal pharmacological action), which constitutes the dose-limiting toxicity for this compound. Based on the mean  $AUC_{0-24h}$  values at the no observed adverse effect level (NOAEL) in the repeated-dose toxicity studies, the cynomolgus monkey was approximately 2-10 times more sensitive to the drug-related hemorrhagic effects than the rat. Pharmacology studies demonstrated that in vitro inhibitory activity of DU-176b for cynomolgus monkey factor Xa and anticoagulant activity of DU-176b in cynomolgus monkey plasma were approximately 10 times and 2 times, respectively, more potent than those for the rat factor Xa and in rat plasma. These data support the interpretation that the species difference in drug potency leads to, at least in part, the difference in the frequency of DU-176b hemorrhagic events in the two toxicity species. Since the pharmacological activity of DU-176b in the cynomolgus monkey was comparable to that in humans, safety margins for hemorrhagic risk were estimated by comparison of exposures between cynomolgus monkeys and humans. The mean  $AUC_{0-24h}$  values at NOAEL (5 mg/kg/day) in the 52-week repeated dose oral toxicity study in cynomolgus monkeys were approximately 1.5 times the exposures in human subjects given DU-176b at the maximum recommended human dose (MRHD) of 60 mg/day.

DU-176b was embryo-fetal toxic and developmental toxic in both rats and rabbits: higher post-implantation loss in rat at DU-176b  $\geq 300$  mg/kg/day [ $\sim 48$  times the human exposure at MRHD of 60 mg/day based on total body surface area in  $mg/m^2$ ]; more post-implantation loss, less live fetuses, lower fetal weight, and increased variation in the gall bladder in rabbits at  $\geq 200$  mg/kg/day ( $\sim 63$  times the human exposure at MRHD of 60 mg/day based on  $mg/m^2$ ), and increased 13th full ribs and 27 presacral vertebrae in rabbits at 600 mg/kg/day ( $\sim 190$  times the human exposure at MRHD of 60 mg/day based on  $mg/m^2$ ); delayed avoidance response during a learning test in F1 rats at 30 mg/kg/day ( $\sim 2.9$  times the human exposure at adult MRHD of 60 mg/day based on  $AUC_{0-24h}$ ), and moderately lower body weight in juvenile rats at 20 mg/kg/day ( $\sim 2.2$  times the human exposure at adult MRHD of 60 mg/day based on  $AUC_{0-24h}$ ). Maternal toxicity including dam deaths and abortion, decreased food consumption and body weight, hemorrhage in uterus, or vaginal hemorrhage occurred at the same or lower DU-176b doses that led to embryo-fetal/developmental toxicity. Thus, DU-176b-associated embryo-fetal toxicity in rats and rabbits and developmental toxicity in rats were considered to be secondary effects of maternal toxicity, rather than a direct DU-176b effect.

DU-176 systemic exposures following repeated oral daily doses of DU-176b were slightly higher in female rats and mice than in male rats and mice ( $\sim 2$  fold). In a 2-year rat carcinogenicity study, DU-176 systemic exposures were still higher in females than in males, although lower dose levels were used for females. While systemic DU-176 exposure, especially  $C_{max}$ , was lower in males than in females, male rats showed higher mortality in the 2-year carcinogenicity studies, which was associated with higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis. Compared to females, higher mortality with lower DU-176 exposure levels in male rats may not be explained as parent drug toxicity, but may likely represent metabolism-related toxicity. Since the gender difference in DU-176 systemic exposure was not seen

in rats following single and repeated daily IV doses, lower DU-176 systemic exposure in male rats than in female rats following oral doses indicates higher first-pass effect in males. Histological findings of higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis in prematurely dead rats indicated liver toxicity of the drug in rats, although such changes were not presented in monkeys or mice. Together these data imply that: (1) male rats had higher liver DU-176 metabolite rate (first-pass) which led to low systemic exposure; (2) DU-176 metabolic processes in liver were toxic, and (3) long term, persistent, and excessive DU-176 metabolic processes in liver led to centrilobular hepatocellular degeneration/necrosis that contributed to higher mortality. Therefore, liver toxicity may be a potential safety issue for long-term high dose DU-176b along with increased liver metabolism, although such findings were not seen in mice and monkeys orally administrated with DU-176b .

**1.3 Recommendations**

**1.3.1 Approvability**

Yes

**1.3.2 Additional Non Clinical Recommendations**

None

**1.3.3 Labeling**

Dr. Shwu Luan Lee of Division of Hematology Oncology Toxicology (DHOT) reviewed primary and secondary pharmacology studies for this NDA (206316). Comments for labeling under Section 12.1 Mechanism of Action can be found in Dr. Lee’s review under NDA 206316.

Some of the findings in the reproductive and developmental studies and carcinogenicity studies were ignored from the label. Revised wording (additions in bold; deletions in ~~strikeout~~) are recommended in the following boxes:

8.1. Pregnancy

Pregnancy C:  (b) (4)

There are no adequate and well-controlled studies in pregnant women. SAVAYSA should be used during pregnancy only if the potential benefit justifies the potential risk to the  (b) (4) fetus.

 (b) (4)

 (b) (4)

(b) (4)

### 8.2. Labor and Delivery

Safety and effectiveness of SAVAYSA during labor and delivery have not been studied in clinical (b) (4). The risks of bleeding should be balanced with the risk of thrombotic events when considering the use of SAVAYSA in this setting.

(b) (4)

### 8.3. Nursing Mothers

It is not known if edoxaban (b) (4) are excreted in human milk. (b) (4) edoxaban was excreted in the (b) (4) milk of rats.

(b) (4) taking into account the importance of the drug to the mother.

### 8.4. Pediatric Use

Safety and effectiveness in pediatric patients has not been established. (b) (4)

(b) (4)

### 13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

#### Carcinogenesis

Edoxaban was not carcinogenic when administered to mice and rats by oral gavage daily for up to 104 weeks. The highest dose tested (500 mg/kg/day) in **male and female**

mice was (b) (4) 3 and 6 (b) (4) times, **respectively**, the human exposure (**AUC**) at the (b) (4) of 60 mg/day (b) (4) and (b) (4) the highest doses tested in **male** (600/400 mg/kg/day) **and female (200 mg/kg/day)** (b) (4) rats were (b) (4) **8 and** (b) (4) times, **respectively**, the human exposure at the (b) (4) of 60 mg/day (b) (4)

(b) (4)

Edoxaban showed no effects on fertility and early embryonic development (b) (4) 162 times (b) (4) the (b) (4) dose of 60 mg/day (b) (4) total body surface area (b) (4)

(b) (4)

## 2 Drug Information

### 2.1 Drug

CAS Registry Number : 480449-71-6

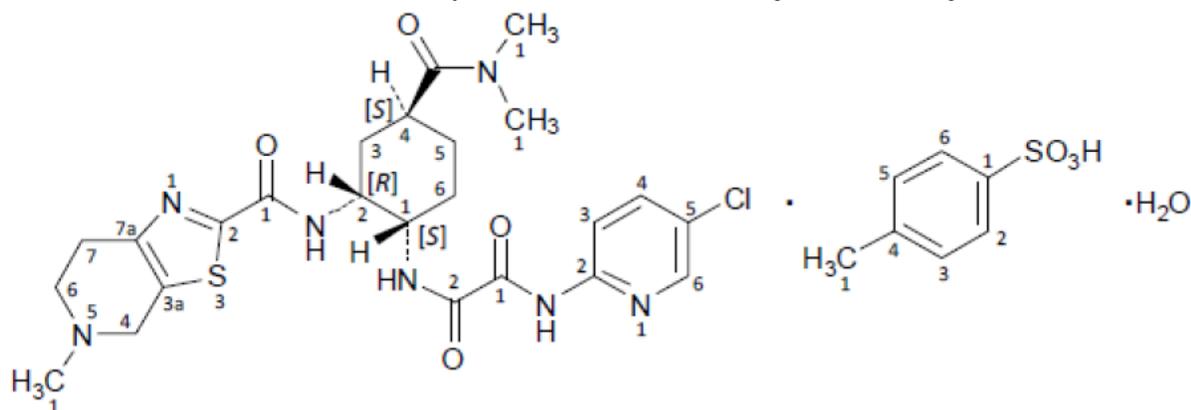
Generic Names: Edoxaban tosylate monohydrate  
Edoxaban tosylate (anhydrous)

Code Name: DU-176b, D11-4176b

Chemical Name: N-(5-chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]oxamide mono(4-methylbenzenesulfonate) monohydrate

Molecular Formula/Molecular Weight: C<sub>24</sub>H<sub>30</sub>ClN<sub>7</sub>O<sub>4</sub>S•H<sub>2</sub>O / 738.27

Structure or Biochemical Description: Edoxaban tosylate monohydrate



Pharmacologic Class: Factor Xa inhibitor

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

IND077254, IND063266, MF- (b) (4) MF- (b) (4) MF- (b) (4)

### 2.3 Drug Formulation

Edoxaban Tablets 15 mg, 30 mg, and 60 mg, with following quantitative compositions

| Ingredient                                   | Function       | 15 mg tablets |         | 30mg tablets  |         | 60 mg tablets |         |
|--|----------------|---------------|---------|---------------|---------|---------------|---------|
|  |                | mg/tablet     | wt/wt%  | mg/tablet     | wt/wt%  | mg/tablet     | wt/wt%  |
| Edoxaban tosylate<br>(as edoxaban free base) | Drug substance | 20.20<br>(15) | (b) (4) | 40.41<br>(30) | (b) (4) | 80.82<br>(60) | (b) (4) |
| Mannitol                                     | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Pregelatinized starch                        | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Croscopovidone                               | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Hydroxypropyl cellulose<br>(b) (4)           | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Magnesium stearate<br>(b) (4)                | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| (b) (4)<br>(Orange)                          | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| (b) (4)<br>(Pink)                            | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| (b) (4)<br>(Yellow)                          | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Carnauba wax                                 | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| Talc   | (b) (4)        | (b) (4)       | (b) (4) | (b) (4)       | (b) (4) | (b) (4)       |         |
| <b>Total Tablet Weight (mg)</b>              |                | <b>105.0</b>  |         | <b>210.0</b>  |         | <b>420.0</b>  |         |

q.s.: quantum sufficit

(b) (4)

### 2.4 Comments on Novel Excipients

None

## 2.5 Comments on Impurities/Degradants of Concern

None

## 2.6 Proposed Clinical Population and Dosing Regimen

Proposed clinical populations include patients with nonvalvular atrial fibrillation, patients with deep vein thrombosis (DVT), and patients with pulmonary embolism (PE). The recommended dose is 60 mg once daily. For the above mentioned patients with moderate to severe renal impairment (CrCL 15-50 mL/min), low body weight ≤60 kg (132 lbs), and/or concomitant use of P glycoprotein (P-gp) inhibitors except amiodarone, the recommended dose is 30 mg once daily.

## 2.7 Regulatory Background

The sponsor submitted study protocols for 2-year carcinogenicity studies in rats and mice in Nov, 2006. The Executive CAC meeting on December 19, 2006 discussed the protocols and approved the proposed dose levels. A type B pre-NDA meeting regarding CMC was held on May 17, 2013. No nonclinical issues were discussed in this meeting.

## 3 Review Information of Submitted Studies

### 3.1 Studies Not Reviewed

#### Primary and secondary pharmacology studies

Studies for primary and secondary pharmacology are reviewed by Dr. Shwu Luan Lee of Division of Hematology Oncology Toxicology (DHOT). The review is filed separately under this NDA.

#### Analytical methods and validation reports:

|                |                |                |           |
|----------------|----------------|----------------|-----------|
| AN07-C0122-R01 | AN07-C0123-R01 | AN08-C0048-R01 | R20020652 |
| R20030101      | R20030102      | R20030336      | R20040462 |

#### Local tolerance study reports:

AN08-C0125-R01, Hemolysis test of DU-176b for Injection 10 mg using human peripheral blood (noteworthy findings: no hemolytic action up to 1 mg/ml)  
AN08-H0084-R01, Local vascular irritation study of DU-176b for Injection 10 mg in rabbits (noteworthy findings: no irritation potential up to 1 mg/mL)

#### Studies with DU-176 analogue

R20010114, D01-8831a: Single oral toxicity in monkeys (noteworthy findings: native)  
R20010124, D01-8831a: Four-week oral toxicity in monkeys (noteworthy findings: native)  
R20010110, D01-8831a and D01-8834a: Two-week oral toxicity in rats (noteworthy findings: native)

#### Non-GLP studies

R20020282, D11-4176b: Single oral toxicity in cynomolgus monkeys – a dose-finding study (noteworthy findings: n=1, negative)  
R20010649, D11-4176a: Single oral toxicity in monkeys (noteworthy findings: negative)

R20010708, D11-4176a: Two-week intravenous toxicity in rats (noteworthy findings: tail injuries at the injection site)

R20010578, D11-4176a: Single oral toxicity in rats (noteworthy findings: negative)

R20020517, D11-4176b: Liver micronucleus test in rats following a single oral administration (noteworthy findings: negative)

### **3.2 Previous Reviews Referenced**

- 3.2.1 IND063266, JOSEPH, DAVID B, 04/04/2007, REV-NONCLINICAL-03 (General Review) Original-1
- 3.2.2 IND063266, SEIFRIED, ADELE S, 12/21/2006 Executive CAC Minutes for SPA-3 (Carcinogenicity) and SPA-2 (Carcinogenicity)
- 3.2.3 IND063266, HONCHEL, RONALD, 03/23/2009 REV-NONCLINICAL-03 (General Review) Original-1
- 3.2.4 IND063266, HONCHEL, RONALD, 04/13/2009 REV-NONCLINICAL-03 (General Review) Original-1
- 3.2.5 IND077254, HARLOW, PATRICIA P, 06/22/2007 REV-NONCLINICAL-03 (General Review) Original-1
- 3.2.6 IND077254, HARLOW, PATRICIA P, 06/03/2013 REV-NONCLINICAL-21 (Primary Review) Original-1
- 3.2.7 IND077254, HARLOW, PATRICIA P, 09/03/2008 N/A 09/03/2008 REV-NONCLINICAL-03 (General Review) Original-1
- 3.2.8 IND077254, CHEN, ZHOU, 09/26/2008 REV-NONCLINICAL-03 (General Review) Original-1

### **3.3 Disclaimer**

Tabular and graphical information are constructed by the reviewer unless cited otherwise. DU-176b doses/concentrations are expressed as free base.

## **4 Pharmacology**

### **4.1 Primary Pharmacology**

Dr. Shwu Luan Lee of DHOT reviewed studies under the primary pharmacology section. The review is filed separately under this NDA.

### **4.2 Secondary Pharmacology**

Dr. Shwu Luan Lee of DHOT reviewed studies under the secondary pharmacology section. The review is filed separately under this NDA.

### **4.3 Safety Pharmacology**

The potential adverse effects of DU-176b on vital functions were investigated in a battery of safety pharmacology studies (Table 1). Dr. David B. Joseph of the Division of

Gastroenterology and Inborn Errors (DGIE) reviewed these studies (April 4, 2007) under IND 063266 (Appendix I).

Table 1. Summary of safety pharmacology studies (from the submission)

| Organ Systems Evaluated | Species/Strain               | Method of Admin. | Doses or concentration   | Gender and No. per Group | Noteworthy Findings | GLP Compliance | Study Number |
|-------------------------|------------------------------|------------------|--------------------------|--------------------------|---------------------|----------------|--------------|
| Central Nerve System    | Mouse / ddY                  | Single oral      | 0, 20, 60, and 200 mg/kg | M:3 to 10                | No effects          | Yes            | 20020505     |
|                         | Cynomolgus monkey            | Single oral      | 0, 20, 60, and 200 mg/kg | M:2, F:2                 | No effects          | Yes            | 20020556     |
| Cardiovascular System   | hERG transfected HEK293 cell | In vitro         | 0, 2, and 20 µg/mL       | 4 cells                  | No effects          | Yes            | 20020444     |
|                         | Guinea pig / Hartley         | In vitro         | 0, 6, and 20 µg/mL       | M:4 specimens            | No effects          | Yes            | 20020491     |
|                         | Cynomolgus monkey            | Single oral      | 0, 20, 60, and 200 mg/kg | M:2, F:2                 | No effects          | Yes            | 20020556     |
| Respiratory System      | Cynomolgus monkey            | Single oral      | 0, 20, 60, and 200 mg/kg | M:2, F:2                 | No effects          | Yes            | 20020556     |
| Renal Function          | Rat / SD                     | Single oral      | 0, 20, 60, and 200 mg/kg | M:8                      | No effects          | Yes            | 20020492     |

HEK: Human embryonic kidney, hERG: Human ether-a-go-go related gene

Oral administration of DU-176b up to 200 mg/kg had no effects on CNS function in mice. Oral administration of DU-176b up to 200 mg/kg in monkeys had no effects on behavior, cardiovascular parameters (including QTc), or respiratory parameters. DU-176b at concentrations up to 20 µg/mL did not affect the human ether-a-go-go related gene (hERG) potassium channel current (peak tail current) in hERG-transfected human embryonic kidney (HEK) 293 cells or the action potential parameters of isolated guinea pig ventricular papillary muscles. These results indicate that DU-176b is not likely to prolong QT interval in humans. Renal function was unaffected in rats at oral doses of DU-176b up to 200 mg/kg.

The highest dose of 200 mg/kg corresponds to 16 times the recommended clinical dose of 60 mg DU-176b on a body surface basis. The highest concentration of 20 µg/ml in vitro corresponds to 69 times mean human C<sub>max</sub> of 289 ng/ml at the recommended clinical dose of 60 mg (Clinical Study Report DU176b-A-U151, page 80).

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 Pharmacokinetics (PK)/ADME

In vivo nonclinical PK studies using oral or intravenous (IV) doses of unlabeled or <sup>14</sup>C-labeled DU-176b (solution or suspension in 0.5% methylcellulose aqueous solution) were conducted primarily in rats and cynomolgus monkeys, the same species used in the in vivo nonclinical pharmacology and toxicology studies. In vitro nonclinical PK studies were performed with <sup>14</sup>C-DU-176b mainly using animal and human plasma/blood. D21-2393, a main metabolite in human plasma and a human specific metabolite, was investigated in terms of plasma protein binding in rats and humans, and tissue distribution in juvenile and adult rats.

## 5.1.1 Absorption

### 5.1.1.1 DU-176b: Blood and plasma concentrations of radioactivity after a single oral administration of <sup>14</sup>C-DU-176b to male rats (Study R20020653)

After a single oral administration of <sup>14</sup>C-labeled DU-176b at a dose of 3 mg/kg (11.6 MBq/kg) to fasting male rats (Wistar, 7 weeks old), blood and plasma concentrations of radioactivity were determined. The plasma radioactivity reached a peak at 0.67 h post-dose and then declined in a biphasic manner. The whole blood radioactivity reached a peak at 0.5 h post-dose and then declined in parallel with that in the plasma. The PK parameters are summarized in Table 2. The C<sub>max</sub> and AUC values for total radioactivity were similar in blood and plasma, indicating extensive radioactivity distributed in the red blood cells (Appendix I).

Table 2. PK parameters of radioactivity in blood and plasma (from the submission)

| PK parameters                         | Blood        | Plasma               |
|---------------------------------------|--------------|----------------------|
| t <sub>max</sub> (h)                  | 0.50 ± 0.00  | 0.67 ± 0.29          |
| C <sub>max</sub> (ng eq./mL)          | 426.8 ± 31.2 | 417.2 ± 72.4         |
| t <sub>1/2</sub> (h)                  | 3.2 ± 0.2    | 3.2 ± 0.4            |
|                                       | (1-12 h)     | (1-12 h or 2-12 h)   |
| t <sub>1/2</sub> (h)                  | 65 ± 23      | 11 ± 6               |
|                                       | (24-72 h)    | (12-24 h or 12-48 h) |
| AUC <sub>0-finite</sub> (µg eq.·h/mL) | 1.82 ± 0.21  | 1.67 ± 0.16          |
| AUC <sub>0-∞</sub> (µg eq.·h/mL)      | 2.16 ± 0.40  | 1.76 ± 0.20          |

### 5.1.1.2 Single-dose oral gavage and intravenous injection pharmacokinetic study with DU-176b in rats (Study AM08-C0010-R01).

This GLP study (AM08-C0010-R01) was performed in (b) (4) 6/26/2008 – 3/3/2009.

Male Wistar Han IGS rats (~10 weeks old with body weights 286-317 g) were dosed with DU-176b via oral gavage at doses 0.3, 1.0, 3.0, or 10 mg/kg, or intravenous (IV) injection of DU-176b at doses 0.3 or 1.0 mg/kg (n=4/dose at each route). Blood samples (~0.3 ml/rat/time-point) were collected from a jugular vein of conscious rats at pre-dose, 0.083 (5 min; IV rats only), 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose with sodium fluoride solution as the anticoagulant. Plasma samples were harvested and analyzed for DU-176 using high performance liquid chromatograph (HPLC) with tandem mass spectrometric (MS/MS) detection.

After oral gavage of DU-176b at doses of 0.3 mg/kg to 10 mg/kg, plasma DU-176 concentrations increased and reached  $C_{max}$  at 0.5 to 0.813 h, then declined.  $T_{1/2}$  ranged from 0.683 to 5.05 hours (Figure 1 and Table 3). For all doses tested, the plasma DU-176 concentration at 24 h post-dose was below the limit of quantification (5 ng/ml). DU-176  $C_{max}$  and the area under curve up to 24 h post-dose ( $AUC_{0-24h}$ ) generally increased in a dose-proportional manner.

After intravenous injection of DU-176b at doses of 0.3 and 1 mg/kg, plasma DU-176 concentrations decreased rapidly with a  $t_{1/2}$  of 0.694 and 1.32 hours, respectively (Figure 1 and Table 3). The total body clearance (CL) of DU-176 was similar at the two dose levels, being 1.97 and 1.86 L/h/kg, respectively, and the volume of distribution at a steady state ( $V_{ss}$ ) was 1.60 and 2.14 L/kg, respectively.

The bioavailability of DU-176 in male rats dosed via oral gavage ranged from 0.370 to 0.409 when compared to the IV 0.3 mg/g group and from 0.352 to 0.389 when compared to the IV 1.0 mg/kg group. Values were similar among all dose groups.

Figure 1. Plasma DU-176 concentrations following a single oral or IV DU-176b dose in male rats (modified from the submission)

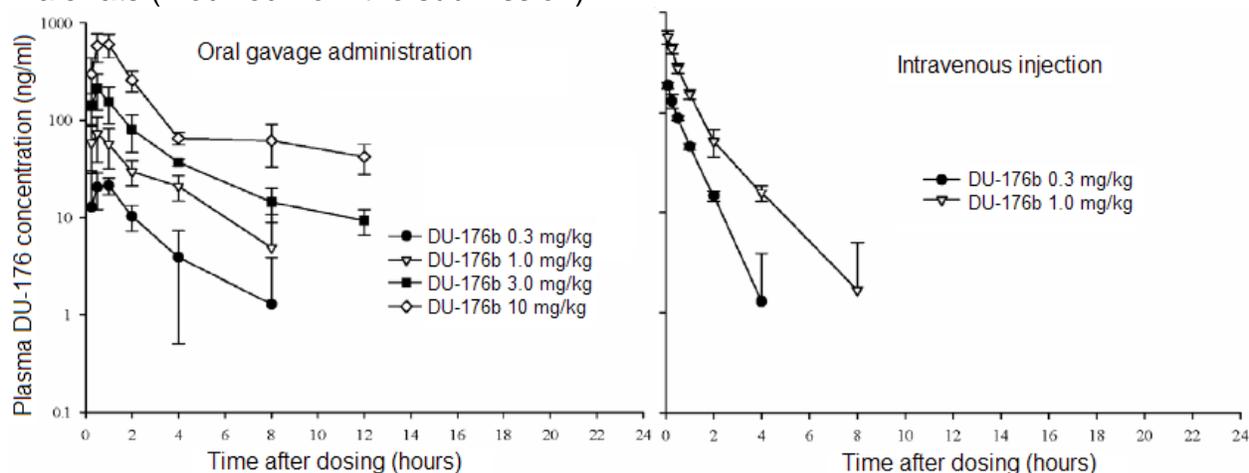


Table 3. PK parameters of DU-176 after single oral and IV administrations to rats (from the submission, Mean  $\pm$  standard deviation, n=4)

| Route                          | Oral                |                               |                   |                    | Intravenous       |                 |
|--------------------------------|---------------------|-------------------------------|-------------------|--------------------|-------------------|-----------------|
|                                | 0.3                 | 1                             | 3                 | 10                 | 0.3               | 1               |
| Dose (mg/kg)                   |                     |                               |                   |                    |                   |                 |
| AUC <sub>last</sub> (ng·h/mL)  | 52.9 $\pm$ 18.7     | 184 $\pm$ 94                  | 540 $\pm$ 149     | 1662 $\pm$ 431     | 141 $\pm$ 14      | 516 $\pm$ 29    |
| AUC <sub>0-24h</sub> (ng·h/mL) | 63.2 $\pm$ 19.1     | 212 $\pm$ 87                  | 596 $\pm$ 160     | 1915 $\pm$ 475     | 155 $\pm$ 12      | 545 $\pm$ 21    |
| AUC <sub>0-inf</sub> (ng·h/mL) | 47.5 <sup>a)</sup>  | 301 <sup>b)</sup>             | 600 $\pm$ 177     | 1671 <sup>a)</sup> | 153 $\pm$ 12      | 539 $\pm$ 29    |
| C <sub>max</sub> (ng/mL)       | 25.2 $\pm$ 8.2      | 72.7 $\pm$ 35.7               | 213 $\pm$ 86      | 643 $\pm$ 160      | NA                | NA              |
| t <sub>max</sub> (h)           | 0.813 $\pm$ 0.375   | 0.500 $\pm$ 0.000             | 0.500 $\pm$ 0.000 | 0.750 $\pm$ 0.289  | NA                | NA              |
| t <sub>1/2</sub> (h)           | 0.683 <sup>a)</sup> | 4.85 $\pm$ 2.88 <sup>c)</sup> | 4.15 $\pm$ 1.25   | 5.05 <sup>a)</sup> | 0.694 $\pm$ 0.205 | 1.32 $\pm$ 0.69 |
| CL (L/h/kg)                    | NA                  | NA                            | NA                | NA                 | 1.97 $\pm$ 0.15   | 1.86 $\pm$ 0.10 |
| V <sub>ss</sub> (L/kg)         | NA                  | NA                            | NA                | NA                 | 1.60 $\pm$ 0.19   | 2.14 $\pm$ 0.36 |

a: n = 1, b: n = 2, c: n = 3; NA: Not applicable

### 5.1.1.3 Plasma concentrations of radioactivity after a 14-day period of repeated oral administration of [<sup>14</sup>C]DU-176b to male rats (AM09-C0117-R01)

This study (AM09-C0117-R01) was conducted in (b) (4) during Sept 14 -Dec 24, 2009.

Three male Slc:Wistar rats (7 weeks old) were orally administered with [<sup>14</sup>C]DU-176b at a dose of 3 mg/kg (5.98 MBq/kg/day), once daily, for 14 days. Blood samples were collected from the jugular vein of non-fasted conscious rats using a heparinized syringe at 0.5, 1, 2, 4, 8, 12, and 24 h after the first dosing, 24 h after the 3rd, 5th, 7th, 9th, 11th, and 13th dosing, and 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after the 14th dosing. The radioactivity (dpm) in plasma samples were measured using a liquid scintillation counter.

Following the first dose on Day 1 and the last dose on Day 14, the plasma radioactivity declined with a t<sub>1/2</sub> of 5.3 h (Day 1) or 5.0 h (Day 14) (Figure 2). DU-176 exposures (C<sub>max</sub> and AUC<sub>0-inf</sub>) were similar between Day 1 and Day 14 (Table 4). The plasma radioactivity concentrations at 24 h post-dose on Days 1, 3, 5, 7, 9, 11, 13, and 14 ranged from 2.08 ng/ml to 5.28 ng /ml (Figure 2). The trough plasma concentrations were consistent through the repeated doses, except for a transiently low level (2.08 ng eq./ml) on Day 11 (Figure 2).

Figure 2. Plasma radioactivity following oral [ $^{14}\text{C}$ ]DU-176b dosing (modified from the submission)

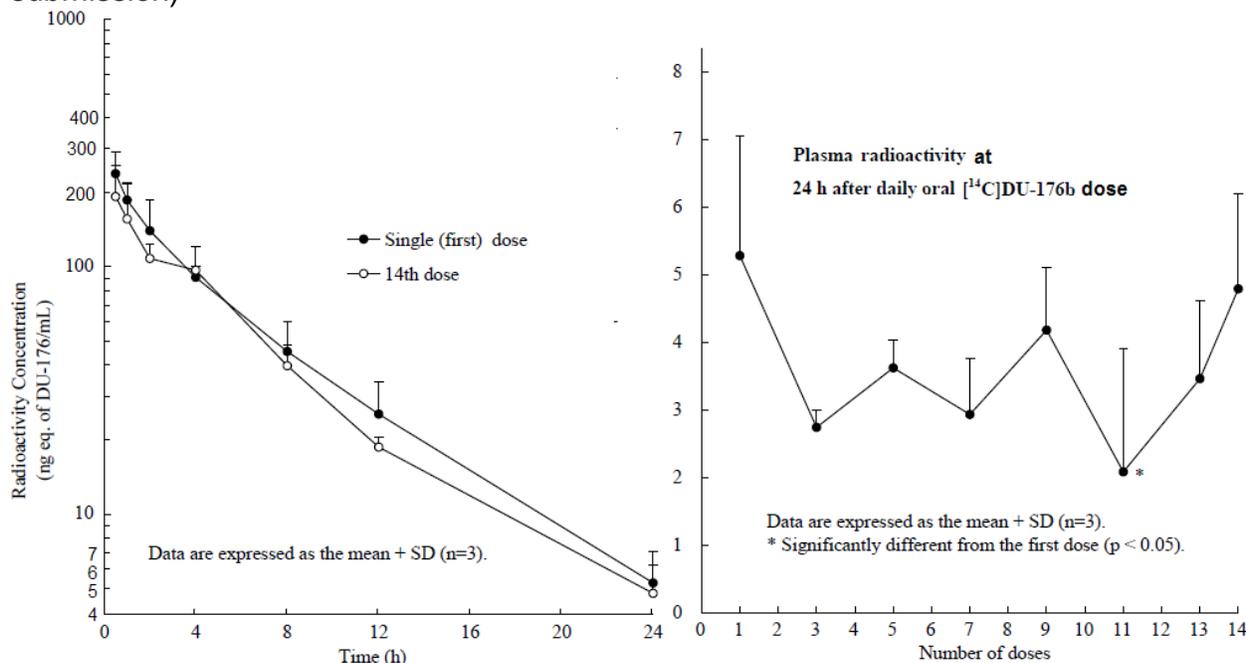


Table 4. PK parameters of radioactivity after a single or a repeated daily oral [ $^{14}\text{C}$ ]DU-176b at a dose of 3 mg/kg/day to rats (modified from the submission)

|        | $\text{AUC}_{0-24\text{h}}$<br>(ng eq.-h/mL) | $\text{AUC}_{0-\text{inf}}$<br>(ng eq.-h/mL) | $\text{C}_{\text{max}}$<br>(ng eq./mL) | $t_{1/2}$<br>(h) |
|--------|--|--|--|------------------|
| Day 1  | 1160 ± 160                                   | 1200 ± 170                                   | 238 ± 54                               | 5.3 ± 1.4        |
| Day 14 | 1000 ± 190                                   | 1040 ± 180                                   | 192 ± 65                               | 5.0 ± 0.9        |

Mean ± standard deviation, n = 3

#### 5.1.1.4 DU-176b: Blood and plasma concentrations of radioactivity, and excretion of radioactivity into urine and feces after a single oral administration of $^{14}\text{C}$ -DU-176b to male cynomolgus monkeys (Study R20020685)

Male cynomolgus monkeys (3 years old, n=3) were orally administered with a single dose of  $^{14}\text{C}$  DU-176b at 1 mg/kg (3.88 MBq/kg) 4 hours before feeding. Blood and plasma concentrations of radioactivity, and radioactivity excretion in urine and feces were determined. The tissue distribution of radioactivity at 336 h (14 days) after administration was also investigated.

As shown in Figure 3 and Table 5, the plasma concentration of radioactivity reached the  $\text{C}_{\text{max}}$  ( $297.9 \pm 141.3$  ng eq./mL) at 1 to 4 h post-dose and then declined. The whole blood concentration of radioactivity declined after reaching a peak in parallel with that in the plasma until 120 h post-dose. The excretion of administered radioactivity into urine and feces was almost complete by 96 h post-dose (Figure 3). Of the administered radioactivity,  $42.0 \pm 5.0\%$  was excreted in urine, and  $51.0 \pm 6.1\%$  was excreted in feces

during the 336 h post-dose. At 336 h post-dose, radioactivity in the eyeballs and skin were still high (Table 6).

Figure 3. Radioactivity in monkey plasma, blood, urine, and feces after a single oral dose of  $^{14}\text{C}$ -DU-176b (modified from the submission)

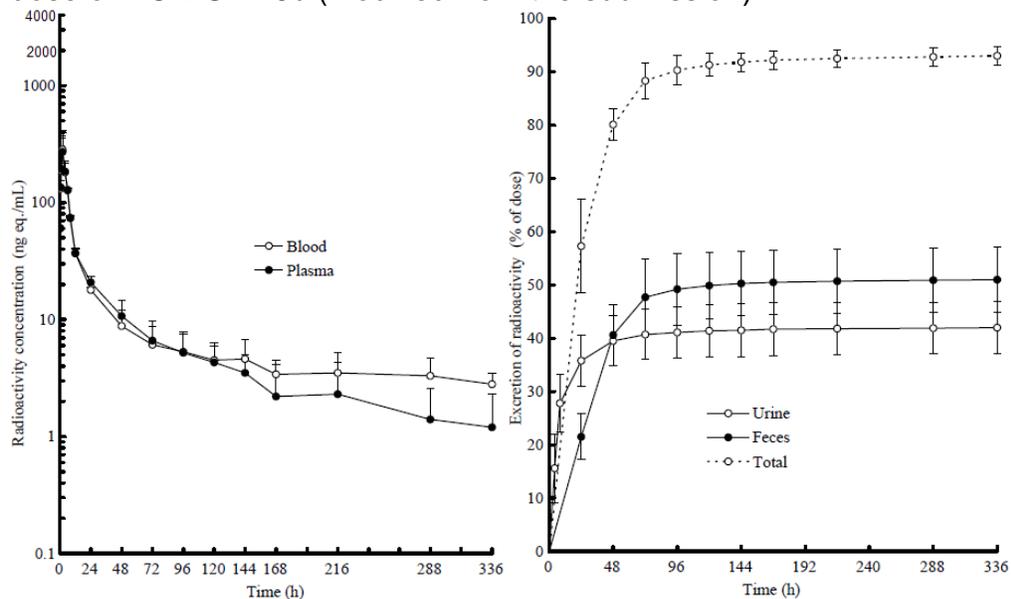


Table 5. PK parameters of radioactivity in monkeys after a single oral dose of  $^{14}\text{C}$ -DU-176b (from the submission)

| PK parameters  | Blood             | Plasma                   |
|--|-------------------|--------------------------|
| $t_{\max}$ (h)   | $1.7 \pm 0.6$     | $2.3 \pm 1.5$            |
| $C_{\max}$ (ng eq./mL)                                 | $297.0 \pm 137.1$ | $297.9 \pm 141.3$        |
| (h)  | $3.4 \pm 0.3$     | $3.2 \pm 0.2$            |
| $t_{1/2}$ (h)  | (2 or 4-12 h)     | (2, 4 or 6-12 h)         |
|  | $32 \pm 12$       | $32 \pm 13$              |
|  | (24-72 h)         | (24-72 or 120 h)         |
| (day)  | $15 \pm 5$        | $5.3 \pm 1.6$            |
|  | (96-336 h)        | (96 or 144-144 or 336 h) |
| $AUC_{0-\text{finite}}$                                | $3.40 \pm 0.93$   | $3.18 \pm 1.01$          |
| $AUC_{0-\infty}$ ( $\mu\text{g eq.}\cdot\text{h/mL}$ ) | $4.76 \pm 0.86$   | $3.51 \pm 1.13$          |

Table 6. Radioactivity in monkey tissues at 336 hours post a single oral dose of <sup>14</sup>C-DU-176b (modified from the submission)

| Tissue               | Radioactivity concentration<br>(ng eq./g or mL) | Radioactivity content<br>(% of dose) |
|----------------------|---|--------------------------------------|
| Blood                | 2.8 ± 0.7                                       | 0.02 ± 0.01                          |
| Brain                | 2.4 ± 0.6                                       | 0.00 ± 0.01                          |
| Eyeball              | 509.0 ± 148.2                                   | 0.09 ± 0.02                          |
| Heart                | 4.7 ± 0.9                                       | 0.00 ± 0.00                          |
| Lung                 | 5.0 ± 1.6                                       | 0.00 ± 0.00                          |
| Liver                | 14.5 ± 2.6                                      | 0.03 ± 0.01                          |
| Kidney               | 6.7 ± 1.2                                       | 0.00 ± 0.00                          |
| Skeletal muscle      | 3.7 ± 2.1                                       | 0.18 ± 0.10                          |
| Fat                  | 2.0 ± 0.2                                       | 0.02 ± 0.01                          |
| Skin                 | 259.9 ± 69.5                                    | 2.41 ± 0.76                          |
| Testis               | 3.4 ± 1.1                                       | 0.00 ± 0.00                          |
| Epididymis           | 2.2 ± 0.8                                       | 0.00 ± 0.00                          |
| Bile in gall bladder | 20.6 ± 5.4                                      | 0.00 ± 0.00                          |

Data are expressed as the mean values ± S.D. of three animals.

The compartment sizes of blood, skeletal muscle, fat and skin were assumed to be 6.0%, 50.0%, 7.8%, and 9.4% of body weight, respectively.

#### 5.1.1.5 Single-dose oral gavage and intravenous injection PK study with DU-176b in cynomolgus monkeys (AM08-C0011-R01)

This GLP study (AM08-C0011-R01) was conducted in [REDACTED] (b) (4) during Jun 26, 2008 – Mar 4, 2009.

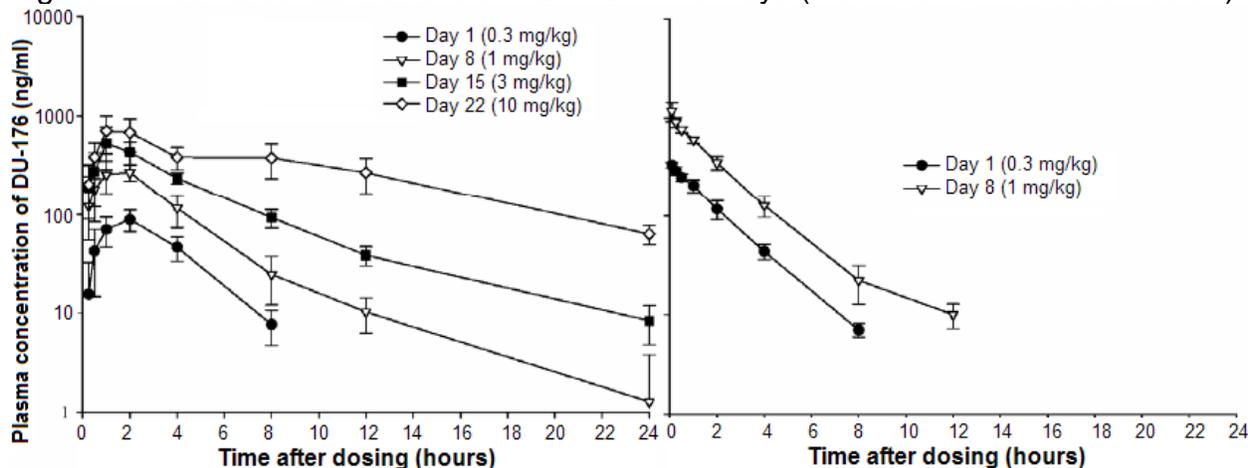
Four fasted male cynomolgus monkeys (2.8 - 3.8 years old, body weight 2.8 - 3.8 kg) were orally administered with DU-176b at doses of 0.3, 1, 3, and 10 mg/kg on days 1, 8, 15, and 22, respectively. Parallel four male monkeys were IV injected with DU-176b once on day 1 and once on day 8 at doses of 0.3 and 1 mg/kg, respectively. Blood samples were collected from the femoral vein with sodium heparin being the anticoagulant at pre-dose, 0.083 (5 min; IV animals only), 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h post-dose on days 1, 8, 15, and 22, respectively. Derived plasma samples were analyzed for DU-176 using HPLC-MS/MS

As shown in Figure 4 and Table 7, DU-176 was readily absorbed following oral administration at doses of 0.3 to 10 mg/kg, with mean  $T_{max}$  values ranging from 1.1 to 3.3 hours. After reaching  $C_{max}$ , DU-176 concentrations readily declined, with mean  $t_{1/2}$  values ranging from 1.55 to 6.59 hours. Exposures ( $C_{max}$  and  $AUC_{0-24}$ ) to DU-176 following oral administration of DU-176b were roughly dose-proportional from 0.3 to 1.0 mg/kg and under dose-proportional from 1.0 to 10.0 mg/kg.

Exposures ( $C_{max}$  and  $AUC_{0-24}$ ) to DU-176 following IV injection of DU-176b at 0.3 to 1.0 mg/kg were roughly dose proportional. Mean  $CL_{tot}$  values were similar between two dose levels, indicating linear PKs for the dose levels tested. DU-176 may be highly distributed to the tissues after intravenous injection of DU-176b (Figure 4, Table 7).

The mean bioavailability of DU-176 in male monkeys ranged from 0.298 to 0.565, and results were generally consistent between the different IV doses used in the calculation. Mean bioavailability appeared to decrease with increasing oral dose levels (Table 7).

Figure 4. Plasma concentrations of DU-176 in monkeys (modified from the submission)



No data at the scheduled time points reflected DU-176 levels under the detect limit of 5 ng/ml.

Table 7. Summary of mean PKs for DU-176 in monkeys

| Day                            | Dose<br>mg/kg | $C_{max}$<br>ng/ml | $T_{max}$<br>hours | AUC       |           | $t_{1/2}$<br>hours | $k_{el}$<br>$hr^{-1}$ | $CL_{tot}/F$<br>L/hr/kg | $V_{ss}/F$<br>L/kg | Bioavailability F (%) |            |
|--------------------------------|---------------|--------------------|--------------------|-----------|-----------|--------------------|-----------------------|-------------------------|--------------------|-----------------------|------------|
|                                |               |                    |                    | 0-t       | 0-24      |                    |                       |                         |                    | vs 0.3 mg/kg          | vs 1 mg/kg |
| Oral administration of DU-176b |               |                    |                    |           |           |                    |                       |                         |                    |                       |            |
| 1                              | 0.3           | 93.8±20.9          | 1.75±0.50          | 360±69    | 376±73    | 1.55               | 0.447                 | 0.792                   | 2.22               | 0.54±0.10             | 0.57±0.11  |
| 8                              | 1             | 289±72             | 1.13±0.63          | 1189±104  | 1233±141  | 3.67±2.94          | 0.258±0.115           | 0.82±0.07               | 3.25±1.48          | 0.53±0.06             | 0.56±0.06  |
| 15                             | 3             | 563±204            | 1.25±0.50          | 2643±542  | 2643±542  | 4.64±0.47          | 0.15±0.015            | 1.15±0.24               | 6.15±1.69          | 0.38±0.08             | 0.40±0.08  |
| 22                             | 10            | 757±233            | 3.25±3.20          | 6967±1484 | 6967±1484 | 6.59±1.73          | 0.11±0.026            | 1.36±0.28               | 13.1±4.7           | 0.30±0.06             | 0.31±0.07  |
| IV injection of DU-176b        |               |                    |                    |           |           |                    |                       |                         | $CL_{tot}$         |                       |            |
| 1                              | 0.3           | 333±21             | 0.083±0            | 687±82    | 701±84    | 1.5±0.08           | 0.464±0.026           | 0.43±0.05               | 0.83±0.07          |                       |            |
| 8                              | 1             | 1183±258           | 0.083±0            | 2157±272  | 2217±276  | 2.22±0.35          | 0.318±0.048           | 0.46±0.06               | 0.99±0.13          |                       |            |

## 5.1.2 Distribution

### 5.1.2.1 DU-176b: Whole-body autoradiography after a single oral administration of $^{14}C$ -DU-176b to male rats (Study R20020655)

Two fasting male Wistar SPF rats (7 weeks old) were orally administered with  $^{14}C$ -DU-176b at a dose of 3 mg/kg (11.6 MBq/kg). The rats were sacrificed at 1 and 24 hr (1 rat/time-point). Whole-body sectioning was performed and autoradiograms were prepared.

At 1 h post-dose, the highest levels of radioactivity were present in gastrointestinal (GI) and bladder contents. The radioactivity levels in kidney, preputial gland, liver, intestine, Harderian gland, pituitary gland, and nasal cavity were much higher than that in blood. The radioactivity levels in adrenals, mandibular glands, spleen, pancreas, mandibular lymph nodes, thyroid, epididymis, thymus, prostate, stomach, and bone marrow were somewhat higher than blood radioactivity levels. The radioactivity levels in lung, heart, brown fat, skin, and skeletal muscle were comparable to blood radioactivity levels. The radioactivity levels in testes and fat were lower than that in blood, and only trace levels were found in eye and brain.

At 24 hr post-dose, the radioactivity levels were decreased in most organs/tissues. However, a relatively high level of radioactivity was still present in the intestinal contents. Low levels were present in bladder contents, nasal cavity, thyroid, liver, and stomach. Only trace levels were observed in the remaining organs/tissues (Appendix I).

#### **5.1.2.2 DU-176b: Tissue distribution of radioactivity after a single oral administration of <sup>14</sup>C-DU-176b to male rats (Study R20040211)**

Eighteen fasting male Slc: Wistar SPF rats (7 weeks old) were orally administered with <sup>14</sup>C-DU-176b at a dose of 3 mg/kg (11.6 MBq/kg). The rats were sacrificed at 1, 4, 8, 24, 72, and 168 hr (3 rats/time-point). Blood and tissues/organs were collected and prepared (homogenized and/or solubilized). Radioactivity in each sample was measured with liquid scintillation counting.

After a single oral administration, radioactivity was maximal at 1 h post-dose in all tissues except for the large intestine. The peak levels of radioactivity in the small intestine, stomach, urinary bladder, kidneys, and liver were high, being 6 to 24 times higher than in the plasma. By contrast, peak radioactivity was very low in the brain, being 20 times lower than in the plasma. Tissue levels of radioactivity generally decreased in parallel with that in the plasma. At 168 h post-dose, the radioactivity in the cerebellum was only 5.8% of its peak level, while the radioactivity in the cerebrum, fat, thyroid gland, brown fat, skin, testis, and eyeball were not more than 2.4% of the respective peak levels. Radioactivity in the other tissues/organs did not exceed 0.6% of the peak level (Figure 5, Table 8).

Figure 5. Meant tissue concentrations of radioactivity (modified from the submission)

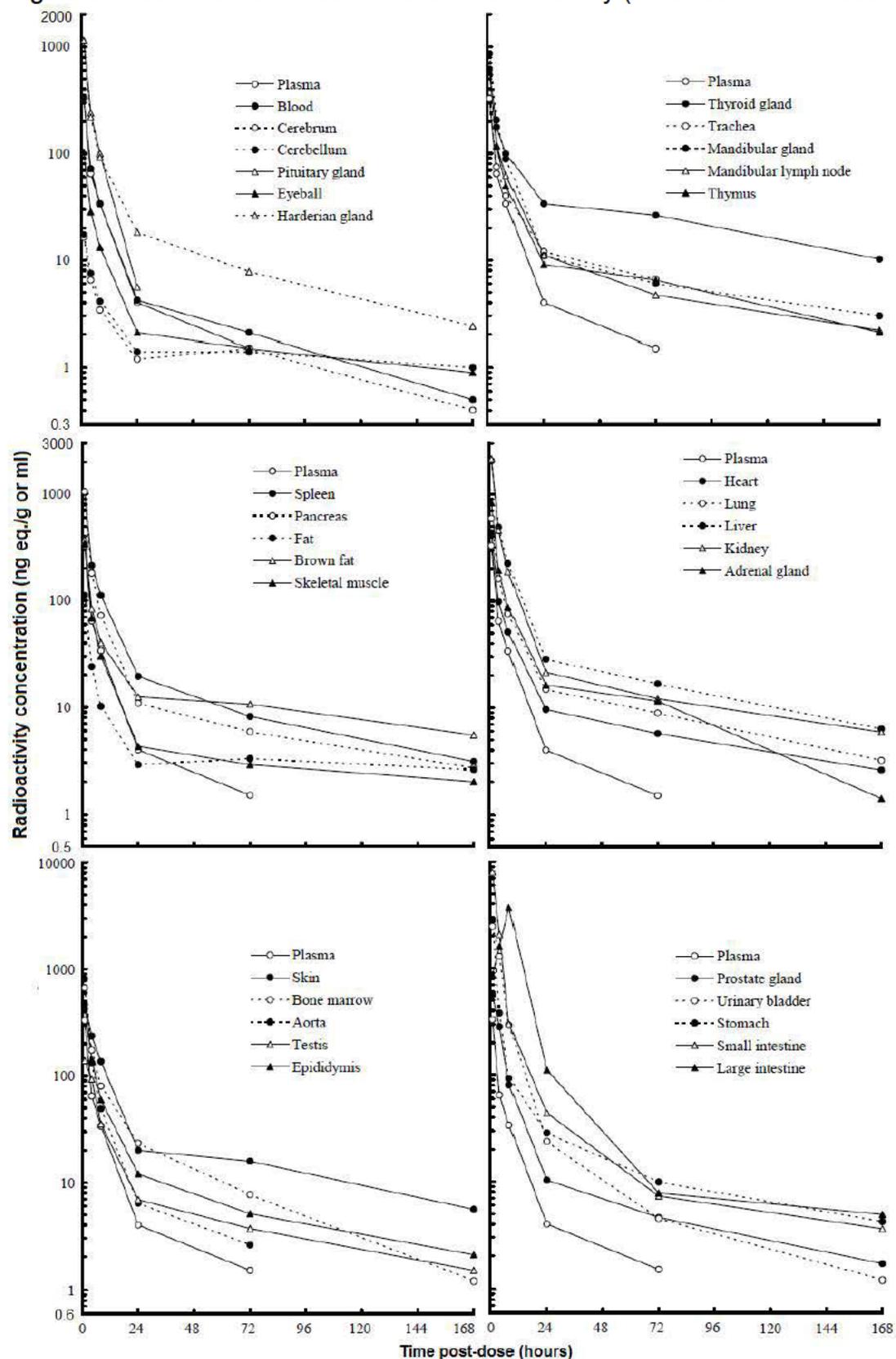


Table 8. Tissue concentrations of radioactivity at 1 (Cmax) and 168 hours (modified from the submission)

| Tissue                | Radioactivity Concentration (ng eq. of DU-176/g or mL) |            | Tissue          | 1 h                          | 168 h     |
|-----------------------|--|------------|-----------------|------------------------------|-----------|
|                       | 1 h  | 168 h      |                 |                              |           |
| Plasma                | 329.6 ± 17.4<br>(1.00)                                 | 0.0 ± 0.0  | Thymus          | 579.5 ± 24.6<br>(1.76)       | 2.1 ± 0.4 |
| Blood                 | 337.8 ± 36.6<br>(1.02)                                 | 0.5 ± 0.8  | Heart           | 435.0 ± 73.7<br>(1.32)       | 2.6 ± 0.4 |
| Cerebrum              | 16.4 ± 1.0<br>(0.05)                                   | 0.4 ± 0.7  | Lung            | 594.2 ± 63.7<br>(1.80)       | 3.2 ± 0.6 |
| Cerebellum            | 17.3 ± 0.9<br>(0.05)                                   | 1.0 ± 0.2  | Liver           | 2059.1 ± 146.0<br>(6.25)     | 6.3 ± 1.0 |
| Pituitary gland       | 1148.8 ± 336.6<br>(3.49)                               | 0.0 ± 0.0  | Kidney          | 2147.0 ± 259.7<br>(6.51)     | 5.9 ± 0.9 |
| Eyeball               | 102.8 ± 11.8<br>(0.31)                                 | 0.9 ± 0.1  | Adrenal gland   | 836.8 ± 101.1<br>(2.54)      | 1.4 ± 2.2 |
| Harderian gland       | 1146.8 ± 159.7<br>(3.48)                               | 2.4 ± 0.8  | Spleen          | 1037.6 ± 147.7<br>(3.15)     | 3.1 ± 0.7 |
| Thyroid gland         | 617.2 ± 112.9<br>(1.87)                                | 10.2 ± 2.4 | Pancreas        | 1052.5 ± 576.0<br>(3.19)     | 2.7 ± 0.4 |
| Trachea               | 326.8 ± 19.4<br>(0.99)                                 | 0.0 ± 0.0  | Fat             | 112.1 ± 1.1<br>(0.34)        | 2.6 ± 0.4 |
| Mandibular gland      | 864.2 ± 104.8<br>(2.62)                                | 3.0 ± 0.6  | Brown fat       | 373.2 ± 33.3<br>(1.13)       | 5.5 ± 1.4 |
| Mandibular lymph node | 581.1 ± 68.6<br>(1.76)                                 | 2.2 ± 0.2  | Skeletal muscle | 341.7 ± 48.8<br>(1.04)       | 2.0 ± 0.5 |
| Skin                  | 470.8 ± 36.4<br>(1.43)                                 | 5.6 ± 2.8  | Prostate gland  | 559.9 ± 149.7<br>(1.70)      | 1.7 ± 0.4 |
| Bone marrow           | 669.7 ± 115.0<br>(2.03)                                | 1.2 ± 2.1  | Urinary bladder | 2475.4 ± 706.2<br>(7.51)     | 1.2 ± 2.1 |
| Aorta                 | 809.5 ± 290.4<br>(2.46)                                | 0.0 ± 0.0  | Stomach         | 2816.0 ± 257.2<br>(8.54)     | 4.2 ± 0.9 |
| Testis                | 138.9 ± 18.7<br>(0.42)                                 | 1.5 ± 0.2  | Small intestine | 8019.3 ± 1694.3<br>(24.33)   | 3.6 ± 0.4 |
| Epididymis            | 456.1 ± 24.7<br>(1.38)                                 | 2.1 ± 0.3  | Large intestine | 3676.1 ± 990.4 *<br>(108.76) | 4.9 ± 0.8 |

\* at 8 hours post-dose

Values in parentheses are expressed as the ratio of the tissue concentration to the plasma concentration.

### 5.1.2.3 The tissue distribution of total radioactivity in the pigmented rat following oral administration of [<sup>14</sup>C]DU-176b (Study R20040290)

Six fasting male Lister Hooded (pigmented) rats (age 8-9 weeks) were orally gavaged with a single dose of [<sup>14</sup>C]-DU-176b 3 mg/kg (11.6 MBq/kg). Rats (1 per each time point) were then euthanized at 1, 8, 24, 96, 168, or 336 hours post-dose. Blood, plasma, eyes, kidneys, liver, muscle, and skin (pigmented and non-pigmented) were collected. Levels of total radioactivity were measured in all samples by liquid scintillation counting.

The highest concentrations of radioactivity were in the liver and kidney at 1 hour post dose, the next highest concentration was in the eyes. From 24 to 336 hours post-dose, the concentration of radioactivity was highest in the eye and next highest in the pigmented skin. The elimination half-life in the eye was 260 hours, suggesting an affinity of DU-176b and/or its metabolite(s) for melanin-containing tissues (Appendix II).

#### 5.1.2.4 Quantitative whole-body autoradiography after a single oral administration of <sup>14</sup>C-DU-176b to juvenile and adult rats (Study AM10-C0055-R01)

Male Brown Norway pigmented rats and male Wistar rats were orally dosed with [<sup>14</sup>C]-DU-176b and subjected to quantitative whole-body autoradiography as the following -

| Species                          | Age                | <sup>14</sup> C-DU-176b dose                           | Number of rat for quantitative whole-body autoradiography at post-dose |       |       |        |
|----------------------------------|--------------------|--|--|-------|-------|--------|
|                                  |                    |  | 1 hr   | 4 hr  | 24 hr | 168 hr |
| Male Wistar rats                 | 4 days (infant)    | A single oral dose of 3 mg/kg (free base, 4.04 MBq/kg) | 1 rat  | 1 rat | 1 rat | 1 rat  |
|                                  | 3 weeks (juvenile) |  | 1 rat  | 1 rat | 1 rat | 1 rat  |
|                                  | 6 weeks (adult)    |  | 1 rat  | 1 rat | 1 rat | 1 rat  |
| Male Brown Norway pigmented rats | 4 days (infant)    |  |  |       | 1 rat | 1 rat  |
|                                  | 3 weeks (juvenile) |  |  |       | 1 rat | 1 rat  |
|                                  | 6 weeks (adult)    |  |  |       | 1 rat | 1 rat  |

In infant albino rats (PND4), at 1 h and 4 h post-dose, the blood concentration was 7.5- and 93.0-fold, respectively, higher than that of adult rats, and overall, tissue concentrations in infant albino rats were higher than those of adult rats. In juvenile albino rats (3 weeks old), at 1 h post-dose, the blood and tissue concentrations were similar to those in adult rats (6 weeks old); however, at 4 h post-dose, the blood concentration was 8.3-fold higher than that of adult rats, and overall, tissue concentrations in juvenile albino rats were higher than those of adult rats. In the pigmented infant and juvenile rats, the long retention of radioactivity in the melanin containing tissues, eyeball and skin (infant rats only), was also observed, which was similar to the adult rats (Table 9, Appendix III).

Table 9. Radioactivity concentrations in rat tissues (modified from the submission)

| Age     | Tissue               | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>b</sub> ) |             |             |             |   |       |                |             |  |
|---------|----------------------|--|-------------|-------------|-------------|---|-------|----------------|-------------|--|
|         |                      | Albino rats <sup>*1</sup> (Wistar)   |             |             |             | Pigmented rats <sup>*2</sup> (Brown Norway) |       |                |             |  |
|         |                      | 1 h  | 4 h         | 24 h        | 168 h       | 24 h  | 168 h |                |             |  |
| 4 days  | Blood                | 2050 (1.00)  | 1850 (1.00) | 166 (1.00)  |             |   |       | 62.8 (1.00)    | N.A.        |  |
|         | Brain                | 139 (0.07)   | 162 (0.09)  | BLQ (N.C.)  |             |   |       | BLQ (N.C.)     | N.A.        |  |
|         | Eyeball              | 471 (0.23)   | 951 (0.51)  | 136 (0.82)  |             |   |       | 21300 (339.17) | 3030        |  |
|         | Heart                | 2290 (1.12)  | 2010 (1.09) | 186 (1.12)  | N.A.        |   |       | 110 (1.75)     | N.A.        |  |
|         | Lung                 | 2140 (1.04)  | 1580 (0.85) | 166 (1.00)  |             |   |       | 93.6 (1.49)    | N.A.        |  |
|         | Liver                | 5850 (2.85)  | 4110 (2.22) | 403 (2.43)  |             |   |       | 197 (3.14)     | N.A.        |  |
|         | Adrenal              | 3820 (1.86)  | 3500 (1.89) | 431 (2.60)  |             |   |       | 143 (2.28)     | N.A.        |  |
|         | Kidney               | 6840 (3.34)  | 3180 (1.72) | 451 (2.72)  | 16.5 (N.C.) |   |       | 262 (4.17)     | BLQ (N.C.)  |  |
|         | Spleen               | 3240 (1.58)  | 2780 (1.50) | 255 (1.54)  | N.A.        |   |       | 157 (2.50)     | N.A.        |  |
|         | Testis               | 1430 (0.70)  | 1970 (1.06) | 204 (1.23)  | N.A.        |   |       | 97.0 (1.54)    | BLQ         |  |
|         | Skin                 | 1470 (0.72)  | 1950 (1.05) | 205 (1.23)  | BLQ         |   |       | 3880 (61.78)   | 1700        |  |
|         | Skeletal muscle      | 1790 (0.87)  | 1960 (1.06) | 172 (1.04)  | N.A.        |   |       | 97.3 (1.55)    | N.A.        |  |
|         | White adipose tissue | 604 (0.29)   | 678 (0.37)  | 49.9 (0.30) | N.A.        |   |       | N.A. (N.C.)    | N.A.        |  |
|         | Brown adipose tissue | 2240 (1.09)  | 2590 (1.40) | 222 (1.34)  | N.A.        |   |       | 117 (1.86)     | N.A.        |  |
| 3 weeks | Blood                | 251 (1.00)   | 165 (1.00)  |             |             |   |       | N.A.           | N.A.        |  |
|         | Brain                | BLQ (N.C.)   | BLQ (N.C.)  |             |             |   |       | N.A.           | N.A.        |  |
|         | Eyeball              | 52.3 (0.21)  | 71.0 (0.43) |             |             |   |       | 2760           | 974         |  |
|         | Heart                | 257 (1.02)   | 165 (1.00)  |             |             |   |       |                |             |  |
|         | Lung                 | 327 (1.30)   | 212 (1.28)  |             |             |   |       |                |             |  |
|         | Liver                | 724 (2.88)   | 515 (3.12)  |             |             |   |       |                |             |  |
|         | Adrenal              | 576 (2.29)   | 273 (1.65)  |             |             |   |       |                |             |  |
|         | Kidney               | 590 (2.35)   | 411 (2.49)  | N.A. (N.C.) | N.A. (N.C.) |   |       | N.A. (N.C.)    | N.A. (N.C.) |  |
|         | Spleen               | 473 (1.88)   | 259 (1.57)  |             |             |   |       |                |             |  |
|         | Testis               | 139 (0.55)   | 199 (1.21)  |             |             |   |       |                |             |  |
|         | Skin                 | 176 (0.70)   | 134 (0.81)  |             |             |   |       |                |             |  |
|         | Skeletal muscle      | 220 (0.88)   | 143 (0.87)  |             |             |   |       |                |             |  |
|         | White adipose tissue | 106 (0.42)   | 39.5 (0.24) |             |             |   |       |                |             |  |
|         | Brown adipose tissue | 216 (0.86)   | 132 (0.80)  |             |             |   |       |                |             |  |
| 6 weeks | Blood                | 275 (1.00)   | 19.9 (1.00) |             |             |   |       | N.A.           | N.A.        |  |
|         | Brain                | BLQ (N.C.)   | BLQ (N.C.)  |             |             |   |       | N.A.           | N.A.        |  |
|         | Eyeball              | BLQ (N.C.)   | BLQ (N.C.)  |             |             |   |       | 889            | 712         |  |
|         | Heart                | 303 (1.10)   | 38.5 (1.93) |             |             |   |       |                |             |  |
|         | Lung                 | 353 (1.28)   | 52.4 (2.63) | N.A.        |             |   |       |                |             |  |
|         | Liver                | 922 (3.35)   | 129 (6.48)  |             |             |   |       |                |             |  |
|         | Adrenal              | 569 (2.07)   | 80.6 (4.05) |             |             |   |       | N.A.           |             |  |
|         | Kidney               | 822 (2.99)   | 195 (9.80)  |             | (N.C.)      | N.A. (N.C.)                                 |       | (N.C.)         | N.A. (N.C.) |  |
|         | Spleen               | 628 (2.28)   | 76.7 (3.85) |             |             |   |       |                |             |  |
|         | Testis               | 107 (0.39)   | 45.1 (2.27) |             |             |   |       |                |             |  |
|         | Skin                 | 261 (0.95)   | 134 (6.73)  | 40.3        |             |   |       | 124            |             |  |
|         | Skeletal muscle      | 244 (0.89)   | N.A. (N.C.) | N.A.        |             |   |       | N.A.           |             |  |
|         | White adipose tissue | 78.5 (0.29)  | N.A. (N.C.) | N.A.        |             |   |       | N.A.           |             |  |
|         | Brown adipose tissue | 226 (0.82)   | N.A. (N.C.) | N.A.        |             |   |       | N.A.           |             |  |

Values in parentheses are expressed as the ratio of the tissue concentration to the blood concentration.

When the radioactivity was not detected by visual observation, the radioactivity was judged as not quantifiable and was not subjected to the calculation and was expressed as N.A. (not applicable).

When the radioactivity concentrations in the blood and/or tissue were N.A. or BLQ, the tissue/blood ratio were not calculated and are shown as N.C. (not calculated).

\*1: BLQ < 31.4 ng eq./g    \*2: BLQ < 30.9 ng eq./g

### 5.1.2.5 DU-176b: Placental transfer after a single oral administration of $^{14}\text{C}$ -DU-176b to pregnant rats (Study R20040804)

Fasted pregnant female rats (Sprague-Dawley, mated at the age of 9 weeks old) were treated with  $^{14}\text{C}$ -DU-176b at a single oral dose of 3 mg/kg (11.8 MBq/kg) on Day 13 or Day 18 of pregnancy. Animals (4/time point) were sacrificed at 0.5, 24, and 48 hours post-dose, and the tissue distribution of radioactivity in pregnant animal and fetuses was investigated by quantification of radioactivity in tissues isolated from animals using a liquid scintillation counter (LSC) (n=3/time point) and by whole-body autoradioluminography (n=1/time point).

Representative whole-body autoradioluminograms are shown in Figure 6, and tissue concentrations of radioactivity determined with whole-body autoradiography are shown in Table 10. Concentrations of radioactivity in tissues isolated from animals determined using LSC are shown in Table 11. Tissue concentrations of radioactivity were higher when measured using whole-body autoradioluminography than using LSC. The levels of radioactivity in most of the tissues markedly declined at 24 hours post-dose, but radioactivity in fetal membrane remained high at 24 and 48 hours post-dose in both rats at pregnancy day 13 (PGD 13) and 18 (PGD 18) (Table 10).

Figure 6. Representative whole-body autoradioluminograms at 30 min post-dose

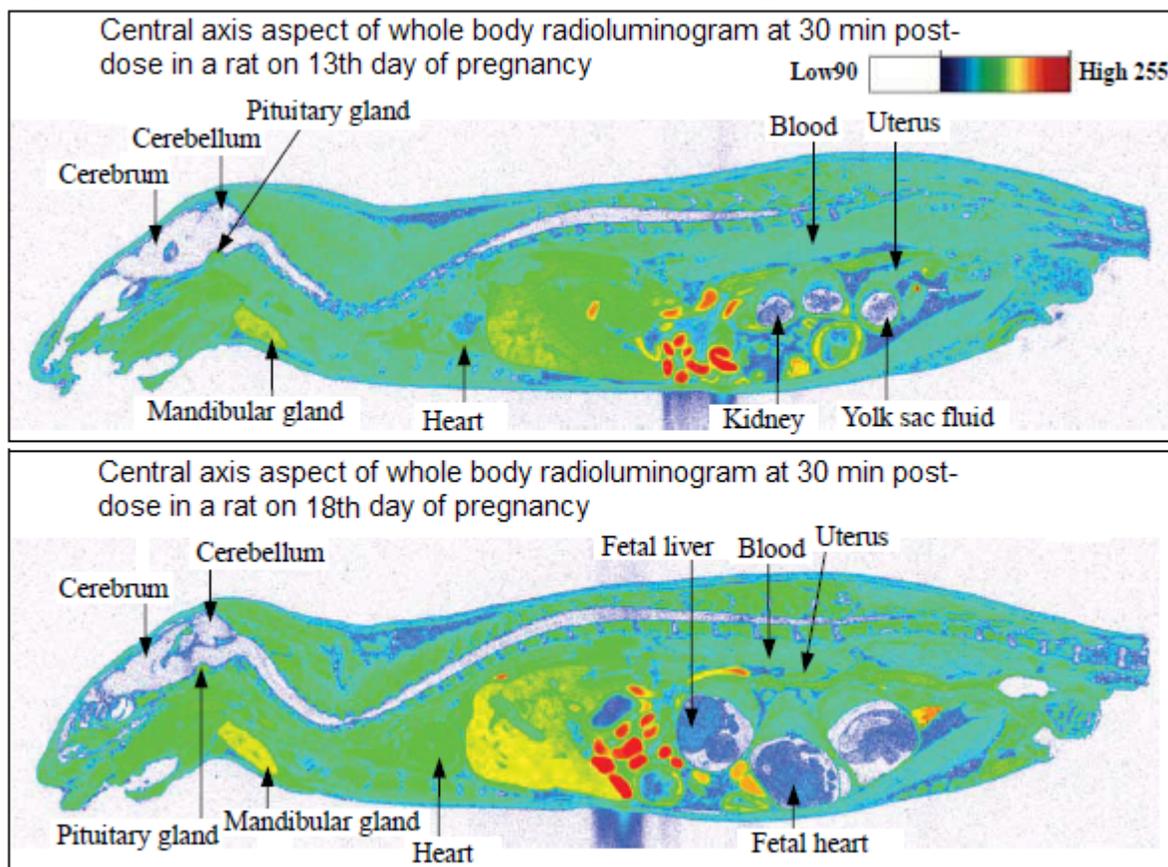


Table 10. Tissue concentrations of radioactivity in pregnant rats determined with whole-body autoradiography (n=1)

| Fasting Rats on the 13th day of pregnancy |   |                  |        | Fasting Rats on the 18th day of pregnancy |   |                  |         |
|---|---|------------------|--------|---|---|------------------|---------|
| Tissue                                    | Radioactivity Concentration (ng eq. of DU-176/g tissue) |                  |        | Tissue                                    | Radioactivity Concentration (ng eq. of DU-176/g tissue) |                  |         |
|   | 30 min  | 24 h             | 48 h   |   | 30 min  | 24 h             | 48 h    |
| Blood                                     | 1303.15 (1.00)  | 12.28 (1.00)     | BLQ    | Blood                                     | 1550.97 (1.00)  | 23.50 (1.00)     | BLQ     |
| Adrenal gland                             | 2441.22 (1.87)  | 33.78 (2.75)     | 25.06  | Adrenal gland                             | 5757.16 (3.71)  | 77.49 (3.30)     | 27.27   |
| Cerebrum                                  | 27.41 (0.02)  | BLQ              | BLQ    | Amniotic fluid                            | 40.78 (0.03)  | BLQ              | BLQ     |
| Cerebellum                                | 45.47 (0.03)  | BLQ              | BLQ    | Cerebrum                                  | 40.15 (0.03)  | BLQ              | BLQ     |
| Fetal membrane                            | 1342.39 (1.03)  | 2108.71 (171.72) | 257.69 | Cerebellum                                | 72.15 (0.05)  | BLQ              | BLQ     |
| Fetus                                     | 231.73 (0.18)   | 15.97 (1.30)     | BLQ    | Fetal blood                               | 492.52 (0.32)   | BLQ              | BLQ     |
| Heart                                     | 2266.18 (1.74)  | 14.74 (1.20)     | BLQ    | Fetal brain                               | 285.47 (0.18)   | BLQ              | BLQ     |
| Kidney                                    | 4043.36 (3.10)  | 27.64 (2.25)     | 33.42  | Fetal heart                               | 394.64 (0.25)   | BLQ              | BLQ     |
| Liver                                     | 3654.04 (2.80)  | 41.77 (3.40)     | 37.91  | Fetal kidney                              | 419.74 (0.27)   | 33.03 (1.41)     | BLQ     |
| Lung                                      | 1979.64 (1.52)  | 18.43 (1.50)     | 9.64   | Fetal liver                               | 574.71 (0.37)   | 25.41 (1.08)     | BLQ     |
| Mammary gland                             | 1434.58 (1.10)  | 18.43 (1.50)     | BLQ    | Fetal lung                                | 479.97 (0.31)   | BLQ              | BLQ     |
| Mandibular gland                          | 6338.82 (4.86)  | 19.04 (1.55)     | BLQ    | Fetal membrane                            | 3835.39 (2.47)  | 2626.33 (111.76) | 3097.42 |
| Ovary                                     | 731.93 (0.56)   | 22.73 (1.85)     | 18.64  | Fetus                                     | 353.86 (0.23)   | 19.05 (0.81)     | 13.63   |
| Pituitary gland                           | 3512.01 (2.70)  | 28.26 (2.30)     | BLQ    | Heart                                     | 2727.38 (1.76)  | 28.58 (1.22)     | BLQ     |
| Placenta                                  | 1091.98 (0.84)  | 21.50 (1.75)     | BLQ    | Kidney                                    | 7673.29 (4.95)  | 94.00 (4.00)     | 44.00   |
| Uterus                                    | 1479.43 (1.14)  | 28.26 (2.30)     | BLQ    | Liver                                     | 10899.46 (7.03)   | 98.45 (4.19)     | 53.92   |
| Yolk sac fluid                            | 82.85 (0.06)  | BLQ              | BLQ    | Lung                                      | 3098.18 (2.00)  | 31.12 (1.32)     | 13.63   |
|   |   |                  |        | Mammary gland                             | 2207.88 (1.42)  | 71.14 (3.03)     | 23.55   |
|   |   |                  |        | Mandibular gland                          | 8286.90 (5.34)  | 47.00 (2.00)     | 15.49   |
|   |   |                  |        | Ovary                                     | 1180.17 (0.76)  | 27.31 (1.16)     | 19.21   |
|   |   |                  |        | Pituitary gland                           | 4758.32 (3.07)  | 34.93 (1.49)     | 42.14   |
|   |   |                  |        | Placenta                                  | 1663.91 (1.07)  | 33.66 (1.43)     | 14.87   |
|   |   |                  |        | Uterus                                    | 2057.92 (1.33)  | 66.06 (2.81)     | BLQ     |

Numbers in parentheses are expressed as the ratio of concentration in tissue relative to maternal blood.

BLQ : below lower limit of quantification.  
(6.65 ng eq. of DU-176/g tissue)

In rats treated on PGD 13 shown in Table 11, the radioactivity at 0.5 h post-dose in the placenta was 1.9-fold higher than that in the plasma, but those in fetal tissues and yolk sac fluid were lower than that in the plasma. At 24 h post-dose, the radioactivity concentration decreased in all tissues, while the concentrations in the uterus, ovary, placenta, and fetal tissues were higher than that in the plasma. At 48 h post-dose, radioactivity decreased in all tissues.

In rats treated on Day 18 of pregnancy, the level of radioactivity at 0.5 h post-dose was 1.6-fold higher in the placenta than in the plasma, while it was lower in the fetal tissues (liver, kidney, heart, lung, blood, and brain) and amniotic fluid than in the plasma. At 24 h post-dose, the radioactivity was lower in all tissues. Radioactivity in the placenta, uterus, ovary, and fetal tissues (liver, kidney, lung, and heart) was higher than that in the plasma. The radioactivity in all tissues was even lower at 48 h post-dose (Table 11).

These findings indicate that DU-176b and/or its metabolite(s) crossed the placenta and distributed to the fetal tissues after administration of a single oral dose of  $^{14}\text{C}$ -DU-176b to pregnant rats.

Table 11. Concentrations of radioactivity in isolated tissues determined using LSC

| Tissue                            | Radioactivity Concentration (ng eq. of DU-176/g or mL, Mean $\pm$ SD, n=3) |                              |                          |                         |
|-----------------------------------|--|------------------------------|--------------------------|-------------------------|
|                                   | 30 min   | 24 h                         | 48 h                     |                         |
| Rats on the 13th day of Pregnancy | Plasma   | 519.96 $\pm$ 261.00 (1.00)   | 9.45 $\pm$ 2.23 (1.00)   | 4.59 $\pm$ 0.96 (1.00)  |
|                                   | Blood  | 593.90 $\pm$ 290.15 (1.14)   | 8.97 $\pm$ 1.50 (0.95)   | 4.60 $\pm$ 0.49 (1.00)  |
|                                   | Cerebrum   | 23.53 $\pm$ 9.25 (0.05)      | 2.42 $\pm$ 0.37 (0.26)   | 1.97 $\pm$ 0.24 (0.43)  |
|                                   | Cerebellum   | 28.64 $\pm$ 12.54 (0.06)     | 2.76 $\pm$ 0.38 (0.29)   | 2.37 $\pm$ 0.57 (0.52)  |
|                                   | Pituitary gland  | 1480.49 $\pm$ 693.28 (2.85)  | 29.75 $\pm$ 6.90 (3.15)  | 15.02 $\pm$ 3.20 (3.27) |
|                                   | Heart  | 797.12 $\pm$ 414.70 (1.53)   | 18.27 $\pm$ 4.56 (1.93)  | 10.51 $\pm$ 1.46 (2.29) |
|                                   | Lung   | 1028.04 $\pm$ 478.21 (1.98)  | 26.25 $\pm$ 5.52 (2.78)  | 13.74 $\pm$ 3.08 (2.99) |
|                                   | Liver  | 3119.40 $\pm$ 1598.13 (6.00) | 55.31 $\pm$ 8.51 (5.85)  | 29.90 $\pm$ 6.11 (6.51) |
|                                   | Kidney   | 2789.76 $\pm$ 1350.54 (5.37) | 36.07 $\pm$ 9.74 (3.82)  | 18.63 $\pm$ 3.60 (4.06) |
|                                   | Adrenal gland  | 1723.04 $\pm$ 878.72 (3.31)  | 37.04 $\pm$ 9.85 (3.92)  | 20.37 $\pm$ 2.89 (4.44) |
|                                   | Uterus   | 529.37 $\pm$ 198.65 (1.02)   | 38.49 $\pm$ 5.20 (4.07)  | 15.66 $\pm$ 4.10 (3.41) |
|                                   | Ovary  | 492.11 $\pm$ 182.15 (0.95)   | 19.99 $\pm$ 5.06 (2.12)  | 14.84 $\pm$ 4.11 (3.23) |
|                                   | Placenta   | 967.90 $\pm$ 450.02 (1.86)   | 65.56 $\pm$ 17.26 (6.94) | 15.58 $\pm$ 2.33 (3.39) |
|                                   | Yolk sac fluid   | 28.36 $\pm$ 12.70 (0.05)     | 6.75 $\pm$ 2.22 (0.71)   | 2.96 $\pm$ 0.91 (0.64)  |
| Fetus                             | 67.53 $\pm$ 23.10 (0.13)   | 13.13 $\pm$ 2.22 (1.39)      | 12.95 $\pm$ 7.08 (2.82)  |                         |
| Rats on the 18th day of Pregnancy | Plasma   | 682.74 $\pm$ 95.82 (1.00)    | 9.60 $\pm$ 2.52 (1.00)   | 4.41 $\pm$ 0.53 (1.00)  |
|                                   | Blood  | 825.74 $\pm$ 110.52 (1.21)   | 7.72 $\pm$ 1.06 (0.80)   | 3.92 $\pm$ 0.46 (0.89)  |
|                                   | Cerebrum   | 31.64 $\pm$ 3.39 (0.05)      | 2.02 $\pm$ 0.28 (0.21)   | 1.68 $\pm$ 0.21 (0.38)  |
|                                   | Cerebellum   | 41.00 $\pm$ 8.07 (0.06)      | 2.45 $\pm$ 0.42 (0.26)   | 2.01 $\pm$ 0.13 (0.46)  |
|                                   | Pituitary gland  | 2204.47 $\pm$ 512.92 (3.23)  | 51.51 $\pm$ 5.79 (5.37)  | 13.18 $\pm$ 3.84 (2.99) |
|                                   | Heart  | 1100.15 $\pm$ 163.16 (1.61)  | 16.41 $\pm$ 1.54 (1.71)  | 12.10 $\pm$ 4.00 (2.74) |
|                                   | Lung   | 1416.04 $\pm$ 174.48 (2.07)  | 20.99 $\pm$ 2.45 (2.19)  | 14.30 $\pm$ 2.24 (3.24) |
|                                   | Liver  | 4070.83 $\pm$ 303.12 (5.96)  | 59.37 $\pm$ 9.43 (6.18)  | 32.62 $\pm$ 2.71 (7.40) |
|                                   | Kidney   | 3462.95 $\pm$ 540.45 (5.07)  | 31.80 $\pm$ 5.38 (3.31)  | 21.04 $\pm$ 2.18 (4.77) |
|                                   | Adrenal gland  | 2654.41 $\pm$ 347.30 (3.89)  | 31.25 $\pm$ 6.34 (3.26)  | 24.15 $\pm$ 3.15 (5.48) |
|                                   | Uterus   | 694.05 $\pm$ 200.85 (1.02)   | 25.54 $\pm$ 7.50 (2.66)  | 22.20 $\pm$ 9.47 (5.03) |
|                                   | Ovary  | 620.69 $\pm$ 64.63 (0.91)    | 16.97 $\pm$ 5.48 (1.77)  | 12.83 $\pm$ 0.27 (2.91) |
|                                   | Placenta   | 1071.25 $\pm$ 204.58 (1.57)  | 40.68 $\pm$ 15.64 (4.24) | 24.08 $\pm$ 7.14 (5.46) |
|                                   | Amniotic fluid   | 19.43 $\pm$ 1.53 (0.03)      | 10.90 $\pm$ 1.00 (1.14)  | 7.58 $\pm$ 1.31 (1.72)  |
|                                   | Fetus  | 113.60 $\pm$ 23.92 (0.17)    | 11.67 $\pm$ 2.95 (1.22)  | 9.58 $\pm$ 0.55 (2.17)  |
|                                   | Fetal blood  | 109.94 $\pm$ 23.10 (0.16)    | 9.64 $\pm$ 2.84 (1.00)   | 7.94 $\pm$ 0.57 (1.80)  |
|                                   | Fetal brain  | 39.56 $\pm$ 5.00 (0.06)      | 7.46 $\pm$ 2.07 (0.78)   | 5.82 $\pm$ 0.24 (1.32)  |
| Fetal heart                       | 158.86 $\pm$ 19.07 (0.23)  | 13.65 $\pm$ 3.65 (1.42)      | 8.59 $\pm$ 0.34 (1.95)   |                         |
| Fetal lung                        | 153.08 $\pm$ 20.72 (0.22)  | 14.99 $\pm$ 3.35 (1.56)      | 10.28 $\pm$ 1.13 (2.33)  |                         |
| Fetal liver                       | 226.83 $\pm$ 41.05 (0.33)  | 22.37 $\pm$ 5.24 (2.33)      | 12.65 $\pm$ 0.31 (2.87)  |                         |
| Fetal kidney                      | 160.58 $\pm$ 26.14 (0.24)  | 16.72 $\pm$ 3.44 (1.74)      | 12.01 $\pm$ 1.04 (2.72)  |                         |

Numbers in parentheses are expressed as the ratio of concentration in tissue relative to plasma.

### 5.1.2.6 DU-176b: In vitro plasma protein binding of <sup>14</sup>C-DU-176b in rats, dogs, monkeys, and humans (Study R20040272)

Fasting blood samples were collected from 3 male Wistar rats (7 weeks old), 3 male beagle dogs (12 months old), 3 male cynomolgus monkey (3 years old), and 3 male humans (25-38 years old). Derived plasma samples were incubated with <sup>14</sup>C-DU176b (final concentration of 0.2, 1, or 5 µg/ml) at 37°C for 10 min. Free <sup>14</sup>C-DU176b was separated using an ultrafiltration device plus centrifugation. Radioactivity in plasma incubations and plasma filtrates was measured by LSC. Ratios of plasma protein binding of radioactivity (%) were calculated by following equation.

$$\text{Protein Binding (\%)} = \left[ 1 - \frac{\text{Radioactivity Concentration in plasma filtrate}}{\text{Radioactivity Concentration in plasma incubation}} \right] \times 100$$

At <sup>14</sup>C-DU-176b concentrations 0.2- 5 µg/ml, the percentage of radioactivity bound to plasma proteins at each <sup>14</sup>C-DU-176b concentration was 31.6% to 34.5% in rats, 44.9% to 46.4% in dogs, 48.0% to 50.2% in cynomolgus monkeys, and 54.3% to 56.6% in humans.

### 5.1.2.7 DU-176b: In vitro distribution of <sup>14</sup>C-DU-176b to blood cells in rats, dogs, monkeys, and humans (Study R20040273)

Fasting blood samples were collected from 3 male Wistar rats (7 weeks old), 3 male beagle dogs (12 months old), 3 male cynomolgus monkey (3 years old), and 3 male humans (25-38 years old), using heparin sodium as the anticoagulant, and incubated with <sup>14</sup>C-DU-176b (final concentration of 0.2, 1, or 5 µg/ml) at 37°C for 10 min. Aliquot of the incubation was centrifuged for obtaining plasma. Radioactivity in blood incubations and plasma was measured by LSC.

In rat, dog, monkey, and human blood samples incubated with <sup>14</sup>C-DU-176b at 0.2, 1, and 5 µg/ml, the distribution of radioactivity to blood cells at each <sup>14</sup>C-DU-176b concentration was 56.3% to 57.8% in rats, 52.3% to 55.4% in dogs, 38.0% to 38.2% in monkeys, and 45.7% to 47.4% in humans.

## 5.1.3 Metabolism

### 5.1.3.1 DU-176b: Metabolism of <sup>14</sup>C-DU-176b in rats and monkeys after a single oral administration (Study R20020973)

Samples of plasma, urine, and feces were collected in studies of absorption (Studies R20020653 and R20020685) and/or excretion (Study R20020654) in rats and Cynomolgus monkeys. The radioactivity in the samples of plasma, urine, and feces was analyzed using an HPLC method. Radioactive compounds were identified based on a comparison to retention times observed for unlabeled DU-176b and several synthetic metabolite standards.

After a single oral administration of 3 mg/kg <sup>14</sup>C-DU-176b to rats, D21-3231 was the main metabolite of DU-176 in plasma. However, unchanged DU-176 was the most abundant compound in both urine (accounting for 73.7% of the total peak area) and feces (accounting for 79.0% of the total peak area) collected within 24 h after administration. D21-3231 was the second-most predominant compound in urine, accounting for 24.5% of the total peak area. In feces collected within 24 h after administration, D21-3231, D21-1402, and an unknown metabolite (RF-3) were observed as the minor metabolites.

After a single oral administration of <sup>14</sup>C-DU-176b 1 mg/kg to monkeys, most of the radioactivity in plasma was associated with unchanged drug at 1 hr post-dose, whereas unidentified metabolites composed most of the radioactivity in plasma at 8 hr post-dose. D21-3231, D21-1402, and several unknown metabolites were detected in monkey urine and feces, although 55-58% of radioactivity in excreta was associated with unchanged drug during the first 24 hr.

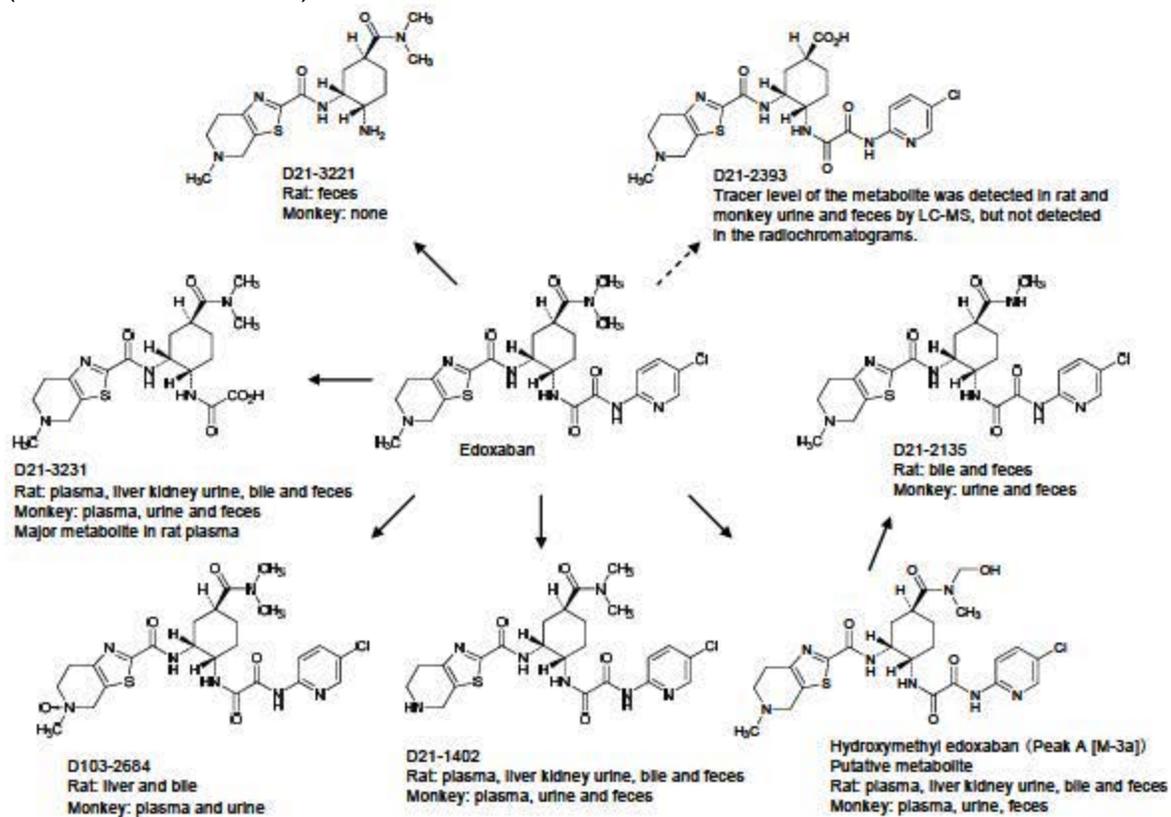
Enzymatic treatment of rat and monkey urine with  $\beta$ -glucuronidase/arylsulfatase did not alter the metabolic profile. These results suggest that <sup>14</sup>C-DU-176 was excreted into urine and feces mainly as the unchanged form in both rats and monkeys (Appendix I).

#### **5.1.3.2 DU-176b: Structure elucidation of <sup>14</sup>C-DU-176b metabolites in urine and feces after a single oral administration to rats and monkeys (Study R20030202)**

Samples of urine and feces were collected in studies of absorption (Study R20020685) and excretion (Study R20020654). Selected reaction monitoring (SRM) of liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis was used to determine the DU-176b metabolites present in urine and feces collected after a single oral administration of <sup>14</sup>C-DU-176b to rats (3 mg/kg) and monkeys (1 mg/kg). Identification of metabolites was based on comparison with the chromatographic retention times and SRM mass chromatograms of available synthetic compounds.

Unchanged DU-176, D21-2135, D21-1402, D21-2393, D21-3221 (feces only), and D21-3231 were found in the urine and feces of both rats and monkeys. LC/MS/MS analysis with radiochemical detection revealed unknown metabolites in the monkey feces. Full-scan and collision-induced dissociation spectra suggested that the unknown metabolite, M-3a, is a hydroxymethyl derivative of DU-176, which is thought to be generated by mono-hydroxylation of the N, N-dimethylamide moiety. A metabolic pathway of DU-176 was proposed in Figure 7 (Appendix I).

Figure 7. Proposed metabolic pathway of DU-176 in rats and cynomolgus monkeys (from the submission)



### 5.1.3.3 DU-176b: Metabolism of DU-176b in male rats after a single oral administration of <sup>14</sup>C-DU-176b (Study R20061350)

Fasted male Wistar rats (7 weeks old) were orally gavaged with <sup>14</sup>C-DU-176b at 3 mg/kg (11.96 MBq/kg). Plasma, urine, feces, bile, liver, and kidney were then collected as indicated in Table 12. Radioactivity levels were determined via liquid scintillation counting. Plasma, bile and urine were directly mixed with scintillation fluid. Liver, kidney and fecal homogenates were first solubilized then mixed with scintillation fluid. Metabolite analyses were performed using LC/MS.

Table 12. Drug administration and sampling schedule

| Group | Test substance          | Dose                 | Volume  | Frequency | Route | Number of animals | Biological sample               | Sampling time |
|-------|-------------------------|----------------------|---------|-----------|-------|-------------------|---------------------------------|---------------|
| 1     | <sup>14</sup> C-DU-176b | 3 mg/kg<br>Free base | 6 mL/kg | Single    | po    | 3                 | Plasma,<br>liver, and<br>kidney | 1 h           |
| 2     |                         |                      |         |           |       | 3                 |                                 | 4 h           |
| 3     |                         |                      |         |           |       | 3                 | Urine and<br>feces              | 0-24 h        |
| 4 *   |                         |                      |         |           |       | 3                 | Bile                            | 0-24 h        |
| 5     |                         |                      |         |           |       | 3                 | Plasma                          | 1 h           |
| 6     |                         |                      |         |           |       | 3                 |                                 | 4 h           |

\* Animals in this group were surgically inserted a cannula into the bile duct prior to drug administration.

As shown in Table 13 and Table 14, DU-176 was the major radioactive component in all plasma, liver, kidney, feces, urine, and bile samples collected post-dose, accounting for 51-72% of the radioactivity in plasma, liver, kidney, feces, and urine samples, and for 36% of the radioactivity in bile samples. Metabolites of next high levels in all samples were D21-3231 and D21-1402. While levels of DU-176, D21-3231 and D21-1402 in plasma at 1 hour post-dose were similar to those at 4 hours post-dose, levels of DU-176 in liver and kidney samples collected at 4 hours post-dose were lower than those at 1 hour post-dose, being opposite to the changes of D21-3231 and D21-1402. No D21-2393 was detected in any of the rat samples. By 24 hours post-dose, total radioactivity excreted into the feces, urine, and bile were about 64, 25, and 23% of dose, respectively.

Table 13. DU-176 and its metabolites in rat tissues samples

| Specimens     | Metabolite     | Proportion of DU-176 metabolite (%) |            | Concentration (ng eq. of DU-176/mL) |               |
|---------------|----------------|-------------------------------------|------------|-------------------------------------|---------------|
|               |                | 1 h                                 | 4 h        | 1 h                                 | 4 h           |
| <b>Plasma</b> | RP1 (D21-3231) | 32.4 ± 5.9                          | 26.7 ± 2.8 | 86.8 ± 24.4                         | 21.8 ± 5.7    |
|               | RP2 (D21-1402) | 0.9 ± 0.1                           | 1.2        | 2.3 ± 0.3                           | 0.9           |
|               | RP3            | 0.8 ± 0.3                           | 1.0        | 2.2 ± 1.0                           | 0.8           |
|               | RP4 (DU-176)   | 50.9 ± 7.1                          | 52.7 ± 6.3 | 133.6 ± 6.9                         | 44.4 ± 19.4   |
|               | D21-3221       | Not detected (ND)                   |            | ND                                  | ND            |
|               | D21-2393       | ND                                  | ND         | ND                                  | ND            |
|               | D21-2135       | ND                                  | ND         | ND                                  | ND            |
|               | Others         | 8.1 ± 1.4                           | 14.4 ± 3.4 |                                     |               |
|               | Total          |                                     |            | 264.8 ± 25.2                        | 82.5 ± 26.2   |
|               | Recovery (%)   | 93.1 ± 1.4                          | 95.3 ± 0.8 |                                     |               |
| <b>Liver</b>  | RL1 (D21-3231) | 4.5 ± 0.8                           | 8.2 ± 0.2  | 78.6 ± 18.2                         | 38.4 ± 3.3    |
|               | RL2            | 0.9 ± 0.3                           | 2.7 ± 0.7  | 16.4 ± 4.8                          | 12.5 ± 4.0    |
|               | RL3 (D21-1402) | 11.1 ± 1.1                          | 11.5 ± 0.5 | 194.1 ± 22.9                        | 53.9 ± 3.1    |
|               | RL4            | 7.4 ± 1.2                           | 4.1 ± 0.7  | 129.6 ± 19.5                        | 19.5 ± 4.6    |
|               | RL5 (DU-176)   | 63.3 ± 2.5                          | 55.5 ± 0.5 | 1106.5 ± 71.2                       | 259.6 ± 19.9  |
|               | D21-3221       | ND                                  | ND         | ND                                  | ND            |
|               | D21-2393       | ND                                  | ND         | ND                                  | ND            |
|               | D21-2135       | ND                                  | ND         | ND                                  | ND            |
|               | Others         | 7.1 ± 0.5                           | 9.4 ± 1.0  |                                     |               |
|               | Total          |                                     |            | 1748.3 ± 96.1                       | 468.0 ± 38.5  |
| Recovery (%)  | 94.3 ± 0.6     | 91.4 ± 0.7                          |            |                                     |               |
| <b>Kidney</b> | RK1 (D21-3231) | 12.8 ± 1.4                          | 15.0 ± 2.5 | 201.1 ± 8.0                         | 73.0 ± 3.3    |
|               | RK2            | ND                                  | 1.6        | ND                                  | 7.0           |
|               | RK3 (D21-1402) | 2.6 ± 0.5                           | 3.6 ± 1.8  | 41.2 ± 11.5                         | 17.0 ± 5.3    |
|               | RK4            | 0.7 ± 0.2                           | ND         | 11.3 ± 4.1                          | ND            |
|               | RK5 (DU-176)   | 71.8 ± 1.3                          | 56.4 ± 5.1 | 1142.5 ± 153.6                      | 284.3 ± 85.4  |
|               | D21-3221       | ND                                  | ND         | ND                                  | ND            |
|               | D21-2393       | ND                                  | ND         | ND                                  | ND            |
|               | D21-2135       | ND                                  | ND         | ND                                  | ND            |
|               | Others         | 5.1 ± 0.4                           | 13.8 ± 2.1 |                                     |               |
|               | Total          |                                     |            | 1588.3 ± 186.8                      | 497.9 ± 103.6 |
| Recovery (%)  | 93.0 ± 0.6     | 89.9 ± 1.1                          |            |                                     |               |

Table 14. DU-176 and its metabolites in rat urine, feces and bile collected over 24 hours post-dose.

| Specimens    | Proportion of DU-176 metabolite (%) | Excretion (% of dose) | Specimens  | Proportion of DU-176 metabolite (%) | Excretion (% of dose) |            |
|--------------|-------------------------------------|-----------------------|------------|-------------------------------------|-----------------------|------------|
| Urine        | Metabolite                          |                       | Metabolite |                                     |                       |            |
|              | RU1 (D21-3231)                      | 19.7 ± 1.0            | 5.0 ± 0.5  | RB1 (D21-3231)                      | 17.5 ± 1.8            | 4.1 ± 1.1  |
|              | RU2 (D21-1402)                      | 1.4 ± 0.1             | 0.4 ± 0.1  | RB2                                 | 1.2 ± 0.2             | 0.3 ± 0.1  |
|              | RU3                                 | 0.6 ± 0.1             | 0.1 ± 0.1  | RB3                                 | 4.2 ± 0.4             | 1.0 ± 0.2  |
|              | RU4 (DU-176)                        | 70.7 ± 3.4            | 17.9 ± 2.3 | RB4                                 | 1.3 ± 0.5             | 0.3 ± 0.2  |
|              | D21-3221                            | Not detected (ND)     | ND         | RB5 (D21-1402)                      | 6.8 ± 2.1             | 1.6 ± 0.9  |
|              | D21-2393                            | ND                    | ND         | RB6                                 | 1.2 ± 0.2             | 0.3 ± 0.1  |
|              | D21-2135                            | ND                    | ND         | RB7                                 | 17.1 ± 3.5            | 3.8 ± 0.3  |
|              | Others                              | 6.6 ± 4.6             |            | RB8 (D21-2135)                      | 1.3 ± 0.2             | 0.3 ± 0.1  |
|              | Total                               |                       | 25.3 ± 2.4 | RB9 (DU-176)                        | 35.5 ± 1.7            | 8.3 ± 2.4  |
| Recovery (%) | 99.0 ± 0.5                          |                       | RB10       | 1.8 ± 0.3                           | 0.4 ± 0.1             |            |
| Feces        | RF1 (D21-3231)                      | 5.8 ± 0.2             | 3.7 ± 0.2  | RB11                                | 1.5 ± 0.5             | 0.3 ± 0.0  |
|              | RF2 (D21-3221)                      | 0.5 ± 0.1             | 0.4 ± 0.1  | D21-3221                            | ND                    | ND         |
|              | RF3 (D21-1402)                      | 3.9 ± 0.4             | 2.5 ± 0.3  | D21-2393                            | ND                    | ND         |
|              | RF4                                 | 5.6 ± 0.1             | 3.6 ± 0.2  | Others                              | 7.2 ± 0.6             |            |
|              | RF5 (D21-2135)                      | 0.9 ± 0.1             | 0.6 ± 0.1  | Total                               |                       | 23.1 ± 5.7 |
|              | RF6 (DU-176)                        | 69.7 ± 1.0            | 44.6 ± 1.0 | Recovery (%)                        | 96.5 ± 1.0            |            |
|              | D21-2393                            | ND                    | ND         |                                     |                       |            |
|              | Others                              | 6.7 ± 0.5             |            |                                     |                       |            |
|              | Total                               |                       | 64.0 ± 2.3 |                                     |                       |            |
|              | Recovery (%)                        | 93.2 ± 1.1            |            |                                     |                       |            |

#### 5.1.3.4 DU-176b: Metabolism of DU-176b in male monkeys after a single oral administration of <sup>14</sup>C-DU-176b (Study R20061351)

Three fasted male cynomolgus monkeys (3 years old) were orally gavaged with <sup>14</sup>C-DU-176b at 1 mg/kg (3.99 MBq/kg). Blood samples were collected at 2 and 4 hours post-dose for obtaining plasma. Urine was collected at 0-8 and 8-48 hours post-dose, and feces was collected at 0-24 and 24-72 hours post-dose. Radioactivity levels were determined via liquid scintillation counting. Plasma and urine were directly mixed with scintillation fluid. Fecal homogenates were first solubilized then mixed with scintillation fluid. Metabolite analyses were performed using LC/MS.

As shown in Table 15, DU-176 was a main radioactive component in the monkey plasma, urine, and feces samples, accounting for 60.7% and 56.0% of the radioactivity in plasma collected at 2 and 4 h post-dose, respectively; accounting for 52.7% and 58.5% of the radioactivity in urine collected at 0–8 and 8–48 h after administration, respectively, and accounting for 34.6% and 27.8% of the radioactivity in feces collected at 0–24 and 24–72 h after administration, respectively. Other major metabolites were MP1, D21-3231, and MP6 in plasma; MU1, MU7, D21-1402, and D21-3231 in urine, and MF5, D21-2135, D21-1402, and D21-3231 in feces.

Table 15. DU-176 and its metabolites in monkey plasma, urine, and feces collected over 24 hours post-dose.

| Specimens    | Metabolite     | Proportion of DU-176 metabolite (% in sample) |            | Concentration (ng eq. of DU-176/mL) |            |
|--------------|----------------|---|------------|-------------------------------------|------------|
|              |                | Sampling time 2 h                             | 4 h        | 2 h                                 | 4 h        |
| Plasma       | MP1            | 7.4   | 5.5        | 12.1                                | 4.5        |
|              | MP2 (D21-3231) | 5.0 ± 2.5                                     | 5.4 ± 2.1  | 8.4 ± 3.8                           | 4.3 ± 1.5  |
|              | MP3            | 0.9 ± 0.4                                     | 1.6        | 1.6 ± 0.9                           | 1.3        |
|              | MP4            | 2.2 ± 0.6                                     | 1.4 ± 0.4  | 4.0 ± 2.0                           | 1.2 ± 0.4  |
|              | MP5 (D21-1402) | 3.9 ± 0.9                                     | 4.6 ± 0.9  | 7.1 ± 3.6                           | 3.7 ± 0.8  |
|              | MP6            | 7.2 ± 1.1                                     | 8.3 ± 1.4  | 12.5 ± 3.3                          | 6.7 ± 0.9  |
|              | MP7 (DU-176)   | 60.7 ± 6.9                                    | 56.0 ± 3.4 | 109.5 ± 42.8                        | 45.4 ± 5.9 |
|              | D21-3221       | ND  | ND         | ND                                  | ND         |
|              | D21-2393       | ND  | ND         | ND                                  | ND         |
|              | D21-2135       | ND  | ND         | ND                                  | ND         |
|              | Others         | 11.7 ± 3.1                                    | 16.3 ± 1.8 |                                     |            |
|              | Total          |   |            | 176.7 ± 51.5                        | 80.9 ± 6.2 |
| Recovery (%) | 96.5 ± 1.5     | 96.8 ± 0.8                                    |            |                                     |            |
| Urine        |                | Sampling time 0-8 h                           | 8-48 h     | 0-8 h                               | 8-48 h     |
|              | MU1            | 17.7 ± 15.0                                   | 8.0 ± 6.1  | 4.8 ± 4.2                           | 1.0 ± 0.8  |
|              | MU2 (D21-3231) | 5.5 ± 1.8                                     | 5.6 ± 0.9  | 1.7 ± 0.8                           | 0.6 ± 0.2  |
|              | MU3            | 0.6 ± 0.2                                     | 0.8 ± 0.3  | 0.2 ± 0.1                           | 0.1 ± 0.0  |
|              | MU4            | 1.1 ± 0.3                                     | 1.2 ± 0.2  | 0.3 ± 0.2                           | 0.1 ± 0.1  |
|              | MU5            | 0.6 ± 0.2                                     | 0.8        | 0.2 ± 0.1                           | 0.1        |
|              | MU6 (D21-1402) | 5.5 ± 2.0                                     | 4.5 ± 0.5  | 1.7 ± 1.0                           | 0.5 ± 0.2  |
|              | MU7            | 9.1 ± 1.7                                     | 6.5 ± 0.7  | 2.8 ± 1.1                           | 0.7 ± 0.3  |
|              | MU8 (D21-2135) | 1.0 ± 0.3                                     | 1.7 ± 0.7  | 0.3 ± 0.2                           | 0.2 ± 0.0  |
|              | MU9 (DU 176)   | 52.7 ± 10.9                                   | 58.5 ± 4.5 | 16.6 ± 7.1                          | 6.6 ± 3.4  |
|              | D21-3221       | ND  | ND         | ND                                  | ND         |
|              | D21-2393       | ND  | ND         | ND                                  | ND         |
| Others       | 5.4 ± 1.7      | 11.9 ± 3.9                                    |            |                                     |            |
| Total        |                |   | 30.6 ± 6.9 | 11.2 ± 4.8                          |            |
| Recovery (%) | 99.2 ± 0.4     | 99.1 ± 0.3                                    |            |                                     |            |
| Feces        |                | Sampling time 0-24 h                          | 24-72 h    | 0-24 h                              | 24-72 h    |
|              | MF1            | 1.2 ± 0.4                                     | 1.2 ± 0.2  | 0.2 ± 0.1                           | 0.3 ± 0.1  |
|              | MF2 (D21-3231) | 4.7 ± 0.8                                     | 4.0 ± 0.8  | 0.9 ± 0.4                           | 1.0 ± 0.3  |
|              | MF3            | 3.7 ± 1.1                                     | 3.8 ± 0.9  | 0.7 ± 0.4                           | 0.9 ± 0.1  |
|              | MF4 (D21-1402) | 12.1 ± 2.8                                    | 13.5 ± 1.3 | 2.2 ± 1.0                           | 3.4 ± 1.1  |
|              | MF5            | 19.7 ± 4.5                                    | 20.6 ± 3.4 | 3.6 ± 1.7                           | 5.2 ± 1.6  |
|              | MF6 (D21-2135) | 5.0 ± 1.3                                     | 7.2 ± 1.1  | 0.9 ± 0.4                           | 1.8 ± 0.6  |
|              | MF7 (DU-176)   | 34.6 ± 11.6                                   | 27.8 ± 8.5 | 6.0 ± 2.2                           | 7.5 ± 3.8  |
|              | D21-3221       | ND  | ND         | ND                                  | ND         |
|              | D21-2393       | ND  | ND         | ND                                  | ND         |
|              | Others         | 9.8 ± 1.1                                     | 11.3 ± 2.1 |                                     |            |
|              | Total          |   |            | 18.0 ± 5.0                          | 25.7 ± 8.6 |
| Recovery (%) | 90.7 ± 1.1     | 89.3 ± 0.3                                    |            |                                     |            |

## 5.1.4 Excretion

### 5.1.4.1 DU-176b: Excretion of radioactivity in urine, feces, and expired air after a single oral administration of <sup>14</sup>C-DU-176b to male rats (Study R20020654)

Three fasted male Wistar rats (7 weeks old) were orally gavaged with <sup>14</sup>C-DU-176b at a dose of 3 mg/kg (11.6 MBq/kg). Radioactivity in feces during 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post-dose, in urine and expired air during 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post-dose, and in carcass at 168 hours post-dose was measured using liquid scintillation counter.

More than 95% of the administered radioactivity was excreted by 24 h post-dose. Most of the administered radioactivity was excreted into urine and feces. During 168 h post-dose, 24.8% of the dose was excreted in the urine, 72.5% in the feces, and 0.3% in the expired air. Only 0.1% of the dose was remained in the carcass at 168 h. The administered radioactivity was excreted mainly in the feces (Table 16).

Table 16. Cumulative excretion of radioactivity in rat urine, feces and expired air during 168 hours after a single oral dose of <sup>14</sup>C-DU-176b (from the submission)

| Time (h)        | Excretion of radioactivity (% of dose, mean ± S.D.) |                |             |                |
|-----------------|---|----------------|-------------|----------------|
|                 | Urine   | Feces          | Expired air | Total          |
| 0 - 4           | 14.1 ± 3.5  | Not determined | 0.1 ± 0.0   | Not determined |
| 8               | 18.9 ± 6.7  | Not determined | 0.2 ± 0.1   | Not determined |
| 24              | 24.5 ± 3.7  | 71.4 ± 1.9     | 0.3 ± 0.1   | 96.2 ± 4.5     |
| 48              | 24.5 ± 3.7  | 72.3 ± 1.5     | 0.3 ± 0.1   | 97.2 ± 4.3     |
| 72              | 24.7 ± 3.7  | 72.4 ± 1.5     | 0.3 ± 0.1   | 97.4 ± 4.3     |
| 96              | 24.7 ± 3.7  | 72.4 ± 1.5     | 0.3 ± 0.1   | 97.5 ± 4.3     |
| 120             | 24.8 ± 3.7  | 72.5 ± 1.5     | 0.3 ± 0.1   | 97.6 ± 4.2     |
| 144             | 24.8 ± 3.7  | 72.5 ± 1.5     | 0.3 ± 0.1   | 97.6 ± 4.2     |
| 168             | 24.8 ± 3.7  | 72.5 ± 1.5     | 0.3 ± 0.1   | 97.6 ± 4.2     |
| Carcass (168 h) |   |                |             | 0.1 ± 0.0      |

### 5.1.4.2 DU-176b: Excretion of radioactivity in bile after a single oral administration of <sup>14</sup>C-DU-176b to the bile duct-cannulated male rats (Study R20040274)

Three fasted male Wistar rats (Wistar, 7 weeks old, with bile duct cannulation) were orally gavaged with <sup>14</sup>C-DU-176b at a dose of 3 mg/kg (11.6 MBq/kg). Radioactivity in bile during 0-1, 1-2, 2-4, 4-8, 8-24, and 24-48 h post-dose, in urine during 0-4, 4-8, 8-24, and 24-48 h post-dose, in feces during 0-24 and 24-48 h post-dose, in gastro-intestinal contents at 48 h post-dose, and in carcasses (except gastro-intestinal contents) at 48 h post-dose was measured using liquid scintillation counting.

Excretion of radioactivity following a single oral dose of  $^{14}\text{C}$ -DU-176b is summarized in Table 17. By 48 h post-dose, 24.9%, 35.0%, and 24.4% of the administered radioactivity was recovered in the bile, urine, and feces, respectively. Thus, the administered radioactivity was excreted into urine more than bile.

Table 17. Excretion of radioactivity after a single oral dose of  $^{14}\text{C}$ -DU-176b to fasting male rats

| Excretion of Radioactivity (% of Dose)* |           |                        |                        | Cumulative Excretion of Radioactivity (% of Dose)* |            |            |            |                    |
|---|-----------|------------------------|------------------------|--|------------|------------|------------|--------------------|
| Time (h)                                | Bile      | Urine                  | Feces                  | Time (h)   | Bile       | Urine      | Feces      | Total              |
| 0 - 1                                   | 1.5 ± 1.2 | not sampled (NS)       | NS                     | 0 - 1  | 1.5 ± 1.2  | NS         | NS         | NA, not applicable |
| 1 - 2                                   | 4.2 ± 1.6 | NS                     | NS                     | 0 - 2  | 5.7 ± 2.8  | NS         | NS         | NA                 |
| 2 - 4                                   | 6.5 ± 0.6 | 9.7 ± 0.7 <sup>a</sup> | NS                     | 0 - 4  | 12.3 ± 2.5 | 9.7 ± 0.7  | NS         | NA                 |
| 4 - 8                                   | 6.8 ± 2.1 | 12.4 ± 1.3             | NS                     | 0 - 8  | 19.0 ± 2.5 | 22.0 ± 0.8 | NS         | NA                 |
| 8 - 24                                  | 3.9 ± 1.9 | 8.1 ± 3.0              | 9.8 ± 6.8 <sup>b</sup> | 0 - 24   | 22.9 ± 3.1 | 30.1 ± 2.4 | 9.8 ± 6.8  | 62.8 ± 6.2         |
| 24 - 48                                 | 2.0 ± 0.1 | 4.9 ± 1.7              | 14.6 ± 2.4             | 0 - 48   | 24.9 ± 3.0 | 35.0 ± 1.6 | 24.4 ± 6.1 | 84.3 ± 4.0         |
| Gastro-intestinal contents (48 h)       |           |                        |                        |  |            |            |            | 15.5 ± 2.0         |
| Carcass (48 h)                          |           |                        |                        |  |            |            |            | 0.6 ± 0.2          |
| Total recovery                          |           |                        |                        |  |            |            |            | 100.3 ± 2.1        |

<sup>a</sup> Time, 0-4 h; <sup>b</sup> Time, 0-24 h. \* Mean ± SD (n = 3)

#### 5.1.4.3 DU-176b: Entero-hepatic circulation after a single oral administration of $^{14}\text{C}$ -DU-176b to male rats (Study R20040275)

Three fasting male rats (Wistar, 7 weeks old) were surgically inserted with bile duct cannula, and then given a single oral dose of  $^{14}\text{C}$ -DU-176b 3 mg/kg (11.6 MBq/kg). Bile was collected for 8 hours from these 3 rats, pooled, and intraduodenally injected to other 3 fasting male rats (Wistar, 7 weeks old, also fitted with bile duct cannula) at 1 ml/animal. From the 3 bile receivers, bile collected during 0-1, 1-2, 2-4, 4-8, 8-24, and 24 -48 h after bile injection, urine collected during 0-4, 4-8, 8-24, and 24-48 h after bile injection, feces collected during 0-24 and 24-48 h after bile injection, gastro-intestinal contents and carcasses (except gastro-intestinal contents) at 48 h after bile injection were determined for radioactivity by an LSC.

Excretion of radioactivity in the bile receivers following an intraduodenal bile injection is summarized in Table 18. By 48 h after intraduodenal injection of the pooled bile sample, radioactivity recovered in bile, urine, and feces of bile receivers was 11.2, 13.2, and 55.7% of dose, respectively. Thus, at least about 24% (11.2% in the bile and 13.2% in the urine) of radioactivity excreted into the bile was reabsorbed in rats.

Table 18. Excretion of radioactivity in rats after an intraduodenal bile injection

| Time (h)                          | Excretion of Radioactivity (% of Dose)* |                        |                         | Time (h) | Cumulative Excretion of Radioactivity (% of Dose)* |            |            |                    |  |
|-----------------------------------|---|------------------------|-------------------------|----------|--|------------|------------|--------------------|--|
|                                   | Bile                                    | Urine                  | Feces                   |          | Bile   | Urine      | Feces      | Total              |  |
| 0 - 1                             | 0.3 ± 0.2                               | not sampled (NS)       | NS                      | 0 - 1    | 0.3 ± 0.2  | NS         | NS         | NA, not applicable |  |
| 1 - 2                             | 0.9 ± 0.4                               | NS                     | NS                      | 0 - 2    | 1.3 ± 0.5  | NS         | NS         | NA                 |  |
| 2 - 4                             | 2.1 ± 0.3                               | 2.4 ± 1.1 <sup>a</sup> | NS                      | 0 - 4    | 3.4 ± 0.5  | 2.4 ± 1.1  | NS         | NA                 |  |
| 4 - 8                             | 3.3 ± 1.6                               | 2.4 ± 0.6              | NS                      | 0 - 8    | 6.7 ± 1.8  | 4.8 ± 0.7  | NS         | NA                 |  |
| 8 - 24                            | 3.3 ± 0.7                               | 5.9 ± 1.7              | 35.1 ± 0.4 <sup>b</sup> | 0 - 24   | 10.0 ± 1.4   | 10.7 ± 2.4 | 35.1 ± 0.4 | 55.8 ± 1.9         |  |
| 24 - 48                           | 1.2 ± 0.8                               | 2.5 ± 0.5              | 20.5 ± 6.5              | 0 - 48   | 11.2 ± 2.2   | 13.2 ± 1.9 | 55.7 ± 6.9 | 80.0 ± 7.7         |  |
| Gastro-intestinal contents (48 h) |   |                        |                         |          |  |            |            | 19.3 ± 6.3         |  |
| Carcass (48 h)                    |   |                        |                         |          |  |            |            | 0.0 ± 0.0          |  |
| Total Recovery                    |   |                        |                         |          |  |            |            | 99.3 ± 2.5         |  |

\* Mean ± SD (n = 3). <sup>a</sup> Time, 0-4 h; <sup>b</sup> Time, 0-24 h.

#### 5.1.4.4 DU-176b: Milk and plasma concentrations of radioactivity after a single oral administration of <sup>14</sup>C-DU-176b to nursing rats (Study R20040803)

Three Sprague-Dawley (SD) female rats on Day 9 of lactation were orally dosed with <sup>14</sup>C-DU-176b at 3 mg/kg (11.8 MBq/kg). Blood and milk samples were collected from the dams at 1, 2, 4, 6, 8, 24, 48 h post-dose. The dams were treated intraperitoneally with 1 U/kg oxytocin at 30 min before the milk-sampling. Radioactivity in the plasma and milk samples was determined using an LSC.

Radioactivity concentrations in milk and plasma are summarized in Table 19. The highest radioactivity concentrations in milk and plasma were at 1 hour post-dose, being 894 and 321 ng eq./ml, respectively. At all time-points, the level of radioactivity in the milk was higher than that in the plasma. The level of radioactivity in the milk decreased over time to reach 53.54 ng eq./mL at 48 hour post-dose. The radioactivity decline in milk was much slower than that in plasma.

Table 19. Milk and plasma concentrations of radioactivity in nursing rats (modified from the submission)

| Time (h) | Radioactivity Concentration (ng eq. of DU-176/mL. Mean ± SD n = 3) |   |        |        |   |       |
|----------|--|---|--------|--------|---|-------|
|          | Milk   |   |        | Plasma |   |       |
| 1        | 894.20   | ± | 505.16 | 321.26 | ± | 15.00 |
| 2        | 827.28   | ± | 277.30 | 181.70 | ± | 18.07 |
| 4        | 627.58   | ± | 90.20  | 64.68  | ± | 8.54  |
| 6        | 526.19   | ± | 180.64 | 46.83  | ± | 11.84 |
| 8        | 406.34   | ± | 134.49 | 32.32  | ± | 2.22  |
| 24       | 259.42   | ± | 60.97  | 10.13  | ± | 0.65  |
| 48       | 53.54  | ± | 40.72  | 1.91   | ± | 1.66  |

#### 5.1.5 Other Studies

### 5.1.5.1 Quantitative whole-body autoradiography after a single intravenous administration of <sup>14</sup>C-D21-2393 to juvenile and adult rats (Study AM10-C0056-R01)

D21-2393 is the main metabolite (M4, submission Section 2.7.2) in the human plasma but not detected in rat and cynomolgus monkey plasma after administration of DU-176b. In this study (Study AM10-C0056-R01), tissue distribution of D21-2393 was investigated in rats following a single IV injection of <sup>14</sup>C- D21-2393 as the following -

| Species                          | Age                | <sup>14</sup> C-D21-2393 dose             | Number of rat for quantitative whole-body autoradiography at post-dose |       |       |        |
|----------------------------------|--------------------|---|--|-------|-------|--------|
|                                  |                    |   | 0.25 hr  | 2 hr  | 24 hr | 168 hr |
| Male Wistar rats                 | 4 days (infant)    | A single IV dose of 1 mg/kg (4.10 MBq/kg) | 1 rat  | 1 rat | 1 rat | 1 rat  |
|                                  | 3 weeks (juvenile) |   | 1 rat  | 1 rat | 1 rat | 1 rat  |
|                                  | 6 weeks (adult)    |   | 1 rat  | 1 rat | 1 rat | 1 rat  |
| Male Brown Norway pigmented rats | 4 days (infant)    |   | 1 rat  | 1 rat | 1 rat |        |
|                                  | 3 weeks (juvenile) |   |  | 1 rat | 1 rat |        |
|                                  | 6 weeks (adult)    |   |  | 1 rat | 1 rat |        |

Adult rats exhibited rapid elimination of radioactivity in the blood and tissues. However, the concentrations of radioactivity in the blood and tissues in infant and juvenile rats were higher than those in adult rats. The radioactivity was still present in the eyeball at 24 h post-dose in infant and juvenile pigmented rats indicating affinity of D21-2393 to melanin-containing tissues (Table 20, Appendix III).

Table 20. Radioactivity concentrations in rat tissues (modified from the submission)

| Age     | Tissue               | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>b</sub> ) |             |             |             |             |             |             |  |                               |             |      |        |
|---------|----------------------|--|-------------|-------------|-------------|-------------|-------------|-------------|--|-------------------------------|-------------|------|--------|
|         |                      | Albino rats (Wistar)   |             |             |             |             |             |             |  | Pigmented rats (Brown Norway) |             |      |        |
|         |                      | 15 min   |             | 2 h         |             | 24 h        |             | 168 h       |  | 2 h                           |             | 24 h |        |
| 4 days  | Blood                | 1990 (1.00)  | 567 (1.00)  |             |             |             |             |             |  | 874 (1.00)                    | N.A.        |      |        |
|         | Brain                | 44.7 (0.02)  | 45.0 (0.08) |             |             |             |             |             |  | 72.1 (0.08)                   | N.A.        |      |        |
|         | Eyeball              | 479 (0.24)   | 280 (0.49)  |             |             |             |             |             |  | 405 (0.46)                    | 120         |      |        |
|         | Heart                | 651 (0.33)   | 189 (0.33)  |             |             |             |             |             |  | 352 (0.40)                    | N.A.        |      |        |
|         | Lung                 | 1450 (0.73)  | 281 (0.50)  |             |             |             |             |             |  | 502 (0.57)                    | N.A.        |      |        |
|         | Liver                | 2570 (1.29)  | 765 (1.35)  |             |             |             |             |             |  | 1100 (1.26)                   | N.A.        |      |        |
|         | Adrenal              | 1110 (0.56)  | 222 (0.39)  |             |             |             |             |             |  | 364 (0.42)                    | N.A.        |      |        |
|         | Kidney               | 2420 (1.22)  | 2920 (5.15) | N.A. (N.C.) |             | N.A. (N.C.) |             |             |  | 735 (0.84)                    | 17.3 (N.C.) |      |        |
|         | Spleen               | 402 (0.20)   | 128 (0.23)  |             |             |             |             |             |  | 171 (0.20)                    | N.A.        |      |        |
|         | Testis               | 986 (0.50)   | 393 (0.69)  |             |             |             |             |             |  | 671 (0.77)                    | N.A.        |      |        |
|         | Skin                 | 1160 (0.58)  | 316 (0.56)  |             |             |             |             |             |  | 535 (0.61)                    | 11.4        |      |        |
|         | Skeletal muscle      | 1110 (0.56)  | 282 (0.50)  |             |             |             |             |             |  | 474 (0.54)                    | N.A.        |      |        |
|         | White adipose tissue | 561 (0.28)   | 131 (0.23)  |             |             |             |             |             |  | N.A. (N.C.)                   | N.A.        |      |        |
|         | Brown adipose tissue | 845 (0.42)   | 255 (0.45)  |             |             |             |             |             |  | 472 (0.54)                    | N.A.        |      |        |
| 3 weeks | Blood                | 539 (1.00)   | N.A.        |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Brain                | 3.68 (N.C.)  | N.A.        |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Eyeball              | 36.6 (0.07)  | N.A.        |             |             |             |             |             |  | 23.4                          | 10.4        |      |        |
|         | Heart                | 153 (0.28)   | N.A.        |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Lung                 | 405 (0.75)   | N.A.        |             |             |             |             |             |  | N.A.                          |             |      |        |
|         | Liver                | 1840 (3.41)  | BLQ         |             |             |             |             |             |  | 8.23                          |             |      |        |
|         | Adrenal              | 231 (0.43)   | N.A.        |             |             |             |             |             |  | N.A.                          |             |      |        |
|         | Kidney               | 603 (1.12)   |             | (N.C.)      |             | N.A. (N.C.) |             | N.A. (N.C.) |  |                               | (N.C.)      | N.A. | (N.C.) |
|         | Spleen               | 92.4 (0.17)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Testis               | 171 (0.32)   |             |             |             |             |             |             |  |                               |             |      |        |
|         | Skin                 | 218 (0.40)   |             |             |             |             |             |             |  |                               |             |      |        |
|         | Skeletal muscle      | 135 (0.25)   |             |             |             |             |             |             |  |                               |             |      |        |
|         | White adipose tissue | 39.8 (0.07)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Brown adipose tissue | 140 (0.26)   | N.A.        |             |             |             |             |             |  | N.A.                          |             |      |        |
| 6 weeks | Blood                | 152 (1.00)   |             |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Brain                | BLQ (N.C.)   |             |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Eyeball              | 20.5 (0.13)  |             |             |             |             |             |             |  | 13.3                          | BLQ         |      |        |
|         | Heart                | 56.0 (0.37)  |             |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |
|         | Lung                 | 126 (0.83)   |             |             |             |             |             |             |  | N.A.                          |             |      |        |
|         | Liver                | 729 (4.80)   |             |             |             |             |             |             |  | BLQ                           |             |      |        |
|         | Adrenal              | 95.6 (0.63)  |             |             |             |             |             |             |  | N.A.                          |             |      |        |
|         | Kidney               | 369 (2.43)   | N.A. (N.C.) |             | N.A. (N.C.) |             | N.A. (N.C.) |             |  |                               | (N.C.)      |      | (N.C.) |
|         | Spleen               | 49.0 (0.32)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Testis               | 94.8 (0.62)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Skin                 | 85.7 (0.56)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Skeletal muscle      | 36.9 (0.24)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | White adipose tissue | 19.9 (0.13)  |             |             |             |             |             |             |  |                               |             |      |        |
|         | Brown adipose tissue | 70.3 (0.46)  |             |             |             |             |             |             |  | N.A.                          | N.A.        |      |        |

Values in parentheses are expressed as the ratio of the tissue concentration to the blood concentration.

When the radioactivity was not detected by visual observation, the radioactivity was judged as not quantifiable and was not subjected to the calculation and was expressed as N.A. (not applicable).

When the radioactivity concentrations in the blood and/or tissue were N.A. or BLQ, the tissue/blood ratio were not calculated and are shown as N.C. (not calculated).

BLQ < 9.92 ng eq./g

### 5.1.5.2 In vitro plasma protein binding of [<sup>14</sup>C]D21-2393 in rats and humans (AM10-C0090-R01)

Fasting blood samples were collected from male Wistar rats (8 weeks old) and male humans (30-49 years old). Derived plasma samples were incubated with <sup>14</sup>C- D21-2393 (final concentration 0.02, 0.2, or 2 µg/ml) at 37°C for 10 min. Protein binding of <sup>14</sup>C-D21-2393 in plasma was assessed by ultrafiltration- LSC.

The adsorption ratios of <sup>14</sup>C-D21-2393 to the ultrafiltration device at <sup>14</sup>C-D21-2393 0.02 µg/ml were about -1.1%, indicating minimal <sup>14</sup>C-D21-2393 adsorption to the ultrafiltration device. In male rat and human plasma samples incubated with <sup>14</sup>C- D21-2393 at 0.02, 0.2, or 2 µg/ml, the mean plasma protein binding ratios of <sup>14</sup>C- D21-2393 at each concentration were 71.2-74.9% and 80.0-81.9%, respectively.

### 5.1.5.3 DU-176b: Pharmacokinetic study in mdr1a/1b knockout mice after a single intravenous administration of <sup>14</sup>C-DU-176b (Study AM07-C0269-R01)

This study (AM07-C0269-R01) was to assess pharmacokinetics of radioactivity and parent compound DU-176 in mdr1a/1b knockout (KO) mice and wild type (WT) mice after a single intravenous administration of <sup>14</sup>C-DU-176b.

Twenty one male FVB mdr-1a/1b KO and 21 male FVB WT mice (7 weeks old) were intravenously dosed with <sup>14</sup>C-DU-176b at 1 mg/kg (3.99 MBq/kg). Blood samples were collected at 5, 15, 30 min, 1, 2, 4, and 8 hours post-dose (n=3/time point). After blood collection at 30 min, 1, and 2 hours post-dose, the brain, liver, and kidney were collected (n=3/time point). The radioactivity concentrations in blood, plasma and tissues were determined using an LSC. The plasma, brain homogenate, liver homogenate, and kidney homogenate were processed and subjected to HPLC analysis. The concentrations of the parent compound (DU-176) in the plasma, brain, liver, and kidney were calculated from the proportion of DU-176 in the sample.

Concentrations of radioactivity in blood, plasma, brain, liver, and kidney of fasting male KO mice and WT mice are shown in Figure 8 and Table 21. Following a single IV dose of <sup>14</sup>C-DU-176b, KO mice had higher concentrations of radioactivity in blood, plasma, brain, liver, and kidney than WT mice. PK parameters of radioactivity were similar between KO and WT mice (Table 22).

Plasma concentrations of DU-176 in KO and WT mice are shown in Table 23. PK parameters of DU-176 following an IV dose of <sup>14</sup>C-DU-176b were similar between KO and WT mice (Table 24). DU-176 concentrations in plasma and tissues, and DU-176 tissue/plasma ratios following an IV dose of <sup>14</sup>C-DU-176b are shown in Table 25. No marked differences in DU-176 tissue/plasma ratios between KO and WT mice were identified in the liver and kidney. However, the DU-176 brain/plasma ratios in KO mice (0.6-1.8) were markedly higher than in WT mice (0-0.11). Thus, DU-176 was proved to be a substrate of mdr1, since the transfer to the brain in KO mice was higher than that in WT mice. The contribution of mdr1 in the elimination processes of DU-176 was

suggested to be minor, since no remarkable differences in the  $t_{1/2}$  and  $CL_{tot}$  of DU-176 were identified between KO and WT mice.

Figure 8. Blood and plasma radioactivity in mice following an IV dose of  $^{14}C$ -DU-176 (modified from the submission)

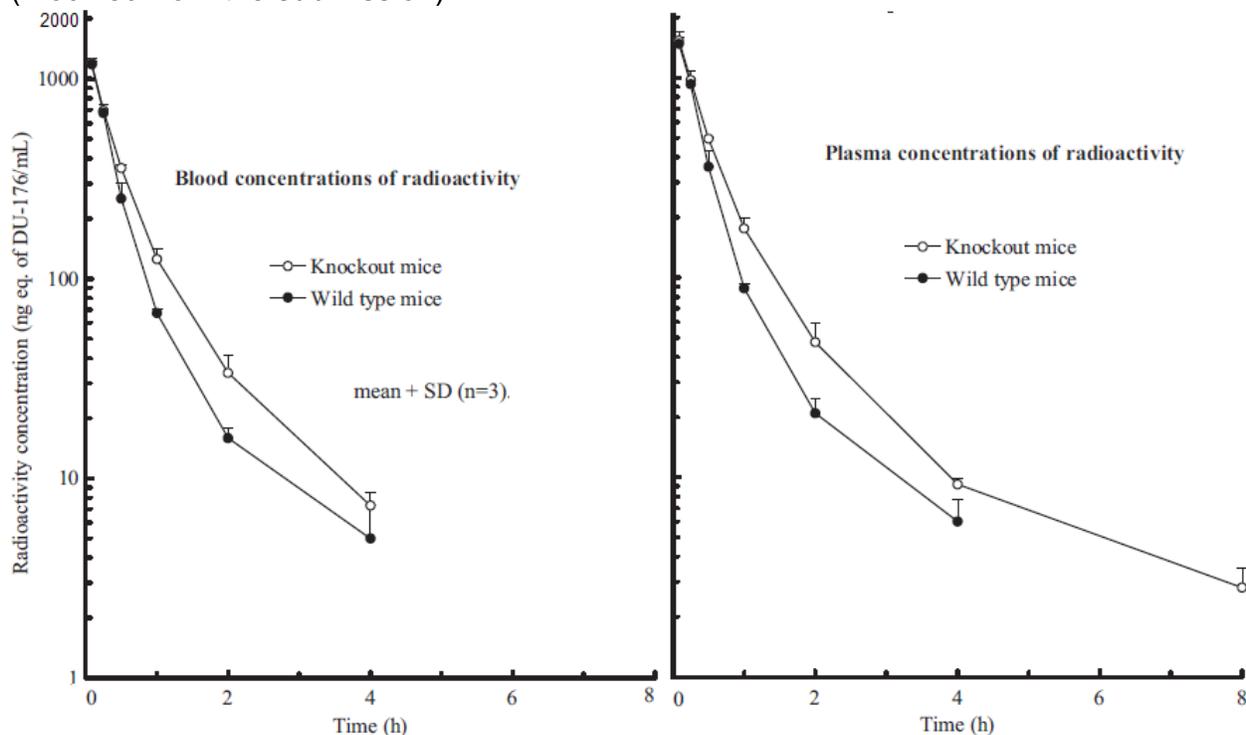


Table 21. Radioactivity concentrations in mouse tissues following an IV dose of  $^{14}C$ -DU-176b

| Tissue | Radioactivity Concentration (ng eq. of DU-176/g, Mean ± SD, n=3) |              |            |                     |             |              |
|--------|--|--------------|------------|---------------------|-------------|--------------|
|        | Male Knockout Mice   |              |            | Male Wild Type Mice |             |              |
|        | 0.5 h  | 1 h          | 2 h        | 0.5 h               | 1 h         | 2 h          |
| Brain  | 63.0±5.6   | 39.8±3.3     | 13.8±7.0   | 21.0±6.7            | 5.2±0.5     | Not detected |
| Liver  | 1964.8±153.1   | 780.9±128.5  | 319.8±87.7 | 1247.0±291.7        | 407.8±61.7  | 103.5±6.7    |
| Kidney | 2086.1±158.4   | 1769.2±813.1 | 372.7±74.9 | 1292.9±399.5        | 717.1±200.8 | 209.4±80.4   |
| Tissue | Radioactivity Content (% of Dose, Mean ± SD, n=3)                |              |            |                     |             |              |
|        | Male Knockout Mice   |              |            | Male Wild Type Mice |             |              |
|        | 0.5 h  | 1 h          | 2 h        | 0.5 h               | 1 h         | 2 h          |
| Brain  | 0.11±0.01  | 0.07±0.02    | 0.02±0.02  | 0.04±0.01           | 0.01±0.00   | ND           |
| Liver  | 8.11±0.90  | 3.28±0.70    | 1.38±0.43  | 5.65±1.68           | 1.70±0.37   | 0.42±0.03    |
| Kidney | 2.55±0.23  | 2.10±1.01    | 0.46±0.09  | 1.73±0.74           | 0.82±0.23   | 0.27±0.13    |

Table 22. PK parameters of radioactivity in mice following an IV dose of  $^{14}\text{C}$ -DU-176b

| PK Parameter                             | Knockout Mice   |                 |                | Wild Type Mice  |                 |
|--|-----------------|-----------------|----------------|-----------------|-----------------|
|  | Blood           | Plasma          |                | Blood           | Plasma          |
| $t_{1/2}$ (h)                            | 0.75<br>(1-4 h) | 0.72<br>(1-4 h) | 1.2<br>(1-8 h) | 0.84<br>(1-4 h) | 0.81<br>(1-4 h) |
| $C_0$ (ng eq./mL)                        | 1572.8          | 1925.0          |                | 1558.4          | 1861.9          |
| $AUC_{0\text{-last}}$<br>(ng eq.·h/mL)   | 645<br>(0-4 h)  | 896<br>(0-8 h)  |                | 527<br>(0-4 h)  | 693<br>(0-4 h)  |
| $AUC_{0\text{-}\infty}$<br>(ng eq.·h/mL) | 653             | 901             |                | 533             | 700             |
| $CL_{\text{tot}}$ (mL/min/kg)            | 25.5            | 18.5            |                | 31.3            | 23.8            |
| $Vd_{\text{ss}}$ (L/kg)                  | 0.891           | 0.786           |                | 0.861           | 0.652           |

Numbers in parentheses represent the time ranges for calculation.

Table 23. Plasma DU-176 concentration in mice following an IV dose of  $^{14}\text{C}$ -DU-176

| Time (h)        | Proportion of DU-176 (% in sample, Mean $\pm$ SD, n=3) |                            |                |                            | Plasma Concentration<br>(ng of DU-176/mL, Mean $\pm$ SD, n=3) |                  |
|-----------------|--|----------------------------|----------------|----------------------------|---|------------------|
|                 | Knockout Mice  |                            | Wild Type Mice |                            | Knockout Mice   | Wild Type Mice   |
|                 | Plasma   | Recovery (%) <sup>2)</sup> | Plasma         | Recovery (%) <sup>2)</sup> |   |                  |
| 0.0833          | 30.2 $\pm$ 12.2  | 77.9 $\pm$ 1.9             | 31.5 $\pm$ 3.1 | 75.6 $\pm$ 3.1             | 453.2 $\pm$ 141.2   | 463.9 $\pm$ 47.7 |
| 0.25            | 19.6 $\pm$ 1.6   | 76.8 $\pm$ 2.4             | 15.5 $\pm$ 1.6 | 78.9 $\pm$ 0.7             | 189.7 $\pm$ 10.8  | 143.2 $\pm$ 13.7 |
| 0.5             | 9.6 $\pm$ 2.4  | 73.1 $\pm$ 6.6             | 6.3 $\pm$ 3.6  | 77.2 $\pm$ 0.1             | 47.7 $\pm$ 12.1   | 23.9 $\pm$ 18.1  |
| 1               | 8.9 $\pm$ 1.2  | 77.5 $\pm$ 0.6             | 4.4 $\pm$ 0.6  | 69.7 $\pm$ 7.6             | 15.5 $\pm$ 0.6  | 3.8 $\pm$ 0.6    |
| 2 <sup>1)</sup> | 3.2  | 64.3                       | 3.0            | 55.1                       | 1.5   | 0.6              |
| 4 <sup>1)</sup> | Not detected.  | 66.3                       | ND             | 60.1                       | ND  | ND               |
| 8 <sup>1)</sup> | ND   | ND                         | ND             | ND                         | ND  | ND               |

<sup>1)</sup>: Data are determined from the pooled plasma samples of three animals.

<sup>2)</sup>: Recovery of radioactivity extracted from the plasma.

Table 24. PK parameters of DU-176 in mice following an IV dose of  $^{14}\text{C}$ -DU-176b

| PK Parameter                      | Knockout Mice  | Wild Type Mice |
|-----------------------------------|----------------|----------------|
| $t_{1/2}$ (h)                     | 0.30 (0.5-2 h) | 0.29 (0.5-2 h) |
| $C_0$ (ng/mL)                     | 700.3          | 834.7          |
| $AUC_{0\text{-last}}$ (ng·h/mL)   | 156 (0-2 h)    | 135 (0-2 h)    |
| $AUC_{0\text{-}\infty}$ (ng·h/mL) | 156            | 135            |
| $CL_{\text{tot}}$ (mL/min/kg)     | 107            | 124            |
| $Vd_{\text{ss}}$ (L/kg)           | 1.57           | 1.14           |

Numbers in parentheses represent the time ranges for calculation.

Table 25. DU-176 concentrations in plasma and tissues and DU-176 tissue/plasma ratios following an IV dose of <sup>14</sup>C-DU-176b

| Concentration (ng of DU-176/g or mL, Mean ± SD, n=3) |                                |                                |                                |                                  |                                 |                              |
|--|--------------------------------|--------------------------------|--------------------------------|----------------------------------|---------------------------------|------------------------------|
| Tissue   | Male Knockout Mice             |                                |                                | Male Wild Type Mice              |                                 |                              |
|  | 0.5 h                          | 1 h                            | 2 h                            | 0.5 h                            | 1 h                             | 2 h                          |
| Plasma   | 47.7 ± 12.1<br>(1.000)         | 15.5 ± 0.6<br>(1.000)          | 1.5*<br>(1.000)                | 23.9 ± 18.1<br>(1.000)           | 3.8 ± 0.6<br>(1.000)            | 0.6*<br>(1.000)              |
| Brain  | 29.0*<br>(0.608)               | 17.9*<br>(1.155)               | 2.7*<br>(1.800)                | 0.8*<br>(0.033)                  | 0.4*<br>(0.105)                 | ND*<br>(0.000)               |
| Liver  | 550.7 ± 141.9<br>(11.5 ± 0.88) | 195.3 ± 51.8<br>(12.6 ± 3.8)   | 39.2 ± 15.1<br>(26.16 ± 10.06) | 207.4 ± 66.2<br>(10.518 ± 3.748) | 55.0 ± 12.7<br>(14.427 ± 3.387) | 10.3 ± 3.1<br>(17.17 ± 5.2)  |
| Kidney   | 390.4 ± 155.6<br>(7.97 ± 1.4)  | 219.1 ± 97.3<br>(14.04 ± 6.05) | 20.2 ± 9.7<br>(13.44 ± 6.446)  | 205.7 ± 84.2<br>(10.09 ± 3.34)   | 47.7 ± 30.6<br>(11.968 ± 6.044) | 12.0 ± 4.1<br>(20.06 ± 6.82) |

\* Data from the pooled samples of three animals.

Numbers in parentheses are expressed as the ratio of the concentration in tissue relative to plasma.

#### 5.1.5.4 DU-176b: Quantitative whole-body autoradiography in *mdr1a/1b* knockout mice after a single intravenous administration of <sup>14</sup>C-DU-176b (AM07-C0270-R01)

This study (AM07-C0270-R01) was to assess the tissue distribution of radioactivity in *mdr1a/1b* KO mice and WT mice after an IV dose of <sup>14</sup>C-DU-176b.

Three male FVB *mdr-1a/1b* KO and 3 male FVB WT mice (8 weeks old) were injected IV with <sup>14</sup>C-DU-176b at 1 mg/kg (3.99 MBq/kg). Animals (1 KO and 1 WT mouse/time point) were sacrificed at 0.5, 1, and 2 hours post-dose, and tissue distribution of radioactivity in these mice was determined by quantitative whole-body autoradiography.

Representative whole-body autoradiograms are shown in Figure 9, and tissue concentrations of radioactivity determined with whole-body autoradiography are shown in Table 26. Following an IV dose of <sup>14</sup>C-DU-176b at 1 mg/kg, radioactivity in bile of both KO and WT mice at 0.5, 1, and 2 hours post-dose, in urine of KO mice at 0.5 and 1 hour post-dose and in urine of WT mice at 0.5 hour post-dose, in small intestine contents of both KO and WT mice at 0.5 and 1 hour post-dose, and in large intestine contents of both KO and WT mice at 2 hours post-dose was above the upper limit of quantification of 12866.7 ng eq. of DU-176/g tissue, indicating large amount of radioactivity was rapidly excreted into bile, urine and feces. Radioactivity in small intestine contents of WT mice at 2 hours post-dose and in large intestine contents of WT mice at 0.5 and 1 hour post-dose was higher than those of KO mice, indicating more excretion of radioactivity into feces in WT mice than in KO mice. Radioactivity in all tissues except prostate gland was higher in KO mice than in WT mice.

No marked difference of tissue/blood radioactivity ratios between KO and WT mice was identified in the liver and kidney. The tissue/blood radioactivity ratios in the cerebrum, cerebellum, and adrenal gland of KO mice at 0.5 h post-dose were 6.0, 3.8, and 1.8 times those in WT mice. These findings indicated that DU-176 is a substrate of *mdr1a/1b* (mouse P-gp) and that brain efflux of DU-176 is regulated by *mdr1a/1b*.

Figure 9. Representative whole-body autoradiograms at 30 min post-dose

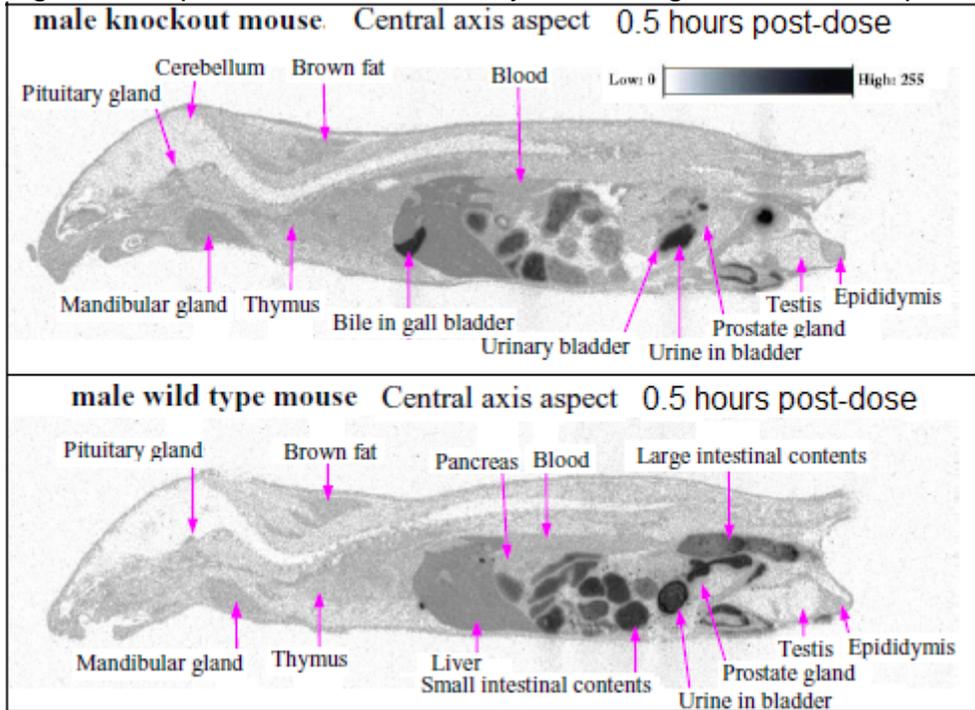


Table 26. Tissue concentrations of radioactivity (ng eq. of DU-176/g tissue) after an IV <sup>14</sup>C-DU-176b dose (modified from the submission)

| Tissue                    | Fasting Male Knockout Mice |              |         | Fasting Male Wild Type Mice |                |         |
|---------------------------|----------------------------|--------------|---------|-----------------------------|----------------|---------|
|                           | 0.5 h                      | 1 h          | 2 h     | 0.5 h                       | 1 h            | 2 h     |
| Blood                     | 733.5 (1.00)               | 200.7 (1.00) | -       | 483.3 (1.00)                | 106.1 (1.00)   | -       |
| Cerebrum                  | 86.8 (0.12)                | 52.6 (0.26)  | BLQ     | 8.5 (0.02)                  | BLQ            | BLQ     |
| Cerebellum                | 108.1 (0.15)               | 42.9 (0.21)  | BLQ     | 18.4 (0.04)                 | BLQ            | BLQ     |
| Pituitary gland           | 744.2 (1.01)               | 307.9 (1.53) | 148.5   | 432.1 (0.89)                | 302.3 (2.85)   | 94.5    |
| Eyeball                   | 162.9 (0.22)               | 63.6 (0.32)  | -       | 103.1 (0.21)                | 40.9 (0.39)    | -       |
| Harderian gland           | 875.0 (1.19)               | 179.9 (0.90) | -       | 330.9 (0.68)                | 125.9 (1.19)   | -       |
| Mandibular gland          | 795.6 (1.08)               | 220.2 (1.10) | 64.1    | 409.1 (0.85)                | 86.9 (0.82)    | 53.9    |
| Thymus                    | 523.3 (0.71)               | 155.9 (0.78) | -       | 292.9 (0.61)                | 65.8 (0.62)    | -       |
| Heart                     | 474.5 (0.65)               | 120.2 (0.60) | 24.3    | 216.0 (0.45)                | 50.5 (0.48)    | 26.6    |
| Lung                      | 663.4 (0.90)               | 202.6 (1.01) | 60.1    | 407.8 (0.84)                | 90.8 (0.86)    | 49.2    |
| Liver                     | 2000.3 (2.73)              | 732.0 (3.65) | 178.2   | 1596.9 (3.30)               | 446.7 (4.21)   | 151.1   |
| Kidney                    | 2846.6 (3.88)              | 887.8 (4.42) | 281.5   | 1499.7 (3.10)               | 863.5 (8.14)   | 214.9   |
| Adrenal gland             | 1222.1 (1.67)              | 445.5 (2.22) | -       | 442.6 (0.92)                | 129.7 (1.22)   | -       |
| Spleen                    | 612.7 (0.84)               | 184.5 (0.92) | 43.9    | 309.3 (0.64)                | 56.2 (0.53)    | -       |
| Pancreas                  | 738.8 (1.01)               | 328.0 (1.63) | 187.0   | 429.4 (0.89)                | 127.8 (1.20)   | 112.5   |
| Bile in gall bladder      | AUQ                        | AUQ          | AUQ     | AUQ                         | AUQ            | AUQ     |
| Fat                       | 56.1 (0.08)                | 27.3 (0.14)  | -       | 44.7 (0.09)                 | -              | -       |
| Brown fat                 | 531.9 (0.73)               | 368.9 (1.84) | 163.3   | 399.9 (0.83)                | 84.4 (0.80)    | 55.9    |
| Skeletal muscle           | 253.0 (0.34)               | 63.6 (0.32)  | 20.9    | 120.2 (0.25)                | 23.6 (0.22)    | -       |
| Skin                      | 450.5 (0.61)               | 115.6 (0.58) | -       | 306.0 (0.63)                | 57.5 (0.54)    | -       |
| Bone marrow               | 476.5 (0.65)               | -            | -       | 245.6 (0.51)                | -              | -       |
| Testis                    | 133.5 (0.18)               | 121.5 (0.61) | 44.5    | 61.1 (0.13)                 | 27.5 (0.26)    | 18.0    |
| Epididymis                | 359.7 (0.49)               | 226.7 (1.13) | 110.0   | 318.5 (0.66)                | 145.1 (1.37)   | 68.5    |
| Prostate gland            | 518.6 (0.71)               | -            | -       | 581.1 (1.20)                | 232.6 (2.19)   | -       |
| Urinary bladder           | 1136.6 (1.55)              | 544.9 (2.71) | -       | 1528.0 (3.16)               | -              | -       |
| Urine in bladder          | AUQ                        | AUQ          | 11576.2 | AUQ                         | 3771.4 (35.55) | 1008.2  |
| Stomach                   | 523.3 (0.71)               | 228.6 (1.14) | -       | 342.1 (0.71)                | 77.3 (0.73)    | -       |
| Gastric contents          | 1705.3 (2.32)              | 511.8 (2.55) | 78.3    | 1520.1 (3.15)               | 889.0 (8.38)   | 190.3   |
| Small intestinal contents | AUQ                        | AUQ          | 4065.5  | AUQ                         | AUQ            | 10515.5 |
| Large intestinal contents | 1079.2 (1.47)              | 667.0 (3.32) | AUQ     | 7262.9 (15.03)              | 6429.5 (60.60) | AUQ     |

Numbers in parentheses are expressed as the ratio of the concentration in tissue relative to blood. -: Not determined.

AUQ: above the upper limit of quantification of 12866.7 ng eq. of DU-176/g tissue.

BLQ: below the lower limit of quantification of 6.5 ng eq. of DU-176/g tissue.

## 5.2 Toxicokinetics

Toxicokinetics (TK) was included in the toxicity studies.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

#### 6.1.1 D11-4176b: Single oral toxicity in cynomolgus monkeys (R20020307, R20020282)

This GLP study (R20020307) was conducted with D11-4176b (lot # CZ001; purity 100.3%) in Daiichi, Tokyo, Japan during May 13 - Oct 4, 2002, and previously reviewed by Dr. David B. Joseph of DGIE (April 4, 2007) under IND063266 (Appendix I)

Female monkeys (age 4-5 years, 2.7-3.4 kg) were treated with a single oral dose of vehicle (0.5% methyl cellulose solution, MC) or D11-4176b at 200 or 400 mg/kg (n=2 /group). The animals were observed for 14 days. Dose selection in this study was based on no toxicological findings in a preliminary study with an oral D11-4176b dose at 200 or 400 mg/kg (n=1/dose level) in cynomolgus monkeys (**Study R20020282**).

Slight reductions in red blood cell counts, hematocrit, hemoglobin, and platelet count were observed through 14 days post-dose in monkeys at D11-4176b 400 mg/kg. Anticoagulant activity was detected at D11-4176b 200 and 400 mg/kg. No limiting toxicity occurred at doses of up to 400 mg/kg. A minimum lethal dose was not established, and this study was deficient in low monkey numbers.

#### 6.1.2 D11-4176b: Single oral toxicity in rats (R20020310)

This GLP study (R20020310) was conducted with D11-4176b (lot # CZ001; purity 100.3%) in Daiichi, Tokyo, Japan during May 14 - Oct 21, 2002, and previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND063266 (Appendix I).

Male and female Crj:CD(SD)IGS rats (6 weeks old) were orally gavaged with a single dose of vehicle (0.5% MC) or D11-4176b at 1000 or 2000 mg/kg (n=5/sex /group). The animals were observed for 15 days. Dose selection in this study was based on no death and abnormal clinical signs in a preliminary bone marrow micronucleus test in rats with oral D11-4176b 2000 mg/kg/day or IV D11-4176b 100 mg/kg/day for 2 days (**Study R20020063**).

A minimum lethal dose was not identified. No adverse effects were observed at oral doses up to D11-4176b 2000 mg/kg.

### 6.2 Repeat-Dose Toxicity

#### 6.2.1 D11-4176b: Four-week oral toxicity in rats (Study R20020612)

This GLP study (R20020612) was conducted with D11-4176b (lot # CZ002; purity 100.6%) in (b) (4) during Apr 24 – Dec 10, 2002, and

previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND063266 (Appendix I).

CD(SD)IGS rats (7 weeks old) were orally gavaged with vehicle (0.5% MC), or D11-4176b at 20, 60, or 200 mg/kg/day for 4 weeks (10 rats/sex/group for toxicity part, and 6 rats/sex/each D11-4176b group for TK part). The dose levels in this study were selected based on a preliminary 2-week oral toxicity study in male rats with D11-4176b at 20, 50, and 200 mg/kg/day (**Study R20010606**). In study R20010606, the only adverse finding was crystalluria (derived from D11-4176b) in all rats at D11-4176b doses  $\geq 20$  mg/kg/day. In the present study, animals were checked for mortality, clinical signs, ophthalmic changes, body weight, food consumption, TKs, clinical pathology, organ weight, and macroscopic and microscopic (in all rats of toxicity part) changes.

Findings included a small number of lesions occurred in the lungs, pancreas, and thymus: focal pneumonitis with hemoglobin crystals and/or phagocytosis of erythrocytes in 1/10 males and 1/10 females at 200 mg/kg/day, and in 2/10 males at 60 mg/kg/day; focal hemorrhage and chronic inflammation in the pancreas of 1/10 males at each dose level; and focal hemorrhage with hemoglobin crystals in the thymus of 1/10 females at 200 mg/kg/day. The significance of these lesions was unclear, since the incidence was low (1-3 out of 20 rats) and was not dose-dependent. In a follow-up 4-week oral toxicity study in male rats (**Study R20030041**) using lower dose D11-4176b, the same lesions occurred without any sign of dose-dependency. Therefore, these lesions were not considered as drug-related in the present study. The NOAEL in this study was 200 mg/kg/day.

After oral administration, D11-4176b was readily absorbed with a  $T_{max}$  of 1 hour. System exposure to D11-4176 ( $C_{max}$  and  $AUC_{0-24}$ ) increased with the dose increases in an under dose-proportional manner. Values for  $C_{max}$  and  $AUC_{0-24}$  on day 28 were about 2 times those on day 1, indicating accumulation of the test article after multiple dosing (Table 27).

Table 27. Summary of TK parameters

| Dose Level<br>(mg/kg/day) | Gender | Day 1                |                      |                           |                            | Day 28               |                      |                           |                            |
|---------------------------|--------|----------------------|----------------------|---------------------------|----------------------------|----------------------|----------------------|---------------------------|----------------------------|
|                           |        | $C_{max}$<br>(ng/mL) | $T_{max}$<br>(Hours) | $AUC_{0-t}$<br>(ng·hr/mL) | $AUC_{0-24}$<br>(ng·hr/mL) | $C_{max}$<br>(ng/mL) | $T_{max}$<br>(Hours) | $AUC_{0-t}$<br>(ng·hr/mL) | $AUC_{0-24}$<br>(ng·hr/mL) |
| 20                        | M      | 691                  | 1.00                 | 1297                      | 2418                       | 1140                 | 1.00                 | 5702                      | 5702                       |
|                           | F      | 820                  | 1.00                 | 2390                      | 2390                       | 1290                 | 1.00                 | 6673                      | 6673                       |
| 60                        | M      | 1380                 | 1.00                 | 6636                      | 6636                       | 2200                 | 1.00                 | 12123                     | 12123                      |
|                           | F      | 2410                 | 1.00                 | 7708                      | 7708                       | 2910                 | 1.00                 | 12956                     | 12956                      |
| 200                       | M      | 1710                 | 1.00                 | 18243                     | 18243                      | 2520                 | 2.00                 | 23745                     | 23745                      |
|                           | F      | 3330                 | 1.00                 | 21699                     | 21699                      | 4520                 | 1.00                 | 44808                     | 44808                      |

### 6.2.2 D11-4176b: Four-week oral toxicity in male rats – Additional study (Study R20030041)

This GLP study (R20030041) was conducted with D11-4176b (lot # CZ002; purity 100.6%) in (b) (4) during Nov 11, 2002 – Apr 15, 2003,

and previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND063266 (Appendix I).

This was a supplemental study of Study R20020612 reviewed above. In Study R20020612, hemorrhagic lesions in lungs, pancreas, and thymus were observed in a small number of rats at doses of 20, 60, and 200 mg/kg/day. Hemorrhagic lesions at doses 20 and 60 mg/kg/day only occurred in males. Therefore, the present study was limited to males only and utilized lower D11-4176b doses (6, 12, and 18 mg/kg/day). Other parts of the study design and methods were similar to those in Study R20020612.

There were no D11-4176b-related findings in this study. Minimal to slight focal hemorrhage, pneumonitis, erythrophagocytosis, and/or hemoglobin crystals occurred sporadically in the lungs of control and treated rats (Table 28), which were not dose-related, were similar to those pulmonary findings in Study R20020612, and not of toxicological significance. The NOAEL in this study was 18 mg/kg/day.

Table 28. Incidence (and severity in parenthesis) of lung findings in male rats (from the submission)

| D11-4176b (mg/kg/day) | 0       | 6       | 12      | 18      |
|-----------------------|---------|---------|---------|---------|
| Number Examined       | 10      | 10      | 10      | 10      |
| Hemorrhage            | 2 (0.2) | 3 (0.4) | 2 (0.2) | 1 (0.1) |
| Focal Pneumonitis     | 0       | 2 (0.3) | 2 (0.2) | 0       |
| Erythrophagocytosis   | 1 (0.1) | 2 (0.2) | 1 (0.1) | 0       |
| Hemoglobin Crystals   | 0       | 1 (0.1) | 1 (0.1) | 0       |

D11-4176 was readily absorbed following oral dosing with a T<sub>max</sub> of 1 hour. Systemic exposures (C<sub>max</sub> and AUC<sub>0-24</sub>) were approximately dose-proportional. Values for C<sub>max</sub> and AUC<sub>0-24</sub> on day 28 were about 2 times those on day 1, indicating accumulation of the test article after multiple dosing.

### 6.2.3 DU-176b: 26-week oral toxicity in rats with a 4-week recovery

Conducting laboratory and location: (b) (4)  
 Study number(s): R20050334  
 Date of study initiation: 18 October 2004  
 Drug lot/batch number/Purity: DU-176b / BB202 / 99.6%  
 GLP compliance: Yes  
 QA statement: Yes

#### Key Study Findings

There were no remarkable findings at any tested dose level (6, 18, or 54 mg/kg/day). The NOAEL was 54 mg/kg/day in this study. This study was deficient in not using a high enough dose level.

Plasma DU-176 concentrations increased dose-proportionally. There were no marked differences in C<sub>max</sub> and AUC values for Days 1 and 178 at 6, 18 and 54 mg/kg/day indicating no accumulation of the drug after repeated dosing. Females generally had slightly higher C<sub>max</sub> and AUC values (1 to 2-fold) than males. At the NOAEL of 54 mg/kg/day, mean C<sub>max</sub> and AUC values were 1856 ng/ml and 7237 ng\*h/ml, respectively.

## Methods

Male and female Crl:CD®(SD)IGS BR rats (7 weeks of age) were orally gavaged with vehicle 0.5% MC (control), or DU-176b at dose levels of 6, 18, or 54 mg/kg/day (15/sex/dose in the control and high-dose groups and 10/sex/dose in low- and mid-dose groups) for 26 weeks (Days 1 through 183), and were euthanized on Days 184 (end of dosing period) or 212 (end of recovery period). Parallel rats for TK (8/sex/dose) were orally dosed with DU-176b at dose levels of 6, 18, or 54 mg/kg/day for up to 178 days. The dose selection was based on the results of previous four-week oral toxicity studies in rats (R20020612 and R20030041). In Studies R20020612 and R20030041, minimal to slight focal hemorrhage, pneumonitis, erythrophagocytosis, and/or hemoglobin crystals occurred sporadically in the lungs of control and treated rats, and the NOAEL was interpreted to be 18 and 60 mg/kg/day for males and females, respectively at that time. Therefore, the high dose in the present study was set at 54 mg/kg/day. Because of the incorrect interpretation of NOAEL in the previous 4-week rat study, this study was deficient in not using a high enough dose level. Samples of the dose formulations from weeks 1, 13, 16, and 26 of treatment were analyzed to verify the homogeneity, stability, and/or concentrations of DU-176b in the dosing solution.

For the toxicity groups, clinical signs and morbidity/death were inspected visually during the study at least twice daily. Detailed observations were performed weekly. Ophthalmic examinations were performed prior to treatment and on Days 88, 179, and 205. Body weight was recorded prior to treatment, on day 1, and then twice weekly. Individual food and water consumptions were measured and recorded weekly. Blood samples were collected from a jugular vein of all surviving animals at scheduled sacrifice following overnight fast, using potassium EDTA as the anticoagulant for hematology (Table 29) and lithium heparin as the anticoagulant for plasma chemistry (Table 30). Blood samples were also collected from any animals sacrificed at an unscheduled interval. Urine sampling was conducted prior to scheduled sacrifice for urinalysis and urine chemistry (Table 31).

Table 29. Parameters of hematology and coagulation

|   |                                       |
|---|---------------------------------------|
| red blood cell (erythrocyte) count        | white blood cell (leukocyte) count    |
| hemoglobin                                | differential blood cell count         |
| hematocrit                                | blood cell morphology                 |
| mean corpuscular volume                   | reticulocyte count                    |
| mean corpuscular hemoglobin               |                                       |
| mean corpuscular hemoglobin concentration | prothrombin time                      |
| platelet count                            | activated partial thromboplastin time |

Table 30. Parameters of plasma chemistry

|                   |                            |                                |
|-------------------|----------------------------|--------------------------------|
| glucose           | albumin/globulin ratio     | lactate dehydrogenase          |
| urea nitrogen     | total cholesterol          | calcium                        |
| creatinine        | triglycerides              | inorganic phosphorus           |
| creatinine kinase | total bilirubin            | sodium                         |
| total protein     | alanine aminotransferase   | potassium                      |
| albumin           | alkaline phosphatase       | chloride                       |
| globulin          | aspartate aminotransferase | plasma protein electrophoresis |

Table 31. Parameters of urinalysis and urine chemistry

| Urinalysis       |                      | Urine chemistry *         |
|------------------|----------------------|---------------------------|
| appearance/color | ketones              | total urine volume        |
| specific gravity | bilirubin            | urine sodium excretion    |
| pH               | occult blood         | urine potassium excretion |
| protein          | microscopic sediment | urine chloride excretion  |
| glucose          | urobilinogen         | urine protein excretion   |

\* Urine levels of sodium, potassium, chloride, and protein were measured, but not presented in the final report. These values were used to calculate the excretion rates.

A detailed necropsy was performed on each animal that died prematurely or at scheduled sacrifice. Weights of organs listed in Table 32 were recorded. Bone marrow smears from the femur of each animal were prepared and examined. The following tissues (when present) from each animal were preserved, histologically processed, and examined microscopically (Table 32).

Table 32. List of organ for weighing and histological process and examination

|   |  |
|---|--|
| adrenal (2)*  | ovary (2)*                                     |
| aorta   | pancreas                                       |
| brain *   | pharynx  |
| cecum   | pituitary gland*                               |
| colon   | prostate *                                     |
| duodenum  | rectum   |
| epididymis (2)  | salivary gland [mandibular (2)]                |
| esophagus   | sciatic nerve                                  |
| eye (2)   | seminal vesicle (2)                            |
| femur with bone marrow<br>(articular surface of the distal end) | skeletal muscle (thigh)                        |
| Harderian gland   | skin   |
| heart *   | spinal cord<br>(cervical, thoracic and lumbar) |
| ileum   | spleen*  |
| jejunum   | sternum with bone marrow                       |
| kidney (2)*   | stomach  |
| lacrimal gland  | testis (2)*                                    |
| larynx  | thymus *                                       |
| gross lesions   | thyroid (2) with parathyroid *                 |
| liver*  | tongue   |
| lung with mainstem bronchi                                      | trachea  |
| lymph node (mesenteric)   | urinary bladder                                |
| mammary gland (females)   | uterus *                                       |
| optic nerve (2)   | vagina   |

\* Weight of organ was measured.

For TK groups, mortality and signs of pain or distress were recorded twice daily. Individual body weights were recorded prior to treatment, before dosing on Day 1, then weekly thereafter. Blood samples were collected from a jugular vein of conscious rats on Day 1 (1, 2, 4, and 24 hours postdose), Day 87 (pre-dose and 2 hours postdose), and Day 178 (1, 2, 4, and 24 hours postdose) using sodium fluoride as the anticoagulant (n=4/dose/time point). Plasma was harvested and analyzed for DU-176 concentrations using an LC/MS/MS method. All TK animals were discarded without necropsy after their scheduled sacrifice or premature death.

## Results

One control male rat was sacrificed on Day 101 due to a moribund condition related to gavage trauma. One female rat in the high-dose group was found dead approximately 30 minutes after the dosing on Day 163 that was likely to be due to a dosing error.

There were no test article-related findings in clinical signs, body weights and food consumption, ophthalmic evaluations, hematology, coagulation parameters, plasma chemistry, urinalysis and urine chemistry, bone marrow cytology, organ weights, necropsy, and histopathology.

Homogeneity and concentrations of DU-176b dosing solutions at 0.6, 1.8, and 5.4 mg/mL during Weeks 1, 13, 16, and 26 were within  $\pm 10\%$  of the target and acceptable for the study.

As shown in Figure 10, plasma DU-176 concentrations increased with the increase in dose levels from 6 to 54 mg/kg/day. The C<sub>max</sub> and AUC values on Day 178 at 6 mg/kg/day dose level were about 2 folds those on Day 1, while there were no marked differences in C<sub>max</sub> and AUC values after multiple dosing at 18 and 54 mg/kg/day. Females generally had higher C<sub>max</sub> and AUC values (1 to 2-fold) than males (Figure 10, Table 33).

Figure 10. Plasma concentrations of DU-176 following oral dosing in rats

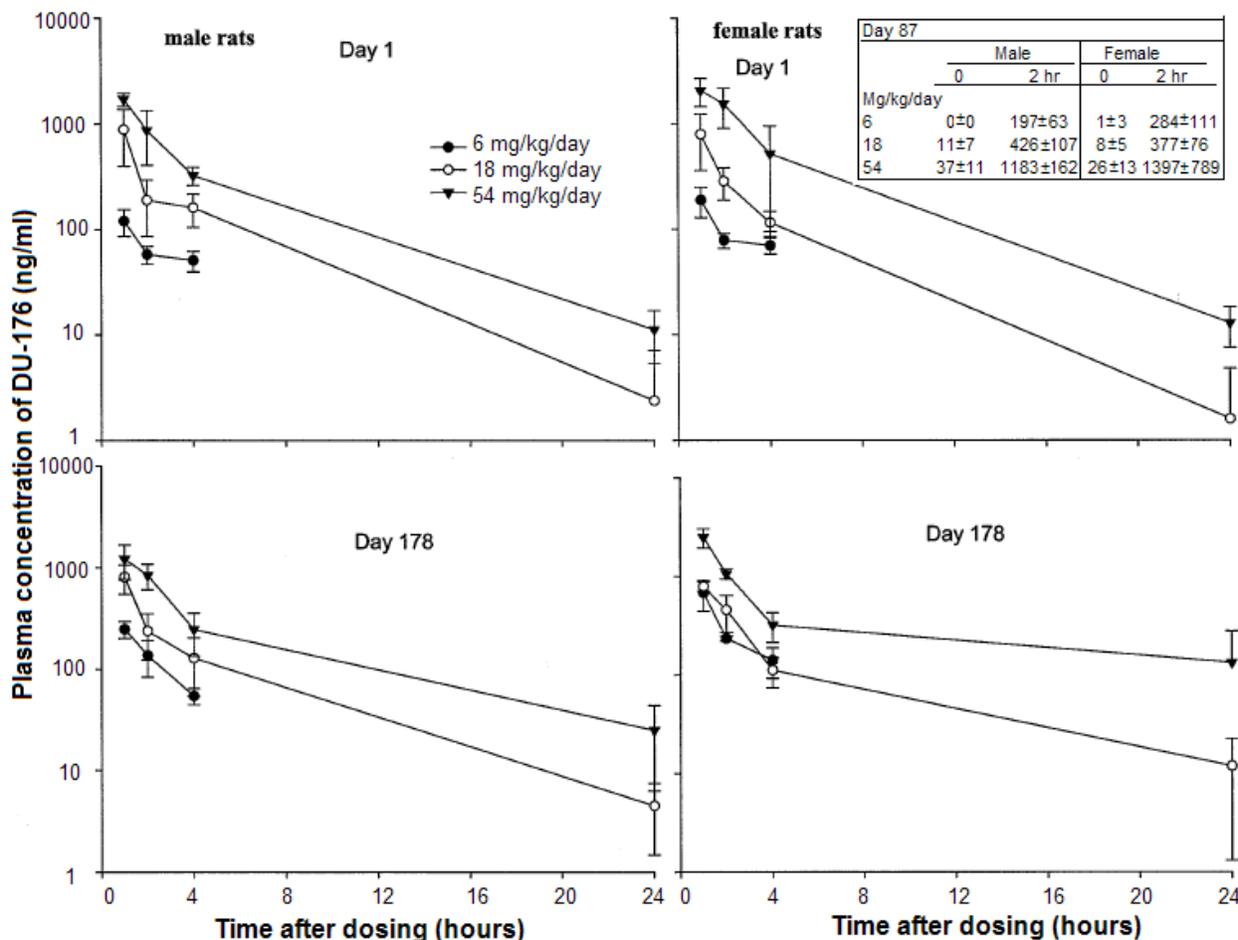


Table 33. DU-176 TK parameters in rats following oral doses (from the submission)

| Dose Level<br>(mg/kg/day) | Sex | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | AUC <sub>0-1</sub><br>(ng•hr/mL) | AUC <sub>0-24</sub><br>(ng•hr/mL) | AUC <sub>0-∞</sub><br>(ng•hr/mL) | t <sub>1/2</sub><br>(hr) |
|---------------------------|-----|-----------------------------|--------------------------|----------------------------------|-----------------------------------|----------------------------------|--------------------------|
| Day 1                     |     |                             |                          |                                  |                                   |                                  |                          |
| 6                         | M   | 121                         | 1.00                     | 258                              | 768                               | NC                               | NC                       |
|                           | F   | 189                         | 1.00                     | 377                              | 1080                              | NC                               | NC                       |
| 18                        | M   | 888                         | 1.00                     | 2968                             | 2968                              | 2980                             | 3.40                     |
|                           | F   | 795                         | 1.00                     | 2514                             | 2514                              | 2521                             | 3.05                     |
| 54                        | M   | 1708                        | 1.00                     | 6706                             | 6706                              | 6767                             | 3.73                     |
|                           | F   | 2060                        | 1.00                     | 10243                            | 10243                             | 10306                            | 3.39                     |
| Day 178                   |     |                             |                          |                                  |                                   |                                  |                          |
| 6                         | M   | 248                         | 1.00                     | 509                              | 1053                              | NC                               | NC                       |
|                           | F   | 680                         | 1.00                     | 1173                             | 2581                              | NC                               | NC                       |
| 18                        | M   | 808                         | 1.00                     | 2624                             | 2624                              | NC                               | NC                       |
|                           | F   | 792                         | 1.00                     | 2833                             | 2833                              | NC                               | NC                       |
| 54                        | M   | 1222                        | 1.00                     | 5459                             | 5459                              | NC                               | NC                       |
|                           | F   | 2490                        | 1.00                     | 9015                             | 9015                              | NC                               | NC                       |

#### 6.2.4 Repeated dose toxicity study in rats with intravenously injection of DU-176b for 14 days

Conducting laboratory and location:

(b) (4)

Study number(s): AN08-C0123-R01  
 Date of study initiation: May 11, 2009  
 Drug lot/batch number: DU-176b for Injection 10 mg, L008SG01, 109.63% (amount of DU-176 vs. labeling)  
 GLP compliance: Yes  
 QA statement: Yes

#### Key Study Findings

No treatment-related changes were noted in any examinations during the course of the study. Plasma concentrations of DU-176 increased dose-proportionally at doses of 5 and 20 mg/kg/day. There was no sex difference in DU-176 systemic exposure, and no accumulation after multiple dosing. At the NOAEL of 20 mg/kg/day, the mean C<sub>0</sub> and AUC<sub>all</sub> values on Day 14 were 11100 ng/mL and 10300 ng•h/mL in males, respectively, and 10700 ng/mL and 10000 ng•h/mL in females, respectively.

#### Methods

Male and female CrI:CD(SD)IGS rats (6 weeks old) were injected IV with DU-176b at 0 (vehicle, saline), 5, or 20 mg/kg/day, QD, for 14 days (n = 10/sex/group for toxicity and 4/sex/group for TK). This study was performed precede a planned clinical bioavailability study with IV infusion of DU-176b at 30 mg (total dose) for 30 minutes. The exposure levels at 5 and 20 mg/kg/day were expected to be comparable to about 1 and 5 times, respectively, those in human subjects with an intravenous infusion at 30 mg for 30 min.

For the toxicity groups, rats were checked for morbidity/death and clinical signs (1-4 times daily), body weight (on days 1, 3, 7, 10, and 14), and food consumption (on days 2, 6, 9, and 13). Urine samples were collected on day 11 for urinalysis and urine chemistry (Table 31). At the end of dosing period, blood samples were collected for hematology and plasma chemistry (Table 29 and Table 30, respectively), rats were then euthanized and necropsy. Organs/tissues listed in Table 34 were pathologically examined.

Table 34. List of organs/tissues

| Organs and tissues        | Organ weight        | Fixation | Histopathology examination | Organs and tissues          | Organ weight                     | Fixation                                     | Histopathology examination |                 |
|---------------------------|---------------------|----------|----------------------------|-----------------------------|----------------------------------|--|----------------------------|-----------------|
| Trachea                   | -                   | o        | o                          | Brain                       | cerebrum                         | o  | o                          |                 |
| Lungs (including bronchi) | left                | o        | o                          |                             | cerebellum                       |  | o                          |                 |
|                           | right               |          | o                          |                             | pons                             |  | o                          |                 |
| Tongue                    | -                   | o        | o                          |                             | medulla oblongata                |  | o                          |                 |
| Submandibular glands      | left                | -        | o                          | Spinal cord                 | thorax                           | -  | o                          |                 |
|                           | right               | -        | o                          |                             | Sciatic nerves                   | left   | -                          | o               |
| Esophagus                 | thorax              | -        | o                          | right                       | -                                | o  | -                          |                 |
| Stomach                   | fore                | -        | o                          | Sternum/Sternal bone marrow | -                                | o  | o                          |                 |
|                           | glandular           | -        | o                          | Femurs/ Femoral bone marrow | left                             | -  | o                          |                 |
| Small intestine           | duodenum            | -        | o                          |                             | right                            | -  | o                          | o               |
|                           | jejunum             | -        | o                          | Mesenteric lymph nodes      |                                  | -  | o                          |                 |
|                           | ileum <sup>a)</sup> | -        | o                          |                             | Spleen                           | o  | o                          | o               |
| Large intestine           | cecum               | -        | o                          | Thymus                      | o                                | o  | o                          |                 |
|                           | colon               | -        | o                          | Pituitary                   | o                                | o  | o                          |                 |
|                           | rectum              | -        | o                          | Thyroids/ Parathyroids      | left                             | o  | o                          |                 |
| Pancreas                  | -                   | o        | right                      |                             | o                                | o  | o                          |                 |
| Liver                     | o                   | o        | o                          | Adrenals                    | left                             | o  | o                          |                 |
| Aorta                     | thorax              | -        | o                          |                             | right                            | o  | o                          |                 |
| Heart                     | o                   | o        | o                          | Eyeballs/ Optic nerves      | left                             | -  | o                          |                 |
| Kidneys                   | left                | o        | o                          |                             | right                            | -  | o                          | o <sup>b)</sup> |
|                           | right               | o        | o                          | o                           | Harderian glands                 | left   | -                          | o               |
| Urinary bladder           |                     | -        | o                          | right                       |                                  | -  | o                          | -               |
|                           | Testes              | left     | o                          | o                           | o                                | Skeletal muscles (quadriceps femoris muscle) | left                       | -               |
| right                     |                     | o        | o                          | o                           | right                            |  | -                          | o               |
| Epididymides              | left                | o        | o                          | o                           | Mammary glands /Skin (abdominal) | left   | -                          | o               |
|                           | right               | o        | o                          | o                           |                                  | right  | -                          | o               |
| Prostate                  | o                   | o        | o                          | o                           | Skin (dorsal) <sup>d)</sup>      |  | -                          | o               |
| Seminal vesicles          | left                | -        | o                          | o                           |                                  | Injection site (tail)                        | -                          | o               |
|                           | right               |          | -                          | o                           | Gross lesions                    | -  | o                          |                 |
| Ovaries                   | left                | o        | o                          | o                           |                                  |  |                            |                 |
|                           | right               | o        | o                          | o                           |                                  |  |                            |                 |
| Uterus                    | left                | o        | o                          |                             |                                  |  |                            |                 |
|                           | right               |          | -                          |                             |                                  |  |                            |                 |
| Vagina                    | -                   | o        | o                          |                             |                                  |  |                            |                 |

o: Performed -: Not performed a) Including Peyer's patches  
b) Only control and 20 mg/kg toxicity groups  
c) Mammary glands: only females d) Interscapular area

For TK groups, clinical sign observations, body weight measurements, and TK assessments were performed. Plasma concentrations of DU-176 were determined at 0.083, 0.5, 2, 7, and 24 h post-dose on Days 1 and 14 (n = 4/sex/group/sampling point) using an LCMS/MS method.

## Results

No animal died during the study period. Some values of hematology, plasma chemistry, and urine chemistry were higher or lower than controls ( $p < 0.05$ ), but the differences were minor, not dose-related, mostly within the physiological variation, and not of toxicological significance. Thus, No test article-related changes were noted in clinical signs, body weight, food consumption, urinalysis, hematology, plasma chemistry, gross pathology, organ weight, or histopathology. NOAEL was 20 mg/kg/day.

Plasma concentrations of DU-176 and TK parameters following IV injection are shown in Table 35. Initial plasma concentrations extrapolated to time 0 ( $C_0$ ) and  $AUC_{all}$  increased dose-proportionally. There were no clear differences in any toxicokinetic parameter after repeated dosing or between sexes.

Table 35. DU-176 plasma concentrations and TK parameters following IV injection to rats (from the submission)

| Day of dosing | Dose (mg/kg) | Sex    | Plasma concentration (ng/mL) |          |       |      |      |       | $t_{1/2}$ (h) | $C_0$ (ng/mL) | $AUC_{all}$ (ng·h/mL) |       |
|---------------|--------------|--------|------------------------------|----------|-------|------|------|-------|---------------|---------------|-----------------------|-------|
|               |              |        | Pre                          | 0.0833 h | 0.5 h | 2 h  | 7 h  | 24 h  |               |               |                       |       |
| 1             | 5            | Male   | Mean                         | 1990     | 780   | 64.9 | 8.80 | 0.298 | 2.54          | 2410          | 1660                  |       |
|               |              |        | SD                           | 146      | 139   | 22.1 | 2.14 | 0.595 | 1.78          | 191           | 229                   |       |
|               |              | Female | Mean                         | 2260     | 852   | 82.1 | 13.0 | 0.318 | 2.67          | 2760          | 1910                  |       |
|               |              |        | SD                           | 183      | 55.4  | 19.6 | 2.91 | 0.635 | 1.68          | 271           | 111                   |       |
|               | 20           | Male   | Mean                         | 8660     | 4600  | 507  | 44.2 | 1.75  | 3.18          | 9850          | 9140                  |       |
|               |              |        | SD                           | 637      | 699   | 202  | 11.6 | 1.98  | 1.46          | 708           | 1540                  |       |
|               | Female       | Mean   | 8970                         | 4820     | 540   | 42.5 | 5.42 | 5.68  | 10200         | 9540          |                       |       |
|               |              | SD     | 496                          | 689      | 102   | 5.65 | 3.27 | 1.71  | 793           | 948           |                       |       |
| 14            | 5            | Male   | Mean                         | 0.000    | 2500  | 1120 | 117  | 16.5  | 0.643         | 2.98          | 2940                  | 2390  |
|               |              |        | SD                           | 0.000    | 191   | 121  | 26.7 | 4.53  | 0.742         | 1.63          | 228                   | 240   |
|               |              | Female | Mean                         | 1.59     | 2480  | 1120 | 133  | 18.8  | 2.04          | 5.30          | 2910                  | 2470  |
|               |              |        | SD                           | 1.12     | 202   | 99.5 | 18.6 | 5.40  | 0.463         | 0.601         | 334                   | 155   |
|               | 20           | Male   | Mean                         | 1.03     | 9600  | 4780 | 713  | 44.1  | 3.47          | 4.57          | 11100                 | 10300 |
|               |              |        | SD                           | 1.18     | 511   | 471  | 120  | 12.7  | 1.84          | 1.00          | 526                   | 759   |
|               |              | Female | Mean                         | 4.37     | 9250  | 4490 | 678  | 66.4  | 5.21          | 4.53          | 10700                 | 10000 |
|               |              |        | SD                           | 1.53     | 640   | 710  | 81.0 | 22.9  | 2.62          | 0.625         | 1010                  | 892   |

### 6.2.5 D11-4176b: 4-week oral toxicity in cynomolgus monkeys (R20020606)

The GLP study **R20020606** was conducted with D11-4176b (lot # CZ002; purity 100.6%) in (b) (4) during Apr 12 – Dec 12, 2002, and previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND 063266 (Appendix I).

Cynomolgus monkeys (*Macaca fascicularis*, 2 - 3.4 years old) were orally gavaged with vehicle (0.5% MC), or D11-4176b at 10, 30, or 100 mg/kg/day for 4 weeks (4

monkeys/sex/group). The dose selection in this study were based on a preliminary 4-week oral toxicity study in female cynomolgus monkeys (n=2 /dose) with D11-4176a (a hydrous form of DU-176) at 0, 5, or 30 mg/kg/day (**Study R20010689**). No D11-4176a-related adverse effects were noted up to 30 mg/kg/day in Study R20010689. In a previous 4-week oral toxicity with DT-831j, an analogue of D11-4176b, in cynomolgus monkeys, three dose levels of 10, 30, and 100 mg/kg/day were employed. In the present study, these dose levels were selected to compare the toxicities between D11-4176b and DT-831j. In the present study, observations/examinations conducted for all animals included clinical observation for mortality and clinical signs, physical examinations, ophthalmic examinations, electrocardiograms, body weight, food consumption, hematology and coagulation, plasma chemistry, urine chemistry, urinalysis, TKs, organ weight, bone marrow cytologic evaluation, and macroscopic and microscopic examinations. In addition, bone marrow samples were obtained from each monkey at the end of 4-week dosing period and prepared for micronucleus assay.

There were no D11-4176b-related effects on body weights, ophthalmic and electrocardiographic parameters, urinalysis, or macroscopic observations.

Two out of 4 female monkeys at D11-4176b 100 mg/kg/day died prematurely. Death in one of the 2 animals was attributed to hemorrhage in multiple tissues, particularly in the lungs and thymus. Higher incidence of hemorrhage in lungs, thymus, heart, thyroid, trachea, adrenals, and uterus was seen at 100 mg/kg/day group, and hemorrhage in stomach was observed in the 30 mg/kg/day group. The hemorrhagic effect was probably due to exaggerated pharmacological activity. Slight reductions in red blood cell (RBC) count, hemoglobin, and hematocrit, slight prolongation of prothrombin time (PT) and activated partial thromboplastin time (APTT), and slight increases in platelets and reticulocyte counts were seen in the 30 and 100 mg/kg/day groups, which were correlated with the incidence of hemorrhage in histopathological examination. Pulmonary lesions of acute inflammation, focal interstitial pneumonitis, fibrinous bronchi, neutrophilic infiltration, and inflammation in pleura were observed in the 100 mg/kg/day group. Higher urine protein was seen at 30 and 100 mg/kg/day. The NOAEL was 10 mg/kg/day.

4-week treatment with D11-4176b did not produce a significant increase in the incidence of micronucleated polychromatic erythrocytes (PCEs) in bone marrow of the monkeys. However, this study was deficient due to the absence of a positive control group. No criteria for a valid study or a positive response were stated.

After oral administration, D11-4176b was readily absorbed with Tmax of 1 – 3.5 hours. System exposure to D11-4176 (Cmax and AUC<sub>0-24</sub>) increased in an under dose-proportional manner. Values for Cmax and AUC<sub>0-24</sub> on day 27 were lower than those on day 1, especially at the high dose. No sex-related differences were observed.

#### **6.2.6 DU-176b: 13-week oral toxicity in cynomolgus monkeys (R20050333)**

The GLP study R20050333 was conducted with DU-176b (Lot BB202, purity 99.6%) in (b) (4) during Aug 8, 2004 – Sept 22, 2005, and

previously reviewed by Dr. Patricia P Harlow of DRCP (06/22/2007) under IND 077254 (Appendix II).

Cynomolgus monkeys (*Macaca fascicularis*, 2 - 3.2 years old) were orally gavaged with vehicle (0.5% MC), or DU-176b at 6, 18, or 54 mg/kg once daily for 13 weeks (4 monkeys/sex/group). The high dose selected in the current study was based on a 4-week repeated dose study in which hemorrhage in the gastrointestinal tract, pleural cavity or adrenal gland was observed at 100 mg/kg (Study R20020606). In the present study, observations/examinations conducted for all animals included clinical observation for mortality and clinical signs, physical examinations, ophthalmic examinations, electrocardiograms, body weight, food consumption, hematology and coagulation, plasma chemistry, urine chemistry, urinalysis, TKs, organ weight, bone marrow cytologic evaluation, and macroscopic and microscopic examinations.

There were no DU-176b-related effects on mortality and clinical signs, body weights, ophthalmic and electrocardiographic parameters, plasma chemistry, urine chemistry, urinalysis, or macroscopic and microscopic observations.

Slight prolongation of APTT and PT was observed at 18 and 54 mg/kg/day at 24 hours post-dose on Days 28 and 90 (Table 36) that was likely due to the extended pharmacological action of DU-176b. Some individual mid- and high-dose animals showed DU-176b-related changes on hematology parameters most likely in response to hemorrhagic events (Table 37). The NOAEL in this study was 6 mg/kg (NOAEL for toxicity other than bleeding was 54 mg/kg/day).

Table 36. Coagulation times (seconds, n=4)

| DU-176b Dose<br>(mg/kg/day) |      | Males  |        |        |        |        |        | Females |        |        |        |        |        |
|-----------------------------|------|--------|--------|--------|--------|--------|--------|---------|--------|--------|--------|--------|--------|
|                             |      | APTT   |        |        | PT     |        |        | APTT    |        |        | PT     |        |        |
|                             |      | Day -7 | Day 28 | Day 90 | Day -7 | Day 28 | Day 90 | Day -7  | Day 28 | Day 90 | Day -7 | Day 28 | Day 90 |
| 0                           | Mean | 17.4   | 16.2   | 16.8   | 10.1   | 9.7    | 9.9    | 16.7    | 17.1   | 18.0   | 10.4   | 10.1   | 10.5   |
|                             | SD   | 1.66   | 1.22   | 1.59   | 0.14   | 0.13   | 0.14   | 0.70    | 0.49   | 0.50   | 0.43   | 0.51   | 0.44   |
| 6                           | Mean | 17.5   | 18.3   | 18.0   | 10.6   | 10.3*  | 10.4   | 16.9    | 17.7   | 19.0   | 10.3   | 10.0   | 10.4   |
|                             | SD   | 0.85   | 1.03   | 1.11   | 0.19   | 0.29   | 0.25   | 0.84    | 0.98   | 1.07   | 0.28   | 0.05   | 0.29   |
| 18                          | Mean | 18.7   | 20.0*  | 21.8*  | 10.2   | 10.4   | 11.8*  | 17.6    | 20.4   | 20.7*  | 10.5   | 10.7   | 11.1   |
|                             | SD   | 1.55   | 1.45   | 2.31   | 0.34   | 0.59   | 1.05   | 1.23    | 3.02   | 1.17   | 0.14   | 0.51   | 0.10   |
| 54                          | Mean | 16.8   | 19.2*  | 19.7   | 10.3   | 11.2*  | 11.3*  | 16.4    | 19.9   | 21.1*  | 10.1   | 11.5*  | 11.2   |
|                             | SD   | 2.06   | 1.75   | 1.74   | 0.57   | 0.98   | 0.85   | 0.44    | 1.28   | 0.62   | 0.34   | 0.87   | 0.60   |

\* = P < or = 0.05

Table 37. summary of hematology effects (bold type signifies a value outside normal range) (from Dr. Harlow's review)

| Parameter                         | Day -14/Day -17/Day 28/ Day 90 |                           |                            |                             | Normal range |            |
|-----------------------------------|--------------------------------|---------------------------|----------------------------|-----------------------------|--------------|------------|
|                                   | Male 57147<br>(Mid dose)       | Male 57151<br>(High dose) | Female 57160<br>(Mid dose) | Female 57164<br>(High dose) | Male         | Female     |
| RBC, 10 <sup>6</sup> /μL          | 5.7/5.6/4.9/5.4                | 5.3/5.1/4.8/4.8           | 5.1/4.9/4.9/3.45           | 5.3/5.2/3.6/5.6             | 4.86-7.81    | 4.89-6.98  |
| HGB, gm/dL                        | 13.5/12.8/11.4/12.1            | 13.3/12.5/11.8/11.7       | 12.1/11.5/11.7/9.4         | 13.3/12.9/8.7/13.3          | 10.6-15.4    | 11.7-15.2  |
| HCT, %                            | 44.7/41.4/35.7/40.9            | 44.0/41.2/39.0/40.1       | 39.5/36.1/38.5/32.6        | 43.7/41.2/29.4/46.5         | 36.7-52.6    | 38.6-50.6  |
| Reticulocyte, %                   | 1.3/2.0/2.1/2.0                | 0.9/1.7/1.6/1.5           | 0.8/1.8/2.3/13.6           | 1.1/1.4/6.3/0.7             | 0.2-2.8      | 0.3-2.9    |
| Reticulocyte, 10 <sup>3</sup> /μL | 76/112/103/110                 | 48/85/79/73               | 42/88/111/471              | 60/71/226/37                | 16.9-142.6   | 20.3-166.2 |
| MCV, fL                           | 78.8/74.0/72.3/76.0            | 84/81/81/83               | 78/74/79/95                | 82/80/81/83                 | 63.1-90.1    | 65.0-85.8  |
| MCH, pg                           | 23.7/23.0/23.2/22.5            | 25.2/24.5/24.5/24.3       | 23.9/23.7/24.1/27.3        | 25.0/25.1/24.1/23.6         | 18.7-26.3    | 18.7-26.0  |
| Platelet, 10 <sup>3</sup> /μL     | 369/445/407/513                | 266/296/281/278           | 440/481/439/689            | 158/256/282/229             | 208-701      | 232-542    |

Following oral dosing, T<sub>max</sub> values for DU-176b were 1 to 4 hours in all groups. Plasma DU-176 concentrations on Days 1 and 91 generally increased with dose, but were less than dose-proportional. No consistent differences in C<sub>max</sub> and AUC were observed after repeated dosing, indicating no marked accumulation after the 13 weeks of daily dosing. No marked (>2-fold) gender differences were observed in C<sub>max</sub> and AUC values, except the 18 mg/kg/day dose level where males had a 2.3-fold higher mean C<sub>max</sub> than females.

### **6.2.7 DU-176b: Fifty-two-week oral toxicity in cynomolgus monkeys with a thirteen-week recovery**

Conducting laboratory and location: (b) (4)

Study number(s): AN07-C0008-R01

Date of study initiation: Aug 2, 2005

Drug, lot/batch number, purity: DU-176b, LB207, 98.6%

GLP compliance: Yes

QA statement: Yes

#### **Key Study Findings**

There were 7 premature deaths during the study: one male and one female at 45 mg/kg/day due to unknown reason, one female at 15 mg/kg/day and 2 females at 45 mg/kg/day due to primary hemorrhage, one female at 5 mg/kg/day and one male at 15 mg/kg/day due to hemorrhage secondary to accident trauma. One of the moribund females at 45 mg/kg/day showed prolonged menses and was extremely pale.

In the surviving animals during dosing, there were more animals with various swelling, pale skin, black or red feces and/or urine, and hypoactivity at 45 mg/kg/day, slight prolongation of PT and APTT at 15 and 45 mg/kg/day, and slightly lower RBC counts, hemoglobin (HGB), and mean corpuscular hemoglobin concentration (MCHC) values associated with higher absolute reticulocyte count (ARET) at 15 and 45 mg/kg/day. At the end of dosing phase, one male at 15 mg/kg/day had a deeply ulcerated hemorrhagic skin lesion over its eye, and one female at 15 mg/kg/day had diffused pulmonary hemorrhage and a large necrotic bronchus within the hemorrhagic lobe; various degrees of neutrophil infiltrations were present in/around the hemorrhagic area, in the necrotic bronchus and the surrounding lung parenchyma. There were no DU-176b-related findings at the end of recovery period.

These findings of hemorrhage, anemia, and anemia-related compensative response were due to the anticoagulant property of DU-176b. NOAEL in this study was 5 mg/kg/day.

After oral dosing, DU-176 readily appeared in plasma, with T<sub>max</sub> value range of mostly 1- 4 hours. Increases in DU-176 C<sub>max</sub> and AUC<sub>0-24</sub> for both sexes were under dose-proportional. There was no apparent sex difference in the mean C<sub>max</sub> and AUC<sub>0-24h</sub> values or accumulation after multiple dosing. At the NOAEL 5 mg/kg/day, mean C<sub>max</sub>

and AUC<sub>0-24h</sub> values on Day 364 were 263 ng/mL and 3370 ng•h/mL, respectively, in males, and 327 ng/mL and 3237 ng•h/mL, respectively, in females.

## Methods

Cynomolgus monkeys (1.8 - 5 years old) were orally gavaged with vehicle (0.5% MC, control) or DU-176b at 5, 15, or 45 mg/kg/day for 52 weeks (n = 6/sex/group in the control and high dose groups, n = 4/sex/group in the low and mid dose groups). Two monkeys/sex/group in the control and high dose groups were followed up for additional 13 weeks. One male and one female in the high dose group were found dead on Days 3 and 10, respectively, and were replaced by other monkeys (with the same animal numbers and dosing period). Dose selection in the current study was based on previous 4- and 13-week repeated-dose oral toxicity study in monkeys (**Studies R20020606 and R20050333**). In Study R20020606 with daily repeated oral dose DU-176b for 4 weeks, two females at 100 mg/kg/day were prematurely sacrificed or died due to hemorrhage in the gastrointestinal tract, pleural cavity or adrenal gland. In Study R20050333 with daily repeated oral dose DU-176b for 13 weeks, one female at 54 mg/kg/day showed transient anemia including decreases in red blood cell count, hemoglobin and hematocrit and an increase in reticulocyte count on Day 28. Therefore, the highest dose level of 45 mg/kg/day was selected in the present study. Samples of dosing formulations for Weeks 1, 2+3 (mix), 5, 13, 26, 39, and 52 were analyzed, and DU-176b stability, concentration and homogeneity were confirmed to be within  $\pm 6.6\%$  of target and acceptable for study.

During the course of the study, animals were observed for mortality and clinical signs (twice daily), and assessed for qualitative food consumption once daily. Body weights were recorded at least once pre-dose, on the first day of treatment, and weekly thereafter. Ophthalmic examinations [including the anterior portion (eyelids, conjunctiva, cornea, anterior chamber, iris, and lens), optic media (vitreous), and ocular fundus] were conducted once pre-dose, on dosing day 357, and on recovery day 89 for all surviving animals. Electrocardiogram examinations were conducted once pre-dose, on dosing days 8 and 357 (2-4 and 24 hours postdose), and recovery day 89 for all surviving animals. Routine measurements of electrocardiograms, including heart rate, were done using 10 leads. Fasting blood samples were collected from a femoral vein of all surviving monkeys twice during the pre-dose phase, on Days 9 (high dose only) and 179 and 360 of the dosing phase, and Day 91 of the recovery phase, and if possible from animals of unscheduled death, for determining hematology (with potassium EDTA as anticoagulant), coagulation (with sodium citrate as anticoagulant), and plasma chemistry (with lithium heparin as anticoagulant) (Table 29, Table 30). Fasted overnight urine samples were collected once prior to study initiation on Days 360 (half of animals) and 361 (remaining animals) of the dosing phase, and on Day 91 of the recovery phase for urinalysis (Table 31).

All monkeys were bled from a femoral vein at 1, 2, 4, and 24 h after dosing on Days 1, 182, and 364 for TK using sodium heparin as the anticoagulant. Plasma concentrations of DU-176 were determined with an LC-MS/MS method.

At scheduled terminations by the end of dosing or recovery periods, necropsies were performed on all surviving animals. Terminal body weights and organ weights were recorded (Table 38). Necropsies were also done on animals of premature death. Bone marrow smears were prepared from the sternum of each animal at sacrifice (including moribund, terminal and recovery animals), and stained for cytologic evaluation. Tissues/organs listed in Table 38 were sampled from all moribund, terminal and recovery animals, preserved, histologically processed, and microscopically examined.

Table 38. List of tissues/organs for weight (#) and microscopic examination

|  |   |
|--|---|
| adrenal (2) #  | optic nerve (2)                             |
| aorta  | ovary (2) #                                 |
| brain #  | pancreas                                    |
| cecum  | pharynx                                     |
| cervix   | pituitary gland #                           |
| colon  | prostate #                                  |
| duodenum   | rectum                                      |
| epididymis (2)   | salivary gland [mandibular (2)] #           |
| esophagus  | sciatic nerve                               |
| eye (2)  | seminal vesicle (2)                         |
| femur with bone marrow (articular surface of the distal end) | skeletal muscle (thigh)                     |
| gallbladder  | skin  |
| heart #  | spinal cord (cervical, thoracic and lumbar) |
| ileum  | spleen #                                    |
| jejunum  | sternum with bone marrow                    |
| kidney (2) #   | stomach                                     |
| lacrimal gland (2)   | testis (2) #                                |
| larynx   | thymus #                                    |
| gross lesions  | thyroid (2) with parathyroid #              |
| liver #  | tongue                                      |
| lung with mainstem bronchi #                                 | trachea                                     |
| lymph node (mesenteric)                                      | urinary bladder                             |
| mammary gland (females)                                      | uterus #                                    |
|  | vagina                                      |

## Results

There were 7 premature deaths during the study (Table 39). The cause of death was unknown for one male and one female at 45 mg/kg/day, and was due to hemorrhage either as a primary occurrence for one female at 15 mg/kg/day and 2 females at 45 mg/kg/day or secondary to accident trauma for one female at 5 mg/kg/day and one male at 15 mg/kg/day. Histopathological examination revealed pulmonary or GI hemorrhage in some of the prematurely dead animals. One of the moribund females at

45 mg/kg/day showed prolonged menses and was extremely pale. These findings were attributable to the anticoagulant properties of DU-176b.

Table 39. Summary of premature deaths

| Animal number | Sex    | Dose level mg/kg/day | Day of death | Death description | Death reason/ Comments                           |
|---------------|--------|----------------------|--------------|-------------------|--|
| I58755        | Female | 5                    | 178          | Moribund          | Severe hemorrhage due to dosing - gastric trauma |
| I58739        | Male   | 15                   | 364          | Moribund          | Severe hemorrhage due to self-mutilation         |
| I58760        | Female | 15                   | 314          | Found dead        | Pulmonary hemorrhage                             |
| I58767        | Female | 45                   | 72           | Moribund          | Anemia due to extended menses                    |
| I58768        | Female | 45                   | 197          | Moribund          | Unknown  |
| I58744*       | Male   | 45                   | 3            | Moribund          | Unknown, Animal had been replaced                |
| I58766*       | Female | 45                   | 10           | Moribund          | GI hemorrhage, Animal had been replaced          |

\* These were replaced by other monkeys with the same animal numbers, which were treated with the test article from Days 4 and 11 to Day 367 and Day 374, respectively.

More animals in the 45 mg/kg/day group had various swelling, pale skin, black or red feces and/or urine, and hypoactivity during dosing phase (Table 40). These findings were considered secondary occurrences attributed to the anticoagulant-hemorrhage property of DU-176b.

Table 40. Number of animals with clinical signs

| Category                         | Dose Level mg/kg/day: | 0 | 5 | 15 | 45 | Category                         | Dose Level mg/kg/day: | 0 | 5 | 15 | 45 |
|----------------------------------|-----------------------|---|---|----|----|----------------------------------|-----------------------|---|---|----|----|
| Sign                             | Number in Group:      | 6 | 4 | 4  | 6  | Sign                             | Number in Group:      | 6 | 4 | 4  | 6  |
| <b>M a l e s</b>                 |                       |   |   |    |    | <b>F e m a l e s</b>             |                       |   |   |    |    |
| <b>Appearance</b>                |                       |   |   |    |    | <b>Appearance</b>                |                       |   |   |    |    |
| Swollen, Left Periorbital        |                       | 0 | 0 | 0  | 2  | Hunched Posture                  |                       | 0 | 0 | 0  | 2  |
| Swollen, Penis                   |                       | 1 | 0 | 0  | 0  | Swollen, Left Inguinal Area      |                       | 0 | 0 | 1  | 0  |
| Swollen, Right Arm               |                       | 0 | 0 | 1  | 1  | Thin                             |                       | 0 | 0 | 0  | 1  |
| Swollen, Right Periorbital       |                       | 0 | 0 | 1  | 0  | <b>Behavior</b>                  |                       |   |   |    |    |
| <b>Discharge-Unknown source</b>  |                       |   |   |    |    | Ataxic                           |                       | 0 | 0 | 0  | 1  |
| Found in Pan, Red in Color       |                       | 0 | 0 | 0  | 1  | Hypoactive                       |                       | 0 | 1 | 0  | 2  |
| <b>Excretion</b>                 |                       |   |   |    |    | Recumbent                        |                       | 0 | 0 | 0  | 1  |
| Discolored Feces, Black in Color |                       | 0 | 0 | 0  | 1  | Recumbent, Lateral               |                       | 0 | 0 | 1  | 1  |
| Discolored Feces, Red in Color   |                       | 0 | 0 | 0  | 1  | <b>Excretion</b>                 |                       |   |   |    |    |
| Discolored Urine, Red in Color   |                       | 0 | 0 | 0  | 1  | Discolored Feces, Black in Color |                       | 0 | 0 | 0  | 1  |
| Liquid Feces                     |                       | 0 | 0 | 0  | 2  | Discolored Feces, Red in Color   |                       | 0 | 0 | 1  | 1  |
| Nonformed Feces                  |                       | 2 | 4 | 4  | 4  | Discolored Urine, Red in Color   |                       | 0 | 0 | 0  | 0  |
| <b>Skin &amp; Pelage</b>         |                       |   |   |    |    | Liquid Feces                     |                       | 2 | 2 | 0  | 2  |
| Pale Skin, Entire Body           |                       | 0 | 0 | 0  | 1  | Mucoid Feces                     |                       | 0 | 0 | 1  | 0  |
|                                  |                       |   |   |    |    | Mucoid Feces, Red in Color       |                       | 0 | 0 | 0  | 1  |
|                                  |                       |   |   |    |    | Nonformed Feces                  |                       | 3 | 2 | 1  | 4  |
|                                  |                       |   |   |    |    | <b>Skin &amp; Pelage</b>         |                       |   |   |    |    |
|                                  |                       |   |   |    |    | Pale Skin, Entire Body           |                       | 0 | 1 | 0  | 0  |
|                                  |                       |   |   |    |    | Pale Skin, Gums                  |                       | 0 | 0 | 0  | 2  |

There were no DU-176b-related adverse effects on body weight and food consumption. No visible DU-176b-related ophthalmic lesions were noted. All electrocardiograms were qualitatively and quantitatively within normal limits. No arrhythmias were found.

In the surviving animals, slightly prolongation of PT and APTT was seen in the 15 and 45 mg/kg/day groups (Table 41) that was attributable to the anticoagulant property of DU-176b. Slightly lower RBC counts, HGB, and MCHC values (Table 42) were associated with higher ARET (Table 43) were seen at doses 15 and 45 mg/kg/day.

These findings of anemia and anemia-related compensative response were secondary to bleeding due to the anticoagulant property of DU-176b. These changes were not observed at the end of recovery period.

Table 41. Prothrombin time (PT) and activated partial thromboplastin time (APTT) in monkeys (modified from the submission)

| DU-176b<br>mg/kg/day |    | PT S (Seconds) |          |          |         | APTT S (Seconds) |          |          |         |      |
|----------------------|----|----------------|----------|----------|---------|------------------|----------|----------|---------|------|
|                      |    | PRED 29        | DSNG 179 | DSNG 360 | RECO 91 | PRED 29          | DSNG 179 | DSNG 360 | RECO 91 |      |
| Male                 | 0  | Mean           | 9.9      | 10.0     | 13.8    | 13.5             | 16.1     | 16.1     | 25.3    | 24.5 |
|                      |    | SD             | 0.26     | 0.38     | 0.69    | 0.07             | 2.98     | 2.75     | 3.26    | 1.77 |
|                      |    | N              | 6        | 6        | 6       | 2                | 6        | 6        | 6       | 2    |
|                      | 5  | Mean           | 9.9      | 10.1     | 14.5    | -                | 15.8     | 17.0     | 25.8    | -    |
|                      |    | SD             | 0.06     | 0.15     | 0.36    | -                | 1.66     | 1.42     | 1.99    | -    |
|                      |    | N              | 4        | 4        | 4       | 0                | 4        | 4        | 4       | 0    |
|                      | 15 | Mean           | 10.3     | 12.0*    | 19.6*   | -                | 19.7     | 22.4*    | 30.7*   | -    |
|                      |    | SD             | 0.45     | 1.61     | 5.21    | -                | 1.67     | 2.70     | 3.81    | -    |
|                      |    | N              | 4        | 4        | 4       | 0                | 4        | 4        | 4       | 0    |
|                      | 45 | Mean           | 9.8      | 12.8*    | 21.2*   | 13.0             | 17.1     | 21.0*    | 30.7*   | 28.0 |
|                      |    | SD             | 0.25     | 1.33     | 1.52    | 0.92             | 1.66     | 1.19     | 2.23    | 1.77 |
|                      |    | N              | 6        | 6        | 6       | 2                | 6        | 6        | 6       | 2    |
| Female               | 0  | Mean           | 9.7      | 9.6      | 12.8    | 12.2             | 16.7     | 16.2     | 25.6    | 29.1 |
|                      |    | SD             | 0.37     | 0.37     | 0.79    | 0.21             | 0.65     | 0.47     | 0.95    | 0.14 |
|                      |    | N              | 6        | 6        | 6       | 2                | 6        | 6        | 6       | 2    |
|                      | 5  | Mean           | 9.5      | 9.7      | 12.9    | -                | 16.2     | 17.7     | 27.0    | -    |
|                      |    | SD             | 0.35     | 0.29     | 0.90    | -                | 2.43     | 2.59     | 2.86    | -    |
|                      |    | N              | 4        | 3        | 3       | 0                | 4        | 3        | 3       | 0    |
|                      | 15 | Mean           | 10.0     | 10.8*    | 16.5*   | -                | 17.8     | 19.2*    | 27.4    | -    |
|                      |    | SD             | 0.13     | 0.30     | 1.01    | -                | 1.83     | 0.91     | 2.38    | -    |
|                      |    | N              | 4        | 4        | 3       | 0                | 4        | 4        | 3       | 0    |
|                      | 45 | Mean           | 10.1     | 13.3*    | 20.6*   | 12.8             | 16.6     | 22.5*    | 30.1*   | 27.4 |
|                      |    | SD             | 0.16     | 0.93     | 0.81    | -                | 1.08     | 2.02     | 2.15    | -    |
|                      |    | N              | 6        | 5        | 4       | 1                | 6        | 5        | 4       | 1    |

\* p<0.05 vs control (0 mg/kg/day). PRED: predose day. DSNG: dosing day. RECO: recovery day.

Table 42. Findings in hematology – anemia in monkeys (modified from the submission)

| DU-176b<br>mg/kg/day |    | RBC 10 <sup>6</sup> /μL |          |          |         | HGB G/DL |          |          |         | MCHC G/DL |          |          |         |      |
|----------------------|----|-------------------------|----------|----------|---------|----------|----------|----------|---------|-----------|----------|----------|---------|------|
|                      |    | PRED 29                 | DSNG 179 | DSNG 360 | RECO 91 | PRED 29  | DSNG 179 | DSNG 360 | RECO 91 | PRED 29   | DSNG 179 | DSNG 360 | RECO 91 |      |
| Male                 | 0  | Mean                    | 5.36     | 5.67     | 5.89    | 6.12     | 13.2     | 14.2     | 14.3    | 15.6      | 30.9     | 32.2     | 32.3    | 32.2 |
|                      |    | SD                      | 0.25     | 0.28     | 0.28    | 0.02     | 0.90     | 0.85     | 0.62    | 0.42      | 0.91     | 0.56     | 1.01    | 0.42 |
|                      |    | N                       | 6        | 6        | 6       | 2        | 6        | 6        | 6       | 2         | 6        | 6        | 6       | 2    |
|                      | 5  | Mean                    | 5.36     | 5.51     | 5.75    | -        | 12.9     | 13.1     | 13.6    | -         | 30.9     | 30.3*    | 31.5    | -    |
|                      |    | SD                      | 0.43     | 0.45     | 0.49    | -        | 0.92     | 0.94     | 1.05    | -         | 1.25     | 1.31     | 0.82    | -    |
|                      |    | N                       | 4        | 4        | 4       | 0        | 4        | 4        | 4       | 0         | 4        | 4        | 4       | 0    |
|                      | 15 | Mean                    | 5.64     | 5.68     | 5.74    | -        | 13.3     | 13.4     | 13.3    | -         | 30.6     | 30.4*    | 30.7    | -    |
|                      |    | SD                      | 0.71     | 0.62     | 0.66    | -        | 1.19     | 1.07     | 1.00    | -         | 1.26     | 1.40     | 1.73    | -    |
|                      |    | N                       | 4        | 4        | 4       | 0        | 4        | 4        | 4       | 0         | 4        | 4        | 4       | 0    |
|                      | 45 | Mean                    | 5.52     | 5.12     | 5.18    | 5.85     | 13.5     | 12.3*    | 12.2*   | 13.9      | 30.8     | 29.9*    | 30.1*   | 32.7 |
|                      |    | SD                      | 0.41     | 0.49     | 0.41    | 0.04     | 0.45     | 0.98     | 0.69    | 0.49      | 0.82     | 0.61     | 0.65    | 0.14 |
|                      |    | N                       | 6        | 6        | 6       | 2        | 6        | 6        | 6       | 2         | 6        | 6        | 6       | 2    |
| Female               | 0  | Mean                    | 5.17     | 5.33     | 5.52    | 5.53     | 12.4     | 12.9     | 13.1    | 13.0      | 24.1     | 24.2     | 23.8    | 23.5 |
|                      |    | SD                      | 0.38     | 0.29     | 0.30    | 0.30     | 0.89     | 0.76     | 0.67    | 0.85      | 0.63     | 0.84     | 0.69    | 0.14 |
|                      |    | N                       | 6        | 6        | 6       | 2        | 6        | 6        | 6       | 2         | 6        | 6        | 6       | 2    |
|                      | 5  | Mean                    | 5.31     | 5.53     | 5.63    | -        | 12.7     | 13.0     | 12.9    | -         | 23.9     | 23.6     | 22.8    | -    |
|                      |    | SD                      | 0.06     | 0.34     | 0.20    | -        | 0.48     | 0.29     | 0.35    | -         | 1.05     | 0.89     | 0.92    | -    |
|                      |    | N                       | 4        | 3        | 3       | 0        | 4        | 3        | 3       | 0         | 4        | 3        | 3       | 0    |
|                      | 15 | Mean                    | 5.20     | 5.20     | 4.59*   | -        | 12.5     | 12.4     | 10.8*   | -         | 24.0     | 23.8     | 23.4    | -    |
|                      |    | SD                      | 0.48     | 0.369    | 0.31    | -        | 1.03     | 0.88     | 0.80    | -         | 1.28     | 1.39     | 0.31    | -    |
|                      |    | N                       | 4        | 4        | 3       | 0        | 4        | 4        | 3       | 0         | 4        | 4        | 3       | 0    |
|                      | 45 | Mean                    | 5.35     | 5.14     | 4.99    | 5.38     | 13.0     | 12.6     | 11.7    | 13.6      | 24.3     | 24.5     | 23.5    | 25.2 |
|                      |    | SD                      | 0.29     | 0.63     | 0.61    | -        | 0.83     | 1.50     | 1.23    | -         | 0.60     | 1.01     | 0.87    | -    |
|                      |    | N                       | 6        | 5        | 4       | 1        | 6        | 5        | 4       | 1         | 6        | 5        | 4       | 1    |

\* p<0.05 vs control (0 mg/kg/day)

Table 43. Findings in hematology – reticulocyte count ( $10^3 /\mu\text{l}$ ), modified from the submission)

| DU-176b<br>mg/kg/day |      | Male    |          |          |         | Female  |          |          |         |
|----------------------|------|---------|----------|----------|---------|---------|----------|----------|---------|
|                      |      | PRED 29 | DSNG 179 | DSNG 360 | RECO 91 | PRED 29 | DSNG 179 | DSNG 360 | RECO 91 |
| 0                    | Mean | 86.9    | 43.7     | 42.6     | 52.2    | 90.5    | 57.8     | 66.7     | 53.2    |
|                      | SD   | 22.28   | 10.36    | 9.24     | 13.22   | 27.31   | 16.87    | 30.57    | 28.57   |
|                      | N    | 6       | 6        | 6        | 2       | 6       | 6        | 6        | 2       |
| 5                    | Mean | 82.0    | 65.5     | 62.2     | -       | 86.3    | 55.6     | 72.0     | -       |
|                      | SD   | 6.52    | 9.22     | 11.74    | -       | 15.93   | 8.11     | 18.30    | -       |
|                      | N    | 4       | 4        | 4        | 0       | 4       | 3        | 3        | 0       |
| 15                   | Mean | 81.3    | 73.9     | 73.6     | -       | 86.4    | 66.9     | 148.6    | -       |
|                      | SD   | 17.13   | 28.08    | 36.76    | -       | 12.85   | 31.73    | 68.32    | -       |
|                      | N    | 4       | 4        | 4        | 0       | 4       | 4        | 3        | 0       |
| 45                   | Mean | 70.9    | 102.9    | 108.6*   | 41.2    | 82.3    | 75.6     | 111.2*   | 54.9    |
|                      | SD   | 15.75   | 89.91    | 45.35    | 10.04   | 23.89   | 32.17    | 7.28     | -       |
|                      | N    | 6       | 6        | 6        | 2       | 6       | 5        | 4        | 1       |

\*  $p < 0.05$  vs control (0 mg/kg/day)

DU-176b did not produce any remarkable or notable effects in plasma chemistry, urinalysis, macroscopic observation, and organ weight.

Three monkeys with premature death had abnormal histopathological findings.

One male (I58739) of the 15 mg/kg/day group had a deeply ulcerated hemorrhagic skin lesion over its eye. Contamination of the wound was indicated by large numbers of neutrophils, present both superficially and deep within the tissue.

One female (I58760) at the 15 mg/kg/day dose had large amounts of free blood within the pulmonary airways. Hemorrhage was diffusely distributed throughout one examined lobe, filling most of the distal airways, distributed in a more patchy fashion in the other examined lobe, and scattered with neutrophil infiltration. There was a large necrotic bronchus within the hemorrhagic lobe densely infiltrated by neutrophils, and neutrophils were also present in large numbers within the surrounding lung parenchyma.

One female (I58755) at the dose 5 mg/kg/day had a large hematoma within the submucosa of the stomach, with hemorrhage into the surrounding tissue and large amounts of blood within the lumen of the gastrointestinal tract. The original gastric lesion was more likely a result of trauma; however, the severe outcome of the episode was due, at least in part, to the anticoagulant action of the test article.

Hemorrhages in all the 3 monkeys were attributable to the anticoagulative properties of the test article, but hemorrhage was not reported at sites of femoral venipuncture.

At the end of dosing period, 1/4, 1/4, 0/3, and 2/4 males were testicular immature (diagnosed histologically as juvenile) at control, DU-176b 5, 15, and 45 mg/kg/day, respectively. All males with testes identified as juvenile were approximately 4 years of age or younger at the time of euthanasia (the age of sexual maturity in cynomolgus monkeys is approximately 6 years of age). One male at 45 mg/kg/day, a little over 5

years of age, was diagnosed histologically with immature spermatogenesis. This immaturity is due to age.

There were no DU-176b-related histological findings in eyes.

After the 14-week recovery period, there were no drug-related findings in any animals examined.

After oral dosing, DU-176 readily appeared in plasma, with T<sub>max</sub> value range of mostly 1- 4 hours (Figure 11, Table 44). Increases in DU-176 C<sub>max</sub> and AUC<sub>0-24</sub> for both sexes were under dose-proportional. There was no apparent sex difference in the mean C<sub>max</sub> and AUC<sub>0-24h</sub> values or accumulation after multiple dosing (Table 44).

Figure 11. Plasma Du-176 concentrations following oral dosing to monkeys (modified from the submission)

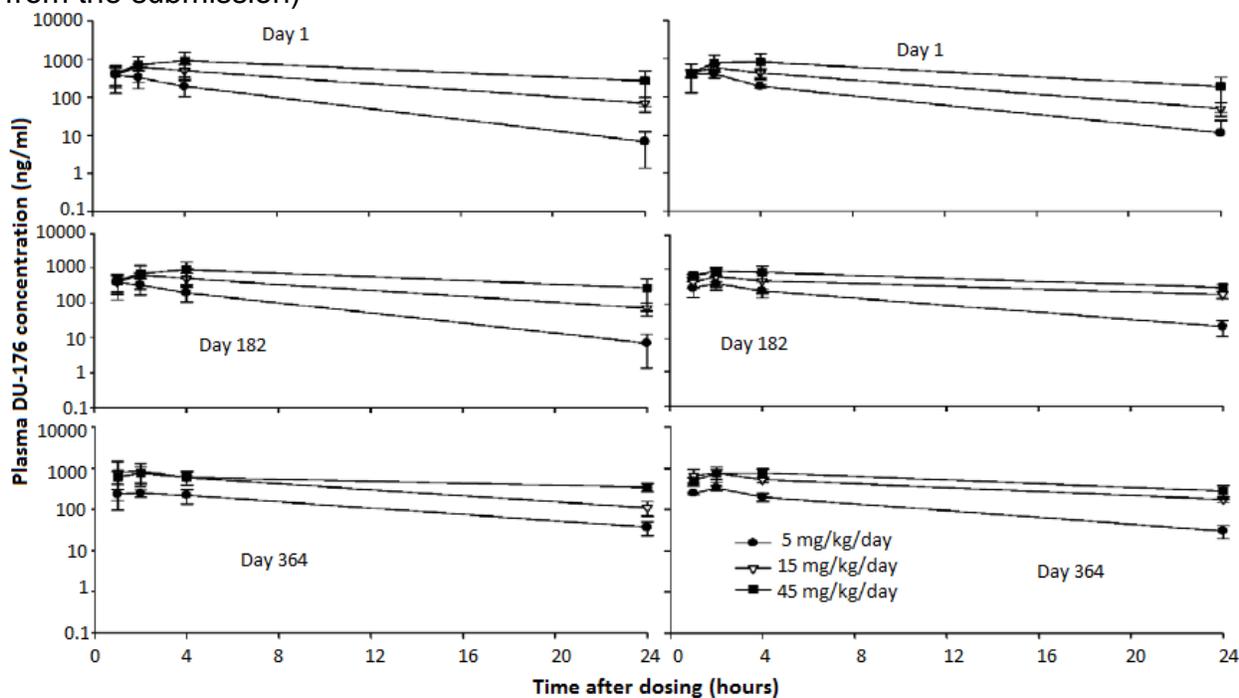


Table 44. Summary of TK parameters following DU-176b oral dosing to monkeys

| Dose Level<br>(mg/kg/day) | Sex |      | Day 1                       |                          |                                   | Day 182                     |                          |                                   | Day 364                     |                          |                                   |
|---------------------------|-----|------|-----------------------------|--------------------------|-----------------------------------|-----------------------------|--------------------------|-----------------------------------|-----------------------------|--------------------------|-----------------------------------|
|                           |     |      | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | AUC <sub>0-24</sub><br>(ng•hr/mL) | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | AUC <sub>0-24</sub><br>(ng•hr/mL) | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | AUC <sub>0-24</sub><br>(ng•hr/mL) |
| 5                         | M   | Mean | 412                         | 1.25                     | 3609                              | 222                         | 2.50                     | 2703                              | 263                         | 2.00                     | 3370                              |
|                           |     | SD   | 269                         | 0.50                     | 1157                              | 58                          | 1.00                     | 617                               | 61                          | 1.41                     | 961                               |
|                           |     | N    | 4                           | 4                        | 3                                 | 4                           | 4                        | 4                                 | 4                           | 4                        | 4                                 |
|                           | F   | Mean | 440                         | 1.50                     | 3380                              | 398                         | 1.67                     | 3772                              | 327                         | 2.00                     | 3237                              |
|                           |     | SD   | 81                          | 0.58                     | 260                               | 76                          | 0.58                     | 34                                | 43                          | 0                        | 481                               |
|                           |     | N    | 4                           | 4                        | 3                                 | 3                           | 3                        | 3                                 | 3                           | 3                        | 3                                 |
| 15                        | M   | Mean | 701                         | 2.50                     | 7550                              | 642                         | 2.25                     | 7728                              | 922                         | 2.75                     | 8469                              |
|                           |     | SD   | 81                          | 1.00                     | 1972                              | 246                         | 1.26                     | 3857                              | 584                         | 1.50                     | 3076                              |
|                           |     | N    | 4                           | 4                        | 4                                 | 4                           | 4                        | 4                                 | 4                           | 4                        | 3                                 |
|                           | F   | Mean | 625                         | 2.50                     | 6457                              | 621                         | 2.00                     | 8576                              | 763                         | 2.67                     | 9345                              |
|                           |     | SD   | 68                          | 1.00                     | 1179                              | 367                         | 0                        | 4498                              | 274                         | 1.15                     | 1662                              |
|                           |     | N    | 4                           | 4                        | 4                                 | 4                           | 4                        | 4                                 | 3                           | 3                        | 3                                 |
| 45                        | M   | Mean | 994                         | 6.29                     | 14236                             | 1115                        | 2.83                     | 16622                             | 759                         | 2.33                     | 11797                             |
|                           |     | SD   | 524                         | 7.87                     | 6650                              | 1241                        | 1.33                     | 15214                             | 297                         | 0.82                     | 2765                              |
|                           |     | N    | 7                           | 7                        | 7                                 | 6                           | 6                        | 6                                 | 6                           | 6                        | 6                                 |
|                           | F   | Mean | 1027                        | 2.86                     | 12428                             | 991                         | 2.60                     | 14243                             | 843                         | 3.00                     | 12998                             |
|                           |     | SD   | 564                         | 1.07                     | 6807                              | 303                         | 1.34                     | 4457                              | 180                         | 1.15                     | 2290                              |
|                           |     | N    | 7                           | 7                        | 7                                 | 5                           | 5                        | 5                                 | 4                           | 4                        | 4                                 |

In conclusion, the NOAEL was 5 mg/kg/day, with mean C<sub>max</sub> and AUC<sub>0-24h</sub> values on Day 364 of 263 ng/mL and 3370 ng•h/mL, respectively, in males, and 327 ng/mL and 3237 ng•h/mL, respectively, in females.

### 6.2.8 Repeated Dose Toxicity Study in Monkeys Treated Intravenously with DU-176b for Injection 10 mg for 14 Days

Conducting laboratory and location: Daiichi Sankyo Co., Ltd., Japan (b) (4)  
 Study number(s): AN08-H0083-R01  
 Date of study initiation: May 20, 2009  
 Drug/lot/batch number: DU-176b for Injection 10 mg / L008SG01 / 109.63% (amount of DU-176 vs. labeling)  
 GLP compliance: Yes  
 QA statement: Yes

### Key Study Findings

There was no premature death during the study. At DU-176 dose of 4 mg/kg/day, 1/3 males had bloody stool; 1/3 males had purpura around the blood sampling site; and females showed anemia (including decreases in RBC, HGB, and HCT), an increase in RETIC, and slight higher platelet count and fibrinogen (FIB). These findings were consistent with hemorrhage and attributable to the anticoagulant property of DU-176b. The NOAEL was 1 mg/kg/day in this study.

Systemic DU-176 exposures ( $C_0$  and  $AUC_{all}$ ) were generally dose-proportional following IV injection, with no apparent sex difference or accumulation after multiple dosing. The mean  $C_0$  and  $AUC_{all}$  values on Day 14 at the NOAEL of 1 mg/kg/day were 782 ng/mL and 1950 ng•h/mL in males, respectively, and 807 ng/mL and 1890 ng•h/mL in females, respectively.

## Methods

Male and female Cynomolgus monkeys (4-6 years old) were injected IV with DU-176b at 0 (vehicle, saline), 1, or 4 mg/kg/day, QD, for 14 days ( $n = 3/\text{sex}/\text{group}$ ). This study was performed precede a planned clinical bioavailability study with IV infusion of DU-176b at 30 mg (total dose) for 30 minutes. Dose levels selected in the current study was based on a preliminary 14-day repeated dose toxicity study in 2 monkeys with IV D11-4176a (anhydrous edoxaban) at 10 mg/kg/day (**Study R20020002**). In Study R20020002, vascular injury at the administration site, subcutaneous hemorrhage, decreases in RBC, HGB and HCT, increases in mean corpuscular volume (MCV), platelet count (PLT), reticulocyte count (RETIC), FIB, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acidophilic change in hepatocytes were noted in 1 of 2 females at 10 mg/kg/day (with  $C_{5 \text{ min}}$  52.6 times human exposure at the MRHD of 60 mg). Slight prolongations of PT and/or APTT occurred in the 2 D11-4176a-treated monkeys. The exposure level in monkeys at 1 mg/kg (AM08-C0011-R01) had been expected to be equivalent to or more than those in human subjects with an intravenous infusion at 30 mg for 30 min.

Monkeys were checked for morbidity/death and clinical signs (1-2 times daily), occult blood test of feces (once on Day 1 for 1 male in the 4 mg/kg group), body weight (weekly), and food consumption (daily). Urine samples were collected on Days -9 and 9 for urinalysis (including color, turbidity, specific gravity, pH, protein, glucose, ketone bodies, occult blood, urobilinogen, and microscopic sediment examination). Blood samples were collected from femoral vein on Days -13, -5, 7, and 14 for hematology (Table 29 plus FIB) and plasma chemistry (Table 30); on Day 1 at 0.083, 0.5, 2, 7, and 24 h post-dose, and on Day 14 prior to dosing, and at 0.083, 0.5, 2, 7, and 24 h post-dose for TK. Plasma concentrations of DU-176 was determined using an LC-MS/MS method.

At the end of dosing period, all surviving monkeys were weighed, euthanized, and necropsied. Weights of brain, pituitary, thyroids (including parathyroids), heart, lung, liver, spleen, kidneys, pancreas, adrenals, prostate, seminal vesicle, testes, epididymides, ovaries and uterus were recorded. The brain, pituitary, thyroids (including parathyroids), heart, lung, liver, spleen, kidneys, pancreas, adrenals, prostate, seminal vesicle, testes, epididymides, ovaries, uterus, salivary glands (right and left), stomach, duodenum, jejunum, ileum, cecum, colon, rectum, gallbladder, mesenteric lymph node, thymus, urinary bladder, skeletal muscle (right rectus femoris), bone (sternum), bone marrow (sternum), spinal cord (lumbar part), eyes (right and left), lacrimal glands (right and left), tongue, trachea, esophagus, aorta (thoracic), skin (right thorax), vagina,

mammary gland (only female), sciatic nerve (right), injection site and skin (left upper arm) were collected, fixed, histologically processed and examined. It was stated that the kidney, testis, epididymis, and salivary gland from the left side and the eye and lacrimal gland from the right side were not examined, but no rationale was provided.

## Results

No animal died prematurely during the study.

At the DU-176 dose of 4 mg/kg/day, 1/3 males had bloody stool, and 1/3 males had purpura around the blood sampling site. Histopathological examination revealed cell infiltration, edema, and hemorrhage in the subcutis of the male showing purpura. Females at the dose of 4 mg/kg/day showed anemia including decreases in RBC, HGB, and HCT, and an increase in RETIC (Table 45), and slightly higher platelet count (PLT) and fibrinogen (FIB) (Table 46). These findings were consistent with hemorrhage and attributable to the anticoagulation property of DU-176b.

There were no treatment-related changes in other examinations.

Table 45. Changes indicating anemia in monkeys received IV DU-176b

| DU-176b   |     | RBC (M/ $\mu$ L) |      |        | HGB (g/dL) |       |        | HCT (%) |       |        | RETIC (%) |      |        |        |
|-----------|-----|------------------|------|--------|------------|-------|--------|---------|-------|--------|-----------|------|--------|--------|
| mg/kg/Day | Day | -5               | 7    | 14     | -5         | 7     | 14     | -5      | 7     | 14     | -5        | 7    | 14     |        |
| Male      | 0   | Mean             | 5.70 | 5.36   | 5.35       | 13.43 | 12.73  | 12.50   | 43.43 | 41.10  | 41.93     | 0.93 | 1.73   | 2.17   |
|           |     | S.D.             | 0.29 | 0.26   | 0.42       | 0.67  | 0.95   | 0.40    | 1.12  | 2.19   | 1.66      | 0.42 | 0.60   | 0.35   |
|           | 1   | Mean             | 5.45 | 4.76   | 5.14       | 12.90 | 11.50  | 12.23   | 42.20 | 37.63  | 41.67     | 0.87 | 2.93   | 2.37   |
|           |     | S.D.             | 0.19 | 0.48   | 0.43       | 0.36  | 1.30   | 0.65    | 1.65  | 3.99   | 3.51      | 0.25 | 2.59   | 0.40   |
|           | 4   | Mean             | 5.44 | 4.73   | 4.93       | 12.93 | 11.43  | 11.50   | 41.63 | 37.27  | 39.07     | 1.07 | 4.97   | 2.57   |
|           |     | S.D.             | 0.47 | 0.01   | 0.40       | 0.25  | 1.12   | 0.56    | 0.29  | 3.77   | 3.17      | 0.42 | 4.03   | 0.35   |
| Female    | 0   | Mean             | 5.43 | 5.27   | 5.30       | 12.67 | 12.53  | 12.37   | 42.53 | 41.10  | 41.73     | 1.17 | 1.57   | 1.97   |
|           |     | S.D.             | 0.15 | 0.23   | 0.11       | 0.23  | 0.90   | 0.60    | 0.86  | 3.44   | 1.56      | 0.31 | 0.47   | 0.65   |
|           | 1   | Mean             | 5.51 | 4.99   | 5.10       | 13.33 | 12.13  | 12.17   | 43.30 | 39.43  | 40.37     | 1.13 | 2.57   | 2.30   |
|           |     | S.D.             | 0.14 | 0.25   | 0.36       | 0.51  | 0.76   | 0.76    | 1.91  | 2.86   | 2.68      | 0.45 | 0.70   | 0.70   |
|           | 4   | Mean             | 5.12 | 3.79** | 3.84**     | 12.47 | 9.20** | 9.27**  | 41.33 | 31.03* | 32.60**   | 1.00 | 4.77** | 7.50** |
|           |     | S.D.             | 0.50 | 0.40   | 0.19       | 0.85  | 0.60   | 0.59    | 3.26  | 2.85   | 1.74      | 0.60 | 0.86   | 1.40   |

Significant difference from 0 mg/kg/Day: \* p <0.05; \*\*p <0.01 by the Dunnett test

Table 46. Effects of IV DU-176b on coagulation-related parameters in monkeys

| DU-176b   |     | PLT (K/ $\mu$ L) |       |       | PT (sec) |       |       | APTT (sec) |       |       | FIB (mg/dL) |        |        |        |
|-----------|-----|------------------|-------|-------|----------|-------|-------|------------|-------|-------|-------------|--------|--------|--------|
| mg/kg/Day | Day | -5               | 7     | 14    | -5       | 7     | 14    | -5         | 7     | 14    | -5          | 7      | 14     |        |
| Male      | 0   | Mean             | 315.7 | 334.0 | 320.7    | 9.80  | 9.77  | 9.77       | 23.37 | 23.03 | 23.30       | 232.20 | 268.13 | 250.27 |
|           |     | S.D.             | 44.06 | 78.3  | 50.5     | 0.36  | 0.32  | 0.35       | 0.46  | 0.55  | 0.70        | 36.99  | 49.94  | 51.22  |
|           | 1   | Mean             | 296.0 | 322.0 | 316.0    | 10.07 | 10.10 | 10.03      | 23.13 | 23.53 | 24.33       | 228.13 | 279.13 | 269.83 |
|           |     | S.D.             | 77.6  | 77.3  | 64.9     | 0.12  | 0.10  | 0.12       | 0.55  | 0.41  | 1.01        | 57.67  | 59.57  | 45.44  |
|           | 4   | Mean             | 362.0 | 405.7 | 369.0    | 9.73  | 9.87  | 9.83       | 22.87 | 24.23 | 24.13       | 220.33 | 288.53 | 260.13 |
|           |     | S.D.             | 84.9  | 36.5  | 83.0     | 0.153 | 0.12  | 0.06       | 1.89  | 2.97  | 2.51        | 27.66  | 44.71  | 41.07  |
| Female    | 0   | Mean             | 375.7 | 375.7 | 362.3    | 9.83  | 9.73  | 9.67       | 23.03 | 22.63 | 23.13       | 190.03 | 222.80 | 223.10 |
|           |     | S.D.             | 62.36 | 39.3  | 67.7     | 0.21  | 0.29  | 0.25       | 0.96  | 0.74  | 0.60        | 40.85  | 55.31  | 39.11  |
|           | 1   | Mean             | 370.7 | 384.7 | 390.0    | 10.07 | 9.87  | 9.93       | 21.53 | 22.00 | 22.77       | 173.83 | 209.43 | 197.97 |
|           |     | S.D.             | 43.9  | 93.9  | 87.5     | 0.38  | 0.38  | 0.32       | 1.65  | 1.56  | 2.27        | 31.22  | 35.81  | 28.64  |
|           | 4   | Mean             | 448.3 | 449.0 | 554.7*   | 9.87  | 9.83  | 9.87       | 22.93 | 23.20 | 24.50       | 175.60 | 277.23 | 252.27 |
|           |     | S.D.             | 38.9  | 36.8  | 70.5     | 0.35  | 0.20  | 0.50       | 3.07  | 3.68  | 3.51        | 22.93  | 21.66  | 41.79  |

Significant difference from 01 group: \* p <0.05 by the Dunnett test

Plasma concentrations of DU-176 increased dose-proportionally following IV injection (Table 47). There was no apparent sex difference in the mean  $C_0$  and  $AUC_{all}$  values or accumulation after multiple dosing (Table 47). Plasma DU-176 levels in the control group was less than the lower limit of quantitation for DU-176 (5.00 ng/mL).

Table 47. Plasma DU-176 concentrations and TKs following IV DU-in monkeys (n=3/group, from the submission)

| Day of dosing | Dose (mg/kg) | Sex    |      | Plasma concentration (ng/mL) at: |          |       |      |      |       | $t_{1/2}$ (h) | $C_0$ (ng/mL) | $AUC_{all}$ (ng-h/mL) |
|---------------|--------------|--------|------|----------------------------------|----------|-------|------|------|-------|---------------|---------------|-----------------------|
|               |              |        |      | pre                              | 0.0833 h | 0.5 h | 2 h  | 7 h  | 24 h  |               |               |                       |
| 1             | 1            | Male   | Mean | -                                | 812      | 582   | 262  | 23.6 | 1.88  | 3.96          | 868           | 1930                  |
|               |              |        | SD   | -                                | 64.2     | 47.2  | 51.6 | 3.99 | 3.26  | 4.40          | 75.5          | 243                   |
|               |              | Female | Mean | -                                | 839      | 592   | 249  | 22.6 | 0.00  | 1.43          | 899           | 1870                  |
|               |              |        | SD   | -                                | 11.0     | 0.577 | 10.2 | 1.78 | 0.00  | 0.0252        | 14.2          | 57.7                  |
|               | 4            | Male   | Mean | -                                | 2940     | 2380  | 1140 | 174  | 16.6  | 5.12          | 3070          | 8900                  |
|               |              |        | SD   | -                                | 358      | 243   | 317  | 109  | 10.1  | 1.10          | 391           | 2260                  |
|               |              | Female | Mean | -                                | 3240     | 2730  | 1200 | 106  | 18.3  | 6.57          | 3350          | 8790                  |
|               |              |        | SD   | -                                | 188      | 159   | 221  | 31.5 | 7.13  | 0.380         | 218           | 1250                  |
| 14            | 1            | Male   | Mean | 8.13                             | 749      | 603   | 251  | 24.5 | 7.17  | 9.60          | 782           | 1950                  |
|               |              |        | SD   | 0.421                            | 54.2     | 78.7  | 62.1 | 5.77 | 0.327 | 1.25          | 52.0          | 348                   |
|               |              | Female | Mean | 6.60                             | 769      | 602   | 250  | 22.8 | 2.43  | 3.58          | 807           | 1890                  |
|               |              |        | SD   | 1.70                             | 54.4     | 37.3  | 38.0 | 7.02 | 4.21  | 3.86          | 61.5          | 243                   |
|               | 4            | Male   | Mean | 16.2                             | 2830     | 2330  | 1090 | 103  | 17.5  | 6.38          | 2940          | 7870                  |
|               |              |        | SD   | 9.71                             | 240      | 359   | 227  | 34.9 | 10.5  | 0.868         | 232           | 1530                  |
|               |              | Female | Mean | 23.3                             | 3100     | 2700  | 1410 | 114  | 22.0  | 7.05          | 3190          | 9510                  |
|               |              |        | SD   | 6.13                             | 208      | 332   | 173  | 31.2 | 7.91  | 0.352         | 183           | 1290                  |

-: Not calculated

In conclusion, the NOAEL was 1 mg/kg/day in this study. The mean  $C_0$  and  $AUC_{all}$  values on Day 14 at the NOAEL of 1 mg/kg/day were 782 ng/mL and 1950 ng-h/mL in males, respectively, and 807 ng/mL and 1890 ng-h/mL in females, respectively.

## 7 Genetic Toxicology

Potential genotoxicity of DU-176b was evaluated in the in vitro assay systems (bacterial reverse mutation test, chromosomal aberration tests in CHL cells, and polyploidy and micronucleus tests in human peripheral lymphocytes) and in vivo assay systems (bone marrow micronucleus tests in rats and monkeys, liver micronucleus test in rats, and unscheduled DNA synthesis test in rats). These studies were summarized in Table 48 and Table 49, and previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND 063266 (Appendix I).

Table 48. Summary of in vitro GLP genotoxicity studies

| Study number | Type of Study          | Cells/Strain  | Concentration (µg/mL)     | Metabolic Activation | Exposure time (recovery)    | Noteworthy Findings (µg/mL)   |
|--------------|------------------------|---|---------------------------|----------------------|-----------------------------|---|
| 20020251     | Reverse Mutation       | <i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537) and <i>E. coli</i> (WP2 <i>uvrA</i> ) | 78.1 to 5000 (µg/plate)   | ±S9                  | –                           | Negative  |
| 20020321     | Chromosomal Aberration | Chinese hamster lung (CHL) cells  | 313 to 2500               | -S9                  | 6 h (18 h)                  | Negative  |
|              |                        |   | 313 to 2500               | +S9                  | 6 h (18 h)                  | ↑polyploidy at 1250 and 2500 with cell proliferation ratio of 55 & 49%, respectively          |
|              |                        |   | 19.5 to 156               | -S9                  | 24 h                        | Negative  |
| 20030641     | Polyploidy             | Human peripheral lymphocytes  | 313 to 1250 <sup>a)</sup> | -S9                  | 3 h (19 h)                  | ↑polyploidy at 625 & 1250 with mitotic index reduction of 51 & 48%, respectively              |
|              |                        |   | 313 to 1250 <sup>a)</sup> | +S9                  | 3 h (19 h)                  | ↑polyploidy at 313, 625, and 1250 with mitotic index reduction of 22, 66, & 69%, respectively |
|              |                        |   | 157 to 2500 <sup>b)</sup> | -S9                  | 22 h                        | Negative <sup>b)</sup>  |
|              |                        |   | 313 to 1250 <sup>a)</sup> | -S9                  | 46 h                        | ↑polyploidy at 313, 625, and 1250 with mitotic index reduction of 76, 71, & 64%, respectively |
|              |                        |   | 313 to 1250 <sup>a)</sup> | -S9                  | 3 h (43 h)                  | ↑polyploidy at 313, 625, and 1250 with mitotic index reduction of 56, 69, & 83%, respectively |
|              |                        |   | 313 to 1250 <sup>a)</sup> | +S9                  | 3 h (43 h)                  | ↑polyploidy at 313, 625, and 1250 with mitotic index reduction of 6, 68, & 65%, respectively  |
| 20030528     | In Vitro Micronucleus  | Human peripheral lymphocytes  | 185.6 to 1113.6           | +S9                  | 3 h (0+21 h) <sup>c)</sup>  | Negative  |
|              |                        |   | 185.6 to 1113.6           | +S9                  | 3 h (28+17 h) <sup>c)</sup> |   |
|              |                        |   | 46.4 to 1856              | -S9                  | 48 h (0+17 h) <sup>c)</sup> |   |

a: Confirmatory assay, b: Initial assay, d: Recovery time + treatment time with cytochalasin B

Table 49. Summary of In Vivo Genotoxicity Studies

| Type of Study             | Species/Strain    | Route    | Dose mg/kg/day     | Sex (No. /group)             | Dosing duration (Sampling time)                                   | Noteworthy Findings | GLP Compliance | Study Number |
|---------------------------|-------------------|----------|--------------------|------------------------------|---|---------------------|----------------|--------------|
| Bone marrow micronucleus  | Rat/SD            | po       | 0, 500, 1000, 2000 | M (5)                        | Single (24 and 48 h after dosing)                                 | Negative            | Yes            | 20020578     |
|                           | Cynomolgus monkey | po       | 0, 10, 30, 100     | M (4)<br>F (4) <sup>a)</sup> | 4 weeks (24 h after the final dosing)                             | Negative            | Yes            | 20020606     |
|                           | Rat/SD            | IV or po | IV 100<br>PO 2000  | M (3)                        | 2 days (24 h after the final dosing)                              | Negative            | NO             | 20020063     |
| Liver micronucleus        | Rat/SD            | po       | 0, 2000            | M (5)                        | Single (3 or 5 days <sup>b)</sup> and 5 or 7 days <sup>c)</sup> ) | Negative            | Yes            | 20030465     |
| Unscheduled DNA synthesis | Rat/SD            | po       | 0, 500, 1000, 2000 | M (3)                        | Single (2 h to 4 h or 14-16 h after dosing)                       | Negative            | Yes            | 20020498     |

Vehicle: 0.5% Methylcellulose, M: Male, F: Female. a: Number of females at 100 mg/kg/day was two.

b: Hepatectomy was done on the day before administration of DU-176b.

c: Hepatectomy was done on the day after administration of DU-176b.

DU-176b was negative in a bacterial reverse mutation test, an in vitro micronucleus test in human peripheral lymphocytes, bone marrow micronucleus tests in rats using a single or repeated oral administration or repeated IV administration and in a 4-week repeated oral toxicity study in monkeys, a hepatocyte micronucleus test and a unscheduled DNA synthesis test in rats using a single oral administration. However, DU-176b increased polyploidy in a chromosomal aberration test using CHL cells at 1250 and 2500 µg/ml in the presence of S9 liver fraction, and increased polyploidy in a follow up study focused solely on numerical chromosomal aberrations using human peripheral lymphocytes at ≥313 µg/ml in the presence or absent of S9 liver fraction. DU-

176b did not affect the incidence of structural aberrations in the two chromosomal aberration test systems, and the induction of polyploidy was associated with mild (for CHL, ~50% cell proliferation ratio) to strong (for human peripheral lymphocytes, mitotic index reduction up to 83%) toxicity.

## 8 Carcinogenicity

### 8.1 DU-176b: 104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study in Mice

|                                     |                          |         |
|-------------------------------------|--------------------------|---------|
| Study no.:                          | AN07-C0019-R01           |         |
| Study report location:              |                          | (b) (4) |
| Conducting laboratory and location: |                          | (b) (4) |
| Date of study initiation:           | Oct 8, 2007              |         |
| GLP compliance:                     | Yes                      |         |
| QA statement:                       | Yes                      |         |
| Drug, lot #, and % purity:          | DU-176b, KD302, 98.8%    |         |
| CAC concurrence:                    | Yes                      |         |
| Deviation from study protocol:      | No impact to the results |         |

#### Key Study Findings

CD-1 mice were orally gavaged with DU-176b at doses of 0, 50, 150, 500 mg/kg/day, once daily for up to 104 weeks. There were higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice (4/65) and whole body cavity hemangiosarcoma in medium dose female mice (4/65) when compared to their respective controls, but none of the tumor incidences showed statistically significant dose-response relationships. Since higher incidences of these two tumors were not dose-dependent, not seen in the high dose groups, not seen in both sexes, and the incidence of whole body cavity hemangiosarcoma (4/65) was within the vendor's histological background range of 1.67-12% (2), higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice and whole body cavity hemangiosarcoma in medium dose female mice were not considered to be toxicologically significant. No other tumor types had p-values  $\leq 0.05$  for dose-response relationships or pairwise comparisons of treated groups and controls. Executive CAC concluded that the study was acceptable, and concurred that there were no drug-related neoplasms in the study.

A significant increase in mortality was noted in the 500-mg/kg/day males and in the 150-mg/kg/day females. Higher incidences of thin appearance, tremor body, hypoactivity, and audible or irregular breath were seen in males at 500 mg/kg/day. For both males and females, higher incidences of pale ears/entire body, red ears and lower body weight were observed at 500 mg/kg/day. Findings in clinical signs were associated with moribundity. A marginal increase in ovarian hematocysts occurred in all DU-176b-treated female groups compared with the control group, which was considered to be the

cause of 4 female premature deaths at 500-mg/kg/day, and was at least in part attributed to the pharmacological action of the test article as an anticoagulant agent (factor Xa inhibitor). NOAELs for carcinogenicity were 500 mg/kg/day for males and females with C<sub>max</sub> of 829 and 1707 ng/ml, respectively, and AUC<sub>0-24</sub> of 6003 and 12320 ng•hr/ml, respectively, during Week 26. NOAELs for general toxicity were 150 mg/kg/day for males with C<sub>max</sub> of 634 ng/mL and AUC<sub>0-24</sub> of 2036 ng•hr/ml, and 50 mg/kg/day for females with C<sub>max</sub> of 308 ng/ml and AUC<sub>0-24</sub> of 1356 ng•hr/ml during Week 26.

## Methods

CrI:CD1 (ICR) mice (b) (4) initial age of 6 weeks (body weights 24.2 - 36.6 g for males and 19.7 - 29.3 g for females), were orally gavaged with DU-176b at 50, 150, 500 mg/kg/day or vehicle (an aqueous solution of 0.5% methylcellulose), once daily for up to 104 weeks (n = 65/sex/group for toxicity, and 16-36/sex/group for toxicokinetics) (Table 50).

Doses for the present study were selected based on an MTD derived from a 13-week oral gavage study in mice (Study 20050792). In this study (R20050792), mice (10/sex/group for toxicity and 32/sex/group for toxicokinetics) were dosed with DU-176b at 60, 200, 600, or 1500 mg/kg/day by oral gavage for 13 weeks. Animals in the 1500 mg/kg/day dose group were terminated early due to excessive mortality (3/10 males & 2/10 females). At this high-dose, red oral or nasal discharge, irregular or labored respiration, hunched posture, tremors, hypoactivity, hypothermia, squinted eyes, swollen abdomen, rough hair coat and pale skin were observed. Necropsy and histopathological examination revealed congestion in the lung, zymogen granule depletion of the pancreas, lymphocyte depletion in thymus and hyperplasia in the adrenal cortex. Decreases in body weight, food consumption, erythrocyte count, lymphocyte count, hemoglobin and hematocrit were observed in the high-dose group animals. At the dose 600 mg/kg/day, less body weight gain and less food consumption were observed and persisted throughout the study. Treatment-related effects including hunched posture, squinted eyes were also observed at 600 mg/kg/day. At 200 mg/kg/day, low incidence of hunched posture was observed. The NOAEL was established at 60 mg/kg/day. Therefore, the high dose 500 mg/kg/day was selected for both males and females in the present study, which is 1/3rd of lethal dose. Low- and mid-doses were set as 50 and 150 mg/kg/day, respectively.

Table 50. Experimental design/schedule

| Group                        | No. of Animals |        | Dose Level <sup>a</sup><br>(mg/kg/day) | Dose duration<br>(week) |        | Week of<br>Sacrifice/Necropsy |        |
|------------------------------|----------------|--------|--|-------------------------|--------|-------------------------------|--------|
|                              | Male           | Female |  | Male                    | Female | Male                          | Female |
| <b>Toxicity Animals</b>      |                |        |  |                         |        |                               |        |
| 1 Control (Vehicle)          | 65             | 65     | 0                                      | 104                     | 102    | 105                           | 103    |
| 2 Low                        | 65             | 65     | 50                                     | 104                     | 102    | 105                           | 103    |
| 3 Mid                        | 65             | 65     | 150                                    | 104                     | 95     | 105                           | 96     |
| 4 High                       | 65             | 65     | 500                                    | 104                     | 102    | 105                           | 103    |
| <b>Toxicokinetic Animals</b> |                |        |  |                         |        |                               |        |
| 5 Control (Vehicle)          | 16             | 16     | 0                                      |                         |        |                               |        |
| 6 Low                        | 36             | 36     | 50                                     | 9                       | 9      | N/A                           | N/A    |
| 7 Mid                        | 36             | 36     | 150                                    |                         |        |                               |        |
| 8 High                       | 36             | 36     | 500                                    |                         |        |                               |        |

<sup>a</sup> Doses and concentrations of DU-176b were expressed as the amount of anhydrous free base, DU-176 (conversion factor: DU-176/DU-176b = 0.7424).

The study was designed to continue dosing for 104 weeks. However, the study with female mice was terminated early (Table 50) because of poor survival in mid dose group (survival in this group by week 96 reached 16 mice). Samples of the dose formulations were analyzed during Weeks 1, 13, 26, 39, 52, 65, 78, 91 and 104 of treatment to verify the homogeneity, stability, and/or concentrations of DU-176b in the dosing solution.

For the mice in the toxicity arm, clinical signs and morbidity/death were inspected visually during the study at least twice daily. Detailed observations, including palpation, were performed at least once during the pre-dose phase, before dosing on Day 1 and weekly thereafter, and on the day of scheduled sacrifice on each animal. Body weight was recorded prior to treatment, weekly for Weeks 1 through 14 and once every 4 weeks thereafter, and at Week 105 during the dosing phase. Individual food consumption was measured and recorded weekly for Weeks 1 through 13 and once every 4 weeks thereafter and for Week 104. Blood samples were collected via cardiac puncture from all surviving animals at scheduled sacrifice (not fasted) using potassium EDTA as the anticoagulant. Blood smears were also prepared (if possible) during the necropsy procedure from animals sacrificed at an unscheduled interval. Blood samples were determined for hematology parameters including red blood cell (erythrocyte) count, white blood cell (leukocyte) count, differential blood cell count, and blood smear.

A detailed necropsy was performed on each animal that died prematurely or sacrificed either prematurely or at the end of scheduled treatment period. The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin.

|                                       |  |
|---------------------------------------|--|
| adrenal (2)                           | optic nerve (2) <sup>a</sup>                 |
| aorta                                 | ovary (2)                                    |
| brain                                 | pancreas                                     |
| cecum                                 | pituitary gland                              |
| cervix                                | prostate                                     |
| colon                                 | rectum                                       |
| duodenum                              | salivary gland [mandibular (2)]              |
| epididymis (2) <sup>a</sup>           | sciatic nerve                                |
| esophagus                             | seminal vesicle                              |
| eye (2) <sup>a</sup>                  | skeletal muscle (thigh)                      |
| femur with bone marrow – stifle joint | skin/subcutis                                |
| gallbladder                           | spinal cord (cervical, thoracic, and lumbar) |
| Harderian gland (2) <sup>a</sup>      | spleen                                       |
| heart                                 | sternum with bone marrow                     |
| ileum                                 | stomach                                      |
| jejunum                               | testis (2) <sup>a</sup>                      |
| kidney (2)                            | thymus                                       |
| lesions                               | thyroid (2 lobes) with parathyroid           |
| liver                                 | tongue                                       |
| lung with large bronchi               | trachea                                      |
| lymph node (mandibular)               | urinary bladder                              |
| lymph node (mesenteric)               | uterus                                       |
| mammary gland (females)               | vagina                                       |

<sup>a</sup> Preserved in modified Davidson's fixative

Preserved tissues listed above from each animal at terminal sacrifice and each animal that died or sacrificed at an unscheduled time were histologically processed and microscopically examined. Macroscopic lesions from each animal were also histologically processed and microscopically examined.

For TK mice, mortality and signs of pain or distress were observed twice daily. Individual body weights were recorded prior to treatment, before dosing on Day 1 of the dosing phase, and weekly for Weeks 1-14 and every 4 weeks thereafter. Blood samples were collected on Day 1 (1, 2, 4, and 24 hours postdose) and during Week 26 (pre-dose and 1, 2, 4, and 24 hours postdose) using sodium fluoride as the anticoagulant. Plasma was harvested and analyzed for DU-176 concentrations. TK parameters including highest concentration (C<sub>max</sub>), time to peak concentration (T<sub>max</sub>), and area under the concentration-time curve (AUC) were obtained. All TK animals were discarded without necropsy after their scheduled sacrifice or premature death.

## Results

DU-176b concentrations in all dosing solutions were within 92.5 - 109% of the respective theoretical values and met acceptance criteria (mean value was within  $\pm 10\%$  of the theoretical value and individual results were within  $\pm 5\%$  from the mean of the replicates). There was no detectable DU-176 in the control samples. All homogeneity results met acceptance criteria, mean value of each location (top, middle, and bottom)

was within  $\pm 3\%$  of the overall mean and individual results were within  $\pm 1.4\%$  from the mean of the replicates.

Higher mortality was noted for males at the dose 500 mg/kg/day that was statistically significant during weeks 52-90. For females at the dose 150 mg/kg/day, higher mortality was statistically significant during weeks 78-102. In Week 96, the number of surviving females in the 150 mg/kg/day group reached 16 (25%). At Week 103, the survival for control, 50 and 500 mg/kg/day females was 35, 32, and 25%, respectively. For the control, 50, 150, and 500 mg/kg/day males, survival at Week 105 was 42, 35, 38, and 29%, respectively (Table 51, Figure 12). Main findings/possible causes for the 166 male and 190 female premature deaths are summarized in Table 52. Although there were 2 more accidental/gavage-related deaths in the high dose males than control, the higher mortality in high dose males cannot be excluded as being due to DU-176b. There were also 2 more accidental/gavage-related deaths in the mid dose females than control. Since the higher mortality was in mid dose females and was not dose-related, the relationship of the higher mortality in mid dose females and DU-176b was not clear.

Table 51. Survival through the study period with initial number of 65/sex/group

| Week | Males (mg/kg/day) |    |     |     | Females (mg/kg/day) |     |      |     |
|------|-------------------|----|-----|-----|---------------------|-----|------|-----|
|      | Control           | 50 | 150 | 500 | Control             | 50  | 150  | 500 |
| 2    | 65                | 65 | 65  | 65  | 65                  | 65  | 65   | 65  |
| 4    | 65                | 65 | 65  | 65  | 65                  | 65  | 65   | 65  |
| 13   | 65                | 65 | 64  | 63  | 65                  | 65  | 65   | 65  |
| 26   | 65                | 64 | 63  | 60  | 64                  | 65  | 63   | 65  |
| 39   | 62                | 61 | 62  | 58  | 62                  | 64  | 60   | 62  |
| 52   | 61                | 60 | 56  | 53* | 59                  | 62  | 54   | 59  |
| 65   | 55                | 54 | 54  | 43* | 56                  | 56  | 49   | 54  |
| 78   | 46                | 45 | 47  | 35* | 53                  | 45  | 35** | 48  |
| 82   | 43                | 43 | 44  | 34  | 50                  | 39  | 33** | 46  |
| 86   | 41                | 37 | 42  | 30  | 42                  | 36  | 27** | 44  |
| 90   | 39                | 32 | 41  | 27* | 36                  | 32  | 22*  | 39  |
| 94   | 37                | 28 | 38  | 26  | 32                  | 29  | 17** | 34  |
| 96   | 35                | 28 | 36  | 26  | 29                  | 27  | 16*  | 31  |
| 102  | 28                | 23 | 28  | 19  | 23                  | 21  | N/A  | 16  |
| 104  | 28                | 23 | 26  | 19  | N/A                 | N/A | N/A  | N/A |

\* p<0.05 vs control; \*\* p<0.01 vs control

Figure 12. Survival over the study period for mice in toxicity arm (from the submission)

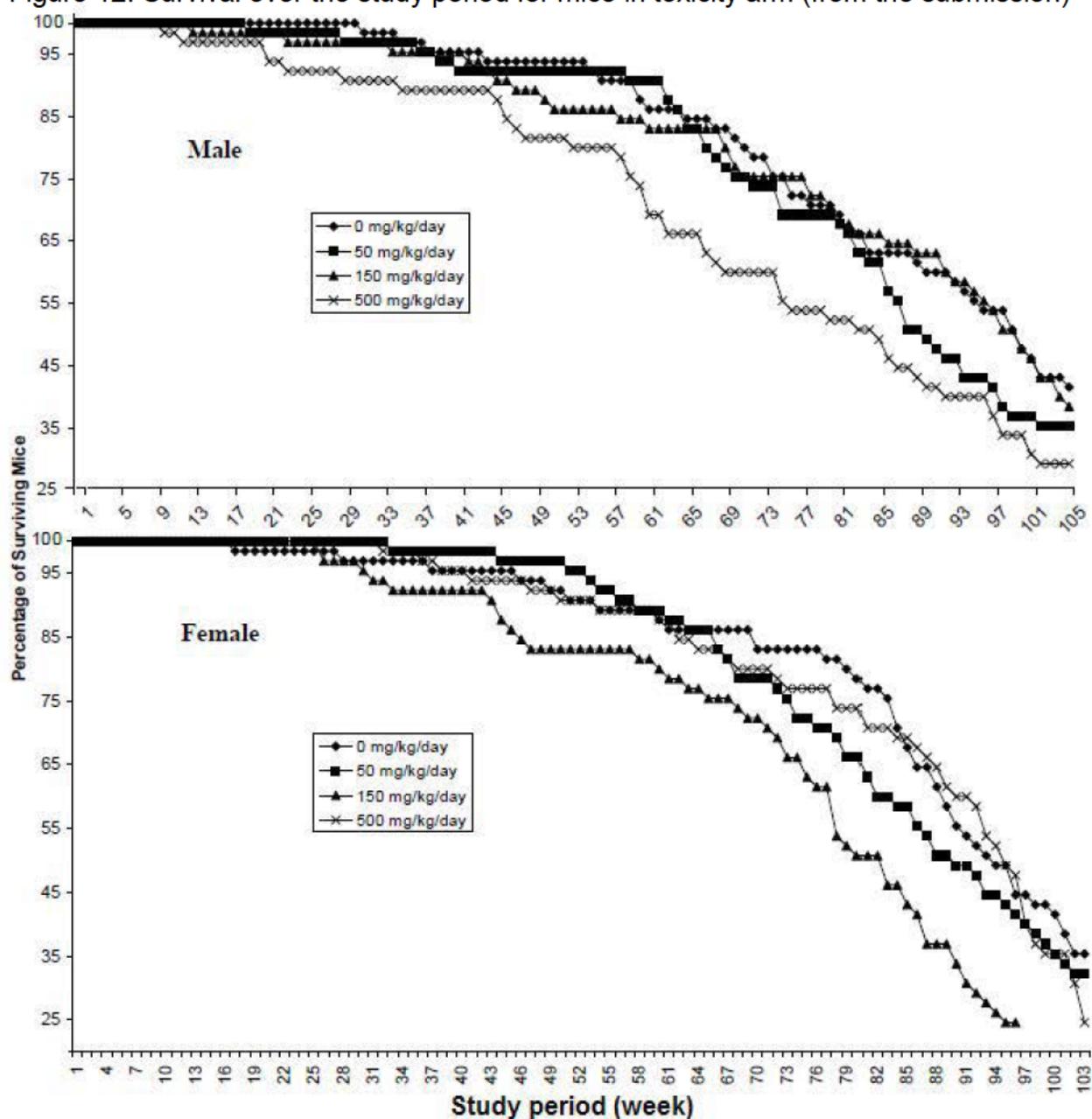


Table 52. Number of premature deaths with main findings/possible causes (modified from the submission)

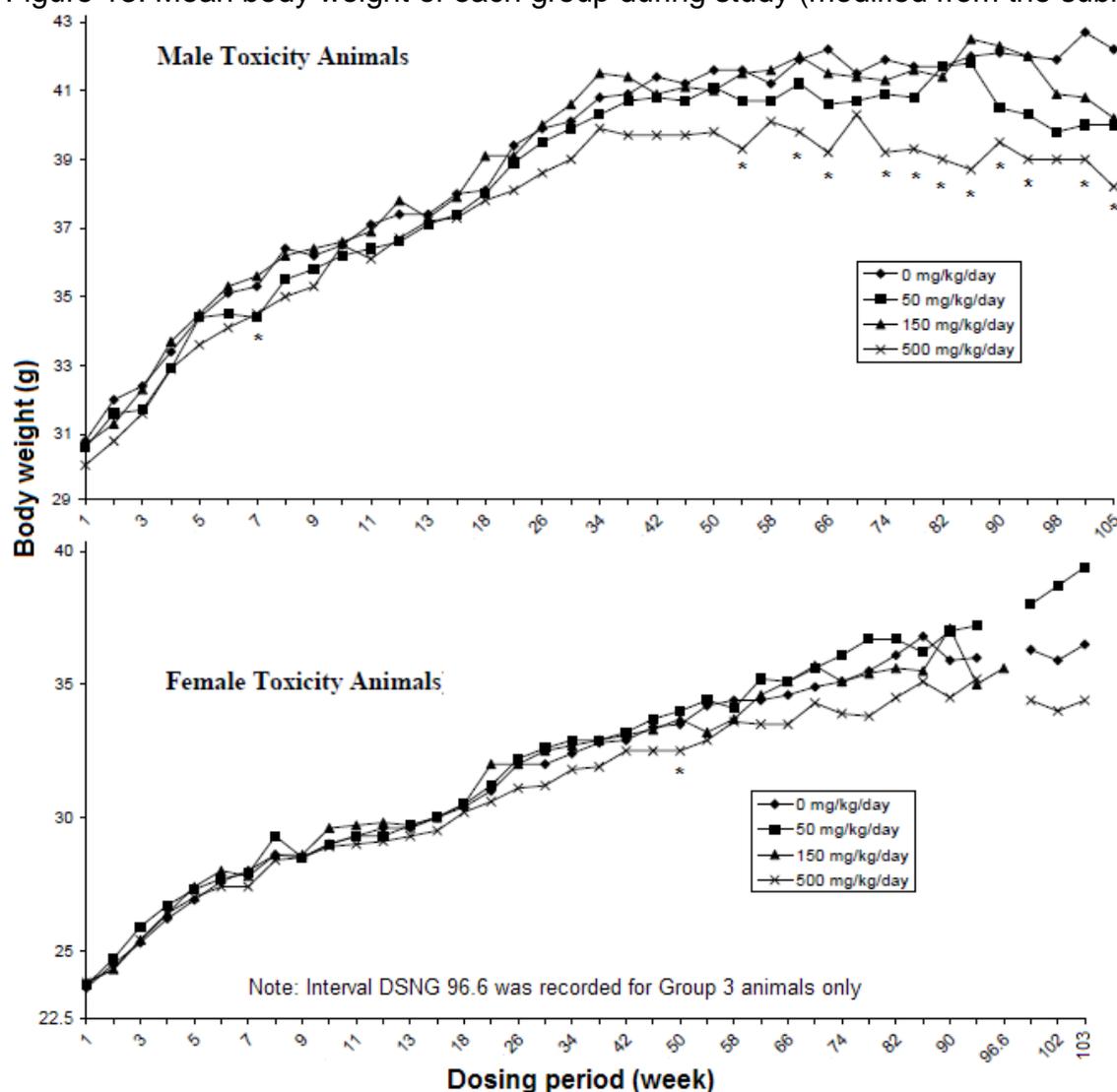
| Tissues                             | Animal sex:<br>Dose level mg/kg/day:<br>Diagnoses<br>No. in group: | Males |    |     |     | Females |    |     |     |
|-------------------------------------|--|-------|----|-----|-----|---------|----|-----|-----|
|                                     |  | Ctls  | 50 | 150 | 500 | Ctls    | 50 | 150 | 500 |
| Death Comment                       | Number examined:   | 38    | 42 | 40  | 46  | 42      | 47 | 50  | 51  |
| Undetermined                        |  | 3     | 6  | 10  | 13  | 4       | 6  | 7   | 13  |
| Accidental                          |  |       |    |     | 1   |         |    | 1   |     |
| Gavage Related Death                |  | 1     |    |     | 2   | 1       |    | 2   | 1   |
| Cystic Liver related Death          |  | 2     |    |     |     |         |    |     |     |
| Ovarian Hematocyst Related Death    |  |       |    |     |     |         |    |     | 4   |
| Hemorrhage Related Death            |  |       | 1  |     |     |         | 1  |     |     |
| Amyloid Related Death               |  | 1     | 1  | 6   | 3   | 7       | 9  | 5   | 7   |
| skin erosion/ulcer related death    |  | 6     | 6  | 4   | 8   | 2       | 2  | 2   | 4   |
| Penile inflammation related death   |  | 2     | 1  | 3   | 2   |         |    |     |     |
| Inflammation related death          |  | 3     | 3  | 6   | 5   | 2       | 5  | 1   |     |
| Kidney Disease related death        |  | 2     | 6  | 2   | 3   | 5       | 2  | 5   | 3   |
| Rectal Prolapse related Death       |  |       |    |     |     | 1       |    |     |     |
| Uterine Prolapse Related Death      |  |       |    |     |     |         |    | 1   | 2   |
| Vaginal prolapse related death      |  |       |    |     |     | 1       |    |     |     |
| Carcinoma related death             |  |       | 1  | 1   |     | 1       |    |     |     |
| Harderian gland tumor related death |  |       |    |     | 1   |         |    |     |     |
| Liver Tumor Related Death           |  | 3     | 1  | 2   | 1   |         | 1  |     |     |
| lung tumor related death            |  | 7     | 3  | 1   | 2   | 2       | 4  | 1   | 1   |
| Mammary tumor related death         |  |       |    |     |     |         | 2  | 3   |     |
| Ovarian Tumor Related Death         |  |       |    |     |     |         |    | 1   |     |
| Pituitary Tumor Related Death       |  |       |    |     |     |         |    | 2   |     |
| Sarcoma Related Death               |  | 2     | 2  | 1   | 1   | 3       |    | 1   | 1   |
| Skin Neopl related death            |  |       | 1  |     | 1   |         |    | 1   | 1   |
| Thymic Tumor related death          |  | 1     |    |     |     |         |    |     |     |
| Uterine Polyp Related Death         |  |       |    |     |     | 1       | 1  |     | 1   |
| symbol tumor related death          |  |       |    |     | 1   |         |    |     |     |
| Hema Neopl Related Death            |  | 4     | 8  | 4   | 1   | 12      | 13 | 15  | 12  |
| Vasc Neopl Related Death            |  | 1     | 2  |     | 1   |         | 1  | 2   | 1   |

Higher incidences of thin appearance, body tremor, hypoactivity, and audible or irregular breath were seen in males at 500 mg/kg/day. For both males and females, higher incidences of pale ears/entire body and red ears were observed at 500 mg/kg/day (Table 53). These clinical signs were either associated with early deaths or attributed to bleeding secondary to the pharmacological effect of DU-176b. About 10% lower mean body weight was observed in males at 500 mg/kg/day, and a lesser extent of lower body weight was also seen in females at 500 mg/kg/day (Figure 13). Food consumption of the groups varied during the study period, and was considered not to be adverse because it was not consistent, not-dose-related, and had no impact on body weight or on the overall survival for these groups.

Table 53. Findings in clinical signs: Number of positive animals

| Category            | Sex:<br>Sign<br>Dose Level mg/kg/day:<br>Number in Group: | Males |    |     |     | Females |    |     |     |
|---------------------|---|-------|----|-----|-----|---------|----|-----|-----|
|                     |   | 0     | 50 | 150 | 500 | 0       | 50 | 150 | 500 |
| Appearance          |   | 65    | 65 | 65  | 65  | 65      | 65 | 65  | 65  |
| Hunched             |   | 9     | 8  | 9   | 15  | 19      | 22 | 9   | 22  |
| Thin                |   | 6     | 2  | 4   | 11  | 15      | 24 | 10  | 12  |
| Tremors Body        |   | 3     | 1  | 3   | 7   | 2       | 3  | 2   | 3   |
| Behavior            |   |       |    |     |     |         |    |     |     |
| Hypoactive          |   | 4     | 4  | 11  | 14  | 10      | 17 | 14  | 17  |
| Eye(s)              |   |       |    |     |     |         |    |     |     |
| Opaque-Eyes         |   | 16    | 15 | 20  | 11  | 12      | 16 | 14  | 22  |
| Respiration         |   |       |    |     |     |         |    |     |     |
| Audible             |   | 0     | 2  | 0   | 3   | 1       | 0  | 0   | 0   |
| Irregular           |   | 7     | 8  | 10  | 15  | 15      | 18 | 10  | 12  |
| Skin & Pelage       |   |       |    |     |     |         |    |     |     |
| Pale, Ears          |   | 3     | 5  | 8   | 14  | 6       | 10 | 13  | 17  |
| Pale, Entire Body   |   | 6     | 6  | 10  | 9   | 14      | 15 | 9   | 17  |
| Red Skin, Ears      |   | 1     | 3  | 0   | 3   | 0       | 1  | 2   | 1   |
| Red Skin, Left Ear  |   | 1     | 4  | 3   | 2   | 0       | 0  | 1   | 9   |
| Red Skin, Right Ear |   | 2     | 4  | 6   | 8   | 0       | 3  | 4   | 7   |

Figure 13. Mean body weight of each group during study (modified from the submission)



There were no DU-176b-related effects on hematology. The only macroscopic finding at necropsy was a trend of more animals with distended gallbladder in treated groups (1/65, 1/65, 2/65, 3/65 males and 2/65, 4/65, 11/65, and 10/65 females, at control, 50, 150, and 500 mg/kg/day, respectively). This was not associated with histological changes, and was not of toxicological significance.

There were higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice and whole body cavity hemangiosarcoma in medium dose female mice when compared to their respective controls, but none of the tumor incidences showed statistically significant dose-response relationships. Since higher incidences of these two tumors were not dose-dependent, not seen in the high dose groups, not seen in both sexes, and the incidence of whole body cavity hemangiosarcoma (4/65) was within the vendor's histological background range of 1.67-12% (2), higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice and whole body cavity

hemangiosarcoma in medium dose female mice were not considered to be toxicologically significant (Table 54).

A marginal increase in ovarian hematocysts occurred in all DU-176b-treated female groups compared with the control group (Table 55). Although the higher incidences of ovarian hematocysts were not statistically significant and not dose-related, it was considered to be the cause of 4 female premature deaths at 500 mg/kg/day (Table 52). Other trends in higher incidences of liver and kidney findings at 500 mg/kg/day were not statistically or toxicologically significant (Table 55).



| Tissues                        | Animal sex: - Males -<br>Dose level mg/kg/day:<br>No. in group: | Unscheduled death |    |     |     |         |    |     |     | Terminal sacrifice |    |     |     |         |    |     |     |
|--------------------------------|---|-------------------|----|-----|-----|---------|----|-----|-----|--------------------|----|-----|-----|---------|----|-----|-----|
|                                |   | Males             |    |     |     | Females |    |     |     | Males              |    |     |     | Females |    |     |     |
|                                |   | Ctl               | 50 | 150 | 500 | Ctl     | 50 | 150 | 500 | Ctl                | 50 | 150 | 500 | Ctl     | 50 | 150 | 500 |
| Colon                          | Number examined:  | 37                | 41 | 40  | 45  | 42      | 47 | 50  | 50  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 0                 | 0  | 0   | 0   | 0       | 0  | 2   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Cecum                          | Number examined:  | 37                | 40 | 40  | 44  | 42      | 47 | 49  | 50  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 1                 | 1  | 0   | 0   | 0       | 0  | 1   | 1   | 0                  | 0  | 0   | 0   | 0       | 1  | 0   | 0   |
| Jejunum                        | Number examined:  | 37                | 41 | 39  | 44  | 41      | 46 | 49  | 49  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 0                 | 0  | 0   | 0   | 0       | 1  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Rectum                         | Number examined:  | 38                | 41 | 39  | 45  | 42      | 46 | 49  | 50  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| I-Sarcoma                      |   | 0                 | 0  | 1   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   | 1                 | 0  | 0   | 0   | 0       | 1  | 0   | 1   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| LN, Mesenteric                 | Number examined:  | 38                | 41 | 39  | 46  | 40      | 45 | 49  | 48  | 27                 | 23 | 24  | 19  | 22      | 18 | 15  | 13  |
| N-Sarcoma                      |   | 0                 | 0  | 1   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   | 3                 | 6  | 5   | 1   | 6       | 10 | 11  | 6   | 1                  | 1  | 0   | 0   | 3       | 3  | 0   | 0   |
| C-Vascular Neoplasm *          |   | 0                 | 1  | 0   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| LN, Mandibular                 | Number examined:  | 38                | 40 | 39  | 44  | 41      | 45 | 50  | 50  | 25                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 2                 | 4  | 3   | 0   | 4       | 9  | 9   | 7   | 0                  | 1  | 0   | 1   | 1       | 3  | 0   | 0   |
| GI, Mandib Saliv               | Number examined:  | 38                | 42 | 40  | 46  | 41      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 2                 | 3  | 2   | 0   | 2       | 2  | 6   | 5   | 0                  | 0  | 0   | 0   | 2       | 1  | 0   | 0   |
| Pancreas                       | Number examined:  | 38                | 42 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| B-Adenoma, Islet Cell          |   | 0                 | 0  | 0   | 0   | 0       | 0  | 0   | 1   | 1                  | 0  | 1   | 0   | 1       | 0  | 0   | 0   |
| M-Carcinoma                    |   | 0                 | 0  | 0   | 0   | 0       | 1  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   | 2                 | 2  | 1   | 1   | 2       | 5  | 9   | 5   | 1                  | 1  | 0   | 0   | 0       | 0  | 0   | 0   |
| Nerve, Optic                   | Number examined:  | 35                | 35 | 35  | 41  | 36      | 42 | 41  | 48  | 24                 | 23 | 22  | 15  | 22      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 1                 | 0  | 0   | 0   | 0       | 0  | 3   | 1   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Eye                            | Number examined:  | 38                | 42 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 24  | 19  | 23      | 18 | 15  | 14  |
| I-Carcinoma                    |   | 0                 | 0  | 0   | 0   | 1       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   | 1                 | 1  | 0   | 0   | 0       | 1  | 3   | 2   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Skin/Subcutis                  | Number examined:  | 38                | 42 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| B-Papilloma, Squamous Cell     |   | 0                 | 0  | 0   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 1  | 0   | 0   |
| B-Keratocanthoma               |   | 0                 | 0  | 0   | 0   | 0       | 0  | 0   | 1   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| B-Trichoepithelioma            |   | 0                 | 0  | 0   | 0   | 0       | 0  | 1   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| M-Sarcoma                      |   | 1                 | 3  | 1   | 2   | 3       | 0  | 1   | 1   | 0                  | 0  | 0   | 0   | 0       | 0  | 1   | 0   |
| C-Hematopoietic Neoplasm *     |   | 2                 | 1  | 0   | 0   | 0       | 5  | 5   | 2   | 0                  | 0  | 0   | 0   | 1       | 0  | 0   | 0   |
| C-Vascular Neoplasm *          |   | 1                 | 0  | 0   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 1  | 0   | 0   |
| GI, Harderian                  | Number examined:  | 38                | 42 | 40  | 46  | 42      | 46 | 49  | 51  | 27                 | 23 | 24  | 19  | 23      | 18 | 15  | 14  |
| B-Adenoma                      |   | 4                 | 2  | 3   | 3   | 2       | 0  | 0   | 1   | 3                  | 4  | 1   | 3   | 0       | 1  | 2   | 0   |
| M-Carcinoma                    |   | 0                 | 0  | 0   | 0   | 1       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   | 1                 | 2  | 0   | 1   | 0       | 2  | 3   | 3   | 0                  | 0  | 0   | 0   | 1       | 0  | 0   | 0   |
| Seminal Vesicle                | Number examined:  | 38                | 42 | 40  | 46  |         |    |     |     | 27                 | 23 | 25  | 19  |         |    |     |     |
| C-Hematopoietic Neoplasm *     |   | 2                 | 1  | 1   | 0   |         |    |     |     | 0                  | 0  | 0   | 0   |         |    |     |     |
| Prostate                       | Number examined:  | 38                | 42 | 40  | 46  |         |    |     |     | 27                 | 23 | 25  | 19  |         |    |     |     |
| C-Hematopoietic Neoplasm *     |   | 2                 | 3  | 1   | 0   |         |    |     |     | 0                  | 0  | 0   | 0   |         |    |     |     |
| Testis                         | Number examined:  | 38                | 42 | 40  | 46  |         |    |     |     | 27                 | 23 | 25  | 19  |         |    |     |     |
| B-Interstitial Cell Tumor      |   | 3                 | 0  | 0   | 0   |         |    |     |     | 1                  | 1  | 0   | 1   |         |    |     |     |
| C-Hematopoietic Neoplasm *     |   | 1                 | 0  | 1   | 0   |         |    |     |     | 0                  | 0  | 0   | 0   |         |    |     |     |
| Epididymis                     | Number examined:  | 38                | 42 | 40  | 46  |         |    |     |     | 27                 | 23 | 25  | 19  |         |    |     |     |
| N-Sarcoma                      |   | 1                 | 0  | 1   | 0   |         |    |     |     | 0                  | 0  | 0   | 0   |         |    |     |     |
| C-Hematopoietic Neoplasm *     |   | 3                 | 4  | 0   | 0   |         |    |     |     | 0                  | 0  | 0   | 0   |         |    |     |     |
| Mammary, Female                | Number examined:  |                   |    |     |     | 40      | 45 | 46  | 50  |                    |    |     |     | 22      | 18 | 15  | 14  |
| M-Adenoacanthoma               |   |                   |    |     |     | 0       | 0  | 1   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| M-Carcinoma                    |   |                   |    |     |     | 0       | 2  | 3   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   |                   |    |     |     | 0       | 0  | 2   | 1   |                    |    |     |     | 1       | 0  | 0   | 0   |
| Ovary                          | Number examined:  |                   |    |     |     | 42      | 47 | 50  | 51  |                    |    |     |     | 23      | 18 | 15  | 14  |
| B-Adenoma                      |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 1  | 1   | 0   |
| B-Cystadenoma                  |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 0  | 2   | 0   |
| B-Granulosa/Theca Cell Tumor   |   |                   |    |     |     | 0       | 0  | 1   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| B-Luteoma                      |   |                   |    |     |     | 1       | 0  | 0   | 0   |                    |    |     |     | 2       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   |                   |    |     |     | 3       | 5  | 6   | 4   |                    |    |     |     | 2       | 1  | 0   | 0   |
| C-Vascular Neoplasm *          |   |                   |    |     |     | 1       | 0  | 0   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| Uterus                         | Number examined:  |                   |    |     |     | 42      | 47 | 50  | 51  |                    |    |     |     | 23      | 18 | 15  | 14  |
| B-Polyp, Endometrial Stromal   |   |                   |    |     |     | 5       | 3  | 3   | 3   |                    |    |     |     | 0       | 1  | 1   | 2   |
| M-Carcinoma                    |   |                   |    |     |     | 0       | 0  | 0   | 0   |                    |    |     |     | 0       | 0  | 0   | 1   |
| B-Schwannoma                   |   |                   |    |     |     | 0       | 0  | 0   | 0   |                    |    |     |     | 0       | 1  | 0   | 0   |
| M-Leiomyosarcoma               |   |                   |    |     |     | 0       | 0  | 0   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| M-Sarcoma, Endometrial Stromal |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   |                   |    |     |     | 0       | 1  | 1   | 0   |                    |    |     |     | 0       | 2  | 0   | 0   |
| C-Vascular Neoplasm *          |   |                   |    |     |     | 1       | 3  | 2   | 3   |                    |    |     |     | 0       | 0  | 0   | 0   |
| Cervix                         | Number examined:  |                   |    |     |     | 40      | 46 | 50  | 51  |                    |    |     |     | 23      | 18 | 15  | 14  |
| B-Granular Cell Tumor          |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 0  | 0   | 0   |
| B-Schwannoma                   |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 1       | 1  | 0   | 0   |
| B-Leiomyoma                    |   |                   |    |     |     | 1       | 1  | 0   | 3   |                    |    |     |     | 0       | 1  | 0   | 1   |
| M-Leiomyosarcoma               |   |                   |    |     |     | 1       | 1  | 0   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| M-Sarcoma, Endometrial Stromal |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 0  | 0   | 0   |
| C-Hematopoietic Neoplasm *     |   |                   |    |     |     | 2       | 2  | 4   | 4   |                    |    |     |     | 1       | 0  | 0   | 0   |
| C-Vascular Neoplasm *          |   |                   |    |     |     | 0       | 0  | 2   | 0   |                    |    |     |     | 0       | 0  | 0   | 0   |
| Vagina                         | Number examined:  |                   |    |     |     | 42      | 47 | 50  | 51  |                    |    |     |     | 23      | 18 | 15  | 14  |
| B-Fibroma                      |   |                   |    |     |     | 0       | 0  | 0   | 1   |                    |    |     |     | 0       | 0  | 0   | 1   |
| C-Hematopoietic Neoplasm *     |   |                   |    |     |     | 0       | 5  | 4   | 2   |                    |    |     |     | 0       | 0  | 0   | 0   |
| Bone, Femur                    | Number examined:  | 38                | 42 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 1                 | 2  | 1   | 0   | 0       | 0  | 2   | 1   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| C-Vascular Neoplasm *          |   | 1                 | 0  | 1   | 0   | 0       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Marrow, Femur                  | Number examined:  | 38                | 41 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 2                 | 2  | 3   | 1   | 0       | 4  | 1   | 1   | 0                  | 0  | 0   | 1   | 0       | 1  | 0   | 0   |
| C-Vascular Neoplasm *          |   | 0                 | 0  | 0   | 0   | 1       | 0  | 0   | 0   | 0                  | 0  | 0   | 0   | 0       | 0  | 0   | 0   |
| Bone, Sternum                  | Number examined:  | 38                | 42 | 39  | 46  | 42      | 47 | 49  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 1                 | 2  | 2   | 0   | 0       | 0  | 3   | 3   | 0                  | 1  | 0   | 0   | 3       | 3  | 3   | 2   |
| Marrow, Sternum                | Number examined:  | 38                | 42 | 39  | 46  | 42      | 47 | 49  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| C-Hematopoietic Neoplasm *     |   | 1                 | 3  | 3   | 1   | 1       | 4  | 2   | 1   | 1                  | 0  | 0   | 1   | 1       | 0  | 0   | 0   |
| Body, Whole/Cav                | Number examined:  | 38                | 42 | 40  | 46  | 42      | 47 | 50  | 51  | 27                 | 23 | 25  | 19  | 23      | 18 | 15  | 14  |
| B-Hemangioma                   |   | 1                 | 1  | 2   | 1   | 2       | 0  | 0   | 1   | 0                  | 0  | 0   | 1   | 1       | 0  | 0   | 0   |
| M-Hemangiosarcoma              |   | 1                 | 2  | 0   | 1   | 0       | 2  | 4   | 0   | 1                  | 0  | 1   | 0   | 0       | 2  | 0   | 0   |
| M-Histiocytic Sarcoma          |   |                   |    |     |     |         |    |     |     |                    |    |     |     |         |    |     |     |

Table 55. Non-neoplastic findings – number of animals with diagnosis (modified from the submission)

| Tissues                                | Animal sex:          |      |    |     |         |      |    |     |     |
|--|----------------------|------|----|-----|---------|------|----|-----|-----|
|  | Males                |      |    |     | Females |      |    |     |     |
| Diagnoses                              | Dose level mg/kg/day | Ctls | 50 | 150 | 500     | Ctls | 50 | 150 | 500 |
|  | Number examined:     | 85   | 85 | 85  | 85      | 85   | 85 | 85  | 85  |
| <b>Liver</b>                           |                      |      |    |     |         |      |    |     |     |
| Vacuolation, Hepatocyte                |                      | 0    | 0  | 0   | 1       | 0    | 0  | 0   | 2   |
| Degeneration/Necrosis                  |                      | 8    | 5  | 5   | 5       | 4    | 4  | 4   | 6   |
| Focus, Cellular Alteration, Basophilic |                      | 1    | 1  | 1   | 3       | 0    | 1  | 0   | 0   |
| <b>Kidney</b>                          |                      |      |    |     |         |      |    |     |     |
| Cyst                                   |                      | 22   | 24 | 35  | 24      | 3    | 10 | 3   | 3   |
| Dilatation, Pelvis                     |                      | 10   | 12 | 12  | 8       | 2    | 2  | 2   | 3   |
| Mineralisation                         |                      | 0    | 1  | 0   | 3       | 1    | 0  | 0   | 2   |
| <b>Ovary</b>                           |                      |      |    |     |         |      |    |     |     |
| Hematocyst                             |                      |      |    |     |         | 6    | 13 | 11  | 14  |

There was no detectable DU-176 in the plasma samples from control animals. As shown in Figure 14 and Table 56, the plasma DU-176 concentrations,  $C_{max}$ , and  $AUC_{0-24}$  increased with the increasing dosages, and were generally less than dose proportional. Exposures to DU-176 were generally similar in females and males on day 1 but slightly higher in females in week 26. Exposures were greatly lower for both males and females in Week 26 than on Day 1, especially in the low and mid dose groups (only 10-20% of day 1), indicating no accumulation of DU-176 after multiple doses. The markedly lower  $C_{max}$  and  $AUC_{0-24}$  in Week 26 may imply higher rate of metabolism or lower bioavailability following repeated dosing. After oral gavage administration of DU-176b, DU-176b was readily absorbed, with  $T_{max}$  values ranging from 1 to 2 hours.

Figure 14. Plasma DU-176 concentrations following oral dosing (modified from the submission)

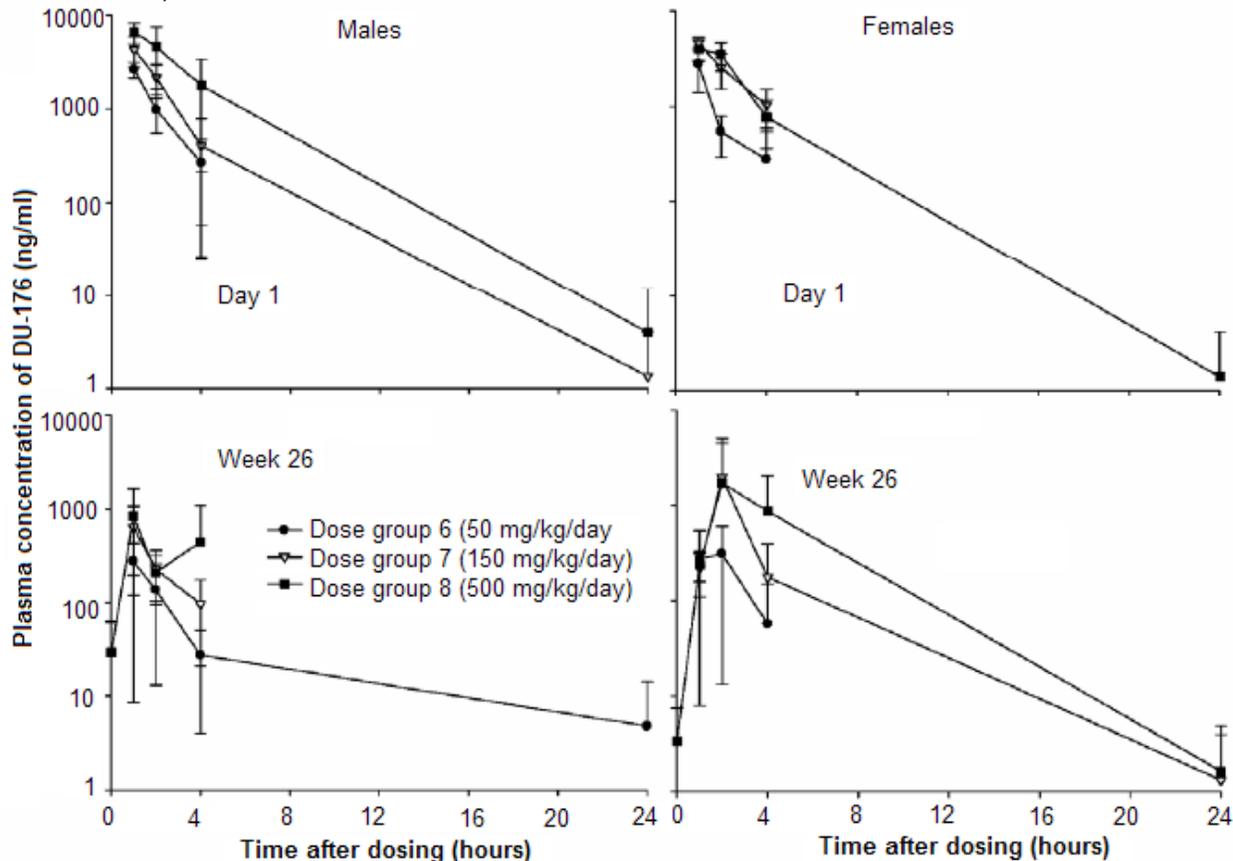


Table 56. TK parameters in mice

| Dose Group     | Dose Level (mg/kg/day) | Sex | C <sub>max</sub> (ng/mL) | T <sub>max</sub> (hr) | AUC <sub>0-24</sub> (ng•hr/mL) | Week 26/Day 1    |                     |
|----------------|------------------------|-----|--------------------------|-----------------------|--------------------------------|------------------|---------------------|
|                |                        |     |                          |                       |                                | C <sub>max</sub> | AUC <sub>0-24</sub> |
| <u>Day 1</u>   |                        |     |                          |                       |                                |                  |                     |
| 6              | 50                     | M   | 2633                     | 1.00                  | 7017                           |                  |                     |
|                |                        | F   | 2768                     | 1.00                  | 6629                           |                  |                     |
| 7              | 150                    | M   | 4325                     | 1.00                  | 11986                          |                  |                     |
|                |                        | F   | 4558                     | 1.00                  | 19677                          |                  |                     |
| 8              | 500                    | M   | 6565                     | 1.00                  | 33046                          |                  |                     |
|                |                        | F   | 3995                     | 1.00                  | 17757                          |                  |                     |
| <u>Week 26</u> |                        |     |                          |                       |                                |                  |                     |
| 6              | 50                     | M   | 274                      | 1.00                  | 826                            | 0.10             | 0.12                |
|                |                        | F   | 308                      | 2.00                  | 1356                           | 0.11             | 0.20                |
| 7              | 150                    | M   | 634                      | 1.00                  | 2036                           | 0.15             | 0.17                |
|                |                        | F   | 1962                     | 2.00                  | 5098                           | 0.43             | 0.26                |
| 8              | 500                    | M   | 829                      | 1.00                  | 6003                           | 0.13             | 0.18                |
|                |                        | F   | 1707                     | 2.00                  | 12320                          | 0.43             | 0.69                |

In conclusion, administration of DU-176b to CrI:CD1 (ICR) mice by oral gavage daily for up to 104 weeks at doses from 50 to 500 mg/kg/day produced no evidence of significantly increased neoplasia at any dose level. Mortality was significantly higher in

males at the 500 mg/kg/day dose and in females at 150 mg/kg/day. Higher incidences of thin appearance, body tremor, hypoactivity, and audible or irregular breath were seen in males at 500 mg/kg/day. For both males and females, higher incidences of pale ears/entire body, red ears and lower body weight were observed at 500 mg/kg/day. Findings in clinical signs were associated with moribundity. A marginal increase in ovarian hematocysts occurred in all DU-176b-treated female groups compared with the control group, which was considered to be the cause of 4 female premature deaths at 500-mg/kg/day, and was at least in part attributed to the pharmacological action of the test article. NOAELs for carcinogenicity were 500 mg/kg/day for males and females with C<sub>max</sub> of 829 and 1707 ng/ml, respectively, and AUC<sub>0-24</sub> of 6003 and 12320 ng•hr/ml, respectively, during Week 26. NOAELs for general toxicity were 150 mg/kg/day for males with C<sub>max</sub> of 634 ng/mL and AUC<sub>0-24</sub> of 2036 ng•hr/ml, and 50 mg/kg/day for females with C<sub>max</sub> of 308 ng/ml and AUC<sub>0-24</sub> of 1356 ng•hr/ml during Week 26.

The proposed maximum recommended human daily dose of DU176b is 60 mg. This dose of 60 mg had a median AUC<sub>0-24ss</sub> of 1940 ng•hr/ml (Clinical Study Report DU176b-A-U151, page 80). Thus, NOAELs for carcinogenicity in male and female mice are estimated to be 3 and 6 times, respectively, the maximum recommended human daily dose of DU-176b based on AUC<sub>0-24</sub> comparisons.

### Summary of FDA statistical analysis on survival rate and tumor findings

FDA statistical analysis by Dr. M.A. Rahman concluded that there were statistically significant dose-response relationships in mortality across the treatment groups in male mice, and the higher mortality in male mice of high dose group than the relevant controls was statistically significant (Table 57).

Table 57. Intercurrent mortality rate and comparison (modified from FDA statistical review)

| Male Mice   | 0 mg kg day |        | 50 mg kg day |        | 150 mg kg day |        | 500 mg kg day |        | Test          | Statistic        | P_Value |
|-------------|-------------|--------|--------------|--------|---------------|--------|---------------|--------|---------------|------------------|---------|
|             | No. of      | Cum. % | No. of       | Cum. % | No. of        | Cum. % | No. of        | Cum. % |               |                  |         |
| Week        | Death       | Cum. % | Death        | Cum. % | Death         | Cum. % | Death         | Cum. % |               |                  |         |
| 0 - 52      | 4           | 6.15   | 5            | 7.69   | 9             | 13.85  | 13            | 20.00  |               |                  |         |
| 53 - 78     | 15          | 29.23  | 15           | 30.77  | 9             | 27.69  | 17            | 46.15  | Dose-Response | Likelihood Ratio | 0.0464  |
| 79 - 91     | 7           | 40.00  | 15           | 53.85  | 8             | 40.00  | 9             | 60.00  | Homogeneity   | Log-Rank         | 0.1536  |
| 92 - 104    | 12          | 58.46  | 7            | 64.62  | 14            | 61.54  | 7             | 70.77  |               |                  |         |
| Ter. Sac.   | 27          | 41.54  | 23           | 35.38  | 25            | 38.46  | 19            | 29.23  |               |                  |         |
| Total       | N=65        |        | N=65         |        | N=65          |        | N=65          |        |               |                  |         |
| Female Mice | 0 mg kg day |        | 50 mg kg day |        | 150 mg kg day |        | 500 mg kg day |        | Test          | Statistic        | P_Value |
|             | No. of      | Cum. % | No. of       | Cum. % | No. of        | Cum. % | No. of        | Cum. % |               |                  |         |
| Week        | Death       | Cum. % | Death        | Cum. % | Death         | Cum. % | Death         | Cum. % |               |                  |         |
| 0 - 52      | 6           | 9.23   | 4            | 6.15   | 11            | 16.92  | 6             | 9.23   |               |                  |         |
| 53 - 78     | 7           | 20.00  | 18           | 33.85  | 20            | 47.69  | 11            | 26.15  | Dose-Response | Likelihood Ratio | 0.6141  |
| 79 - 91     | 18          | 47.69  | 12           | 52.31  | 15            | 70.77  | 10            | 41.54  | Homogeneity   | Log-Rank         | 0.0052  |
| 92 - 102    | 11          | 64.62  | 10           | 67.69  | 3             | 75.38  | 22            | 75.38  |               |                  |         |
| Ter. Sac.   | 23          | 35.38  | 21           | 32.31  | 16            | 24.61  | 16            | 24.62  |               |                  |         |
| Total       | N=65        |        | N=65         |        | N=65          |        | N=65          |        |               |                  |         |

For tumor data analysis, multiple testing adjustment was applied: For dose-response relationship tests,  $\alpha=0.005$  for common tumors and  $\alpha=0.025$  for rare tumors for a submission with two species, and a significance level  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors for a submission with one species; For multiple pairwise comparisons of treated group with control,  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors.

FDA statistical analysis showed higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice and whole body cavity hemangiosarcoma in medium dose female mice when compared to their respective control, but neither of the tumor incidences showed statistically significant dose-response relationships (Table 58). No other tumor types had p-values  $\leq 0.05$  for dose-response relationships or pairwise comparisons of treated groups and control.

Table 58. Tumor Types with P-Values  $\leq 0.05$  for Dose-Response Relationship or Pairwise Comparisons of Treated Groups and Control in Mice (from FDA statistical review)

| Sex    | Organ Name      | Tumor Name                  | Cont<br>N=65 | Low<br>N=65 | Med<br>N=65 | High ...<br>N=65 | Dose Resp | P_Value |         |        |
|--------|-----------------|-----------------------------|--------------|-------------|-------------|------------------|-----------|---------|---------|--------|
|        |                 |                             |              |             |             |                  |           | C vs L  | C vs M  | C vs H |
| Male   | Adrenal, Cortex | B-Adenoma, Subcapsular Cell | 0            | 4           | 2           | 1                | 0.5368    | 0.0500* | 0.2471  | 0.4430 |
| Female | Body, Whole/Cav | M-Hemangiosarcoma           | 0            | 4           | 4           | 0                | 0.8518    | 0.0503  | 0.0287* | .      |

### Executive CAC Conclusions (June 10, 2014)

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in the study.

### 8.2 DU-176b: 104-Week Oral Gavage Carcinogenicity and Toxicokinetic Study in Rats

Study no.: AN07-C0020-R01 (Report No. 6630-172)  
AN11-H7301-R01

Study report location: (b) (4)

Conducting laboratory and location: (b) (4)

Date of study initiation: Sept 6, 2007

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: DU-176b, MH101U, 99.3%; KD302, 98.8%

CAC concurrence: Yes

Deviation from study protocol: No impact to the results

### Key Study Findings

Sprague Dawley rats were orally gavaged with DU-176b at doses of 0, 60, 200, and 600/400 mg/kg/day for males, and 0, 50, 100, and 200 mg/kg/day for females, once daily for up to 104 week. There was no evidence of increased neoplasia at any dose level. Mortality was significantly higher in males at the 600/400 mg/kg/day dose, and marginally higher in females at 200 mg/kg/day. There was a higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis in males at 600/400 mg/kg/day, and liver centrilobular hepatocellular degeneration/necrosis may be the cause for 8 of the 50 unscheduled male deaths in the male 600/400 mg/kg/day group. There were slightly but statistically lower red cell counts in females at 200 mg/kg/day, higher incidences of red oral and nasal discharge, and red haircoat in males at DU-176b 600/400 mg/kg/day. Similar clinical signs of less extent were also seen in females at DU-176b 200 mg/kg/day. The findings in hematology and clinical signs may, at least in part, be attributed to bleeding, the pharmacological action of the test article as an anticoagulant (factor Xa inhibitor). NOAELs for carcinogenicity were 600/400 mg/kg/day for males with C<sub>max</sub> of 1055 ng/mL and AUC<sub>0-24</sub> of 15086 ng•hr/ml, and 200 mg/kg/day for females with C<sub>max</sub> of 4045 ng/ml and AUC<sub>0-24</sub> of 27796 ng•hr/ml during Week 26. NOAELs for general toxicity were 200 mg/kg/day for males with C<sub>max</sub> of 1395 ng/ml and AUC<sub>0-24</sub> of 13763 ng•hr/ml, and 100 mg/kg/day for females with C<sub>max</sub> of 3223 ng/ml and AUC<sub>0-24</sub> of 11782 ng•hr/ml during Week 26.

## Methods

Crl:CD (SD) rats, initial age of 6 weeks with body weights 136 - 225 g for males and 127 - 185 g for females, were orally gavaged with DU-176b at doses of 0, 60, 200, and 600/400 mg/kg/day for males, and 0, 50, 100, and 200 mg/kg/day for females in an aqueous solution of 0.5% methylcellulose, once daily for up to 104 weeks (n = 65/sex/group for toxicity group, and 4-12/sex/group for toxicokinetic group) (Table 59). Doses for the present study were selected based on the results of a 13-week dose range finding study (**Study R20050791**) and a 26-week toxicity study (**Study R20050334**) in rats. In the 13-week study (R20050791), rats (10/sex/group for toxicity and 8/sex/group for toxicokinetics) were dosed with DU-176b at 60, 200, 600, or 1500 mg/kg/day by oral gavage. Animals in the 1500 mg/kg/day dose group were terminated early due to excessive mortality. There was one male death at the 600 mg/kg/day dose (1/10 males). The cause of death was unclear. Body weight, clinical pathology and histopathology parameters (limited tissues examined) were not affected at 60, 200 and 600 mg/kg/day dose levels. At 200 and 600 mg/kg/day, alopecia and sores/scabs were observed in females only, but at a lower incidence as compared to the 1500 mg/kg/day dose group. NOAEL was 60 mg/kg/day. Thus, 200 and 600 mg/kg/day were selected as the high dose for female and male rats, respectively. In the 26-week rat toxicity study with oral DU-176b at 0, 6, 18, and 54 mg/kg/day (Study R20050334), NOAEL was 54 mg/kg/day. Therefore, 50 and 60 mg/kg/day were selected as the low dose for female and male rats, respectively. The mid dose for male rats, 200 mg/kg/day, was based on the results of the 13-week study. The mid dose for female rats, 100 mg/kg/day, was based on the results of the 13-week and 26-week studies.

Table 59. Study design

| Group <sup>a</sup>            | No. of Animals |         | Dose Level <sup>b</sup><br>(mg/kg/day) |          |     |
|-------------------------------|----------------|---------|--|----------|-----|
|                               | Males          | Females |  |          |     |
| <b>Toxicity Animals</b>       |                |         |  |          |     |
| 1 (Control – Females)         |                | 65      | 0                                      | 104      | 105 |
| (Control – Males)             | 65             |         |  | 89       | 90  |
| 2 (Low – Females)             |                | 65      | 50                                     | 104      | 105 |
| 3 (Low – Males)               | 65             |         | 60                                     | 89       | 90  |
| 4 (Mid – Females)             |                | 65      | 100                                    | 104      | 105 |
| 5 (Mid – Males)               | 65             |         | 200                                    | 89       | 90  |
| 6 (High – Females)            |                | 65      | 200                                    | 104      | 105 |
| 7 (High – Males) <sup>c</sup> | 65             |         | 600/400                                | 80       | 88  |
| <b>Toxicokinetic Animals</b>  |                |         |  |          |     |
| 8 (Control)                   | 4              | 4       | 0                                      | 26 weeks | N/A |
| 9 (Low – Females)             |                | 12      | 50                                     |          |     |
| 10 (Low – Males)              | 12             |         | 60                                     |          |     |
| 11 (Mid – Females)            |                | 12      | 100                                    |          |     |
| 12 (Mid – Males)              | 12             |         | 200                                    |          |     |
| 13 (High – Females)           |                | 12      | 200                                    |          |     |
| 14 (High – Males)             | 12             |         | 600                                    |          |     |

a Groups 1 and 8 received control article (aqueous solution of 0.5% methylcellulose) only.

b Doses and concentrations of DU-176b were expressed as the amount of anhydrous free base, DU-176 (conversion factor: DU-176/DU-176b = 0.7424).

c Dose level changed to 400 mg/kg/day on Day 302, Week 44.

d Dosing in male high dose group was terminated early due to high mortality, and dosing duration was shorter than 104 weeks in all males

The carcinogenicity study was designed to continue dosing for 104 weeks. However, the study with male rats was terminated early (Table 59) because of poor survival in high dose group (survival in this group by week 80 reached 20 rats). Samples of the dose formulations for treatment weeks 1, 13, 26, 27, 39, 44, 52, 56, 65, 78, 91, and 104 were analyzed to verify the homogeneity, stability, and/or concentrations of DU-176b in the dosing solution.

For the toxicity group, rats were inspected visually for clinical signs and morbidity/death during the study at least twice daily. Detailed observations, including palpation, were performed at least once during the predose phase, before dosing on Day 1 and weekly thereafter, and on the day of scheduled sacrifice on each animal. Body weight was recorded prior to treatment, weekly for Weeks 1 through 14 and once every 4 weeks thereafter, and at Week 105 during the dosing phase. Individual food consumption was measured and recorded weekly for Weeks 1 through 13 and once every 4 weeks thereafter and for Week 104. Blood samples were collected from a jugular vein of all surviving animals at scheduled sacrifice following overnight fast using potassium EDTA as the anticoagulant. Blood smears were also prepared (if possible) during the necropsy procedure from animals sacrificed at an unscheduled interval. Blood samples were determined for hematology parameters including red blood cell (erythrocyte) count, white blood cell (leukocyte) count, differential blood cell count, and blood smear.

A detailed necropsy was performed on each animal that died prematurely or sacrificed either prematurely or at the end of scheduled treatment period. The following tissues (when present) from each animal were preserved in 10% neutral buffered formalin.

|  |  |
|--|--|
| adrenal (2)  | optic nerve (2) <sup>a</sup>                 |
| aorta  | ovary (2)                                    |
| brain  | pancreas                                     |
| cecum  | pituitary gland                              |
| cervix   | prostate                                     |
| colon  | rectum                                       |
| duodenum   | salivary gland [mandibular (2)]              |
| epididymis (2) <sup>a</sup>  | sciatic nerve                                |
| esophagus  | seminal vesicle                              |
| eye (2) <sup>a</sup>   | skeletal muscle (thigh)                      |
| femur with bone marrow – stifle joint<br>(articular surface of the distal end) | skin/subcutis                                |
| Harderian gland (2) <sup>a</sup>   | spinal cord (cervical, thoracic, and lumbar) |
| heart  | spleen                                       |
| ileum  | sternum with bone marrow                     |
| jejunum  | stomach                                      |
| kidney (2)   | testis (2) <sup>a</sup>                      |
| lesions  | thymus                                       |
| liver  | thyroid (2 lobes) with parathyroid           |
| lung with large bronchi  | tongue                                       |
| lymph node (mandibular)  | trachea                                      |
| lymph node (mesenteric)  | urinary bladder                              |
| mammary gland (females)  | uterus                                       |
|  | vagina                                       |

<sup>a</sup> Preserved in modified Davidson's fixative

Preserved tissues listed above from each animal at terminal sacrifice and each animal that died or sacrificed at an unscheduled time were histologically processed and microscopically examined. Macroscopic lesions from each animal and suspected target organs at the high dose group were also histologically processed and microscopically examined.

For TK rats, mortality and signs of pain or distress were observed twice daily. Individual body weights were recorded prior to treatment, before dosing on Day 1 of the dosing phase, and weekly for Weeks 1-14 and every 4 weeks thereafter. Blood samples were collected on Day 1 (1, 2, 4, and 24 hours postdose) and during Week 26 (predose and 1, 2, 4, and 24 hours postdose) using sodium fluoride as the anticoagulant. Plasma was harvested and analyzed for DU-176 concentrations. TK parameters including highest concentration (C<sub>max</sub>), time to peak concentration (T<sub>max</sub>), and area under the concentration-time curve (AUC) were obtained. All TK animals were discarded without necropsy after their scheduled sacrifice or premature death.

## Results

DU-176b concentrations in all dosing solutions were within 89.8 - 106% of the respective theoretical values and met acceptance criteria (mean value was within  $\pm 10\%$  of the theoretical value and individual results were within  $\pm 5\%$  of the mean of the

replicates). There was no detectable DU-176 in the control samples. All homogeneity results met acceptance criteria, mean value of each location (top, middle, and bottom) was within  $\pm 7\%$  of the overall mean and individual results were within  $\pm 5\%$  from the mean of the replicates.

A trend in higher mortality was noted for males through Week 88 with significantly higher mortality in the 600/400 mg/kg/day group when compared to control ( $p < 0.01$ ), especially during weeks 26 and 39 (9 deaths vs 1 death in control group). In Week 88, survival of males in 600/400 mg/kg/day group was 25% while in Week 90, the survival for control, 60 and 200 mg/kg/day males was 29, 45, and 38%, respectively. A marginal higher mortality was noted in females at the dose 200 mg/kg/day when compared to controls through Week 105. For the female control, 50, 100 and 200 mg/kg/day groups, survival in Week 105 was 34, 26, 28, and 25%, respectively (Table 60, Figure 15). Main findings/possible causes for the 172 male premature deaths and 189 female premature deaths are summarized in Table 61. Although there were 3 more gavage-related deaths in the high dose males than control, the higher mortality in high dose males cannot be excluded as being due to DU-176b. Notably, centrilobular hepatocellular degeneration/necrosis of the liver was considered to be the main finding or possible cause for 8 of the 50 male premature deaths at DU-176b 600/400 mg/kg/day.

Table 60. Survival through the study period with initial number of 65/sex/group

| Week | Males (mg/kg/day) |     |     |         | Females (mg/kg/day) |    |     |     |
|------|-------------------|-----|-----|---------|---------------------|----|-----|-----|
|      | Control           | 60  | 200 | 600/400 | Control             | 50 | 100 | 200 |
| 2    | 65                | 65  | 65  | 65      | 65                  | 65 | 65  | 65  |
| 4    | 65                | 65  | 65  | 63      | 65                  | 65 | 65  | 65  |
| 13   | 65                | 65  | 65  | 61*     | 65                  | 65 | 65  | 65  |
| 26   | 64                | 63  | 63  | 58*     | 65                  | 65 | 65  | 63  |
| 39   | 64                | 61  | 60  | 49**    | 65                  | 65 | 65  | 63  |
| 52   | 58                | 60  | 56  | 41**    | 63                  | 63 | 64  | 58  |
| 78   | 41                | 40  | 41  | 26**    | 46                  | 46 | 51  | 42  |
| 80   | 37                | 39  | 36  | 20**    | 45                  | 40 | 49  | 41  |
| 88   | 24                | 29  | 29  | 16      | 37                  | 33 | 35  | 29  |
| 90   | 19                | 29  | 25  | N/A     | 33                  | 29 | 30  | 27  |
| 104  | N/A               | N/A | N/A | N/A     | 23                  | 17 | 20  | 17  |
| 105  | N/A               | N/A | N/A | N/A     | 22                  | 17 | 18  | 16  |

\*  $p < 0.05$  vs control; \*\*  $p < 0.01$  vs control

Figure 15. Survival over the study period for rats in toxicity arm (from the submission)

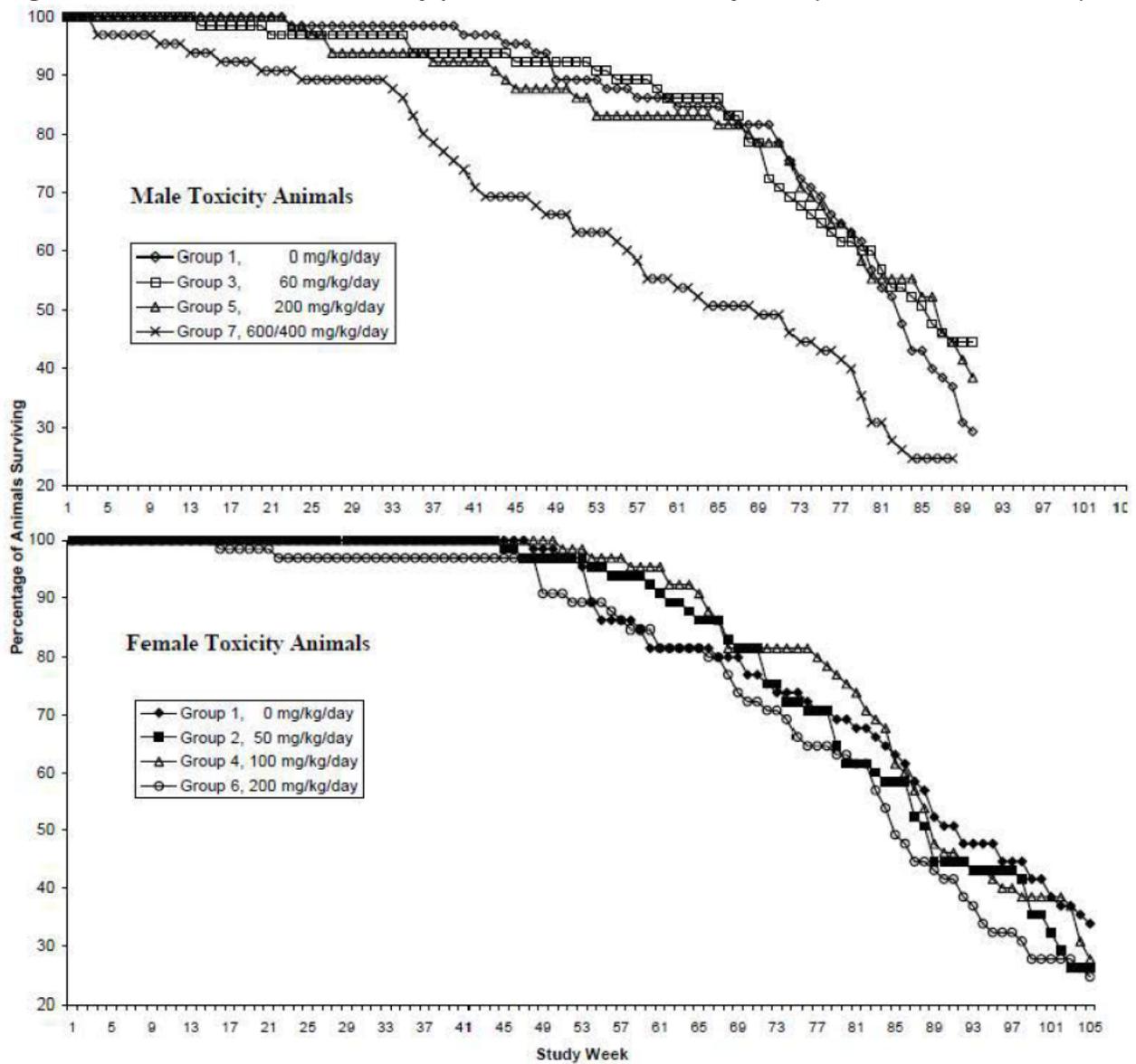


Table 61. Main findings/possible causes for the premature deaths (modified from the submission)

| Tissues<br>Diagnoses                           | Animal sex:<br>Dosage group:<br>No. in group: | Animals of unscheduled death |    |    |    |                     |    |    |    |
|--|---|------------------------------|----|----|----|---------------------|----|----|----|
|  |   | -- M a l e s --              |    |    |    | -- F e m a l e s -- |    |    |    |
|  |   | Ctls                         | 3  | 5  | 7  | Ctls                | 2  | 4  | 6  |
| Controls from group(s): 1                      |   | 46                           | 36 | 40 | 50 | 44                  | 49 | 47 | 49 |
| Death Comment                                  | Number examined:                              | 45                           | 36 | 40 | 50 | 44                  | 49 | 47 | 49 |
|  | Unremarkable:                                 | 0                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Undetermined                                   |   | 6                            | 6  | 12 | 13 | 2                   | 1  | 1  | 4  |
| Gavage Related Death                           |   | 3                            | 1  | 2  | 6  | 5                   | 1  | 0  | 3  |
| Atrial Thrombus                                |   | 2                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Cardiomyopathy                                 |   | 0                            | 1  | 0  | 2  | 0                   | 0  | 0  | 0  |
| Inflammation                                   |   | 3                            | 1  | 3  | 3  | 0                   | 2  | 2  | 0  |
| Liver Degeneration/Necrosis                    |   | 0                            | 0  | 0  | 8  | 0                   | 1  | 0  | 0  |
| Nephropathy, Chronic Progressive               |   | 1                            | 0  | 0  | 1  | 0                   | 0  | 0  | 1  |
| Pyelonephritis                                 |   | 0                            | 2  | 0  | 0  | 0                   | 0  | 0  | 1  |
| Carcinoma, Hepatocellular                      |   | 0                            | 0  | 2  | 1  | 0                   | 0  | 0  | 0  |
| Carcinoma, Uterus                              |   | 0                            | 0  | 0  | 0  | 0                   | 1  | 0  | 1  |
| Acinar Carcinoma, Pancreas                     |   | 0                            | 0  | 0  | 0  | 0                   | 0  | 1  | 0  |
| Carcinoma, Adrenal Cortex                      |   | 0                            | 0  | 0  | 0  | 0                   | 0  | 1  | 0  |
| Carcinoma, Cervix                              |   | 0                            | 0  | 0  | 0  | 0                   | 0  | 1  | 0  |
| Endocardial Schwannoma                         |   | 1                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Hemangiosarcoma                                |   | 0                            | 0  | 1  | 0  | 1                   | 0  | 0  | 1  |
| Hibernoma                                      |   | 0                            | 1  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Histiocytic Sarcoma                            |   | 2                            | 1  | 1  | 0  | 1                   | 1  | 0  | 1  |
| Islet Cell Carcinoma, Pancreas                 |   | 0                            | 0  | 0  | 1  | 0                   | 1  | 0  | 0  |
| Fibroma  |   | 0                            | 0  | 1  | 0  | 0                   | 0  | 0  | 0  |
| Follicular Cell Carcinoma                      |   | 1                            | 0  | 1  | 0  | 0                   | 0  | 0  | 0  |
| Leiomyosarcoma                                 |   | 1                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Liposarcoma                                    |   | 0                            | 0  | 2  | 0  | 0                   | 0  | 1  | 0  |
| Malignant Mesothelioma                         |   | 0                            | 0  | 0  | 0  | 1                   | 0  | 0  | 0  |
| Malignant Pheochromocytoma                     |   | 0                            | 3  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Malignant Astrocytoma                          |   | 3                            | 1  | 0  | 2  | 0                   | 0  | 1  | 0  |
| Malignant Schwannoma                           |   | 2                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Malignant Renal Mesenchymal Tumor              |   | 0                            | 1  | 0  | 0  | 0                   | 0  | 1  | 0  |
| Mammary Fibroadenoma                           |   | 0                            | 0  | 0  | 0  | 2                   | 3  | 3  | 1  |
| Mammary Carcinoma                              |   | 0                            | 0  | 0  | 0  | 9                   | 8  | 5  | 11 |
| Nephroblastoma                                 |   | 0                            | 0  | 1  | 0  | 0                   | 0  | 0  | 1  |
| Neuroblastoma, Adrenal Medulla                 |   | 0                            | 0  | 0  | 0  | 0                   | 0  | 1  | 0  |
| Oligodendroglioma                              |   | 1                            | 0  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Pituitary Neoplasm                             |   | 19                           | 17 | 14 | 11 | 22                  | 30 | 29 | 23 |
| Schwannoma                                     |   | 0                            | 0  | 0  | 0  | 1                   | 0  | 0  | 1  |
| Squamous Cell Papilloma, Non-Glandular Stomach |   | 0                            | 1  | 0  | 0  | 0                   | 0  | 0  | 0  |
| Trichoepithelioma                              |   | 0                            | 0  | 0  | 1  | 0                   | 0  | 0  | 0  |
| Pheochromocytoma                               |   | 0                            | 0  | 0  | 1  | 0                   | 0  | 0  | 0  |

There were higher incidences of clear and red oral and red nasal discharge, rough haircoat, red haircoat, audible and labored breath, cold and pale skin in males at DU-176b 600/400 mg/kg/day. Similar findings of less extent were also seen in females at DU-176b 200 mg/kg/day (Table 62). The red oral/nasal discharge and red haircoat is considered, at least in part, to be associated with bleeding, the pharmacological action of the test article. Other clinical signs were associated with early deaths.

Table 62. Findings of clinical signs (modified from the submission)

| Category<br>Sign                 | Sex:                  |           |           |           |
|----------------------------------|-----------------------|-----------|-----------|-----------|
|                                  | M a l e s             |           |           |           |
|                                  | Group: 1              | 3         | 5         | 7         |
|                                  | Dose Level: 0         | 60        | 200       | 600/400   |
|                                  | Dose Units: mg/kg/day | mg/kg/day | mg/kg/day | mg/kg/day |
|                                  | Number in Group: 65   | 65        | 65        | 65        |
|                                  | N                     | N         | N         | N         |
| Discharge                        |                       |           |           |           |
| Clear Oral                       | 3                     | 6         | 7         | 11        |
| Nasal, Red in Color              | 3                     | 5         | 12        | 23        |
| Red Oral                         | 1                     | 4         | 4         | 9         |
| Excretion(s)                     |                       |           |           |           |
| Discolored Feces, Black in Color | 0                     | 0         | 0         | 1         |
| Respiration                      |                       |           |           |           |
| Audible                          | 5                     | 3         | 3         | 9         |
| Labored                          | 5                     | 6         | 4         | 13        |
| Skin & Pelage                    |                       |           |           |           |
| Cold To Touch                    | 2                     | 4         | 1         | 8         |
| Pale                             | 0                     | 0         | 0         | 2         |
| Pale, Entire Body                | 2                     | 1         | 2         | 9         |
| Red Haircoat, Entire Head        | 2                     | 0         | 1         | 5         |
| Rough Haircoat                   | 48                    | 44        | 46        | 61        |
|                                  |                       |           |           |           |
| Category<br>Sign                 | Sex:                  |           |           |           |
|                                  | F e m a l e           |           |           |           |
|                                  | Group: 1              | 2         | 4         | 6         |
|                                  | Dose Level: 0         | 50        | 100       | 200       |
|                                  | Dose Units: mg/kg/day | mg/kg/day | mg/kg/day | mg/kg/day |
|                                  | Number in Group: 65   | 65        | 65        | 65        |
|                                  | N                     | N         | N         | N         |
| Discharge                        |                       |           |           |           |
| Clear Oral                       | 1                     | 2         | 1         | 4         |
| Nasal, Red in Color              | 2                     | 5         | 1         | 5         |
| Red Oral                         | 0                     | 3         | 1         | 5         |
| Excretion(s)                     |                       |           |           |           |
| Discolored Feces, Blue in Color  | 0                     | 0         | 0         | 1         |
| Skin & Pelage                    |                       |           |           |           |
| Cold To Touch                    | 2                     | 1         | 2         | 6         |
| Pale, Entire Body                | 1                     | 5         | 4         | 6         |
| Rough Haircoat                   | 39                    | 51        | 36        | 50        |

There were no DU-176b-related effects on mean body weight or food consumption. Statistically significant differences in mean body weight change and mean food consumption were sporadic, small and not dose related. The only DU-176b-related finding in hematology at scheduled termination was the moderately lower mean red cell count in females at 200 mg/kg/day (Table 63). There was no evidence of hematologic neoplasia.

Table 63. Hematology at the scheduled termination (modified for the submission)

| Group/ Sex    |      | RBC                 | WBC                 | NEUT                | LYM                 | MONO                | EOS                 | BASO                | LUC                 |
|---------------|------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| mg/kg/day     |      | 10 <sup>6</sup> /μL | 10 <sup>3</sup> /μL |
| 1F<br>0       | Mean | 7.36                | 7.49                | 4.01                | 2.83                | 0.47                | 0.11                | 0.01                | 0.06                |
|               | SD   | 0.508               | 2.927               | 2.375               | 0.715               | 0.180               | 0.045               | 0.009               | 0.065               |
|               | N    | 21                  | 21                  | 21                  | 21                  | 21                  | 21                  | 21                  | 21                  |
| 2F<br>50      | Mean | 6.60                | 8.25                | 4.87                | 2.73                | 0.46                | 0.12                | 0.01                | 0.06                |
|               | SD   | 1.419               | 3.032               | 2.783               | 0.823               | 0.176               | 0.079               | 0.004               | 0.024               |
|               | N    | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  |
| 4F<br>100     | Mean | 7.09                | 8.82                | 4.80                | 3.31                | 0.51                | 0.14                | 0.02                | 0.05                |
|               | SD   | 0.886               | 3.769               | 3.111               | 1.752               | 0.187               | 0.121               | 0.012               | 0.023               |
|               | N    | 18                  | 18                  | 18                  | 18                  | 18                  | 18                  | 18                  | 18                  |
| 6F<br>200     | Mean | 6.05*               | 8.04                | 4.47                | 2.94                | 0.45                | 0.11                | 0.01                | 0.06                |
|               | SD   | 1.407               | 2.983               | 2.701               | 1.042               | 0.152               | 0.062               | 0.006               | 0.031               |
|               | N    | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  | 16                  |
| 1M<br>0       | Mean | 8.57                | 9.80                | 3.40                | 5.69                | 0.47                | 0.18                | 0.01                | 0.05                |
|               | SD   | 0.609               | 2.035               | 1.190               | 1.509               | 0.143               | 0.065               | 0.005               | 0.013               |
|               | N    | 19                  | 19                  | 19                  | 19                  | 19                  | 19                  | 19                  | 19                  |
| 3M<br>60      | Mean | 8.18                | 11.23               | 4.58                | 5.81                | 0.53                | 0.23                | 0.01                | 0.06                |
|               | SD   | 0.838               | 3.517               | 2.788               | 1.858               | 0.214               | 0.127               | 0.005               | 0.027               |
|               | N    | 29                  | 29                  | 29                  | 29                  | 29                  | 29                  | 29                  | 29                  |
| 5M<br>200     | Mean | 7.93                | 9.95                | 3.96                | 5.27                | 0.47                | 0.18                | 0.01                | 0.05                |
|               | SD   | 1.004               | 2.909               | 1.879               | 1.540               | 0.181               | 0.081               | 0.006               | 0.027               |
|               | N    | 25                  | 25                  | 25                  | 25                  | 25                  | 25                  | 25                  | 25                  |
| 7M<br>600/400 | Mean | 7.98                | 12.35               | 5.10                | 6.27                | 0.66                | 0.19                | 0.03                | 0.13                |
|               | SD   | 1.400               | 6.073               | 4.060               | 2.260               | 0.333               | 0.090               | 0.013               | 0.092               |
|               | N    | 15                  | 15                  | 15                  | 15                  | 15                  | 15                  | 15                  | 15                  |

\* P < or = 0.05 vs Control

No DU-176b-related macroscopic changes were observed.

There was no test article-related increase in the incidence of any neoplasm in DU-176b-treated rats compared with control. No statistically significant increase or decrease was noted in any common or rare tumors in either sex of this study (Table 64).

Table 64. Neoplastic findings in animals of both premature death and terminal sacrifice groups (modified from the submission)

| Tissues<br>Diagnoses                | Controls from group(s): 1<br>Animal sex:<br>Dosage group:<br>No. in group: | UNSCHEMULATED DEATHS |          |          |          |          |          |          |          | TERMINAL SACRIFICE |          |          |          |          |          |          |          |
|-------------------------------------|--|----------------------|----------|----------|----------|----------|----------|----------|----------|--------------------|----------|----------|----------|----------|----------|----------|----------|
|                                     |  | Males                |          |          |          | Females  |          |          |          | Males              |          |          |          | Females  |          |          |          |
|                                     |  | Ctl's                | 3        | 5        | 7        | Ctl's    | 2        | 4        | 6        | Ctl's              | 3        | 5        | 7        | Ctl's    | 2        | 4        | 6        |
| Brain                               | Number examined:<br>Unremarkable:  | 45<br>26             | 35<br>19 | 40<br>31 | 50<br>37 | 44<br>14 | 48<br>14 | 47<br>17 | 49<br>26 | 19<br>16           | 29<br>24 | 25<br>22 | 15<br>14 | 21<br>8  | 16<br>8  | 18<br>6  | 16<br>6  |
| B-Astrocytoma                       |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 1        | 0        | 0        | 0        | 0        | 0        |
| B-Oligodendroglioma                 |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| B-Granular Cell Tumor               |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| M-Malignant Astrocytoma             |  | 2                    | 1        | 0        | 2        | 0        | 0        | 0        | 1        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| I-Carcinoma, Pituitary              |  | 0                    | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Spinal Cord                         | Number examined:<br>Unremarkable:  | 45<br>43             | 36<br>36 | 40<br>39 | 50<br>49 | 44<br>44 | 48<br>48 | 47<br>47 | 49<br>49 | 19<br>19           | 29<br>29 | 25<br>25 | 15<br>15 | 21<br>21 | 16<br>16 | 18<br>18 | 16<br>16 |
| M-Malignant Astrocytoma             |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| I-Malignant Astrocytoma             |  | 1                    | 0        | 0        | 1        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Adrenal, Cortex                     | Number examined:<br>Unremarkable:  | 45<br>1              | 36<br>2  | 40<br>0  | 50<br>10 | 44<br>0  | 49<br>0  | 47<br>0  | 49<br>1  | 19<br>1            | 29<br>0  | 25<br>1  | 15<br>0  | 21<br>0  | 16<br>0  | 18<br>1  | 16<br>0  |
| B-Adenoma                           |  | 2                    | 0        | 0        | 0        | 1        | 1        | 2        | 1        | 1                  | 0        | 0        | 1        | 3        | 0        | 2        | 1        |
| M-Carcinoma                         |  | 0                    | 2        | 0        | 0        | 1        | 0        | 1        | 0        | 1                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Malignant Renal Mesenchymal Tumor |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Hematopoietic Neoplasm *          |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 1        | 0        | 0        |
| M-Ganglioneuroma                    |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Adrenal, Medulla                    | Number examined:<br>Unremarkable:  | 45<br>38             | 36<br>31 | 40<br>37 | 50<br>49 | 44<br>39 | 49<br>44 | 47<br>43 | 49<br>47 | 19<br>15           | 29<br>22 | 25<br>23 | 15<br>13 | 21<br>18 | 16<br>10 | 18<br>16 | 16<br>13 |
| B-Ganglioneuroma                    |  | 0                    | 0        | 0        | 0        | 0        | 1        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| B-Pheochromocytoma                  |  | 3                    | 1        | 2        | 1        | 2        | 0        | 0        | 1        | 3                  | 6        | 0        | 0        | 1        | 2        | 2        | 1        |
| M-Malignant Pheochromocytoma        |  | 0                    | 3        | 0        | 0        | 0        | 0        | 0        | 0        | 1                  | 1        | 0        | 2        | 0        | 0        | 0        | 0        |
| M-Neuroblastoma                     |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Pituitary                           | Number examined:<br>Unremarkable:  | 45<br>15             | 36<br>15 | 40<br>14 | 50<br>31 | 44<br>5  | 49<br>3  | 47<br>6  | 49<br>4  | 19<br>1            | 29<br>17 | 25<br>12 | 15<br>6  | 21<br>17 | 16<br>5  | 18<br>15 | 16<br>13 |
| B-Adenoma                           |  | 25                   | 19       | 20       | 13       | 33       | 37       | 38       | 37       | 12                 | 7        | 8        | 6        | 17       | 9        | 15       | 13       |
| M-Carcinoma                         |  | 0                    | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Thyroid                             | Number examined:<br>Unremarkable:  | 45<br>22             | 35<br>16 | 40<br>17 | 50<br>24 | 44<br>14 | 49<br>17 | 47<br>23 | 49<br>18 | 19<br>6            | 29<br>9  | 25<br>9  | 15<br>4  | 21<br>4  | 16<br>3  | 18<br>1  | 16<br>2  |
| B-Adenoma, Follicular Cell          |  | 2                    | 2        | 0        | 2        | 0        | 0        | 0        | 1        | 0                  | 0        | 0        | 0        | 0        | 1        | 0        | 0        |
| B-C-Cell Adenoma                    |  | 4                    | 3        | 3        | 4        | 4        | 7        | 3        | 2        | 3                  | 4        | 5        | 5        | 4        | 3        | 3        | 2        |
| M-Carcinoma, C-cell                 |  | 0                    | 2        | 1        | 0        | 0        | 1        | 0        | 0        | 1                  | 1        | 1        | 2        | 1        | 1        | 0        | 0        |
| M-Carcinoma, Follicular Cell        |  | 1                    | 0        | 1        | 0        | 0        | 0        | 0        | 0        | 1                  | 0        | 0        | 0        | 0        | 0        | 1        | 1        |
| Parathyroid                         | Number examined:<br>Unremarkable:  | 39<br>35             | 33<br>31 | 33<br>30 | 41<br>37 | 41<br>40 | 42<br>41 | 44<br>43 | 37<br>35 | 16<br>9            | 26<br>20 | 24<br>14 | 14<br>10 | 20<br>18 | 10<br>9  | 17<br>16 | 16<br>16 |
| B-Adenoma                           |  | 2                    | 1        | 0        | 1        | 0        | 0        | 0        | 1        | 2                  | 1        | 1        | 1        | 0        | 0        | 0        | 0        |
| Heart                               | Number examined:<br>Unremarkable:  | 45<br>10             | 36<br>10 | 40<br>15 | 50<br>14 | 44<br>16 | 49<br>28 | 47<br>27 | 49<br>23 | 19<br>0            | 29<br>2  | 25<br>1  | 15<br>1  | 21<br>3  | 16<br>2  | 18<br>3  | 16<br>6  |
| M-Endocardial Schwannoma            |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Muscle, Bi Fem                      | Number examined:<br>Unremarkable:  | 45<br>43             | 36<br>35 | 40<br>40 | 50<br>50 | 44<br>43 | 49<br>47 | 47<br>49 | 49<br>49 | 19<br>19           | 29<br>29 | 25<br>24 | 15<br>15 | 21<br>20 | 16<br>15 | 18<br>18 | 16<br>16 |
| M-Osteosarcoma                      |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Liver                               | Number examined:<br>Unremarkable:  | 45<br>2              | 36<br>3  | 40<br>1  | 50<br>5  | 44<br>2  | 49<br>0  | 47<br>3  | 49<br>3  | 19<br>1            | 29<br>1  | 25<br>0  | 15<br>0  | 21<br>3  | 16<br>0  | 18<br>1  | 16<br>0  |
| B-Adenoma, Hepatocellular           |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 1                  | 1        | 0        | 0        | 3        | 0        | 1        | 0        |
| M-Carcinoma, Hepatocellular         |  | 1                    | 0        | 2        | 1        | 0        | 0        | 0        | 0        | 0                  | 0        | 1        | 1        | 0        | 1        | 0        | 0        |
| M-Cholangiocarcinoma                |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 1        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Hematopoietic Neoplasm *          |  | 1                    | 1        | 1        | 0        | 1        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 1        | 1        | 0        |
| C-Vascular Neoplasm *               |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 1        |
| Spleen                              | Number examined:<br>Unremarkable:  | 45<br>15             | 36<br>20 | 40<br>22 | 50<br>28 | 44<br>4  | 49<br>8  | 47<br>10 | 49<br>7  | 19<br>14           | 29<br>27 | 25<br>22 | 15<br>13 | 21<br>5  | 16<br>7  | 18<br>5  | 16<br>7  |
| I-Carcinoma, Uterus                 |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Hematopoietic Neoplasm *          |  | 1                    | 0        | 0        | 0        | 1        | 1        | 0        | 0        | 1                  | 0        | 0        | 0        | 0        | 1        | 1        | 0        |
| Lung                                | Number examined:<br>Unremarkable:  | 45<br>5              | 36<br>11 | 40<br>9  | 50<br>20 | 44<br>13 | 49<br>15 | 47<br>19 | 49<br>25 | 19<br>4            | 29<br>7  | 25<br>9  | 15<br>7  | 21<br>4  | 16<br>5  | 18<br>2  | 16<br>5  |
| N-Malignant Renal Mesenchymal Tumor |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Carcinoma, Mammary                |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Malignant Mesothelioma            |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Acinar Carcinoma                  |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Carcinoma, Adrenal Cortex         |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Hematopoietic Neoplasm *          |  | 2                    | 1        | 1        | 0        | 1        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 1        | 0        | 0        |
| Kidney                              | Number examined:<br>Unremarkable:  | 45<br>2              | 36<br>0  | 40<br>0  | 50<br>7  | 44<br>0  | 49<br>0  | 47<br>2  | 49<br>2  | 19<br>0            | 29<br>0  | 25<br>1  | 15<br>0  | 21<br>0  | 16<br>0  | 18<br>0  | 16<br>0  |
| B-Lipoma                            |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 1        | 1        |
| B-Adenoma, Tubule Cell              |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| M-Carcinoma, Tubule Cell            |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 1        | 0        | 0        |
| M-Malignant Renal Mesenchymal Tumor |  | 0                    | 1        | 0        | 0        | 0        | 0        | 0        | 1        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| M-Liposarcoma                       |  | 0                    | 0        | 1        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 1        | 0        | 0        | 0        | 0        | 0        |
| M-Nephroblastoma                    |  | 0                    | 0        | 1        | 0        | 0        | 0        | 0        | 1        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Carcinoma, Mammary                |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| I-Carcinoma, Uterus                 |  | 0                    | 0        | 0        | 0        | 0        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Vascular Neoplasm *               |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 1        | 0        | 0        | 0        | 0        | 0        | 1        |
| Urinary Bladder                     | Number examined:<br>Unremarkable:  | 44<br>33             | 35<br>29 | 40<br>33 | 50<br>37 | 44<br>41 | 49<br>47 | 47<br>46 | 48<br>46 | 19<br>13           | 29<br>23 | 25<br>21 | 15<br>12 | 21<br>21 | 16<br>15 | 18<br>17 | 16<br>15 |
| C-Vascular Neoplasm *               |  | 0                    | 0        | 0        | 0        | 0        | 0        | 0        | 1        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Stomach, Nongl                      | Number examined:<br>Unremarkable:  | 45<br>41             | 36<br>33 | 40<br>39 | 50<br>47 | 44<br>42 | 49<br>45 | 47<br>44 | 49<br>48 | 19<br>18           | 29<br>29 | 25<br>24 | 15<br>14 | 21<br>21 | 16<br>16 | 18<br>18 | 16<br>16 |
| M-Leiomyosarcoma                    |  | 1                    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 1        | 0        | 0        | 0        | 0        | 0        |
| B-Papilloma, Squamous Cell          |  | 0                    | 1        | 0        | 0        | 0        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| LN, Mesenteric                      | Number examined:<br>Unremarkable:  | 45<br>13             | 36<br>7  | 40<br>8  | 50<br>17 | 43<br>4  | 48<br>4  | 47<br>6  | 49<br>4  | 19<br>1            | 29<br>1  | 25<br>1  | 15<br>0  | 21<br>3  | 16<br>1  | 18<br>0  | 16<br>2  |
| C-Hematopoietic Neoplasm *          |  | 0                    | 0        | 0        | 0        | 1        | 1        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| Pancreas                            | Number examined:<br>Unremarkable:  | 45<br>25             | 36<br>27 | 40<br>25 | 50<br>35 | 44<br>33 | 49<br>39 | 47<br>41 | 49<br>40 | 19<br>1            | 29<br>0  | 25<br>16 | 15<br>8  | 21<br>13 | 16<br>11 | 18<br>12 | 16<br>13 |
| B-Adenoma, Islet Cell               |  | 0                    | 1        | 0        | 0        | 0        | 1        | 0        | 0        | 1                  | 0        | 0        | 1        | 3        | 0        | 0        | 1        |
| M-Carcinoma, Islet Cell             |  | 0                    | 0        | 0        | 1        | 0        | 1        | 0        | 1        | 0                  | 1        | 0        | 0        | 0        | 0        | 1        | 0        |
| B-Adenoma, Acinar Cell              |  | 1                    | 0        | 0        | 1        | 0        | 0        | 0        | 0        | 0                  | 2        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Malignant Renal Mesenchymal Tumor |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| N-Malignant Mesothelioma            |  | 0                    | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| C-Vascular Neoplasm *               |  | 0                    | 0        | 0        | 0        | 1        | 0        | 0        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |
| M-Carcinoma, Acinar Cell            |  | 0                    | 0        | 0        | 0        | 0        | 0        | 1        | 0        | 0                  | 0        | 0        | 0        | 0        | 0        | 0        | 0        |

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| Continued from previous page        |                  | UNSCHEDULED DEATHS |    |    |    |         |    |    |    | TERMINAL SACRIFICE |    |    |    |         |    |    |    |
|-------------------------------------|------------------|--------------------|----|----|----|---------|----|----|----|--------------------|----|----|----|---------|----|----|----|
|                                     |                  | Males              |    |    |    | Females |    |    |    | Males              |    |    |    | Females |    |    |    |
| Controls from group(s): 1           | Animal sex:      | Ctls               | 3  | 5  | 7  | Ctls    | 2  | 4  | 6  | Ctls               | 3  | 5  | 7  | Ctls    | 2  | 4  | 6  |
| Tissues                             | Dosage group:    | 46                 | 36 | 40 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
| Diagnoses                           | No. in group:    | 46                 | 36 | 40 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
| Skin/Subcutis                       | Number examined: | 45                 | 36 | 40 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 34                 | 27 | 31 | 41 | 39      | 41 | 44 | 37 | 16                 | 20 | 19 | 11 | 18      | 16 | 18 | 14 |
| B-Lipoma                            |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 1  | 0  | 0       | 0  | 0  | 0  |
| B-Fibroma                           |                  | 0                  | 0  | 2  | 0  | 0       | 0  | 0  | 0  | 1                  | 0  | 1  | 1  | 0       | 0  | 0  | 0  |
| B-Papilloma, Squamous Cell          |                  | 2                  | 1  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 1  | 0       | 0  | 0  | 0  |
| B-Keratoacanthoma                   |                  | 1                  | 0  | 2  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Trichoepithelioma                 |                  | 0                  | 0  | 0  | 1  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Schwannoma                        |                  | 1                  | 0  | 0  | 0  | 2       | 1  | 1  | 1  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Fibroadenoma, Mammary Gland       |                  | 0                  | 0  | 0  | 1  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Malignant Schwannoma              |                  | 1                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Carcinoma, Sebaceous Gland        |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 1  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Carcinoma, Squamous Cell          |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 1  | 0  | 0  | 0       | 0  | 0  | 1  |
| M-Schwannoma                        |                  | 1                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Carcinoma, Basal Cell             |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 1  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Carcinoma, Mammary Gland          |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 1  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Carcinoma, Baso Squamous Cell     |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| C-Hematopoietic Neoplasm*           |                  | 1                  | 0  | 1  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| C-Vascular Neoplasm*                |                  | 0                  | 0  | 1  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| Testis                              | Number examined: | 45                 | 36 | 40 | 50 |         |    |    |    | 19                 | 29 | 25 | 15 |         |    |    |    |
|                                     | Unremarkable:    | 29                 | 29 | 31 | 43 |         |    |    |    | 13                 | 20 | 18 | 12 |         |    |    |    |
| B-Interstitial Cell Tumor           |                  | 0                  | 0  | 1  | 0  |         |    |    |    | 0                  | 1  | 0  | 0  |         |    |    |    |
| B-Mesothelioma                      |                  | 1                  | 0  | 0  | 0  |         |    |    |    | 0                  | 0  | 0  | 0  |         |    |    |    |
| Mammary, Female                     | Number examined: |                    |    |    |    | 44      | 49 | 47 | 49 |                    |    |    |    | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    |                    |    |    |    | 18      | 17 | 19 | 18 |                    |    |    |    | 3       | 2  | 4  | 5  |
| B-Fibroma                           |                  |                    |    |    |    | 0       | 1  | 0  | 0  |                    |    |    |    | 0       | 0  | 1  | 1  |
| B-Fibroadenoma                      |                  |                    |    |    |    | 15      | 15 | 16 | 12 |                    |    |    |    | 14      | 10 | 8  | 10 |
| B-Lipoma                            |                  |                    |    |    |    | 0       | 0  | 0  | 1  |                    |    |    |    | 0       | 0  | 0  | 0  |
| M-Carcinoma                         |                  |                    |    |    |    | 12      | 9  | 11 | 14 |                    |    |    |    | 5       | 3  | 8  | 4  |
| C-Hematopoietic Neoplasm*           |                  |                    |    |    |    | 0       | 0  | 0  | 0  |                    |    |    |    | 0       | 1  | 0  | 0  |
| Ovary                               | Number examined: |                    |    |    |    | 44      | 49 | 47 | 49 |                    |    |    |    | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    |                    |    |    |    | 17      | 13 | 19 | 21 |                    |    |    |    | 2       | 3  | 0  | 0  |
| B-Lipoma                            |                  |                    |    |    |    | 0       | 0  | 1  | 0  |                    |    |    |    | 0       | 0  | 0  | 0  |
| M-Mesothelioma                      |                  |                    |    |    |    | 1       | 0  | 0  | 0  |                    |    |    |    | 0       | 0  | 0  | 0  |
| M-Malignant Teratoma                |                  |                    |    |    |    | 0       | 0  | 1  | 0  |                    |    |    |    | 0       | 0  | 0  | 0  |
| Uterus                              | Number examined: |                    |    |    |    | 44      | 49 | 47 | 49 |                    |    |    |    | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    |                    |    |    |    | 34      | 41 | 37 | 38 |                    |    |    |    | 13      | 11 | 12 | 8  |
| B-Polyp, Endometrial Stromal        |                  |                    |    |    |    | 1       | 0  | 1  | 0  |                    |    |    |    | 1       | 2  | 1  | 3  |
| M-Carcinoma                         |                  |                    |    |    |    | 0       | 1  | 0  | 1  |                    |    |    |    | 0       | 0  | 1  | 0  |
| C-Vascular Neoplasm*                |                  |                    |    |    |    | 0       | 0  | 0  | 1  |                    |    |    |    | 0       | 0  | 0  | 0  |
| Cervix                              | Number examined: |                    |    |    |    | 44      | 49 | 47 | 49 |                    |    |    |    | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    |                    |    |    |    | 43      | 49 | 45 | 42 |                    |    |    |    | 21      | 15 | 17 | 16 |
| M-Carcinoma                         |                  |                    |    |    |    | 0       | 0  | 1  | 0  |                    |    |    |    | 0       | 0  | 0  | 0  |
| C-Hematopoietic Neoplasm*           |                  |                    |    |    |    | 0       | 0  | 0  | 1  |                    |    |    |    | 0       | 0  | 0  | 0  |
| Vagina                              | Number examined: |                    |    |    |    | 44      | 49 | 46 | 49 |                    |    |    |    | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    |                    |    |    |    | 42      | 45 | 45 | 44 |                    |    |    |    | 20      | 16 | 18 | 15 |
| B-Fibroma                           |                  |                    |    |    |    | 0       | 0  | 0  | 0  |                    |    |    |    | 1       | 0  | 0  | 0  |
| B-Polyp, Stromal                    |                  |                    |    |    |    | 0       | 2  | 0  | 0  |                    |    |    |    | 0       | 0  | 0  | 0  |
| C-Hematopoietic Neoplasm*           |                  |                    |    |    |    | 0       | 0  | 0  | 1  |                    |    |    |    | 0       | 0  | 0  | 0  |
| Bone, Femur                         | Number examined: | 45                 | 36 | 39 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 45                 | 36 | 39 | 50 | 43      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 15 |
| C-Hematopoietic Neoplasm*           |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| Marrow, Femur                       | Number examined: | 45                 | 36 | 39 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 8                  | 9  | 10 | 14 | 20      | 26 | 18 | 27 | 0                  | 2  | 1  | 4  | 0       | 2  | 2  | 5  |
| C-Hematopoietic Neoplasm*           |                  | 0                  | 0  | 0  | 0  | 1       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  |
| Bone, Sternum                       | Number examined: | 45                 | 36 | 39 | 50 | 44      | 49 | 46 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 45                 | 36 | 39 | 50 | 43      | 49 | 45 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
| I-Liposarcoma                       |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| C-Hematopoietic Neoplasm*           |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| Marrow, Sternum                     | Number examined: | 45                 | 36 | 39 | 50 | 44      | 49 | 46 | 48 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 15                 | 14 | 15 | 24 | 21      | 26 | 18 | 27 | 2                  | 4  | 0  | 4  | 0       | 4  | 2  | 5  |
| C-Hematopoietic Neoplasm*           |                  | 0                  | 0  | 0  | 0  | 1       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  |
| Body, Whole/Cav                     | Number examined: | 45                 | 36 | 40 | 50 | 44      | 49 | 47 | 49 | 19                 | 29 | 25 | 15 | 21      | 16 | 18 | 16 |
|                                     | Unremarkable:    | 43                 | 35 | 38 | 50 | 42      | 48 | 47 | 46 | 18                 | 28 | 25 | 15 | 21      | 15 | 17 | 14 |
| B-Hemangioma                        |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 1  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 1  |
| M-Hemangiosarcoma                   |                  | 0                  | 0  | 1  | 0  | 1       | 0  | 0  | 1  | 1                  | 1  | 0  | 0  | 0       | 0  | 0  | 1  |
| M-Histiocytic Sarcoma               |                  | 2                  | 1  | 1  | 0  | 1       | 1  | 0  | 1  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  |
| M-Lymphoma, Lymphocytic             |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  |
| Adipose Tissue                      | Number examined: | 1                  | 0  | 0  | 0  | 0       | 2  | 1  | 0  | 0                  | 0  | 1  | 1  | 0       | 1  | 0  | 0  |
|                                     | Unremarkable:    | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  |
| B-Fibroma                           |                  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| I-Carcinoma, Uterus                 |                  | 0                  | 0  | 0  | 0  | 0       | 1  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| N-Malignant Renal Mesenchymal Tumor |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Lipoma                            |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 1  | 1  | 0  | 0       | 0  | 0  | 0  |
| Cavity, Thoracic                    | Number examined: | 4                  | 3  | 0  | 4  | 4       | 1  | 2  | 3  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 1  |
|                                     | Unremarkable:    | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Benign Mesothelioma               |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Lipoma                            |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Liposarcoma                       |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| C-Hematopoietic Neoplasm*           |                  | 1                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Hibernoma                         |                  | 0                  | 1  | 0  | 1  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| Cavity, Abdomin                     | Number examined: | 3                  | 0  | 1  | 0  | 1       | 0  | 2  | 2  | 0                  | 0  | 1  | 0  | 2       | 0  | 0  | 0  |
|                                     | Unremarkable:    | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| N-Malignant Renal Mesenchymal Tumor |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 1  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| B-Hemangioma                        |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 1  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| M-Liposarcoma                       |                  | 0                  | 0  | 1  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| N-Cholangiocarcinoma                |                  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |
| N-Malignant Mesothelioma            |                  | 0                  | 0  | 0  | 0  | 1       | 0  | 0  | 0  | 0                  | 0  | 0  | 0  | 0       | 0  | 0  | 0  |

\* see Body, Whole for type

DU-176b-related microscopic findings were limited to centrilobular hepatocellular deg

terminal animals exhibited this lesion in the liver. The incidence and severity of liver centrilobular hepatocellular degeneration/necrosis were higher in males with premature deaths at the DU-176b dose of 600/400 mg/kg/day when compared with other treated or control groups. The likely cause of death was identified as centrilobular hepatocellular degeneration/necrosis for 8 of 21 of these animals. An expert report (AN11-H7301-R01) prepared by Dr. Robert R. Maronpot considered that the observed centrilobular hepatocellular degeneration/necrosis was secondary to hypoxia/anoxia secondary to in-life and terminal debility in the affected rats, but no clear evidence was provided. Since the in-life and terminal debility was similar for rats of premature death in other groups and there was not any degenerative or necrotic changes in other tissues such as pancreas and intestinal mucosa that would be susceptible to generalized anoxia or postmortem autolysis, the higher incidence and greater severity of liver centrilobular hepatocellular degeneration/necrosis cannot be attributed to hypoxia/anoxia secondary to in-life and terminal debility, and cannot be excluded as being due to the administration of high dose of DU-176b in males, where there is clear indication that the maximum tolerated dose (MTD) was reached or exceeded.

Table 65. Microscopic liver findings in animals with premature death (from the submission)

| Sex              | Males            |     |     |     | Females |     |     |     |
|------------------|------------------|-----|-----|-----|---------|-----|-----|-----|
|                  | Dose (mg/kg/day) | 0   | 60  | 200 | 600/400 | 0   | 50  | 100 |
| Number Examined  | 45               | 36  | 40  | 50  | 44      | 49  | 47  | 49  |
| Minimal          | 0                | 0   | 1   | 2   | 1       | 0   | 1   | 0   |
| Slight           | 2                | 0   | 0   | 7   | 1       | 1   | 1   | 0   |
| Moderate         | 0                | 3   | 2   | 8   | 1       | 2   | 1   | 2   |
| Marked           | 0                | 0   | 0   | 4   | 0       | 1   | 1   | 1   |
| Number w/Lesion  | 2                | 3   | 3   | 21  | 3       | 4   | 4   | 3   |
| Average Severity | 0.1              | 0.3 | 0.2 | 1.1 | 0.1     | 0.2 | 0.2 | 0.2 |

There was no detectable DU-176 in the plasma samples from control animals. As shown in Figure 16 and Table 66, the plasma DU-176 concentrations, C<sub>max</sub>, and AUC<sub>0-24</sub> increased with the DU-176b dose increases, which were generally less than dose proportional. Exposures to DU-176 were generally higher in females than in males, were similar on Day 1 and Week 26 for females and lower in Week 26 than on Day 1 males (especially at high dose), indicating no accumulation of DU-176 after multiple doses. The markedly lower C<sub>max</sub> and AUC<sub>0-24</sub> of high dose males in Week 26 may imply higher rate of metabolism following repeated dosing. After oral gavage administration of DU-176b, DU-176b was readily absorbed, with T<sub>max</sub> values ranging from 1 to 2 hours.

Figure 16. Plasma DU-176 concentrations on dosing day 1 and in week 26 (modified from the submission)

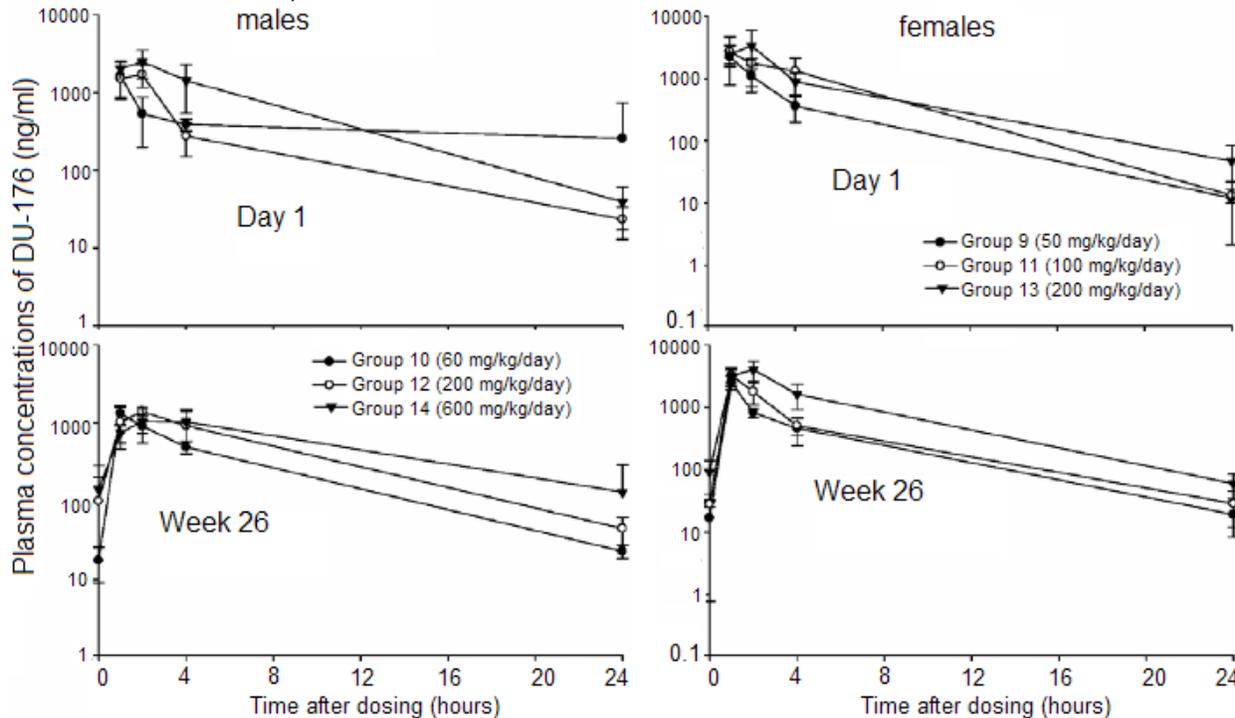


Table 66. TK parameters follow oral administration of DU176b

| Group   | Dose level | Sex | Cmax  | Tmax  | AUC <sub>0-24</sub> | Week 26/Day 1 |                     |
|---------|------------|-----|-------|-------|---------------------|---------------|---------------------|
|         | mg/kg/day  |     | ng/ml | Hours | ng.hr/ml            | Cmax          | AUC <sub>0-24</sub> |
| Day 1   |            |     |       |       |                     |               |                     |
| 9       | 50         | F   | 2210  | 1     | 7927                | N/A           |                     |
| 10      | 60         | M   | 1641  | 1     | 9288                |               |                     |
| 11      | 100        | F   | 2687  | 1     | 19839               |               |                     |
| 12      | 200        | M   | 1698  | 2     | 7335                |               |                     |
| 13      | 200        | F   | 3330  | 2     | 17563               |               |                     |
| 14      | 600        | M   | 2498  | 2     | 21885               |               |                     |
| Week 26 |            |     |       |       |                     |               |                     |
| 9       | 50         | F   | 2505  | 1     | 9047                | 1.1           | 1.1                 |
| 10      | 60         | M   | 1316  | 1     | 8320                | 0.8           | 0.9                 |
| 11      | 100        | F   | 3223  | 1     | 11782               | 1.2           | 0.6                 |
| 12      | 200        | M   | 1395  | 2     | 13763               | 0.8           | 1.9                 |
| 13      | 200        | F   | 4045  | 2     | 27796               | 1.2           | 1.6                 |
| 14      | 600        | M   | 1055  | 2     | 15086               | 0.4           | 0.7                 |

In conclusion, when DU-176b was given to SD rats by oral gavage daily for up to 104 weeks at doses from 50 to 600 mg/kg/day, there was no evidence of increased neoplasia at any dose level. Mortality was significantly higher in males at the dose of

600/400 mg/kg/day, and marginally higher in females at 200 mg/kg/day. There were higher incidences and greater severity of liver centrilobular hepatocellular degeneration/necrosis in males at 600/400 mg/kg/day, and centrilobular hepatocellular degeneration/necrosis may be the cause for 8 of the 50 unscheduled deaths in the 600/400 mg/kg/day males. There were slightly but statistically lower red cell counts in females at 200 mg/kg/day, higher incidences of red oral and nasal discharge, and red haircoat in males at DU-176b 600/400 mg/kg/day. Similar clinical signs of less extent were also seen in females at DU-176b 200 mg/kg/day. The findings in hematology and clinical signs may, at least in part, be attributed to bleeding, the pharmacological action of the test article. NOAELs for carcinogenicity were 600/400 mg/kg/day for males with C<sub>max</sub> of 1055 ng/mL and AUC<sub>0-24</sub> of 15086 ng•hr/mL during Week 26, and 200 mg/kg/day for females with C<sub>max</sub> of 4045 ng/mL and AUC<sub>0-24</sub> of 27796 ng•hr/mL during Week 26. NOAELs for general toxicity were 200 mg/kg/day for males with C<sub>max</sub> of 1395 ng/mL and AUC<sub>0-24</sub> of 13763 ng•hr/mL, and 100 mg/kg/day for females with C<sub>max</sub> of 3223 ng/mL and AUC<sub>0-24</sub> of 11782 ng•hr/mL.

The proposed maximum recommended human daily dose of DU176b is 60 mg. This dose of 60 mg had a median AUC<sub>0-24,ss</sub> of 1940 (Clinical Study Report DU176b-A-U151, page 80). Thus, NOAELs for carcinogenicity in male and female rats are estimated to be 8 and 14 times, respectively, the maximum recommended human daily dose of DU-176b based on AUC<sub>0-24</sub> comparisons.

### **Summary of FDA statistical analysis on survival rate and tumor findings**

FDA statistical analysis by Dr. M.A. Rahman concluded that there were statistically significant dose-response relationships in mortality across control and treated groups in male rats, and statistically significant increase of mortality in the male rats at high dose level compared to their controls (Table 67).

Table 67. Intercurrent Mortality Rate in Rats and Comparison (modified from FDA statistical review)

| Male Rats | 0 mg/kg/day |        | 60 mg/kg/day |        | 200 mg/kg/day |        | 600/400 mg/kg/day |        | Test          | Statistic        | P_Value |
|-----------|-------------|--------|--------------|--------|---------------|--------|-------------------|--------|---------------|------------------|---------|
|           | No. of      | Cum. % | No. of       | Cum. % | No. of        | Cum. % | No. of            | Cum. % |               |                  |         |
| Week      | Death       | Cum. % | Death        | Cum. % | Death         | Cum. % | Death             | Cum. % |               |                  |         |
| 0 - 52    | 7           | 10.77  | 6            | 9.23   | 11            | 16.92  | 24                | 36.92  | Dose-Response | Likelihood Ratio | 0.0016  |
| 53 - 78   | 18          | 38.46  | 20           | 40.00  | 16            | 41.54  | 18                | 64.62  |               |                  |         |
| 79 - 89   | 21          | 70.77  | 10           | 55.38  | 13            | 61.54  | 7                 | 76.92  | Homogeneity   | Log-Rank         | 0.0013  |
| Ter. Sac. | 19          | 29.23  | 29           | 44.62  | 25            | 38.46  | 16                | 23.08  |               |                  |         |
| Total     | N=65        |        | N=65         |        | N=65          |        | N=65              |        |               |                  |         |

| Female Rats | 0 mg/kg/day |        | 50 mg/kg/day |        | 100 mg/kg/day |        | 200 mg/kg/day |        | Test          | Statistic        | P_Value |
|-------------|-------------|--------|--------------|--------|---------------|--------|---------------|--------|---------------|------------------|---------|
|             | No. of      | Cum. % | No. of       | Cum. % | No. of        | Cum. % | No. of        | Cum. % |               |                  |         |
| Week        | Death       | Cum. % | Death        | Cum. % | Death         | Cum. % | Death         | Cum. % |               |                  |         |
| 0 - 52      | 3           | 4.62   | 2            | 3.08   | 1             | 1.54   | 7             | 10.77  | Dose-Response | Likelihood Ratio | 0.2658  |
| 53 - 78     | 17          | 30.77  | 21           | 35.38  | 14            | 23.08  | 17            | 36.92  |               |                  |         |
| 79 - 91     | 14          | 52.31  | 13           | 55.38  | 21            | 55.38  | 16            | 61.54  | Homogeneity   | Log-Rank         | 0.5717  |
| 92 - 104    | 9           | 66.15  | 12           | 73.85  | 11            | 72.31  | 9             | 75.38  |               |                  |         |
| Ter. Sac.   | 22          | 33.85  | 17           | 26.15  | 18            | 27.69  | 16            | 24.62  |               |                  |         |
| Total       | N=65        |        | N=65         |        | N=65          |        | N=65          |        |               |                  |         |

For tumor data analysis, multiple testing adjustment was applied. Briefly, the FDA draft guidance for the carcinogenicity study design and data analysis (May 2001) suggests the use of test levels  $\alpha=0.005$  for common tumors and  $\alpha=0.025$  for rare tumors for a submission with two species, and a significance level  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors for a submission with one species study in order to keep the false-positive rate at the nominal level of approximately 10%. For multiple pairwise comparisons of treated groups with control, the FDA guidance suggested the use of test levels  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors, in order to keep the false-positive rate at the nominal level of approximately 10% for both submissions with two or one species.

For tumor findings, FDA statistical analysis concluded that none of the observed tumors was considered to have statistically significant dose-response relationships in either sex. The pairwise comparison also did not show statistically significant increased incidence in any observed tumor type in any treated group in either sex compared to their respective controls (Table 68).

Table 68. Tumor types and statistical analysis (modified from FDA statistical review)

| Male Rats        |                           | 0 mg                      | 60 mg       | 200 mg      | 600/400 mg   | P_Value      | P_Value       | P_Value       | P_Value       |        |
|------------------|---------------------------|---------------------------|-------------|-------------|--------------|--------------|---------------|---------------|---------------|--------|
| Organ Name       | Tumor Name                | Control<br>N=65           | Low<br>N=65 | Med<br>N=65 | High<br>N=65 | Dose<br>Resp | L vs<br>Com C | M vs<br>Com C | H vs<br>Com C |        |
| Adipose Tissue   | B-Lipoma                  | 0                         | 0           | 1           | 1            | 0.1271       | .             | 0.5055        | 0.3919        |        |
| Adrenal, Cortex  | B-Adenoma                 | 3                         | 0           | 0           | 1            | 0.6074       | 0.8791        | 0.8791        | 0.5036        |        |
|                  | M-Carcinoma               | 1                         | 2           | 0           | 0            | 0.8565       | 0.5165        | 0.5055        | 0.3919        |        |
| Adrenal, Medull  | B-Pheochromocytoma        | 6                         | 7           | 2           | 1            | 0.9754       | 0.5000        | 0.8666        | 0.8470        |        |
|                  | M-Malignant Pheochromocyt | 1                         | 4           | 0           | 2            | 0.3699       | 0.1944        | 0.5055        | 0.3382        |        |
| Body, Whole/Cav  | M-Hemangiosarcoma         | 1                         | 1           | 1           | 0            | 0.6497       | 0.2527        | 0.2527        | 0.3919        |        |
|                  | M-Histiocytic Sarcoma     | 2                         | 1           | 1           | 0            | 0.8171       | 0.5082        | 0.5000        | 0.6270        |        |
| Brain            | B-Astrocytoma             | 0                         | 0           | 1           | 0            | 0.4518       | .             | 0.5055        | .             |        |
|                  | B-Granular Cell Tumor     | 1                         | 0           | 0           | 0            | 0.7289       | 0.5055        | 0.5055        | 0.3919        |        |
|                  | B-Oligodendroglioma       | 1                         | 0           | 0           | 0            | 0.7246       | 0.5000        | 0.5000        | 0.3867        |        |
|                  | M-Malignant Astrocytoma   | 2                         | 1           | 0           | 2            | 0.2657       | 0.5000        | 0.7527        | 0.5317        |        |
| Cavity, Abdomin  | M-Liposarcoma             | 0                         | 0           | 1           | 0            | 0.4518       | .             | 0.5055        | .             |        |
| Cavity, Thoraci  | B-Hibernoma               | 0                         | 1           | 0           | 1            | 0.2299       | 0.5109        | .             | 0.4000        |        |
| Heart            | M-Endocardial Schwannoma  | 1                         | 0           | 0           | 0            | 0.7289       | 0.5055        | 0.5055        | 0.3919        |        |
| Kidney           | B-Adenoma, Tubule Cell    | 1                         | 0           | 0           | 0            | 0.7246       | 0.5000        | 0.5000        | 0.3867        |        |
|                  | M-Liposarcoma             | 0                         | 0           | 2           | 0            | 0.3953       | .             | 0.2527        | .             |        |
|                  | M-Malignant Renal Mesench | 0                         | 1           | 0           | 0            | 0.4491       | 0.5109        | .             | .             |        |
|                  | M-Nephroblastoma          | 0                         | 0           | 1           | 0            | 0.4551       | .             | 0.5109        | .             |        |
| Liver            | B-Adenoma, Hepatocellular | 1                         | 1           | 0           | 0            | 0.7766       | 0.2527        | 0.5055        | 0.3919        |        |
|                  | M-Carcinoma, Hepatocellul | 1                         | 0           | 3           | 2            | 0.0856       | 0.5000        | 0.3083        | 0.3424        |        |
|                  | M-Cholangiocarcinoma      | 0                         | 1           | 0           | 0            | 0.4518       | 0.5055        | .             | .             |        |
| Muscle, Bi Fem   | M-Osteosarcoma            | 1                         | 0           | 0           | 0            | 0.7289       | 0.5055        | 0.5055        | 0.3919        |        |
| Pancreas         | B-Adenoma, Acinar Cell    | 1                         | 2           | 0           | 1            | 0.4718       | 0.5000        | 0.5000        | 0.6368        |        |
|                  | B-Adenoma, Islet Cell     | 1                         | 1           | 0           | 1            | 0.4220       | 0.2527        | 0.5055        | 0.6335        |        |
|                  | M-Carcinoma, Islet Cell   | 0                         | 1           | 0           | 1            | 0.2305       | 0.5055        | .             | 0.4000        |        |
| Parathyroid      | B-Adenoma                 | 4                         | 2           | 1           | 2            | 0.5484       | 0.6718        | 0.8195        | 0.4270        |        |
| Pituitary        | B-Adenoma                 | 37                        | 26          | 28          | 19           | 0.7436       | 0.9718        | 0.8875        | 0.8545        |        |
| Skin/Subcutis    | B-Fibroadenoma, Mammary G | 0                         | 0           | 0           | 1            | 0.1796       | .             | .             | 0.4000        |        |
|                  | B-Fibroma                 | 1                         | 0           | 3           | 1            | 0.2308       | 0.5055        | 0.3166        | 0.6335        |        |
|                  | B-Keratoacanthoma         | 1                         | 0           | 2           | 0            | 0.5239       | 0.5055        | 0.5083        | 0.3919        |        |
|                  | B-Lipoma                  | 0                         | 0           | 1           | 0            | 0.4518       | .             | 0.5055        | .             |        |
|                  | B-Papilloma, Squamous Cel | 2                         | 1           | 0           | 1            | 0.5289       | 0.5000        | 0.7527        | 0.3307        |        |
|                  | B-Schwannoma              | 1                         | 0           | 0           | 0            | 0.7246       | 0.5000        | 0.5000        | 0.3867        |        |
|                  | B-Trichoepithelioma       | 0                         | 0           | 0           | 1            | 0.1796       | .             | .             | 0.4000        |        |
|                  | M-Carcinoma, Basal Cell   | 0                         | 1           | 0           | 0            | 0.4518       | 0.5055        | .             | .             |        |
|                  | M-Carcinoma, Sebaceous Gl | 0                         | 1           | 0           | 0            | 0.4518       | 0.5055        | .             | .             |        |
|                  | M-Carcinoma, Squamous Cel | 0                         | 1           | 0           | 0            | 0.4518       | 0.5055        | .             | .             |        |
|                  | M-Malignant Schwannoma    | 1                         | 0           | 0           | 0            | 0.7246       | 0.5000        | 0.5000        | 0.3867        |        |
|                  | M-Schwannoma              | 1                         | 0           | 0           | 0            | 0.7289       | 0.5055        | 0.5055        | 0.3919        |        |
|                  | Spinal Cord               | M-Malignant Astrocytoma   | 1           | 0           | 0            | 0            | 0.7246        | 0.5000        | 0.5000        | 0.3867 |
|                  | Stomach, Nongl            | B-Papilloma, Squamous Cel | 0           | 1           | 0            | 0            | 0.4491        | 0.5109        | .             | .      |
| M-Leiomyosarcoma |                           | 1                         | 0           | 1           | 0            | 0.5498       | 0.5055        | 0.2527        | 0.3919        |        |
| Testis           | B-Interstitial Cell Tumor | 0                         | 1           | 1           | 0            | 0.3953       | 0.5055        | 0.5055        | .             |        |
|                  | B-Mesothelioma            | 1                         | 0           | 0           | 0            | 0.7289       | 0.5055        | 0.5055        | 0.3919        |        |
| Thyroid          | B-Adenoma, Follicular Cel | 2                         | 2           | 0           | 2            | 0.3428       | 0.3166        | 0.7527        | 0.5179        |        |
|                  | B-C-Cell Adenoma          | 7                         | 7           | 8           | 9            | 0.0568       | 0.4203        | 0.5000        | 0.1352        |        |
|                  | M-Carcinoma, C-cell       | 1                         | 3           | 2           | 2            | 0.2642       | 0.3250        | 0.5083        | 0.3382        |        |
|                  | M-Carcinoma, Follicular C | 2                         | 0           | 1           | 0            | 0.7728       | 0.7527        | 0.5000        | 0.6270        |        |

Continue on next page

| Female Rats     |                           | 0 mg            | 50 mg       | 100 mg      | 200 mg       | P_Value      | P_Value       | P_Value       | P_Value       |
|-----------------|---------------------------|-----------------|-------------|-------------|--------------|--------------|---------------|---------------|---------------|
| Organ Name      | Tumor Name                | Control<br>N=65 | Low<br>N=65 | Med<br>N=65 | High<br>N=65 | Dose<br>Resp | L vs<br>Com C | M vs<br>Com C | H vs<br>Com C |
| Adipose Tissue  | B-Fibroma                 | 0               | 1           | 0           | 0            | 0.4877       | 0.4940        | .             | .             |
| Adrenal, Cortex | B-Adenoma                 | 4               | 1           | 4           | 2            | 0.5843       | 0.8047        | 0.3698        | 0.5997        |
|                 | M-Carcinoma               | 1               | 0           | 1           | 0            | 0.6069       | 0.4878        | 0.2529        | 0.4684        |
|                 | M-Ganglioneuroma          | 0               | 1           | 0           | 0            | 0.4907       | 0.4878        | .             | .             |
| Adrenal, Medull | B-Ganglioneuroma          | 0               | 1           | 1           | 0            | 0.4750       | 0.4878        | 0.5059        | .             |
|                 | B-Pheochromocytoma        | 3               | 2           | 2           | 2            | 0.5549       | 0.4766        | 0.5000        | 0.4393        |
|                 | M-Neuroblastoma           | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
| Body, Whole/Cav | B-Hemangioma              | 0               | 0           | 0           | 2            | 0.0517       | .             | .             | 0.2162        |
|                 | M-Hemangiosarcoma         | 1               | 0           | 0           | 2            | 0.1790       | 0.4878        | 0.5000        | 0.4520        |
|                 | M-Histiocytic Sarcoma     | 1               | 2           | 0           | 1            | 0.5157       | 0.4815        | 0.5000        | 0.7205        |
|                 | M-Lymphoma, Lymphocytic   | 0               | 0           | 1           | 0            | 0.4907       | .             | 0.5000        | .             |
| Brain           | M-Malignant Astrocytoma   | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
| Cavity, Abdomin | B-Hemangioma              | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
| Cavity, Thoraci | B-Lipoma                  | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
|                 | M-Liposarcoma             | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
| Cervix          | M-Carcinoma               | 0               | 0           | 1           | 0            | 0.4907       | .             | 0.5000        | .             |
| Kidnev          | B-Lipoma                  | 0               | 0           | 1           | 1            | 0.1724       | .             | 0.5000        | 0.4684        |
|                 | M-Carcinoma, Tubule Cell  | 0               | 1           | 0           | 0            | 0.4907       | 0.4878        | .             | .             |
|                 | M-Malignant Renal Mesench | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
|                 | M-Nephroblastoma          | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
| Liver           | B-Adenoma, Hepatocellular | 3               | 0           | 1           | 0            | 0.9360       | 0.8704        | 0.6921        | 0.8548        |
|                 | M-Carcinoma, Hepatocellul | 0               | 1           | 0           | 0            | 0.4907       | 0.4878        | .             | .             |
| Mammary, Female | B-Fibroadenoma            | 29              | 25          | 24          | 22           | 0.7207       | 0.6503        | 0.7941        | 0.6656        |
|                 | B-Fibroma                 | 0               | 1           | 1           | 1            | 0.2683       | 0.4940        | 0.5000        | 0.4684        |
|                 | B-Lipoma                  | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
|                 | M-Carcinoma               | 17              | 12          | 19          | 18           | 0.2155       | 0.7530        | 0.4479        | 0.4017        |
| Ovary           | B-Lipoma                  | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
|                 | M-Malignant Teratoma      | 0               | 0           | 1           | 0            | 0.4907       | .             | 0.5000        | .             |
|                 | M-Mesothelioma            | 1               | 0           | 0           | 0            | 0.7391       | 0.4878        | 0.5000        | 0.4684        |
| Pancreas        | B-Adenoma, Islet Cell     | 3               | 1           | 0           | 1            | 0.8125       | 0.6830        | 0.8795        | 0.6428        |
|                 | M-Carcinoma, Acinar Cell  | 0               | 0           | 1           | 0            | 0.4907       | .             | 0.5000        | .             |
|                 | M-Carcinoma, Islet Cell   | 0               | 1           | 1           | 1            | 0.2683       | 0.4940        | 0.5000        | 0.4684        |
| Parathyroid     | B-Adenoma                 | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
| Pituitary       | B-Adenoma                 | 50              | 46          | 53          | 50           | 0.1400       | 0.7692        | 0.5628        | 0.3141        |
|                 | M-Carcinoma               | 1               | 0           | 0           | 0            | 0.7391       | 0.4878        | 0.5000        | 0.4684        |
| Skin/Subcutis   | B-Keratoacanthoma         | 0               | 0           | 1           | 0            | 0.4938       | .             | 0.5059        | .             |
|                 | B-Schwannoma              | 2               | 1           | 1           | 1            | 0.6031       | 0.4726        | 0.5000        | 0.4532        |
|                 | M-Carcinoma, Baso Squamou | 1               | 0           | 0           | 0            | 0.7391       | 0.4878        | 0.5000        | 0.4684        |
|                 | M-Carcinoma, Mammary Glan | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
|                 | M-Carcinoma, Squamous Cel | 0               | 0           | 0           | 1            | 0.2298       | .             | .             | 0.4684        |
|                 | M-Malignant Schwannoma    | 0               | 1           | 0           | 0            | 0.4877       | 0.4940        | .             | .             |
| Thyroid         | B-Adenoma, Follicular Cel | 0               | 1           | 0           | 1            | 0.2873       | 0.4878        | .             | 0.4684        |
|                 | B-C-Cell Adenoma          | 8               | 10          | 6           | 4            | 0.8956       | 0.3740        | 0.6323        | 0.7590        |
|                 | M-Carcinoma, C-cell       | 1               | 2           | 0           | 0            | 0.8312       | 0.4909        | 0.5000        | 0.4684        |
|                 | M-Carcinoma, Follicular C | 0               | 0           | 1           | 1            | 0.1724       | .             | 0.5000        | 0.4684        |
| Uterus          | B-Polyp, Endometrial Stro | 2               | 2           | 2           | 3            | 0.2921       | 0.6735        | 0.3169        | 0.4393        |
|                 | M-Carcinoma               | 0               | 1           | 1           | 1            | 0.2683       | 0.4940        | 0.5000        | 0.4684        |
| Vagina          | B-Fibroma                 | 1               | 0           | 0           | 0            | 0.7391       | 0.4878        | 0.5000        | 0.4684        |
|                 | B-Polyp, Stromal          | 0               | 2           | 0           | 0            | 0.6052       | 0.2349        | .             | .             |

### Executive CAC Conclusions (June 10, 2014)

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in the study.

### 8.3 DU-176b: Oral medium-term liver carcinogenesis bioassay in male F344 rats (Study R20030708) and TK evaluation (Study R20040221)

This GLP study (R20030708) was conducted with DU-176b (lot # CA201; purity 99.8%) in (b) (4) during July 2003 – March 2004, and previously reviewed by Dr. David B. Joseph of DGIE (Apr 4, 2007) under IND 063266 (Appendix I).

Male F344/DuCrj (SPF) rats, 24/group at 6 weeks of age, were given a single intraperitoneal injection of 200 mg/kg N-nitrosodiethylamine (DEN) as the initiation step for hepatocarcinogenesis. On Day 15, the animals were orally gavaged with DU-176b at 0 (vehicle control, 0.5% MC), 5, 10, or 20 mg/kg/day for 6 weeks. On Day 22, the animals were subjected to two-thirds partial hepatectomy. The positive control group was given the diet containing 500 ppm sodium phenobarbital (S.PB) ad libitum instead of DU-176b. Ten male rats per group without DEN initiation (saline was injected instead) also received oral doses of DU-176b (vehicle or 20 mg/kg/day) for 6 weeks. Rats were observed for mortality, clinical sign, body weight, and food consumption during the study period. Blood samples (n = 5/group) were collected 1 h after final DU-176b dose for the determination of plasma concentration of DU-176 (by an LC-MS/MS method). At the end of DU-176 treatment, rats were sacrificed. The main organs and tissues in the thoracic and abdominal cavities were examined macroscopically. Liver was weighed, and histologically processed. Liver sections were immunohistochemically stained for glutathione S-transferase placental form (GST-P, as endpoint marker for preneoplastic lesions), followed by quantitative analysis of preneoplastic GST-P-positive hepatocytic foci.

There were no DU-176b-related effects on mortality, clinical signs, body weight, food consumption, macroscopic observation, and liver weights. Quantitative values for GST-P-positive foci were significantly increased in the S.PB treatment group, in line with its proven tumor promoter nature in the liver, confirming the successful medium-term liver carcinogenesis bioassay (Table 69). The numbers and areas of GST-P-positive foci per liver section in animals exposed to DU-176b after DEN were similar to the carcinogen control group values. In the non-DEN initiation groups, no GST-P-positive foci were observed in rats given 20 mg/kg/day DU-176b or the control group (Table 69). Plasma DU-176 concentrations increased dose-proportionally following repeat oral DU-176b doses (Table 69).

Table 69. Quantitative data for liver GST-P positive foci and plasma DU-176 concentrations

| TREATMENT |               |           | NO. of Examined | GST-P POSITIVE FOCI         |  | Plasma DU-176 Concentration, <sup>a</sup> ng/ml |
|-----------|---------------|-----------|-----------------|-----------------------------|--|---|
| DEN       | TEST CHEMICAL | mg/kg/day |                 | NO. /cm <sup>2</sup>        | AREA (mm <sup>2</sup> /cm <sup>2</sup> ) |   |
| +         | DU-176b       | 0         | 20              | 4.269 ± 2.133               | 0.347 ± 0.188                            | 0.00 ± 0.00                                     |
| +         | DU-176b       | 5         | 20              | 5.076 ± 2.047               | 0.359 ± 0.144                            | 413 ± 86.0                                      |
| +         | DU-176b       | 10        | 20              | 3.697 ± 2.123               | 0.325 ± 0.255                            | 840 ± 251                                       |
| +         | DU-176b       | 20        | 18              | 4.948 ± 2.354               | 0.379 ± 0.234                            | 1810 ± 328                                      |
| +         | S. PB         | 500 ppm   | 19              | 8.872 ± 3.326 <sup>##</sup> | 0.778 ± 0.325 <sup>##</sup>              |   |
| -         | DU-176b       | 0         | 10              | 0.000 ± 0.000               | 0.000 ± 0.000                            | 0.00 ± 0.00                                     |
| -         | DU-176b       | 20        | 10              | 0.000 ± 0.000               | 0.000 ± 0.000                            | 2090 ± 724                                      |

<sup>##</sup> : p<0.01 vs control. a n=5/group

In conclusion, DU-176b was not associated with tumor promotion potential in the current medium-term liver bioassay using male F344 rats, suggesting that DU-176b is not a liver tumor promotor.

## **9 Reproductive and Developmental Toxicology**

### **9.1 Fertility and Early Embryonic Development Studies in Rats, Embryo-Fetal Development Studies in Rats and Rabbits**

Fertility and early embryonic development studies in rats (R20030165 & R20030552), and embryo-fetal development studies in rats (R20030196 & R20030532) and rabbits (R20030648 & R20040042) were performed with oral DU-176b, and are summarized in Table 70. Dr. David B. Joseph of DGIE previously reviewed these studies (April 4, 2007) under IND063266 (Appendix I).

Table 70. Fertility and embryo-fetal development studies with DU-176b

| Type of Study                             | Species /Strain | NO./sex /group | Drug, Lot, Purity     | Treatment regimen  | GLP | Noteworthy Findings  | Study Number |
|---|-----------------|----------------|-----------------------|--|-----|--|--------------|
| Fertility and Early Embryonic Development | Rat/SD          | 7              | DU-176b, JZ101B       | Oral; Vehicle (0.5% MC), 100, 300, 600, or 1000 mg/kg/day for 1 week                                     | No  | No signs of toxicity were observed at oral doses of up to 1000 mg/kg/day   | R20030165 *  |
|   | Rat/SD          | 19-20          | DU-176b, CA201, 99.8% | Oral; vehicle, 100, 300, or 1000 mg/kg/day for 2 weeks before mating to copulation (M), and until G7 (F) | Yes | No effect on mating or fertility at oral doses of up to 1000 mg/kg/day. NOAEL = 1000 for general toxicity and reproduction in parent animals and for the development of next generation  | R20030552    |
| Embryo-Fetal Development                  | Rat/SD          | 7 F            | DU-176b, JZ101A       | Oral; vehicle, 100, 330, 600, or 1000 mg/kg/day for G7 to G17  | No  | Deaths at 1000 mg/kg/day; Vaginal hemorrhage at $\geq 600$ mg/kg/day; Intrauterine hemorrhage or dark greenish substance around the placenta in all treatment groups; No drug-related fetal anomalies.   | R20030196 *  |
|   | Rat/SD          | 20 F           | DU-176b, CA201, 99.8% | Oral; vehicle, 30, 100, or 300 mg/kg/day for G7 to G17   | Yes | Dam: Vaginal hemorrhage at 300 mg/kg/day<br>Fetus: Higher postimplantation loss at 300 mg/kg/day. no teratogenic effects. NOAEL: 100 mg/kg/day for general toxicity and reproduction in dams and for the development of next generation  | R20030532    |
|   | Rabbit/NZW      | 6 F            | DU-176b, CA201, 99.8% | Oral; vehicle, 100, 300, 600, or 1000 mg/kg/day for G7 to G20  | Yes | Dam: Deaths and abortion at 600 mg/kg/day; Decreased body weight gain at $\geq 600$ mg/kg/day; Brown contents in the amniotic sac, vagina and/or uterus at all dose levels. Fetus: Increased postimplantation losses and decreased viable fetuses at all dose levels; Decreased fetal body weights at $\geq 600$ mg/kg/day. No teratogenic effects.  | R20030648 *  |
|   | Rabbit/NZW      | 26-30 F        | DU-176b, CA201, 99.8% | Oral; vehicle, 60, 200, or 600 mg/kg/day for G7 to G20   | Yes | Dam: deaths and abortion, decreased food consumption, body weight, and defecation, and increased dark red contents of the uterus at $\geq 200$ mg/kg/day. Fetus: Increased postimplantation loss, decreased live fetuses, decreased fetal weight, and increased variation in the gall bladder at $\geq 200$ mg/kg/day; Increased 13th full ribs and 27 presacral vertebrae at 600 mg/kg/day. No teratogenic effects. NOAEL: 60 for general toxicity and reproduction in dams and for the development of next generation. | R20040022    |

\* Preliminary dose range-finding study

Oral DU-176b at doses up to 1000 mg/kg/day did not affect mating and fertility parameters in rats. DU-176b was not teratogenic in rats at doses up to 300 mg/kg/day and in rabbits at doses up to 600 mg/kg/day, but was maternal and embryo-fetal toxic at mid and/or high doses in both rats and rabbits. In rat embryo-fetal development studies, dam vaginal hemorrhage and higher post-implantation loss were observed at DU-176b  $\geq 300$  mg/kg/day. Both maternal and embryo-fetal NOAELs were 100 mg/kg/day in rats. In rabbit embryo-fetal development studies, DU-176b at doses  $\geq 200$  mg/kg/day resulted in dam deaths and abortion, decreased food consumption and body weight,

and hemorrhage in uterus, more post-implantation loss, less live fetuses, lower fetal weight, and increased variation in the gall bladder. Increased 13th full ribs and 27 presacral vertebrae occurred in rabbits at DU-176b 600 mg/kg/day. Both maternal and embryo-fetal NOAELs were 60 mg/kg/day in rabbits.

Because of maternal and embryo-fetal toxicities were observed at the same dose level, DU-176b-associated embryo-fetal toxicity in rats and rabbits was considered to be secondary effects of maternal toxicity, rather than a direct DU-176b effect.

## 9.2 Study for Effects of DU-176b Administered Orally on Pre- and Postnatal Development Including Maternal Function in Rats

Conducting laboratory and location: Daiichi Sankyo Co., Ltd., Fukuroi, Japan

Study number(s): AN08-H0090-R01

Date of study initiation: May 11, 2009

Drug, lot/batch number, purity: DU-176b, MH409-U, 100.6%

GLP compliance: Yes

QA statement: Yes

### Key Study Findings

There were no DU-176b-related premature deaths during the course of the study. Vaginal bleeding was noted in 1 dam each on Day 16 or Day 17 of pregnancy in the 10 mg/kg group. F1 females at 30 mg/kg group showed delayed avoidance response in the learning test. DU-176 systemic exposure (C<sub>max</sub> and AUC<sub>0-24h</sub>) in F0 dams was generally dose-proportional with no accumulation. NOAELs in this study were 30 mg/kg/day for maternal general toxicity, 10 mg/kg/day for maternal reproduction and F1 development.

### Methods

As shown in Figure 17, F<sub>0</sub> female Crl:CD® (SD) rats were orally gavaged with DU-176b at 3, 10, 30 mg/kg/day, or vehicle 0.5% MC at 10 ml/kg/day during gestation Day 7 – lactation Day 20 (n=21-22/group for toxicity, n=4-5/group for TK). DU-176b doses used here were based on a preliminary pre- and post-natal rat study with DU-176b performed in the same laboratories (**Study AN08-H0065-R01**). In study AN08-H0065-R01, DU-176b administered during gestation Day 7 – lactation Day 20 at 100 or 300 mg/kg/day resulted in dam deaths or vaginal bleeding. In newborns, there were no significant differences between the control group and treated groups regarding the number of live and dead newborns, sex, external anomalies, clinical signs, body weight, motor function test, physical development, viability, and necropsy findings. NOAEL in study AN08-H0065-R01 was the next dose level, 10 mg/kg/day. Therefore, 30 mg/kg/day was chosen to be the high dose in the current study. The dosing formulations were confirmed to have been prepared accurately (101 - 102%) and uniformly (±0.33%).

F<sub>0</sub> females were allowed to litter and rear their offspring to weaning. These females were observed or examined for clinical signs, body weight, food consumption, gestation length/delivery, and parturition between mating day 0 and lactation day 21. On lactation day 21, all F<sub>0</sub> generation dams were sacrificed and macroscopically examined for gross lesions. The number of implantation sites was counted. The uteri of all animals were collected and preserved in 10 % neutral buffered formalin. All F<sub>1</sub> litters were examined at ~ 24 hours after birth and then daily thereafter; clinical conditions, litter size, survival, sex ratio, and body weight were assessed. On day 4 of age, 4 males and 4 females per dam in toxicity groups were selected. At day 21 of age, 1 male and 1 female per dam in toxicity groups were selected. The selected F<sub>1</sub> rats were continued on evaluations listed in Table 71. Culled F<sub>1</sub> rats at weaning were necropsied and macroscopically examined.

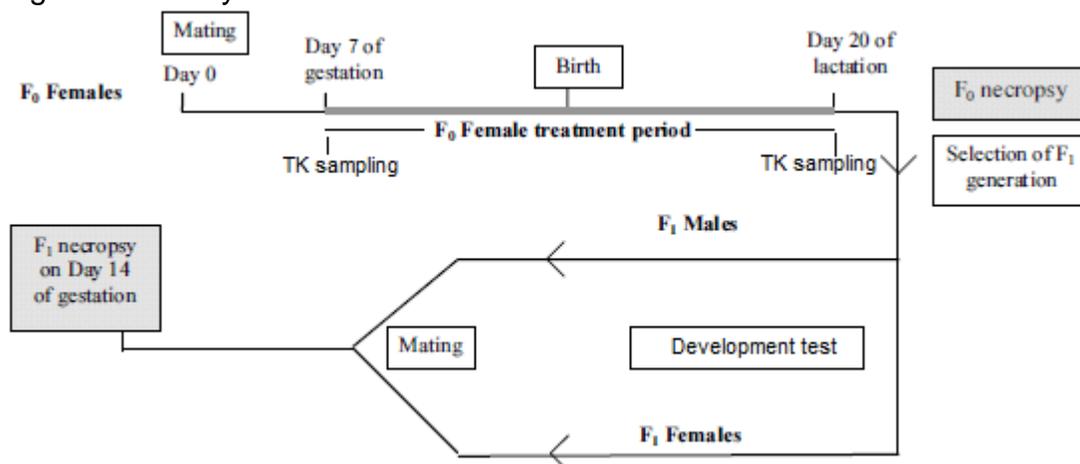
Table 71. Observations on selected F<sub>1</sub> generation

| Observation                           | Examination   | Time  |
|---------------------------------------|---|---|
| Clinical signs and viabilities        |   | From 4 days old to the day of necropsy  |
| Body weight                           | Offspring that were culled at weaning   | Days 0, 4, 7, 10, 14, 18 and 21 after birth   |
|                                       | Offspring that were used for fertility test (male)  | Days 0, 4, 7, 10, 14, 18, 21, 28, 35, 42, 49, 56, 63 and 70 after birth                                   |
|                                       | Offspring that were used for fertility test (female)  | Days 0, 4, 7, 10, 14, 18, 21, 28, 35, 42, 49, 56, 63 and 70 after birth and Days 0, 7 and 14 of pregnancy |
| Food consumption                      |   | Days 28, 35, 42, 49, 56, 63 and 70 after birth  |
| Motor Function test - Righting reflex |   | Day 7 after birth   |
| Physical development                  | Pinna unfolding   | Day 5 after birth   |
|                                       | Incisor eruption  | Day 14 after birth  |
|                                       | Eye opening   | Day 17 after birth  |
|                                       | Vaginal opening (female)  | Day 35 after birth  |
|                                       | Balanopreputial separation (male) on Day 49 after birth   |   |
| Sensory function test                 | Pupillary reflex  | Day 22 after birth  |
|                                       | Corneal reflex and Preyer's reflex on Day 35 after birth  |   |
| Motility test                         | Open field test (total moving time, latent time at the center; number of crossed sections, number of occurrences of rearing, defecation and urination) at 28 days old |   |
| Learning test                         | Shuttle box test (avoidance response) on Day 35 to 38 after birth   |   |

At 10-11 weeks of age, the selected F<sub>1</sub> rats from the same treatment groups were paired on a one-to-one basis for a period of up to 2 weeks. Sibling mating was avoided. Once mating occurred, the males and females were separated. The day on which evidence of mating was found was designated gestation Day 0. F<sub>1</sub> adult females were sacrificed on day 14 of mating. C-section and gross necropsy were then performed. The numbers of corpora lutea, implantations, and live and dead embryos were counted. When implantation sites were macroscopically absent in the uterus, an implantation site test utilizing a modified Salewski's method was performed to determine the presence or absence of implantations. Females that failed to mate were necropsied on day 14 after the last day of pairing. Selected F<sub>1</sub> males were killed and necropsied soon after the necropsy of females with copulation in the fertility test.

F<sub>0</sub> dams in TK groups were observed for clinical signs, body weight, and delivery. Blood samples were collected from jugular vein 1, 2, 4 and 24 h after the first dose, and prior to dosing and 1, 2, 4, 24 h after dosing on Day 20 after delivery (the final day of dosing). Plasma DU-176 concentrations were measured using liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS)

Figure 17. Study scheme



## Results

For F<sub>0</sub> females, one dam in the 3 mg/kg group was sacrificed on Day 18 of pregnancy following mistaken double dosing on Day 17 of pregnancy. Vaginal bleeding was noted in 1 dam each on Day 16 or Day 17 of pregnancy in the 30 mg/kg group. There were no DU-176b-related effects on body weight, food consumption, gestation length, parturition, gestation index, the number of implantation sites, the litter size, and gross pathology in F<sub>0</sub> females.

In the offspring, lower avoidance response rate compared to the control group was noted in the 30 mg/kg group F<sub>1</sub> females in 4 of the 5 trial sets on day 35 of age, but disappeared at 38 days old; this finding was not seen in male offsprings (Table 72). There were no DU-176b-related effects on the numbers of live and dead newborns, sex ratio, external anomalies, clinical signs, body weight, food consumption, motor function test, physical development, sensory function test, motility test, sexual maturation, mating performance, fertility, reproductive capacity, and macroscopic pathology of F<sub>1</sub> and F<sub>2</sub> generation offspring.

Table 72. Learning test: avoidance response rate (% , n=21/group)

| DU-176b<br>mg/kg/Day | Shuttle box test at 35 days old |      |       |       |        | Shuttle box test at 38 days old |        |       |       |       |       |       |
|----------------------|---------------------------------|------|-------|-------|--------|---------------------------------|--------|-------|-------|-------|-------|-------|
|                      |                                 | 1st  | 2nd   | 3rd   | 4th    | 5th                             | 1st    | 2nd   | 3rd   | 4th   | 5th   |       |
| Male                 | Control                         | Mean | 15.71 | 42.14 | 58.57  | 69.52                           | 74.05  | 67.62 | 79.52 | 84.29 | 86.43 | 89.29 |
|                      |                                 | S.D. | 17.84 | 34.04 | 30.50  | 27.15                           | 26.15  | 31.73 | 30.33 | 28.74 | 24.60 | 17.77 |
|                      | 3                               | Mean | 12.38 | 34.29 | 53.10  | 59.52                           | 65.48  | 61.43 | 75.24 | 85.48 | 86.90 | 85.95 |
|                      |                                 | S.D. | 13.84 | 28.52 | 34.55  | 34.17                           | 28.81  | 33.32 | 27.45 | 18.90 | 17.92 | 13.57 |
|                      | 10                              | Mean | 15.95 | 37.38 | 58.57  | 65.71                           | 70.24  | 64.29 | 77.86 | 83.57 | 86.67 | 82.38 |
|                      |                                 | S.D. | 17.79 | 27.28 | 33.17  | 30.34                           | 31.20  | 31.04 | 31.25 | 30.17 | 22.77 | 24.27 |
|                      | 30                              | Mean | 9.52  | 34.29 | 54.52  | 64.52                           | 70.71  | 60.48 | 76.19 | 79.05 | 82.14 | 76.67 |
|                      |                                 | S.D. | 8.20  | 22.43 | 31.97  | 33.65                           | 33.14  | 35.25 | 33.98 | 30.64 | 29.65 | 31.79 |
| Female               | Control                         | Mean | 12.86 | 36.43 | 66.67  | 80.00                           | 81.43  | 74.05 | 84.05 | 90.48 | 88.57 | 90.95 |
|                      |                                 | S.D. | 10.56 | 29.37 | 26.38  | 19.81                           | 20.62  | 30.52 | 28.13 | 21.44 | 21.86 | 14.63 |
|                      | 3                               | Mean | 19.52 | 45.71 | 62.86  | 71.67                           | 69.52  | 74.76 | 85.24 | 90.24 | 91.19 | 89.29 |
|                      |                                 | S.D. | 17.46 | 34.43 | 36.21  | 35.37                           | 34.67  | 32.46 | 27.36 | 16.62 | 15.48 | 20.57 |
|                      | 10                              | Mean | 21.19 | 45.24 | 62.14  | 75.24                           | 75.71  | 70.00 | 84.76 | 89.76 | 90.95 | 91.43 |
|                      |                                 | S.D. | 18.57 | 34.77 | 33.11  | 27.45                           | 27.45  | 30.86 | 22.94 | 19.27 | 16.02 | 15.50 |
|                      | 30                              | Mean | 5.48* | 19.05 | 38.81* | 47.14**                         | 55.24* | 57.38 | 65.48 | 74.52 | 78.57 | 80.95 |
|                      |                                 | S.D. | 7.05  | 21.37 | 35.56  | 35.02                           | 35.30  | 29.90 | 34.82 | 33.83 | 26.37 | 20.65 |

Significant difference from 01 group: \* :p <0.05 \*\* :p <0.01

Following oral gavage with DU-176b, plasma DU-176 concentrations in F0 dams increased in a dose-related manner. DU-176 systemic exposure (C<sub>max</sub> and AUC<sub>0-24h</sub>) in F0 dams was generally dose-proportional. There were no marked differences in any TK parameters after repeated administration, indicating no accumulation (Table 73).

Table 73. Summary of TK data in F0 dams (from the submission)

| Day of administration | Dose (mg/kg) | Plasma concentration (ng/mL) at: |       |      |      |      |       | t <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |
|-----------------------|--------------|----------------------------------|-------|------|------|------|-------|----------------------|--------------------------|--------------------------------|
|                       |              | Pre                              | 1 h   | 2 h  | 4 h  | 24 h |       |                      |                          |                                |
| First day             | 3            | Mean (n=4)                       | -     | 181  | 77.1 | 67.2 | 0.825 | 1.0                  | 181                      | 1040                           |
|                       |              | SD (n=4)                         | -     | 30.5 | 20.1 | 16.5 | 0.960 | 0.000                | 30.5                     | 231                            |
|                       | 10           | Mean (n=5)                       | -     | 739  | 331  | 129  | 4.69  | 1.0                  | 739                      | 2700                           |
|                       |              | SD (n=5)                         | -     | 190  | 129  | 52.7 | 3.70  | 0.000                | 190                      | 920                            |
|                       | 30           | Mean (n=4)                       | -     | 2490 | 1250 | 275  | 15.5  | 1.0                  | 2490                     | 7560                           |
|                       |              | SD (n=4)                         | -     | 1140 | 912  | 155  | 6.02  | 0.000                | 1140                     | 4180                           |
| Final day             | 3            | Mean (n=4)                       | 0.360 | 215  | 82.6 | 44.1 | 0.363 | 1.0                  | 215                      | 827                            |
|                       |              | SD (n=4)                         | 0.720 | 55.3 | 19.8 | 19.5 | 0.725 | 0.000                | 55.3                     | 293                            |
|                       | 10           | Mean (n=5)                       | 0.984 | 817  | 257  | 76.8 | 1.72  | 1.0                  | 817                      | 2060                           |
|                       |              | SD (n=5)                         | 1.53  | 272  | 84.1 | 35.1 | 1.39  | 0.000                | 272                      | 756                            |
|                       | 30           | Mean (n=4)                       | 3.49  | 2090 | 751  | 235  | 7.34  | 1.0                  | 2090                     | 5870                           |
|                       |              | SD (n=4)                         | 1.31  | 690  | 251  | 80.7 | 3.89  | 0.000                | 690                      | 1740                           |

Based on the above results, the no observed adverse-effect levels (NOAELs) were 10 mg/kg/day for general toxicity in dams, and 10 mg/kg/day for reproduction in dams and development of the next generation.

### 9.3 Repeated Dose Toxicity Study in Juvenile Rats Treated Orally with DU-176b for 7 Weeks (Study AN11-C0003-R01)

This GLP study (AN11-C0003-R01) in CrI:CD(SD) juvenile rats was conducted with DU-176b (Lot # MH409-U, purity 100.6%) (b) (4) during June 2011 – Jan, 2012 to investigate the toxic effects of DU-176b on organ development after the completion of dosing period and when animals reached maturity. This study was previously reviewed by Dr. Patricia P Harlow (06/03/2013) under IND 077254 (Appendix III).

CrI:CD(SD) rats (16 rats/sex/group in main study, 48/ rats/sex/group in satellite study) were orally gavaged with vehicle 0.5% MC, or DU-176b at doses 2, 6, or 20 mg/kg/day for 7 weeks from postnatal day (PND) 4 to PND 49. Doses selected in the current study were based on a previous dose-ranging study in juvenile rats (**Study AN10-C0079-R01**) with oral DU-176b doses at 0, 2, 6, or 20 mg/kg/day for 3 weeks from PND 4 to PND 21. No test article-related toxic changes in clinical signs, body weight, or necropsy observations were noted in the dose-ranging study (Study AN10-C0079-R01). The exposure levels on PND 4 to PND 14 were reported to be sufficiently high when compared with adult rats (**Study R 20020612**) or healthy adult men who were treated at 60 mg of DU-176b (**DU176-E-PRT001**). Therefore, the high dose level in the current study was set at 20 mg/kg/day, and the middle and low dose levels were set at 6 and 2 mg/kg/day, respectively, in a common ratio of approximately 3.

Animals were observed or examined for mortality, clinical signs, body weight, food consumption, functional and physical development, morphological differentiation of external genitalia, hematology, and plasma chemistry at specific time points during the study. Rats in main study were sacrificed on PND 50 (10 rats/sex/group) and PND 91 (6 rats/sex/group) for necropsy (macroscopic examination and measurement of bone length), organ weight, and histological examination. Rats of the satellite groups were subjected to blood sampling on PND 4 at 1, 2, 4, and 24 hours post-dose, and on PNDs 14, 21, and 49 at pre-dose, 1, 2, 4, and 24 hours post-dose (3 rats/sex/group/sampling point). Plasma concentrations of DU-176 were determined using LC/MS/MS.

No animal died during the study period. There were no test article-related changes in clinical signs, functional and physical development, morphological differentiation of external genitalia, hematology, blood chemistry, necropsy findings, bone length, organ weight, or histopathology (particularly in the eyes and the skin). However, extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted, and the fertility, mating ability and reproductive performance were not specifically evaluated.

Body weights at the dose 20 mg/kg/day were lower or trended to be lower when compared with controls, and were statistically significant in males at PND 17 and in females during PND 10 -28 (Table 74). The lower body weight in females during PND 10-28 was associated with significantly lower food consumption on PND 21 - 22 when compared with the control group ( $7.6 \pm 1.0$ ,  $7.1 \pm 1.1$ ,  $6.0 \pm 1.3^{**}$ ,  $6.1 \pm 1.1^{**}$  g/day, respectively for control, 2, 6, and 20 mg/kg/day,  $^{**}p < 0.01$  vs control). Although the

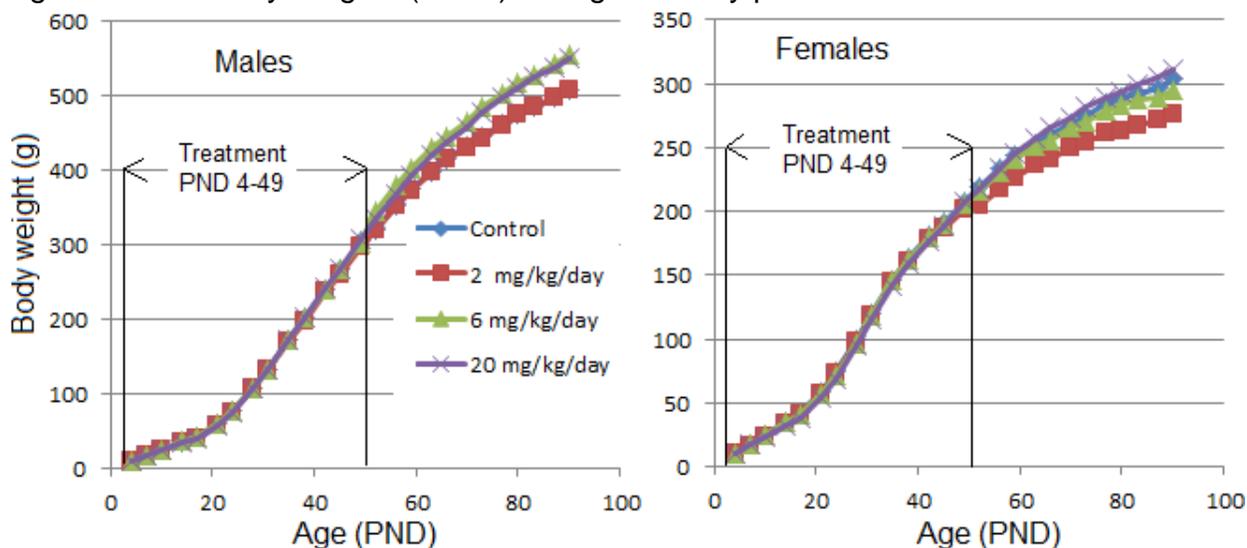
differences in body weight between control and treated rats were moderate (<9%) and transient (Figure 18), it may be drug-related and cannot be ignored.

Table 74. Findings in body weight (g) following oral DU-176b doses

| Sex     | PND | Control |      | 2 mg/kg/day |      | 6 mg/kg/day |      | 20 mg/kg/day |      |
|---------|-----|---------|------|-------------|------|-------------|------|--------------|------|
|         |     | Mean    | SD   | Mean        | SD   | Mean        | SD   | Mean         | SD   |
| Males   | 4   | 11.00   | 0.92 | 10.84       | 1.11 | 11.35       | 1.22 | 11.44        | 1.18 |
|         | 7   | 17.62   | 1.47 | 17.04       | 1.93 | 18.37       | 1.94 | 17.91        | 1.68 |
|         | 10  | 25.22   | 2.14 | 24.03       | 2.51 | 26.04       | 2.56 | 24.28        | 2.26 |
|         | 14  | 35.76   | 3.26 | 34.16       | 2.74 | 36.63       | 2.65 | 34.06        | 3.27 |
|         | 17  | 43.12   | 3.86 | 41.52       | 2.78 | 43.14       | 2.66 | 40.26*       | 3.80 |
|         | 21  | 59.53   | 4.80 | 58.68       | 3.50 | 61.18       | 3.47 | 58.95        | 5.11 |
|         | 24  | 77.19   | 6.22 | 76.50       | 4.19 | 77.83       | 4.19 | 76.21        | 6.18 |
|         | 28  | 107.09  | 8.02 | 106.62      | 5.63 | 106.98      | 6.46 | 107.08       | 9.58 |
| Females | 4   | 11.08   | 0.75 | 10.66       | 0.84 | 10.33       | 1.01 | 10.81        | 0.88 |
|         | 7   | 17.58   | 1.44 | 16.76       | 1.32 | 17.26       | 1.68 | 17.01        | 1.39 |
|         | 10  | 25.04   | 2.52 | 24.05       | 1.72 | 24.63       | 2.07 | 23.21*       | 1.84 |
|         | 14  | 35.13   | 3.90 | 34.14       | 2.06 | 34.64       | 2.12 | 32.35*       | 2.84 |
|         | 17  | 42.33   | 4.46 | 40.90       | 2.68 | 41.36       | 2.58 | 38.24**      | 3.25 |
|         | 21  | 58.63   | 4.39 | 57.41       | 3.32 | 57.44       | 3.53 | 54.84*       | 4.32 |
|         | 24  | 74.63   | 4.73 | 72.43       | 4.26 | 71.66       | 3.79 | 69.05**      | 5.51 |
|         | 28  | 99.37   | 5.46 | 97.94       | 5.83 | 96.82       | 5.11 | 94.33*       | 6.27 |

\* p< 0.05 vs control; \*\* p<0.01 vs control. n=16/sex/group.

Figure 18. Rat body weights (mean) during the study period



Following oral doses, DU-176 C<sub>max</sub> and AUC<sub>0-24h</sub> values increased dose-proportionally with the dose ranging from 2 to 20 mg/kg/day, and were much lower in PND 21 and 49

than in PND 4 and 14. This may imply increased metabolite rate with maturation. There were no apparent sex differences in the toxicokinetic parameters (Table 75).

Table 75. DU-176 TK parameters in juvenile rats (from the submission)

| Days after birth | Dose (mg/kg/day) | Sex        | Plasma concentration (ng/mL) |            |      |       |       | T <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |       |      |
|------------------|------------------|------------|------------------------------|------------|------|-------|-------|----------------------|--------------------------|--------------------------------|-------|------|
|                  |                  |            | Pre                          | 1 h        | 2 h  | 4 h   | 24 h  |                      |                          |                                |       |      |
| 4                | 2                | Male       | Mean (n=3)                   | -          | 257  | 531   | 472   | 70.1                 | 2.0                      | 531                            | 6950  |      |
|                  |                  | SD (n=3)   | -                            | 34.1       | 173  | 130   | 25.9  | -                    | -                        | -                              |       |      |
|                  | Female           | Mean (n=3) | -                            | 378        | 404  | 378   | 52.4  | 2.0                  | 404                      | 5670                           |       |      |
|                  |                  | SD (n=3)   | -                            | 115        | 86.5 | 68.1  | 7.87  | -                    | -                        | -                              |       |      |
|                  | 6                | Male       | Mean (n=3)                   | -          | 1500 | 1150  | 973   | 123                  | 1.0                      | 1500                           | 15200 |      |
|                  |                  | SD (n=3)   | -                            | 80.0       | 140  | 99.6  | 16.9  | -                    | -                        | -                              |       |      |
| Female           | Mean (n=3)       | -          | 1290                         | 999        | 1040 | 141   | 1.0   | 1290                 | 15600                    |                                |       |      |
|                  | SD (n=3)         | -          | 52.9                         | 61.5       | 68.1 | 21.5  | -     | -                    | -                        |                                |       |      |
| 20               | Male             | Mean (n=3) | -                            | 4840       | 4350 | 4270  | 433   | 1.0                  | 4840                     | 62700                          |       |      |
|                  |                  | SD (n=3)   | -                            | 474        | 390  | 498   | 113   | -                    | -                        | -                              |       |      |
|                  | Female           | Mean (n=3) | -                            | 5380       | 4960 | 4270  | 375   | 1.0                  | 5380                     | 63500                          |       |      |
|                  |                  | SD (n=3)   | -                            | 401        | 311  | 161   | 86.9  | -                    | -                        | -                              |       |      |
|                  | 14               | 2          | Male                         | Mean (n=3) | 9.83 | 457   | 524   | 413                  | 15.5                     | 2.0                            | 524   | 5950 |
|                  |                  |            | SD (n=3)                     | 3.84       | 26.0 | 78.8  | 227   | 4.22                 | -                        | -                              | -     |      |
| Female           |                  | Mean (n=3) | 11.9                         | 422        | 417  | 271   | 7.94  | 1.0                  | 422                      | 4110                           |       |      |
|                  |                  | SD (n=3)   | 4.87                         | 28.5       | 36.5 | 89.6  | 0.836 | -                    | -                        | -                              |       |      |
| 6                |                  | Male       | Mean (n=3)                   | 35.9       | 1650 | 1240  | 852   | 23.7                 | 1.0                      | 1650                           | 13100 |      |
|                  |                  | SD (n=3)   | 11.1                         | 130        | 85.0 | 219   | 3.56  | -                    | -                        | -                              |       |      |
| Female           | Mean (n=3)       | 26.6       | 1650                         | 1170       | 847  | 27.2  | 1.0   | 1650                 | 13000                    |                                |       |      |
|                  | SD (n=3)         | 3.68       | 146                          | 17.3       | 144  | 4.76  | -     | -                    | -                        |                                |       |      |
| 20               | Male             | Mean (n=3) | 99.7                         | 4820       | 4710 | 2680  | 88.5  | 1.0                  | 4820                     | 42300                          |       |      |
|                  | SD (n=3)         | 28.3       | 644                          | 90.7       | 15.3 | 22.6  | -     | -                    | -                        |                                |       |      |
| Female           | Mean (n=3)       | 121        | 4830                         | 4160       | 2580 | 108   | 1.0   | 4830                 | 40600                    |                                |       |      |
|                  | SD (n=3)         | 28.0       | 537                          | 136        | 451  | 3.61  | -     | -                    | -                        |                                |       |      |
| 21               | 2                | Male       | Mean (n=3)                   | 0.000      | 107  | 44.0  | 36.0  | 0.000                | 1.0                      | 107                            | 569   |      |
|                  |                  | SD (n=3)   | 0.000                        | 60.6       | 9.46 | 15.0  | 0.000 | -                    | -                        | -                              |       |      |
|                  | Female           | Mean (n=3) | 0.000                        | 90.1       | 59.1 | 39.6  | 0.000 | 1.0                  | 90.1                     | 614                            |       |      |
|                  |                  | SD (n=3)   | 0.000                        | 22.0       | 13.8 | 6.51  | 0.000 | -                    | -                        | -                              |       |      |
|                  | 6                | Male       | Mean (n=3)                   | 1.74       | 261  | 153   | 194   | 0.337                | 1.0                      | 261                            | 2630  |      |
|                  |                  | SD (n=3)   | 1.72                         | 66.2       | 47.1 | 48.5  | 0.583 | -                    | -                        | -                              |       |      |
| Female           | Mean (n=3)       | 1.70       | 252                          | 196        | 191  | 0.853 | 1.0   | 252                  | 2660                     |                                |       |      |
|                  | SD (n=3)         | 0.432      | 27.0                         | 30.5       | 29.5 | 0.754 | -     | -                    | -                        |                                |       |      |
| 20               | Male             | Mean (n=3) | 3.87                         | 1060       | 798  | 567   | 1.00  | 1.0                  | 1060                     | 8510                           |       |      |
|                  | SD (n=3)         | 2.38       | 349                          | 211        | 256  | 1.74  | -     | -                    | -                        |                                |       |      |
| Female           | Mean (n=3)       | 8.16       | 1660                         | 891        | 489  | 5.32  | 1.0   | 1660                 | 8430                     |                                |       |      |
|                  | SD (n=3)         | 5.76       | 274                          | 174        | 71.5 | 4.27  | -     | -                    | -                        |                                |       |      |
| 49               | 2                | Male       | Mean (n=3)                   | 0.000      | 51.6 | 30.5  | 25.0  | 0.000                | 1.0                      | 51.6                           | 372   |      |
|                  |                  | SD (n=3)   | 0.000                        | 16.6       | 13.4 | 9.19  | 0.000 | -                    | -                        | -                              |       |      |
|                  | Female           | Mean (n=3) | 0.000                        | 50.9       | 40.9 | 15.5  | 0.000 | 1.0                  | 50.9                     | 283                            |       |      |
|                  |                  | SD (n=3)   | 0.000                        | 8.92       | 3.27 | 5.59  | 0.000 | -                    | -                        | -                              |       |      |
|                  | 6                | Male       | Mean (n=3)                   | 1.81       | 193  | 121   | 60.7  | 1.65                 | 1.0                      | 193                            | 1060  |      |
|                  |                  | SD (n=3)   | 0.278                        | 58.3       | 34.1 | 10.6  | 2.86  | -                    | -                        | -                              |       |      |
| Female           | Mean (n=3)       | 1.12       | 341                          | 153        | 59.9 | 0.917 | 1.0   | 341                  | 1240                     |                                |       |      |
|                  | SD (n=3)         | 1.02       | 105                          | 48.6       | 14.3 | 1.59  | -     | -                    | -                        |                                |       |      |
| 20               | Male             | Mean (n=3) | 4.00                         | 1230       | 297  | 176   | 5.39  | 1.0                  | 1230                     | 3670                           |       |      |
|                  | SD (n=3)         | 1.90       | 90.7                         | 121        | 82.7 | 2.56  | -     | -                    | -                        |                                |       |      |
| Female           | Mean (n=3)       | 2.51       | 1520                         | 660        | 254  | 4.17  | 1.0   | 1520                 | 5350                     |                                |       |      |
|                  | SD (n=3)         | 1.10       | 310                          | 372        | 16.3 | 0.568 | -     | -                    | -                        |                                |       |      |

-: Not calculated

Therefore, NOAEL of DU-176b in male and female juvenile rats was 6 mg/kg/day, based on lower body weight in females and males at 20 mg/kg/day during PND 10-28.

## 10 Special Toxicology Studies

### 10.1 DU-176b: Photochromosomal aberration test in Chinese hamster lung cells (Study R20060731)

This GLP study (R20060731), an in vitro chromosomal aberration test with DU-176b (Lot # BB202, purity 99.6%), was conducted in (b) (4), during Aug –Dec, 2006. This study was previously reviewed by Dr. Patricia P Harlow (06/22/2007) under IND 077254 (Appendix II).

The photo-cytogenetic effect of DU-176b was evaluated using Chinese hamster lung (CHL/IU) cells at DU-176b concentrations of 156-5000 µg/mL with or without photo-irradiation. Based on the cell growth and the mitotic index in the cytogenetic test, chromosome analysis was performed with DU-176b at 313, 625, 1250 and 2500 µg/mL in the non-irradiated group and at 625, 1250 and 2500 µg/mL in the irradiated group. DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation (Table 76).

Table 76. Summary of photochromosomal aberration test for DU-176b (modified from the submission)

| Photo-irradiation | Treatment Time (min-min-hr) <sup>a</sup> | Test compound | Concentration (µg/mL) <sup>b</sup> | Relative Cell Growth (%) <sup>c</sup> | Mitotic Index (%) <sup>d</sup> | Structural Aberrant Cells (%) <sup>e</sup> | Numerical Aberrant Cells (%) <sup>f</sup> |
|-------------------|--|---------------|------------------------------------|---------------------------------------|--------------------------------|--|---|
| Without           | 110-0-(22)                               | Non-treatment | —                                  | NA                                    | NA                             | 0.5  | 0.9                                       |
|                   |  | DMSO (2 vol%) | 0                                  | 100                                   | NA                             | 0.5  | 1.1                                       |
|                   |  | DU-176b       | 313 <sup>g</sup>                   | 90                                    | NA                             | 2.0  | 1.1                                       |
|                   |  |               | 625 <sup>g</sup>                   | 68                                    | 8.6, 10.2                      | 0.0  | 0.4                                       |
|                   |  |               | 1250 <sup>g</sup>                  | 32                                    | 8.8, 1.8                       | 0.0 <sup>h</sup>                           | 0.0 <sup>i</sup>                          |
|                   |  |               | 2500 <sup>g</sup>                  | 24                                    | 7.4, 6.6                       | 1.5  | 1.6                                       |
|                   |  |               | 5000 <sup>g</sup>                  | 9                                     | NA                             | NA   | NA  |
|                   |  | MNNG          | 1                                  | NA                                    | NA                             | 50.5 <sup>*1</sup>                         | 0.1                                       |
| With              | 60-50-(22)                               | Non-treatment | —                                  | NA                                    | NA                             | 2.5  | 0.8                                       |
|                   |  | DMSO (2 vol%) | 0                                  | 100                                   | NA                             | 1.5  | 1.3                                       |
|                   |  | DU-176b       | 625 <sup>g</sup>                   | 85                                    | NA                             | 0.0  | 0.9                                       |
|                   |  |               | 1250 <sup>g</sup>                  | 69                                    | NA                             | 1.0  | 1.6                                       |
|                   |  |               | 2500 <sup>g</sup>                  | 40                                    | 5.8, 4.6                       | 1.5  | 1.9                                       |
|                   |  |               | 5000 <sup>g</sup>                  | 30                                    | 0.8, 3.6                       | NA   | NA  |
|                   |  | 8-MOP         | 0.01                               | NA                                    | NA                             | 72.0 <sup>*1</sup>                         | 0.4                                       |

DMSO: Dimethyl sulfoxide, MNNG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine, 8-MOP, Methoxsalen, NA: Not analyzed

\*1: Two-tailed Fisher's exact probability test with Bonferroni adjustment ( $P < 0.05$ ).

<sup>a</sup>: Treatment time was expressed as treatment time (min) without photo-irradiation, treatment time (min) with photo-irradiation, and recovery time (hr).

<sup>b</sup>: Concentrations of DU-176b are expressed as the amount of anhydrous free base.

<sup>c</sup>: Based on cell counting with a Coulter Counter.

<sup>d</sup>: The 500 cells per slide were analyzed.

<sup>e</sup>: Excluding gaps. 100 metaphases per culture (200 metaphases per concentration) were analyzed.

<sup>f</sup>: The 400 metaphases/culture (800 metaphases/concentration) were analyzed.

<sup>g</sup>: Precipitation was observed at the beginning and the end of the treatment.

<sup>h</sup>: Only one culture was analyzed (100 metaphases per concentration).

<sup>i</sup>: Only one culture was analyzed (400 metaphases per concentration).

Historical negative control data (n=23/each) of the photochromosomal aberration test using CHL/IU cells in the test facility

| Light irradiation | Incidence (%) of aberrant cells |         |         |
|-------------------|---------------------------------|---------|---------|
|                   | Average                         | Maximum | Minimum |

Structural aberrations

|         |     |     |     |
|---------|-----|-----|-----|
| Without | 1.1 | 3.5 | 0.0 |
| with    | 5.0 | 14  | 0.5 |

Polyploid cells

|         |      |      |      |
|---------|------|------|------|
| Without | 0.22 | 0.63 | 0.00 |
| with    | 0.63 | 1.38 | 0.13 |

## 10.2 DU-176b: Phototoxicity test in BALB/3T3 cells (Study R20060746)

This GLP study (R20060746) was conducted with DU-176b (Lot # BB202, purity 99.6%) in [REDACTED] <sup>(b) (4)</sup> during Aug, 2006 –Jan, 2007. This study was previously reviewed by Dr. Patricia P Harlow (06/22/2007) under IND 077254 (Appendix II).

Using BALB/3T3 cells, DU-176b was evaluated in a neutral red uptake (NRU) phototoxicity test in comparison to dimethyl sulfoxide as a vehicle control and chlorpromazine hydrochloride (CPZ) as a positive control. The cells were pretreated with DU-176b at concentrations 0.0078 to 1.0 mg/mL or control article for 60 minutes, then irradiated with UVA and UVB light for 50 min, while a parallel set of cells were kept in dark for 50 min (not irradiated). Cell survival measured with the NRU assay on the following day revealed that DU-176b at 1.0 mg/ml only caused slight cytotoxic effects (relative survival 64.9 and 78.0% for irradiation and non-irradiation conditions, respectively,  $IC_{50s} > 1$  mg/ml), with confirmation of the assay sensitivity. Thus, DU-176b at concentrations up to 1 mg/ml was non-phototoxic under the present test conditions.

## 10.3 Effects on Eye Function in Monkeys Treated Orally with DU-176b for 9 Months (Study AN07-H0045-R01)

This GLP study (AN07-H0045-R01) was conducted in Daiichi Sankyo Co., Ltd, Shizuoka, Japan, initiated in October 2007, to investigate the potential toxic effects of DU-176b on the eye function following repeated dosing in monkeys for 9 months. This study was to address the concern of DU-176b on eye function, because of prolonged DU-176 retention in melanic tissues, especially the eye of monkeys and pigmented rats. This study was previously reviewed by Dr. Patricia P Harlow (09/02/2008) of DCRP, and consulted with Dr. Zhou Chen (9/18/2008) of the Division of Anti-Infective and Ophthalmology Products under IND 077254.

Male and female cynomolgus monkeys (2 to 5 years old) were orally gavaged with vehicle 0.5% MC or DU-176b (Lot # KD302, purity 100.5%) 15 mg/kg/day once daily for 39 weeks (n = 4/sex/group). The dose selected in the current study was based on a previous 52-week repeated dose toxicity study in monkeys with oral DU-176b (at doses 0, 5, 15 and 45 mg/kg/day) (**Study AN07-C0008-R01**) and human exposure levels from clinical trials. In Study AN07-C0008-R01, deaths occurred at  $\geq 15$  mg/kg/day. Hematological and hemorrhagic changes were noted in the surviving animals at  $\geq 15$  mg/kg/day. NOAEL was 5 mg/kg for both genders. AUC values at 15 mg/kg were 8469 ng·h/mL for males and 9345 ng·h/mL for females. In clinical trials, the AUC value in healthy volunteers receiving 120 mg of DU-176b was 3682 ng·h/mL. The exposure levels in monkeys dosed at 15 mg/kg/day were expected to be significantly higher than those in human subjects given DU-176b at the maximum recommended clinical dose of 60 mg.

During the study period, eye function was evaluated by electroretinography (ERG) at predose on Day -1, and on Days 85, 176, and 267 of dosing and measurement of intra-ocular pressure prior to dosing and after 3, 6 and 9 months of dosing. Other observations/examinations included mortality, clinical signs, body weights, food

consumption, ophthalmology (at pre-dose, 3, 6 and 9 months), and gross pathology of the eye were also performed. Plasma DU-176 concentrations were determined on day 1 at 1, 2, 4, and 24 h post-dose, and on Days 86, 177, and 268 at pre-dose, 1, 2, 4, and 24 h post-dose using an LC-MS/MS method.

There were no deaths during the study. Blood on the surface of feces was observed in one male treated with DU-176b on Days 121 to 123. Pale mucous membrane in the oral cavity associated with menses was observed in one female given 15 mg/kg/day of DU-176b from Days 243 to 252. Body weights in the DU-176b-treated groups trended to be lower than controls; body weight gain of treated males was significantly lower than control males (Figure 19). Food consumption was slightly lower in treated males than controls during first 7 months which was consistent with lower body weight gain, but was higher in treated females than controls being related to more body weight gain and not of toxicological significance (Figure 20).

There were not DU-176b-related findings in electroretinography, intraocular pressure, ophthalmologic evaluation, and macroscopic evaluation of the eye.

Figure 19. Mean body weight and body weight gain during the study

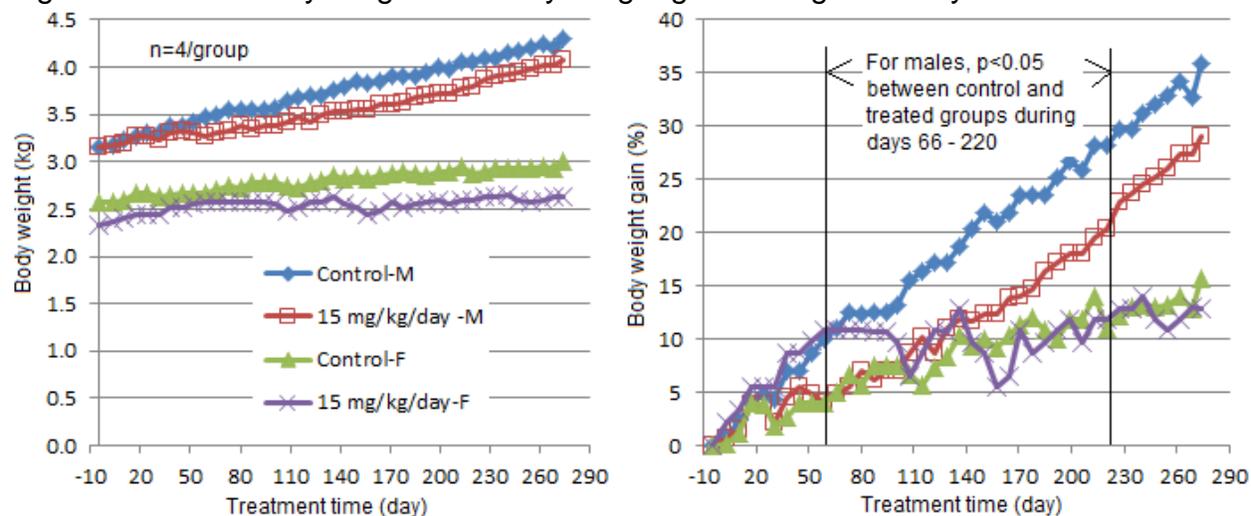
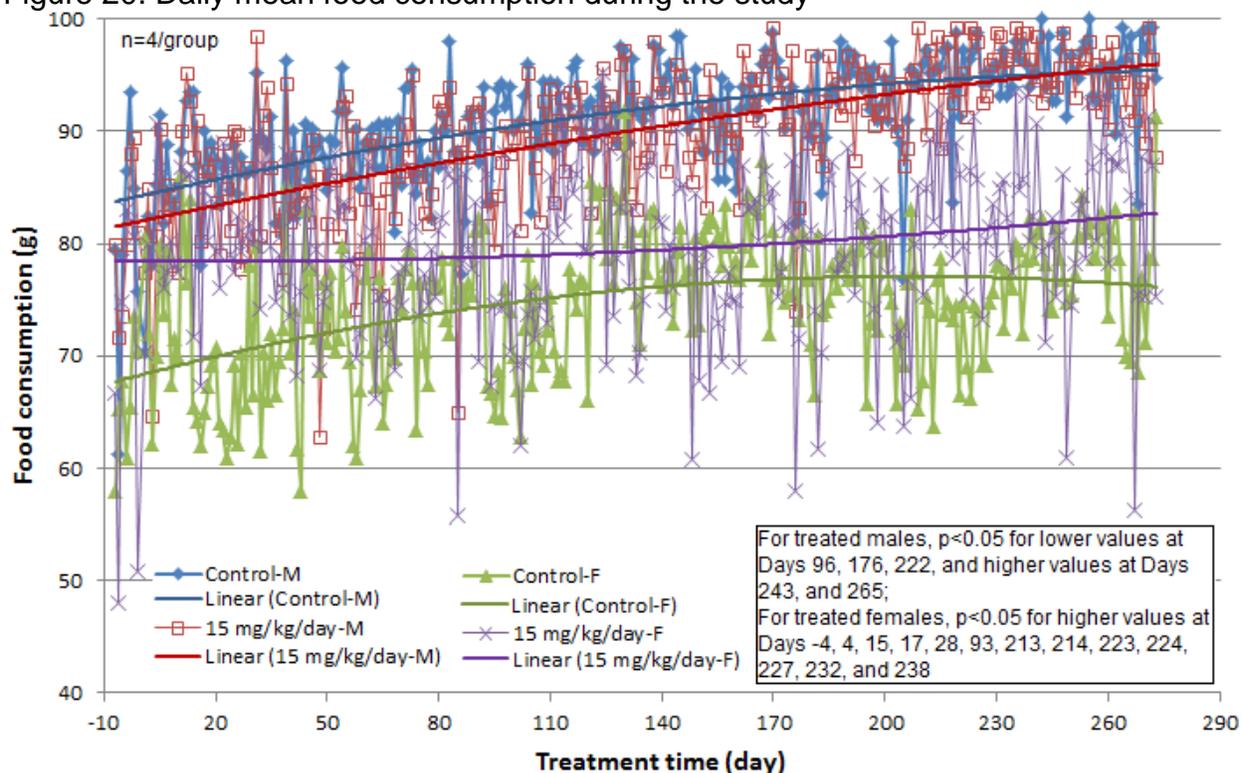


Figure 20. Daily mean food consumption during the study



DU-176 system exposures ( $C_{max}$  and  $AUC_{0-24h}$ ) following repeated oral DU-176b doses were generally similar between males and females.  $C_{max}$  and  $AUC_{0-24h}$  values of DU-176 on Day 268 were 706 ng/mL and 8430 ng·h/mL in males, respectively, and 827 ng/mL and 8550 ng·h/mL in females, respectively. There was no accumulation during the dosing period (Figure 21 and Table 77).

Figure 21. Mean plasma DU-176 concentrations in the 9-month study (n=4)

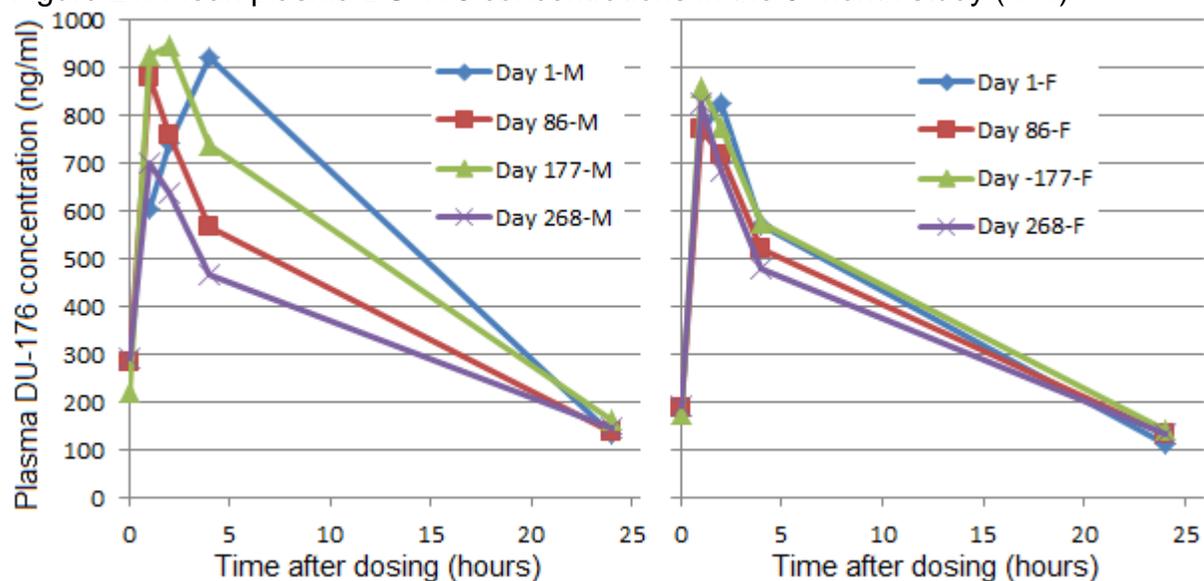


Table 77. Summary of DU-176 TK in monkeys dosed with oral DU-176b 15 mg/kg/day for 9 months (n=4)

| Day | Sex    | Tmax (hours) |     | Cmax (ng/ml) |     | AUC <sub>0-24</sub> (ng.h/ml) |      |
|-----|--------|--------------|-----|--------------|-----|-------------------------------|------|
|     |        | Mean         | SD  | Mean         | SD  | Mean                          | SD   |
| 1   | male   | 3.3          | 1.5 | 995          | 233 | 13200                         | 4470 |
|     | female | 1.5          | 0.6 | 905          | 153 | 9430                          | 3640 |
| 86  | male   | 1.3          | 0.5 | 884          | 165 | 9810                          | 1660 |
|     | female | 2.0          | 1.4 | 815          | 183 | 9040                          | 2820 |
| 177 | male   | 1.3          | 0.5 | 958          | 155 | 12200                         | 3090 |
|     | female | 1.3          | 0.5 | 940          | 86  | 9830                          | 1460 |
| 268 | male   | 1.3          | 0.5 | 706          | 257 | 8430                          | 4180 |
|     | female | 1.0          | 0.0 | 827          | 346 | 8550                          | 2930 |

In conclusion, there was no evidence of adverse effects on eye function or morphology of the eye in cynomolgus monkeys following a 39-week repeated dosing of DU-176b at 15 mg/kg/day. The NOAEL for effects on eye function was 15 mg/kg/day.

#### 10.4 Determination of DU-176 and D21-3231 for toxicokinetic analysis in rats treated orally with DU-176b for 14 days (AN08-C0007-R01)

This GLP study (AN08-C0007-R01) was conducted (b) (4) during May-Sept, 2008, to investigate the plasma concentration profiles of DU-176 and its metabolite D21-3231 and their TK parameters, when DU-176b was repeatedly administered orally to rats for 14 days.

CrI:CD(SD) rats (6 weeks old, 5/sex) were orally gavaged with DU-176b (Lot #KD302, purity 100.5%, suspended in 0.5% MC) at a dose level of 54 mg/kg/day for 14 days. The dose level 54 mg/kg/day in the current study is the NOAEL in a 26-week repeated oral dose toxicity study with DU176b in rats (**Study R20050334**). Rats were checked for clinical signs and body weight during the dosing period. Blood samples were collected on Day 1 at 1, 2, 4, and 24 hours post-dose and on Day 14 at pre-dose and 1, 2, 4, and 24 hours post-dose for analyzing plasma concentrations of DU-176 and its metabolite D21-3231 (using a LC/MS/MS method). At the end of blood sampling, rats were checked for gross pathology.

One female died on day 14 after the 2-hour blood sampling due to hemorrhage secondary to blood sampling-resulted injury (blood clots in the subcutaneous tissues and thoracic cavity). There were no other DU-176b-related findings.

Plasma concentrations of DU-176 and D21-3231 following repeated DU-176b doses are shown in Figure 22, and TK parameters are summarized in Table 78. On both Days 1 and 14 of dosing, Cmax and AUC<sub>0-24h</sub> of DU-176 in females were higher than those in males, and no sex differences were noted for D21-3231. There were no marked differences in Cmax or AUC<sub>0-24h</sub> after repeated dosing, indicating no accumulation. Exposures to D21-3231 were lower than those to DU-176.

Figure 22. Plasma concentrations of DU-176 and D21-3231 in rats (n=4-5/point)

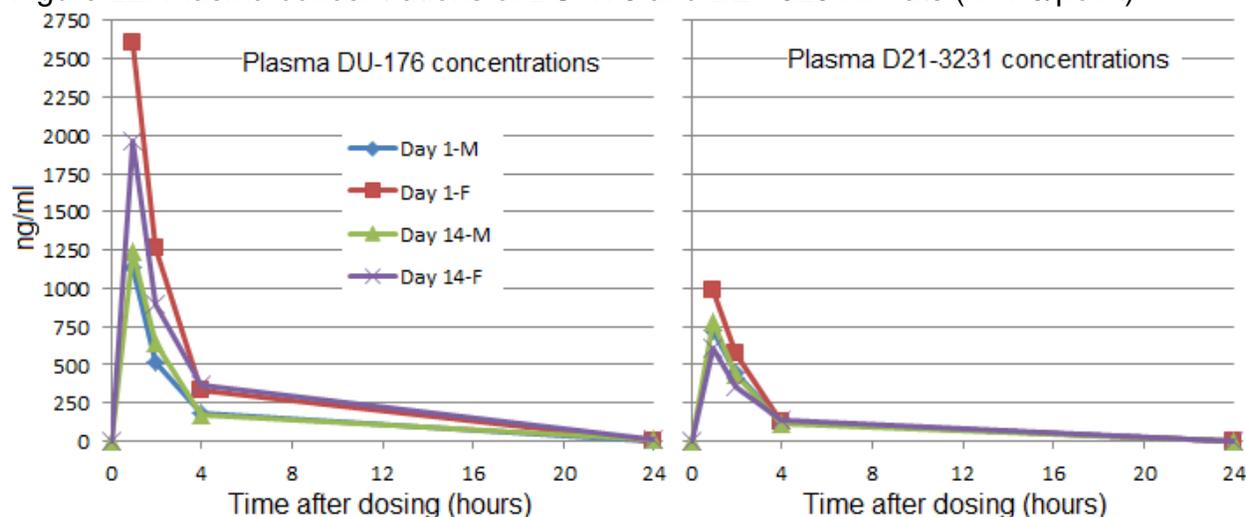


Table 78. Summary of TK parameters in the 14-day rat study (from the submission)

| Day of dosing | Sex    | DU-176        |                   |                                | D21-3231      |                   |                                |       |
|---------------|--------|---------------|-------------------|--------------------------------|---------------|-------------------|--------------------------------|-------|
|               |        | $t_{max}$ (h) | $C_{max}$ (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) | $t_{max}$ (h) | $C_{max}$ (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |       |
| 1             | Male   | Mean (n=5)    | 1.0               | 1140                           | 4020          | 1.0               | 723                            | 2870  |
|               |        | SD (n=5)      | 0.000             | 257                            | 1360          | 0.000             | 232                            | 1190  |
|               | Female | Mean (n=5)    | 1.0               | 2600                           | 8170          | 1.0               | 991                            | 3320  |
|               |        | SD (n=5)      | 0.000             | 699                            | 2870          | 0.000             | 632                            | 2020  |
| 14            | Male   | Mean (n=5)    | 1.0               | 1240                           | 4260          | 1.0               | 778                            | 2750  |
|               |        | SD (n=5)      | 0.000             | 236                            | 1100          | 0.000             | 217                            | 388   |
|               | Female | Mean (n=5)    | 1.0               | 1960                           | 7590*         | 1.0               | 612                            | 2880* |
|               |        | SD (n=5)      | 0.000             | 487                            | 3590*         | 0.000             | 258                            | 1770* |

\*: These values were calculated from four animals.

## 10.5 Safety Testing of the Human Specific Metabolite D21-2393

### 10.5.1 Repeated Dose Toxicity Study in Rats Treated Orally with D21-2393 for 3 Months

Conducting laboratory and location: Daiichi Sankyo Co., Ltd., Japan

Study numbers: AN09-H0089-R01 (and AN07-C0144-R01)

Date of study initiation: May 31, 2010

Drug, lot/batch #, purity: D21-2393, D21-2393-09, 99.94%

GLP compliance: Yes

QA statement: Yes

### Key Study Findings

There were no D21-2393-related deaths or abnormal clinical signs. There were no D21-2393-related effects on body weight, food consumption, urinalysis, ophthalmology,

hematology, blood chemistry or pathology. NOAEL was 600 mg/kg/day. At the NOAEL (600 mg/kg/day), the mean C<sub>max</sub> and AUC<sub>0-24h</sub> values on Day 91 were 314 ng/mL and 1757 ng•h/mL in males, respectively, and 177 ng/mL and 1300 ng•h/mL in females, respectively.

## Methods

CrI:CD(SD) rats (6 weeks old) were orally gavaged with D21-2393 at dose levels of 0 (vehicle 0.5% MC, control), 60, 200, or 600 mg/kg/day for 3 months (n=10/sex/group for toxicity; n=4/sex/group for TK). Doses selected in the current study were based on data from clinical trials and a previous repeated dose toxicity study with D21-2393 in rats (**Study AN07-C0144-R01**). The mean C<sub>max</sub> and AUC<sub>0-24h</sub> values of D21-2393 in healthy subjects receiving 60 mg of DU-176b were 22.2 ng/mL and 147 ng•h/mL, respectively (**DU176-E-PRT001, DU-176b-A-U120, and DU-176b-PRT019**). In the Study AN07-C0144-R01 (previously reviewed by Dr. Ronald Honchel under IND063266, 04/13/2009), SD rats (6 weeks old, 10/sex/group) were gavaged once daily at doses of 0 (vehicle), 200, 600, or 2000 mg/kg/day D21-2393 (lot # D21-2393-07, purity 99.8%) for 14 consecutive days. There were no drug-related adverse effects observed in this study. The C<sub>max</sub> and AUC<sub>0-24h</sub> values of D21-2393 in rats at the D21-2393 dose of 600 mg/kg on day 14 were 171-210 ng/mL and 1020-1190 ng•h/mL, respectively (Table 79). Based on these exposure levels, the highest dose of D21-2393 in the present study was set at 600 mg/kg, at which the exposure level was expected to be 8 folds that in humans at the maximum recommended human dose of 60 mg/day. The middle and lowest doses were set at 200 and 60 mg/kg, respectively, dividing by a common ratio of about 3. Dose formulations were confirmed to acceptable for accuracy (98-102% of target concentrations) and uniformity (-0.66 – 1.02%).

Table 79. Summary of D21-2393 TK data in rats from Study AN07-C0144-R01

| Day of dosing | Dose (mg/kg/day) | Sex        | Plasma concentration (ng/mL) |       |      |      |       | t <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng•h/mL) |      |
|---------------|------------------|------------|------------------------------|-------|------|------|-------|----------------------|--------------------------|--------------------------------|------|
|               |                  |            | pre                          | 1h    | 2 h  | 4 h  | 24 h  |                      |                          |                                |      |
| 1             | 200              | Male       | Mean (n=4)                   | -     | 147  | 72.9 | 55.0  | 2.27                 | 1.0                      | 147                            | 885  |
|               |                  |            | SD (n=4)                     | -     | 49.4 | 33.9 | 24.1  | 0.498                | 0.000                    | 49.4                           | 329  |
|               | Female           | Mean (n=4) | -                            | 80.4  | 29.2 | 16.3 | 3.58  | 1.0                  | 80.4                     | 339                            |      |
|               |                  | SD (n=4)   | -                            | 42.0  | 13.4 | 3.03 | 3.30  | 0.000                | 42.0                     | 71.3                           |      |
|               | 600              | Male       | Mean (n=4)                   | -     | 368  | 192  | 110   | 20.3                 | 1.0                      | 368                            | 2070 |
|               |                  |            | SD (n=4)                     | -     | 82.4 | 66.7 | 43.9  | 6.98                 | 0.000                    | 82.4                           | 714  |
| Female        | Mean (n=4)       | -          | 265                          | 127   | 97.9 | 12.1 | 1.0   | 265                  | 1650                     |                                |      |
|               | SD (n=4)         | -          | 89.5                         | 56.9  | 31.6 | 9.06 | 0.000 | 89.5                 | 380                      |                                |      |
| 2000          | Male             | Mean (n=4) | -                            | 680   | 490  | 285  | 43.6  | 1.0                  | 680                      | 4990                           |      |
|               |                  | SD (n=4)   | -                            | 145   | 189  | 88.2 | 16.3  | 0.000                | 145                      | 1390                           |      |
| Female        | Mean (n=4)       | -          | 429                          | 308   | 265  | 51.4 | 1.0   | 429                  | 4320                     |                                |      |
|               | SD (n=4)         | -          | 184                          | 186   | 122  | 8.62 | 0.000 | 184                  | 1720                     |                                |      |
| 14            | 200              | Male       | Mean (n=4)                   | 1.25  | 65.5 | 26.5 | 25.5  | 1.71                 | 1.0                      | 65.5                           | 404  |
|               |                  |            | SD (n=4)                     | 0.864 | 35.1 | 12.9 | 7.96  | 1.13                 | 0.000                    | 35.1                           | 123  |
|               | Female           | Mean (n=4) | 2.34                         | 39.7  | 16.7 | 16.3 | 12.1  | 6.8                  | 42.4                     | 366                            |      |
|               |                  | SD (n=4)   | 1.48                         | 17.7  | 4.90 | 5.55 | 14.7  | 11.5                 | 14.9                     | 186                            |      |
|               | 600              | Male       | Mean (n=4)                   | 7.13  | 210  | 64.5 | 66.1  | 15.6                 | 1.0                      | 210                            | 1190 |
|               |                  |            | SD (n=4)                     | 4.67  | 93.6 | 10.3 | 22.5  | 8.03                 | 0.000                    | 93.6                           | 278  |
| Female        | Mean (n=4)       | 13.9       | 171                          | 51.7  | 54.1 | 17.2 | 1.0   | 171                  | 1020                     |                                |      |
|               | SD (n=4)         | 10.3       | 50.0                         | 21.0  | 27.5 | 8.90 | 0.000 | 50.0                 | 414                      |                                |      |
| 2000          | Male             | Mean (n=4) | 7.36                         | 941   | 382  | 185  | 28.0  | 1.0                  | 941                      | 3830                           |      |
|               |                  | SD (n=4)   | 4.34                         | 857   | 195  | 60.7 | 17.8  | 0.000                | 857                      | 1850                           |      |
| Female        | Mean (n=4)       | 35.1       | 588                          | 276   | 136  | 39.5 | 1.0   | 588                  | 2910                     |                                |      |
|               | SD (n=4)         | 13.5       | 125                          | 50.3  | 8.26 | 20.5 | 0.000 | 125                  | 234                      |                                |      |

-: Not calculated

For rats in the toxicity groups during the study, clinical signs and morbidity/death were inspected visually at least once daily. Body weight and food consumption were recorded prior to treatment and then once weekly. Ophthalmic examination and urinalysis (Table 31) were performed on day 88. Blood samples were collected from the abdominal aorta of all surviving animals at scheduled sacrifice (on day 92) for blood smear, hematology, and coagulation (Table 29), and plasma chemistry (Table 30). At the end of dosing period, necropsy and macroscopic observation were performed on all animals.

Prematurely died animals were also necropsied. Weights of brain, pituitary, thyroids (including parathyroids), thymus, heart, lung, liver, spleen, adrenals, kidneys, ovaries, uterus, prostate, testes, and epididymides were recorded. The weighed organs and eyes, right sciatic nerve, seminal vesicle, pancreas, mesenteric lymph node, salivary glands, submandibular lymph nodes, skeletal muscle (right rectus femoris), urinary bladder, harderian glands, vagina, tongue, trachea, esophagus, aorta (thoracic), skin, mammary gland, spinal cord (lumbar part), bone and bone marrow (right femur and sternum), stomach (forestomach and glandular stomach), duodenum, jejunum, ileum (including Peyer's patch), cecum, colon, rectum and organs showing abnormalities at necropsy were collected, histologically processed. The histopathological specimens of all the processed organs from animals in the control and 600 mg/kg groups and the organs showing abnormalities at necropsy in the other treatment groups were examined under a light microscope. No peer review was mentioned.

For rats in the TK groups during study, clinical signs and body weight were monitored. Blood samples were collected at 1, 2, 4, and 24 h post-dose on Day 1, and at pre-dose, 1, 2, 4, and 24 hours post-dose on Days 28, and 91 for the determination of plasma D21-2393 concentrations (using an LC-MS/MS method). Because of measured values extraordinarily high or low, or abnormal concentration transition observed, some samples were reanalyzed. Following TK analyzing samples were diluted with blank plasma spiked with sodium fluoride and reanalyzed since first analysis values exceeded the upper limit of calibration curve –

| Day of dosing | Animal No.      | mg/kg/day | Blood sampling point  | Dilution factor at reanalysis |
|---------------|-----------------|-----------|-----------------------|-------------------------------|
| Day 1         | 06F03           | 60        | 1 hour after dosing   | 10-fold                       |
|               | 08M04           | 600       | 24 hours after dosing |                               |
| Day 28        | 08F01           | 600       | Before dosing         | 10-fold                       |
| Day 91        | 06M04 and 06F04 | 60        | 1 hour after dosing   | 100-fold                      |
|               | 06M04 and 06F04 | 60        | 2 hours after dosing  | 10-fold                       |
|               | 08M01           | 600       | 1 hour after dosing   | 500-fold                      |
|               | 08M01           | 600       | 2 hours after dosing  | 100-fold                      |

## Results

At necropsy, perforation of the esophagus was observed in 2 control males, and was diagnosed as changes related to misgavage. These animals had shown some clinical signs including hypoactivity, irregular respiration, smudge of the perinasal area, and

transiently decreased body weight and food consumption during the dosing period. Therefore, the data from these animals were excluded from the study evaluation.

One male in the 600 mg/kg/day group died on Day 27. Prior to death, continuous bleeding due to an accidental avulsion of the nail was observed in one digit of the left hindlimb on Days 25 and 26. At necropsy, discoloration of the liver and spleen was obvious, suggesting abundant blood loss. Since there were no other significant pathological findings in any other organs, the cause of death was identified as substantial blood loss due to an accidental hemorrhage.

During the course of the study, there were no D21-2393-related deaths or abnormal clinical signs. There were no D21-2393-related effects on body weight, food consumption, urinalysis, ophthalmology, hematology, blood chemistry or pathology.

In the TK analysis, D21-2393 plasma concentrations of rats #06M04, #06F04, and #08M01 at post-dose on day 91 were 15-20 times the values of rats in the same group on day 91 (Table 80), and 15-20 times the values of the same rats Days 1 and 28. Since D21-2393 plasma concentrations of rats #06M04, #06F04, and #08M01 at pre-dose on day 91 were similar to (not higher than) the values of rats in the same group on the same day, these values of rats #06M04, #06F04, and #08M01 on day 91 were not due to drug accumulation, but were outliers probably due to higher bioavailability as a consequence of dosing trauma and drug-in-tissues. Therefore, the reviewer re-analyzed the TK data without D21-2393 plasma concentrations of rats #06M04, #06F04, and #08M01 on Day 91.

Table 80. Identified outliers regarding D21-2393 plasma concentrations (ng/ml)

| Dose (mg/kg) | Sex    | Animal No. | Day 1 |      |      |       | Day 28 |      |      |      |       | Day 91 |       |       |      |       |
|--------------|--------|------------|-------|------|------|-------|--------|------|------|------|-------|--------|-------|-------|------|-------|
|              |        |            | 1 h   | 2 h  | 4 h  | 24 h  | Pre    | 1 h  | 2 h  | 4 h  | 24 h  | Pre    | 1 h   | 2 h   | 4 h  | 24 h  |
| 60           | Male   | 06M01      | 36.8  | 23.9 | 19.9 | 2.02  | 2.01   | 17.9 | 7.01 | 5.53 | 1.43  | 1.67   | 13.8  | 6.58  | 5.80 | 2.24  |
|              |        | 06M02      | 37.5  | 22.6 | 13.3 | 0.836 | 1.01   | 27.8 | 12.8 | 3.97 | 1.30  | 1.91   | 42.0  | 11.8  | 7.36 | 1.81  |
|              |        | 06M03      | 44.7  | 31.7 | 12.3 | 0.722 | 1.35   | 36.4 | 9.83 | 4.09 | 1.00  | 2.10   | 23.0  | 12.7  | 5.82 | 2.11  |
|              |        | 06M04      | 44.0  | 22.0 | 17.7 | 1.46  | 1.69   | 17.4 | 6.35 | 5.24 | 2.07  | 2.28   | 791*  | 118*  | 21.3 | 1.42  |
|              | Female | 06F01      | 8.01  | 8.20 | 6.32 | 0.575 | 0.715  | 8.74 | 8.02 | 7.37 | 0.975 | 1.64   | 17.2  | 10.4  | 6.19 | 2.44  |
|              |        | 06F02      | 20.0  | 9.34 | 5.33 | 1.73  | 1.05   | 11.8 | 7.85 | 5.16 | 1.76  | 1.29   | 15.4  | 7.86  | 5.11 | 0.982 |
|              |        | 06F03      | 55.9  | 26.5 | 16.6 | 5.58  | 0.641  | 34.0 | 26.8 | 6.95 | 3.14  | 1.31   | 41.5  | 15.8  | 14.3 | 1.41  |
|              |        | 06F04      | 21.1  | 5.29 | 4.88 | 0.624 | 0.586  | 7.09 | 6.48 | 3.60 | 1.23  | 1.09   | 596*  | 111*  | 13.8 | 1.33  |
| 600          | Male   | 08M01      | 494   | 259  | 125  | 46.7  | 31.7   | 242  | 139  | 104  | 37.3  | 24.2   | 5610* | 1180* | 300  | 42.5  |
|              |        | 08M02      | 460   | 205  | 81.1 | 30.7  | 18.4   | 223  | 120  | 111  | 11.9  | 19.6   | 260   | 97.1  | 47.6 | 10.8  |
|              |        | 08M03      | 261   | 146  | 81.4 | 18.3  | 17.1   | 267  | 91.5 | 33.8 | 16.9  | 27.0   | 248   | 120   | 65.8 | 19.5  |
|              |        | 08M04      | 639   | 218  | 76.6 | 69.2  | 48.3   | 209  | 134  | 91.3 | 37.6  | 121    | 434   | 236   | 153  | 32.7  |

\* outliers

As shown in Figure 23, D21-2393 plasma concentrations generally increased in a dose-dependent manner in a dose ranging from 60 to 600 mg/kg. D21-2393 systemic exposures (C<sub>max</sub> and AUC<sub>0-24h</sub>) were general dose-proportional, slightly higher in males than in females, and no apparently different after repeated doses indicating no accumulation (Table 81). Values of TK parameters in the current study were consistent with those of a previous 14-day repeat dose toxicity study in rats with D21-2393 (Study AN07-C0144-R01).

Figure 23. Mean plasma D21-2393 concentrations following oral D21-2393 in rats

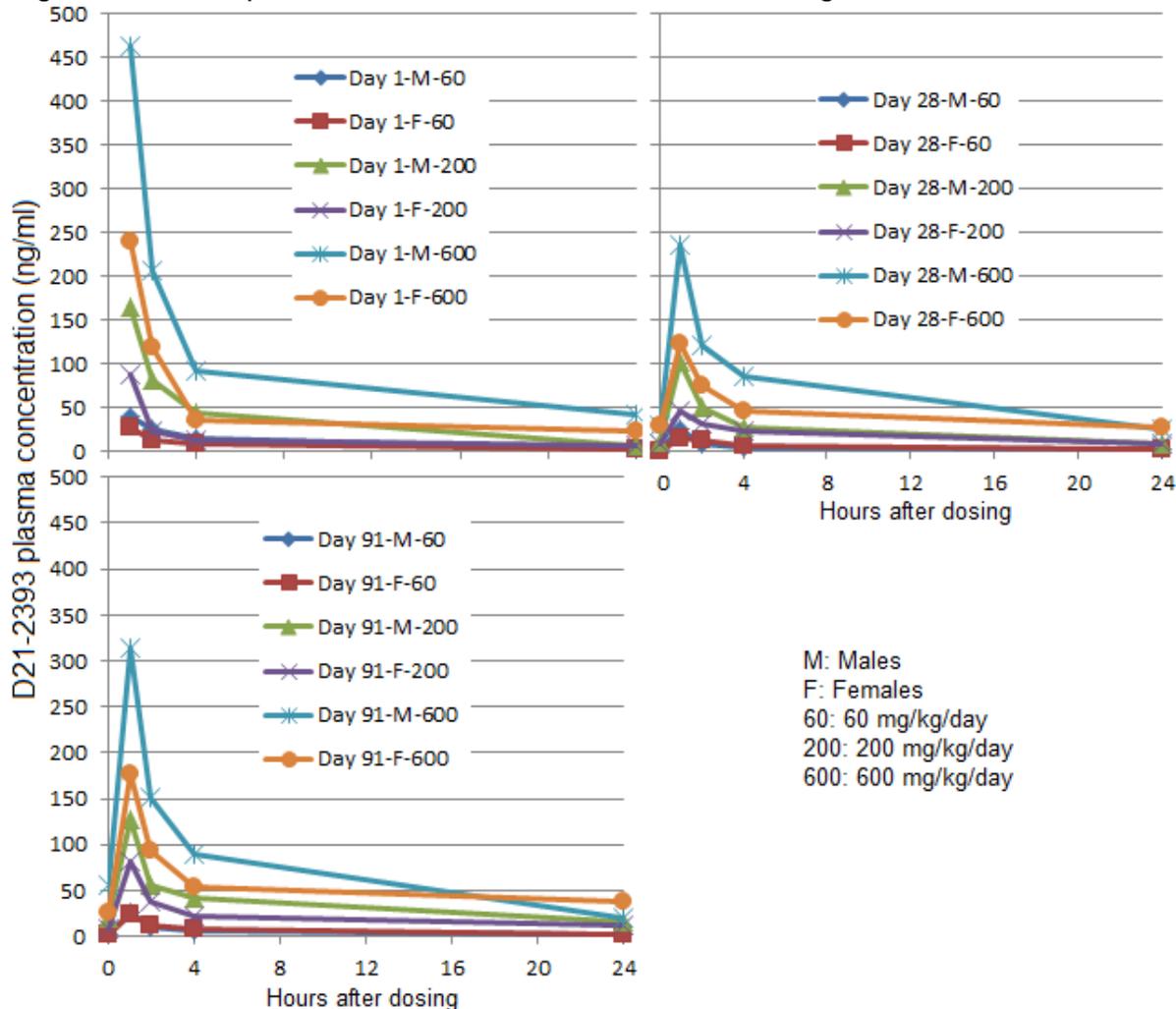


Table 81. Summary of TK parameter in the 3-month toxicity study in rats with D21-2393

| mg/kg/day | Sex |      | Day 1    |              |                     | Day 28   |              |                     | Day 91   |              |                     |
|-----------|-----|------|----------|--------------|---------------------|----------|--------------|---------------------|----------|--------------|---------------------|
|           |     |      | Tmax (h) | Cmax (ng/ml) | AUC0-24 h (ng.h/ml) | Tmax (h) | Cmax (ng/ml) | AUC0-24 h (ng.h/ml) | Tmax (h) | Cmax (ng/ml) | AUC0-24 h (ng.h/ml) |
| 60        | M   | Mean | 1.0      | 41           | 265                 | 1.0      | 25           | 106                 | 1.0      | 26           | 133                 |
|           |     | SD   | 0.0      | 4            | 41                  | 0.0      | 9            | 1                   | 0.0      | 14           | 25                  |
|           | F   | Mean | 1.3      | 26           | 157                 | 1.0      | 15           | 115                 | 1.0      | 25           | 152                 |
|           |     | SD   | 0.5      | 21           | 118                 | 0.0      | 13           | 48                  | 0.0      | 15           | 75                  |
| 200       | M   | Mean | 1.0      | 164          | 845                 | 1.0      | 102          | 567                 | 1.0      | 127          | 839                 |
|           |     | SD   | 0.0      | 48           | 104                 | 0.0      | 41           | 185                 | 0.0      | 56           | 284                 |
|           | F   | Mean | 1.0      | 88           | 338                 | 1.0      | 46           | 435                 | 1.0      | 83           | 504                 |
|           |     | SD   | 0.0      | 85           | 99                  | 0.0      | 14           | 152                 | 0.0      | 19           | 84                  |
| 600       | M   | Mean | 1.0      | 464          | 2190                | 1.0      | 235          | 1620                | 1.0      | 314          | 1757                |
|           |     | SD   | 0.0      | 156          | 524                 | 0.0      | 25           | 457                 | 0.0      | 104          | 968                 |
|           | F   | Mean | 1.3      | 258          | 1030                | 1.0      | 123          | 1020                | 1.0      | 177          | 1300                |
|           |     | SD   | 0.5      | 56           | 121                 | 0.0      | 31           | 277                 | 0.0      | 69           | 605                 |

In conclusion, the NOAEL was 600 mg/kg/day in the current study. At the NOAEL (600 mg/kg/day), the mean C<sub>max</sub> and AUC<sub>0-24h</sub> values on Day 91 were 314 ng/mL and 1757 ng·h/mL in males, respectively, and 177 ng/mL and 1300 ng·h/mL in females, respectively.

### 10.5.2 Bacterial reverse mutation test of D21-2393

Conducting laboratory and location: (b) (4)

Study number(s): AN07-C0145-R01

Date of study initiation: December 27, 2007

Drug, lot/batch number, purity: D21-2393, D21-2393-07, 99.82%

GLP compliance: Yes

QA statement: Yes

### Key Study Findings

D21-2393 up to 5000 µg/plate was negative in all tester strains both with and without metabolic activation.

### Methods

The mutagenic activity of D21-2393 was tested in five histidine-requiring stains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (WP2uvrA) in the absence and presence of metabolic activation (by S-9, an Aroclor 1254-induced rat liver post-mitochondrial fraction). For all assays, bacteria were cultured at 37°C for 10 hours with shaking prior to use. After adding the test compound to the bacterial culture with or without metabolic activation, the test tube was incubated for 20 minutes at 37°C, with shaking. After plated, triplicate plates were incubated at 37°C in dark for 48 hours, then examined for revertant colonies as an indicator of toxicity and mutation. The experiments included:

- A toxicity range-finding experiment was carried out in all strains, in the absence and presence of S-9 using final concentrations of D21-2393 at 9.8, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate, plus negative (vehicle: water for injection) and positive (Table 82) controls.
- Main test in all strains was performed in the absence and presence of S-9 using final concentrations of D21-2393 at 156, 313, 625, 1250, 2500, and 5000 µg/plate, plus negative (vehicle: water) and positive (Table 82) controls.

Table 82. Positive controls for the Ames test

| Test strain     | Without metabolic activation                        | With metabolic activation                    |
|-----------------|---|--|
|                 | Article & Concentration ( $\mu\text{g/mL}$ )        | Article & Concentration ( $\mu\text{g/mL}$ ) |
| TA100           | AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide 0.1 | 2AA: 2-Aminoanthracene 10                    |
| TA1535          | NaN <sub>3</sub> : Sodium azide 5                   | 2AA: 2-Aminoanthracene 20                    |
| WP2 <i>uvrA</i> | AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide 0.2 | 2AA: 2-Aminoanthracene 100                   |
| TA98            | AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide 1   | 2AA: 2-Aminoanthracene 5                     |
| TA1537          | 9AA: 9-Aminoacridine hydrochloride hydrate 800      | 2AA: 2-Aminoanthracene 20                    |

The number of revertant colonies on each plate was counted macroscopically. In the positive control groups, the number of revertant colonies on each plate was measured using a colony analyzer. The background bacterial lawn was inspected for signs of toxicity. The results were judged as positive for inducibility of gene mutation when the number of revertant colonies per plate (mean) increases dose-dependently by 2-fold or greater than that of the negative control, and the results are reproducible between the dose-finding test and the main test.

## Results

The number of revertant colonies in the negative and positive controls (Table 83) was within the range (mean $\pm$ 3S.D.) of the background data of the test facility, and this study was judged to be satisfactory. Test article precipitation was observed at  $\geq 2500 \mu\text{g/plate}$  upon addition of the test article preparation with and without metabolic activation, but was absent on the plates after incubation for 48 hours. Growth inhibition was not observed up to  $5000 \mu\text{g/plate}$  in any test strain with or without metabolic activation.

When compared with the negative control, a 2-fold or greater increase in the number of revertant colonies was not observed in any test strain with or without metabolic activation (Table 83).

Table 83. Mean number of revertant in Ames test with D21-2393

| Metabolic activation                          | Dose (µg/plate)  | Concentration-finding test       |                  |                     |                      |        | Main test                        |                  |                     |                      |        |
|---|------------------|----------------------------------|------------------|---------------------|----------------------|--------|----------------------------------|------------------|---------------------|----------------------|--------|
|   |                  | Base pair substitution mutations |                  |                     | Frameshift mutations |        | Base pair substitution mutations |                  |                     | Frameshift mutations |        |
|   |                  | TA100                            | TA1535           | WP2 <sub>uvrA</sub> | TA98                 | TA1537 | TA100                            | TA1535           | WP2 <sub>uvrA</sub> | TA98                 | TA1537 |
| without metabolic activation                  | Negative control | 102                              | 14               | 25                  | 27                   | 8      | 89                               | 10               | 26                  | 22                   | 5      |
|   | 9.8              | 106                              | 10               | 21                  | 23                   | 9      |                                  |                  |                     |                      |        |
|   | 19.5             | 100                              | 8                | 21                  | 26                   | 8      |                                  |                  |                     |                      |        |
|   | 39.1             | 102                              | 9                | 18                  | 25                   | 5      |                                  |                  |                     |                      |        |
|   | 78.1             | 104                              | 11               | 22                  | 27                   | 7      |                                  |                  |                     |                      |        |
|   | 156              | 101                              | 8                | 22                  | 28                   | 7      | 100                              | 8                | 25                  | 20                   | 3      |
|   | 313              | 103                              | 8                | 17                  | 21                   | 7      | 84                               | 8                | 23                  | 18                   | 3      |
|   | 625              | 98                               | 10               | 21                  | 26                   | 7      | 92                               | 7                | 24                  | 20                   | 4      |
|   | 1250             | 102                              | 9                | 18                  | 27                   | 5      | 93                               | 6                | 22                  | 16                   | 4      |
|   | 2500             | 102                              | 9                | 18                  | 26                   | 5      | 98                               | 7                | 21                  | 27                   | 4      |
| 5000  | 92               | 9                                | 22               | 28                  | 7                    | 98     | 6                                | 23               | 21                  | 3                    |        |
| with metabolic activation                     | Negative control | 120                              | 10               | 22                  | 35                   | 13     | 103                              | 9                | 24                  | 33                   | 6      |
|   | 9.8              | 122                              | 9                | 21                  | 28                   | 16     |                                  |                  |                     |                      |        |
|   | 19.5             | 119                              | 11               | 21                  | 33                   | 14     |                                  |                  |                     |                      |        |
|   | 39.1             | 115                              | 9                | 20                  | 32                   | 14     |                                  |                  |                     |                      |        |
|   | 78.1             | 124                              | 10               | 20                  | 26                   | 15     |                                  |                  |                     |                      |        |
|   | 156              | 124                              | 7                | 21                  | 28                   | 12     | 105                              | 11               | 22                  | 31                   | 6      |
|   | 313              | 112                              | 11               | 22                  | 30                   | 15     | 110                              | 14               | 27                  | 30                   | 8      |
|   | 625              | 119                              | 8                | 20                  | 27                   | 11     | 98                               | 7                | 23                  | 22                   | 8      |
|   | 1250             | 122                              | 9                | 22                  | 28                   | 14     | 98                               | 9                | 23                  | 25                   | 6      |
|   | 2500             | 109                              | 5                | 22                  | 29                   | 14     | 112                              | 9                | 21                  | 32                   | 6      |
| 5000  | 110              | 8                                | 23               | 29                  | 13                   | 98     | 8                                | 24               | 34                  | 5                    |        |
| Positive control without metabolic activation | Article          | AF-2                             | NaN <sub>3</sub> | AF-2                | AF-2                 | 9AA    | AF-2                             | NaN <sub>3</sub> | AF-2                | AF-2                 | 9AA    |
|   | Dose (µg/plate)  | 0.01                             | 0.5              | 0.02                | 0.1                  | 80     | 0.01                             | 0.5              | 0.02                | 0.1                  | 80     |
|   | Colonies/plate   | 357                              | 142              | 360                 | 485                  | 339    | 345                              | 202              | 349                 | 477                  | 324    |
| Positive control with metabolic activation    | Article          | 2AA                              | 2AA              | 2AA                 | 2AA                  | 2AA    | 2AA                              | 2AA              | 2AA                 | 2AA                  | 2AA    |
|   | Dose (µg/plate)  | 1                                | 2                | 10                  | 0.5                  | 2      | 1                                | 2                | 10                  | 0.5                  | 2      |
|   | Colonies/plate   | 564                              | 170              | 116                 | 437                  | 170    | 494                              | 149              | 158                 | 446                  | 146    |

### 10.5.3 Chromosomal aberration test of D21-2393 in cultured mammalian cells

Conducting laboratory and location: (b) (4)

Study number(s): AN01-C0146-R01

Date of study initiation: January 11, 2008

Drug, lot/batch number, purity: D21-2393, D21-2393-07, 99.82%

GLP compliance: Yes

QA statement: Yes

### Key Study Findings

D21-2393 induced numerical aberrations in CHL cells at  $\geq 1250$   $\mu\text{g/ml}$  with and without S9- activation system in both short term and continuous treatment.

## Methods

In dose range-finding experiments, Chinese hamster lung (CHL/IU cell line) cells were pre-incubated at  $37^{\circ}\text{C}$  for 72 hours, then cultured with medium containing vehicle (water for injection), D21-2393 (5-5000  $\mu\text{g/ml}$ ) or positive controls (Table 84) for 6 hours in the absence and presence of S-9, followed by an 18-hour recovery (i.e., short-term treatment), or continuously cultured with medium containing vehicle (water for injection), D21-2393 (5-5000  $\mu\text{g/ml}$ ) or positive controls (Table 84) for 24 hours in the absence of S-9 (i.e., continuous treatment). CHL/IU cells were then harvested. Aliquots of cells were suspended in 0.2% trypan blue for checking viable cells using an inverted culture microscope. The cell proliferation ratio (%) was determined from the viable cell count (mean), while that in the negative control group was taken as 100%.

The chromosomal aberration test was performed with D21-2393 at 625, 1250, 2500, and 5000  $\mu\text{g/mL}$  in both short-term and continuous treatments using CHL/IU cells. Short-term treatments were performed with and without metabolic activation. The cells were treated with D21-2393 and control articles (Table 84) for the first 6 hours of a 24-hour period, and then the chromosome specimens were prepared. For continuous treatment, the chromosome specimens were prepared without metabolic activation after treatment with D21-2393 and control articles (Table 84) for 24 hours (approximately 1.5 cell cycles). In all treatments, the number of viable cells was counted at the point of preparation of chromosome specimens as an indication of cell proliferation.

The chromosome specimens were examined for percentage of cells in mitosis and chromosome aberration. Two hundred well-spread metaphases per dose (100 cells/dish) were observed for structural aberrations. One thousand well-spread metaphases per dose (1000 cells/dish) were observed for numerical aberrations.

Table 84. list of positive controls

| Treatment condition        | Positive control article | Final concentration ( $\mu\text{g/mL}$ ) |
|----------------------------|--------------------------|--|
| Short-term treatment (-S9) | Mitomycin C, MMC         | 0.015                                    |
| Short-term treatment (+S9) | Benzo[a]pyrene, B[a]P    | 20                                       |
| Continuous treatment       | Mitomycin C, MMC         | 0.05                                     |

-S9: Without metabolic activation, +S9: With metabolic activation

## Results

In the dose-finding test, the 50% cell growth inhibition doses were 5000  $\mu\text{g/mL}$  or greater in short-term treatments with and without S-9, and in continuous treatment for 24 hours (Table 85). Test article precipitation in the culture medium was observed at  $\geq 1500$   $\mu\text{g/mL}$  at the start and end of test article treatment in all treatments.

Table 85. The mean relative cell proliferation ratio (%) with the negative control being 100% (from the submission)

| D21-2393<br>Dose<br>(µg/mL) | Short-term treatment         |                           | Continuous treatment |
|-----------------------------|------------------------------|---------------------------|----------------------|
|                             | Without metabolic activation | With metabolic activation | for 24 hour          |
| Negative control            | 100                          | 100                       | 100                  |
| 5                           | 94.0                         | 96.7                      | 107.2                |
| 15                          | 94.0                         | 93.5                      | 109.0                |
| 50                          | 99.1                         | 95.1                      | 104.5                |
| 150                         | 97.4                         | 90.2                      | 97.3                 |
| 500                         | 94.8                         | 92.7                      | 91.9                 |
| 1500                        | 89.7                         | 75.6                      | 75.7                 |
| 5000                        | 48.3                         | 71.5                      | 49.5                 |

The incidence of cells with structure or chromosomal structure aberrations in negative and positive controls in all treatments was within background range of the test facility. D21-2393 did not affect the incidence of cells with structure aberrations, but dose dependently increased the incidence of cells having numerical chromosomal aberrations in short-term treatments with and without metabolic activation (statistical significance at  $\geq 2500$  µg/ml when compared with the negative controls), and increased the incidence of cells having numerical chromosomal aberrations in continuous treatments (statistical significance at 1250 and 2500 µg/ml when compared with the negative control) (Table 86). Thus, D21-2393 induced numerical aberrations in CHL/IU cells under the conditions of this study.

Table 86. Summary of chromosomal aberrations in CHL/IU with D21-2393

| Treatment time (hours)                   | S9 mix | D21-2393 µl/ml   | No. of cells with structural aberrations |     |     |     |     |   |       |      | No. of gap | Cell proliferation ratio (%) | No. of cells with numerical |      |      |       |      |   |
|--|--------|------------------|--|-----|-----|-----|-----|---|-------|------|------------|------------------------------|-----------------------------|------|------|-------|------|---|
|  |        |                  | No. of cells                             | Ctb | Cte | Csb | Cse | O | total | %    |            |                              | No. of cells                | Poly | Endo | Total | %    |   |
| Short term (6 hours + 18 hours recovery) | -      | Negative control | 200                                      | 1   | 0   | 0   | 0   | 0 | 0     | 1    | 0.5        | 0                            | 100                         | 2000 | 19   | 0     | 19   | 1 |
|  |        | 625              | 200                                      | 2   | 0   | 0   | 0   | 0 | 2     | 1    | 0          | 100.4                        | 2000                        | 34   | 1    | 35    | 1.8  |   |
|  |        | 1250             | 200                                      | 1   | 0   | 0   | 0   | 0 | 1     | 0.5  | 0          | 80.7                         | 2000                        | 36   | 1    | 37    | 1.9  |   |
|  |        | 2500             | 200                                      | 0   | 1   | 0   | 0   | 0 | 1     | 0.5  | 0          | 67.5                         | 2000                        | 67   | 1    | 68    | 3.4* |   |
|  |        | 5000             | 0  | 0   | 1   | 0   | 0   | 0 | 1     | 0.5  | 0          | 34.9                         | 2000                        | 138  | 2    | 140   | 7*   |   |
|  |        | MMC, 0.15        | 200                                      | 24  | 39  | 0   | 0   | 0 | 51    | 25.5 | 0          |                              | 2000                        | 19   | 0    | 19    | 1    |   |
|  | +      | Negative control | 200                                      | 0   | 0   | 0   | 0   | 0 | 0     | 0    | 0          | 100                          | 2000                        | 18   | 0    | 18    | 0.9  |   |
|  |        | 625              | 200                                      | 0   | 1   | 0   | 0   | 0 | 1     | 0.5  | 0          | 93.9                         | 2000                        | 26   | 0    | 26    | 1.3  |   |
|  |        | 1250             | 200                                      | 0   | 1   | 0   | 0   | 0 | 1     | 0.5  | 0          | 80.5                         | 2000                        | 32   | 1    | 33    | 1.7  |   |
|  |        | 2500             | 200                                      | 0   | 0   | 0   | 0   | 0 | 0     | 0    | 0          | 77.5                         | 2000                        | 65   | 1    | 66    | 3.3* |   |
|  |        | 5000             | 200                                      | 0   | 0   | 0   | 0   | 0 | 0     | 0    | 0          | 53.7                         | 2000                        | 181  | 2    | 183   | 9.2* |   |
|  |        | B[a]P, 20        | 200                                      | 12  | 34  | 0   | 0   | 0 | 42    | 21   | 0          | 55.8                         | 2000                        | 21   | 0    | 21    | 1.1  |   |
| Continuous treatment for 24 hours        | -      | Negative control | 200                                      | 1   | 0   | 0   | 0   | 0 | 1     | 0.5  | 0          | 100                          | 2000                        | 19   | 0    | 19    | 1    |   |
|  |        | 625              | 200                                      | 0   | 0   | 0   | 0   | 0 | 0     | 0    | 0          | 89.9                         | 2000                        | 31   | 0    | 31    | 1.6  |   |
|  |        | 1250             | 200                                      | 0   | 0   | 0   | 0   | 0 | 0     | 0    | 0          | 81.3                         | 2000                        | 38   | 1    | 39    | 2*   |   |
|  |        | 2500             | 200                                      | 0   | 1   | 0   | 0   | 0 | 1     | 0.5  | 0          | 54.7                         | 2000                        | 54   | 0    | 54    | 2.7* |   |
|  |        | 5000             | 200                                      | 1   | 0   | 0   | 0   | 0 | 1     | 0.5  | 0          | 34.8                         | 2000                        | 32   | 0    | 32    | 1.6  |   |
|  |        | MMC 0.05         | 200                                      | 22  | 33  | 0   | 0   | 0 | 49    | 24.5 | 0          | 78.7                         | 2000                        | 14   | 0    | 14    | 0.7  |   |

Ctb: chromatid break, Cte: chromatid exchange, Csb: chromosome break, Cse: chromosome exchange, O: others, gap: chromatid and chromosome gap, Poly: polyploid, Endo: endoreduplication.

Test article precipitation in the culture medium was observed at  $\geq 625$   $\mu\text{g/mL}$  at the start and end of test article treatment. \*:  $p < 0.05$  when compared with the negative control group

#### 10.5.4 Polyploidy test of D21-2393 in human lymphocytes (AN08-C0006-R01), Micronucleus study of D21-2393 in rats (AN08C0004-R01), Repeated oral dose micronucleus study of D21-29393 for 14 days in rats (AN08-C0005-R01)

Three GLP studies, polyploidy test (AN08-C0006-R01) and micronucleus studies with single (AN08C0004-R01) and repeated doses (AN08C0005-R01) were conducted with D21-2393 (lot # D21-2393-07, purity 99.82%) (b) (4) during 2008. These studies were summarized in Table 87, and previously reviewed by Dr. Ronald Honchel of the Division of Medical Imaging Products (3/23/2009) under IND 063266 (Appendix IV).

Table 87. Summary of polyploidy test and micronucleus studies with D21-2393

| Type of Study               | Species/<br>Strain                 | Route<br>(Vehicle) | Dose<br>(mg/kg/day)<br>or concentration | Sex and<br>No. per<br>Group | Duration<br>of Dosing   | Noteworthy<br>Findings | GLP<br>Compliance | Study<br>Number |
|-----------------------------|------------------------------------|--------------------|---|-----------------------------|---|------------------------|-------------------|-----------------|
| Polyploidy                  | Human<br>peripheral<br>lymphocytes | in vitro           | 625 to 5000<br>$\mu\text{g/mL}$         | NA                          | 3 <sup>a)</sup> , 24 <sup>b)</sup> ,<br>or 48 h <sup>b)</sup> | Negative               | Yes               | AN08-C0006-R01  |
| Bone marrow<br>micronucleus | Rat/SD                             | po<br>(0.5% MC)    | 0, 500,<br>1000, 2000                   | M:6                         | Single  | Negative               | Yes               | AN08-C0004-R01  |
|                             | Rat/SD                             | po<br>(0.5% MC)    | 0, 500,<br>1000, 2000                   | M:6                         | 2 weeks   | Negative               | Yes               | AN08-C0005-R01  |

a: With (+S9) or without (-S9) metabolic activation, b: Without metabolic activation (-S9)

In the polyploidy test, no significant increase in the incidence of cells having numerical aberrations was noted at D21-2393 concentrations up to 5000  $\mu\text{g/mL}$ . D21-2393 precipitation in the culture medium was observed at concentrations  $\geq 156$   $\mu\text{g/mL}$  at the start of test article treatment. At the end of test article treatment, D21-2393 precipitation was observed at  $\geq 625$   $\mu\text{g/mL}$  and  $\geq 2500$   $\mu\text{g/mL}$  in short-term treatments and continuous treatment, respectively.

In the rat micronucleus study with single oral dose of D21-2393, D21-2393 did not increase the incidence of micronucleated immature erythrocytes (MNIE) at any dose level. In addition, D21-2393 at 2000 mg/kg decreased the percentage of immature erythrocytes (IE) at 24 and 48 h post-dose. In the rat micronucleus study with repeated oral dose of D21-2393 for 14 days, D21-2393 did not affect the incidence of MNIE or IE at any dose levels.

Thus, D21-2393 had no in vivo clastogenic or aneugenic potential in rat bone marrow at oral doses up to 2000 mg/kg/day for up to 2 weeks, and did not induce numerical aberrations in human lymphocytes at concentrations up to 5000  $\mu\text{g/mL}$  with or without metabolic activation.

### 10.5.5 Study for Effects of D21-2393 Administered Orally on Embryo-fetal Development in Rats

Conducting laboratory and location: Daiichi Sankyo Co., Ltd, Japan

Study number(s): AN08-H0052-R01

Date of study initiation: January 19, 2009

Drug, lot/batch number, purity: D21-2393, D21-2393-07, 99.82%

GLP compliance: Yes

QA statement: Yes

#### Key Study Findings

There were no D21-2393-related findings at doses up to 1000 mg/kg/day. NOAELs for maternal and embryo toxicity were 1000 mg/kg/day, with Cmax 581 ng/mL and AUC0-24h 4540 ng·h/mL.

#### Methods

Four groups of 22 pregnant female CrI:CD® (SD) rats (11-12 weeks old) received D21-2393 by oral gavage at doses of 0 (0.5% MC), 200, 600 or 1000 mg/kg from gestation Days 7 to 17. Additionally, 5 dams per group were similarly treated for toxicokinetic evaluation. The dosages used in this study were based on data from previous work (Study R20030532 and AN07-C0144-R01) with this compound. D21-2393 oral absorption was estimated to be one-tenth that of DU-176b. In a rat embryo-fetal developmental study with DU-176b (Study R20030532), NOAELs for dams and the next generation were both 100 mg/kg. In a rat 14-day repeated dose toxicity study of D21-2393 (AN07-C0144-R01), NOAEL was 2000 mg/kg for both males and females. Thus, the highest dose in the present study was set at 1000 mg/kg. The low dose was set at 200 mg/kg, at which the exposure level of D21-2393 in rats was expected to be comparable to or higher than that in humans at the intended clinical dose level of DU-176b. The mid dose was set at 600 mg/kg, three times higher than the low dose. Dosing formulations were confirmed to be accurate and uniform: actual concentrations of each dose formulation ranged from 103% to 104%, and uniformity ranged from -1.21% to 0.00%.

During the study, clinical signs were observed twice daily. Bodyweight was recorded on Days 0, 3, 7, 9, 11, 13, 15, 17, and 20 of pregnancy, and food consumption was monitored and recorded for gestation Days 2-3, 6-7, 8-9, 10-11, 12-13, 14-15, 16-17, and 19-20. Animals were killed on gestation Day 20 for necropsy, and fetuses were examined macroscopically, and subsequently by detailed internal visceral examination or skeletal examination. Plasma concentrations of D21-2393 in satellite animals were determined 1, 2, 4, and 24 h after dosing on gestation Days 7 and 17 using an LC-MS/MS method.

#### Results

There were no D21-2393-related findings in clinical signs, body weight, and food consumption during dosing. At termination necropsy, no differences in the number of corpora lutea and implantations, and placentae between control and treated groups were observed (Table 88). Incidences of skeletal variations in the 1000 mg/kg group were higher than those in the control group, being statistically significant for the total incidences of skeletal variations (Table 89). However, the study reported that the background range of skeletal variation in the test facility was 4.6 to 16.4% (total of 9 studies, conducted in 2000 –2005). The incidence (15/157=9.6%) in the 1000 mg/kg group in the current study is within the range of background data, and was considered to be not toxicologically significant. There were no differences in the number of embryo-fetal deaths and live fetuses, sex ratio, fetal body weight, external, visceral and skeletal anomalies, and degree of ossification between control and treated groups (Table 88, Table 89).

Table 88. Observation of cesarean section in dams

| Observations                    | D21-2393 (mg/kg/day) |              |              |              |              |              |
|---------------------------------|----------------------|--------------|--------------|--------------|--------------|--------------|
|                                 | 0                    | 200          | 600          | 1000         |              |              |
| No. of pregnant females         | 20                   | 19           | 20           | 21           |              |              |
| No. of dams with live fetuses   | 20                   | 19           | 20           | 21           |              |              |
| No. of corpora lutea            | Total                | 309          | 299          | 312          | 344          |              |
|                                 | Mean ± SD            | 15.5 ± 1.70  | 15.7 ± 2.10  | 15.6 ± 2.11  | 16.4 ± 1.96  |              |
| No. of implantations            | Total                | 291          | 267          | 296          | 319          |              |
|                                 | Mean ± SD            | 14.6 ± 1.82  | 14.1 ± 3.96  | 14.8 ± 1.91  | 15.2 ± 1.47  |              |
| Pre-implantation loss rate (%)  | Mean ± SD            | 5.6 ± 9.20   | 12.6 ± 19.10 | 4.8 ± 6.65   | 6.5 ± 9.89   |              |
| No. of post-implantation losses | Total                | 7            | 10           | 9            | 12           |              |
|                                 | Mean ± SD            | 0.4 ± 0.49   | 0.5 ± 0.90   | 0.5 ± 0.83   | 0.6 ± 0.98   |              |
| Dead fetuses                    | Total                | 0            | 0            | 0            | 0            |              |
| Late resorptions                | Total                | 1            | 2            | 1            | 0            |              |
| Early resorptions               | Total                | 6            | 8            | 8            | 12           |              |
| Implantation sites              | Total                | 0            | 0            | 0            | 0            |              |
| Post-implantation loss rate (%) | Mean ± SD            | 2.5 ± 3.46   | 4.0 ± 6.49   | 3.0 ± 5.68   | 3.8 ± 6.48   |              |
| No. of live fetuses             | Total                | 284          | 257          | 287          | 307          |              |
|                                 | Mean ± SD            | 14.2 ± 1.91  | 13.5 ± 3.99  | 14.4 ± 1.98  | 14.6 ± 1.77  |              |
| Male                            | Total                | 139          | 142          | 134          | 152          |              |
| Female                          | Total                | 145          | 115          | 153          | 155          |              |
| Sex ratio (Male%)               | Mean ± SD            | 49.4 ± 10.97 | 54.5 ± 17.34 | 46.4 ± 15.05 | 49.6 ± 14.84 |              |
| Body Weight (g)                 | Male                 | Mean ± SD    | 4.07 ± 0.219 | 3.95 ± 0.223 | 4.05 ± 0.317 | 3.93 ± 0.256 |
|                                 | Female               | Mean ± SD    | 3.89 ± 0.262 | 3.77 ± 0.301 | 3.87 ± 0.291 | 3.72 ± 0.270 |

Table 89. Summary of findings in fetuses

| Observations                               | D21-2393 (mg/kg/day) |            |          |              |
|--|----------------------|------------|----------|--------------|
|  | 0                    | 200        | 600      | 1000         |
| <u>External observation</u>                |                      |            |          |              |
| No. of dams                                | 20                   | 19         | 20       | 21           |
| No. of fetuses                             | 284                  | 257        | 287      | 307          |
| No. of fetuses with anomalies              | Total                | 0          | 1        | 0            |
|  | Mean ±SD             | 0.0±0.00   | 0.4±1.54 | 0.0±0.00     |
| Anasarca                                   | Total                | 0          | 1        | 0            |
|  | Mean ±SD             | 0.0±0.00   | 0.4±1.54 | 0.0±0.00     |
| <u>Visceral observation</u>                |                      |            |          |              |
| No. of dams                                | 20                   |            |          | 21           |
| No. of fetuses                             | 137                  |            |          | 150          |
| No. of fetuses with anomalies              | Total                | 9          |          | 5            |
|  | Mean ±SD             | 7.1±12.66  |          | 3.6±8.46     |
| Thymic remnant in neck                     | Total                | 2          |          | 4            |
|  | Mean ±SD             | 1.7± 5.36  |          | 2.8±5.95     |
| Membranous ventricular septum defect       | Total                | 3          |          | 1            |
|  | Mean ±SD             | 2.6± 6.30  |          | 0.8±3.64     |
| Supernumerary coronary ostium              | Total                | 3          |          | 0            |
|  | Mean ±SD             | 2.2± 5.36  |          | 0.0±0.00     |
| Persistent left umbilical artery           | Total                | 1          |          | 0            |
|  | Mean ±SD             | 0.6± 2.80  |          | 0.0±0.00     |
| <u>Skeletal observation</u>                |                      |            |          |              |
| No. of dams                                | 20                   |            |          | 21           |
| No. of fetuses                             | 147                  |            |          | 157          |
| Anomalies                                  |                      |            |          |              |
| No. of fetuses with anomalies              | Total                | 0          |          | 0            |
|  | Mean ±SD             | 0.0 ± 0.00 |          | 0.0 ± 0.00   |
| Variations                                 |                      |            |          |              |
| No. of fetuses with variations             | Total                | 4          |          | 15           |
|  | Mean ±SD             | 2.8 ± 5.70 |          | 9.9 ± 11.99@ |
| Cervical rib                               | Total                | 0          |          | 2            |
|  | Mean ±SD             | 0.0 ± 0.00 |          | 1.2 ± 3.85   |
| Full supernumerary rib                     | Total                | 0          |          | 1            |
|  | Mean ±SD             | 0.0 ± 0.00 |          | 0.7 ± 3.12   |
| Short supernumerary rib                    | Total                | 4          |          | 8            |
|  | Mean ±SD             | 2.8 ± 5.70 |          | 5.2 ± 9.65   |
| Bipartite ossification of thoracic centrum | Total                | 0          |          | 3            |
|  | Mean ±SD             | 0.0 ± 0.00 |          | 2.1 ± 5.24   |
| Unossified thoracic centrum                | Total                | 0          |          | 1            |
|  | Mean ±SD             | 0.0 ± 0.00 |          | 0.8 ± 3.64   |
| Ossifications                              |                      |            |          |              |
| Sacrocaudal vertebrae                      | Mean ±SD             | 8.1 ± 0.36 |          | 7.9 ± 0.44   |

@:p <0.05 vs control

Plasma concentrations of D21-2393 increased in a dose-related manner within dose ranging from 200 to 1000 mg/kg/day. D21-2393 systemic exposures (C<sub>max</sub> and AUC<sub>0-24</sub>) were generally dose-proportional. There was no accumulation after multiple dosing (Table 90).

Table 90. D21-2393 TK parameters

| Day of administration | Dose (mg/kg/day) |            | Plasma concentration (ng/mL) |                 |                  |                  |                   | t <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |
|-----------------------|------------------|------------|------------------------------|-----------------|------------------|------------------|-------------------|----------------------|--------------------------|--------------------------------|
|                       |                  |            | pre <sup>#</sup>             | 1h <sup>#</sup> | 2 h <sup>#</sup> | 4 h <sup>#</sup> | 24 h <sup>#</sup> |                      |                          |                                |
| First day             | 200              | Mean (n=5) | -                            | 138             | 43.0             | 32.2             | 5.99              | 1.0                  | 138                      | 616                            |
|                       |                  | SD (n=5)   | -                            | 55.0            | 20.5             | 15.0             | 3.89              | 0.000                | 55.0                     | 261                            |
|                       | 600              | Mean (n=5) | -                            | 283             | 111              | 52.6             | 25.0              | 1.0                  | 283                      | 1280                           |
|                       |                  | SD (n=5)   | -                            | 30.4            | 22.0             | 13.9             | 10.3              | 0.000                | 30.4                     | 240                            |
|                       | 1000             | Mean (n=5) | -                            | 627             | 220              | 115              | 51.9              | 1.0                  | 627                      | 2740                           |
|                       |                  | SD (n=5)   | -                            | 277             | 96.8             | 24.7             | 12.0              | 0.000                | 277                      | 675                            |
| Final day             | 200              | Mean (n=5) | 4.43                         | 145             | 75.3             | 43.7             | 5.95              | 1.2                  | 145                      | 801                            |
|                       |                  | SD (n=5)   | 1.17                         | 76.8            | 24.1             | 16.5             | 4.35              | 0.447                | 76.7                     | 198                            |
|                       | 600              | Mean (n=5) | 24.1                         | 330             | 192              | 115              | 26.1              | 1.0                  | 330                      | 2150                           |
|                       |                  | SD (n=5)   | 11.6                         | 96.6            | 69.6             | 80.5             | 6.29              | 0.000                | 96.6                     | 1060                           |
|                       | 1000             | Mean (n=5) | 46.7                         | 581             | 266              | 274              | 52.4              | 1.0                  | 581                      | 4540                           |
|                       |                  | SD (n=5)   | 15.2                         | 224             | 171              | 242              | 21.6              | 0.000                | 224                      | 2960                           |

-: Not calculated

#: Time after administration

In conclusion, the NOAEL was 1000 mg/kg/day for general toxicity and reproduction in dams and for development of the next generation. D21-2393 did not display any teratogenic potential in rats. At the NOAEL of 1000 mg/kg/day, the mean C<sub>max</sub> and AUC<sub>0-24h</sub> values on Day 17 of gestation were 581 ng/mL and 4540 ng·h/mL, respectively.

#### 10.5.6 Repeated dose toxicity study in juvenile rats treated orally with D21-2393 for 7 weeks (AN11-C0004-R01)

This GLP study (AN11-C0004-R01) in CrI:CD(SD) juvenile rats was conducted with D21-2393 (Lot # D21-2393-09, purity 99.94%) (b) (4) during June 2011 – Feb, 2012 to investigate the toxic effects of D21-2393 on organ development after the completion of dosing period and when animals reached maturity. This study was previously reviewed by Dr. Patricia P Harlow (06/03/2013) under IND 077254 (Appendix III).

CrI:CD(SD) rats (16 rats/sex/group in main study part, 48/ rats/sex/group in satellite study part) were orally gavaged with vehicle 0.5% MC, or D21-2393 at doses of 20, 60, or 200 mg/kg/day for 7 weeks from postnatal day (PND) 4 to PND 49, followed by a 6-week observation period. Doses selected in the current study were based on a previous dose-range study in juvenile rats (**Study AN10-C0080- R01**) with oral D21-2393 at doses of 0, 19.6, 58.8, or 196 mg/kg/day for 3 weeks from PND 4 to PND 21. In Study AN10-C0080-R01, no test article-related toxic changes in clinical signs, body weight, or necropsy observations were noted; D21-2393 exposure levels in juvenile rats given 196 mg/kg/day from PND 4 to PND 14 (Table 91) were markedly higher than those (C<sub>max</sub> 22.2 ng/mL and AUC<sub>0-24</sub> 147 ng·h/mL) in human subjects given DU-176b at the maximum recommended clinical dose of 60 mg (**Studies DU176-E-PRT001, DU176b-A-U120, and DU176b-PRT019**) or than those in adult rats given D21-2393 at the same dose level (**AN09-H0089-R01**, Table 81). Therefore, the dose level of 200 mg/kg/day was selected as the highest dose for the present study, and the middle and low dose levels were set at 60 and 20 mg/kg/day, respectively, in a common ratio of approximately 3. Dose formulations were confirmed to be stable and homogeneous for 7 days refrigerated followed by 6 hours at room temperature when protected from light

in a tight container. Toxicity and TK were assessed during the study period. Methods details were similar to those described in Study AN11-C0003-R01 - Repeated Dose Toxicity Study in Juvenile Rats Treated Orally with DU-176b for 7 Weeks (under section 9.3).

Table 91. Summary of D21-2393 TK data (mean of 3) in Study AN10-C0080- R01 (modified from the submission)

| Days after birth | D21-2393 (mg/kg/day) | Sex    | Plasma concentration (ng/mL) |      |      |      |       | T <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |
|------------------|----------------------|--------|------------------------------|------|------|------|-------|----------------------|--------------------------|--------------------------------|
|                  |                      |        | Pre                          | 1 h  | 2 h  | 4 h  | 24 h  |                      |                          |                                |
| 4                | 19.6                 | Male   | -                            | 342  | 283  | 332  | 22.0  | 1.0                  | 342                      | 4640                           |
|                  |                      | Female | -                            | 143  | 306  | 267  | 14.6  | 2.0                  | 306                      | 3690                           |
|                  | 58.8                 | Male   | -                            | 576  | 725  | 1030 | 82.9  | 4.0                  | 1030                     | 13800                          |
|                  |                      | Female | -                            | 603  | 665  | 1240 | 89.3  | 4.0                  | 1240                     | 16100                          |
|                  | 196                  | Male   | -                            | 1860 | 1720 | 2170 | 366   | 4.0                  | 2170                     | 32000                          |
|                  |                      | Female | -                            | 1920 | 1570 | 2680 | 280   | 4.0                  | 2680                     | 36600                          |
| 7                | 19.6                 | Male   | 47.4                         | 310  | 411  | 463  | 50.7  | 4.0                  | 463                      | 6550                           |
|                  |                      | Female | 60.5                         | 282  | 365  | 411  | 34.4  | 4.0                  | 411                      | 5720                           |
|                  | 58.8                 | Male   | 119                          | 1080 | 1190 | 906  | 102   | 2.0                  | 1190                     | 13900                          |
|                  |                      | Female | 111                          | 1150 | 1040 | 1340 | 171   | 4.0                  | 1340                     | 19200                          |
|                  | 196                  | Male   | 246                          | 2380 | 3160 | 2340 | 494   | 2.0                  | 3160                     | 37900                          |
|                  |                      | Female | 227                          | 2110 | 2500 | 3250 | 350   | 4.0                  | 3250                     | 45200                          |
| 14               | 19.6                 | Male   | 154                          | 869  | 824  | 570  | 169   | 1.0                  | 869                      | 10100                          |
|                  |                      | Female | 169                          | 847  | 816  | 605  | 166   | 1.0                  | 847                      | 10500                          |
|                  | 58.8                 | Male   | 219                          | 2140 | 1410 | 1760 | 232   | 1.0                  | 2140                     | 26000                          |
|                  |                      | Female | 258                          | 1600 | 1820 | 1300 | 238   | 2.0                  | 1820                     | 21100                          |
|                  | 196                  | Male   | 209                          | 8230 | 3650 | 3520 | 170   | 1.0                  | 8230                     | 54200                          |
|                  |                      | Female | 226                          | 7130 | 5320 | 3190 | 280   | 1.0                  | 7130                     | 53100                          |
| 21               | 19.6                 | Male   | 3.54                         | 87.4 | 82.4 | 49.6 | 0.638 | 1.0                  | 87.4                     | 765                            |
|                  |                      | Female | 2.67                         | 94.2 | 45.5 | 48.2 | 0.684 | 1.0                  | 94.2                     | 701                            |
|                  | 58.8                 | Male   | 7.90                         | 312  | 183  | 112  | 1.68  | 1.0                  | 312                      | 1840                           |
|                  |                      | Female | 6.44                         | 251  | 153  | 103  | 2.72  | 1.0                  | 251                      | 1640                           |
|                  | 196                  | Male   | 12.1                         | 1150 | 467  | 390  | 19.4  | 1.0                  | 1150                     | 6340                           |
|                  |                      | Female | 6.86                         | 745  | 260  | 368  | 12.8  | 1.0                  | 745                      | 5310                           |

There were no premature deaths in this study. There were no D21-2393-related toxic findings in clinical signs, body weight, food consumption, functional and physical development, morphological differentiation of external genitalia, hematology, blood chemistry, necropsy observation, bone length, organ weight, or histopathology. However, extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted. Fertility, mating ability, and reproductive performance were not specifically evaluated.

Following oral doses, plasma concentrations of D21-2393 increased with dose ranging from 20 mg/kg/day to 200 mg/kg/day. Systemic exposures (C<sub>max</sub> and AUC<sub>0-24h</sub>) were generally dose-proportional, and decreased after repeated dosing on PND 21 and PND49, which may imply increased metabolite rate with maturation. There were no marked sex differences in toxicokinetic parameters (Table 92).

Table 92. Summary of D21-2393 TK data (mean of 3) in juvenile rats

| Age    | D21-2393<br>mg/kg/day | Sex    | Plasma concentraion (ng/ml) |           |           |           |           | Tmax<br>(h) | Cmax<br>(ng/ml) | AUC0-24<br>(ng.h/ml) |
|--------|-----------------------|--------|-----------------------------|-----------|-----------|-----------|-----------|-------------|-----------------|----------------------|
|        |                       |        | Pre-dose                    | 1 h       | 2 h       | 4 h       | 24 h      |             |                 |                      |
| PND 4  | 20                    | Male   | -                           | 203±16.9  | 260±23.8  | 399±71.5  | 19.1±8.6  | 4.0         | 399             | 5170                 |
|        |                       | Female | -                           | 223±28.2  | 309±79.2  | 343±61    | 21.3±16   | 4           | 343             | 4670                 |
|        | 60                    | Male   | -                           | 781±206   | 844±128   | 1320±535  | 69.7±15.2 | 4.0         | 1320            | 17300                |
|        |                       | Female | -                           | 872±238   | 767±245   | 1270±173  | 58.6±4.3  | 4           | 1270            | 16600                |
|        | 200                   | Male   | -                           | 1160±365  | 3750±670  | 2780±780  | 135±23.9  | 2.0         | 3750            | 38700                |
|        |                       | Female | -                           | 1380±276  | 3090±672  | 2750±773  | 235±160   | 2           | 3090            | 38600                |
| PND 14 | 20                    | Male   | 192±74.6                    | 549±89.2  | 633±148   | 268±52.8  | 155±44.5  | 2.0         | 633             | 6090                 |
|        |                       | Female | 95.5±71                     | 650±158   | 584±62.1  | 343±54    | 175±34.4  | 1           | 650             | 7100                 |
|        | 60                    | Male   | 224±25.2                    | 1330±5.8  | 1350±271  | 954±267   | 195±56    | 2.0         | 1350            | 15900                |
|        |                       | Female | 249±27.2                    | 1100±185  | 1100±52.9 | 1240±195  | 198±29.5  | 4           | 1240            | 18500                |
|        | 200                   | Male   | 202±48.8                    | 5230±303  | 3530±369  | 3970±1270 | 214±18.5  | 1.0         | 5230            | 56400                |
|        |                       | Female | 203±49.5                    | 5820±1230 | 3330±558  | 3270±465  | 183±25.5  | 1           | 5820            | 48700                |
| PND 21 | 20                    | Male   | 1.76±0.35                   | 96±17.3   | 49.7±2.3  | 52.8±16.4 | 2.79±2.12 | 1.0         | 96.0            | 780                  |
|        |                       | Female | 1.04±0.55                   | 75.4±14.0 | 51.1±2.0  | 48.2±6.1  | 1.5±1.0   | 1           | 75.4            | 698                  |
|        | 60                    | Male   | 2.6±1.85                    | 182±14.6  | 112±21.5  | 54.7±4.7  | 4.1±3.2   | 1.0         | 182             | 994                  |
|        |                       | Female | 3.5±2.7                     | 163±16.7  | 86.9±28.3 | 126±50.6  | 1.4±0.4   | 1           | 163             | 1690                 |
|        | 200                   | Male   | 24.2±8.6                    | 661±240   | 322±111   | 412±92.3  | 1.8±1.0   | 1.0         | 661             | 5710                 |
|        |                       | Female | 21.4±3.6                    | 763±169   | 418±78.3  | 266±101   | 8.9±5.9   | 1           | 763             | 4420                 |
| PND 49 | 20                    | Male   | 0.36±0.26                   | 19.2±7.9  | 6.7±3.4   | 7.5±5.9   | 0.3±0.2   | 1.0         | 19.2            | 115                  |
|        |                       | Female | 0.32±0.16                   | 17±11.4   | 8.4±8.4   | 3.0±0.4   | 0.2±0.1   | 1           | 17              | 65                   |
|        | 60                    | Male   | 0.92±0.76                   | 41.8±6.4  | 38.9±35.5 | 24.4±8.3  | 1.2±1.0   | 1.0         | 41.8            | 381                  |
|        |                       | Female | 0.8±0.8                     | 35.7±26.3 | 30.3±7.6  | 11.0±4.4  | 0.7±0.2   | 1           | 35.7            | 210                  |
|        | 200                   | Male   | 4.1±3.9                     | 163±26    | 101±77    | 62.7±13.7 | 1.2±0.7   | 1.0         | 163             | 1020                 |
|        |                       | Female | 1.8±0.6                     | 85.8±43.3 | 41.9±0.9  | 34.5±23.7 | 2.5±0.6   | 1           | 85.8            | 554                  |

In conclusion, the NOAEL was 200 mg/kg/day for male and female juvenile rats. At the NOAEL (200 mg/kg/day), the highest mean Cmax and AUC0-24h of D21-2393 (on PND 14) were 5515 ng/mL and 52550 ng.h/ml, respectively.

## 11 Integrated Summary and Safety Evaluation

### Brief Background / Introduction

Edoxaban tosylate hydrate (DU-176b), N-(5-Chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]oxamide mono(4-methylbenzenesulfonate) monohydrate, is a direct, selective, and reversible inhibitor of activated coagulation factor X (factor Xa) and has been developed as an oral anticoagulant agent. Indications of DU-176b include: 1) Reduce the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation; 2) Treatment of deep vein thrombosis and pulmonary embolism; <sup>(b) (4)</sup>

(b) (4) The recommended maximal human dose is 60 mg once daily.

## Pharmacology

DU-176b inhibited human factor Xa in a competitive and selective manner with an inhibition constant (K<sub>i</sub>) of 0.561 nM. It inhibited factor Xa of rabbits and cynomolgus monkeys with similar K<sub>i</sub> values (0.457 and 0.715 nM, respectively), but its inhibition on rat factor Xa was less potent with a K<sub>i</sub> about 10 times higher (6.98 nM). DU-176b also inhibited the protease activity of the prothrombinase complex (composed of factor Xa, activated factor V, Ca<sup>2+</sup>, and phospholipids) with a chromogenic substrate S-2222 or a physiological substrate prothrombin with K<sub>i</sub> of 0.903 and 2.98 nM, respectively.

DU-176b prolonged the clotting time of human plasma in a concentration-dependent manner. The concentrations required to double prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time were 0.256, 0.508, and 4.95 μM, respectively. The potency of its anticoagulant activity is in the following order: PT > APTT > thrombin time. The concentrations of DU-176b required to double PT in the plasma of rats, rabbits, and cynomolgus monkeys were 0.647, 0.149, and 0.320 μM, respectively. Thus, the relative potency of the anticoagulant activity of DU-176b in different animal species paralleled that of its factor Xa inhibition activity. DU-176b inhibited the activated forms of mutant (Ala112Thr and Gly152Arg) recombinant human factor X with K<sub>i</sub> of 0.85 and 1.1 nM, respectively, which were similar to that for activated wild type recombinant human factor X (0.89 nM). DU-176b prolonged PT and APTT with a similar potency in factor X deficient human plasma supplemented with wild type or mutant (Ala112Thr or Gly152Arg) factor X.

The three metabolites of DU-176b (D21-1402-0201, D21-2135-0101, D21-2393) shared the parent drug's anti-factor Xa and clotting time prolongation activities. Among these active metabolites, the human specific metabolite D21-2393 (10% of the circulating parent drug) showed comparable anti-coagulant effects as DU-176b.

In various animal models, oral administrations of DU-176b resulted in dose-dependent anti-thrombosis, as manifested by reduced thrombus formation, as well as prolongation of clotting time. DU-176b also induced dose-dependent regression of venous thrombus (i.e., reduced mass/weights of formed thrombi). These antithrombotic effects of DU-176b were comparable with known antithrombotic agents, such as enoxaparin (a low molecular weight heparin, which inhibits both factor Xa and thrombin) and warfarin (vitamin K antagonist). In vitro, hemostatic agents reversed DU-176b-induced PT prolongation, without affecting the anti-factor Xa activity of DU-176b. However, a conclusion cannot be made on the antidote effect of plasma factors due to limitations of an in vitro study, and absence of in vivo evidence. DU-176b inhibited platelet aggregation induced by thrombin, possibly via inhibition of thrombin, since DU-176b did not affect ADP, U46619 or collagen-induced platelet aggregation.

The potential adverse effects of DU-176b on vital functions were investigated in a battery of safety pharmacology studies. Oral administration of DU-176b up to 200 mg/kg had no effects on CNS function in mice, no effects on behavior, cardiovascular parameters (including QTc), and respiratory parameters in monkeys, and no effects on renal function in rats. DU-176b at concentrations up to 20 µg/mL did not affect hERG potassium channel current or the action potential parameters of isolated guinea pig ventricular papillary muscles. The highest dose of 200 mg/kg or the highest concentration of 20 µg/ml corresponds to 16 times or 11 times the recommended clinical dose of a 60 mg of edoxaban tosylate hydrate on a body surface basis or predicted human C<sub>max</sub> basis (216.8 ng/mL, TMPP008, page 81), respectively.

In vivo nonclinical PK studies were conducted primarily in rats and cynomolgus monkeys, the same species used in the in vivo nonclinical pharmacology and toxicology studies. After a single oral administration of DU-176b to rats or cynomolgus monkeys, the plasma concentration of DU-176 increased rapidly with a mean T<sub>max</sub> of 0.5-3 hours; DU-176 C<sub>max</sub> and AUC<sub>0-24h</sub> were generally dose-proportional in rats, but under dose-proportional in cynomolgus monkeys; t<sub>1/2</sub> ranged from 0.7 to 5 hours in rats and 1.6 to 6.6 hours in cynomolgus monkeys; bioavailability was ~40% in rats and 55% in cynomolgus monkeys. After a single IV dose, plasma DU-176 concentrations rapidly declined with t<sub>1/2</sub> of 0.7 - 1.3 hours in rats and 1.5 - 2.2 hours in cynomolgus monkeys, and DU-176 exhibited moderate total body clearance and volume of distribution in both species.

After repeated daily oral doses of DU-176b to rats, monkeys, or mice for 14 days to 1 year in either PK or TK studies, C<sub>max</sub> and AUC were generally similar on day 1 and at the end of dosing period, indicating no accumulation, although DU-176 exposures after four-week repeated dosing in rats were 2 times those on day 1, and DU-176 exposures after 26-week repeated dosing in mice were <0.5 times those on day 1. Repeated daily doses of DU-176b resulted in slightly higher DU-176 exposures in female rats and mice than male rats and mice (1-2 folds), but showed no consistent gender difference in monkeys. There were no differences in C<sub>max</sub> and AUC values after repeated IV dosing or between sexes in rats or monkeys. The gender difference in rats and mice following oral dosing indicated higher first pass/liver metabolism in male vs. female rats and mice.

After a single oral dose of <sup>14</sup>C-DU-176b to albino rats, radioactivity was distributed broadly through the body except for the central nervous system. The level of radioactivity was high in digestive and urinary systems, but was low in the brain. Higher levels of radioactivity in the eyeball and skin than in other tissues were found in pigmented rats at 24 h post-dose or later and in cynomolgus monkeys at 336 h post-dose, and the t<sub>1/2</sub> for radioactivity in the eyeball of pigmented rats was long (approximately 260 h), indicating an affinity of DU-176 and/or its metabolite(s) to melanin-containing tissues. After a single oral dose of <sup>14</sup>C-DU-176b to pregnant rats, radioactivity crossed the placenta and distributed to the fetal tissues. Albino infant rats (postnatal 4 days, PND4) exhibited higher radioactivity concentrations in blood and tissues than juvenile (3 weeks old) and adult rats (6 weeks old) after a single oral dose of <sup>14</sup>C-DU-176b. Similar to adult pigmented rats, an affinity for melanin-containing

tissues in infant and juvenile pigmented rats was observed. In vitro studies demonstrated low protein binding of DU-176b (31.6 - 56.6%) in the plasma, and high red blood cell distribution of DU-176b (38-56%) in blood of rats, dogs, cynomolgus monkeys, and humans.

In rats and cynomolgus monkeys, unchanged DU-176 was the major radioactive compound in the plasma, urine, bile (tested only in rats), and feces after doses of <sup>14</sup>C-DU-176b. D21-3231 was the major metabolite in the plasma of both species. The other metabolites of DU-176 detected in rats were also found in cynomolgus monkeys, except D21-3221 that was found only in rats. However, D21-2393, a major metabolite in human plasma, was not detected in rat and monkey plasma, indicating that D21-2393 is a human-specific metabolite.

After a single oral dose of <sup>14</sup>C-DU176b to rats, 72.5% and 24.8% of the dose was excreted to the feces and urine, respectively, by 168 h post-dose. In bile-duct cannulated rats, 24.9% of dose was excreted to the bile. In rats, at least 24% of the radioactivity (including <sup>14</sup>C-DU-176 and its metabolites) excreted into the bile was reabsorbed, suggesting enterohepatic circulation. In cynomolgus monkeys, 51% and 42% of dose was recovered in the feces and urine, respectively, by 336 h post-dose. After a single oral dose of <sup>14</sup>C-DU-176b to lactating rats, radioactivity in milk was higher than in blood, and declined much slower than that in blood.

Following a single IV dose of <sup>14</sup>C-DU-176b, multi-drug resistance gene (mdr1a/1b) knock-out mice showed higher brain/plasma concentration ratio of DU-176, higher radioactivity concentrations in blood, plasma, and tissues, and less GI radioactivity excretion than those in wild-type mice. These results indicate that DU-176 is a substrate for mouse mdr1a/1b (mouse P-glycoprotein, P-gp) and mouse P-gp plays a role in the efflux of DU-176 from the central nerve system and in DU-176 absorption in GI.

D21-2393, a main metabolite in human plasma and a human specific metabolite, showed moderate plasma protein binding in rats (71.2% to 74.9%) and humans (80.0% to 81.9%), which was higher than those of DU-176. Infant and juvenile rats (PND3 and PND21, respectively) exhibited higher radioactivity concentrations in blood and tissues than those in adult rats after a single intravenous dose of <sup>14</sup>C-D21-2393. A D21-2393 affinity for melanin-containing tissues in infant and juvenile rats was also observed. TK data from studies with repeated oral doses with D21-2393 showed that D21-2393 systemic exposures were generally dose-proportional, similar between males and females, similar after first and last dose in adult animals, but markedly decreased after 7-week dosing in juvenile rats attributable to maturing metabolism associated with age.

## Toxicology

General toxicity of DU-176b was assessed in rats and cynomolgus monkeys following single or repeated oral or IV doses.

A single oral dose of DU-176b did not result in any animal deaths or abnormal clinical signs in rats at doses up to 2000 mg/kg or in cynomolgus monkeys at 400 mg/kg. Thus, the lethal doses of oral DU176b were over 2000 mg/kg for rats and over 400 mg/kg for monkeys. Prolongation of PT and APTT and a decrease in factor Xa activity were evident on the next day following a single oral dose of DU-176b at  $\geq 200$  mg/kg, and was most likely due to the pharmacological action of the test compound.

Repeated-oral dose toxicity studies in cynomolgus monkeys were carried out for 4 weeks with DU-176b doses of 10, 30, and 100 mg/kg/day, for 13 weeks with DU-176b doses of 6, 18, and 54 mg/kg/day, and for 52 weeks with DU-176b doses of 5, 15, and 45 mg/kg/day followed a 13-week recovery period. In the 4-week repeated-oral dose toxicity study, two of four female monkeys at 100 mg/kg/day were found prematurely dead, and this was attributed to hemorrhage in multiple tissues, particularly in the lung and thymus; one male at 100 mg/kg/day showed adrenal hemorrhage; monkeys of 30, and 100 mg/kg/day groups showed slight prolongation of PT and APTT, slight anemic changes, and higher urine protein. NOAEL for the 4-week study was 10 mg/kg/day. In the 13-week repeated-oral dose toxicity study, monkeys at doses 18 and 54 mg/kg/day showed slight prolongation of PT and APTT; 1/4 male and female monkeys at doses 18 and 54 mg/kg/day showed transient anemia, likely in response to hemorrhage events. NOAEL for the 13-week study was 6 mg/kg/day. In the 52-week repeated-oral dose toxicity study with a 13-week recovery period, premature deaths due to hemorrhage occurred 1/4 female monkeys and 1/4 male monkeys at 15 mg/kg/day, and 2/6 female monkeys at 45 mg/kg/day. Another 1/6 males and 1/6 females were prematurely sacrificed due to moribund without clear cause. Histopathological examination revealed pulmonary or GI hemorrhage in some of the prematurely died animals. One of the moribund females at 45 mg/kg/day showed prolonged menses and was extremely pale. These findings were likely attributable to the anticoagulant properties of DU-176b. Monkeys at doses 15 and 45 mg/kg/day showed slight prolongation of PT and APTT and anemic changes. There were no abnormal findings after the 13-week recovery period. NOAEL for the 52-week study was 5 mg/kg/day.

Repeated-oral dose toxicity studies in SD rats were carried out for 4 weeks with DU-176b doses of 6, 12, 18, 20, 60, and 200 mg/kg/day, and for 26 weeks with DU-176b doses of 6, 18, and 54 mg/kg/day followed by a 4-week recovery period. There were no remarkable findings at any tested dose level. NOAELs were 200 mg/kg/day for the 4-week study and 54 mg/kg/day for the 26-week study.

In a 2-week repeated-IV dose toxicity study in SD rats with DU-176b doses of 5 and 20 mg/kg/day, there were no treatment-related toxicities and the NOAEL was 20 mg/kg/day. In a preliminary 14-day repeated dose toxicity study with IV D11-4176a (anhydrous edoxaban) at 10 mg/kg/day, increases in AST, ALT, and ALP, and acidophilic change in hepatocytes were noted in 1 of 2 female monkeys at 10 mg/kg/day (with  $C_{5\text{ min}}$  52.6 times human exposure at the MRHD of 60 mg). In a 2-week repeated-IV dose toxicity study in cynomolgus monkeys with DU-176 doses of 1 and 4 mg/kg/day, bloody stool and purpura with hemorrhage in the subcutis around the blood sampling

site were observed in males at 4 mg/kg/day. Females at 4 mg/kg/day showed anemia. The NOAEL was 1 mg/kg/day.

These hemorrhagic and hemorrhagic-related findings in cynomolgus monkeys following oral and IV administration of DU-176b were the primary adverse effect and the lone dose-limiting toxicity for DU-176b. No ocular anatomical abnormality was observed at any dose level evaluated in the repeated-dose toxicity studies in rats and cynomolgus monkeys.

Potential genotoxicity of DU-176b was evaluated in both in vitro and in vitro assay systems. DU-176b was negative in a bacterial reverse mutation test, an in vitro micronucleus test in human peripheral lymphocytes, bone marrow micronucleus tests in rats using a single or repeated oral administration or repeated IV administration and in a 4-week repeated oral toxicity study in monkeys, a hepatocyte micronucleus test and a unscheduled DNA synthesis test in rats using a single oral administration. However, DU-176b increased polyploidy in a chromosomal aberration test using CHL cells at 1250 and 2500 µg/ml in the presence of S9 liver fraction, and increased polyploidy in a follow up study focused solely on numerical chromosomal aberrations using human peripheral lymphocytes at  $\geq 313$  µg/ml in the presence or absent of S9 liver fraction. DU-176b did not affect the incidence of structural aberrations in the two chromosomal aberration test systems, and the induction of polyploidy was associated with mild (for CHL, ~50% cell proliferation ratio) to strong (for human peripheral lymphocytes, mitotic index reduction up to 83%) toxicity.

The carcinogenic potential of DU-176b was evaluated in a medium-term liver carcinogenesis bioassay in rats, and in standard 2-year mouse and rat carcinogenicity studies. Dose range-finding studies in mice and rats with oral DU-176b up to 1500 mg/kg/day did not identify toxicity other than hemorrhage-related deaths and findings which was attributable to the excessive pharmacological anticoagulant effect of the test article. Oral DU-176b did not increase the number or area of GST-P-positive foci per liver section in rats at doses up to 20 mg/kg/day, and showed no evidence of increased neoplasia in the 2-year mouse study with doses of 50, 150, and 500 mg/kg/day or in the 2-year rat study with doses of 60, 200, and 600/400 mg/kg/day for males and 50, 100, and 200 mg/kg/day for females. NOAELs for carcinogenicity were 500 mg/kg/day for mice, 400 mg/kg/day for male rats, and 200 mg/kg/day for female rats -

- In the 2-year mouse carcinogenicity study with oral daily dosing of DU-176b, there were higher incidences of adrenal cortex subcapsular cell adenoma in low dose male mice (4/65) and whole body cavity hemangiosarcoma in medium dose female mice (4/65) when compared to their respective controls, but none of tumor incidences showed statistically significant dose-response relationships. Since higher incidences of these two tumors were not dose-dependent, not seen in the high dose groups, not seen in both sexes, and the incidence of whole body cavity hemangiosarcoma (4/65) was within the vendor's histological background range of 1.67-12% (2), higher incidences of adrenal cortex subcapsular cell adenoma in in low dose male mice and whole body cavity hemangiosarcoma in medium dose

female mice were not considered to be toxicologically significant. No other tumor types had p-values  $\leq 0.05$  for dose-response relationships or pairwise comparisons of treated groups and controls. A significant increase in mortality was noted in the 500-mg/kg/day males and in the 150-mg/kg/day females. Higher incidences of thin appearance, tremor body, hypoactivity, and audible or irregular breath were seen in males at 500 mg/kg/day. For both males and females, higher incidences of pale ears/entire body, red ears and lower body weight were observed at 500 mg/kg/day. Findings in clinical signs were associated with moribundity. A marginal increase in ovarian hematocysts occurred in all DU-176b-treated female groups compared with the control group, which was considered to be the cause of 4 female premature deaths at 500-mg/kg/day, and was at least in part attributed to the pharmacological action of the test article as an anticoagulant agent (factor Xa inhibitor). NOAELs for carcinogenicity were 500 mg/kg/day for males and females. NOAELs for general toxicity were 150 mg/kg/day for males and 50 mg/kg/day for females.

- In the 2-year rat carcinogenicity study with oral daily dosing of DU-176, there was no evidence of increased neoplasia at any dose level. Mortality was significantly higher in males at the 600/400 mg/kg/day dose, and marginally higher in females at 200 mg/kg/day. There was a higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis in males at 600/400 mg/kg/day, and liver centrilobular hepatocellular degeneration/necrosis may have been the cause for 8 of the 50 unscheduled male deaths in the 600/400 mg/kg/day males. There were slightly but statistically lower red cell counts in females at 200 mg/kg/day, higher incidences of red oral and nasal discharge, and red haircoat in males at DU-176b 600/400 mg/kg/day. Similar clinical signs of less extent were also seen in females at DU-176b 200 mg/kg/day. The findings in hematology and clinical signs may, at least in part, be attributed to bleeding, the pharmacological action of the test article as an anticoagulant (factor Xa inhibitor). NOAELs for carcinogenicity were 600/400 mg/kg/day for males and 200 mg/kg/day for females. NOAELs for general toxicity were 200 mg/kg/day for males and 100 mg/kg/day for females.

The reproductive and developmental toxicity potential of DU-176b was evaluated in fertility and early embryonic development studies in rats, embryo-fetal development studies in rats and rabbits, and a pre- and postnatal developmental study and a juvenile toxicity study in rats. Oral DU-176b at doses up to 1000 mg/kg/day did not affect mating and fertility parameters in rats. DU-176b was not teratogenic in rats at doses up to 300 mg/kg/day or in rabbits at doses up to 600 mg/kg/day, but was maternal and embryo-fetal toxic at mid and/or high doses in both rats and rabbits. In rat embryo-fetal development studies, dam vaginal hemorrhage and higher post-implantation loss were observed at DU-176b  $\geq 300$  mg/kg/day. Both maternal and embryo-fetal NOAELs were 100 mg/kg/day in rats. In rabbit embryo-fetal development studies, DU-176b at doses  $\geq 200$  mg/kg/day resulted in dam deaths and abortion, decreased food consumption and body weight, and hemorrhage in uterus, more post-implantation loss, less live fetuses, lower fetal weight, and increased variation in the gall bladder. Increased 13th full ribs and 27 presacral vertebrae occurred in rabbits at DU-176b 600 mg/kg/day. Both maternal and embryo-fetal NOAELs were 60 mg/kg/day in rabbits. Because of maternal

and embryo-fetal toxicities were observed at the same dose level, DU-176b-associated embryo-fetal toxicity in rats and rabbits was considered to be secondary effects of maternal toxicity, rather than a direct DU-176b effect.

In the pre- and postnatal development study in rats with oral daily DU-176b doses of 3, 10, and 30 mg/kg/day during gestation day 7 to lactation day 20, vaginal bleeding was noted in 1 F0 dam each on Day 16 or Day 17 of pregnancy in the 30 mg/kg group. F1 females at 30 mg/kg group showed delayed avoidance response in the learning test (the shuttle box test). NOAELs in this study were 10 mg/kg/day for maternal general toxicity, and 10 mg/kg/day for maternal reproduction and F1 development.

Findings in the juvenile rat toxicity study with oral daily DU-176b doses of 2, 6, and 20 mg/kg/day during PND 4 - PND 49 included moderately lower body weight at the dose 20 mg/kg/day compared to controls, which were <9%, and associated with or without less food consumption. NOAEL was 6 mg/kg/day. This study is deficient because extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted, and the fertility, mating ability and reproductive performance were not specifically evaluated.

Photosafety and eye function studies were performed because DU-176b distributed to the ocular and skin tissues and bound to melanin in pigmented rats and monkeys as well as showing some absorption for light wavelengths greater than 290 nm ( $\lambda_{\max}$ : 292 nm). Phototoxic potential was evaluated in an in vitro photochromosomal aberration test in CHL cells with DU-176b up to 2.5 mg/ml and in an in vitro phototoxicity test in BALB/3T3 cells with DU-176b up to 1 mg/ml. DU-176b did not show any phototoxic potential in either study. In a 39-week eye function test in male and female cynomolgus monkeys orally given DU-176b at 15 mg/kg/day, DU-176b did not affect any eye function parameters. But, general toxicity including bleeding-related findings (blood on the surface of feces and pale mucous membrane in the oral cavity associated with menses) and lower body weight gain were observed at 15 mg/kg/day of DU-176b in the 39-week monkey study.

Metabolite D21-2393, a major metabolite in humans but only traceable in animals, was tested for potential toxicity in general, embryo-fetal developmental, and juvenile toxicity studies in rats, and in genotoxicity studies. There were no D21-2393 findings in repeated oral dose toxicity studies in rats with doses up to 600 mg/kg/day for 3 months. D21-2393 induced numerical aberrations in CHL cells at  $\geq 1250$   $\mu\text{g/ml}$  with or without S9-activation system, but was negative in Ames test at concentrations up to 5000  $\mu\text{g/ml}$  with or without metabolic activation, in polyploidy test using human peripheral lymphocytes, and in in vivo bone marrow micronucleus studies with single or repeated oral D21-2393 doses up to 2000 mg/kg/day for up to 14 days. There were no D21-2393-related findings in rat embryo-fetal studies at doses up to 1000 mg/kg/day or in juvenile rat studies at doses up to 200 mg/kg/day for up to 7 weeks. However, extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted, and fertility, mating ability, and reproductive performance were not specifically evaluated in juvenile rat studies.

## Conclusions

The nonclinical profile of DU-176b and its main human specific metabolite D21-2393 were investigated in a series of pharmacological, pharmacokinetic, and toxicological studies. The nonclinical studies were generally well designed and conducted, except for the following defects: 1) in the bone marrow micronucleus assay for DU-176b in the 4-week repeated oral dose toxicity study in monkey due to the absent of positive control and relevant criteria, 2) in the 26-week repeated oral dose toxicity study with DU-176b in rats due to the insufficient dose level, and 3) in the juvenile rat studies with DU-176b or D21-2393 due to no comprehensive neurological and reproductive assessments. These defects do not have a significant impact on the preclinical safety profile of DU-176b since relevant data are available from bone marrow micronucleus tests with DU-176b in rat repeated oral dose toxicity studies, a 2-year carcinogenicity study in rats, and pre- and post-neonatal studies with DU-176b in rats.

Findings and NOAELs from pivotal GLP toxicological studies are summarized in Table 93. DU-176b and its main human specific metabolite D21-2393 showed similar safety profiles. Issues identified included (I) increased polyploidy in chromosomal aberration tests that associated with cell toxicity; (II) hemorrhage secondary to exaggerated anticoagulant effects of DU-176b, which is the intended pharmacological action; (III) more post-implantation loss, less live fetuses, lower fetal weight, increased gall bladder and skeletal variations, and delayed avoidance response in a learning test in F1 females, which were associated with maternal hemorrhagic toxicity; and (IV) higher mortality in male animals at high dose in the 2-year carcinogenicity studies that was associated with higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis in rats.

Table 93. Summary of pivotal GLP toxicological studies and safety margins

| Test article                | Type of Study                             | Species /Strain                     | Dose (mg/kg/day) and Duration   | Noteworthy Findings and NOAEL (Cmax in ng/ml, AUC <sub>0-24</sub> in ng.hr/ml)   | Safety Margin*   |                      |
|-----------------------------|---|-------------------------------------|---|--|--|----------------------|
|                             |   |                                     |   |  | /Cmax  | /AUC <sub>0-24</sub> |
| DU-176b (oral)              | Single dose toxicity                      | Rat/SD                              | 0, 1000, or 2000, once  | No findings. NOAEL 2000 mg/kg  | 323 <sup>§</sup>   |                      |
|                             |   | cynomolgus Monkey                   | 0, 200, or 400, once  | Anticoagulant activity and related hematology findings at 400 mg/kg. NOAEL 200 mg/kg   | 65 <sup>§</sup>  |                      |
|                             | Repeated dose toxicity                    | Rat/SD                              | 0, 20, 60, or 200 for 4 weeks   | No findings. NOAEL 200 mg/kg/day (Cmax M2520 and F4520, AUC M23745 and F44808)   | M: 9<br>F: 16  | M: 13<br>F: 23       |
|                             |   | Rat/SD                              | 0, 6, 18, or 54 for 26 weeks  | No findings. NOAEL 54 mg/kg (Cmax M1222 and F2490, AUC M5459 and F9015)  | M: 4.5<br>F: 8.3   | M: 2.8<br>F: 4.6     |
|                             |   | cynomolgus Monkey                   | 0, 10, 30, or 100 for 4 weeks   | Deaths at 100 mg/kg/day, hemorrhage and related findings at ≥ 30 mg/kg/day. NOAEL 10 mg/kg/day (Cmax M358 and F328, AUC M3273 and F3269)   | M: 1.3<br>F: 1.1   | M: 1.7<br>F: 1.7     |
|                             |   | cynomolgus Monkey                   | 0, 6, 18, or 54 for 13 weeks  | Anticoagulant activity and related hematology findings at ≥18 mg/kg/day. NOAEL 6 mg/kg/day (Cmax M357 and F366, AUC M3322 and F3326)   | M: 0.9<br>F: 1.1   | M: 1.8<br>F: 1.8     |
|                             |   | cynomolgus Monkey                   | 0, 5, 15, or 45 for 52 weeks  | Deaths, hemorrhage and related findings at ≥ 15 mg/kg/day. NOAEL 5 mg/kg/day (Cmax M263 and F327, AUC M3370 and F3237)   | M: 0.9<br>F: 1.1   | M: 1.8<br>F: 1.7     |
|                             | Carcinogenic Studies                      | Rat/SD                              | 0, 60, 200, or 600/400 for males; 0, 50, 100, or 200 for females, 2 year                          | Carcinogenicity: negative. NOAEL: M 600/400 mg/kg/day and F 200 mg/kg/day (Cmax M1055 and F4045, AUC M15086 and F27796)<br>General toxicity: Higher mortality, centrilobular hepatocellular degeneration/necrosis, and hemorrhage-related findings at high dose. NOAEL: 200 and 100 mg/kg/day respectively for males and females (Cmax M1395 and F3223, AUC M13763 and F11782)   | M: 3.8<br>F: 14  | M: 7.8<br>F: 15      |
|                             |   | Mouse/CD1                           | 0, 50, 150, or 500 for 2 year   | Carcinogenicity: negative. NOAEL: 500 mg/kg/day for both sexes (Cmax M829 and F1707, AUC M6003 and F12320)<br>General toxicity: Higher mortality in males at 500 mg/kg/day and in females at 150 mg/kg/day; hemorrhage-related findings at 500 mg/kg/day. NOAEL: 150 and 50 mg/kg/day respectively for males and females (Cmax M634 and F308, AUC M2036 and F1356)   | M: 3<br>F: 6   | M: 3.1<br>F: 6.3     |
|                             |   | Rat/F344                            | 0, 5, 10, or 20 for 6 weeks   | Negative in rat medium-term liver bioassay. NOAEL 20 mg/kg/day (C <sub>11r</sub> M1810)  | 6  |                      |
|                             | Fertility and Early Embryonic Development | Rat/SD                              | 0, 100, 300, or 1000 for 2 weeks before mating to copulation (M), and until GD7 (F)               | Negative. NOAEL 1000 mg/kg/day   | M: 160 <sup>§</sup><br>F: 160 <sup>§</sup>                   |                      |
|                             | Embryo-Fetal Development                  | Rat/SD                              | 0, 30, 100, or 300 for GD7 to GD17  | No teratogenic effects. Dam vaginal hemorrhage at 300 mg/kg/day, higher post-implantation loss at 300 mg/kg/day. NOAEL 100 mg/kg/day for maternal and fetal toxicity.  | Maternal: 16 <sup>§</sup><br>Fetal toxicity: 16 <sup>§</sup> |                      |
|                             |   | Rabbit/NZW                          | 0, 60, 200, or 600 for GD7 to GD20  | No teratogenic effects. Dam deaths and abortion, decreased food consumption, body weight, and defecation, and increased dark red contents of the uterus at ≥ 200 mg/kg/day (Cmax 7180, AUC 39473). Increased postimplantation loss, decreased live fetuses, decreased fetal weight, and increased variation at ≥ 200 and/or 600 mg/kg/day (AUC 95025). NOAEL 60 mg/kg/day (Cmax 5830, AUC 15468) for maternal and fetal toxicity . | 20   | 8                    |
|                             | Pre- and Postnatal Development            | Rat/SD                              | 0, 3, 10, or 30 from GD 7 – lactation Day 20  | F0 vaginal bleeding at 30 mg/kg/day. Delayed avoidance response in F1 females at 30 mg/kg/day. NOAEL 10 mg/kg/day for maternal and fetal toxicity (Cmac 817, AUC 2060)   | 2.9  | 1                    |
| Juvenile Study <sup>†</sup> | Rat/SD                                    | 0, 2, 6, or 20 from PND 4 to PND 49 | Lower body weight at 20 mg/kg/day. NOAEL 6 mg/kg/day (Cmax M193 and F341, AUC M1060 and F1240)    | M: 0.7<br>F: 1.2   | M: 0.5<br>F: 0.6   |                      |
| Eye function study          | cynomolgus Monkey                         | 0 or 15 for 9 months                | Negative in eye function assessment. NOAEL 15 mg/kg/day (Cmax M706 and F827, AUC M8430 and F8550) | M: 2.5<br>F: 2.9   | M: 3.8<br>F: 3.9   |                      |
| D21-2393 (oral)             | Repeated dose toxicity                    | Rat/SD                              | 0, 60, 200, or 600 for 3 months   | Negative. NOAEL 600 mg/kg/day (Cmax M314 and F177, AUC M1757 and F1300)  | M: 10<br>F: 6  | M: 12<br>F: 9        |
|                             | Embryo-fetal Development                  | Rat/SD                              | 0, 200, 600, or 1000 from GDs 7 to 17   | Negative. NOAELs 1000 mg/kg/day for maternal and embryo toxicity (Cmax 581 and AUC 4540)   | 20   | 33                   |
|                             | Juvenile Study <sup>†</sup>               | Rat/SD                              | 0, 20, 60, or 200 during PNDs 4-49  | NOAEL 200 mg/kg/day (Cmax M163 and F86, AUC M1020 and F554)  | M: 5<br>F: 3   | M: 7<br>F: 4         |

\* Based on (mean) DU-176 Cmax 289 ng/ml, AUC<sub>0-24</sub> 1940 ng.h/ml (Clinical Study Report DU176b-A-U151, page 80), D21-2393 Cmax 22.2 ng/ml, and AUC<sub>0-24</sub> 147 ng.h/ml (Clinical Study Report DU176b-PRT019, page 61) in human subjects given DU-176b at the maximum recommended clinical dose of 60 mg. M: males. F: females. §: based on body surface. † Defects due to incomprehensive neurological and reproductive assessments. GD: gestation day. PND: postnatal day.

Numerical chromosome aberrations (polyploidy) observed in DU-176b or D21-2393-treated CHL cells and human peripheral lymphocytes were the only positive finding among a battery tests for genotoxicity. The increased polyploidy in chromosomal aberration test using human peripheral lymphocytes was associated with cell toxicity (mitotic index reduction up to 83%), which lowered the weight for genotoxic potential. Meanwhile, DU-176b did not show any genotoxic potential in both in vitro micronucleus test using human peripheral lymphocytes and in vivo bone marrow micronucleus tests in rats and liver micronucleus test in rats, indicating that DU-176b has little potential to induce aneuploidy. Although aneuploidy is known to be correlated with cancer and birth defect (1), negative results in several micronucleus tests and in an unscheduled DNA synthesis test in rat liver demonstrated no damage to chromosomes or DNA in these in vitro and in vivo studies. Based upon a weight of evidence approach, DU-176b is not considered to pose a genotoxic risk.

In repeated-dose oral toxicity studies, 2-year carcinogenicity studies, and reproductive and developmental studies, hemorrhagic findings and anemia were noted in monkeys at DU-176b doses of  $\geq 15$  mg/kg/day, in mice at 500 mg/kg/day, in rats at  $\geq 200$  mg/kg/day, and in rabbits at  $\geq 30$  mg/kg/day, leading to deteriorated animal condition or animal deaths. These findings are thought to be the exaggerated anticoagulant effect of DU-176b (its principal pharmacological action), which constitutes the dose-limiting toxicity for this compound. Based on the mean  $AUC_{0-24h}$  values at the NOAELs in the repeated-dose toxicity studies, the cynomolgus monkey was approximately 2-10 times more sensitive to the drug-related hemorrhagic effects than the rat. Pharmacology studies demonstrated that in vitro inhibitory activity of DU-176b for cynomolgus monkey factor Xa and anticoagulant activity of DU-176b in cynomolgus monkey plasma were approximately 10 times and 2 times more potent than those for the rat factor Xa and in rat plasma, respectively. These data support the interpretation that the species difference in drug potency leads, at least in part, to the difference in the frequency of DU-176b hemorrhagic events in the two toxicity species. Since the pharmacological activity of DU-176b in the cynomolgus monkey was comparable to that in humans, safety margins for hemorrhagic risk were estimated by comparison of exposures between cynomolgus monkeys and humans. The mean  $AUC_{0-24h}$  values at NOAEL in the 52-week repeated dose oral toxicity study in cynomolgus monkeys were approximately 1.5 times the exposures in human subjects given DU-176b at the maximum recommended clinical dose of 60 mg.

DU-176b was embryo-fetal toxic and developmental toxic in both rats and rabbits: higher post-implantation loss in rats at DU-176b  $\geq 300$  mg/kg/day [ $\sim 48$  times the human exposure at maximum recommended human dose (MRHD) of 60 mg/day based on total body surface area in  $mg/m^2$ ]; more post-implantation loss, less live fetuses, lower fetal weight, and increased variation in the gall bladder in rabbits at  $\geq 200$  mg/kg/day ( $\sim 63$  times the human exposure at MRHD of 60 mg/day based on  $mg/m^2$ ), and increased 13th full ribs and 27 presacral vertebrae in rabbits at 600 mg/kg/day ( $\sim 190$  times the human exposure at MRHD of 60 mg/day based on  $mg/m^2$ ); delayed avoidance response during a learning test in F1 female rats at 30 mg/kg/day ( $\sim 2.9$  times the

human exposure at adult MRHD of 60 mg/day based on  $AUC_{0-24h}$ ), and moderately lower body weight in juvenile rats at 20 mg/kg/day (~2.2 times the human exposure at adult MRHD of 60 mg/day based on  $AUC_{0-24h}$ ). Maternal toxicity including dam deaths and abortion, decreased food consumption and body weight, hemorrhage in uterus, or vaginal hemorrhage occurred at the same or lower DU-176b doses that led to embryo-fetal/developmental toxicity. Thus, DU-176b-associated embryo-fetal toxicity in rats and rabbits and developmental toxicity in rats were considered to be secondary effects of maternal toxicity, rather than a direct DU-176b effect.

DU-176 systemic exposures following repeated oral daily doses of DU-176b were slightly higher in female rats and mice than in male rats and mice (~2 folds). In the 2-year rat carcinogenicity study, DU-176 systemic exposures were still higher in females than in males although low dose levels were used for females. While systemic DU-176 exposure, especially  $C_{max}$ , was lower in males than in females, male rats showed higher mortality in the 2-year carcinogenicity study, which was associated with higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis. Compared to females, higher mortality with lower DU-176 exposure level in male rats could not be explained by direct drug toxicity other than metabolism-related toxicity. Since the gender difference in DU-176 systemic exposure was not seen in rats following single and repeated daily IV doses, lower DU-176 systemic exposure in male rats than in female rats following oral doses indicates higher first-pass effect in males. Histological findings of higher incidence and greater severity of centrilobular hepatocellular degeneration/necrosis in prematurely died rats indicated liver toxicity of the drug in rats, although centrilobular hepatocellular degeneration/necrosis was not presented in monkeys or mice. These data together imply that male rats had higher liver DU-176 metabolite rate (first-pass), which led to low systemic exposure; DU-176 metabolic processes in liver were toxic, long term, persistent, and excessive DU-176 metabolic processes in liver led to centrilobular hepatocellular degeneration/necrosis, and contributed to higher mortality. Therefore, liver toxicity is a potential safety issue for long-term high dose DU-176b along with increased liver metabolism, although liver toxicity was not seen in mice or monkeys orally administered with DU-176b.

## 12 References

1. Aardema MJ, Albertini S, Arni P, et al. Aneuploidy: a report of an ECETOC task force. *Mutat Res.* 1998; 410: 3-79.
2. Charles River Laboratories: Spontaneous Neoplastic Lesions in the Crl:CD-1(ICR) Mouse in Control Groups from 18 Month to 2 year Studies. 2005.  
[http://www.criver.com/files/pdfs/rms/cd1/rm\\_rm\\_r\\_lesions\\_crlcd\\_1\\_icr\\_mouse.aspx](http://www.criver.com/files/pdfs/rms/cd1/rm_rm_r_lesions_crlcd_1_icr_mouse.aspx)

## 13 Appendix / Attachments

Appendix I, pages 149-232  
Appendix II, pages 233-271  
Appendix III, pages 272-298  
Appendix IV, pages 299-325

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**IND number:** 63,266

**Review number:** 1

**Sequence number/date/type of submission:** 000/May 27, 2004/Initial

**Date of FDR receipt:** June 3, 2004

**Information to sponsor:** Yes (x) No ( )

**Sponsor and/or agent:** Daiichi Medical Research, Inc.  
Montvale, New Jersey

**Manufacturer for drug substance:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Reviewer name:** David B. Joseph, Ph.D.

**Division name:** Gastrointestinal Products

**HFD #:** 180

**Review completion date:** April 4, 2007

#### Drug:

Trade name: none

Generic name: none

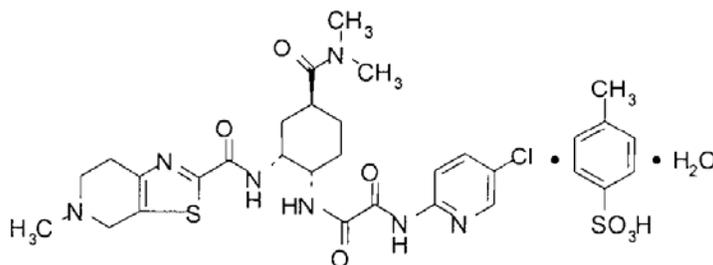
Code names: DU-176b; D11-4176b

Chemical name: N-(5-Chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]ethanediamide p-toluenesulfonate monohydrate

CAS registry number: 480449-71-6 (anhydrous free base)

Molecular formula/molecular weight:  $C_{24}H_{30}ClN_7O_4S \cdot C_7H_8O_3S \cdot H_2O/738.27$

Structure:



**Relevant INDs/NDAs/DMFs:** none

**Drug class:** anticoagulant/anti-thrombotic

**Intended clinical population:** [REDACTED] (b) (4)

**Clinical formulation:** Tablets containing 40.4 mg DU-176b (30 mg DU-176). The ingredients are shown in the table below.

|                                      | mg/tablet         |
|--------------------------------------|-------------------|
| <b>Active Ingredient</b>             |                   |
| DU-176b                              | 40.4 <sup>a</sup> |
| <b>Inactive Ingredients</b>          |                   |
| (b) (4) Mannitol                     | (b) (4)           |
| (b) (4) Pregelatinized Starch        | (b) (4)           |
| Crospovidone                         | (b) (4)           |
| Hydroxypropylcellulose               | (b) (4)           |
| Magnesium Stearate                   | (b) (4)           |
| Hydroxypropylmethylcellulose (b) (4) | (b) (4)           |
| Talc <sup>b</sup>                    | (b) (4)           |
| Macrogol (b) (4)                     | (b) (4)           |
| Titanium Oxide <sup>b</sup>          | (b) (4)           |

a: equivalent to 30 mg anhydrous free base (DU-176)

b: Present in coating

**Route of administration:** oral

**Proposed clinical protocol:**

The Sponsor will perform a Phase 1, open-label study in healthy volunteers to assess the pharmacodynamic effects of DU-176b, using an *ex vivo* model of thrombosis. The study will include 12 healthy male volunteers, age 20-50 years. Each subject will be treated with a single oral administration of 80.8 mg DU-176b (60 mg DU-176). The primary objective will be the measurement of thrombogenic activity using the Badimon chamber perfusion method, in which venous blood from the antecubital vein of the subject's arm is allowed to flow directly into a perfusion chamber containing a thrombogenic surface (porcine aorta tunica media). Measurement of thrombogenesis will be performed at 0 (pre-dose), 1.5, 5, and 12 hr post-dose. Secondary objectives will include safety evaluation and the measurement of thrombin generation, anti-Xa activity, PT/INR, aPTT, and plasma drug concentration. Safety parameters will include physical examination, vital signs, ECG, hematology, clinical chemistry, and fecal occult blood test.

**Previous clinical experience:**

Two Phase 1 studies have been conducted on DU-176b in the United Kingdom. The initial study was performed on healthy male volunteers, age 18-55 years old, and was placebo-controlled. This study included a dose-escalation single-dose phase and an ascending multiple dose phase. The objectives were to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of a single oral dose of 10, 30, 60, 90, 120, or 150 mg in the first phase, and of 90 mg od, 60 mg bid, or 120 mg od given for 10 days in the second phase. The drug was well tolerated at all dose levels in both phases of the study. A Phase I study was performed on healthy postmenopausal or surgically sterile female volunteers and healthy elderly male volunteers. The objectives of this study were to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of DU-176b using repeated dosing. Females were age 18-65 years, and males were age 65 years and over. The study subjects were treated orally with placebo or 90 mg/day on day 1 and on days 3-10. No serious adverse events occurred. Minor bleeding episodes were observed in the drug-treated volunteers. Headache was the most frequent adverse event.

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

| Preclinical Study   | Testing Laboratory  | Study #                     | Lot #  | Page |
|---|---|-----------------------------|--|------|
| <b>PHARMACOLOGY</b>   |   |                             |  | 8    |
| <b>SAFETY<br/>PHARMACOLOGY</b>  |   |                             |  | 15   |
| <b>PHARMACOKINETICS/<br/>TOXICOKINETICS</b>                                       |   |                             |  |      |
| <b>Absorption Study of<br/>[<sup>14</sup>C]DU-176b in Male Rats</b>               | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3435-G<br>(MET-20020653) | CP-2667                                      | 19   |
| <b>Absorption of [<sup>14</sup>C]DU-176b<br/>in Male Cynomolgus<br/>Monkeys</b>   | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3437-G<br>(MET-20020685) | CP-2667                                      | 20   |
| <b>Distribution of [<sup>14</sup>C]DU-176b<br/>in Male Cynomolgus<br/>Monkeys</b> | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3437-G<br>(MET-20020685) | CP-2667                                      | 21   |
| <b>Whole-Body<br/>Autoradiography Study in<br/>Male Rats</b>                      | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3448-G<br>(MET-20020655) | CP-2667                                      | 22   |
| <b>Metabolism Study in Rats<br/>and Monkeys</b>                                   | (b) (4)   | 03-043<br>(MET-20020973)    | CP-2667                                      | 23   |
| <b>Structural Characterization<br/>of Metabolites in Rats and<br/>Monkeys</b>     | (b) (4)   | 03-140<br>(MET-20030202)    | CP-2667<br>(labeled)<br>CZ001<br>(unlabeled) | 26   |
| <b>Excretion Study in Male Rats</b>   | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3436-G<br>(MET-20020654) | CP-2667                                      | 28   |
|   |   |                             |  |      |
| <b>Excretion of [<sup>14</sup>C]DU-176b in<br/>Male Cynomolgus Monkeys</b>        | ADME/TOX Research<br>Institute<br>Daiichi Pure Chemicals<br>Co., Ltd.<br>Ibaraki, Japan | AE-3437-G<br>(MET-20020685) | CP-2667                                      | 28   |
| <b>GENERAL TOXICOLOGY</b>   |   |                             |  |      |
| <b>D11-4176b: Single Oral<br/>Toxicity in Rats</b>                                | Daiichi Pharmaceutical<br>Co., Ltd.<br>Tokyo, Japan                                     | 20020310<br>(0996)          | CZ001  | 31   |
| <b>D11-4176b: Single Oral<br/>Toxicity in Cynomolgus<br/>Monkeys</b>              | Daiichi Pharmaceutical<br>Co., Ltd.<br>Tokyo, Japan                                     | 20020307<br>(0997)          | CZ001  | 32   |
| <b>D11-4176b: Four-Week Oral<br/>Toxicity in Rats</b>                             | (b) (4)   | 6630-119                    | DZ002  | 33   |
|   |   |                             |  |      |

|  |  |  |           |    |
|--|--|--|-----------|----|
| <b>D11-4176b: Four-Week Oral Toxicity in Male Rats – Additional Study</b>  | (b) (4)  | 6630-132                                       | DZ002     | 37 |
| <b>D11-4176b: 4-Week Oral Toxicity in Cynomolgus Monkeys</b>   | (b) (4)  | 6630-120                                       | DZ002     | 39 |
| <b>GENETIC TOXICOLOGY</b>  |  |  |           |    |
| <b>D11-4176b: Bacterial Reverse Mutation Test</b>  | (b) (4)  | SBL63-54                                       | CZ001     | 45 |
| <b>D11-4176b: In Vitro Chromosomal Aberration Test in CHL Cells</b>  | (b) (4)  | SBL63-55                                       | CZ001     | 47 |
| <b>DU-176b: Polyploidy Test in Cultured Human Peripheral Blood Lymphocytes</b>   | (b) (4)  | 6630-142<br>(23563-0-449OECD)                  | CA201     | 50 |
| <b>In Vitro Micronucleus Test in Cultured Human Lymphocytes</b>  | (b) (4)  | ORE 065/033611                                 | CA201     | 55 |
| <b>D11-4176b: Micronucleus Test in Rats Following Oral Administration</b>  | (b) (4)  | 6630-123<br>(23563-0-454OECD)                  | DZ002     | 57 |
| <b>D11-4176b: Bone Marrow Micronucleus Test in Rats Following Twice Intravenous or Oral Administration</b>                   | Daiichi Pharmaceutical Co., Ltd.<br>Tokyo, Japan | 20020063<br>(1314-105)                         | MO2427-01 | 58 |
| <b>DU-176b: Liver Micronucleus Test in Rats Following a Single Oral Administration</b>                                       | Daiichi Pharmaceutical Co., Ltd.<br>Tokyo, Japan | 20030465<br>(1036)                             | CA201     | 59 |
| <b>Micronucleus Test in a 4-Week Oral Toxicity Study in Cynomolgus Monkeys</b>   | (b) (4)  | 6630-120                                       | DZ002     | 61 |
| <b>D11-4176b: Unscheduled DNA Synthesis Test in Rat Liver Following Oral Administration</b>                                  | (b) (4)  | 6630-125<br>(23563-0-494OECD;<br>TOX-20020498) | DZ002     | 62 |
| <b>REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY</b>   |  |  |           |    |
| <b>DU-176b: Oral Study of Effects on Fertility and Early Embryonic Development to Implantation in Rats (Segment I Study)</b> | Daiichi Pharmaceutical Co., Ltd.<br>Tokyo, Japan | 1035<br>(20030552)                             | CA201     | 64 |
| <b>DU-176b: Oral Study for Effects on Embryo-Fetal Development in Rats (Segment II Study)</b>                                | Daiichi Pharmaceutical Co., Ltd.<br>Tokyo, Japan | 1034<br>(20030532)                             | CA201     | 66 |
| <b>DU-176b: A Study of the Effects on Embryo/Fetal Development in Rabbits (Segment II Study)</b>                             | (b) (4)  | (b) (4)-147011<br>(TOX-20040022)               | CA201     | 68 |
|  |  |  |           |    |

|  |         |                        |       |    |
|--|---------|------------------------|-------|----|
| <b>SPECIAL TOXICOLOGY STUDIES</b>  |         |                        |       |    |
| <b>DU-176b: Oral Medium-Term Liver Carcinogenesis Bioassay in Male F344 Rats</b> | (b) (4) | 0332<br>(TOX-20030708) | CA201 | 75 |

Studies not reviewed within this submission: None.

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## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

DU-176b is a highly potent inhibitor of factor Xa in humans, Cynomolgus monkeys, rabbits, and rats. The drug exhibited anti-thrombotic activity in two models of rat venous thrombosis and in a rat A-V shunt model. Anti-thrombotic activity occurred at oral dose levels as low as 0.5 mg/kg (depending on the model), and was associated with inhibition of factor Xa. Thus, factor Xa inhibition appears to be the primary mechanism of action. Although the drug exhibited anticoagulant activity *in vitro* (increases in PT, APTT, and TT), the anti-thrombotic activity that occurred in rats was not associated with clinically significant increases in PT (the only coagulation parameter measured). DU-176b also produced attenuation of tissue factor-induced disseminated intravascular coagulation in rats. Oral administration of up to 200 mg/kg had no effects on CNS function in mice. Oral administration of up to 200 mg/kg in monkeys had no effects on behavior, cardiovascular parameters (including QT<sub>c</sub>), or respiratory parameters.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action:

#### **In Vitro Anticoagulant Activity and Inhibition of Factor Xa in Humans, Rats, Cynomolgus Monkeys, and Rabbits**

**Methods:** Blood samples were collected from healthy volunteers, male Wistar rats, Cynomolgus monkeys, and rabbits (strain was not stated), followed by preparation of plasma. Anticoagulant activity was assessed by adding DU-176b to plasma samples. Anti-Xa activity and inhibition of other serine proteases was measured using purified enzymes.

**Results:** The results are shown in the following tables.

| <b>Factor Xa Inhibition</b> |                           |
|-----------------------------|---------------------------|
| <b>Species</b>              | <b>K<sub>i</sub> (nM)</b> |
| Human                       | 0.561                     |
| Rat                         | 6.96                      |
| Cynomolgus Monkey           | 0.715                     |
| Rabbit                      | 0.457                     |

| Serine Protease Inhibition |                     |
|----------------------------|---------------------|
| Enzyme                     | K <sub>i</sub> (μM) |
| Thrombin                   | 6.0                 |
| Trypsin                    | > 100               |
| Chymotrypsin               | > 100               |
| Plasmin                    | > 100               |
| rtPA                       | > 100               |
| rh-Factor VIIa/sTF         | > 100               |

| Anticoagulant Activity in Human Plasma |          |                         |       |
|--|----------|-------------------------|-------|
|  |          | 95% Confidence Interval |       |
| Parameter                              | CT2 (μM) | Lower                   | Upper |
| PT                                     | 0.256    | 0.230                   | 0.281 |
| APTT                                   | 0.508    | 0.485                   | 0.532 |
| TT                                     | 4.95     | 4.46                    | 5.40  |

CT2: concentration of DU-176b required to increase clotting time by 2-fold

| Anticoagulant Activity in Animals (Prothrombin Time) |          |                         |       |
|--|----------|-------------------------|-------|
|  |          | 95% Confidence Interval |       |
| Species  | CT2 (μM) | Lower                   | Upper |
| Rat  | 0.647    | 0.623                   | 0.671 |
| Cynomolgus monkey                                    | 0.320    | 0.303                   | 0.337 |
| Rabbit   | 0.149    | 0.133                   | 0.164 |

CT2: concentration of DU-176b required to increase clotting time by 2-fold

DU-176b was highly potent in producing inhibition of factor Xa in humans, Cynomolgus monkeys, and rabbits, with similar K<sub>i</sub> values observed in these species. The anti-Xa potency was about 10-fold lower in rats. The drug exhibited low potency as a thrombin inhibitor, and no inhibition was observed for other serine proteases. DU-176b produced increases in PT (prothrombin time), APTT (activated partial thromboplastin time), and TT (thrombin time) in human plasma. The most sensitive coagulation parameter was PT, which was also affected in rats, Cynomolgus monkeys, and rabbits.

**Conclusions:** DU-176b is a highly potent inhibitor of factor Xa in humans, Cynomolgus monkeys, rabbits, and rats.

Drug activity related to proposed indication:

**Effect of D11-4176b in a Rat Venous Thrombosis Model**

**Methods:** Male Wistar rats (age 10 weeks) were treated with a single oral administration of 0 (vehicle), 0.1, 0.5, or 2.5 mg/kg D11-4176b (8 rats/group). D11-4176b is identical to DU-176b. Dose levels are expressed as the anhydrous free base. The vehicle was 0.5% methylcellulose. Fifteen minutes after dosing, the rats were anesthetized with thiopental sodium (100 mg/kg ip). At 30 min after administration of D11-4176b, a platinum wire (2 cm) sharpened at one tip was inserted into the abdominal vein below the renal vessel. The wire remained in place for 60 min. One ml of 1% glutaraldehyde in 10 mM PBS was injected into the lower vena cava to fix the thrombus. Next, 1 ml of 3.13% sodium citrate was injected to block additional thrombus formation. The wire was removed and the weight of thrombus was measured. Blood was collected at 30 and 90 min after dosing for measurement of plasma drug concentrations. PT and inhibition of human factor Xa was measured in plasma from the 90-min blood sample.

**Results:** The results are shown in the following tables.

| <b>Dose (mg/kg)</b> | <b>Thrombus Weight (mg)</b> | <b>PT (sec)</b> | <b>Human Xa Activity (mOD/min at 405 nm)</b> |
|---------------------|-----------------------------|-----------------|--|
| Vehicle             | 2.45 ± 0.38                 | 19.0 ± 0.1      | 7.595 ± 0.048                                |
| 0.1                 | 2.60 ± 0.39                 | 19.2 ± 0.2      | 7.850 ± 0.119                                |
| 0.5                 | 1.91 ± 0.20                 | 18.4 ± 0.1      | 6.429 ± 0.120                                |
| 2.5                 | 0.73 ± 0.21                 | 18.7 ± 0.2      | 2.197 ± 0.162                                |

Values are the mean ± S.E. (n=8)

| <b>Dose (mg/kg)</b> | <b>Plasma Drug Concentration<sup>a</sup> (ng/ml)</b> |               |
|---------------------|--|---------------|
|                     | <b>30 min</b>  | <b>90 min</b> |
| 0.1                 | < 5.0  | < 5.0         |
| 0.5                 | 19.2 ± 1.4   | 12.9 ± 0.8    |
| 2.5                 | 188 ± 9.6  | 106 ± 9.1     |

Values are the mean ± S.E. (n=8)

a: anhydrous free base

D11-4176b produced a dose-dependent reduction in thrombus weight, and a dose-dependent inhibition of human Xa activity. However, PT was unaffected. The magnitude of the anti-thrombotic activity was correlated with plasma drug levels.

**Anti-thrombotic Effect of D11-4176b in a Rat A-V Shunt Model**

**Methods:** Male Wistar rats (age 10 weeks) were treated with a single oral administration of 0 (vehicle), 0.5, 2.5, or 12.5 mg/kg D11-4176b (6 rats/group). Dose levels are expressed as the anhydrous free base. The vehicle was 0.5% methylcellulose. Thirteen minutes after dosing, the rats were anesthetized with thiopental sodium (100 mg/kg ip). A shunt was constructed between the left carotid artery and right jugular vein. The shunt consisted of a polyethylene catheter containing a copper wire. Blood was allowed to flow through the shunt for 12 min, followed by removal of the shunt and measurement of protein content in the thrombus on the copper wire. Blood was collected from the inferior vena cava for measurement of plasma drug concentration, human factor Xa inhibition, and PT.

**Results:** The results are shown in the following tables.

| Dose (mg/kg) | Protein Content in Thrombus ( $\mu\text{g}$ ) | Human Xa Activity (mOD/min at 405 nm) | Prothrombin Time (sec) |
|--------------|---|---------------------------------------|------------------------|
| Vehicle      | 577.4 $\pm$ 72.2                              | 7.3 $\pm$ 0.1                         | 18.3 $\pm$ 0.1         |
| 0.5          | 509.7 $\pm$ 38.3                              | 4.7 $\pm$ 0.2                         | 17.5 $\pm$ 0.2         |
| 2.5          | 338.6 $\pm$ 60.7                              | 1.0 $\pm$ 0.1                         | 18.4 $\pm$ 0.4         |
| 12.5         | 269.0 $\pm$ 19.4                              | 0.49 $\pm$ 0.03                       | 21.3 $\pm$ 0.6         |

Values are the mean  $\pm$  S.E. (n=6)

| Dose (mg/kg) | Plasma Drug Concentration <sup>a</sup> (ng/ml) |
|--------------|--|
| 0.5          | 20.6 $\pm$ 2.7                                 |
| 2.5          | 172 $\pm$ 11.5                                 |
| 12.5         | 396 $\pm$ 35.1                                 |

Values are the mean  $\pm$  S.E. (n=6)

a: anhydrous free base

D11-4176b produced a dose-dependent anti-thrombotic effect and inhibition of Xa activity, with no clinically significant change in PT. The magnitude of anti-thrombotic activity was correlated with plasma drug levels.

**Anti-thrombotic Effect of DU-176b in a Rat Venous Stasis Model**

**Methods:** Male Wistar rats (age 10 weeks) were treated with a single oral administration of 0 (vehicle), 0.5, 2.5, or 12.5 mg/kg DU-176b (8 rats/group). Dose levels are expressed as the anhydrous free base. The vehicle was 0.5% methylcellulose. Thirteen minutes after dosing, the rats were anesthetized with thiopental sodium (100 mg/kg ip). At 29 min after treatment with DU-176b, blood was collected from the jugular vein, followed immediately by infusion of hypotonic saline into the femoral vein for 2 min (5 ml/kg/min). These blood samples were used for measurement of plasma drug concentration, human factor Xa inhibition, and PT. The inferior vena cava was ligated with a cotton thread below the left renal vein. Ten minutes later, the inferior vena cava was ligated at 1.5 cm below the first ligature. Sixty minutes later, the thrombus was removed and weighed.

**Results:** The results are shown in the following tables.

| <b>Dose (mg/kg)</b> | <b>Protein Content in Thrombus (mg)</b> | <b>Human Xa Activity (mOD/min at 405 nm)</b> | <b>Prothrombin Time (sec)</b> |
|---------------------|---|--|-------------------------------|
| Vehicle             | 4.38 ± 0.53                             | 13.8 ± 0.2                                   | 18.2 ± 0.1                    |
| 0.5                 | 2.03 ± 0.49                             | 6.6 ± 0.3                                    | 17.9 ± 0.1                    |
| 2.5                 | 0.71 ± 0.29                             | 0.84 ± 0.10                                  | 20.6 ± 0.7                    |
| 12.5                | 0.58 ± 0.16                             | 0.35 ± 0.03                                  | 24.0 ± 0.4                    |

Values are the mean ± S.E. (n=8)

| <b>Dose (mg/kg)</b> | <b>Plasma Drug Concentration<sup>a</sup> (ng/ml)</b> |
|---------------------|--|
| 0.5                 | 20.5 ± 1.4   |
| 2.5                 | 194 ± 27.1   |
| 12.5                | 449 ± 37.6   |

Values are the mean ± S.E. (n=8)

a: anhydrous free base

DU-176b produced a dose-dependent anti-thrombotic effect and inhibition of Xa activity, but only a slight increase in PT. The magnitude of anti-thrombotic activity was correlated with plasma drug levels.

### 2.6.2.3 Secondary pharmacodynamics

#### Effect of DU-176b on Recombinant Factor VIIa-Induced Reduction of Prothrombin Time

**Methods:** Blood was obtained from human volunteers. Plasma was prepared from blood samples. The effects of NovoSeven (recombinant human factor VIIa) and DU-176b on PT were measured, either alone or in combination.

**Results:** The results are shown in the following figures (taken from the study report).

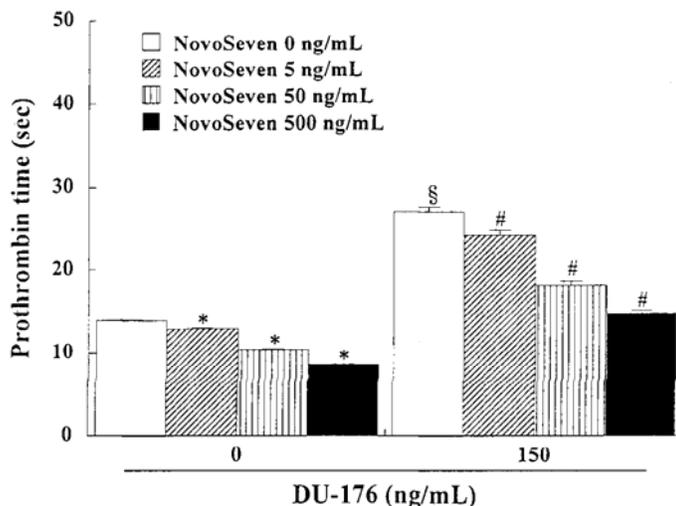


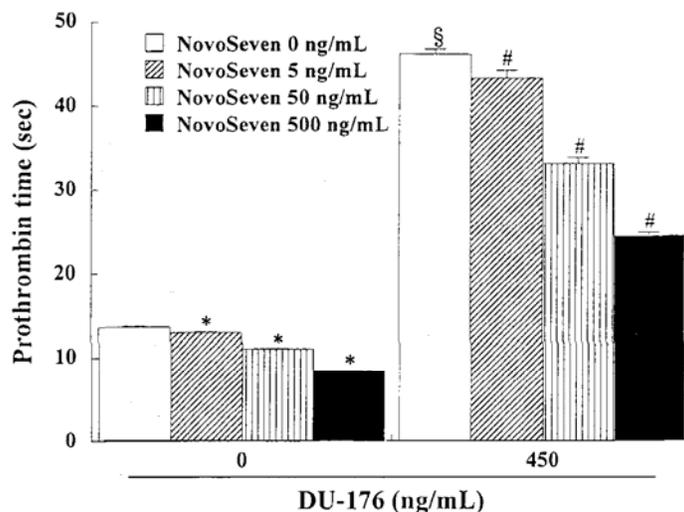
Figure 1 Effect of NovoSeven on prolonged prothrombin time induced by DU-176 at a concentration of 150 ng/mL.

Data represents the mean  $\pm$  S.E.M. (n=6). The statistical significance of the data was analyzed by Aspin-Welch t test (between control and DU-176 alone) and parametric Dunnett's multiple comparison test.

\*:  $p < 0.05$  (vs. control, parametric Dunnett's multiple comparison test)

§:  $p < 0.05$  (vs. control, Aspin-Welch t test)

#:  $p < 0.05$  (vs. DU-176 alone, parametric Dunnett's multiple comparison test)



**Figure 2** Effect of NovoSeven on prolonged prothrombin time induced by DU-176 at a concentration of 450 ng/mL.

Data represents the mean  $\pm$  S.E.M.(n=6). The statistical significance of the data was analyzed by Aspin-Welch t test (between control and DU-176 alone) and parametric Dunnett's multiple comparison test.

\*:  $p < 0.05$  (vs. control, parametric Dunnett's multiple comparison test)

§:  $p < 0.05$  (vs. control, Aspin-Welch t test)

#:  $p < 0.05$  (vs. DU-176 alone, parametric Dunnett's multiple comparison test)

NovoSeven produced a dose-dependent decrease in PT in the absence and presence of DU-176. However, PT values in the DU-176-treated samples were greater than those observed in the absence of DU-176.

### **Effect of D11-4176b on Tissue Factor-Induced DIC in Rats**

**Methods:** Male Wistar rats (age 11 weeks) were treated with a single oral administration of 0 (vehicle, saline sham), 0 (vehicle), 0.1, 0.5, or 2.5 mg/kg D11-4176b (6 rats/group). Dose levels are expressed as the anhydrous free base. The vehicle was 0.5% methylcellulose. Fifteen minutes after dosing, the rats were anesthetized with thiopental sodium (100 mg/kg ip). At 30 min after administration of D11-4176b, 0.8 U/ml tissue factor (Thromboplastin C Plus) in saline was infused into the femoral vein for 1 min (3.1 ml/kg) to produce DIC (disseminated intravascular coagulation). The sham group was infused with saline. Ten minutes later, blood was collected from the inferior vena cava. Platelet count was measured in blood, and plasma was prepared. Inhibition of human factor Xa was measured in plasma samples. Plasma concentrations of TAT (thrombin-antithrombin III complex), fibrinogen, and D11-4176b were also measured.

**Results:** The results are shown in the following tables.

| Dose (mg/kg)                     | Platelet Count (x 10 <sup>4</sup> /μl) | Plasma TAT (ng/ml) | Plasma Fibrinogen (mg/dl) | Human Xa Activity (mOD/min at 405 nm) |
|----------------------------------|--|--------------------|---------------------------|---------------------------------------|
| Vehicle (sham)                   | 64.6 ± 1.6                             | 3.4 ± 0.7          | 202.2 ± 2.8               | 8.6 ± 0.1                             |
| <b>Tissue Factor-Induced DIC</b> |  |                    |                           |                                       |
| Vehicle                          | 39.8 ± 1.0                             | 290.5 ± 26.4       | 124.4 ± 7.7               | 8.7 ± 0.2                             |
| 0.1                              | 47.8 ± 2.8                             | 173.1 ± 22.0       | 157.3 ± 9.1               | 8.4 ± 0.1                             |
| 0.5                              | 50.2 ± 1.2                             | 108.3 ± 17.2       | 182.8 ± 5.0               | 6.1 ± 0.2                             |
| 2.5                              | 56.9 ± 2.4                             | 38.6 ± 3.2         | 197.4 ± 5.1               | 1.5 ± 0.1                             |

Values are the mean ± S.E. (n=6)

| Dose (mg/kg) | Plasma Drug Concentration <sup>a</sup> (ng/ml) |
|--------------|--|
| 0.1          | < 5.0  |
| 0.5          | 16.2 ± 2.0                                     |
| 2.5          | 163 ± 16.7                                     |

Values are the mean ± S.E. (n=6)  
a: anhydrous free base

D11-4176b prevented the DIC-induced decrease in platelet count, increase in TAT, and decrease in fibrinogen. These effects were dose-dependent, and were correlated with plasma drug levels.

#### 2.6.2.4 Safety pharmacology

Neurological effects:

#### CNS Study in Male Mice

**Methods:** Male Slc:ddY mice (age 4-5 weeks, 22-33 g) were used. For each test, the mice were treated orally with 0 (vehicle), 20, 60, or 200 mg/kg DU-176b. The dose levels are expressed as the anhydrous free base equivalent. The drug was administered as a suspension in 0.5% methylcellulose using a dose volume of 10 ml/kg. The dose groups included 10 mice in all tests, except for the Irwin test, for which 3 mice/group were used.

#### Results:

**Irwin Test:** Huddling behavior was absent in 1/3 mice treated with 20 mg/kg at 60 min post-dose. This effect was probably not related to treatment. Vocalization occurred in the 200 mg/kg group at 30 min post-dose (1/3 mice).

**Motor Coordination (rota-rod test):** This test was performed at 1 hr post-dose. There were no effects.

**Spontaneous Locomotor Activity (wheel cage test):** Activity was measured in revolutions per 10 minute interval starting at 1 hr post-dose. In the initial test, locomotor activity was reduced in the 60 mg/kg group at each 10-min interval during 60-120 min after dosing. However, no significant changes occurred in the 20 or 200 mg/kg groups. In the repetition of this test, no significant changes in activity were detected in any treatment group.

**Electroshock-Induced Convulsion:** Electroshock was administered at 1 hr post-dose. The results from two experiments are shown below.

|                     | <b>Experiment # 1</b>                 |
|---------------------|---------------------------------------|
| <b>Dose (mg/kg)</b> | <b>Seizure Threshold Current (mA)</b> |
| 0                   | 13.8 ± 0.3                            |
| 20                  | 13.1 ± 0.3                            |
| 60                  | 12.5 ± 0.3*                           |
| 200                 | 13.3 ± 0.3                            |

Values are the mean ± S.E.

\*p<0.05

|                     | <b>Experiment # 2</b>                 |
|---------------------|---------------------------------------|
| <b>Dose (mg/kg)</b> | <b>Seizure Threshold Current (mA)</b> |
| 0                   | 13.0 ± 0.3                            |
| 20                  | 12.5 ± 0.3                            |
| 60                  | 12.8 ± 0.3                            |
| 200                 | 12.6 ± 0.4                            |

Values are the mean ± S.E.

No drug-related effect was observed. The positive result (i.e. decreased threshold current) in the first experiment was not dose-dependent, and was not reproduced in the second experiment.

**Pentylentetrazole-Induced Convulsion:** This test was performed at 1 hr after treatment with DU-176b, using intravenous infusion of pentylentetrazole (10 mg/min). No effects were observed.

**Conclusions:** Oral administration of up to 200 mg/kg had no effects on CNS function in mice.

## **CNS, Cardiovascular, and Respiratory Study in Cynomolgus Monkeys**

**Methods:** Two male and two female Cynomolgus monkeys (age 4 years) were implanted with a telemetric transmitter. The animals were treated orally with 0 (vehicle), 20, 60, and 200 mg/kg DU-176b. The dose levels are expressed as the anhydrous free base equivalent. Dosing was performed on four occasions using a crossover study design. The doses were separated by six days. The dose volume was 2 ml/kg. The drug was administered as a suspension in 0.5% methylcellulose. ECG was recorded prior to dosing at 0.25, 0.5, 1, 1.5, 2, 4, 6, and 8 hr post-dose.

**Results:** The drug had no effects on behavior (0-24 hr), spontaneous locomotor activity (0-8 hr), temperature (0-8 hr), systolic pressure (0-8 hr), diastolic pressure (0-8 hr), heart rate (0-8 hr), RR, PR, QRS, QT, QT<sub>c</sub>, respiration rate (0-8 hr), blood pH (0-8 hr), blood pO<sub>2</sub>, blood pCO<sub>2</sub>, and sO<sub>2</sub> (hemoglobin oxygen saturation). Plasma levels of histamine were measured before dosing and at 60 and 240 min post-dose. Histamine levels were below the limit of quantification (1 nMol/L) for every sample collected in each animal.

### **Cardiovascular effects:**

See study in monkeys under the “Neurological effects” subsection for ECG effects.

## **Effects on HERG Channel Current in HEK293 Cells**

**Methods:** HEK293 cells were stably transfected with HERG cDNA. Currents mediated by HERG channel activity were recorded using the whole-cell patch clamp method. Cells were initially clamped at -70 mV. Outward current was elicited by changing the potential to 0 mV for 0.75 sec, followed by repolarization to -50 mV for 0.75 sec to produce the tail current. The potential was then returned to -70 mV. HERG channel current was measured prior to drug exposure and at 10 min of exposure to drug solution. The vehicle was 0.1% DMSO in physiological buffer. The cells were exposed to 0 (vehicle), 2, or 20 µg/ml DU-176b (concentrations expressed as anhydrous free base). E-4031 (100 nM) was tested as a positive control compound.

**Results:** DU-176b had no effect on tail current. E-4031 produced a strong inhibition (84% less than the pre-exposure value) of tail current.

## **Effects on the Electrophysiological Properties of Isolated Guinea Pig Papillary Muscle**

**Methods:** Male Hartley guinea pigs (age 4-6 weeks) were used. The animals were sacrificed and the heart was removed and placed in a physiological buffer. The papillary muscles were removed from the right ventricle, and suspended in physiological buffer. Action potentials were evoked by electrical stimulation (1 Hz for 1 msec) using microelectrodes. After a 30-60 min equilibration period during which baseline measurements were obtained, the muscle preparations were exposed to cumulative concentrations of drug. The effect of each concentration was

measured at 30 min of exposure. The muscle strips were treated with 0 (vehicle), 6, and 20 µg/ml DU-176b (concentrations expressed as anhydrous free base). E-4031 (100 nM) was tested as a positive control compound. The vehicle was 0.03-0.07% DMSO in physiological buffer.

**Results:** DU-176b had no effects on the following parameters: resting membrane potential, action potential amplitude, overshoot, action potential duration at 20%, 50%, and 90% of repolarization (APD<sub>20</sub>, APD<sub>50</sub>, and APD<sub>90</sub>, respectively), and V<sub>max</sub> (dV/dT<sub>max</sub>). E-4031 produced prolongation of APD<sub>20</sub>, APD<sub>50</sub>, and APD<sub>90</sub>.

Pulmonary effects: See study in monkeys under the “Neurological effects” subsection.

Renal effects:

### **Renal Function Study in Rats**

**Methods:** Male Crj:CD(SD)IGS rats (age 6 weeks, 192-210 g) were fasted for 18-24 hr and deprived of water during the final 2 hr before the experiment. The rats were treated orally with 25 ml/kg saline, followed immediately by a single oral administration of 0 (vehicle), 20, 60, or 200 mg/kg DU-176b (8 rats/group). The dose levels are expressed as anhydrous free base. The drug was administered as a suspension in 0.5% methylcellulose, using a dose volume of 5 ml/kg. Urine was collected for 5 hr after dosing. Pooled urine samples were analyzed for sodium, potassium, and chloride.

**Results:** Urine volume and the excretion of sodium, potassium, and chloride (µEq/5 hr) were unaffected.

Gastrointestinal effects: No studies were submitted.

Abuse liability: No studies were submitted.

Other: No studies were submitted.

#### **2.6.2.5 Pharmacodynamic drug interactions**

No studies were submitted.

#### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

Not applicable.

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Studies were performed to characterize the absorption, distribution, metabolism, and excretion of [<sup>14</sup>C]DU-176b in male rats and male Cynomolgus monkeys. Oral administration was used exclusively in these studies.

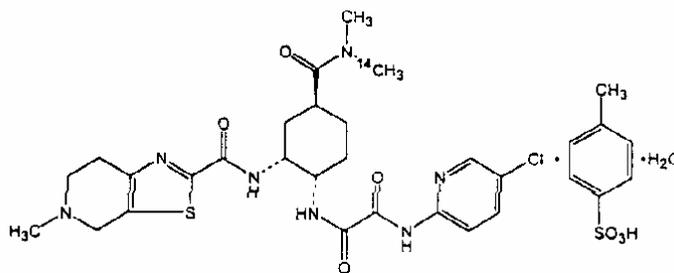
### 2.6.4.2 Methods of Analysis

[see under individual study reviews]

### 2.6.4.3 Absorption

#### Absorption Study of [<sup>14</sup>C]DU-176b in Male Rats

**Methods:** Three male Wistar SPF rats (age 7 weeks) were treated with a single oral administration of 3 mg/kg [<sup>14</sup>C]DU-176b. The dose level is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 6 ml/kg. Blood was collected from each animal at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 hr after dosing. The structure of [<sup>14</sup>C]DU-176b is shown below.



**Results:** Pharmacokinetic parameters for total radioactivity in blood and plasma are shown in the table below (taken from the study report).

| PK parameters                         | Blood                 | Plasma                          |
|---------------------------------------|-----------------------|---------------------------------|
| $t_{\max}$ (h)                        | 0.50 ± 0.00           | 0.67 ± 0.29                     |
| $C_{\max}$ (ng eq./mL)                | 426.8 ± 31.2          | 417.2 ± 72.4                    |
| $t_{1/2}$ (h)                         | 3.2 ± 0.2<br>(1-12 h) | 3.2 ± 0.4<br>(1-12 h or 2-12 h) |
| $t_{1/2}$ (h)                         | 65 ± 23<br>(24-72 h)  | 11 ± 6<br>(12-24 h or 12-48 h)  |
| $AUC_{0-\text{finite}}$ (µg eq.·h/mL) | 1.82 ± 0.21           | 1.67 ± 0.16                     |
| $AUC_{0-\infty}$ (µg eq.·h/mL)        | 2.16 ± 0.40           | 1.76 ± 0.20                     |

Dose: 3 mg/kg as DU-176.

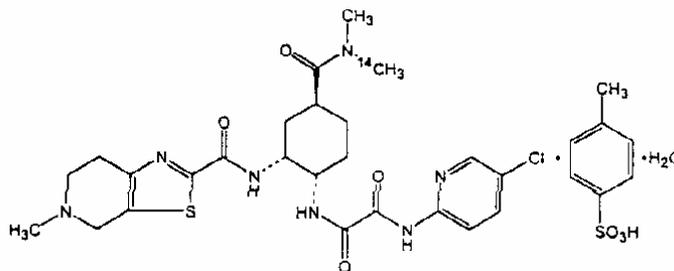
Data are expressed as the mean values ± S.D. of three animals.

Figures in parentheses represent time for calculation.

The  $C_{max}$  and AUC values for total radioactivity were similar in blood and plasma, which indicates that radioactivity was extensively distributed in the red blood cells. Radioactivity was detectable for up to 72 hr in blood, and 48 hr in plasma (detection limit was 2.7 ng eq in blood and 3.4 ng eq in plasma). Elimination of radioactivity from blood and plasma was rapid during the initial 12 hr after dosing. The initial rapid phase of elimination was followed by a much slower phase.

### Absorption of [ $^{14}$ C]DU-176b in Male Cynomolgus Monkeys

**Methods:** Three male Cynomolgus monkeys (age 3 years) were treated with a single oral administration of 1 mg/kg [ $^{14}$ C]DU-176b. The dose is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 2 ml/kg. Blood was collected from each animal at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 216, 288, and 336 hr after dosing. The structure of [ $^{14}$ C]DU-176b is shown below.



**Results:** Pharmacokinetic parameters for blood and plasma are shown in the table below (taken from the study report).

| PK parameters                  | Blood         | Plasma                   |
|--------------------------------|---------------|--------------------------|
| $t_{max}$ (h)                  | 1.7 ± 0.6     | 2.3 ± 1.5                |
| $C_{max}$ (ng eq./mL)          | 297.0 ± 137.1 | 297.9 ± 141.3            |
| (h)                            | 3.4 ± 0.3     | 3.2 ± 0.2                |
|                                | (2 or 4-12 h) | (2, 4 or 6-12 h)         |
| $t_{1/2}$ (h)                  | 32 ± 12       | 32 ± 13                  |
|                                | (24-72 h)     | (24-72 or 120 h)         |
| (day)                          | 15 ± 5        | 5.3 ± 1.6                |
|                                | (96-336 h)    | (96 or 144-144 or 336 h) |
| $AUC_{0-finite}$ (μg eq.·h/mL) | 3.40 ± 0.93   | 3.18 ± 1.01              |
| $AUC_{0-∞}$                    | 4.76 ± 0.86   | 3.51 ± 1.13              |

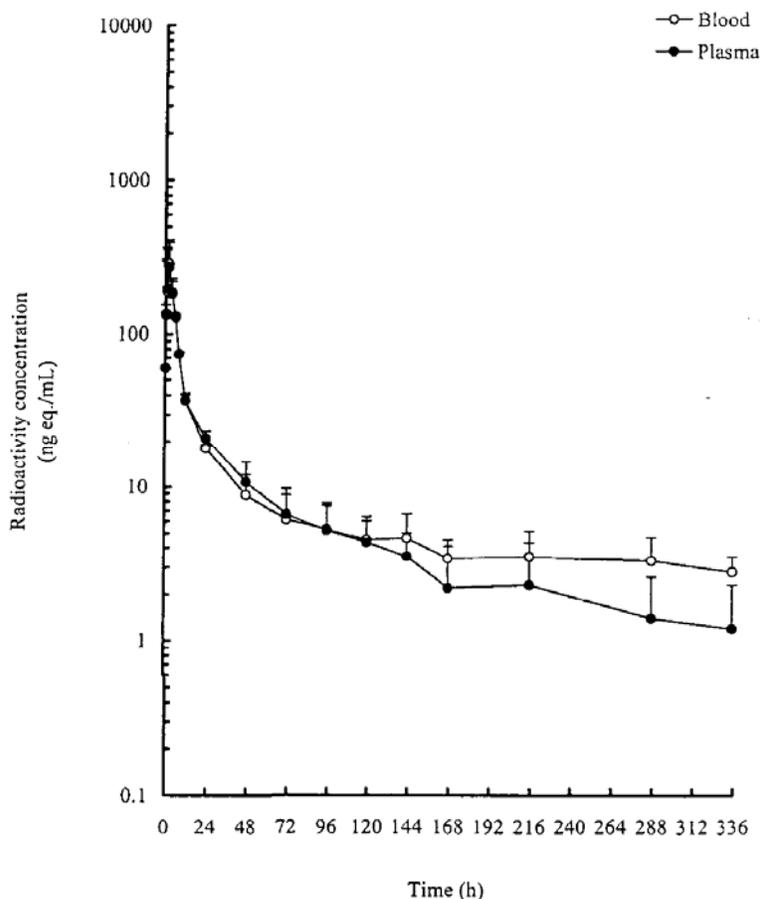
Dose: 1 mg/kg as DU-176.

Data are expressed as the mean values ± S.D. of three animals.

Figures in parentheses represent time for calculation.

$^{14}$ C-DU-176b was administered 4 h before feeding.

The  $C_{max}$  and AUC values for radioactivity were similar in blood and plasma, which indicates that radioactivity was extensively distributed in red blood cells. Elimination of radioactivity from blood and plasma was rapid during the initial 12 hr after dosing, but was much slower thereafter. Radioactivity was still detectable in blood and plasma at 336 hr (14 days) post-dose, as shown in the figure below (taken from the study report).



#### 2.6.4.4 Distribution

##### Distribution of [ $^{14}$ C]DU-176b in Male Cynomolgus Monkeys

**Methods:** Three male Cynomolgus monkeys (age 3 years) were treated with a single oral administration of 1 mg/kg [ $^{14}$ C]DU-176b. The dose is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 2 ml/kg. The animals were sacrificed at 336 hr (14 days) post-dose, and radioactivity was measured in a limited number of organs/tissues.

**Results:** The distribution results are shown in the table below (taken from the study report).

Table 4. Tissue concentrations of radioactivity 336 h after a single oral administration of  $^{14}\text{C}$ -DU-176b to male cynomolgus monkeys.

| Tissue               | Radioactivity concentration<br>(ng eq./g or mL) |
|----------------------|---|
| Blood                | 2.8 ± 0.7                                       |
| Brain                | 2.4 ± 0.6                                       |
| Eyeball              | 509.0 ± 148.2                                   |
| Heart                | 4.7 ± 0.9                                       |
| Lung                 | 5.0 ± 1.6                                       |
| Liver                | 14.5 ± 2.6                                      |
| Kidney               | 6.7 ± 1.2                                       |
| Skeletal muscle      | 3.7 ± 2.1                                       |
| Fat                  | 2.0 ± 0.2                                       |
| Skin                 | 259.9 ± 69.5                                    |
| Testis               | 3.4 ± 1.1                                       |
| Epididymis           | 2.2 ± 0.8                                       |
| Bile in gall bladder | 20.6 ± 5.4                                      |

Dose: 1 mg/kg as DU-176.

Data are expressed as the mean values ± S.D. of three animals.

$^{14}\text{C}$ -DU-176b was administered 4 h before feeding.

Radioactivity was highly concentrated in eyes and skin. This observation and the presence of low concentrations in multiple organs are noteworthy, given that the animals were sacrificed 14 days after dosing. The highest proportion of the radioactive dose was present in skin, skeletal muscle, and eye ( $2.41 \pm 0.76\%$ ,  $0.18 \pm 0.1\%$ , and  $0.09 \pm 0.02\%$ , respectively).

**Conclusions:** This study was flawed due to the absence of early time-points and the limited number of organs/tissues that were analyzed. However, clearance of radioactivity from organs and tissues in Cynomolgus monkeys appears to occur very slowly following oral administration of [ $^{14}\text{C}$ ]DU-176b, based on the presence of detectable concentrations at 14 days after dosing. Radioactivity was highly concentrated in eyes and skin.

### **Whole-Body Autoradiography Study in Male Rats**

**Methods:** Male Wistar SPF rats (age 7 weeks) were treated with a single oral administration of 3 mg/kg [ $^{14}\text{C}$ ]DU-176b. The dose is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 6 ml/kg. The rats were sacrificed at 1 and 24 hr (1 rat/time-point). Whole-body sectioning was performed and autoradiograms were prepared.

**Results:** At 1 hr after administration, the highest levels of radioactivity were present in gastrointestinal and bladder contents. The radioactivity levels in kidney, preputial gland, liver, intestine, Harderian Gland, pituitary gland, and nasal cavity were much higher than that in blood. The radioactivity levels in adrenals, mandibular glands, spleen, pancreas, mandibular lymph

nodes, thyroid, epididymis, thymus, prostate, stomach, and bone marrow were somewhat higher than blood radioactivity levels. The radioactivity levels in lung, heart, brown fat, skin, and skeletal muscle were comparable to blood radioactivity levels. The radioactivity levels in testes and fat were lower than that in blood, and only trace levels were found in eye and brain.

At 24 hr after administration, the radioactivity levels were decreased in most organs/tissues. However, a relatively high level of radioactivity was still present in the intestinal contents. Low levels were present in bladder contents, nasal cavity, thyroid, liver, and stomach. Only trace levels were observed in the remaining organs/tissues.

## 2.6.4.5 Metabolism

### Metabolism Study in Rats and Monkeys

**Methods:** Samples of plasma, urine, and feces were collected in studies of absorption and/or excretion in rats and Cynomolgus monkeys (these studies are included in the present submission). In these studies, rats and Cynomolgus monkeys were treated with a single oral administration of 3 and 1 mg/kg [<sup>14</sup>C]DU-176b, respectively. The radioactivity in the samples of plasma, urine, and feces was analyzed using an HPLC method. Radioactive compounds were identified based on a comparison to retention times observed for unlabelled DU-176b and several synthetic metabolite standards.

**Results:** Metabolite analysis for rats and monkeys is shown in the following tables (taken from the study report).

| <b>Rat plasma</b> |                              |       |
|-------------------|------------------------------|-------|
| Metabolite        | % of radioactivity in sample |       |
|                   | 15 min-4 h                   | 6-8 h |
| RP-1 (D21-3231)   | 75.8                         | 18.1  |
| RP-2 (DU-176)     | 3.62                         | 7.73  |
| Others            | 20.5                         | 74.2  |

| <b>Rat urine</b> |                      |
|------------------|----------------------|
| Metabolite       | % of total peak area |
|                  | 0-24 h               |
| RU-1 (D21-3231)  | 24.5                 |
| RU-2 (D21-1402)  | 0.846                |
| RU-3             | 0.986                |
| RU-4 (DU-176)    | 73.7                 |

| <b>Rat feces</b> |                      |         |
|------------------|----------------------|---------|
| Metabolite       | % of total peak area |         |
|                  | 0-24 h               | 24-48 h |
| RF-1 (D21-3231)  | 7.31                 | 44.2    |
| RF-2 (D21-1402)  | 4.12                 | —       |
| RF-3             | 9.57                 | —       |
| RF-4 (DU-176)    | 79.0                 | 55.8    |

—: Not detected.

**Monkey plasma**

| Metabolite    | % of radioactivity in sample |      |
|---------------|------------------------------|------|
|               | 1 h                          | 8 h  |
| MP-1 (DU-176) | 83.0                         | 22.7 |
| Others        | 17.0                         | 77.3 |

**Monkey urine**

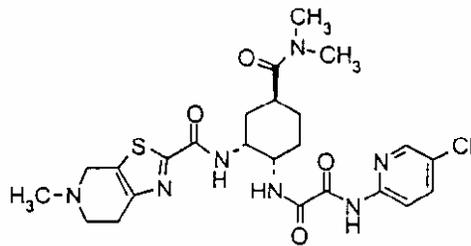
| Metabolite      | % of total peak area |
|-----------------|----------------------|
|                 | 0-24 h               |
| MU-1            | 1.96                 |
| MU-2            | 15.8                 |
| MU-3 (D21-3231) | 5.30                 |
| MU-4            | 0.420                |
| MU-5            | 1.79                 |
| MU-6            | 1.52                 |
| MU-7 (D21-1402) | 5.23                 |
| MU-8            | 9.61                 |
| MU-9 (DU-176)   | 58.4                 |

**Monkey feces**

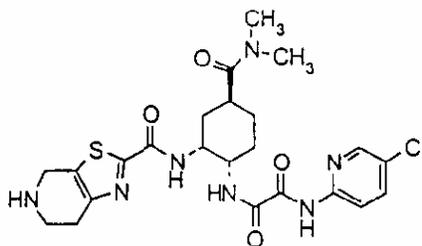
| Metabolite      | % of total peak area |         |
|-----------------|----------------------|---------|
|                 | 0-24 h               | 24-48 h |
| MF-1            | 2.86                 | 3.78    |
| MF-2 (D21-3231) | 5.17                 | 8.96    |
| MF-3 (D21-3221) | 0.772                | 0.489   |
| MF-4            | 4.30                 | 4.08    |
| MF-5 (D21-1402) | 12.9                 | 13.4    |
| MF-6            | 19.1                 | 24.4    |
| MF-7 (DU-176)   | 54.8                 | 44.9    |

( ) Name of standard metabolite with a similar retention time.

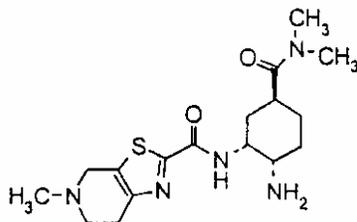
D21-3231 was a major metabolite in rat plasma. This metabolite was present at much higher levels as compared to the unchanged drug (DU-176). D21-3231 was detected in urine and feces, but accounted for a lower proportion of radioactivity. In monkey plasma, most of the radioactivity was associated with unchanged drug at 1 hr post-dose, whereas unidentified metabolites composed most of the radioactivity at 8 hr. Several metabolites were detected in monkey urine and feces, although 55-58% of radioactivity in excreta was associated with unchanged drug during the first 24 hr. Enzymatic treatment of rat and monkey urine with  $\beta$ -glucuronidase/arylsulfatase did not alter the metabolic profile. This result indicates that glucuronide and sulfate conjugates were not present in urine. The structures of DU-176 and its metabolites are shown below.



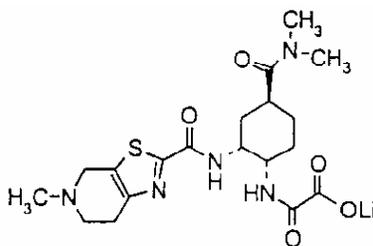
**DU-176**



**D21-1402**



**D21-3221**

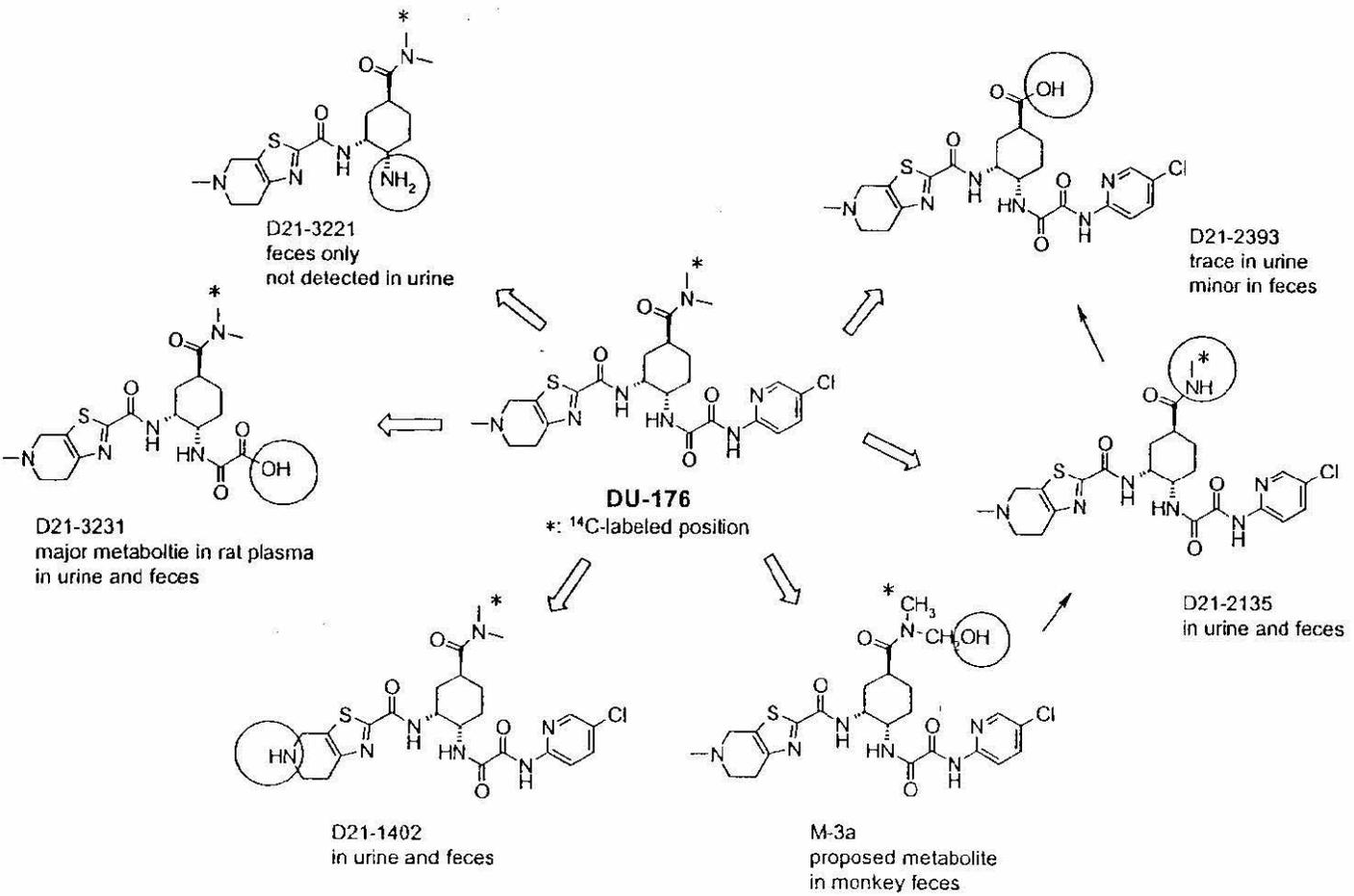


**D21-3231 (lithium salt)**

### **Structural Characterization of Metabolites in Rats and Monkeys**

**Methods:** Samples of urine and feces were collected in studies of excretion in rats and Cynomolgus monkeys (these studies are included in the present submission). In these studies, rats and Cynomolgus monkeys were treated with a single oral administration of 3 and 1 mg/kg [<sup>14</sup>C]DU-176b, respectively. Radioactivity in urine and feces was analyzed using an HPLC method. Radioactive compounds were identified based on a comparison to HPLC retention times observed for unlabeled DU-176b and several synthetic metabolite standards. Additional analysis of urinary and fecal radioactivity was performed using LC/MS/MS, to confirm the metabolite structures and to characterize any metabolites not identified through HPLC.

**Results:** The authors proposed a metabolic pathway based on the results of HPLC and LC/MS/MS analysis. The proposed pathway is shown in the figure below (taken from the study report).



The M-3a metabolite is proposed to be a metabolite in monkey feces. A relatively high proportion of unidentified metabolites were found in monkey feces in a previous study (reviewed above).

## 2.6.4.6 Excretion

### Excretion Study in Male Rats

**Methods:** Three male Wistar SPF rats (age 7 weeks) were treated with a single oral administration of 3 mg/kg [ $^{14}\text{C}$ ]DU-176b. The dose is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 6 ml/kg. Urine, feces, and expired air were collected for 168 hr after administration.

**Results:** Excretion data are shown in the table below (taken from the study report).

Table 1. Cumulative excretion of radioactivity in urine, feces and expired air as  $^{14}\text{CO}_2$  up to 168 h after a single oral administration of  $^{14}\text{C}$ -DU-176b to fasting male rats (dose: 3 mg/kg as DU-176).

| Time (h)        | Excretion of radioactivity (% of dose) |            |             |            |
|-----------------|--|------------|-------------|------------|
|                 | Urine                                  | Feces      | Expired air | Total      |
| 0 - 4           | 14.1 ± 3.5                             | –          | 0.1 ± 0.0   | –          |
| 8               | 18.9 ± 6.7                             | –          | 0.2 ± 0.1   | –          |
| 24              | 24.5 ± 3.7                             | 71.4 ± 1.9 | 0.3 ± 0.1   | 96.2 ± 4.5 |
| 48              | 24.5 ± 3.7                             | 72.3 ± 1.5 | 0.3 ± 0.1   | 97.2 ± 4.3 |
| 72              | 24.7 ± 3.7                             | 72.4 ± 1.5 | 0.3 ± 0.1   | 97.4 ± 4.3 |
| 96              | 24.7 ± 3.7                             | 72.4 ± 1.5 | 0.3 ± 0.1   | 97.5 ± 4.3 |
| 120             | 24.8 ± 3.7                             | 72.5 ± 1.5 | 0.3 ± 0.1   | 97.6 ± 4.2 |
| 144             | 24.8 ± 3.7                             | 72.5 ± 1.5 | 0.3 ± 0.1   | 97.6 ± 4.2 |
| 168             | 24.8 ± 3.7                             | 72.5 ± 1.5 | 0.3 ± 0.1   | 97.6 ± 4.2 |
| Carcass (168 h) |  |            |             | 0.1 ± 0.0  |

Data are expressed as the mean values ± S.D. of three animals.

– : Not determined

Radioactivity was excreted primarily in feces. Almost all of the radioactive dose was excreted at 24 hr after dosing.

### Excretion of [ $^{14}\text{C}$ ]DU-176b in Male Cynomolgus Monkeys

**Methods:** Three male Cynomolgus monkeys (age 3 years) were treated with a single oral administration of 1 mg/kg [ $^{14}\text{C}$ ]DU-176b. The dose is expressed as the anhydrous free base equivalent. The drug was administered as a solution in water with pH adjusted to 4.0 with HCl. The dose volume was 2 ml/kg. Urine and feces were collected through 336 hr (14 days) post-dose.

**Results:** Cumulative excretion data are shown below (taken from the study report).

| Time (h)              | Excretion of radioactivity (% of dose) |            |            |
|-----------------------|--|------------|------------|
|                       | Urine                                  | Feces      | Total      |
| 0 - 4                 | 15.6 ± 6.5                             | -          | -          |
| 8                     | 27.8 ± 5.5                             | -          | -          |
| 24                    | 35.8 ± 4.8                             | 21.5 ± 4.3 | 57.3 ± 8.8 |
| 48                    | 39.5 ± 4.7                             | 40.6 ± 5.7 | 80.1 ± 2.9 |
| 72                    | 40.7 ± 4.7                             | 47.7 ± 7.2 | 88.3 ± 3.4 |
| 96                    | 41.1 ± 4.8                             | 49.2 ± 6.7 | 90.3 ± 2.7 |
| 120                   | 41.4 ± 4.9                             | 49.9 ± 6.3 | 91.3 ± 2.1 |
| 144                   | 41.5 ± 4.9                             | 50.3 ± 6.1 | 91.8 ± 1.8 |
| 168                   | 41.7 ± 5.0                             | 50.5 ± 6.1 | 92.2 ± 1.8 |
| 216                   | 41.8 ± 4.9                             | 50.7 ± 6.1 | 92.5 ± 1.7 |
| 288                   | 41.9 ± 4.9                             | 50.9 ± 6.1 | 92.8 ± 1.7 |
| 336                   | 42.0 ± 5.0                             | 51.0 ± 6.1 | 93.0 ± 1.7 |
| Cage washings (336 h) |  |            | 2.2 ± 1.6  |

Dose: 1 mg/kg as DU-176.

Data are expressed as the mean values ± S.D. of three animals.

<sup>14</sup>C-DU-176b was administered 4 h before feeding.

- : Not determined

Both urine and feces were major routes of excretion, although feces contained a slightly higher proportion of the radioactive dose. Excretion occurred slowly, particularly after the first 24 hr post-dose. Approximately 5% of the radioactivity was not recovered in excreta at 336 hr post-dose. Detectable levels of radioactivity remained in several organs/tissues from these animals (see study in the "Distribution" subsection above). Excretion in bile was demonstrated in the distribution measurements.

#### 2.6.4.7 Pharmacokinetic drug interactions

No studies were submitted.

#### 2.6.4.8 Other Pharmacokinetic Studies

No studies were submitted.

#### 2.6.4.9 Discussion and Conclusions

[<sup>14</sup>C]DU-176b was absorbed in rats and Cynomolgus monkeys following oral administration. In both species, elimination of radioactivity from blood and plasma was rapid during the initial 12 hr after dosing, but was much slower thereafter. In monkeys, radioactivity was still detectable in blood and plasma at 336 hr (14 days) post-dose. The C<sub>max</sub> and AUC values for total radioactivity were similar in blood and plasma, which indicates that radioactivity was extensively distributed in the cellular compartment of blood. At 336 hr after oral administration of [<sup>14</sup>C]DU-176b in Cynomolgus monkeys, radioactivity was highly concentrated in eyes and skin, and was still detectable in several organs/tissues. Thus, clearance of radioactivity from organs and tissues in Cynomolgus monkeys appears to occur very slowly. This study was flawed due to the absence

of early time-points and the limited number of organs/tissues that were analyzed. Results from a whole-body autoradiography study in rats indicate that the drug is highly concentrated in urine, kidney, preputial gland, liver, intestine, Harderian Gland, pituitary gland, and nasal cavity. A major metabolite (D21-3231) was identified in rat plasma. This metabolite was present at much higher levels as compared to the unchanged drug. Excretion occurred primarily in feces following oral administration of [<sup>14</sup>C]DU-176b in rats. Almost all radioactivity was excreted at 24 hr after dosing. In monkeys, both urine and feces were major routes of excretion, although feces contained a slightly higher proportion of the radioactive dose. Excretion occurred slowly, particularly after the first 24 hr post-dose. Approximately 5% of the radioactivity was not recovered in excreta at 336 hr post-dose.

#### **2.6.4.10 Tables and figures**

Not applicable.

### **2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

Not applicable.

### **2.6.6 TOXICOLOGY**

#### **2.6.6.1 Overall toxicology summary**

General toxicology: DU-176b was not lethal at oral doses of up to 2000 mg/kg (anhydrous free base equivalent) in rats or 400 mg/kg in monkeys. The NOAEL (no observed adverse effect level) in a 4-week oral toxicity study in rats was 60 mg/kg/day. The only adverse effect was impaired weight gain at 200 mg/kg/day. In a 4-week oral toxicity study in monkeys, the NOAEL was 10 mg/kg/day. Deaths due to hemorrhage occurred in the 100 mg/kg/day group. Hemorrhage in several organs was observed at this dose level. However, target organ toxicity that did not involve hemorrhage was observed in kidneys (30 and 100 mg/kg/day), lungs (100 mg/kg/day), liver (30 mg/kg/day), and spleen (100 mg/kg/day).

Genetic toxicology: DU-176b was negative in the bacterial reverse mutation test, *in vitro* micronucleus test in human lymphocytes, micronucleus test in rats using a single oral administration, micronucleus test in rats using repeated oral and intravenous administration, hepatocyte micronucleus test in rats using a single oral administration, micronucleus test in the 4-week oral toxicity study in monkeys, and the unscheduled DNA synthesis test in rats. However, the drug did produce polyploidy in the chromosomal aberration test in CHL cells, without affecting the incidence of structural aberrations. In a follow-up study which focused solely on numerical chromosomal aberrations, DU-176b produced an increase in polyploidy in human lymphocytes.

Carcinogenicity: No studies were submitted.

**Reproductive toxicology:** In a Segment I reproductive study in rats, mating and fertility was unaffected at oral doses of up to 1000 mg/kg/day. The use of a 2-week treatment period for males was a major deficiency in this study. The drug was not teratogenic or embryo-fetotoxic in rats or rabbits.

**Special toxicology:** DU-176b did not promote N-nitrosodiethylamine-induced liver carcinogenesis in male F344 rats, as indicated by GST-P positive staining.

### 2.6.6.2 Single-dose toxicity

#### **D11-4176b: Single Oral Toxicity in Rats**

**Key study findings:** not lethal at oral doses of up to 2000 mg/kg

**Study no.:** 20020310 (0996)

**Volume #, page #:** Vol. 7, Pg. 1

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** May 14, 2002 (report dated October 21, 2002)

**GLP compliance:** A statement of compliance was included.

**QA report:** yes (x) no ( )

**Drug:** lot # CZ001; 100.3% pure

**Animals:** Crj:CD(SD)IGS rats, age 6 weeks  
Males: 181-195 g  
Females: 136-146 g

**Methods:** Rats were treated with a single oral administration of 0 (vehicle), 1000, or 2000 mg/kg D11-4176b (5 rats/sex/group). Dose levels are expressed as the anhydrous free base equivalent. The dose volume was 20 ml/kg. The drug was administered as a suspension in 0.5% methylcellulose. The animals were observed for 15 days.

#### **RESULTS:**

**Mortality:** None.

**Clinical Signs:** None.

**Bodyweight:** On day 8, bodyweight was increased by 7% and 5% in the 1000 and 2000 mg/kg males, respectively. These changes were not observed at day 15.

**Gross Pathology:** No abnormalities.

**Conclusions:** A minimum lethal dose was not identified. No adverse effects were observed at oral doses of up to 2000 mg/kg (anhydrous free base equivalent).

**D11-4176b: Single Oral Toxicity in Cynomolgus Monkeys**

**Key study findings:** not lethal at oral dose levels of up to 400 mg/kg

**Study no.:** 20020307 (0997)

**Volume #, page #:** Vol. 7, Pg. 61

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** May 13, 2002 (report dated October 4, 2002)

**GLP compliance:** A statement of compliance was included.

**QA report:** yes (x) no ( )

**Drug:** lot # CZ001; 100.3% pure

**Animals:** female Cynomolgus monkeys, age 4-5 years, 2.73-3.38 kg

**Methods:** Female monkeys were treated with a single oral administration of 0 (vehicle), 200, or 400 mg/kg D11-4176b (2 monkeys/group). The doses are expressed as the anhydrous free base equivalent. The drug was administered as a suspension in 0.5% methylcellulose. The dose volume was 4 ml/kg. The animals were observed for 14 days.

**RESULTS:**

**Mortality:** None.

**Clinical Signs:** Menses was observed in the 0 and 200 mg/kg groups (1/2 and 2/2 monkeys, respectively).

**Bodyweight:** No effects.

**Hematology:** Blood was collected on days -15, -8, 2, 8, and 15 (dosing day was day 1). RBCs were reduced by 4-7% relative to the vehicle control values, and by 10-11% relative to the baseline value (day -8) in the 400 mg/kg group on days 2, 8, and 15. Hemoglobin was decreased by 9-11% relative to the vehicle control values, and by 15-16% relative to the baseline value (day -8) in the 400 mg/kg group on days 2, 8, and 15. Hematocrit was reduced by 5-8% relative

to the vehicle control values, and by 10-11% relative to the baseline value in the 400 mg/kg group on days 2, 8, and 15. Platelets were decreased by 54% in one of the 400 mg/kg monkeys on day 2, relative to the baseline value. The vehicle had no effect on platelet count. PT (prothrombin time) was increased by 64% and 60% in the 200 and 400 mg/kg groups, respectively, on day 2. APTT (activated partial thromboplastin time) was increased by 27% and 59% in the 200 and 400 mg/kg groups, respectively, on day 2. Factor X was reduced by 26% in the 200 mg/kg group on day 2, relative to the vehicle control value (38% reduction relative to the group baseline value). Factor X was decreased by 33% in one of the 400 mg/kg monkeys on day 2, relative to the baseline value. The vehicle had no effect.

**Clinical Chemistry:** One female in the 400 mg/kg group exhibited changes, as listed below.

Albumin: 22% reduction on day 2, relative to the baseline value (day -8). The vehicle had no effect.

Albumin/Globulin: 40% reduction on day 2, relative to the baseline value (day -8). The vehicle had no effect.

Phospholipids: 3-fold increase on day 2, relative to the baseline value (day -8). The vehicle had no effect.

Calcium: 13% reduction on day 2 relative to the baseline value (day -8). The vehicle had no effect.

**Conclusions:** This study was deficient in the number of animals used. A minimum lethal dose was not established. No limiting toxicity occurred at doses of up to 400 mg/kg. Slight reductions in RBCs, hematocrit, and hemoglobin were observed through 14 days post-dose in monkeys treated with 400 mg/kg. Anticoagulant activity was detected in the 200 and 400 mg/kg groups. A transient reduction in platelet count occurred in one animal treated with 400 mg/kg.

### 2.6.6.3 Repeat-dose toxicity

#### **D11-4176b: Four-Week Oral Toxicity in Rats**

**Key study findings:** NOAEL was 60 mg/kg/day; no target organ toxicity

**Study no.:** 6630-119

**Volume #, page #:** Vol. 7, Pg. 129

**Conducting laboratory and location:**  (b) (4)

**Date of study initiation:** April 24, 2002 (report dated December 10, 2002)

**GLP compliance:** A statement of compliance was included.

**QA report:** yes (x) no ( )

**Drug:** lot # DZ002; 100.6% pure

**Animals:** CD(SD)IGS rats, age 7 weeks

Males: 241-316 g

Females: 146-215 g

**Methods:** CD(SD)IGS rats were treated orally with 0 (vehicle), 20, 60, or 200 mg/kg/day D11-4176b for 4 weeks (10 rats/sex/group). The dose levels are expressed as the anhydrous free base equivalent. Toxicokinetic groups were treated with 20, 60, or 200 mg/kg/day (6 rats/sex/group). The drug was administered (gavage) as a suspension in 0.5% methylcellulose, using a dose volume of 10 ml/kg. Dose selection was based on results of preliminary 2-week oral toxicity study in male rats. The only adverse event in that study was crystalluria, which occurred at doses of up to 200 mg/kg/day (lower dose levels were not stated). An additional basis of dose selection was a 4-week oral toxicity study in rats using an analogue of D11-4176b. The dose levels selected for the present study are the same as those used in the 4-week study of the analogue. In the present study, complete histopathologic examination was performed for all dose groups.

## **RESULTS:**

**Mortality:** None.

**Clinical Signs:** Alopecia occurred in the 200 mg/kg/day group (1/20 rats).

**Bodyweight:** Weight gain was reduced by 7% and 11% in the 20 and 200 mg/kg/day males, respectively (not significant).

**Food Consumption:** No effects.

**Ophthalmoscopy:** No lesions were observed.

**ECG:** Not performed.

**Hematology:** No changes in hematology parameters were observed. PT and APTT were unaffected. Myeloid/erythroid ratio in bone marrow was unchanged.

**Clinical Chemistry:** The results are shown in the following table.

| <b>Parameter</b>  | <b>Dose(s)<br/>(mg/kg/day)</b>     | <b>Change</b>     | <b>Week</b> |
|-------------------|------------------------------------|-------------------|-------------|
| Total Cholesterol | 60 <sup>m</sup>                    | 32% increase      | 4           |
| LDH               | 60 <sup>m</sup> , 200 <sup>m</sup> | 34-36% decrease   | 4           |
| Creatine Kinase   | 60 <sup>m</sup> , 200 <sup>m</sup> | 35%, 45% decrease | 4           |
| ALP               | 200 <sup>f</sup>                   | 28% decrease      | 4           |

m: males  
f: females

No clinically significant changes were observed.

**Urinalysis:** The following parameters were reported: specific gravity, pH, urobilinogen, clarity, color, glucose, ketones, bilirubin, blood, RBC, WBC, epithelial cells, crystals, bacteria, casts, yeast, spermatozoa, mucus, volume, sodium concentration, sodium excretion (mEq/time), potassium concentration, potassium excretion, chloride concentration, chloride excretion, total protein concentration, and total protein excretion. Total protein (mg/ml) was increased by 55%, 89%, and 98% in the 20, 60, and 200 mg/kg/day males, respectively (not significant).

**Organ Weights:** Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Adrenal: Organ/brain weight ratio was increased 13% in the 200 mg/kg/day females.

Thyroid/Parathyroid (left): Absolute weight was increased by 25% and 50% in the 60 and 200 mg/kg/day males, respectively. Organ/bodyweight ratio was increased by 23% and 63% in the 60 and 200 mg/kg/day males, respectively. Organ/brain weight ratio was increased by 26% and 58% in the 60 and 200 mg/kg/day males, respectively. Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 27%, 28%, and 23%, respectively, in the 200 mg/kg/day females.

Thyroid/Parathyroid (right): Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 15%, 21%, and 18%, respectively, in the 200 mg/kg/day males. Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 20%, 24%, and 18%, respectively, in the 200 mg/kg/day females.

### **Gross Pathology:**

Diaphragm: A mass was observed in the 200 mg/kg/day group (1/20 rats).

Uterus: Distension with fluid in lumen occurred in the 20 and 60 mg/kg/day groups (1/10 and 3/10 females, respectively).

**Histopathology:**

Lungs: Focal pneumonitis with hemoglobin crystals and/or phagocytosis of erythrocytes occurred in the 60 and 200 mg/kg/day groups (2/20 rats in each group). Hemorrhage occurred in the 60 and 200 mg/kg/day groups (1/20 rats in both groups, males only).

Pancreas: Focal hemorrhage and chronic inflammation occurred in the 20, 60, and 200 mg/kg/day groups (1/20 rats in each group, males only).

Thymus: Focal hemorrhage with hemoglobin crystals was observed in the 200 mg/kg/day group (1/10 females).

**Toxicokinetics:** Plasma kinetic parameters are shown in the table below (taken from the study report).

Summary of Toxicokinetic Parameters for D11-4176 in Rat Plasma

| Dose Group    | Dose Level (mg/kg/day) | Gender | C <sub>max</sub> (ng/mL) | T <sub>max</sub> (Hours) | AUC <sub>0-4</sub> (ng·hr/mL) | AUC <sub>0-24</sub> (ng·hr/mL) |
|---------------|------------------------|--------|--------------------------|--------------------------|-------------------------------|--------------------------------|
| <u>Day 1</u>  |                        |        |                          |                          |                               |                                |
| 5             | 20                     | M      | 691                      | 1.00                     | 1297                          | 2418                           |
|               |                        | F      | 820                      | 1.00                     | 2390                          | 2390                           |
| 6             | 60                     | M      | 1380                     | 1.00                     | 6636                          | 6636                           |
|               |                        | F      | 2410                     | 1.00                     | 7708                          | 7708                           |
| 7             | 200                    | M      | 1710                     | 1.00                     | 18243                         | 18243                          |
|               |                        | F      | 3330                     | 1.00                     | 21699                         | 21699                          |
| <u>Day 28</u> |                        |        |                          |                          |                               |                                |
| 5             | 20                     | M      | 1140                     | 1.00                     | 5702                          | 5702                           |
|               |                        | F      | 1290                     | 1.00                     | 6673                          | 6673                           |
| 6             | 60                     | M      | 2200                     | 1.00                     | 12123                         | 12123                          |
|               |                        | F      | 2910                     | 1.00                     | 12956                         | 12956                          |
| 7             | 200                    | M      | 2520                     | 2.00                     | 23745                         | 23745                          |
|               |                        | F      | 4520                     | 1.00                     | 44808                         | 44808                          |

Values were determined from 3 rats/group/time-point.

The increases in AUC and C<sub>max</sub> values were disproportionately low relative to dose increment, with the exception of females on day 1. AUC and C<sub>max</sub> values were substantially increased on day 28 relative to day 1, suggestive of plasma drug accumulation.

**Conclusions:** The authors considered the lesions in lungs (60 and 200 mg/kg/day), pancreas (20, 60, and 200 mg/kg/day), and thymus at (200 mg/kg/day) as drug-related, although the incidence was low (1-3 out of 20 rats). In a follow-up 4-week oral toxicity study in male rats using lower dose levels (reviewed below), the same lesions occurred without any sign of dose-dependency. Therefore, these lesions should not be viewed as drug-related in the present study. The NOAEL is considered to be 60 mg/kg/day, since weight gain was reduced in the 200 mg/kg/day group.

**D11-4176b: Four-Week Oral Toxicity in Male Rats – Additional Study**

**Key study findings:** NOAEL was 18 mg/kg/day; no target organ toxicity

**Study no.:** 6630-132

**Volume #, page #:** Vol. 9, Pg. 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** November 11, 2002 (report dated April 15, 2003)

**GLP compliance:** A statement of compliance was included.

**QA report:** yes (x) no ( )

**Drug:** DZ002; 100.6%

**Animals:** male CD(SD)IGS rats, age 6 weeks, 160-203 g

**Methods:** Male rats were treated orally with 0 (vehicle), 6, 12, or 18 mg/kg/day D11-4176b for 29 days (10 rats/group). The dose levels are expressed as the anhydrous free base equivalent. Toxicokinetic groups were treated with 6, 12, or 18 mg/kg/day (6 rats/group). The drug was administered as a suspension in 0.5% methylcellulose, using a dose volume of 10 ml/kg. Complete histopathologic examination was performed in all groups. Dose selection was based on results of the 4-week oral toxicity study in rats (reviewed above), in which hemorrhagic lesions in lungs, pancreas, and thymus were observed in a small number of rats at doses of 20, 60, and 200 mg/kg/day. These lesions occurred in males only in the 20 and 60 mg/kg/day groups. Therefore, the present study was limited to males only and utilized doses that were lower than those of the previous study.

**RESULTS:**

**Mortality:** None.

**Clinical Signs:** No signs were observed.

**Bodyweight:** Weight gain was not affected. The mean bodyweight was  $177 \pm 5.7$  g on day 1, and  $382 \pm 26.4$  on day 29.

**Food Consumption:** No effects.

**Ophthalmoscopy:** Not performed.

**ECG:** Not performed.

**Hematology:** No effects were observed in blood samples collected on the day of sacrifice. PT and APTT were unaffected.

**Clinical Chemistry:** Total protein and albumin were increased by 5% in the 18 mg/kg/day group. Triglyceride concentration was increased by 32% in the 12 mg/kg/day group. One rat in the 12 mg/kg/day group exhibited a 2.8-fold increase in LDH, relative to the mean control value. Creatine kinase was increased by 2.9- and 6.4-fold in two individual animals in the 6 and 12 mg/kg/day groups, respectively.

**Urinalysis:** Not performed.

**Organ Weights:** The absolute weight, organ/bodyweight ratio, and organ/brain weight ratio of thyroid/parathyroid (right) were reduced by 25-26% in the 12 mg/kg/day group.

**Gross Pathology:** No abnormalities were observed.

**Histopathology:**

Lungs: Focal pneumonitis occurred in the 6 and 12 mg/kg/day groups (2/10 rats in both groups). Hemoglobin crystals were observed in the 6 and 12 mg/kg/day groups (1/10 rats in both groups). Erythrophagocytosis occurred in the control, 6, and 12 mg/kg/day groups (1/10, 2/10, and 1/10 rats, respectively). These pulmonary lesions were similar to those observed in the previous 4-week oral toxicity study in rats. In the present study, these lesions were not considered as drug-related, based on the minimal severity and the absence of a dose relationship. In addition, a sporadic incidence of focal pneumonitis was noted in a review of the testing laboratory's historical database.

Pancreas: Inflammation occurred in the control and 6 mg/kg/day groups (1/10 rats in both groups). A low incidence of pancreatic inflammation was observed in the previous 4-week oral toxicity study in rats at dose levels of 20, 60, and 200 mg/kg/day (males only).

**Toxicokinetics:** Plasma kinetic parameters are shown in the following table.

| Dose<br>(mg/kg/day) | t <sub>max</sub><br>(hr) | C <sub>max</sub><br>(ng/ml) | AUC <sub>0-24 hr</sub><br>(ng•hr/ml) |
|---------------------|--------------------------|-----------------------------|--------------------------------------|
| <b>Day 1</b>        |                          |                             |                                      |
| 6                   | 1                        | 105                         | 1063                                 |
| 12                  | 1                        | 288                         | 1810                                 |
| 18                  | 1                        | 504                         | 2563                                 |
| <b>Day 28</b>       |                          |                             |                                      |
| 6                   | 1                        | 279                         | 1051                                 |
| 12                  | 1                        | 717                         | 3619                                 |
| 18                  | 1                        | 1365                        | 5786                                 |

Values were determined from 3 rats/time-point.

The AUC and C<sub>max</sub> values were increased with dose level. These parameters were increased by approximately two-fold on day 28 in the 12 and 18 mg/kg/day groups, relative to the respective day 1 values.

**Conclusions:** The NOAEL was 18 mg/kg/day, the highest dose tested. A sporadic, non-dose-dependent incidence of lesions in the lungs and pancreas was observed. A low incidence of similar lesions was observed in a previous 4-week oral toxicity study in rats using a dose range of 20-200 mg/kg/day. However, the results of this study indicate that the pulmonary and pancreatic lesions in the initial 4-week oral toxicity study in rats should not be considered as drug-related.

**D11-4176b: 4-Week Oral Toxicity in Cynomolgus Monkeys**

**Key study findings:** NOAEL was 10 mg/kg/day; mortality occurred at 100 mg/kg/day; hemorrhage occurred in several organs at 100 mg/kg/day; target organ toxicity that did not involve hemorrhage was observed in kidneys, lungs, liver, and spleen

**Study no.:** 6630-120

**Volume #, page #:** Vol. 10, Pg. 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** April 12, 2002 (report dated December 4, 2002)

**GLP compliance:** A statement of compliance was included.

**QA report:** yes (x) no ( )

**Drug:** lot# DZ002; 100.6% pure

**Animals:** Cynomolgus monkeys, age 24-41 months  
Males: 1.8-3.5 kg  
Females: 1.8-2.4 kg

**Methods:** Cynomolgus monkeys were treated orally with 0 (vehicle), 10, 30, or 100 mg/kg/day D11-4176b for a duration of four weeks (4 monkeys/sex/group). Dose levels are expressed as the anhydrous free base equivalent. The drug was administered as a suspension in 0.5% methylcellulose, using a dose volume of 5 ml/kg. A complete histopathologic examination was performed in all groups. Dose selection was based on results from a preliminary 4-week oral toxicity study in female Cynomolgus monkeys. No drug-related adverse effects were observed at doses of up to 30 mg/kg/day in this study. In an acute toxicity study, no signs of toxicity were observed at 100 mg/kg.

## **RESULTS:**

**Mortality:** One high-dose female was sacrificed in moribund condition on day 3 and another high-dose female died on day 27 during the blood collection procedure. The cause of death was not identified in the female sacrificed on day 3. This animal exhibited loose black feces, red vomitus, aspiration of deep red stomach contents following drug administration, red oral discharge, lateral recumbency, and hypothermia. In the female that died on day 27, audible respiration and red discharge from the oral and nasal passages were observed just before death. White vomitus was observed on day 21. Death was attributed to hemorrhage in multiple tissues, which was most severe in the lungs and thymus. Both of these deaths were considered to be drug-related.

**Clinical Signs:** In the surviving animals, swelling of right maxillary area occurred in the 100 mg/kg/day group (1/6 monkeys). White vomitus was observed in the 30 mg/kg/day group (1/8 monkeys). Loose feces occurred in the 100 mg/kg/day group (1/6 monkeys). Red skin in the maxillary and/or inguinal regions was observed in the 30 and 100 mg/kg/day groups (2/8 monkeys in both groups).

**Bodyweight:** Weight gain was unaffected.

**Food Consumption:** No data was provided.

**Ophthalmoscopy:** No effects.

**ECG:** The authors stated that no abnormalities were observed at pre-study or on week 4. However, no data was provided.

**Hematology:** Blood was collected on day 27. The results are shown in the table below.

| Parameter             | Dose(s)<br>(mg/kg/day)             | Change                 | Week |
|-----------------------|------------------------------------|------------------------|------|
| RBC                   | 30 <sup>m</sup> , 100 <sup>m</sup> | 7-8% decrease (ns)     | 4    |
|                       | 30 <sup>f</sup>                    | 9% decrease (ns)       | 4    |
| Reticulocytes (count) | 100 <sup>m</sup>                   | 43% increase (ns)      | 4    |
|                       | 30 <sup>f</sup> , 100 <sup>f</sup> | 83%, 59% increase (ns) | 4    |
| Hemoglobin            | 30 <sup>m</sup> , 100 <sup>m</sup> | 7%, 10% decrease*      | 4    |
|                       | 30 <sup>f</sup>                    | 12% decrease (ns)      | 4    |
| Hematocrit            | 100 <sup>m</sup>                   | 7% decrease (ns)       | 4    |
|                       | 30 <sup>f</sup>                    | 12% decrease (ns)      | 4    |

\*Significant at 100 mg/kg/day  
n=2 for 100 mg/kg/day females  
ns: not significant  
m: males  
f: females

Mild anemia occurred in the 30 and 100 mg/kg/day males, and in the 30 mg/kg/day females. The proportion of reticulocytes was increased to 2.5% (not significant) in the 100 mg/kg/day males, as compared to 1.6% in the control males and the baseline value of 0.8%. The proportion of reticulocytes was increased to 2.7% and 2.0% (not significant) in the 30 and 100 mg/kg/day females, respectively, as compared to 1.3% in the control females.

PT was increased by 15% and 28% in the 30 and 100 mg/kg/day males, respectively. APTT was increased by 15%, 22%, and 26% in the 10, 30, and 100 mg/kg/day males, respectively. PT was increased by 15% and 35% in the 30 and 100 mg/kg/day females. APTT was increased by 12%, 22%, and 25% in the 10, 30, and 100 mg/kg/day females, respectively.

M/E ratio in bone marrow was reduced by 8%, 20%, and 36% in the 10, 30, and 100 mg/kg/day males (not significant), respectively, and by 18% and 12% in the 10 and 100 mg/kg/day females (not significant), respectively. Mild erythroid hyperplasia occurred in one female in the 30 mg/kg/day group. This effect was associated with an increased relative and absolute reticulocyte count in peripheral blood. The authors suggested that this effect was probably a normal bone marrow response to the collection of multiple blood samples for toxicokinetic analysis.

**Clinical Chemistry:** Globulin was increased by 21% in the 100 mg/kg/day females (not significant). A/G ratio was reduced by 11% in the 100 mg/kg/day females (not significant).

**Urinalysis:** Total protein was increased by 2.1- and 2.7-fold in the 30 and 100 mg/kg/day males, respectively. Protein excretion (mg/hr) was increased by 57% (not significant) in the 100 mg/kg/day males. Total protein was increased by 40% in the 100 mg/kg/day females (not significant), and protein excretion was increased by 68% and 24% in the 30 and 100 mg/kg/day females (not significant). Chloride concentration and excretion (mEq/hr) was decreased by 28% and 30%, respectively, in the 100 mg/kg/day females (not significant).

**Organ Weights:** Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported. Organs in the 100 mg/kg/day females that died prematurely were not weighed.

Lungs: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 18-29%, 7-18%, and 14-23% in the 10, 30, and 100 mg/kg/day males, respectively (not significant). Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 22%, 23%, and 24%, respectively, in the 30 mg/kg/day females.

Prostate: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 30%, 54%, and 40%, respectively, in the 100 mg/kg/day males (not significant).

Spleen: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 33-42%, 22-33%, and 29-43% in the 10, 30, and 100 mg/kg/day males, respectively. Absolute weight was increased by 17% and 24% in the 30 and 100 mg/kg/day females, respectively. Organ/bodyweight ratio was increased by 14% and 29% in the 30 and 100 mg/kg/day females, respectively. None of the above changes were significant.

Thymus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio in males were reduced by 19-32%, 12-19%, and 14-32% at 10, 30, or 100 mg/kg/day, respectively (not significant).

Uterus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were decreased by 41%, 36%, and 45%, respectively, in the 100 mg/kg/day females (not significant).

### **Gross Pathology:**

#### **Premature Deaths (2 high-dose females)**

Cecum: Dark brown material in lumen was observed in the female sacrificed on day 3. Dark red fluid in lumen was observed in the female that died on day 27.

Colon: Dark brown material in lumen was observed in the female sacrificed on day 3.

Duodenum: Dark brown material in lumen was observed in the female sacrificed on day 3. Dark red fluid in lumen was observed in the female that died on day 27.

Ileum: Dark brown material in lumen was observed in the female sacrificed on day 3. Dark red fluid in lumen was observed in the female that died on day 27.

Jejunum: Dark red fluid in lumen was observed in the female that died on day 27.

Kidneys: Pale cortices were observed in the female sacrificed on day 3.

Liver: Pallor in all lobes was observed in the female sacrificed on day 3.

Lungs: All lobes were mottled, firm, and red in the female that died on day 27.

Rectum: Dark brown material in lumen was observed in the female sacrificed on day 3.

Stomach: Dark brown material in lumen was observed in the female sacrificed on day 3. Dark red fluid in lumen was observed in the female that died on day 27.

Thymus: Dark red discoloration occurred in the female that died on day 27.

### **Terminal Sacrifice**

No treatment-related changes were observed.

### **Histopathology:**

#### **Premature Deaths (2 high-dose females)**

Cecum: Pigmented material in lumen was observed in both females. Free blood was associated with the pigmented material in the female that died on day 27.

Colon: Pigmented material in lumen was observed in both females.

Heart: Hemorrhage was observed in the female that died on day 27.

Ileum: Pigmented material in lumen was observed in the female sacrificed on day 3.

Jejunum: Pigmented material with the appearance of free blood in lumen was observed in the female sacrificed on day 3. The mucosa was intact.

Kidney: Minimal or slight tubular dilatation occurred in both females. Chronic inflammation (minimal) with some protein deposits in dilated tubules occurred in the female that died on day 27.

Liver: Slight vacuolization was observed in the female sacrificed on day 3. Hepatocellular enlargement also occurred in this animal. Chronic inflammation (minimal) occurred in the female that died on day 27.

Lungs: Severe hemorrhage, acute inflammation, focal interstitial pneumonitis, fibrinous bronchi, neutrophilic infiltration, and inflammation in pleura occurred in the female that died on day 27.

Rectum: Pigmented material in lumen was observed in the female sacrificed on day 3.

Spleen: Lymphoid necrosis (moderate) and lymphoid hyperplasia (slight) was observed in the female sacrificed on day 3.

Stomach: Focal degeneration in fundic region occurred in the female sacrificed on day 3.

Thymus: Severe hemorrhage occurred in the female that died on day 27.

Thyroid: Microcysts were observed in the female sacrificed on day 3. Slight hemorrhage occurred in the female that died on day 27.

Trachea: Moderate hemorrhage occurred in the female that died on day 27.

**Terminal Sacrifice**

Adrenals: Hemorrhage in cortex occurred in the 100 mg/kg/day group (1/6 monkeys).

Liver: Focal vacuolization was observed in the 30 mg/kg/day group (1/8 monkeys).

Lungs: Focal interstitial pneumonitis was observed in the 100 mg/kg/day group (1/6 monkeys).

Spinal Cord (thoracic): Perivascular mononuclear infiltrate was observed in the 100 mg/kg/day group (1/6 monkeys).

Stomach: Hemorrhage occurred in the 30 mg/kg/day group (1/8 monkeys).

Uterus: Hemorrhage was observed in the 100 mg/kg/day group (1/2 females).

**Toxicokinetics:** Plasma kinetic parameters are shown in the following table.

| Dose (mg/kg/day) | t <sub>max</sub> (hr)          | C <sub>max</sub> (ng/ml)        | AUC <sub>0-24 hr</sub> (ng•hr/ml)   |
|------------------|--------------------------------|---------------------------------|-------------------------------------|
| <b>Day 1</b>     |                                |                                 |                                     |
| 10               | M: 1.75 ± 0.5<br>F: 1.0 ± 0.0  | M: 397 ± 114<br>F: 457 ± 31     | M: 4061 ± 872<br>F: 4187 ± 1172     |
| 30               | M: 3.5 ± 1.0<br>F: 2.0 ± 0.0   | M: 722 ± 336<br>F: 688 ± 82     | M: 10051 ± 4171<br>F: 6860 ± 1435   |
| 100              | M: 3.5 ± 1.0<br>F: 3.0 ± 1.15  | M: 1630 ± 731<br>F: 1646 ± 1104 | M: 21206 ± 9772<br>F: 23365 ± 11670 |
| <b>Day 27</b>    |                                |                                 |                                     |
| 10               | M: 1.5 ± 0.58<br>F: 1.25 ± 0.5 | M: 358 ± 61<br>F: 328 ± 31      | M: 3273 ± 310<br>F: 3269 ± 568      |
| 30               | M: 2.0 ± 0.0<br>F: 1.75 ± 0.5  | M: 495 ± 77<br>F: 553 ± 153     | M: 5963 ± 606<br>F: 6213 ± 1448     |
| 100              | M: 2.5 ± 1.0<br>F: 3.0*        | M: 701 ± 201<br>F: 691*         | M: 11307 ± 3939<br>F: 10229*        |

Values are the mean ± S.D. of 4 monkeys, except where noted.

\* n=2

M: males

F: females

The increases in  $C_{max}$  and AUC were disproportionately small relative to the dose increment. These parameters were lower on day 27, as compared to day 1. No sex-related differences were observed.

**Other:** Fecal samples were tested for occult blood on day 2. One female in the 100 mg/kg/group (the animal sacrificed on day 3) tested positive for fecal occult blood. All other monkeys were negative.

**Conclusions:** Administration of 100 mg/kg/day produced mortality (2/8 monkeys). Death in one animal was attributed to hemorrhage in multiple tissues, particularly in the lungs and thymus. Based on the incidence of hemorrhage alone in the 100 mg/kg/day group, the target organs of toxicity include lungs, thymus, heart, thyroid, trachea, adrenals, and uterus. In addition, hemorrhage in stomach was observed in the 30 mg/kg/day group. The hemorrhagic effect was probably due to exaggerated pharmacological activity. Pulmonary lesions other than hemorrhage were observed in the 100 mg/kg/day group (i.e. acute inflammation, focal interstitial pneumonitis, fibrinous bronchi, neutrophilic infiltration, and inflammation in pleura). Other target organs of toxicity include kidneys (tubular dilatation and inflammation in premature deaths, increased urine protein at 30 and 100 mg/kg/day), liver (vacuolization and inflammation in premature deaths and the 30 mg/kg/day group), and spleen (lymphoid necrosis and hyperplasia in a premature death). Slight reductions in RBC count, hemoglobin, and hematocrit in the 30 and 100 mg/kg/day groups were correlated with the incidence of hemorrhage in histopathologic examination. The NOAEL was 10 mg/kg/day.

#### 2.6.6.4 Genetic toxicology

##### **D11-4176b: Bacterial Reverse Mutation Test**

**Key findings:** negative

**Study no.:** SBL63-54

**Volume #, page #:** Vol. 11, Pg. 186

**Conducting laboratory and location:**  (b) (4)

**Date of study initiation:** April 1, 2002 (report dated July 18, 2002)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CZ001; 100.3% pure

**Methods:** The mutagenic potential of D11-4176b was evaluated using the Ames test (Ames et al., Mutation Res, 31, 1975).

**Strains/species/cell line:** The histidine-dependent *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537, as well as *E. coli* WP2uvrA were tested.

**Doses used in definitive study:** 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/plate D11-4176b (3 plates/group); dose levels are expressed as the anhydrous free base equivalent

**Basis of dose selection:** preliminary toxicity test

**Negative controls:** DMSO

**Positive controls:**

| Tester Strain          | - S9                         | + S9                            |
|------------------------|------------------------------|---------------------------------|
| TA-98                  | AF-2, 0.1 µg/plate           | 2-aminoanthracene, 0.5 µg/plate |
| TA-100                 | AF-2, 0.01 µg/plate          | 2-aminoanthracene, 1 µg/plate   |
| TA-1535                | ENNG, 5 µg/plate             | 2-aminoanthracene, 2 µg/plate   |
| TA-1537                | 9-aminoacridine, 80 µg/plate | 2-aminoanthracene, 2 µg/plate   |
| <i>E. coli</i> WP2uvrA | ENNG, 2 µg/plate             | 2-aminoanthracene, 10 µg/plate  |

**Incubation and sampling times:** Bacterial suspensions were pre-incubated with the test article for 20 min at 37°C, with or without rat S9 liver fraction. Agar was added to the bacterial suspensions. The mixtures were transferred to plates, which were then incubated for 48 hr at 37°C. The mutation assay was performed twice.

**Results:**

**Study validity:** The criteria for a valid study were not stated, although the authors claim that the study fulfilled these criteria. The criterion for a positive result for all strains was a dose-dependent increase of at least 2-fold in the revertant colony count.

**Study outcome:** In the preliminary toxicity test, decreased background lawn density was observed at 5000 µg/plate in the TA-98 and TA-100 strains, and at 2500 µg/plate in the TA-1535 and TA-1537 strains. Precipitation of D11-4176b occurred at 156 µg/plate and higher. D11-4176b tested negative. The positive control compounds produced increases in revertant colonies.

**Conclusions:** D11-4176b was negative under the study conditions.

**D11-4176b: In Vitro Chromosomal Aberration Test in CHL Cells**

**Key findings:** polyploidy was increased in the presence of S9

**Study no.:** SBL63-55

**Volume #, page #:** Vol. 11, Pg. 222

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** April 1, 2002 (report dated August 23, 2002)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CZ001; 100.3% pure

**Methods:**

**Strains/species/cell line:** Chinese hamster lung (CHL/IU) cells

**Doses used in definitive study:** 313, 625, 1250, and 2500 µg/ml D11-4176b for the 6-hr treatments in the absence or presence of rat S9 liver fraction (duplicate cultures); 19.5, 39.1, 78.1, and 156 µg/ml D11-4176b for the 24-hr treatment without S9 (duplicate cultures); dose levels are expressed as the anhydrous free base equivalent

**Basis of dose selection:** preliminary toxicity test

**Negative controls:** DMSO

**Positive controls:** benzo[a]pyrene (20 µg/ml) for the 6-hr treatment in the presence of S9; mitomycin C for the 6- and 24-hr treatment without S9 (0.15 and 0.05 µg/ml, respectively)

**Incubation and sampling times:** CHL/IU cells were treated for 6 or 24 hr with the test or control articles. In the 6-hr treatment assays, the cells were washed and resuspended in fresh medium at the end of treatment, and the incubation was continued for 18 hr. To arrest the cells in metaphase, 0.2 µg/ml Colcemid was added to the cell suspensions at 2 hr before harvesting. One hundred metaphase cells were observed for each culture.

**Results:**

**Study validity:** The criteria for a valid study were fulfilled (i.e. frequency of aberrant cells in the negative and positive control groups was within the historical control range). The criterion for a positive result was an incidence of at least 10% for structural or numerical aberrations. An

aberration incidence of <5% was considered negative, and an incidence of at least 5% but less than 10% was considered as equivocal.

Study outcome: In the preliminary toxicity test, cell proliferation was reduced by about 50% at 2500 µg/ml in the 6-hr treatment with or without S9, and at 156 µg/ml in the 24-hr treatment. Toxicity was dose-dependent. In the aberration assay, the incidence of structural chromosomal aberrations was not affected by the test article. However, a marked increase in numerical aberrations was observed at 1250 and 2500 µg/ml in the 6-hr treatment in the presence of S9. Each of these aberrations was classified as polyploidy (defined as 3n or more). Test article precipitation occurred at concentrations of 78.1 µg/ml and higher. The results from the 6-hr treatment are shown in the table below.

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Table 4 Results of Chromosomal Aberration Test (Short-term Treatment)

Article: D11-4176b

| Test | S 9 Mix | Dose levels (µg/mL)         | Number of cells with structural aberrations (frequency %) |                 |                    |                  |                     |        |             | Judgement* | Number of gaps | Cell Proliferation Ratio (%)# | Number of cells with numerical aberrations (frequency %) |            |                     |             | Judgement* |
|------|---------|-----------------------------|---|-----------------|--------------------|------------------|---------------------|--------|-------------|------------|----------------|-------------------------------|--|------------|---------------------|-------------|------------|
|      |         |                             | No. of cells observed                                     | Chromatid break | Chromatid exchange | Chromosome break | Chromosome exchange | Others | Total       |            |                |                               | No. of cells observed                                    | Polyploidy | endo-reduplications | Total       |            |
| 18   | -       | Negative control DMSO       | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           | /          | 0              | 100                           | 100  | 1          | 0                   | 1           | /          |
|      |         |                             | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           |            |                |                               | 100  | 1          | 0                   | 1           |            |
|      |         |                             | 200   | 1               | 0                  | 0                | 0                   | 0      | 1 ( 0.5 )   |            |                |                               | 200  | 2          | 0                   | 2 ( 1.0 )   |            |
| 18   | -       | 313                         | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           | Negative   | 0              | 80.6                          | 100  | 2          | 0                   | 2           | Negative   |
|      |         |                             | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           |            |                |                               | 100  | 1          | 0                   | 1           |            |
|      |         |                             | 200   | 1               | 0                  | 0                | 0                   | 0      | 1 ( 0.5 )   |            |                |                               | 200  | 3          | 0                   | 3 ( 1.5 )   |            |
| 18   | -       | 625                         | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           | Negative   | 1              | 70.1                          | 100  | 1          | 0                   | 1           | Negative   |
|      |         |                             | 100   | 0               | 0                  | 1                | 0                   | 0      | 1           |            |                |                               | 100  | 1          | 0                   | 1           |            |
|      |         |                             | 200   | 0               | 0                  | 1                | 0                   | 0      | 1 ( 0.5 )   |            |                |                               | 200  | 2          | 0                   | 2 ( 1.0 )   |            |
| 18   | -       | 1250                        | 100   | 2               | 0                  | 0                | 0                   | 0      | 2           | Negative   | 1              | 56.7                          | 100  | 2          | 0                   | 2           | Negative   |
|      |         |                             | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           |            |                |                               | 100  | 2          | 0                   | 2           |            |
|      |         |                             | 200   | 3               | 0                  | 0                | 0                   | 0      | 3 ( 1.5 )   |            |                |                               | 200  | 4          | 0                   | 4 ( 2.0 )   |            |
| 18   | -       | 2500                        | 100   | 0               | 1                  | 0                | 0                   | 0      | 1           | Negative   | 0              | 34.3                          | 100  | 1          | 0                   | 1           | Negative   |
|      |         |                             | 100   | 1               | 1                  | 0                | 0                   | 0      | 2           |            |                |                               | 100  | 2          | 0                   | 2           |            |
|      |         |                             | 200   | 1               | 2                  | 0                | 0                   | 0      | 3 ( 1.5 )   |            |                |                               | 200  | 3          | 0                   | 3 ( 1.5 )   |            |
| 18   | -       | Positive control (MMC) 0.15 | 100   | 5               | 18                 | 0                | 0                   | 0      | 19          | /          | 0              | 67.2                          | 100  | 0          | 0                   | 0           | /          |
|      |         |                             | 100   | 6               | 21                 | 0                | 0                   | 0      | 22          |            |                |                               | 100  | 0          | 0                   | 0           |            |
|      |         |                             | 200   | 11              | 39                 | 0                | 0                   | 0      | 41 ( 20.5 ) |            |                |                               | 200  | 0          | 0                   | 0 ( 0.0 )   |            |
| 18   | +       | Negative control DMSO       | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           | /          | 1              | 100                           | 100  | 1          | 0                   | 1           | /          |
|      |         |                             | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           |            |                |                               | 100  | 1          | 0                   | 1           |            |
|      |         |                             | 200   | 1               | 0                  | 0                | 0                   | 0      | 1 ( 0.5 )   |            |                |                               | 200  | 2          | 0                   | 2 ( 1.0 )   |            |
| 18   | +       | 313                         | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           | Negative   | 0              | 82.2                          | 100  | 2          | 0                   | 2           | Negative   |
|      |         |                             | 100   | 0               | 0                  | 0                | 0                   | 0      | 0 ( 0.0 )   |            |                |                               | 100  | 2          | 0                   | 2           |            |
|      |         |                             | 200   | 0               | 0                  | 0                | 0                   | 0      | 0 ( 0.0 )   |            |                |                               | 200  | 4          | 0                   | 4 ( 2.0 )   |            |
| 18   | +       | 625                         | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           | Negative   | 0              | 83.6                          | 100  | 3          | 0                   | 3           | Negative   |
|      |         |                             | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           |            |                |                               | 100  | 3          | 0                   | 3           |            |
|      |         |                             | 200   | 1               | 0                  | 0                | 0                   | 0      | 1 ( 0.5 )   |            |                |                               | 200  | 6          | 0                   | 6 ( 3.0 )   |            |
| 18   | +       | 1250                        | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           | Negative   | 0              | 54.8                          | 100  | 10         | 0                   | 10          | Positive   |
|      |         |                             | 100   | 0               | 0                  | 0                | 0                   | 0      | 0           |            |                |                               | 100  | 11         | 0                   | 11          |            |
|      |         |                             | 200   | 0               | 0                  | 0                | 0                   | 0      | 0 ( 0.0 )   |            |                |                               | 200  | 21         | 0                   | 21 ( 10.5 ) |            |
| 18   | +       | 2500                        | 100   | 1               | 0                  | 0                | 0                   | 0      | 1           | Negative   | 0              | 49.3                          | 100  | 27         | 0                   | 27          | Positive   |
|      |         |                             | 100   | 1               | 1                  | 0                | 0                   | 0      | 2           |            |                |                               | 100  | 30         | 0                   | 30          |            |
|      |         |                             | 200   | 2               | 1                  | 0                | 0                   | 0      | 3 ( 1.5 )   |            |                |                               | 200  | 57         | 0                   | 57 ( 28.5 ) |            |
| 18   | +       | Positive control [B(a)P] 20 | 100   | 2               | 19                 | 0                | 0                   | 0      | 20          | /          | 0              | 65.8                          | 100  | 0          | 0                   | 0           | /          |
|      |         |                             | 100   | 4               | 20                 | 0                | 0                   | 0      | 21          |            |                |                               | 100  | 0          | 0                   | 0           |            |
|      |         |                             | 200   | 6               | 39                 | 0                | 0                   | 0      | 41 ( 20.5 ) |            |                |                               | 200  | 0          | 0                   | 0 ( 0.0 )   |            |

Final concentration of S 9 was 5%, gaps: chromatid and chromosome gaps, Treatment time: treatment time + recovery time,

# of each plate per dose fill in lines 1 & 2, and the total in line 3. #: ratio of viable cell count %, DMSO: Dimethylsulfoxide, MMC: Mitomycin C, B[a]P: Benzo(a)pyrene,

x: without metabolic activation, + S 9 Mix: with metabolic activation, \*: Ishikawa's criteria (Negative; frequency% < 5%, Equivocal; 5% ≤ frequency% < 10%, Positive; 10% ≤ frequency%)

no precipitation in the culture medium was observed at all doses at the start and end of treatment.

Aberrations of D11-4176b were expressed in terms of anhydrous free base.

Polyploidy occurred at dose levels that produced strong inhibition of cell growth (45-51%), but only in the presence of S9. In the absence of S9, the inhibition of cell growth was similar to that observed in the presence of S9, but the incidence of polyploidy was not affected.

**Conclusions:** The authors considered D11-4176b to be positive for the induction of chromosomal aberrations. However, this conclusion was based solely on the increase in frequency of polyploidy. The incidence of structural aberrations was not affected by the drug. The polyploidy-inducing effect appears to be mediated by a metabolite, since the effect occurred only in the presence of S9. The dose levels which produced polyploidy were associated with strong toxicity. However, a similar level of toxicity was observed in the absence of S9 without an increase in polyploidy, which suggests that the mechanism of polyploidy-induction was not related to toxicity. Given that the biological significance of polyploidy is unknown, the study results should be considered as negative.

**DU-176b: Polyploidy Test in Cultured Human Peripheral Blood Lymphocytes**

**Key findings:** positive in the absence or presence of S9 at concentrations of 313 µg/ml and higher; the dose levels which produced polyploidy were associated with mild to strong toxicity

**Study no.:** 6630-142 (23563-0-449OECD)

**Volume #, page #:** Vol. 12, Pg. 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** June 9, 2003 (report dated February 19, 2004)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

**Methods:**

**Strains/species/cell line:** human peripheral blood lymphocytes

**Doses used in definitive study:** 157, 313, 938, and 1880 µg/ml DU-176b for the 3-hr treatment in the absence of rat S9 liver fraction with 22-hr recovery (duplicate cultures); 157, 625, 1250, and 2500 µg/ml for the 22-hr treatment in the absence of rat S9 liver fraction (duplicate cultures); 157, 313, 625, and 1250 µg/ml for the 3-hr treatment in the absence of S9 with 43-hr recovery (duplicate cultures); 78.5, 157, 313, and 1250 µg/ml for the 46-hr treatment in the absence of S9 (duplicate cultures); 157, 313, 625, and 1880 µg/ml for the 3-hr treatment in the presence of S9 with 19-hr recovery (duplicate cultures); 157, 313, 625, and 1250 µg/ml for the

3-hr treatment in the presence of S9 with 43-hr recovery (duplicate cultures); dose levels are expressed as the anhydrous free base equivalent

Basis of dose selection: dose range-finding study using 3-hr, 22-hr, and 46-hr treatments

Negative controls: DMSO

Positive controls: noscapine HCl (used only in the 22-hr treatment without S9; there are no known positive control compounds that induce polyploidy after a short treatment period with or without metabolic activation)

Incubation and sampling times: Lymphocytes were treated for 3, 22, or 46 hr with the test or control articles. In the 3-hr treatment assays with and without S9, the cells were washed and resuspended in fresh medium at the end of treatment, and the incubation was continued for 19 or 43 hr. In the 22- and 46-hr treatment assays, the cells were harvested at the end of the treatment period. To arrest the cells in metaphase, 0.1 µg/ml Colcemid® was added to the cell suspensions at 2 hr before harvesting. One thousand metaphase cells were observed for each culture. The assays were performed twice.

### **Results:**

Study validity: The criteria for a valid study were fulfilled (i.e. the vehicle control cultures were within the historical control range, the positive control induced a statistically significant increase over the vehicle control cultures, and the highest applicable dose was used as determined from the preliminary toxicity assay). The criteria for a positive response were a significant and reproducible increase in the number of cells with polyploidy/endoreduplication at one or more dose levels, with evidence of a dose-dependent effect. The results were considered as negative if no significant increase in polyploidy/endoreduplication occurred at any dose level.

Study outcome: The results of the dose range-finding study were not reported. The effects of DU-176b on polyploidy frequency are shown in the following tables.

Table 1 Summary data of polyploidy test

| Initial Assay        |                        |                          |                   |                       |                           |                                     |                        |
|----------------------|------------------------|--------------------------|-------------------|-----------------------|---------------------------|-------------------------------------|------------------------|
| Metabolic Activation | Treatment-Recovery (h) | Harvest After Dosing (h) | Test Article      | Concentration (µg/mL) | % Mitotic Index Reduction | Total Number of Metaphases Analyzed | Polyploid Cells Mean % |
| Without Activation   | 3-19                   | 22                       | DMSO              | 1.2%                  | 0                         | 1519                                | 0.72                   |
|                      |                        |                          | DU-176b           | 157                   | 0                         | 2000                                | 0.05                   |
|                      |                        |                          |                   | 313 <sup>c</sup>      | 23                        | 2000                                | 0.25                   |
|                      |                        |                          |                   | 938 <sup>d</sup>      | 37                        | 2000                                | 0.85                   |
|                      |                        |                          |                   | 1880 <sup>d</sup>     | 52                        | 990                                 | 0.50                   |
|                      | 22-0                   | 22                       | DMSO              | 1.2%                  | 0                         | 2000                                | 0                      |
|                      |                        |                          | DU-176b           | 157                   | 0                         | 2000                                | 0.05                   |
|                      |                        |                          |                   | 625 <sup>e</sup>      | 4                         | 2000                                | 0.15                   |
|                      |                        |                          |                   | 1250 <sup>e</sup>     | 43                        | 2000                                | 0.05                   |
|                      |                        |                          |                   | 2500 <sup>e</sup>     | 43                        | 2000                                | 0                      |
|                      |                        |                          | Positive control  | 90                    | -                         | 2000                                | 6.25 <sup>*</sup>      |
|                      | 3-43                   | 46                       | DMSO              | 1.2%                  | 0                         | 2000                                | 0.25                   |
|                      |                        |                          | DU-176b           | 157                   | 13                        | 2000                                | 0.35                   |
|                      |                        |                          |                   | 313 <sup>a</sup>      | 32                        | 2000                                | 0.65 <sup>*</sup>      |
|                      |                        |                          |                   | 625 <sup>a</sup>      | 42                        | 1980                                | 2.88 <sup>*</sup>      |
|                      |                        |                          |                   | 1250 <sup>d</sup>     | 45                        | 2000                                | 1.25 <sup>*</sup>      |
|                      | 46-0                   | 46                       | DMSO              | 1.2%                  | 0                         | 2000                                | 0.05                   |
|                      |                        |                          | DU-176b           | 78.5                  | ND                        | 2000                                | 0                      |
|                      |                        |                          |                   | 157                   | 32                        | 2000                                | 0.10                   |
|                      |                        |                          |                   | 313 <sup>e</sup>      | 45                        | 2000                                | 1.10 <sup>*</sup>      |
|                      |                        |                          | 1250 <sup>e</sup> | 53                    | 2000                      | 0.55 <sup>*</sup>                   |                        |

<sup>a</sup>-Precipitate after dose, <sup>b</sup>-Precipitate after dose and prior to wash, <sup>c</sup>-Precipitate after dose and prior to harvest, <sup>d</sup>-Precipitate after d ND: No data<sup>e</sup> Exceeded the historical control and significantly greater numerical aberrations than the vehicle controls,  $P < 0.05$  (One-tailed Fisher's exact test, vs DMSO control). <sup>\*</sup> Significant dose-related increase,  $P < 0.05$  (One-tailed Cochran-Armitage trend test). DMSO: Dimethyl sulphoxide, Positive control: Noscapine hydrochloride

Table 1 Summary data of polyploidy test (continued)

## Initial Assay (Continued)

| Metabolic Activation | Treatment-Recovery (h) | Harvest After Dosing (h) | Test Article | Concentration (µg/mL) | % Mitotic Index Reduction | Total Number of Metaphases Analyzed | Polyploid Cells Mean % |
|----------------------|------------------------|--------------------------|--------------|-----------------------|---------------------------|-------------------------------------|------------------------|
| With Activation      | 3-19                   | 22                       | DMSO         | 1.2%                  | 0                         | 2000                                | 0.05                   |
|                      |                        |                          | DU-176b      | 157                   | 3                         | 2000                                | 0.20                   |
|                      |                        |                          |              | 313 <sup>a</sup>      | 13                        | 2000                                | 0.35 <sup>a</sup>      |
|                      |                        |                          |              | 625 <sup>c</sup>      | 39                        | 2000                                | 0.75 <sup>a</sup>      |
|                      |                        |                          |              | 1880 <sup>d</sup>     | 52                        | 2000                                | 0.60 <sup>a</sup>      |
|                      | 3-43                   | 46                       | DMSO         | 1.2%                  | 0                         | 2000                                | 0.25                   |
|                      |                        |                          | DU-176b      | 157                   | 0                         | 2000                                | 0.20                   |
|                      |                        |                          |              | 313 <sup>c</sup>      | 25                        | 2000                                | 0.65 <sup>a</sup>      |
|                      |                        |                          |              | 625 <sup>c</sup>      | 40                        | 2000                                | 1.30 <sup>a</sup>      |
|                      |                        |                          |              | 1250 <sup>d</sup>     | 56                        | 2000                                | 0.65 <sup>a</sup>      |

<sup>a</sup> -Precipitate after dose, <sup>b</sup> -Precipitate after dose and prior to wash, <sup>c</sup> -Precipitate after dose and prior to harvest, <sup>d</sup> -Precipitate after d ND: No data<sup>a</sup> Exceeded the historical control and significantly greater numerical aberrations than the vehicle controls,  $P < 0.05$  (One-tailed Fisher's exact test, vs DMSO control). <sup>a</sup> Significant dose-related increase,  $P < 0.05$  (One-tailed Cochran-Armitage trend test).  
DMSO: Dimethyl sulphoxide, Positive control: Noscapine hydrochloride

Table 1 Summary data of polyploidy test (continued)

## Confirmation Assay

| Metabolic Activation | Treatment-Recovery (h) | Harvest After Dosing (h) | Test Article             | Concentration (µg/mL) | % Mitotic Index Reduction | Total Number of Metaphases Analyzed | Polyploid Cells Mean % |
|----------------------|------------------------|--------------------------|--------------------------|-----------------------|---------------------------|-------------------------------------|------------------------|
| Without Activation   | 3-19                   | 22                       | DMSO<br>DU-176b          | 1.0%                  | 0                         | 2000                                | 0.05                   |
|                      |                        |                          |                          | 313 <sup>a</sup>      | 31                        | 2000                                | 0.20                   |
|                      |                        |                          |                          | 625 <sup>d</sup>      | 51                        | 2000                                | 0.55 <sup>*</sup>      |
|                      |                        |                          |                          | 1250 <sup>d</sup>     | 48                        | 2000                                | 0.65 <sup>*</sup>      |
|                      | 22-0                   | 22                       | DMSO<br>Positive control | 1.0%                  | 0                         | 2000                                | 0.2                    |
|                      |                        |                          |                          | 90                    | -                         | 2000                                | 1.35 <sup>*</sup>      |
|                      |                        |                          |                          | 120                   | -                         | 2000                                | 2.85 <sup>*</sup>      |
|                      | 3-43                   | 46                       | DMSO<br>DU-176b          | 1.0%                  | 0                         | 2000                                | 0                      |
|                      |                        |                          |                          | 313 <sup>a</sup>      | 56                        | 2000                                | 1.80 <sup>*</sup>      |
|                      |                        |                          |                          | 625 <sup>c</sup>      | 69                        | 2000                                | 2.20 <sup>*</sup>      |
|                      |                        |                          |                          | 1250 <sup>c</sup>     | 83                        | 2000                                | 2.40 <sup>*</sup>      |
|                      | 46-0                   | 46                       | DMSO<br>DU-176b          | 1.0%                  | 0                         | 2000                                | 0.05                   |
| 313 <sup>a</sup>     |                        |                          |                          | 76                    | 2000                      | 0.75 <sup>*</sup>                   |                        |
| 625 <sup>c</sup>     |                        |                          |                          | 71                    | 2000                      | 1.35 <sup>*</sup>                   |                        |
| 1250 <sup>c</sup>    |                        |                          |                          | 64                    | 2000                      | 1.40 <sup>*</sup>                   |                        |
| With Activation      | 3-19                   | 22                       | DMSO<br>DU-176b          | 1.0%                  | 0                         | 2000                                | 0                      |
|                      |                        |                          |                          | 313 <sup>a</sup>      | 22                        | 2000                                | 0.65 <sup>*</sup>      |
|                      |                        |                          |                          | 625 <sup>d</sup>      | 66                        | 2000                                | 1.90 <sup>*</sup>      |
|                      |                        |                          |                          | 1250 <sup>d</sup>     | 69                        | 2000                                | 1.90 <sup>*</sup>      |
|                      | 3-43                   | 46                       | DMSO<br>DU-176b          | 1.0%                  | 0                         | 2000                                | 0.05                   |
|                      |                        |                          |                          | 313 <sup>a</sup>      | 6                         | 2000                                | 0.95 <sup>*</sup>      |
|                      |                        |                          |                          | 625 <sup>c</sup>      | 68                        | 2000                                | 2.40 <sup>*</sup>      |
|                      |                        |                          |                          | 1250 <sup>c</sup>     | 65                        | 2000                                | 2.70 <sup>*</sup>      |

<sup>a</sup>-Precipitate after dose, <sup>b</sup>-Precipitate after dose and prior to wash, <sup>c</sup>-Precipitate after dose and prior to harvest, <sup>d</sup>-Precipitate after d ND: No data<sup>†</sup> Exceeded the historical control and significantly greater numerical aberrations than the vehicle controls,  $P < 0.05$  (One-tailed Fisher's exact test, vs DMSO control). <sup>\*</sup> Significant dose-related increase,  $P < 0.05$  (One-tailed Cochran-Armitage trend test). DMSO: Dimethyl sulphoxide, Positive control: Noscapine hydrochloride

In the initial assay, the incidence of polyploidy cells was increased in the 3-hr treatment with 43-hr recovery and the 46-hr treatment (absence of S9). Polyploidy was also increased in the 3-hr treatments in the presence of S9, with recovery periods of 19 or 43 hr. The highest dose levels tested in the initial assay produced a decrease in mitotic index of about 50%. In the confirmatory assay, an increased incidence of polyploidy occurred in the 3-hr treatment in the absence of S9, with recovery periods of 19 or 43 hr, and in the 46-hr treatment. Polyploidy was also increased in the 3-hr treatments in the presence of S9, with recovery periods of 19 or 43 hr. The highest dose levels tested in the confirmatory assay produced a decrease of 48-83% in mitotic index. For all treatment procedures used, the lowest dose that induced polyploidy was 313 µg/ml. The incidence of endoreduplication was not affected by DU-176b. Precipitate was observed at drug concentrations of 313 µg/ml and higher, in both the absence and presence of S9.

**Conclusions:** DU-176b produced an increase incidence of polyploidy cells in the absence and presence of S9 liver fraction, but had no effect on the incidence of endoreduplication. The dose levels that induced polyploidy were associated with mild to strong toxicity.

### **In Vitro Micronucleus Test in Cultured Human Lymphocytes**

**Key findings:** negative

**Study no.:** ORE 065/033611

**Volume #, page #:** Vol. 12, Pg. 93

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** August 5, 2003 (report dated December 18, 2003)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

### **Methods:**

**Strains/species/cell line:** human peripheral blood lymphocytes

**Doses used in definitive study:** 31.25, 62.5, 125, 250, 500, 750, 1000, 1500, 2000, and 2500 µg/ml DU-176b for the 3-hr treatment in the presence of S9 rat liver fraction, followed by harvest at 24 or 48 hr (duplicate cultures); 31.25, 62.5, 125, 250, 500, 750, 1000, 1500, 2000, and 2500 µg/ml DU-176b for the 48-hr treatment in the absence of S9, followed immediately by harvesting (duplicate cultures)

Basis of dose selection: preliminary toxicity test

Negative controls: DMSO

Positive controls: 0.015, 0.02, and 0.025 µg/ml Colcemid in the 3-hr treatment with 24-hr harvest; 5, 10, and 15 µg/ml cyclophosphamide in the 3-hr treatment with 48-hr harvest; 0.05, 0.1, and 0.15 µg/ml mitomycin C in the 48-hr treatment

Incubation and sampling times: Lymphocytes were treated with the test or control articles for 3 hr in the presence of S9, or for 48 hr in the absence of S9. In the 3-hr treatment assay, the cells were washed and resuspended in fresh medium at the end of treatment, and the incubation was continued for 21 or 45 hr. In the 48-hr treatment assays, the cells were harvested at the end of the treatment period. Cell division was inhibited by the addition of cytochalasin B (6 µg/ml) at 17 hr before harvest.

### **Results:**

Study validity: The criteria for a valid study were fulfilled (i.e. the positive control compounds produced a significant increase in the proportion of micronucleated cells and Colcemid produced inhibition of cell proliferation). The criteria for a positive result were the following: a statistically significant and reproducible increase in the incidence of micronucleated cells at one or more drug concentrations; the increase in micronuclei is not associated with large changes in pH or osmolality of the treatment medium, or extreme toxicity; evidence of a dose-response relationship. The results were considered as negative if no significant increase in the incidence of micronucleated cells was observed.

Study outcome: In the preliminary toxicity test, drug concentrations of 625, 1250, and 2500 µg/ml produced an increased proportion of dead cells in the 3-hr treatment/24-hr harvest assay. Drug concentrations of 312.5 µg/ml and higher produced toxicity (decrease in viable cells) in the 3-hr treatment/48-hr harvest assay and in the 48-hr treatment assay. DU-176b produced a 3-fold increase ( $p < 0.05$ ) in micronucleated cells at 250 µg/ml in the 3-hr treatment with 48-hr harvest. However, this effect occurred only at 250 µg/ml, whereas no significant increase was observed at 750 or 1500 µg/ml (the only other concentrations used for micronuclei evaluation). Thus, the increased incidence of micronuclei was not dose-related. No significant increases in micronuclei were observed in the 3-hr treatment with 24-hr harvest or in the 48-hr treatment. Precipitate was observed at drug concentrations of 250 µg/ml and higher.

**Conclusions:** DU-176b was negative under the study conditions.

**D11-4176b: Micronucleus Test in Rats Following Oral Administration**

**Key findings:** negative

**Study no.:** 6630-123 (23563-0-454OECD)

**Volume #, page #:** Vol. 12, Pg. 147

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** July 12, 2002 (report dated November 25, 2002)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # DZ002; 100.6% pure

**Methods:**

**Strains/species/cell line:** male Crl:CD(SD)IGS BR rats, age 8 weeks, 250-296 g

**Doses used in definitive study:** single oral administration of 500, 1000, and 2000 mg/kg D11-4176b; an additional group was treated with 2000 mg/kg for measurement of toxicokinetics; the dose levels are expressed as the anhydrous free base equivalent

**Basis of dose selection:** available information on drug toxicity in rats

**Negative controls:** 0.5% methylcellulose

**Positive controls:** 60 mg/kg cyclophosphamide (oral)

**Incubation and sampling times:** rats were sacrificed at 24 and 48 hr after dosing (6 rats/group/time-point); bone marrow was removed from the tibias of the first five animals in each group

**Results:**

**Study validity:** The criteria for a valid study were fulfilled (i.e. the vehicle control value was less than 0.4% micronucleated polychromatic erythrocytes and was consistent with historical control data; the positive control group exhibited a statistically significant increase in micronucleated polychromatic erythrocytes that was consistent with historical control data). The criteria for a positive response were a statistically significant increase in micronucleated PCEs (polychromatic erythrocytes) for at least one dose level, and a statistically significant dose-dependent response.

**Study outcome:** No overt signs of toxicity were observed. The incidence of micronucleated PCEs and the proportion of PCEs in bone marrow cells was unaffected by D11-4176b. The AUC<sub>0-48hr</sub> value at 2000 mg/kg was 19,962 ng•hr/ml, and the C<sub>max</sub> value was 1323 ng/ml.

**Conclusions:** D11-4176b was negative under the assay conditions.

**D11-4176b: Bone Marrow Micronucleus Test in Rats Following Twice Intravenous or Oral Administration**

**Note:** This study was submitted as a draft report.

**Key findings:** negative

**Study no.:** 20020063 (1314-105)

**Volume #, page #:** Vol. 12, Pg. 239

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** January 28, 2002

**GLP compliance:** No statement of compliance was included.

**QA reports:** yes ( ) no (x)

**Drug:** lot # MO2427-01; purity not stated

**Methods:**

**Strains/species/cell line:** male Crj:CD(SD)IGS rats, age 6 weeks (weight was not stated)

**Doses used in definitive study:** Rats were treated with the test or control articles for two days (two doses separated by 24 hr), as described in the table below.

| <b>Treatment</b> | <b>Dose*<br/>(mg/kg/day)</b> | <b>Route</b> | <b>Dose Volume<br/>(ml/kg)</b> | <b>n</b> |
|------------------|------------------------------|--------------|--------------------------------|----------|
| 5% Glucose       | -                            | iv           | 80                             | 3        |
| 0.5% MC          | -                            | po           | 20                             | 3        |
| D11-4176b        | 100                          | iv           | 80                             | 3        |
| D11-4176b        | 2000                         | po           | 20                             | 3        |

\*Dose levels are expressed as the anhydrous free base equivalent.

MC: methylcellulose

Basis of dose selection: 100 mg/kg was thought to be the highest non-lethal intravenous dose. 2000 mg/kg was used for the oral dose because it is the highest recommended dose in the regulatory guidelines.

Negative controls: 0.5% methylcellulose (oral) and 5% glucose (intravenous)

Positive controls: none

Incubation and sampling times: Rats were sacrificed at 24 hr after the final dose. Bone marrow was collected from the femur.

### **Results:**

Study validity: The criteria for a valid study were not stated. The criterion for a positive result was a statistically significant increase in the incidence of micronucleated PCEs.

Study outcome: No clinical signs were observed. The incidence of micronucleated PCEs was not significantly increased. The proportion of PCEs in bone marrow cells was not affected.

Conclusions: This study was deficient due to the absence of a positive control compound. D11-4176b tested negative under the study conditions.

### **DU-176b: Liver Micronucleus Test in Rats Following a Single Oral Administration**

**Key findings:** negative

**Study no.:** 20030465 (1036)

**Volume #, page #:** Vol. 12, Pg. 251

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** July 30, 2003 (report dated November 28, 2003)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

### **Methods:**

Strains/species/cell line: male Crj:CD(SD)IGS rats, age 7-8 weeks, 255-340 g

Doses used in definitive study: Rats were treated with a single administration of the test or control articles, either before or after performance of a partial hepatectomy (removal of the left and quadrate lobes). The study design is shown in the following table.

| <b>Time of Treatment</b>           | <b>Treatment</b> | <b>Dose* (mg/kg)</b> | <b>Route</b> | <b>Sampling Day</b> | <b>n</b> |
|------------------------------------|------------------|----------------------|--------------|---------------------|----------|
| 1 day prior to partial hepatectomy | 0.5% MC          | -                    | po           | 5                   | 5        |
|                                    | 0.5% MC          | -                    | po           | 7                   | 5        |
|                                    | DU-176b          | 2000                 | po           | 5                   | 5        |
|                                    | DU-176b          | 2000                 | po           | 7                   | 5        |
|                                    | Taxol            | 12.5                 | ip           | 5                   | 5        |
| 1 day after partial hepatectomy    | 0.5% MC          | -                    | po           | 5                   | 5        |
|                                    | 0.5% MC          | -                    | po           | 7                   | 5        |
|                                    | DU-176b          | 2000                 | po           | 5                   | 5        |
|                                    | DU-176b          | 2000                 | po           | 7                   | 5        |
|                                    | Taxol            | 12.5                 | ip           | 5                   | 5        |

\*Dose levels of DU-176b are expressed as the anhydrous free base equivalent  
MC: methylcellulose

Basis of dose selection: In a previous liver micronucleus test, no change in cell proliferation indicators was observed in rats following a single oral administration of 1000 or 2000 mg/kg. No signs of toxicity were observed.

Negative controls: 0.5% methylcellulose

Positive controls: taxol

Incubation and sampling times: Rats were sacrificed on day 5 or 7. The day of partial hepatectomy was considered to be day 1 for all study animals. The liver was removed and hepatocytes were isolated on the day of sacrifice.

### **Results:**

Study validity: The criteria for a valid study were fulfilled (i.e. the incidence of micronucleated hepatocytes in the control groups was within the mean  $\pm$  3 standard deviations of the historical control data, and the incidence of micronucleated hepatocytes in the positive control groups was significantly increased). The criterion for a positive result was an incidence of MNH (micronucleated hepatocytes) that exceeded the historical control range and was significantly greater than the control group value. The results were considered to be negative when MNH was within the historical control range and did not increase significantly.

Study outcome: No clinical signs were observed. The incidence of MNH was not significantly increased by DU-176b.

**Conclusions:** DU-176b tested negative under the study conditions.

**Micronucleus Test in a 4-Week Oral Toxicity Study in Cynomolgus Monkeys**

**Key findings:** negative

**Study no.:** 6630-120

**Volume #, page #:** Vol. 10, Pg. 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** April 12, 2002 (report dated December 4, 2002)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # DZ002; 100.6% pure

**Methods:** This study was incorporated in a general toxicology study (reviewed above).

**Strains/species/cell line:** Cynomolgus monkeys, age 24-41 months

**Doses used in definitive study:** Cynomolgus monkeys were treated orally with 0 (vehicle), 10, 30, or 100 mg/kg/day D11-4176b for a duration of four weeks (4 monkeys/sex/group). Dose levels are expressed as the anhydrous free base equivalent. The drug was administered as a suspension in 0.5% methylcellulose, using a dose volume of 5 ml/kg.

**Basis of dose selection:** In a preliminary 4-week oral toxicity study in female Cynomolgus monkeys, no drug-related adverse effects were observed at doses of up to 30 mg/kg/day. In an acute toxicity study, no signs of toxicity were observed at 100 mg/kg po.

**Negative controls:** 0.5% methylcellulose

**Positive controls:** none

**Incubation and sampling times:** Monkeys were sacrificed on the day after the final dose. Bone marrow was collected from the femur. Marrow was not collected from the two high-dose females that died before study termination. At least 2000 PCEs (polychromatic erythrocytes) per monkey were evaluated for micronuclei.

**Results:**

**Study validity:** No criteria for a valid study were stated, presumably because this study was part of a general toxicology study. The criteria for a positive response were not defined. However, statistical analysis of the incidence of micronuclei in the treatment and control groups was performed.

**Study outcome:** Two 100-mg/kg/day females died before study termination. D11-4176b produced no significant increase in the incidence of micronucleated PCEs. No significant change in the proportion of PCEs in bone marrow cells was observed.

**Conclusions:** D11-4176b did not produce a significant increase in the incidence of micronucleated PCEs in Cynomolgus monkeys. However, this study was deficient due to the absence of a positive control group. Furthermore, no criteria for a valid study or a positive response were stated.

**D11-4176b: Unscheduled DNA Synthesis Test in Rat Liver Following Oral Administration**

**Key findings:** negative

**Study no.:** 6630-125 (23563-0-494OECD; TOX-20020498)

**Volume #, page #:** Vol. 13, Pg. 1

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** June 26, 2002 (report dated October 18, 2002)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # DZ002; 100.6% pure

**Methods:**

**Strains/species/cell line:** male CrI:CD®(SD)IGS BR rats, age 8-9 weeks (weight was not stated)

**Doses used in definitive study:** The study design is shown in the following table.

| Treatment | Dose (mg/kg) | Route | Number of Animals/Sacrifice |                        |
|-----------|--------------|-------|-----------------------------|------------------------|
|           |              |       | Sacrificed at 2-4 hr        | Sacrificed at 14-16 hr |
| Vehicle   | 20 ml/kg     | po    | 4                           | 4                      |
| D11-4176b | 500          | po    | 4                           | 4                      |
| D11-4176b | 1000         | po    | 4                           | 4                      |
| D11-4176b | 2000         | po    | 4                           | 4                      |
| DMN*      | 10           | ip    | 4                           | -                      |
| DMN*      | 15           | ip    | -                           | 4                      |

\*N-dimethylnitrosamine (positive control)

A single administration was used in each group. The dose volume was 20 ml/kg in the vehicle control and test article groups, and 1 ml/kg in the positive control groups. The vehicle for D11-4176b was 0.5% methylcellulose.

Basis of dose selection: No toxicity was observed in rats following oral administration of 2000 mg/kg/day for two days in a previous micronucleus test.

Negative controls: 0.5% methylcellulose

Positive controls: N-dimethylnitrosamine

Incubation and sampling times: Rats were sacrificed at 2-4 hr and 14-16 hr after dosing. The liver was removed and hepatocytes were isolated.

**Results:**

Study validity: The criteria for a valid study were fulfilled (i.e. the viability of the vehicle control hepatocytes was at least 50%; the average net nuclear labeling in control hepatocytes was in the range of -5.00 to 1.00; no more than 10% of control hepatocytes exhibited DNA repair; the positive control compound produced unscheduled DNA synthesis). The criteria for a positive response was an increase of at least three net grains per nucleus above the concurrent vehicle control value, resulting in a positive value, or an increase in the proportion of nuclei with five or more net grains such that the percentage of these nuclei is 10% above the concurrent vehicle control value.

Study outcome: Red discharge from rectum occurred in one rat in the 2000 mg/kg group (14-16 hr sacrifice). No other clinical signs were observed. D11-4176b tested negative for induction of UDS (unscheduled DNA synthesis). The proportion of cells in S-phase was similar in all groups (about 1%). The viability of hepatocytes was unaffected by treatment with D11-4176b or the positive control article.

**Conclusions**: D11-4176b tested negative for induction of UDS under the study conditions.

### 2.6.6.5 Carcinogenicity

No studies were submitted.

### 2.6.6.6 Reproductive and developmental toxicology

#### *Fertility and early embryonic development*

#### **DU-176b: Oral Study of Effects on Fertility and Early Embryonic Development to Implantation in Rats (Segment I Study)**

**Key study findings:** no effect on mating or fertility at oral doses of up to 1000 mg/kg/day

**Study no.:** 1035 (20030552)

**Volume #, page #:** Vol. 14, Pg. 1

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** July 28, 2003 (report dated March 17, 2004)

**GLP compliance:** Yes.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

#### **Methods:**

**Doses:** 0 (vehicle), 100, 300, and 1000 mg/kg/day DU-176b (dose levels are expressed as the free base equivalent)

**Species/strain:** rat/Crj:CD(SD)IGS

**Number/sex/group:** 20

**Route, formulation, volume:** oral; suspension in 0.5% methylcellulose; 10 ml/kg

**Satellite groups used for toxicokinetics:** none

**Study design:** Males were treated starting at two weeks before mating through the end of mating. Females were treated starting at two weeks before mating through day 7 of gestation. Males and females were repeatedly cohabitated overnight until mating was confirmed through observation of a copulation plug. The mating period was two weeks.

Males were sacrificed at the end of mating. Females were sacrificed on day 14 of gestation, and C-sections were performed. Dose selection was based on results from a preliminary 1-week oral toxicity study in rats, in which no signs of toxicity were observed at oral doses of up to 1000 mg/kg/day.

**Parameters and endpoints evaluated:** clinical signs, bodyweight, food consumption, necropsy, sperm count, sperm motility, sperm abnormalities, estrous cycle, mating parameters, fertility parameters.

## **RESULTS:**

**Mortality:** None.

**Clinical Signs:** None.

**Bodyweight:** Weight gain during the pre-mating period was decreased by 21% and 14% in the 300 and 1000 mg/kg/day males, respectively. Mean bodyweight in the control males was increased by 28.9 g during the pre-mating period. Weight gain was unaffected in females.

**Food Consumption:** No effects were observed.

**Toxicokinetics:** Not performed.

**Estrous Cycle:** No effect.

**Necropsy:** No treatment-related changes were observed.

**Sperm Examination:** The number, motility, and morphology of sperm were unaffected.

**Fertility Parameters:** The drug had no effect on mating, mating period duration, proportion of pregnant females, proportion of males that impregnated, number of dams with live embryos, corpora lutea, implantations, live embryos, or dead embryos.

**Conclusions:** DU-176b had no effect on mating or fertility in male and female rats at oral doses of up to 1000 mg/kg/day. Embryonic development was also unaffected. Dose selection was adequate.

*Embryofetal development*

**DU-176b: Oral Study for Effects on Embryo-Fetal Development in Rats (Segment II Study)**

**Key study findings:** no teratogenic effects; slight embryotoxicity and maternal toxicity at 300 mg/kg/day

**Study no.:** 1034 (20030532)

**Volume #, page #:** Vol. 15, Pg. 1

**Conducting laboratory and location:** Daiichi Pharmaceutical Co., Ltd.  
Tokyo, Japan

**Date of study initiation:** June 3, 2003 (report dated February 10, 2004)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

**Methods:**

**Doses:** 0 (vehicle), 30, 100, and 300 mg/kg/day DU-176b (dose levels are expressed as the free base equivalent)

**Species/strain:** rat/Crj:CD(SD)IGS

**Females/group:** 20

**Route, formulation, volume:** oral; suspension in 0.5% methylcellulose; 10 ml/kg

**Satellite groups used for toxicokinetics:** none

**Study design:** Mating was confirmed through observation of a copulation plug following overnight cohabitation of male and female rats in 1:1 ratio. The day of mating confirmation was designated as day 0. Mated females were treated orally with 0 (vehicle), 30, 100, or 300 mg/kg/day DU-176b on days 7-17 of gestation. Females were sacrificed on day 21, and the contents of the reproductive tract were examined during C-sections. Dose selection was based on results of a dose range-finding embryo-fetal developmental study in rats using dose levels of 300, 600, and 1000 mg/kg/day. Deaths occurred at 1000 mg/kg/day, and vaginal hemorrhage was observed at 600 and 1000 mg/kg/day. Intrauterine hemorrhage or dark greenish substance around the placenta were observed in all treatment groups. Maternal bodyweight was similar in the control and treatment groups, and no drug-related fetal anomalies were observed.

**Parameters and endpoints evaluated:** clinical signs, maternal bodyweight, food consumption, necropsy (liver, kidney, heart, spleen, lung, ovary, and uterus), contents of reproductive tract, fetal bodyweight, placenta, external examination of fetuses, skeletal examination of fetuses (about ½ of fetuses in each litter), and visceral examination of fetuses (about ½ of fetuses in each litter).

**RESULTS:**

**Mortality:** None.

**Clinical Signs:** Vaginal hemorrhage occurred at 300 mg/kg/day group on days 17 and 18 of gestation (2/20 females). One female in the 300 mg/kg/day exhibited piloerection, pale skin, vaginal discharge, and soiled perineal region on days 17 and 18. A mass in the right axillary region was observed in one high-dose female. No signs were observed at 30 or 100 mg/kg/day.

**Bodyweight:** Weight gain during days 7-21 was decreased by 9.5% in the 300 mg/kg/day group. The mean control bodyweight was increased by 150.5 g during this period.

**Food Consumption:** Food intake was not affected.

**Toxicokinetics:** Not performed.

**Terminal and Necroscopic Evaluations:** A mass in the right axillary region was observed in one high-dose female.

**C-Section Data:** All females were pregnant with live fetuses at sacrifice. The following parameters were unaffected: number of corpora lutea, number of implantations, number of dead fetuses, proportion of male fetuses, fetal weight, and appearance of placenta. However, the incidence of early resorptions (described as remnants of implantation) was increased in the 300 mg/kg/day group, as shown in the table below.

| Early Resorptions        | Dose (mg/kg/day) |           |           |           |
|--------------------------|------------------|-----------|-----------|-----------|
|                          | 0                | 30        | 100       | 300       |
| Total (%)                | 7 (2.4)          | 8 (2.9)   | 10 (3.4)  | 21 (7.5)* |
| Mean ± S.D. (per litter) | 0.4 ± 0.5        | 0.4 ± 0.6 | 0.5 ± 0.7 | 1.1 ± 1.1 |

\* p < 0.05

**Offspring:** The incidence of external anomalies, visceral malformations and variations, skeletal malformations and variations, and delayed ossification was not affected by DU-176b.

**Conclusions:** DU-176b did not produce teratogenic effects. However, embryotoxicity was observed at 300 mg/kg/day po, as indicated by a slight increase in resorptions. This dose level

also produced mild maternal toxicity. Therefore, the increase in resorptions is considered to be related to maternal toxicity, rather than a direct drug effect. The dose selection was adequate.

**DU-176b: A Study of the Effects on Embryo/Fetal Development in Rabbits (Segment II Study)**

**Key study findings:** no teratogenic effects; embryo-fetotoxicity was observed at 200 and 600 mg/kg/day, doses which produced strong signs of maternal toxicity

**Study no.:** (b) (4)-147011 (TOX-20040022)

**Volume #, page #:** Vol. 16, Pg. 1

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** July 30, 2003 (report dated April 2, 2004)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

**Methods:**

**Doses:** 0 (vehicle), 60, 200, and 600 mg/kg/day DU-176b (free base equivalent)

**Species/strain:** rabbit/Hra:(NZW)SPF

**Females/group:** 22 females/group were assigned at study initiation. Additional females were later assigned due to the failure to achieve a minimum of 16 litters in the 200 and 600 mg/kg/day groups. The 0, 60, 200, and 600 mg/kg/day groups were assigned an additional 4, 4, 8, and 8 females, respectively.

**Route, formulation, volume:** oral; suspension in 0.5% methylcellulose; 6 ml/kg

**Satellite groups used for toxicokinetics:** 60, 200, and 600 mg/kg/day (4 females/group)

**Study design:** Females were artificially inseminated using semen from the same rabbit strain. Immediately following insemination, each doe was treated intravenously with 100 USP units of human chorionic gonadotropin to induce ovulation. The day of insemination was designated as day 0 of gestation. Females were treated orally with 0 (vehicle), 60, 200, and 600 mg/kg/day DU-176b on days 7-20 of gestation. Does were sacrificed on day 29, and the contents of the reproductive tract were examined during C-sections. Dose selection was based on results of a dose range-finding study in

pregnant rabbits using dose levels of 100, 300, 600, 1000 mg/kg/day. In that study, decreased weight gain occurred in the 600 and 1000 mg/kg/day groups. No adverse effects were observed at 100 and 300 mg/kg/day.

**Parameters and endpoints evaluated:** clinical signs, maternal bodyweight, food consumption, necropsy, gravid uterine weight, toxicokinetics, contents of reproductive tract, fetal bodyweight, placentae, external examination of fetuses, visceral examination of fetuses (including a mid-coronal head slice), and skeletal examination of fetuses.

**RESULTS:**

**Mortality:** Deaths occurred in the 200 and 600 mg/kg/day groups on days 9-21 (3/30 and 4/30 females respectively). No significant clinical signs were observed in these animals prior to death. One control female was sacrificed *in extremis* as the result of a fractured pelvis.

**Clinical Signs:** Abortions occurred at 200 mg/kg/day (2/30 females, days 20 and 28) and 600 mg/kg/day (3/20 females, days 18, 23, and 24). No significant clinical signs were observed in these animals prior to abortion. Decreased defecation was more frequently observed in the 600 mg/kg/day group.

**Bodyweight:** DU-176b produced a dose-dependent reduction in weight gain. The results observed for the intervals of days 7-21 and 0-29 are shown in the table below.

| Interval | Weight Gain (g) |              |               |               |
|----------|-----------------|--------------|---------------|---------------|
|          | Control         | 60 mg/kg/day | 200 mg/kg/day | 600 mg/kg/day |
| Day 7-21 | 332 ± 136       | 234 ± 128*   | 134 ± 89*     | -6 ± 157*     |
| Day 0-29 | 798 ± 230       | 709 ± 194    | 571 ± 244*    | 515 ± 257*    |

Values are the mean ± S.D. of 18-21 females.

\* p < 0.05

On day 0, the mean bodyweight (± S.D.) in the control group was 3165 ± 149 g, with similar values observed in the treatment groups.

**Food Consumption:** DU-176b produced a dose-dependent reduction in food intake. The results observed for the intervals of days 7-21 and 0-29 are shown in the table below.

| Interval | Food Consumption (g/animal/day) |              |               |               |
|----------|---------------------------------|--------------|---------------|---------------|
|          | Control                         | 60 mg/kg/day | 200 mg/kg/day | 600 mg/kg/day |
| Day 7-21 | 214 ± 40                        | 196 ± 30     | 159 ± 30*     | 138 ± 37*     |
| Day 0-29 | 202 ± 37                        | 193 ± 29     | 168 ± 28*     | 167 ± 35*     |

Values are the mean ± S.D. of 18-21 females.

\* p < 0.05

**Toxicokinetics:** Toxicokinetic parameters were not calculated. Plasma drug concentrations at individual time-points are shown in the table below.

| Time (hr)                  | Plasma Drug Concentration (ng/ml) |               |               |
|----------------------------|-----------------------------------|---------------|---------------|
|                            | 60 mg/kg/day                      | 200 mg/kg/day | 600 mg/kg/day |
| <b>Day 7 of Gestation</b>  |                                   |               |               |
| 1                          | 5820 ± 1350                       | 7770 ± 2180   | 4820 ± 1630   |
| 2                          | 4820 ± 507                        | 8320 ± 2160   | 6380 ± 1170   |
| 4                          | 1670 ± 676                        | 6760 ± 1420   | 8690 ± 1870   |
| 6                          | 469 ± 196                         | 1690 ± 863    | 7700 ± 2430   |
| 12                         | 25.6 ± 12.4                       | 1760 ± 1540   | 3910 ± 1540   |
| 24                         | 45.5 ± 31.3                       | 501 ± 497     | 1990 ± 1370   |
| <b>Day 20 of Gestation</b> |                                   |               |               |
| 1                          | 5830 ± 1040                       | 6030 ± 350    | 5500 ± 1570   |
| 2                          | 3860 ± 591                        | 7180 ± 802    | 6150 ± 1450   |
| 4                          | 875 ± 71.3                        | 4460 ± 994    | 6850 ± 2400   |
| 6                          | 226 ± 43.1                        | 1090 ± 641    | 6810 ± 2900   |
| 12                         | 94.1 ± 76.4                       | 933 ± 791     | 2760 ± 3290   |
| 24                         | 57.8 ± 4.9                        | 166 ± 106     | 2420 ± 3420   |

Peak drug levels were generally increased with dose level, and occurred at later time-points in the high-dose group.

**Terminal and Necroscopic Evaluations:** All females were examined, regardless of pregnancy status (26 females in each of the 0 and 60 mg/kg/day group, 30 females in each of the 200 and 600 mg/kg/day groups). The intermediate-dose female that died on day 9 exhibited dark red discoloration of thymus, pericardium, mediastinal lymph node, and lungs. Ovarian cysts were also observed. Dark red discoloration and enlargement of thymus occurred in the three high-dose females that died on day 10. Dark red areas in lungs were observed in the intermediate-dose female that died on day 14. The high-dose female that died on day 21 exhibited pale liver, dark red areas in lungs, and thick red uterine contents. The high-dose female that aborted on day 24 had red areas in the lungs. In the surviving animals, the most common observation was thymus nodules (1, 3, and 3 females in the 60, 200, and 600 mg/kg/day groups, respectively). Dark red area in lungs occurred in two surviving high-dose females.

**Gravid Uterine Weight:** The results are shown in the table below. The data include only measurements from pregnant rabbits that survived through study termination.

| Parameter                   | Dose (mg/kg/day) |            |            |            |
|-----------------------------|------------------|------------|------------|------------|
|                             | 0                | 60         | 200        | 600        |
| N                           | 20               | 21         | 17         | 16         |
| Initial Bodyweight (g)      | 3170 ± 151       | 3208 ± 200 | 3190 ± 174 | 3218 ± 197 |
| Terminal Bodyweight (g)     | 3968 ± 280       | 3917 ± 273 | 3761 ± 332 | 3733 ± 344 |
| Gravid Uterine Weight (g)   | 415 ± 142        | 341 ± 152  | 363 ± 112  | 224 ± 157* |
| Terminal Net Bodyweight (g) | 3553 ± 298       | 3576 ± 324 | 3398 ± 321 | 3509 ± 389 |
| Net Bodyweight Gain (g)     | 383 ± 259        | 368 ± 230  | 208 ± 249  | 291 ± 297  |

Values are the mean ± S.D.

\* p<0.05

Gravid uterine weight was decreased in all treatment groups, although this change was not significant in the 60 and 200 mg/kg/day groups due to data variability. Net bodyweight gain tended to be reduced in the 200 and 600 mg/kg/day groups.

**C-Section Data:** The uterine contents were examined for each animal. The results are listed in the following table.

| Parameter                                  | Dose (mg/kg/day)         |                           |                             |                             |
|--|--------------------------|---------------------------|-----------------------------|-----------------------------|
|  | 0                        | 60                        | 200                         | 600                         |
| Inseminated Females                        | 26                       | 26                        | 30                          | 30                          |
| # Pregnant                                 | 20                       | 21                        | 17                          | 16                          |
| # Pregnant with complete litter resorption | 0                        | 1                         | 0                           | 3                           |
| Corpora Lutea                              | 8.4 ± 1.8                | 7.7 ± 2.0                 | 9.8 ± 2.5                   | 8.4 ± 3.5                   |
| Implantations                              | 6.5 ± 2.7                | 5.7 ± 2.6                 | 7.5 ± 2.2                   | 5.8 ± 3.1                   |
| Preimplantation Loss (%) <sup>a</sup>      | 23.4                     | 25.2                      | 22.7                        | 30.6                        |
| Live Fetuses                               | 6.3 ± 2.6                | 5.3 ± 2.8                 | 5.9 ± 2.4                   | 3.7 ± 3.0*                  |
| Dead Fetuses (total)                       | 0                        | 0                         | 0                           | 0                           |
| Early Resorptions (% per litter)           | 0.2 ± 0.4<br>(2.5 ± 5.1) | 0.4 ± 1.0<br>(8.0 ± 23.1) | 1.4 ± 1.8<br>(17.4 ± 21.7)* | 1.9 ± 1.6<br>(41.2 ± 36.7)* |
| Late Resorptions                           | 0 ± 0                    | 0 ± 0                     | 0.2 ± 0.6                   | 0.2 ± 0.5                   |
| Post-implantation Loss (% per litter)      | 0.2 ± 0.4<br>(2.5 ± 5.1) | 0.4 ± 1.0<br>(8.0 ± 23.1) | 1.6 ± 2.1<br>(20.3 ± 25.4)* | 2.1 ± 1.6<br>(43.1 ± 35.3)* |
| Male Fetuses (%) <sup>b</sup>              | 50                       | 46.4                      | 49                          | 49.1                        |

Values are the mean ± S.D. per litter, unless stated otherwise.

a: Based on total number of implantations and corpora lutea in each group.

b: Based on total number of males and females in each group.

\* p<0.05

The number of live fetuses was significantly decreased in the 600 mg/kg/day group. The incidence of resorptions was increased in the 200 and 600 mg/kg/day groups.

**Offspring:** Fetal weights were reduced in the 200 and 600 mg/kg/day groups, as shown in the following table.

| Sex     | Fetal Weight (g) |              |               |               |
|---------|------------------|--------------|---------------|---------------|
|         | Vehicle          | 60 mg/kg/day | 200 mg/kg/day | 600 mg/kg/day |
| Males   | 50.1 ± 5.9       | 47.1 ± 6.9   | 41.0 ± 8.7*   | 40.5 ± 10.7*  |
| Females | 48.9 ± 6.8       | 46.0 ± 7.0   | 41.0 ± 7.0*   | 39.4 ± 7.7*   |

External observations are shown in the table below.

| EXTERNAL OBSERVATIONS                | DOSE (mg/kg/day) |         |     |          |     |
|--------------------------------------|------------------|---------|-----|----------|-----|
|                                      |                  | 0       | 60  | 200      | 600 |
| Litters Evaluated                    | N                | 20      | 20  | 17       | 13  |
| Fetuses Evaluated                    | N                | 126     | 112 | 100      | 59  |
| <b>MALFORMATIONS</b>                 |                  |         |     |          |     |
| Gastroschisis                        |                  |         |     |          |     |
| Fetal Incidence                      | N                | 1       | 0   | 0        | 0   |
| Litter Incidence                     | N                | 1       | 0   | 0        | 0   |
| Cleft lip                            |                  |         |     |          |     |
| Fetal Incidence                      | N                | 0       | 0   | 1        | 0   |
| Litter Incidence                     | N                | 0       | 0   | 1        | 0   |
| Cleft palate                         |                  |         |     |          |     |
| Fetal Incidence                      | N                | 0       | 0   | 1        | 0   |
| Litter Incidence                     | N                | 0       | 0   | 1        | 0   |
| Omphalocele                          |                  |         |     |          |     |
| Fetal Incidence                      | N                | 0       | 0   | 1        | 0   |
| Litter Incidence                     | N                | 0       | 0   | 1        | 0   |
| <b>TOTAL MALFORMATIONS:</b>          |                  |         |     |          |     |
| Fetal Incidence                      | N (%)            | 2 (1.6) | 0   | 2 (2)    | 0   |
| Litter Incidence                     | N (%)            | 2 (10)  | 0   | 2 (11.8) | 0   |
| <b>ABNORMALITIES</b>                 |                  |         |     |          |     |
| Disseminated subcutaneous hemorrhage |                  |         |     |          |     |
| Fetal Incidence                      | N                | 1       | 0   | 0        | 0   |
| Litter Incidence                     | N                | 1       | 0   | 0        | 0   |

One fetus in the 200 mg/kg/day group exhibited cleft lip and cleft palate. Another fetus in this group exhibited omphalocele. These anomalies are not considered to be drug-related, given the absence of dose-dependency.

Soft tissue observations are shown in the table below.

| SOFT TISSUE<br>OBSERVATIONS       | DOSE (mg/kg/day) |          |           |          |          |
|-----------------------------------|------------------|----------|-----------|----------|----------|
|                                   |                  | 0        | 60        | 200      | 600      |
| Litters Evaluated                 | N                | 20       | 20        | 17       | 13       |
| Fetuses Evaluated                 | N                | 126      | 112       | 100      | 59       |
| <b>MALFORMATIONS</b>              |                  |          |           |          |          |
| Retroesophageal aortic arch       |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 1 (0.8)  | 0         | 0        | 0        |
| Litter Incidence                  | N (%)            | 1 (5)    | 0         | 0        | 0        |
| Bulbous aorta                     |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 0         | 2 (2)    | 0        |
| Litter Incidence                  | N (%)            | 0        | 0         | 2 (11.8) | 0        |
| Stenotic pulmonary trunk          |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 0         | 1 (1)    | 0        |
| Litter Incidence                  | N (%)            | 0        | 0         | 1 (5.9)  | 0        |
| Heart and/or great vessel anomaly |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 0         | 1 (1)    | 0        |
| Litter Incidence                  | N (%)            | 0        | 0         | 1 (5.9)  | 0        |
| Diaphragmatic hernia              |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 0         | 1 (1)    | 0        |
| Litter Incidence                  | N (%)            | 0        | 0         | 1 (5.9)  | 0        |
| Hydrocephaly                      |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 0         | 0        | 1 (1.7)  |
| Litter Incidence                  | N (%)            | 0        | 0         | 0        | 1 (7.7)  |
| <b>TOTAL MALFORMATIONS:</b>       |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 1 (0.8)  | 0         | 3 (3)    | 1 (1.7)  |
| Litter Incidence                  | N (%)            | 1 (5)    | 0         | 3 (17.6) | 1 (7.7)  |
| <b>VARIATIONS</b>                 |                  |          |           |          |          |
| Accessory spleen                  |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 8 (6.3)  | 8 (7.1)   | 8 (8)    | 4 (6.8)  |
| Litter Incidence                  | N (%)            | 5 (25)   | 5 (25)    | 7 (41)   | 3 (23)   |
| Small spleen                      |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 0        | 2 (1.8)   | 0        | 0        |
| Litter Incidence                  | N (%)            | 0        | 1 (5)     | 0        | 0        |
| Hemorrhagic thymus                |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 1 (0.8)  | 0         | 0        | 0        |
| Litter Incidence                  | N (%)            | 1 (5)    | 0         | 0        | 0        |
| Retrocaval ureter                 |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 1 (0.8)  | 0         | 4 (4)    | 1 (1.7)  |
| Litter Incidence                  | N (%)            | 1 (5)    | 0         | 3 (17.6) | 1 (7.7)  |
| Major blood vessel variation      |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 10 (7.9) | 17 (15.2) | 11 (11)  | 4 (6.8)  |
| Litter Incidence                  | N (%)            | 5 (25)   | 8 (40)    | 6 (35)   | 3 (23)   |
| Gallbladder absent or small       |                  |          |           |          |          |
| Fetal Incidence                   | N (%)            | 3 (2.4)  | 3 (2.7)   | 3 (3)    | 7 (11.9) |
| Litter Incidence                  | N (%)            | 1 (5)    | 3 (15)    | 3 (17.6) | 4 (30.8) |

Malformations occurred sporadically in the 0, 200, and 600 mg/kg/day groups. However, these effects do not appear to be drug-related, given the absence of dose-dependency. No drug-related variations were observed.

Skeletal malformations, variations, and delayed ossification are listed in the following tables.

| SKELETAL MALFORMATIONS               | DOSE (mg/kg/day) |         |     |         |     |
|--------------------------------------|------------------|---------|-----|---------|-----|
|                                      |                  | 0       | 60  | 200     | 600 |
| Litters Evaluated                    | N                | 20      | 20  | 17      | 13  |
| Fetuses Evaluated                    | N                | 126     | 112 | 100     | 59  |
| <b>SKULL:</b>                        |                  |         |     |         |     |
| Unspecified anomaly                  |                  |         |     |         |     |
| Fetal Incidence                      | N                | 3 (2.4) | 0   | 0       | 0   |
| Litter Incidence                     | N                | 1 (5)   | 0   | 0       | 0   |
| <b>VERTEBRAE:</b>                    |                  |         |     |         |     |
| Unspecified anomaly                  |                  |         |     |         |     |
| Fetal Incidence                      | N (%)            | 1 (0.8) | 0   | 0       | 0   |
| Litter Incidence                     | N (%)            | 1 (5)   | 0   | 0       | 0   |
| <b>STERNEBRAE:</b>                   |                  |         |     |         |     |
| Sternoschisis                        |                  |         |     |         |     |
| Fetal Incidence                      | N (%)            | 1 (0.8) | 0   | 0       | 0   |
| Litter Incidence                     | N (%)            | 1 (5)   | 0   | 0       | 0   |
| Fused                                |                  |         |     |         |     |
| Fetal Incidence                      | N (%)            | 0       | 0   | 1 (1)   | 0   |
| Litter Incidence                     | N (%)            | 0       | 0   | 1 (5.9) | 0   |
| <b>TOTAL SKELETAL MALFORMATIONS:</b> |                  |         |     |         |     |
| Fetal Incidence                      | N (%)            | 5 (4.0) | 0   | 1 (1)   | 0   |
| Litter Incidence                     | N (%)            | 3 (15)  | 0   | 1 (5.9) | 0   |

| SKELETAL VARIATIONS (INCLUDING DELAYED OSSIFICATION) | DOSE (mg/kg/day) |           |           |           |           |
|--|------------------|-----------|-----------|-----------|-----------|
|  |                  | 0         | 60        | 200       | 600       |
| Litters Evaluated                                    | N                | 20        | 20        | 17        | 13        |
| Fetuses Evaluated                                    | N                | 126       | 112       | 100       | 59        |
| <b>SKULL:</b>  |                  |           |           |           |           |
| Accessory bone(s)                                    |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 1 (0.8)   | 4 (3.6)   | 2 (2)     | 0         |
| Litter Incidence                                     | N (%)            | 1 (5)     | 1 (5)     | 2 (11.8)  | 0         |
| <b>VERTEBRAE:</b>                                    |                  |           |           |           |           |
| 27 presacral vertebrae                               |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 19 (15.1) | 17 (15.2) | 26 (26)   | 16 (27.1) |
| Litter Incidence                                     | N (%)            | 8 (40)    | 6 (30)    | 11 (64.7) | 6 (46.1)  |
| Hyoid arch bent                                      |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 2 (1.6)   | 9 (8.0)   | 16 (16)*  | 8 (13.6)  |
| Litter Incidence                                     | N (%)            | 2 (10)    | 5 (25)    | 9 (52.9)  | 4 (30.8)  |
| Hyoid body and arches unossified                     |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 0         | 0         | 1 (1)     | 0         |
| Litter Incidence                                     | N (%)            | 0         | 0         | 1 (5.9)   | 0         |
| <b>STERNEBRAE:</b>                                   |                  |           |           |           |           |
| Sternebra(e) #5 and/or #6 unossified                 |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 13 (10.3) | 6 (5.4)   | 5 (5)     | 6 (10.2)  |
| Litter Incidence                                     | N (%)            | 5 (25)    | 4 (20)    | 3 (17.6)  | 4 (30.8)  |
| Misaligned (slight or moderate)                      |                  |           |           |           |           |
| Fetal Incidence                                      | N (%)            | 1 (0.8)   | 1 (0.9)   | 3 (3)     | 0         |

|                                       |       |                   |           |           |            |
|---------------------------------------|-------|-------------------|-----------|-----------|------------|
| Litter Incidence                      | N (%) | 1 (5)             | 1 (5)     | 3 (17.6)  | 0          |
| 7 <sup>th</sup> sternebra             |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | 0                 | 0         | 2 (2)     | 0          |
| Litter Incidence                      | N (%) | 0                 | 0         | 1 (5.9)   | 0          |
| <b>RIBS:</b>                          |       |                   |           |           |            |
| Full extra (13 <sup>th</sup> ) rib(s) |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | 34 (27.0)         | 49 (34.8) | 41 (41)   | 28 (47.5)* |
| Litter Incidence                      | N (%) | 12 (60)           | 15 (75)   | 12 (70.6) | 11 (84.6)  |
| 13 <sup>th</sup> rudimentary rib(s)   |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | 31 (24.6)         | 19 (17.0) | 18 (18)   | 7 (11.9)*  |
| Litter Incidence                      | N (%) | 14 (70.0)         | 12 (60)   | 9 (52.9)  | 5 (38.5)   |
| 7 <sup>th</sup> cervical rib          |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | 0                 | 1 (0.9)   | 0         | 0          |
| Litter Incidence                      | N (%) | 0                 | 1 (5.0)   | 0         | 0          |
| <b>PELVIS:</b>                        |       |                   |           |           |            |
| Pubis unossified                      |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | 0                 | 0         | 1 (100)   | 0          |
| Litter Incidence                      | N (%) | 0                 | 0         | 1 (5.9)   | 0          |
| <b>TOTAL SKELETAL VARIATIONS:</b>     |       |                   |           |           |            |
| Fetal Incidence                       | N (%) | Data not provided |           |           |            |
| Litter Incidence                      | N (%) | Data not provided |           |           |            |

\*p < 0.05

The incidence of full extra (13<sup>th</sup>) ribs was increased in 600 mg/kg/day group. This effect was associated with a decreased incidence of rudimentary ribs in the 13<sup>th</sup> position. The incidence of bent hyoid arch was increased in the 200 mg/kg/day group.

**Conclusions:** DU-176b did not produce teratogenic effects. Embryo-fetotoxicity was observed at 200 and 600 mg/kg/day. However, these doses were associated with strong signs of maternal toxicity, such as death, abortion, reduced weight gain, and reduced food intake. Therefore, the embryo-fetotoxic effects are not considered as drug-related. The dose selection is acceptable, although lower doses should have been used.

#### 2.6.6.7 Local tolerance

No studies were submitted.

#### 2.6.6.8 Special toxicology studies

##### **DU-176b: Oral Medium-Term Liver Carcinogenesis Bioassay in Male F344 Rats**

**Key study findings:** DU-176b did not promote liver carcinogenesis

**Study no.:** 0332 (TOX-20030708)

**Volume #, page #:** Vol. 13, Pg. 84

**Conducting laboratory and location:** [REDACTED] (b) (4)

**Date of study initiation:** July 10, 2003 (report dated March 5, 2004)

**GLP compliance:** A statement of compliance was included.

**QA reports:** yes (x) no ( )

**Drug:** lot # CA201; 99.8% pure

**Formulation/vehicle:** suspension/0.5% methylcellulose

**Animals:** Male F344/DuCrj(SPF) rats, age 6 weeks, 101-116 g

**Methods:** The objective of this study was to evaluate the tumor-promoting ability of DU-176b in a hepatocarcinogenesis model in rats. The study design is shown in the following table.

| Group # | Day 1 Treatment       | Daily Treatment      | Dose (mg/kg/day) | N  |
|---------|-----------------------|----------------------|------------------|----|
| 1       | N-nitrosodiethylamine | Vehicle              | 0                | 20 |
| 2       | N-nitrosodiethylamine | DU-176b              | 5                | 20 |
| 3       | N-nitrosodiethylamine | DU-176b              | 10               | 20 |
| 4       | N-nitrosodiethylamine | DU-176b              | 20               | 20 |
| 5       | N-nitrosodiethylamine | Sodium phenobarbital | 500 ppm (diet)   | 20 |
| 6       | Saline                | Vehicle              | 0                | 10 |
| 7       | Saline                | DU-176b              | 20               | 10 |

Dose levels of DU-176b are expressed as the anhydrous free base equivalent.

Male rats were given a single dose of N-nitrosodiethylamine (200 mg/kg ip) on day 1 to induce hepatocarcinogenesis. Rats in the comparator groups were treated with saline ip. Daily dosing with the control or test articles was initiated two weeks later (day 15), and continued for six weeks. DU-176b was administered by oral gavage using a dose volume of 10 ml/kg. Dose selection was based on results of the 4-week oral toxicity study in rats (# 6630-119), in which hemorrhagic lesions in lungs, pancreas, and thymus were observed in a small number of rats at doses of 20, 60, and 200 mg/kg/day. The positive control group was given a diet containing 500 ppm sodium phenobarbital, a known tumor promoter, starting on day 15. After one week of dosing (day 22), all rats were subjected to 2/3 partial hepatectomy (2/3 of liver).

**RESULTS:**

**Mortality:** Deaths occurred in groups 4 and 5 (2/20 and 1/20 rats, respectively). Each of these deaths was considered to be the result of complications of the surgical procedure. Two deaths occurred on day 22, and one death occurred on day 55.

**Clinical Signs:** Depression was observed in all animals after surgery on day 22.

**Bodyweight:** The drug had no effect on weight gain. Weight gain was increased by 10% in the phenobarbital group, relative to the vehicle control group.

**Food Consumption:** Food intake tended to be increased in the phenobarbital group during weeks 4-8 (drug treatment began on week 3).

**Liver Weight:** Absolute weight and liver/bodyweight ratio were increased by 44% and 40%, respectively, in group 5 (phenobarbital-treated).

**Gross Pathology:**

Abdominal Cavity: Red fluid was observed in groups 4 and 5 (dead animals only).

Liver: A single discolored spot was observed in groups 2, 3, 4, and 5 (1/20, 1/20, 1/20, and 5/20 rats, respectively). Three rats in group 5 exhibited multiple discolored spots. A discolored area was also observed in group 5 (2/20 rats). Red discolored area occurred in one dead animal in group 4.

Thoracic Cavity: Hernia and clear fluid were observed in one dead animal in group 4.

**Immunohistochemical Analysis of Liver Sections:**

Liver sections were exposed to antibodies specific to rat glutathione S-transferase placental form (GST-P), followed by staining using an avid-biotin-peroxidase method. GST-P positive foci were detected in all initiated groups (#1-5), indicative of hepatocarcinogenesis. DU-176b had no effect on the number or area of GST-P positive foci in the initiated groups, whereas dietary administration of phenobarbital produced a 2-fold increase in both of these parameters. No GST-P positive foci were detected in the uninitiated groups (# 6 and 7).

**Conclusions:** DU-176b was not a promoter of liver carcinogenesis in this study, in which GST-P positive staining was used as a surrogate endpoint.

**2.6.6.9 Discussion and Conclusions**

DU-176b was not lethal at oral doses of up to 2000 mg/kg (anhydrous free base equivalent) in rats or 400 mg/kg in monkeys. The NOAEL in a 4-week oral toxicity study in rats was 60 mg/kg/day. The only adverse effect was impaired weight gain at 200 mg/kg/day. In a 4-week oral toxicity study in monkeys, the NOAEL was 10 mg/kg/day. Deaths due to hemorrhage occurred in the 100 mg/kg/day group. Hemorrhage in several organs was observed at this dose level. However, target organ toxicity that did not involve hemorrhage was observed in kidneys (30 and 100 mg/kg/day), lungs (100 mg/kg/day), liver (30 and 100 mg/kg/day), and spleen (100 mg/kg/day). DU-176b was negative in the bacterial reverse mutation test, *in vitro* micronucleus test in human lymphocytes, micronucleus test in rats using a single oral

administration, micronucleus test in rats using repeated oral and intravenous administration, hepatocyte micronucleus test in rats using a single oral administration, micronucleus test in the 4-week oral toxicity study in monkeys, and the unscheduled DNA synthesis test in rats. However, the drug did produce polyploidy in the chromosomal aberration test in CHL cells, without affecting the incidence of structural aberrations. In a follow-up study which focused solely on numerical chromosomal aberrations, DU-176b produced an increase in polyploidy in human lymphocytes. The induction of polyploidy occurred only in the presence of S9 liver fraction in CHL cells, whereas this effect was observed in both the absence and presence of S9 in human lymphocytes. In both test systems, induction of polyploidy was associated with mild to strong toxicity. In a Segment I reproductive study in rats, mating and fertility were unaffected at oral doses of up to 1000 mg/kg/day. The drug was not teratogenic in rats or rabbits. Oral administration of 300 mg/kg/day in pregnant rats produced a slight increase in resorptions, but this effect was associated with maternal toxicity. Treatment of pregnant rabbits with 200 or 600 mg/kg/day po produced strong signs of maternal toxicity (e.g. death, abortion), along with embryo-fetotoxicity. Therefore, DU-176b is not considered to be embryo-fetotoxic in rats or rabbits. DU-176b did not promote N-nitrosodiethylamine-induced liver carcinogenesis in male F344 rats, as indicated by GST-P positive staining.

#### **2.6.6.10 Tables and Figures**

Not applicable.

#### **2.6.7 TOXICOLOGY TABULATED SUMMARY**

Not applicable.

### **OVERALL CONCLUSIONS AND RECOMMENDATIONS**

DU-176b is an anticoagulant that acts through potent inhibition of clotting factor Xa. This drug has been shown to suppress thrombus formation in animal studies, and is orally active. Previous human experience is limited to two Phase 1 studies in healthy volunteers. Both of these studies were conducted in the United Kingdom. DU-176b (also referred to as D11-4176b) is the p-toluenesulfonate monohydrate salt of DU-176 (anhydrous free base). The Sponsor intends to develop DU-176b for prevention of thrombosis in patients with total knee or hip replacement.

The Sponsor will perform a Phase 1, open-label study in healthy volunteers to assess the pharmacodynamic effects of DU-176b, using an *ex vivo* model of thrombosis. The study will include 12 healthy male volunteers, age 20-50 years. Each subject will be treated with a single oral administration of 80.8 mg DU-176b (60 mg DU-176). The primary objective will be the measurement of thrombogenic activity using the Badimon chamber perfusion method, in which venous blood from the antecubital vein of the subject's arm is allowed to flow directly into a perfusion chamber containing a thrombogenic surface (porcine aorta tunica media). Measurement of thrombogenesis will be performed at 0 (pre-dose), 1.5, 5, and 12 hr post-dose. Secondary objectives will include safety evaluation and the measurement of thrombin generation, anti-Xa activity, PT/INR, aPTT, and plasma drug concentration. Safety parameters

will include physical examination, vital signs, ECG, hematology, clinical chemistry, and fecal occult blood test.

The Sponsor submitted the following studies in support of the proposed clinical study: pharmacology; CNS function study in male mice; CNS, cardiovascular, and respiratory function study in Cynomolgus monkeys; HERG channel inhibition study; electrophysiology study in isolated guinea pig papillary muscle; renal function study in rats; absorption study of [<sup>14</sup>C]DU-176b in male rats; absorption study of [<sup>14</sup>C]DU-176b in male Cynomolgus monkeys; distribution study in male Cynomolgus monkeys; whole-body autoradiography study in male rats; metabolism study in rats and monkeys; study of metabolite structure in rats and monkeys; excretion study in male rats; excretion study in male Cynomolgus monkeys; acute oral toxicity study in rats; acute oral toxicity study in Cynomolgus monkeys; 4-week oral toxicity study in rats; 4-week oral toxicity study in male rats; 4-week oral toxicity study in Cynomolgus monkeys; bacterial reverse mutation test; chromosomal aberration test in CHL cells; polyploidy test in human lymphocytes; *in vitro* micronucleus test in human lymphocytes; micronucleus test in rats using oral administration; micronucleus test in rats using intravenous and oral administration; liver micronucleus test in rats; micronucleus test in Cynomolgus monkeys; unscheduled DNA synthesis test in rats; fertility and early embryonic development study in rats (Segment I study); embryo-fetal development study in rats (Segment II study); embryo-fetal development study in rabbits (Segment II study); liver tumor promotion study in male rats.

DU-176b is a highly potent inhibitor of factor Xa in humans, Cynomolgus monkeys, rabbits, and rats. The inhibitory activity of DU-176b against thrombin, plasmin, or factor VIIa is weak or completely absent. However, the selectivity for factor Xa was not established since no other enzyme in the coagulation and fibrinolytic pathways was tested. The drug was active in two models of rat venous thrombosis and in a rat A-V shunt model. Anti-thrombotic activity occurred at oral dose levels as low as 0.5 mg/kg (depending on the model), and was associated with inhibition of factor Xa. Thus, factor Xa inhibition appears to be the primary mechanism of action. Although the drug exhibited anticoagulant activity *in vitro* (increases in PT, APTT, and TT), the anti-thrombotic activity that occurred in rats was not associated with clinically significant increases in PT (the only coagulation parameter measured). DU-176b also produced attenuation of tissue factor-induced disseminated intravascular coagulation in rats.

Oral administration of up to 200 mg/kg had no effects on CNS function in mice. Oral administration of up to 200 mg/kg in monkeys had no effects on behavior, cardiovascular parameters (including QT<sub>c</sub>), or respiratory parameters. DU-176b had no effects on HERG channel function or the electrophysiological properties of isolated guinea pig papillary muscle. Renal function was unaffected in rats at oral doses of up to 200 mg/kg.

Studies were performed to characterize the absorption, distribution, metabolism, and excretion of [<sup>14</sup>C]DU-176b in male rats and male Cynomolgus monkeys. Oral administration was used exclusively in these studies. In rats, elimination of radioactivity from plasma was rapid during the initial 12 hr after administration of 3 mg/kg ( $t_{1/2} = 3.2$  hr). The rapid elimination phase was followed by a much slower phase ( $t_{1/2} = 11$  hr). Radioactivity was extensively distributed in red blood cells. Monkeys exhibited similar plasma kinetics for radioactivity following treatment with 1 mg/kg, with the addition of a third elimination phase that was extremely slow ( $t_{1/2} = 5.3$

days starting at 96 hr post-dose). Radioactivity was still detectable in blood and plasma at 336 hr (14 days) post-dose. As observed in rats, radioactivity was extensively distributed in red blood cells. In a distribution study in monkeys, radioactivity was highly concentrated in eyes and skin at 14 days after oral administration of 1 mg/kg [<sup>14</sup>C]DU-176b. This study was flawed due to the use of only one time-point and the limited number of organs/tissues that were analyzed. However, clearance of radioactivity from organs and tissues in monkeys appears to occur very slowly, based on the presence of detectable concentrations at 14 days after dosing. A distribution study was performed in rats using whole-body autoradiography, with rats sacrificed at 1 and 24 hr post-dose. At 1 hr, the highest levels of radioactivity were present in gastrointestinal and bladder contents. Radioactivity was also concentrated in kidney, preputial gland, liver, intestine, Harderian Gland, pituitary gland, and nasal cavity, relative to blood. At 24 hr after administration, the radioactivity levels were decreased in most organs/tissues. However, a relatively high level of radioactivity was still present in the intestinal contents. D21-3231 was a major metabolite in rat plasma during the first 4 hr after dosing. This metabolite was present at much higher levels as compared to the unchanged drug. In monkey plasma, most of the radioactivity was associated with unchanged drug at 1 hr post-dose, whereas unidentified metabolites composed most of the radioactivity at 8 hr. Excretion in rats occurred primarily in feces. In contrast, both urine and feces were major routes of excretion in monkeys, with a slightly higher proportion of radioactivity present in feces.

In an acute oral toxicity study in rats, no mortality or adverse effects were observed at doses of up to 2000 mg/kg D11-4176b (anhydrous free base equivalent). In female Cynomolgus monkeys, oral administration of up to 400 mg/kg (anhydrous free base equivalent) was not lethal. Slight reductions in RBCs, hematocrit, and hemoglobin were observed through 14 days post-dose in monkeys treated with 400 mg/kg. Anticoagulant activity was detected in the 200 and 400 mg/kg groups. This study was deficient in the number of animals used (2 monkeys/group).

A 4-week oral toxicity study in rats was performed using dose levels of 0 (vehicle), 20, 60, and 200 mg/kg/day D11-4176b (anhydrous free base equivalent). No target organs of toxicity were identified. The NOAEL (no observed adverse effect level) was considered to be 60 mg/kg/day, since weight gain was reduced in the 200 mg/kg/day group.

A 4-week oral toxicity study in male rats was performed using dose levels of 0 (vehicle), 6, 12, and 18 mg/kg/day D11-4176b (anhydrous free base equivalent). The NOAEL was 18 mg/kg/day. This study was performed as a follow-up to the initial 4-week oral toxicity study in rats, as summarized above. In that study, the authors concluded that a NOAEL in males was not established due to the low incidence of microscopic focal hemorrhage and chronic inflammation in pancreas at 20, 60, and 200 mg/kg/day (1/10 males in each group). Other lesions that were considered as drug-related included focal pneumonitis associated with hemoglobin crystals and/or phagocytosis of erythrocytes in the 60 and 200 mg/kg/day groups (2/20 rats in each group), and focal hemorrhage with hemoglobin crystals in thymus at 200 mg/kg/day (1/10 females). Since none of these lesions occurred in females at 20 and 60 mg/kg/day, the authors considered the NOAEL in females to be 60 mg/kg/day. The results of this follow-up study indicate that the organ lesions that were considered as drug-related by the

authors were probably incidental, with the possible exception of hemorrhage with hemoglobin crystals in thymus.

A 4-week oral toxicity study in *Cynomolgus* monkeys was performed using dose levels of 0 (vehicle), 10, 30, and 100 mg/kg/day D11-4176b (anhydrous free base equivalent). The NOAEL was 10 mg/kg/day. Administration of 100 mg/kg/day produced mortality (2/8 monkeys). Death in one animal was attributed to hemorrhage in multiple tissues, particularly in the lungs and thymus. Based on the incidence of hemorrhage alone in the 100 mg/kg/day group, the target organs of toxicity included lungs, thymus, heart, thyroid, trachea, adrenals, and uterus. In addition, hemorrhage in stomach was observed in the 30 mg/kg/day group. The hemorrhagic effect was probably due to exaggerated pharmacological activity. Pulmonary lesions other than hemorrhage were observed in the 100 mg/kg/day group (i.e. acute inflammation, focal interstitial pneumonitis, fibrinous bronchi, neutrophilic infiltration, and inflammation in pleura). Other target organs of toxicity included kidneys (tubular dilatation and inflammation in premature deaths, increased urine protein at 30 and 100 mg/kg/day), liver (vacuolization and inflammation in premature deaths and the 30 mg/kg/day group), and spleen (lymphoid necrosis and hyperplasia in a premature death). Slight reductions in RBC count, hemoglobin, and hematocrit in the 30 and 100 mg/kg/day groups were correlated with the incidence of hemorrhage in histopathologic examination.

DU-176b was negative in the bacterial reverse mutation test, *in vitro* micronucleus test in human lymphocytes, micronucleus test in rats using a single oral administration, micronucleus test in rats using repeated oral and intravenous administration, hepatocyte micronucleus test in rats using a single oral administration, micronucleus test in the 4-week oral toxicity study in monkeys, and the unscheduled DNA synthesis test in rats. However, the drug did produce polyploidy in the chromosomal aberration test in CHL cells, without affecting the incidence of structural aberrations. In a follow-up study which focused solely on numerical chromosomal aberrations, DU-176b produced an increase in polyploidy in human lymphocytes. The induction of polyploidy occurred only in the presence of S9 liver fraction in CHL cells, whereas this effect was observed in the absence and presence of S9 in human lymphocytes. In both test systems, induction of polyploidy was associated with mild to strong toxicity. Given that the biological significance of polyploidy is unknown, no recommendations for additional studies are warranted.

A fertility and early embryonic development study in rats was performed using oral dose levels of 0 (vehicle), 100, 300, and 1000 mg/kg/day DU-176b (anhydrous free base equivalent). Mating and fertility parameters in both sexes and embryonic development were unaffected. A teratogenicity study in rats was performed using oral dose levels of 0 (vehicle), 30, 100, and 300 mg/kg/day DU-176b (anhydrous free base equivalent). DU-176b did not produce teratogenic effects. However, embryotoxicity was observed at 300 mg/kg/day, as indicated by a slight increase in resorptions. This dose level also produced mild maternal toxicity. Therefore, the increase in resorptions is considered to be the result of maternal toxicity, rather than a direct drug effect.

A teratogenicity study in rabbits was performed using oral dose levels of 0 (vehicle), 60, 200, and 600 mg/kg/day DU-176b (anhydrous free base equivalent). DU-176b did not produce teratogenic effects. Embryo-fetotoxicity was observed at 200 and 600 mg/kg/day. However,

these doses were associated with strong signs of maternal toxicity, such as death, abortion, reduced weight gain, and reduced food intake. Therefore, the embryo-fetotoxic effects are not considered as drug-related.

A study was performed in male F344 rats to evaluate the tumor-promoting ability of DU-176b. The animals were treated once with N-nitrosodiethylamine to induce liver carcinogenesis. Two weeks later, daily treatment with DU-176b or control articles was initiated. Oral administration of up to 20 mg/kg/day DU-176b for six weeks did not produce promotion of liver carcinogenesis in this model, in which GST-P (glutathione S-transferase placental form) positive staining of liver was used as a surrogate endpoint.

In the proposed clinical study, healthy volunteers will be treated with a single oral administration 80.8 mg DU-176b, equivalent to 60 mg anhydrous free base (DU-176). This will result in a dose level of 1.2 mg/kg anhydrous free base, based on a 50-kg bodyweight. Safety assessment of the proposed study is based on the 4-week oral toxicity studies of DU-176b in rats and monkeys. The NOAEL in the 4-week oral study in rats was 60 mg/kg/day (anhydrous free base equivalent), which yields a safety margin of 50. The NOAEL in the 4-week oral study in monkeys was 10 mg/kg/day (anhydrous free base equivalent), resulting in a safety margin of 8. Therefore, the 4-week oral toxicity studies in rats and monkeys provide adequate support for the safety of the proposed study.

Two Phase 1 studies have been conducted on DU-176b in the United Kingdom. The initial study was performed on healthy male volunteers, 18-55 years old, and was placebo-controlled. The drug was well tolerated at single oral doses of up to 150 mg in the initial part of the study, and at 90 mg od, 60 mg bid, and 120 mg od given for 10 days in the second part. A Phase 1 study was performed on healthy postmenopausal or surgically sterile female volunteers and healthy elderly male volunteers. The study subjects were treated orally with placebo or 90 mg/day on day 1 and on days 3-10. No serious adverse events occurred. Minor bleeding episodes were observed in the drug-treated volunteers. Headache was the most frequent adverse event.

Human pharmacokinetic information with relevance to the proposed clinical study is available from the initial Phase 1 study. The  $AUC_{0-48 \text{ hr}}$  value in male volunteers after administration of 60 mg, the dose to be used in the proposed study, was 1760 ng•hr/ml. The  $AUC_{0-24 \text{ hr}}$  values associated with the NOAEL in the 4-week oral toxicity studies in rats and monkeys were 9856 ng•hr/ml and 3698 ng•hr/ml, respectively. Both of the animal AUC values exceeded the human AUC value, which further supports the safety of the proposed study. The  $C_{\text{max}}$  values that were associated with the rat and monkey NOAEL also exceeded the human value for a 60-mg dose.

## **RECOMMENDATIONS:**

From a preclinical viewpoint, it is safe to proceed with the proposed clinical study.

Reviewer Signature \_\_\_\_\_  
David B. Joseph, Ph.D.  
Pharmacologist, HFD-180

Supervisor Signature \_\_\_\_\_ Concurrency Yes \_\_\_ No \_\_\_  
Jasti B. Choudary, B.V.Sc., Ph.D.  
Supervisory Pharmacologist, HFD-180

cc:  
IND  
HFD-160  
HFD-161/CSO  
HFD-180/Dr. Choudary  
HFD-180/Dr. Joseph  
HFD-045/Dr. Viswanathan  
HFD-160/Dr. Laniyonu

R/D Init.: J. Choudary 12/15/06

DJ/dbj: 4/4/07  
C:\DATA\I63266703.0DJ

**APPENDIX/ATTACHMENTS**

None

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/s/

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4/4/2007 01:20:57 PM  
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DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

IND NUMBER: **77254**  
SERIAL NUMBER: N000  
DATE RECEIVED BY CENTER: 3/26/07  
DRUG NAME: **DU-176b**  
PROPOSED USE: Anti-thrombotic for prevention of stroke and thromboembolic events in patients with non-valvular atrial fibrillation  
SPONSOR: Daiichi Medical Research, Inc.  
DOCUMENTS REVIEWED: Vol 1-6  
REVIEW DIVISION: Cardiovascular and Renal Products, HFD-110  
PHARM/TOX REVIEWER: Patricia P. Harlow, Ph.D.  
PHARM/TOX SUPERVISOR: Albert Defelice, Ph.D.  
DIVISION DIRECTOR: Norman Stockbridge, M.D., Ph.D.  
PROJECT MANAGER: Margaret Pease-Fye

Date of review submission to Division File System (DFS): 6/22/07

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## **EXECUTIVE SUMMARY**

### **I. Recommendations**

1. The sponsor has previously evaluated a maximum daily dose of 120 mg of DU-176B, a FXa inhibitor, in human subjects for a period of 10 weeks. The proposed trial will evaluate doses of 30 mg qd, 30 mg, bid, 60 mg, qd and 60 mg bid in patients with atrial fibrillation for three months. The human equivalent doses of the NOEL's in the 6 month rat study and the 3 month monkey study are 9.7 and 17.5 mg/kg, respectively, or 4.9- and 8.8-fold higher than the highest proposed human dose. These NOEL's provide a reasonable safety margin for the proposed trial to proceed.
2. Given the retention of DU-176B in the melaniferous tissues of the eye, the sponsor should submit the absorption spectrum of DU-176B between 325 and 700 nm. Since the sponsor has conduct an *in vitro* phototoxicity assays as specified in the Guidance for Photosafety Testing, CDER (May 2003), no further phototoxicity testing is required.
3. The sponsor should monitor intraocular pressure and obtain electroretinograms, in addition to standard slit-lamp biomicroscopy and funduscopy, before the treatment initiation and at multiple intervals in the longer toxicity studies in monkeys or pigmented animals. Careful histopathology of the eye should be performed on all animals. The Division has previously consulted with the Division of Anti-Infective and Ophthalmology Products concerning similar findings for other products. The Division of Anti-Infective and Ophthalmology Products recommended close ocular monitoring needs to be performed in human subjects receiving repeat doses of DU-176B in the absence of longer term animal studies demonstrating no ocular anatomic or functional pathology.

### **II. Summary of nonclinical findings**

DU-176b, an orally active inhibitor of activated Factor X (FXa), prolongs standard *in vitro* coagulation assays in a concentration-dependent manner, but is more potent in plasma from humans, rabbits, and monkeys than in plasma from rats. *In vivo*, DU-176b inhibited thrombosis in three rat models (venous thrombosis, venous stasis, and an arterio-venous shunt) in association with FXa inhibition but not clinically significant increases in prothrombin time. The effect of DU-176b on bleeding times or platelet aggregation was not monitored. The sponsor has not evaluated DU-176b for secondary pharmacodynamic effects such as in receptor binding studies *in vitro*.

DU176b did not show adverse effects in a battery of safety pharmacology studies, including *in vitro* and *in vivo* cardiovascular system studies and *in vivo* respiratory, renal, and central nervous system studies.

Following oral administration, [<sup>14</sup>C]-DU-176b was rapidly absorbed having a Tmax of 0.7 and 2.3 hours in rats and cynomolgus monkeys, respectively. The initial elimination half-life (t<sub>1/2</sub>) was about 3.2 hours in both species, followed by a slower elimination phase with t<sub>1/2</sub> of 11 and 32 hours in the rat and monkey, respectively.

Protein binding of [<sup>14</sup>C]-DU-176b *in vitro* was approximately 33, 45, 49, and 55% in plasma from rats, dogs, monkeys, and humans, respectively. The *in vitro* distribution of radioactivity to blood cells was 57, 54, 38, and 47% in rat, dog, monkey, and human,

respectively. A whole-body autoradiography study in albino rats indicated that the highest concentrations of DU-176 were found in the gastrointestinal and bladder contents, followed by the kidney, preputial gland, and liver with only trace levels found in the brain and the eye. However, at 336 hr after oral administration of [<sup>14</sup>C]-DU-176b to cynomolgus monkeys, radioactivity was highly concentrated in eyes and skin. A tissue distribution study in pigmented rats showed the maximum concentration of [<sup>14</sup>C]-DU-176b radioactivity in the eye was similar to that in the kidneys and liver. The half-life of elimination from the rat eye was 260 hours.

Following oral administration of DU-176b to monkeys, unchanged DU-176 was the principal component in plasma. In contrast, a major metabolite (D21-3231, a hydrolysis product of DU-176b) was present in rat plasma at higher levels than the unchanged drug. The assessment of metabolites in the urine and feces of rats and monkeys indicated that DU-176 is excreted primarily unchanged and not conjugated.

In the rat, most of the administered dose was eliminated within 24 h, with 71 % of the dose excreted via the bile into the feces and 25% in the urine. In the monkey, most of administered dose was eliminated within 96 h, with 49% of the dose excreted in the feces and 41 % in the urine. DU-176 can cross the placenta in rats and is excreted into breast milk of lactating rats,

Various *in vitro* studies showed that DU-176b is primarily metabolized by CYP3A4. However, an *in vitro* inhibition study indicated that the IC<sub>50</sub> values of DU-176 were >100 μM for all CYP 450 isozymes investigated.

In a 13-week repeated-dose toxicity study, male and female cynomolgus monkeys received DU-176b doses of 0, 6, 18, and 54 mg/kg once daily by oral gavage. Slight prolongation of coagulation times consistent with the pharmacodynamic effect of DU-176B was observed at 24 hours after dosing. Some individual mid- and high-dose animals showed effects on hematology parameters most likely in response to hemorrhagic events. The sponsor's NOAEL was 54 mg/kg.

DU-176b was administered for 26-weeks at 0, 6, 18, and 54 mg/kg to rats. The previous reviewer indicated that no remarkable findings were observed at any of the dose levels tested. The NOAEL was 54 mg/kg. However, the 4-week toxicity study in rats (20, 60 and 200 mg/kg) showed dose-related hemorrhage (pancreas, thymus and lungs) at doses of 20 mg/kg and higher, but no mortality at any dose level.

Although the Ames assays were negative, the chromosomal aberration assay in Chinese hamster lung cells (CHL) and the human lymphocyte polyploidy assay were considered positive. DU-176b had no effect on the incidence of chromosomal aberrations, but increased the incidence of polyploidy CHL cells in the presence of metabolic activation. In human lymphocytes, DU-176b increased the incidence of polyploidy cells in the absence and presence metabolic activation at dose levels that produced precipitate formation. However, all *in vivo* genotoxicity assays were negative.

The mouse and rat carcinogenicity protocols were reviewed by the Exec CAC in December 2006. Based on MTD's in the 13-week oral gavage studies in rats and mice, the Exec CAC recommended doses of 0, 50, 150 and 500 mg/kg for male and female mice, doses of 0, 60, 200, and 600 mg/kg for male rats, doses of 0, 50, 100 and 200

mg/kg in female rats. Previously, the sponsor showed that DU-176b at 20 mg/kg did not enhance liver carcinogenesis in rats exposed to a single dose of N-nitrosodiethylamine.

DU-176b produced no effect on mating or fertility in male and female rats at oral doses of up to 1000 mg/kg/day. In rats, DU-176b produced no teratogenic effects, although slight embryotoxicity and maternal toxicity was observed at 300 mg/kg/day. In rabbits, DU176b produced no teratogenic effects, although embryo-fetotoxicity was observed at doses (200 and 600 mg/kg/day) that produced maternal toxicity.

The *in vitro* phototoxicity of DU-176b in BALB/3T3 cells was judged as incapable of being evaluated, since the IC<sub>50</sub> value was greater than 1 mg/mL in the presence and absence of photo-irradiation,. However, DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation.

### III. **Nonclinical safety issues relevant to clinical use:**

The distribution studies in pigmented rats and monkeys clearly showed retention of DU-176B in the eye for more than 168 hours following a single dose. Although the sponsor has demonstrated that DU-176B lacks significant phototoxicity potential, the sponsor has not evaluated the effect of DU-176b on retinal function. The sponsor needs to perform electroretinogram evaluations in longer term toxicity studies.

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

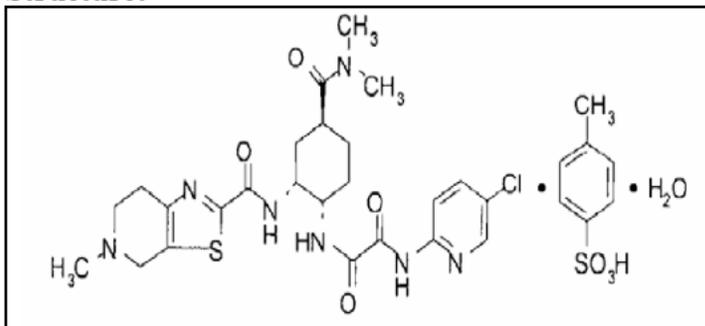
**IND number:** 77254  
 Sequence number, date, type of submission: N000, 5/15/07, Initial submission  
 Information to sponsor: Yes (X) No ( )

**Sponsor and/or agent:** Daiichi Medical Research, Inc. Montvale, N.J.  
 Manufacturer for drug substance: Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan,  
 Manufacturer for drug product: Daiichi Pharmaceutical Co., Ltd., Shizuoka, Japan,

Reviewer name: Patricia P. Harlow, Ph.D.  
 Division name: Cardiovascular and Renal Drug Products, HFD-110  
 Review completed: 06/20/07

**Drug (Generic name):** Not available  
**Trade name:** Not available  
**Code name:** DU-176b  
 Other names (list alphabetically): D11-4176b  
 CAS registry number/mole file number: 480449-71-6  
 Molecular formula:  $C_{24}H_{30}ClN_7O_4S$ ,  $C_7H_8O_3S \cdot H_2O$   
 Molecular weight: 738.27 (toluene sulfonate salt); 548.06 (anhydrous free base)  
 Chemical name: N-(5-Chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]ethanediamide p-toluenesulfonate monohydrate

#### Structure:



Relevant INDs/NDAs/DMFs: IND 63, 266, DMF# (b) (4) DMF# (b) (4)

Drug class: Anti-thrombotic, FXa inhibitor

Proposed use: Anti-thrombotic, Prevention of thromboembolic events and stroke in patients with nonvalvular atrial fibrillation (b) (4)

Route of administration: Oral tablet

Clinical formulation: Tablets contain 40.4 mg DU-176b (30 mg DU-176). Excipients in the unlabeled drug product include mannitol, pregelatinized starch, crospovidone, magnesium stearate, hydroxypropylcellulose, hydroxypropylmethylcellulose, talc, macrogol and titanium oxide, yellow ferric oxide.

### **Proposed clinical protocol (Study DU176b-PRT018)**

*Protocol Title:* A Phase 2, randomized, parallel group, multi-center, multi-national study for the evaluation of safety of four fixed dose regimens of DU-176b in subjects with non-valvular atrial fibrillation.

#### *Protocol objectives:*

The primary objective is to evaluate the safety of four fixed dose regimens in subjects with non-valvular atrial fibrillation. Emphasis is placed on liver function abnormalities and clinically relevant bleeding events.

The secondary objective is to evaluate the four dose regimens with respect to

1. incidence of major adverse cardiovascular events (MACE),
2. effects on biomarkers of thrombus formation,
3. pharmacokinetics of DU176b and metabolites in patients, and
4. effects on pharmacodynamic biomarkers.

*Study design:* In this multi-center (100 sites), multi-national, randomized double blind study of DU-176b, subjects will receive one of four blinded fixed dose regimens of DU-176B or open-labeled warfarin for a period of three months as out-patients. The doses of DU-176b will be 30 mg qd, 30 mg bid, 60 mg qd, and 60 mg bid. Warfarin dosing will be adjusted to achieve an INR of 2.0 to 3.0.

*Study Population:* Approximately 2000 patients (400 per group) with documented persistent non-valvular atrial fibrillation and a CHADS<sub>2</sub> index score of at least 2 will participate in the study. Subjects must be between 18 and 80 years of age. Females must be post-menopausal, surgically sterile or using accepted barrier contraception measures. Exclusion criteria include mitral valve disease, previous valvular heart surgery, acute coronary syndromes, conditions associated with high bleeding risk, recent (< 30 days) PCI, MI, stroke, or other major surgery or life expectancy of < 12 months. Other exclusion criteria include a history of hepatitis or impaired hepatic or renal function. Subjects cannot be taking other anticoagulants, thrombolytic agents, clopidogrel or ticlopidine.

#### *Safety monitoring:*

Clinical safety parameters include vital signs (HR, BP, respiratory rate and body temperature), 12-lead ECGs, physical examination, adverse event reporting, and clinical laboratory tests as indicated in the schedules below. Clinical laboratory tests will include hematology (RBC count, hematocrit, hemoglobin, white blood cell with differential count, and platelet count), clinical chemistry (sodium, potassium, chloride, calcium, bicarbonate, glucose, albumin, total protein, total bilirubin, phosphorus, total cholesterol, triglycerides, BUN, creatinine, uric acid, CK, AST (SGOT), ALT (SGPT), GGT, alkaline phosphatase, and LDH), and urinalysis (protein, albumin, glucose, creatinine, uric acid, and microscopic examination). Clinical laboratory tests will be performed at screening,

Days 1, 7, 14, 21, 28, 42, 56, 70 and 84 (or the end of treatment) and at the end of the 30 day follow-up period.

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| Sponsor's schedule of events                             |                |                    |                     |    |                 |                 |    |    |    |                                     |                   |
|--|----------------|--------------------|---------------------|----|-----------------|-----------------|----|----|----|-------------------------------------|-------------------|
| Study Period   | Screening      | Baseline Randomize | On-Treatment Visits |    |                 |                 |    |    |    | End of Treatment Visit <sup>a</sup> | Follow-up         |
| Visit  | 1              | 2                  | 3                   | 4  | 5               | 6               | 7  | 8  | 9  | 10                                  | 11                |
| Day  | ≤ 30           | 1 <sup>b</sup>     | 7                   | 14 | 21 <sup>c</sup> | 28 <sup>d</sup> | 42 | 56 | 70 | 84                                  | Post-LD 30 day ±5 |
| Visit Window (days)                                      |                | ±2                 | ±2                  | ±2 | ±2              | ±2              | ±3 | ±3 | ±3 | ±3                                  |                   |
| Study Informed Consent                                   | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |
| Inclusion/Exclusion Criteria                             | X              | X                  |                     |    |                 |                 |    |    |    |                                     |                   |
| Demographic Information                                  | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |
| Medical/Surgical History                                 | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |
| Alcohol and Tobacco Use                                  | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |
| Physical Examination                                     | X <sup>e</sup> | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   | X                 |
| Vital Signs <sup>f</sup>                                 | X              | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   | X                 |
| 12-lead ECG  | X              | X                  |                     |    |                 |                 |    |    |    | X                                   | X                 |
| Clinical Safety Laboratory Tests                         | X <sup>g</sup> | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   | X                 |
| Pregnancy Test <sup>h</sup>                              | X              | X                  |                     |    |                 |                 |    |    |    | X                                   |                   |
| AE Reporting   |                | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   | X                 |
| Prior and Concomitant Medication <sup>i</sup>            | X <sup>j</sup> | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   | X                 |
| In-clinic dose administration <sup>k</sup>               |                | X <sup>l</sup>     |                     |    |                 | X <sup>m</sup>  |    |    |    |                                     |                   |
| Study drug dispensing <sup>n</sup>                       |                | X                  |                     |    |                 | X               |    | X  |    |                                     |                   |
| Study drug compliance <sup>o</sup>                       |                |                    | X                   | X  | X               | X               | X  | X  | X  | X                                   |                   |
| Contact IVRS   | X              | X                  |                     |    |                 | X               |    | X  |    | X                                   | X                 |
| D-dimer and prothrombin fragments F1 and F2              | X              | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   |                   |
| INR and PT for warfarin-treated subjects <sup>p</sup>    | X              | X                  | X                   | X  | X               | X               | X  | X  | X  | X                                   |                   |
| PK/PD Sampling for DU-176b treated subjects <sup>q</sup> |                | X                  |                     |    |                 | X <sup>r</sup>  |    |    |    |                                     |                   |
| Record time of subject meals                             |                | X                  |                     |    |                 | X               |    |    |    |                                     |                   |
| Pharmacogenomics informed consent (optional)             | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |
| Pharmacogenomic sampling (optional)                      | X              |                    |                     |    |                 |                 |    |    |    |                                     |                   |

a: Early termination visit procedures are the same as End-of-Treatment visit procedures. Collect drug diary.  
b: Day 1 is the day of the first dose of study drug.  
c: Instruct subjects randomized to DU-176b dose regimens to record date/time of the evening dose on the day before the Day 28 visit, to record date/time of the morning meal on the day of the Day 28 visit, and not to take the Day 28 morning dose until administered the dose at the clinic.  
d: Record time of the subject's evening dose of the day before.  
e: Record height and body weight at Screening visit only.  
f: Includes sitting blood pressure, heart rate, respiratory rate, and body temperature.  
g: Hepatitis B antigen and Hepatitis C antibody tests also.  
h: Serum pregnancy tests for women of childbearing potential at screening visit and at end of treatment visit or early termination visit. Urine pregnancy tests for women of childbearing potential at baseline.  
i: Prior medications are recorded only at the screening visit. Concomitant medication recorded at each subsequent visit.  
j: Medications taken within 30 days prior to screening visit.  
k: Record actual dosing time of the morning dose on day of visit (for example 09:02 h instead of 09:00 h if the sample is taken at 09:02 h).  
l: All subjects  
m: Subjects on DU-176b dose regimens only.  
n: Dosing will continue for 84 days. The subject will be provided study drug, drug diary, and instructions for dosing and diary use. Subjects will be instructed to bring all study medications to each visit. Last drug diary collection will be at the End-of-Treatment or Early Termination visit.  
o: Count all unused tablets. On drug dispensing visits, collect and count unused tablets from the previous kit.  
p: INR and PT done for dose adjustment purposes, not as pharmacodynamic variables, for subjects on warfarin.  
q: INR, PT, anti-Factor Xa activity, endogenous Factor Xa activity, PiCT, and CAT-TG done as pharmacodynamic variables for subjects on DU-176b dose regimens.  
r: Collect blood samples for PK and PD analyses before the morning dose and between 1 and 3 hours post-dose.  
Abbreviations: AE = adverse event, CAT-TG = thrombin generation using the calibrated automated thrombogram, ECG = electrocardiogram, INR = international normalized ratio, IVRS = interactive voice response system, PD = pharmacodynamic, PiCT = prothrombinase induced clotting time, PK = pharmacokinetic, and PT = prothrombin time.

**Assessment:**

Pharmacokinetic assessment will be performed before the morning dose and between 1 to 3 hours post dose. Pharmacodynamic assessment includes D-dimer, prothrombin fragments F1 and F2, INR, PT, anti-FXa activity, endogenous FXa activity, Prothrombin-induced clotting time (PiCT), and thrombin generation using a calibrated automated thrombogram (CAT-TG).

**Drug history/Previous clinical experience with DU-176B:**

DU-176B has been administered to at least 1300 subjects in sixteen Phase 1 trials, three Phase 2 trials in patients undergoing orthopedic surgery and two Phase 2 trials in Japanese patients with atrial fibrillation. Doses up to a maximum of 120 mg qd or 60 mg bid were administered for 10 days in healthy subjects and patients undergoing hip-

replacement surgery. The atrial fibrillation studies involved dose escalation (30 mg bid to 60 mg bid) over a period of ten weeks. The sponsor maintains that DU-176b was well-tolerated with no evidence of major bleeding adverse events. In the Phase 2 trials the incidence of adverse effects on liver function was approximately 2%.

The clinical pharmacology studies are summarized in the table below. Renal clearance was lower in elderly males than in young males resulting in a slightly higher exposure in the elderly ( $AUC_{(0-\tau)}$  of 3032 and 3582 ng.hr/mL versus  $AUC_{(0-\tau)}$  of 2549 and 2806 ng.hr/mL on Day 1 and Day 10, respectively). The studies in the United States were conducted under IND 63, 266.

**Sponsor's summary of clinical pharmacology studies.**

| Study No.               | Study title   | Region | Duration  | Exposed to DU-176b              | Doses  | Status                      | Date Submitted to IND 63,266    |
|-------------------------|---|--------|-----------|---------------------------------|--|-----------------------------|---------------------------------|
| <b>U.S./EU Studies</b>  |   |        |           |                                 |  |                             |                                 |
| PRT001                  | DU-176b – A Phase I, Single-Blind, Randomised, Placebo-Controlled Study in Healthy Male Subjects to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Ascending Single and Multiple Oral Doses of DU-176b, Incorporating a Comparison of Fed / Fasted Pharmacokinetics and an Assessment of the Relative Bioavailability of Two Formulations | UK     | 1 day     | 58                              | 10mg(qd), 30mg(qd), 60mg(qd), 90mg(qd), 120mg(qd), 150mg(qd) | Completed (CSR available)   | Serial # 002 September 19, 2004 |
|                         |   |        | 1 day     | 10                              | 60mg(qd)   |                             |                                 |
|                         |   |        | 10 days   | 27                              | 60mg(bid), 90mg(qd), 120mg(qd)                               |                             |                                 |
| PRT002                  | A Phase I, Single Blinded, Randomised, Placebo Controlled Multiple Oral Dose Study to Assess Safety, Tolerability, Pharmacokinetic and Pharmacodynamic Profiles of an Oral Anticoagulant, DU-176b, in Healthy Post-Menopausal or Surgically Sterile Females and Healthy Elderly Male Subjects   | UK     | 10 days   | 18                              | 90mg(qd)   | Completed (CSR available)   | Serial # 002 September 19, 2004 |
| PRT003                  | An Open Label, Randomised, Placebo and Positive Controlled (Fondaparinux) Single Dose Study in Healthy Male Subjects to Assess the Pharmacokinetic and Pharmacodynamic Profile of the Oral Anticoagulant DU-176b in Venous and Shed Blood   | UK     | 1 day     | 60                              | 30mg(qd), 60mg(qd), 120mg(qd)                                | Completed (CSR available)   | Serial # 002 September 19, 2004 |
| PRT004                  | A Phase I, open label, randomised, single dose pharmacoscintigraphic four-way crossover study to evaluate the regional drug absorption of DU-176b in healthy male subjects  | UK     | 1 day     | 8                               | 60mg(qd)   | Completed (CSR available)   | Serial # 002 September 19, 2004 |
| PRT005                  | A Phase I, Open Label Study in Healthy Adult Male and Female Subjects to Assess the Antithrombotic Effect Following a Single Dose of DU-176b, an Oral Direct Factor Xa Inhibitor, Using an ex vivo Human Model of Thrombosis  | US     | 1 day     | 12                              | 60mg(qd)   | Completed (CSR available)   | Serial # 025 September 1, 2005  |
| PRT008                  | A Phase I, Open-Label, Randomised, Two-Treatment, Two-Period, Two-Sequence Crossover Study in Healthy Asian (Japanese) and Non-Asian (Caucasian) Subjects to Investigate the Fed/Fasted Pharmacokinetics of Single Oral Doses of DU-176b  | US     | 1 day     | 16 (Japanese)<br>16 (Caucasian) | 60mg(qd)   | Completed (CSR available)   | Serial # 101 April 17, 2007     |
| PRT009                  | An Open Label, Randomised, Non-Treatment and Active Controlled (Dalteparin Sodium, Ximelagatran) Multiple Dose Study in Fed Elderly Healthy Subjects to Assess the Pharmacodynamics of the oral Anticoagulant DU-176b in ex vivo Thrombin Generation and Platelet Activation  | UK     | 4 days    | 10                              | 60mg(bid)  | Completed (CSR in progress) | Not applicable                  |
| PRT012                  | A Randomized, Open-Label, Crossover Study Assessing the Effect of Esomeprazole on DU-176b Pharmacokinetics and Pharmacodynamics When Administered Orally as Tablet and as Solution  | US     | 4 days    | 32                              | 60mg(qd)   | Completed (CSR available)   | Serial # 101 April 17, 2007     |
| PRT013                  | A Phase I, open-label, randomized, single dose pharmacoscintigraphic two-way crossover study to evaluate the colonic permeability of DU-176b in healthy male subjects   | UK     | 4 days    | 6                               | 30mg(qd)   | Completed (CSR available)   | Serial # 101 April 17, 2007     |
| PRT014                  | A Randomized, Open-Label, Dual Sequence, Parallel Group Study Assessing the Pharmacokinetic and Pharmacodynamic Effects of the Co-administration of Digoxin and DU-176b   | US     | 7-14 days | 48                              | 60mg(qd)   | Completed (CSR in progress) | Not applicable                  |
| PRT017                  | A Two-Cohort, Double-Blind, Randomized, 2-Way Crossover Study to Assess the Effect of Aspirin on the Pharmacokinetics and Pharmacodynamics of DU-176b and the Effect of DU-176b on the Pharmacodynamics of Aspirin  | US     | 5 days    | 56                              | 60mg(qd)   | Completed (CSR in progress) | Not applicable                  |
| PRT019                  | An Open-Label Study Assessing the Mass Balance and Metabolite Profile of a Single Oral DU-176b dose   | UK     | 1 day     | 6                               | 60mg(qd)   | Completed (CSR in progress) | Not applicable                  |
| A-U120                  | An Open-Label, Single-Dose, Study to Assess the Impact of Renal Function on the Pharmacokinetics of DU-176b in Subjects and to Assess the Impact of Peritoneal Dialysis on the Pharmacokinetics of DU-176b in Subjects with End-Stage Renal Disease   | EU     | 1 day     | 33*                             | 15mg(qd)   | Ongoing                     | Not applicable                  |
| C-U122                  | A Randomized, Double Blind Study to Assess the Safety, Pharmacokinetics and Pharmacodynamics of DU-176b in Healthy Subjects who have recently discontinued Warfarin   | US     | 5 days    | 48**                            | 60mg(qd)   | Completed (CSR in progress) | Not applicable                  |
| <b>Japanese Studies</b> |   |        |           |                                 |  |                             |                                 |
| PRT020 (J-01)           | DU-176b Clinical Pharmacology Examination (Phase I) -Single-Dose Test in Healthy Adult Males-   | JP     | 1 day     | 45(Japanese)<br>27(Caucasian)   | 30mg(qd), 60mg(qd), 90mg(qd), 120mg(qd), 150mg(qd)           | Completed (CSR available)   | Serial # 002 September 19, 2004 |
| PRT010 (J-02)           | DU-176b Clinical Pharmacological Study (Phase I) -Repeated dose study using healthy adult males as subjects-  | JP     | 10 days   | 18                              | 60mg(bid), 120mg(qd)   | Completed (CSR available)   | In progress                     |

**Disclaimer:** Tabular and graphical depiction of information is provided by the reviewer unless cited otherwise.

**Studies reviewed within this submission:** The studies in the original submission (5/27/04) to IND 63, 266 reviewed by David Joseph included 4-week toxicology studies in rats and monkeys. Subsequently, Tushar Kokate reviewed 13- and 26-week studies in rats and 13-week studies in mice in December 2006 for the Exec CAC evaluation of proposed carcinogenicity protocols in rats and mice, respectively. Since the sponsor did not want to resubmit to IND 77, 254 all the previously submitted nonclinical studies, the reviewer determined that sixteen studies had not been previously reviewed. Of these studies, six were deemed most important. The sponsor agreed to submit the study reports

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for the six studies listed below. The sponsor submitted only summaries for the remaining nonclinical studies submitted to IND 63, 266.

| Studies reviewed – IND 77, 254 |   |                                     | IND 77, 254 location |        |
|--------------------------------|---|-------------------------------------|----------------------|--------|
| Report Number                  | Report Title  | IND 63,266 Serial #, Date Submitted | Vol.                 | Page   |
| 20040290                       | The Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Oral Administration of [ <sup>14</sup> C] DU-176b | Serial # 019<br>Aug 16, 2005        | 2                    | 92     |
| 20050248                       | DU-176b: <i>In Vitro</i> Reaction Phenotyping of DU-176 (B041111)   | Serial # 099<br>April 2, 2007       | 2                    | 127    |
| 20040467                       | DU-176b: Effects on Specific Metabolic Activities of Human Cytochrome P450 Isozymes.  | Serial # 019<br>Aug 16, 2005        | 2                    | 182    |
| 20050333                       | DU-176b: 13 week oral toxicity in cynomolgus monkeys.   | Serial # 099<br>April 2, 2007       | 3<br>4               | 2<br>2 |
| 20060731                       | DU-176b: Photochromosomal aberration test in Chinese hamster lung cells   | Serial # 099<br>April 2, 2007       | 4                    | 307    |
| 20060746                       | DU-176b: Phototoxicity test in BALB/3T3 cells   | Serial # 099<br>April 2, 2007       | 5                    | 6      |

**Studies not reviewed within these submissions: None**

[Note: The brief summaries below of the pharmacology, pharmacokinetic and toxicology studies are based on the previous reviews of studies submitted to IND 63, 266 and the study reports and summaries included in the current submission.]

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

DU-176b, an orally active inhibitor of activated Factor X (FXa), is more than 100-fold selective against most other serine proteases, except thrombin (6-fold). Using a low molecular weight chromogenic substrate (S-2222), DU-176b competitively inhibited prothrombinase activity ( $K_i = 0.903$  nM) and free FXa ( $K_i = 0.561$  nM), but acted as a non-competitive/mixed-type inhibitor of prothrombinase ( $K_i = 2.98$  nM) when prothrombin was used as a substrate. DU-176b prolongs standard *in vitro* coagulation assays (PT and aPTT) in a concentration-dependent manner, but is more potent in plasma from humans, rabbits, and monkeys than in plasma from rats. Recombinant Factor VIIa neutralized the anti-coagulant effects of DU-176b *in vitro*. *In vivo*, DU-176b inhibited thrombosis in three rat models, including venous thrombosis, venous stasis, and an arterio-venous shunt models. The dose-dependent anti-thrombotic activity of DU-176b was associated with inhibition of factor Xa, but not clinically significant increases in PT. The effect of DU-176b on bleeding or blood loss was not monitored. In a model of tissue factor-induced disseminated intravascular coagulation (DIC) in rats, DU-176b prevented DIC-induced decrease in platelet count, decrease in fibrinogen, and increase in thrombin-antithrombin complex. The effect of DU-176b on platelet aggregation was not directly monitored.

In mice, DU-176b administration of up to 200 mg/kg did not induce significant adverse effects on CNS function monitored in an Irwin functional observational battery, rota-rod motor coordination, spontaneous locomotor activity, electroshock-induced convulsion or pentylenetetrazole-induced convulsion. A maximum concentration of 20 µg/mL DU-176b did not inhibit hERG tail current in HEK293 cells or inhibit resting membrane potential and action potential parameters in guinea pig papillary muscle *in vitro*. In saline-loaded rats, DU-176b administration of up to 200 mg/kg did not induce significant effects on urine volume and excretion of sodium, potassium and chloride. In Cynomolgus monkeys, DU-176b administration of up to 200 mg/kg did not induce significant effects on behavior, spontaneous locomotor activity, body temperature, systolic and diastolic blood pressure, heart rate, ECG parameters (RR, PR, and QT intervals, QRS duration, and QTc), respiration rate, blood pH, pO<sub>2</sub>, pCO<sub>2</sub>, and sO<sub>2</sub> (hemoglobin oxygen saturation).

#### **2.6.2.2 Primary pharmacodynamics**

No new study report was submitted.

#### **2.6.2.3 Secondary pharmacodynamics**

The sponsor has examined the specificity of DU-176b for FXa versus other serine proteases, but not against other physiological receptors.

#### **2.6.2.4 Safety pharmacology**

No new study report was submitted.

#### **2.6.2.5 Pharmacodynamic drug interactions:**

No study was submitted.

#### **2.6.2.6 Discussion and Conclusions**

*In vitro*, DU-176b inhibits activated Factor X (FXa) and prolongs standard coagulation assays (PT and aPTT) in a concentration-dependent manner. *In vivo* DU-176b inhibited thrombosis in several rat models. However, the effect of DU-176b on bleeding times or platelet aggregation was not monitored. The sponsor has not evaluated DU-176b for secondary pharmacodynamic effects, such as in receptor binding studies *in vitro*.

DU176b did not show adverse effects in a battery of safety pharmacology studies, including *in vitro* and *in vivo* cardiovascular system studies and *in vivo* respiratory, renal, and central nervous system studies.

### **2.6.3 PHARMACOLOGY TABULATED SUMMARY**

The following table was provided by the sponsor.

| Sponsor's table          |                    |                                   |                  |                          |   |            |                               |
|--------------------------|--------------------|-----------------------------------|------------------|--------------------------|---|------------|-------------------------------|
| Organ Systems Evaluated  | Species/ Strain    | Route of Administration (Vehicle) | Dose (mg/kg)     | Gender and No. per Group | Noteworthy Findings   | Report No. | Status of Submission          |
| Central Nervous System   | Mouse/ddY          | PO (0.5% MC <sup>a</sup> )        | 0, 20, 60, 200   | 3M - 10M                 | <b>Multiple neurobehavioral sings</b><br>No effect<br><b>Spontaneous locomotor activity</b><br>No effect<br><b>Motor Coordination</b><br>No effect<br><b>Increasing current method</b><br>No effect<br><b>Pentyletetrazole infusion method</b><br>No effect | 20050505   | Serial #000<br>May 27<br>2004 |
|                          | Cynomolgus Monkey  | PO (0.5% MC <sup>a</sup> )        | 0, 20, 60, 200   | 2M<br>2F                 | No effect on behavior, spontaneous motor activity and body temperature by telemetry in conscious  | 20020556   | Serial #000<br>May 27<br>2004 |
| hERG Current             | HEK293 cells       | In vitro                          | 0, 2, 20 (µg/mL) | 4 cells                  | No effect   | 20020444   | Serial #000<br>May 27<br>2004 |
| Cardiac Action Potential | Guinea Pig/Hartley | In vitro                          | 0, 6, 20 (µg/mL) | 4                        | No effect   | 20020491   | Serial #000<br>May 27<br>2004 |
| Cardiovascular Systems   | Cynomolgus Monkey  | PO (0.5% MC <sup>a</sup> )        | 0, 20, 60, 200   | 2M<br>2F                 | No effect by telemetry in conscious   | 20020556   | Serial #000<br>May 27<br>2004 |
| Respiratory Function     | Cynomolgus Monkey  | PO (0.5% MC <sup>a</sup> )        | 0, 20, 60, 200   | 2M<br>2F                 | No effect by telemetry in conscious   | 20020556   | Serial #000<br>May 27<br>2004 |
| Renal Function           | Rat/Sprague Dawley | PO (0.5% MC <sup>a</sup> )        | 0, 20, 60, 200   | 8M                       | No effect on urine volume and Na <sup>+</sup> , K <sup>+</sup> and Cl <sup>-</sup> excretion  | 20020492   | Serial #000<br>May 27<br>2004 |

<sup>a</sup> Methylcellulose

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Following oral administration, [<sup>14</sup>C]-DU-176b was rapidly absorbed having a T<sub>max</sub> of 0.7 and 2.3 hours in rats and cynomolgus monkeys, respectively. The initial elimination half-life (t<sub>1/2</sub>) was about 3.2 hours in both species, followed by a slower elimination phase with t<sub>1/2</sub> of 11 and 32 hours in the rat and monkey, respectively.

Protein binding of [<sup>14</sup>C]-DU-176b *in vitro* was approximately 33, 45, 49, and 55% in plasma from rats, dogs, monkeys, and humans, respectively. The *in vitro* distribution of radioactivity to blood cells was 57, 54, 38, and 47% in rat, dog, monkey, and human, respectively. A whole-body autoradiography study in albino rats indicated that the highest concentrations of DU-176 were found in the gastrointestinal and bladder contents, followed by the kidney, preputial gland, and liver with only trace levels found in the brain and the eye. However, at 336 hr after oral administration of [<sup>14</sup>C]-DU-176b to cynomolgus monkeys, radioactivity was highly concentrated in eyes and skin, and was still detectable in bile, liver and kidney at levels more than twice the level in plasma. A tissue distribution study in pigmented rats showed the maximum concentration of [<sup>14</sup>C]-DU-176b radioactivity in the eye was similar to that in the kidneys and liver. The half-life of elimination from the rat eye was 260 hours.

Following oral administration of DU-176b to monkeys, unchanged DU-176 was the principal component in plasma. In contrast, in rat plasma a major metabolite (D21-3231) was present at higher levels than the unchanged drug. Since DU-176 is unstable in rat plasma, the sponsor maintains that D21-3231 results from hydrolysis of DU-176. The

assessment of metabolites in the urine and feces of rats and monkeys indicated that DU-176 is excreted primarily unchanged and not conjugated.

In the rat, most of the administered dose was eliminated within 24 h, with 71 % of the dose excreted via the bile into the feces and 25% in the urine. In the monkey, most of administered dose was eliminated within 96 h, with 49% of the dose excreted in the feces and 41 % in the urine. DU-176 can cross the placenta in rats and is excreted into breast milk of lactating rats,

Various *in vitro* studies showed that DU-176b is primarily metabolized by CYP3A4. However, an *in vitro* inhibition study indicated that the IC<sub>50</sub> values of DU-176 were >100 μM for all CYP 450 isozymes investigated.

#### 2.6.4.2 Methods of Analysis

No new study report was submitted.

#### 2.6.4.3 Absorption

No new study report was submitted.

#### 2.6.4.4 Distribution

##### Study title: The Tissue Distribution of Total Radioactivity in the Pigmented Rat Following Oral Administration of [<sup>14</sup>C] DU 176b

Study no.: 20040290

Volume #, and page #: N000, Vol. 2, pg 92

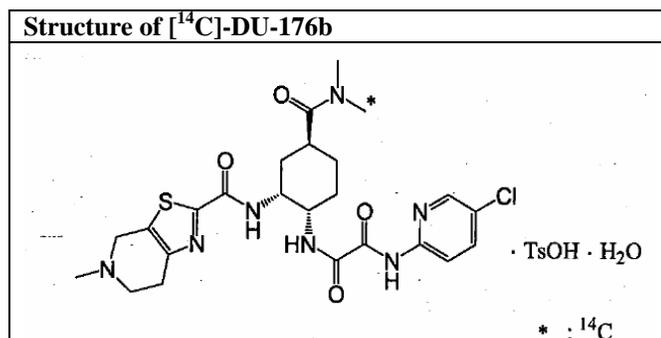
Conducting laboratory and location: (b) (4)

Study Initiation: 2/13/2004

GLP compliance: Indicated

QA reports: Indicated

Drug, lot #, radiolabel, and % purity: [<sup>14</sup>C]-DU-176b, Lot CP-2801, purity >98.0%, specific radioactivity 2.87 MBq/mg.



#### Methods:

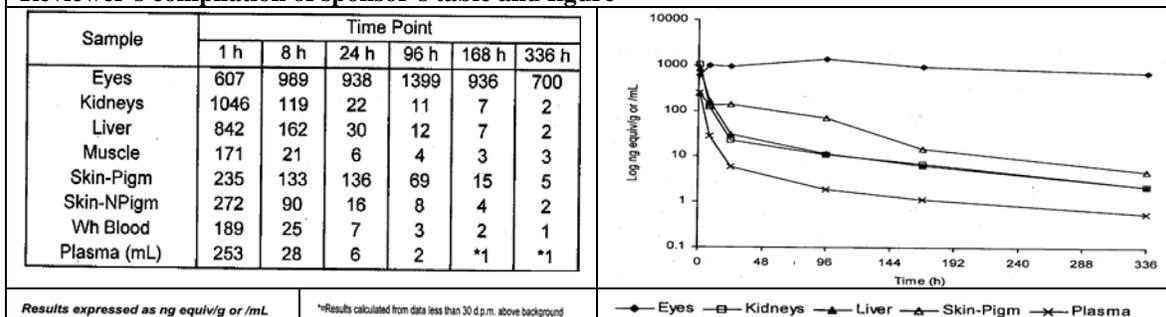
A single oral gavage dose of [<sup>14</sup>C]-DU-176b in water was administered at a target dose level of 3 mg/kg (11.6 MBq/kg) to six male pigmented rats. Lister Hooded rats (8-9 weeks of age) were euthanized at 1, 8, 24, 96, 168, and 336 hours after dosing, respectively. Blood, plasma, eyes, kidneys, liver, muscle and skin (pigmented and non-

pigmented) were collected. Samples were weighed, dried and combusted. The resulting [ $^{14}\text{C}$ ]- $\text{CO}_2$  was collected by absorption and quantified by liquid scintillation counting.

### Results:

Although the highest concentrations of radioactivity were in the liver and kidney at 1 hour post dose, the next highest concentration was in the eyes. From 24 to 336 hours post dose, the concentration of radioactivity was highest in the eye and next highest in the pigmented skin. The elimination half-life in the eye is 260 hours.

#### Reviewer's compilation of sponsor's table and figure



Dosimetry calculations indicated that an oral dose of 1.93 MBq (52.7  $\mu\text{Ci}$ ) of [ $^{14}\text{C}$ ]-DU-176b to human male volunteers would result in a radiation exposure of 0.5 mSv.

### 2.6.4.5 Metabolism

#### *In vitro* studies

#### Study title: DU-176b: *In Vitro* Reaction Phenotyping of DU-176

Study number: 20050248 (B041111)

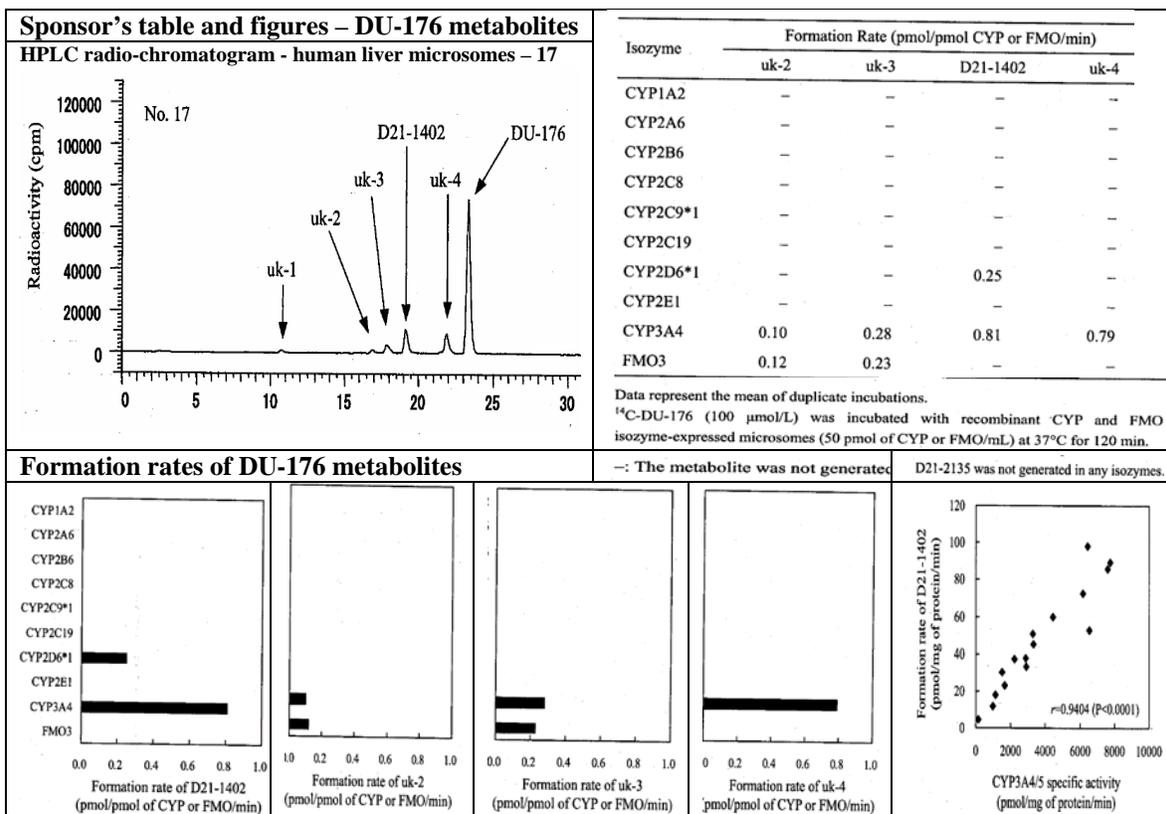
The metabolism of DU-176 was investigated using liver microsomes from 16 different individual human donors as well as recombinant human cytochrome P450 (CYP) and flavin-containing monooxygenase (FMO) isozymes expressed in microsomes.

Each of the 16 individual human liver microsomal systems metabolized DU-176 to D21-1402 (a demethylated metabolite) and three unknown metabolites (uk-2, uk-3, and uk-4). A peak corresponding to D21-2135 was not observed in any of the radio-chromatograms. Although a fifth peak designated uk-1 was observed in all radio-chromatograms, it was also produced in the control microsomal system that does not express any human isozymes. Since analysis by ESI-LC/MS analysis suggested that uk-1 is formed by hydrolysis of amino bond at the chloroaminopyridine position of DU-176, uk-1 was excluded as a target in this study. LC/MS analysis indicated that uk-2 and uk-3 are mono-oxygenated metabolites of the thiazopyridine ring, while uk-4 is a hydroxylated metabolite at the N, N-dimethylcarbamoyl group.

The CYP and FMO isozyme specific activities were determined in liver microsomes from the 16 different human donors. CYP3A4/5-mediated testosterone 6 $\beta$ -hydroxylase specific activity in these microsomes showed the highest correlation ( $r > 0.93$ ,  $p < 0.0001$ ) with the formation rates of D21-1402, uk-2, uk-3, and uk-4.

Among the ten recombinant isozymes tested, only CYP2D6\*1, CYP3A4, and FM03 catalyzed metabolism of DU-176b. Recombinant CYP3A4 catalyzed the formation of all four metabolites (D21-1402, uk-2, uk-3, and uk-4). *In vitro* recombinant CYP2D6\*1 demethylated DU-176 to D21-1402, and recombinant FM03 formed uk-2 and uk-3.

The sponsor concluded that CYP2D6 and FMO are probably not responsible for the metabolism of DU-176 in humans, because no significant correlation was observed between DU-176 metabolite formation and the specific activity of CYP2D6\*1 ( $r = -0.26$  to  $-0.29$ ;  $p > 0.27$ ) and FMO ( $r = 0.22$  to  $0.44$ ;  $P > 0.08$ ) in 16 individual human liver microsomes. The sponsor concluded that CYP3A4 is the major enzyme involved in the formations of the four DU-176 metabolites.



**2.6.4.6 Excretion**

No new study report was submitted.

**2.6.4.7 Pharmacokinetic drug interactions**

**Study title: DU-176b: Effects on Specific Metabolic Activities of Human Cytochrome P450 Isozymes (B031517)**

Study number: 20040467

Pooled human liver microsomes were used to determine the effect of DU-176b on eight CYP P450 isozymes under two incubation conditions in comparison to known inhibitors of each isozyme. At a concentration of 100 μM, DU-176b inhibited (S)-mephenytoin 4'-

hydroxylase (CYP2C19) activity by 40.2%, but showed inhibition of 11.1 % or less for all other CYPs. Pre-incubation of DU-176b with human liver microsomes in the presence of an NADPH-generating system, but absence of CYP substrate, did not significantly alter the effect of DU-176b on each CYP isozyme. The IC<sub>50</sub> values for DU-176b were above 100 μM for CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. The sponsor concluded that DU-176b has a low potential to interact with the metabolism of other drugs by these CYP isozymes.

| Reviewer's compilation of sponsor's tables |   |                                  |      |       |                                  |      |                  |                           |
|--|---|----------------------------------|------|-------|----------------------------------|------|------------------|---------------------------|
| CYP Isozyme                                | Metabolic Reaction                                | Percent Inhibition               |      |       |                                  |      |                  | IC <sub>50</sub> (μmol/L) |
|  |   | Incubation Method 1 <sup>a</sup> |      |       | Incubation Method 2 <sup>a</sup> |      |                  |                           |
|  |   | DU-176b (μmol/L)                 |      |       | DU-176b (μmol/L)                 |      |                  |                           |
|  |   | 1                                | 10   | 100   | Positive Control <sup>b</sup>    | 10   | Positive Control |                           |
| CYP1A2                                     | 7-Ethoxyresorufin O-deethylation                  | 6.5                              | 9.7  | 10.8  | 92.4                             | 1.4  | - <sup>c</sup>   | > 100                     |
| CYP2A6                                     | Coumarin 7-hydroxylation                          | -8.3                             | -2.1 | -0.9  | -                                | 1.2  | > 93.5           | > 100                     |
| CYP2B6                                     | 7-Ethoxy-4-trifluoromethylcoumarin O-deethylation | -0.1                             | -0.2 | 0.8   | -                                | -3.1 | 39.0             | > 100                     |
| CYP2C8/9                                   | Tolbutamide 4-methylhydroxylation                 | -1.5                             | 1.3  | 11.1  | 79.8                             | 2.0  | -                | > 100                     |
| CYP2C19                                    | (S)-Mephenytoin 4'-hydroxylation                  | -0.8                             | 7.7  | 40.2  | 71.1                             | 11.1 | -                | > 100                     |
| CYP2D6                                     | Bufuralol 1'-hydroxylation                        | -5.6                             | -5.9 | -1.2  | 76.5                             | 0.6  | -                | > 100                     |
| CYP2E1                                     | Chlorzoxazone 6-hydroxylation                     | -3.2                             | -7.9 | -13.1 | -                                | -2.7 | 54.9             | > 100                     |
| CYP3A4                                     | Testosterone 6β-hydroxylation                     | 0.0                              | 2.4  | 0.7   | 89.8                             | -1.9 | -                | > 100                     |

Data represent the mean of duplicate incubations.

<sup>a</sup> Incubation method 1: Reaction mixture was pre-incubated for 5 min without NADPH-generating system. Reaction was started by an addition of the NADPH-generating system. Incubation method 2: Reaction mixture without the substrate was pre-incubated for 15 min in the presence of NADPH-generating system. Reaction was started by an addition of the substrate.

<sup>b</sup> Positive control: CYP1A2, α-Naphthoflavone (0.1 μmol/L); CYP2A6, 8-Methoxypsoralen (0.5 μmol/L); CYP2B6, Orphenadrine (300 μmol/L); CYP2C8/9, Sulfaphenazole (50 μmol/L); CYP2C19, Tranilcyproprine (25 μmol/L); CYP2D6, Quinidine (1 μmol/L); CYP2E1, Diethylidithiocarbamate (20 μmol/L); CYP3A4, Ketoconazole (0.5 μmol/L)

<sup>c</sup> -: Not determined

## 2.6.4.8 Other Pharmacokinetic Studies

No new study report was submitted.

## 2.6.4.9 Discussion and Conclusions

The most important PK/ADME issue for the development of DU-176b is the high distribution and retention of DU-176b in the eye and skin of both monkeys and pigmented rats. At 336 hr after oral administration of [<sup>14</sup>C]-DU-176b in Cynomolgus monkeys, radioactivity was highly concentrated in eyes and skin. The tissue distribution study in pigmented rats showed the half-life of elimination from the eye is 260 hours.

Since the sponsor has conduct an *in vitro* phototoxicity assays as specified in the Guidance for Photosafety Testing, CDER (May 2003), no further phototoxicity testing is required. However, based on previous consultation with Division of Anti-Infective and Ophthalmology Products, the sponsor should monitor intraocular pressure and obtain electroretinograms, in addition to standard slit-lamp biomicroscopy and funduscopy, before the treatment initiation and at multiple intervals in the longer toxicity studies in monkeys or pigmented animals.

#### **2.6.4.10 Tables and figures to include comparative TK summary**

The sponsor did not provide a comparative TK table.

#### **2.6.5 PHARMACOKINETICS TABULATED SUMMARY**

The sponsor did not provide tabulated summaries.

#### **2.6.6 TOXICOLOGY**

##### **2.6.6.1 Overall toxicology summary**

Based on single dose studies, the minimum lethal dose for DU-176b is greater than 2000 mg/kg in rats and 400 mg/kg in monkeys.

In a 13-week repeated-dose toxicity study, male and female cynomolgus monkeys received DU-176b doses of 0, 6, 18, and 54 mg/kg once daily by oral gavage. Slight prolongation of coagulation times consistent with the pharmacodynamic effect of DU-176B was observed at 24 hours after dosing. Some individual mid- and high-dose animals showed effects on hematology parameters most likely in response to hemorrhagic events. The sponsor's NOAEL was 54 mg/kg.

DU-176b was administered for 26-weeks at 0, 6, 18, and 54 mg/kg/day dose levels to rats. The previous reviewer indicated that no remarkable findings were observed at any of the dose levels tested. The NOAEL was established at 54 mg/kg/day. However, the 4-week toxicity study in rats (20, 60 and 200 mg/kg/day) showed dose-related hemorrhage (pancreas, thymus and lungs) at doses of 20 mg/kg/day and higher, but no mortality at any dose level. The discrepancy in the hemorrhagic findings in the 4-week and 26-week repeat dose toxicity may be explained by improper handling of the animals exposed to an anticoagulant in the 4-week study.

Although the Ames assays were negative, the chromosomal aberration assay in Chinese hamster lung cells (CHL) and the human lymphocyte polyploidy assay were considered positive. DU-176b had no effect on the incidence of chromosomal aberrations, but increased the incidence of polyploidy CHL cells in the presence of metabolic activation. In human lymphocytes, DU-176b increased the incidence of polyploidy cells in the absence and presence metabolic activation at dose levels that produced precipitate formation. However, all *in vivo* genotoxicity assays were negative.

The mouse and rat carcinogenicity protocols were reviewed by the Exec CAC in December 2006. Based on MTD's in the 13-week oral gavage studies in rats and mice, the Exec CAC recommended doses of 0, 50, 150 and 500 mg/kg for male and female mice, doses of 0, 60, 200, and 600 mg/kg for male rats, doses of 0, 50, 100 and 200 mg/kg in female rats. Previously, the sponsor showed that DU-176b at 20 mg/kg did not enhance liver carcinogenesis in rats exposed to a single dose of N-nitrosodiethylamine (200 mg/kg ip).

DU-176b produced no effect on mating or fertility in male and female rats at oral doses of up to 1000 mg/kg/day. In rats, DU-176b produced no teratogenic effects, although slight embryotoxicity and maternal toxicity was observed at 300 mg/kg/day. In rabbits,

DU176b produced no teratogenic effects, although embryo-fetotoxicity was observed at doses (200 and 600 mg/kg/day) that produced maternal toxicity.

The *in vitro* phototoxicity of DU-176b in BALB/3T3 cells was judged as incapable of being evaluated, since the IC<sub>50</sub> value was greater than 1 mg/mL in the presence and absence of photo-irradiation. However, DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation.

**2.6.6.2 Single-dose toxicity**

The minimum lethal single oral dose of DU-176B in rats and monkeys is greater than 2000 and 400 mg/kg, respectively.

**2.6.6.3 Repeat-dose toxicity**

The 4-week studies in rats and monkeys, the 13-week studies in mice and rats, and the 26-week studies in rats were previously reviewed under IND 63, 266. The sponsor’s tabulated summaries of the toxicology studies in rats and monkeys are in APPENDICES 2 and 3, respectively.

**Sponsor’s summary of repeated dose toxicology studies with DU-176b**

**Table 4.4.2: Summary of Repeated Dose Oral Toxicity Studies with DU-176b**

| Species | Sex | Duration (Weeks) | NOAEL            |                          | GLP | Report Number |
|---------|-----|------------------|------------------|--------------------------|-----|---------------|
|         |     |                  | (mg/kg/day)      | (mg/m <sup>2</sup> /day) |     |               |
| Rat     | M/F | 4                | 60 (F)           | 360                      | Y   | TOX 20020612  |
| Rat     | M   | 4                | 18 (M)           | 108                      | Y   | TOX 20030041  |
| Rat     | M/F | 26               | 54               | 324                      | Y   | TOX           |
| Monkey  | M/F | 4                | 30               | 360                      | Y   | TOX 20020606  |
| Monkey  | M/F | 13               | 18 (M)<br>54 (F) | 216 (M)<br>648 (F)       | Y   | TOX           |

NOAEL=No Observed Adverse Effect Level

**Study title: DU-176b: 13 week oral toxicity in cynomolgus monkeys.**

**Key study findings:** In a 13-week repeated-dose toxicity study, male and female cynomolgus monkeys received DU-176b doses of 0, 6, 18, and 54 mg/kg once daily by oral gavage. Slight prolongation of coagulation times consistent with the pharmacodynamic effect of DU-176B was observed at 24 hours after dosing. Some individual mid- and high-dose animals showed effects on hematology parameters most likely in response to hemorrhagic events. The sponsor’s NOAEL was 54 mg/kg.

**Study no.:** 20050333 (6630-160)

**Volume #, and page #:** N000, Vol. 1.3 and 1.4

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 8/9/04 (Dosing initiated 9/16/04)

**GLP compliance:** Indicated

**QA report:** yes ( X ) no ( )

**Drug, lot #, and % purity:** DU-176b, Lot BB202, purity 99.6%

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## Methods

Doses: 0, 6, 18, and 54 mg/kg once daily for 13 weeks. The high dose was based on a 4-week repeated dose study in which hemorrhage in the gastrointestinal tract, pleural cavity or adrenal gland was observed at 100 mg/kg.

Route, formulation, volume, and infusion rate: Test article formulations in 0.5% methylcellulose were administered by oral gavage in a dose volume of 3 mL/kg.

Test article formulation analysis: The test article formulations were 97.6 to 100% of the nominal concentration during the week 1, 94 to 101.3% of the nominal concentration during week 5, and 100.6 to 101.7% of the nominal concentration during week 13. The formulations were found to be homogeneous on Day 1 and stable for 14 days under refrigeration.

Species/strain: Cynomolgus monkeys (*Macaca fascicularis*, Vietnam)

Number/sex/group or time point (main study): 4/sex/group

Satellite groups used for toxicokinetics or recovery: None.

Age: approximately 24-38 months old at study initiation

Weight: 1.8-3.2 kg for males, 2.0-2.4 kg for females

Unique study design or methodology (if any): None

## Observations and times

| Observation                               | Time (s)   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
|---|--|------------------------------------|-----------------------------|---------------|---------------------------|------------|------------------------------------|-------------------------|-------------------------------|---|-----------------------|---------|-----------------------|----------|----------------------|------------------------|--------|-------------------|-----------|---------------|----------|-----------------|
| Mortality                                 | At least twice daily.  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Clinical observations                     | At least twice daily immediately before dosing and 1 hour after dosing for general clinical signs. Once weekly, detailed observations were made.   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Bodyweight                                | Bodyweight was measured on Day -7, on Day 1, weekly thereafter, and at necropsy  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Food consumption                          | Food consumption was estimated qualitatively daily.  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Ophthalmoscopy                            | Examinations were conducted on Day -6 and on Day 86 at 2-4 hours after dosing by indirect ophthalmoscopy under ketamine anesthesia.  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Electrocardiography                       | Ten lead electrocardiograms were recorded once on Day -13 and on Day 86 at 2-4 hours after dosing under ketamine anesthesia. HR, PR, QT, QRS intervals were determined and the corrected QT interval calculated using the formula, (QTc = QT-(0.87 X {60/HR -1})).   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Hematology                                | Blood was collected from all animals on days -14 and -7 and prior to dosing on days 28 and 90 after overnight fast. The following parameters were measured in plasma.  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
|   | <table border="0"> <tr> <td>red blood cell (erythrocyte) count</td> <td>mean corpuscular hemoglobin</td> </tr> <tr> <td>hemoglobin</td> <td>platelet count</td> </tr> <tr> <td>hematocrit</td> <td>white blood cell (leukocyte) count</td> </tr> <tr> <td>mean corpuscular volume</td> <td>differential blood cell count</td> </tr> <tr> <td>mean corpuscular hemoglobin concentration</td> <td>reticulocyte count</td> </tr> <tr> <td></td> <td>blood cell morphology</td> </tr> </table>   | red blood cell (erythrocyte) count | mean corpuscular hemoglobin | hemoglobin    | platelet count            | hematocrit | white blood cell (leukocyte) count | mean corpuscular volume | differential blood cell count | mean corpuscular hemoglobin concentration | reticulocyte count    |         | blood cell morphology |          |                      |                        |        |                   |           |               |          |                 |
| red blood cell (erythrocyte) count        | mean corpuscular hemoglobin  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| hemoglobin                                | platelet count   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| hematocrit                                | white blood cell (leukocyte) count   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| mean corpuscular volume                   | differential blood cell count  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| mean corpuscular hemoglobin concentration | reticulocyte count   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
|   | blood cell morphology  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Coagulation                               | Prothrombin time and activated partial thromboplastin time were measured in plasma obtained from blood collected from all animals on days -14 and -7 and prior to dosing on days 28 and 90 after overnight fast.   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| Clinical chemistry                        | Blood was collected from all animals on days -14 and -7 and prior to dosing on days 28 and 90 after overnight fast. The following parameters were measured in plasma or serum, as appropriate.   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
|   | <table border="0"> <tr> <td>glucose</td> <td>alanine aminotransferase</td> </tr> <tr> <td>urea nitrogen</td> <td>gamma glutamyltransferase</td> </tr> <tr> <td>creatinine</td> <td>alkaline phosphatase</td> </tr> <tr> <td>creatinine kinase</td> <td>aspartate aminotransferase</td> </tr> <tr> <td>total protein</td> <td>lactate dehydrogenase</td> </tr> <tr> <td>albumin</td> <td>calcium</td> </tr> <tr> <td>globulin</td> <td>inorganic phosphorus</td> </tr> <tr> <td>albumin/globulin ratio</td> <td>sodium</td> </tr> <tr> <td>total cholesterol</td> <td>potassium</td> </tr> <tr> <td>triglycerides</td> <td>chloride</td> </tr> <tr> <td>total bilirubin</td> <td>plasma protein electrophoresis</td> </tr> </table> | glucose                            | alanine aminotransferase    | urea nitrogen | gamma glutamyltransferase | creatinine | alkaline phosphatase               | creatinine kinase       | aspartate aminotransferase    | total protein                             | lactate dehydrogenase | albumin | calcium               | globulin | inorganic phosphorus | albumin/globulin ratio | sodium | total cholesterol | potassium | triglycerides | chloride | total bilirubin |
| glucose                                   | alanine aminotransferase   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| urea nitrogen                             | gamma glutamyltransferase  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| creatinine                                | alkaline phosphatase   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| creatinine kinase                         | aspartate aminotransferase   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| total protein                             | lactate dehydrogenase  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| albumin                                   | calcium  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| globulin                                  | inorganic phosphorus   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| albumin/globulin ratio                    | sodium   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| total cholesterol                         | potassium  |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| triglycerides                             | chloride   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |
| total bilirubin                           | plasma protein electrophoresis   |                                    |                             |               |                           |            |                                    |                         |                               |   |                       |         |                       |          |                      |                        |        |                   |           |               |          |                 |

|   |  |   |  |
|---|--|---|--|
| Urinalysis  | <p>Overnight urine was collected from fasted animals on days -7 and 90. The following parameters were monitored.</p> <table border="0" style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">                     total urine volume<br/>                     urine sodium excretion<br/>                     urine potassium excretion<br/>                     appearance/color<br/>                     specific gravity<br/>                     pH<br/>                     protein<br/>                     glucose                 </td> <td style="width: 50%; vertical-align: top;">                     urine chloride excretion<br/>                     urine protein excretion<br/> <br/>                     ketones<br/>                     bilirubin<br/>                     occult blood<br/>                     microscopic sediment<br/>                     urobilinogen                 </td> </tr> </table>   | total urine volume<br>urine sodium excretion<br>urine potassium excretion<br>appearance/color<br>specific gravity<br>pH<br>protein<br>glucose   | urine chloride excretion<br>urine protein excretion<br><br>ketones<br>bilirubin<br>occult blood<br>microscopic sediment<br>urobilinogen  |
| total urine volume<br>urine sodium excretion<br>urine potassium excretion<br>appearance/color<br>specific gravity<br>pH<br>protein<br>glucose   | urine chloride excretion<br>urine protein excretion<br><br>ketones<br>bilirubin<br>occult blood<br>microscopic sediment<br>urobilinogen  |   |  |
| Toxicokinetic analysis:   | <p>On days 1 and 91 at 1, 2, 4, and 24 hours after dosing and on Day 29 pre-dose and 2 hours post-dose, blood samples were collected from all treated animals/timepoint. Plasma was prepared and used for determination of DU-176b after addition of an internal standard, protein precipitation and LC/MS/MS.</p>   |   |  |
| Necropsy:   | <p>Detailed necropsy on all main animals. A terminal body weight was obtained at necropsy.</p>   |   |  |
| Organ weights   | <p>The following organs were weighed.</p> <table border="0" style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">                     adrenal (2)<br/>                     brain<br/>                     heart<br/>                     kidney (2)<br/>                     liver with gallbladder (drained)<br/>                     lung<br/>                     ovary (2)<br/>                     pituitary gland                 </td> <td style="width: 50%; vertical-align: top;">                     prostate<br/>                     salivary gland [mandibular (2)]<br/>                     spleen<br/>                     testis (2)<br/>                     thymus<br/>                     thyroid (2) with parathyroid<br/>                     uterus                 </td> </tr> </table>  | adrenal (2)<br>brain<br>heart<br>kidney (2)<br>liver with gallbladder (drained)<br>lung<br>ovary (2)<br>pituitary gland   | prostate<br>salivary gland [mandibular (2)]<br>spleen<br>testis (2)<br>thymus<br>thyroid (2) with parathyroid<br>uterus  |
| adrenal (2)<br>brain<br>heart<br>kidney (2)<br>liver with gallbladder (drained)<br>lung<br>ovary (2)<br>pituitary gland   | prostate<br>salivary gland [mandibular (2)]<br>spleen<br>testis (2)<br>thymus<br>thyroid (2) with parathyroid<br>uterus  |   |  |
| Histopathology  | <p>Adequate Battery:        yes ( X ), no ( )—explain.<br/>                 Peer review:            yes ( ), no ( X ) Peer review was not specifically indicated in the report. The reviewer noted that the slides for individual animals were evaluated by two pathologists, although only one of them signed the anatomic pathology report.<br/>                 The following tissues were preserved in 10% neutral formalin from all animals. After sectioning and staining with hematoxylin/eosin the tissues indicated below were examined from all main study animals. Bone marrow smears were prepared for all animals and stained with Wright’s stain.</p>  |   |  |
| Tissues collected, fixed, and examined  | <table border="0" style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;">                     adrenal (2)<br/>                     aorta<br/>                     brain<br/>                     cecum<br/>                     cervix<br/>                     colon<br/>                     duodenum<br/>                     epididymis (2)<br/>                     esophagus<br/>                     eye (2)<br/>                     femur with bone marrow (articular surface of the distal end)<br/>                     gallbladder<br/>                     heart<br/>                     ileum<br/>                     jejunum<br/>                     kidney (2)<br/> <br/>                     lacrimal gland (2)<br/>                     larynx<br/>                     gross lesions<br/>                     liver<br/>                     lung with mainstem bronchi<br/>                     lymph node (mesenteric)<br/>                     mammary gland (females)                 </td> <td style="width: 50%; vertical-align: top;">                     optic nerve (2)<br/>                     ovary (2)<br/>                     pancreas<br/>                     pharynx<br/>                     pituitary gland<br/>                     prostate<br/>                     rectum<br/>                     salivary gland [mandibular (2)]<br/>                     sciatic nerve<br/>                     seminal vesicle (2)<br/>                     skeletal muscle (thigh)<br/>                     skin<br/>                     spinal cord (cervical, thoracic and lumbar)<br/>                     spleen<br/>                     sternum with bone marrow<br/>                     stomach<br/>                     testis (2)<br/> <br/>                     thymus<br/>                     thyroid (2) with parathyroid<br/>                     tongue<br/>                     trachea<br/>                     urinary bladder<br/>                     uterus<br/>                     vagina                 </td> </tr> </table> | adrenal (2)<br>aorta<br>brain<br>cecum<br>cervix<br>colon<br>duodenum<br>epididymis (2)<br>esophagus<br>eye (2)<br>femur with bone marrow (articular surface of the distal end)<br>gallbladder<br>heart<br>ileum<br>jejunum<br>kidney (2)<br><br>lacrimal gland (2)<br>larynx<br>gross lesions<br>liver<br>lung with mainstem bronchi<br>lymph node (mesenteric)<br>mammary gland (females) | optic nerve (2)<br>ovary (2)<br>pancreas<br>pharynx<br>pituitary gland<br>prostate<br>rectum<br>salivary gland [mandibular (2)]<br>sciatic nerve<br>seminal vesicle (2)<br>skeletal muscle (thigh)<br>skin<br>spinal cord (cervical, thoracic and lumbar)<br>spleen<br>sternum with bone marrow<br>stomach<br>testis (2)<br><br>thymus<br>thyroid (2) with parathyroid<br>tongue<br>trachea<br>urinary bladder<br>uterus<br>vagina |
| adrenal (2)<br>aorta<br>brain<br>cecum<br>cervix<br>colon<br>duodenum<br>epididymis (2)<br>esophagus<br>eye (2)<br>femur with bone marrow (articular surface of the distal end)<br>gallbladder<br>heart<br>ileum<br>jejunum<br>kidney (2)<br><br>lacrimal gland (2)<br>larynx<br>gross lesions<br>liver<br>lung with mainstem bronchi<br>lymph node (mesenteric)<br>mammary gland (females) | optic nerve (2)<br>ovary (2)<br>pancreas<br>pharynx<br>pituitary gland<br>prostate<br>rectum<br>salivary gland [mandibular (2)]<br>sciatic nerve<br>seminal vesicle (2)<br>skeletal muscle (thigh)<br>skin<br>spinal cord (cervical, thoracic and lumbar)<br>spleen<br>sternum with bone marrow<br>stomach<br>testis (2)<br><br>thymus<br>thyroid (2) with parathyroid<br>tongue<br>trachea<br>urinary bladder<br>uterus<br>vagina   |   |  |
|   | <p>Samples of the liver, renal cortex and renal medulla were collected from the first two surviving animals/sex in the control and high dose groups at terminal sacrifice and placed in Trump's fixative. Epon blocks of each tissue were prepared and retained.</p>   |   |  |
| <p>Water consumption was not monitored.</p>   |  |   |  |

**Results**

Mortality: No mortality was observed in this study.

Clinical signs: The incidence of black or red feces was slightly higher in the high dose groups.

Body weights: No treatment-related effect was observed.

Food consumption: The sponsor noted low food consumption in all groups including control groups.

Ophthalmoscopy: No treatment-related effect was observed.

Electrocardiography: The sponsor noted that all electrocardiograms were within normal limits. No clear treatment-related effect was observed on heart rate, P-R interval, QRS interval and QTc.

Hematology: Several individual animals showed significant effects on hematology parameters. The sponsor referred to animal 57164 as a male; however, this identification number refers to a high dose female. She showed transient decreases in red blood cell count, hemoglobin and hematocrit and an increase in reticulocyte count on Day 28. This transient anemia may be related to the observation of “black feces” on Day 26. A mid-dose female, 57160, showed similar effects on red blood cell count, hemoglobin, hematocrit, and reticulocyte count in addition to increases in MCH, MCV and platelet count. The sponsor attributed these findings to menstruation, which was observed on Days 91-92. Since other females had similar menstruation cycles without adverse effects on hematology, the reviewer believes the hematology changes in female 57160 are more likely related to the “black feces” observed on Days 84-85 and the macroscopic finding of a red area (2 X 1 cm) in the mucosa of the fundic area of the stomach. The only males showing hematology values outside the laboratory’s normal range were 57147 (decreased hematocrit) and 57151 (decreased hemoglobin). Neither animal had corresponding clinical or macroscopic observations

| <b>Reviewer’s summary of hematology effects (bold type signifies a value outside normal range)</b> |                                |                           |                             |                              |              |            |
|--|--------------------------------|---------------------------|-----------------------------|------------------------------|--------------|------------|
| Parameter  | Day -14/Day -17/Day 28/ Day 90 |                           |                             |                              | Normal range |            |
|  | Male 57147<br>(Mid dose)       | Male 57151<br>(High dose) | Female 57160<br>(Mid dose)  | Female 57164<br>(High dose)  | Male         | Female     |
| RBC, 10 <sup>6</sup> /μL   | 5.7/5.6/4.9/5.4                | 5.3/5.1/ <b>4.8/4.8</b>   | 5.1/4.9/4.9/ <b>3.45</b>    | 5.3/5.2/ <b>3.6</b> /5.6     | 4.86-7.81    | 4.89-6.98  |
| HGB, gm/dL   | 13.5/12.8/11.4/12.1            | 13.3/12.5/11.8/11.7       | 12.1/11.5/11.7/ <b>9.4</b>  | 13.3/12.9/ <b>8.7</b> /13.3  | 10.6-15.4    | 11.7-15.2  |
| HCT, %   | 44.7/41.4/ <b>35.7</b> /40.9   | 44.0/41.2/39.0/40.1       | 39.5/36.1/38.5/ <b>32.6</b> | 43.7/41.2/ <b>29.4</b> /46.5 | 36.7-52.6    | 38.6-50.6  |
| Reticulocyte, %  | 1.3/2.0/2.1/2.0                | 0.9/1.7/1.6/1.5           | 0.8/1.8/2.3/ <b>13.6</b>    | 1.1/1.4/ <b>6.3</b> /0.7     | 0.2-2.8      | 0.3-2.9    |
| Reticulocyte, 10 <sup>3</sup> /μL  | 76/112/103/110                 | 48/85/79/73               | 42/88/111/ <b>471</b>       | 60/71/ <b>226</b> /37        | 16.9-142.6   | 20.3-166.2 |
| MCV, FL  | 78.8/74.0/72.3/76.0            | 84/81/81/83               | 78/74/79/ <b>95</b>         | 82/80/81/83                  | 63.1-90.1    | 65.0-85.8  |
| MCH, pg  | 23.7/23.0/23.2/22.5            | 25.2/24.5/24.5/24.3       | 23.9/23.7/24.1/ <b>27.3</b> | 25.0/25.1/24.1/23.6          | 18.7-26.3    | 18.7-26.0  |
| Platelet, 10 <sup>3</sup> /μL  | 369/445/407/513                | 266/296/281/278           | 440/481/439/ <b>689</b>     | 158/256/282/229              | 208-701      | 232-542    |

Coagulation: Treatment-related effects were observed on PT and aPTT values in both males and females as indicated in the table below. Although prolongation of coagulation times was consistent with the pharmacodynamic effect of DU-176b, a clear dose-dependence was not observed. This is probably attributable to collection of blood samples prior to dosing on Day 28 and 90.

| Reviewer's summary of effects on coagulation parameters (Mean, SD, N ) |    |                   |                   |                    |                    |                   |                   |                    |                    |
|--|----|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|--------------------|--------------------|
| Sex/Dose (mg/kg)   |    | PT (sec)          |                   |                    |                    | aPTT (sec)        |                   |                    |                    |
|  |    | PT S Day -14      | PT S Day -7       | PT S Day 28        | PT S Day 90        | APTT S Day -14    | APTT S Day -7     | APTT S Day 28      | APTT S Day 90      |
| M  | 0  | 10.1<br>0.05<br>4 | 10.1<br>0.14<br>4 | 9.7<br>0.13<br>4   | 9.9<br>0.14<br>4   | 17.2<br>1.63<br>4 | 17.4<br>1.66<br>4 | 16.2<br>1.22<br>4  | 16.8<br>1.59<br>4  |
|  | 6  | 10.6<br>0.21<br>4 | 10.6<br>0.19<br>4 | 10.3*<br>0.29<br>4 | 10.4<br>0.25<br>4  | 17.7<br>0.99<br>4 | 17.5<br>0.85<br>4 | 18.3<br>1.03<br>4  | 18.0<br>1.11<br>4  |
|  | 18 | 10.2<br>0.31<br>4 | 10.2<br>0.34<br>4 | 10.4<br>0.59<br>4  | 11.8*<br>1.05<br>4 | 18.4<br>1.68<br>4 | 18.7<br>1.55<br>4 | 20.0*<br>1.45<br>4 | 21.8*<br>2.31<br>4 |
|  | 54 | 10.6<br>0.75<br>4 | 10.3<br>0.57<br>4 | 11.2*<br>0.98<br>4 | 11.3*<br>0.85<br>4 | 18.5<br>2.98<br>4 | 16.8<br>2.06<br>4 | 19.2*<br>1.75<br>4 | 19.7<br>1.74<br>4  |
| F  | 0  | 10.6<br>0.31<br>4 | 10.4<br>0.43<br>4 | 10.1<br>0.51<br>4  | 10.5<br>0.44<br>4  | 16.7<br>1.13<br>4 | 16.7<br>0.70<br>4 | 17.1<br>0.49<br>4  | 18.0<br>0.50<br>4  |
|  | 6  | 10.4<br>0.21<br>4 | 10.3<br>0.28<br>4 | 10.0<br>0.05<br>4  | 10.4<br>0.29<br>4  | 16.5<br>0.68<br>4 | 16.9<br>0.84<br>4 | 17.7<br>0.98<br>4  | 19.0<br>1.07<br>4  |
|  | 18 | 10.6<br>0.15<br>4 | 10.5<br>0.14<br>4 | 10.7<br>0.51<br>4  | 11.1<br>0.10<br>4  | 17.2<br>1.26<br>4 | 17.6<br>1.23<br>4 | 20.4<br>3.02<br>4  | 20.7*<br>1.17<br>4 |
|  | 54 | 10.2<br>0.34<br>4 | 10.1<br>0.34<br>4 | 11.5*<br>0.87<br>4 | 11.2<br>0.60<br>4  | 16.3<br>1.06<br>4 | 16.4<br>0.44<br>4 | 19.9<br>1.28<br>4  | 21.1*<br>0.62<br>4 |

**Clinical chemistry:** The sponsor maintained that no treatment-related effect was observed. One mid-dose female, two high dose males and one control male had 1.4-1.5 fold increases in BUN on Day 28 in the absence of effects on creatinine. One high dose female (57166) had a 2.2-fold increase in AST on Day 28 in the absence of effects on ALT, alkaline phosphatase, GGT and bilirubin. This female had increased CK (>40-fold) and LDH (2.3 fold) on Day 28, but not Day 90.

**Urinalysis:** No treatment-related effect was observed.

**Organ weights:** No treatment-related effect was observed. The relative testes weight in the high dose group was higher than that in the control and lower dose groups. This was due to the higher testes weight in one high dose male. This male was the only adult male in the study. All the other males were juvenile males based on histopathology.

| Reviewer's compilation from sponsor's tables of relative organ weights in monkeys – Mean (Range) |   |                    |                    |                    |                    |
|--|---|--------------------|--------------------|--------------------|--------------------|
| Organ  | Sex   | Control            | Low dose           | Mid dose           | High dose          |
| Testes, rt or lt   | M   | 0.02 (0.01 - 0.03) | 0.02 (0.01 – 0.04) | 0.02 (0.01 - 0.04) | 0.06 (0.01 – 0.18) |
| Comment:   | High dose male 57149 had relative testes weight of 0.18 |                    |                    |                    |                    |

**Gross pathology:** Although no treatment-related effect was observed, four treated and two control animals showed macroscopic findings related to hemorrhage.

| Reviewer's summary of the most relevant macroscopic findings |         |   |   |                               |
|--|---------|---|---|-------------------------------|
| Dose   | Animal  | Macroscopic                                     | Microscopic   | Comment                       |
| 6 mg/kg  | M 57143 | Dark areas on lungs                             | Lung - Hemorrhage -minimal                          | Black colored feces Day 41-42 |
| 18 mg/kg   | M 57145 | Dark areas on lungs                             | Lung - Hemorrhage –slight                           | Red skin on limb Day 81       |
| 0 mg/kg  | F 57153 | Dark areas on lungs, adhesions                  | Lung - Lymphoid infiltration, interstitial fibrosis |                               |
| 0 mg/kg  | F 57154 | Dark area on one lung lobe                      | Lung Hemorrhage –slight                             |                               |
| 18 mg/kg   | F 57160 | Dark area in mucosa of fundic region of stomach | No microscopic correlate                            | Black colored feces Day 84-85 |
| 54 mg/kg   | F 57167 | Dark areas in mucosa of colon                   | No microscopic correlate                            | Red colored feces on Day 82   |

Histopathology: Adequate Battery: yes ( X ), no ( )—explain

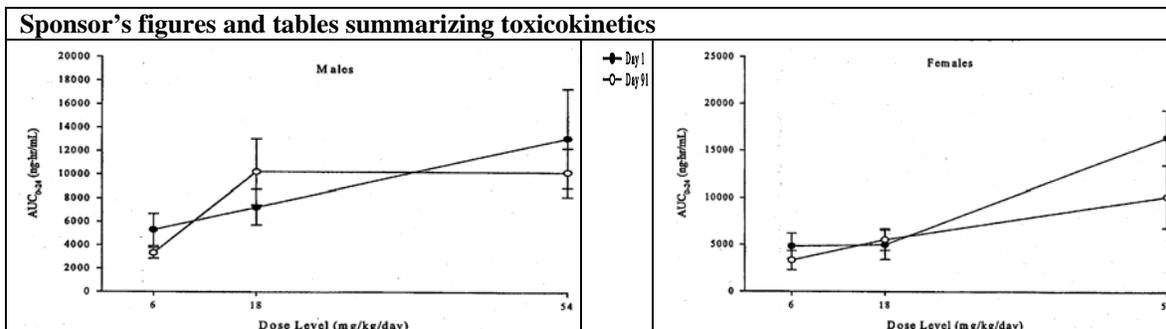
Peer review: yes ( ), no ( X )

The anatomic pathology report concluded that no treatment-related effect was observed. However, the report did comment on the slight focal gliosis and gitter cell accumulation in the white matter of the cerebrum of mid-dose female 157163. The report considered this isolated finding unrelated to DU-176b, because this accumulation and activation of glial cells is a nonspecific inflammatory reaction. No clear treatment-related effect was observed in the liver, kidney or the eye. All males, except one, had juvenile testes.

| Reviewer's compilation from sponsor's tables – treatment – related histopathology |                         |     |     |     |                  |     |     |     |   |
|---|-------------------------|-----|-----|-----|------------------|-----|-----|-----|---|
| ORGAN AND FINDING DESCRIPTION   | -- NUMBER OF ANIMALS -- |     |     |     |                  |     |     |     |   |
|   | SEX: -----MALE-----     |     |     |     | -----FEMALE----- |     |     |     |   |
|   | GROUP: -1-              | -2- | -3- | -4- | -1-              | -2- | -3- | -4- |   |
| *** FROM PREVIOUS PAGE ***  |                         |     |     |     |                  |     |     |     |   |
| LIVER (LI) .....  | NUMBER EXAMINED:        | 4   | 4   | 4   | 4                | 4   | 4   | 4   | 4 |
|   | NOT REMARKABLE:         | 1   | 0   | 0   | 1                | 1   | 1   | 1   | 1 |
| --VACUOLIZATION   |                         | 0   | 1   | 0   | 1                | 0   | 0   | 0   | 0 |
| KIDNEY (KD) .....   | NUMBER EXAMINED:        | 4   | 4   | 4   | 4                | 4   | 4   | 4   | 4 |
|   | NOT REMARKABLE:         | 0   | 1   | 0   | 1                | 1   | 1   | 1   | 1 |
| --INFILTRATES, INTERSTITIAL, LYMPHOHISTIOCYTIC                                    |                         | 4   | 3   | 4   | 2                | 3   | 3   | 3   | 3 |
| --TUBULE, MINERALIZATION  |                         | 0   | 0   | 0   | 2                | 0   | 0   | 0   | 0 |
| --TUBULE, REGENERATION  |                         | 0   | 0   | 0   | 0                | 1   | 0   | 0   | 1 |
| EYE (EY) .....  | NUMBER EXAMINED:        | 4   | 4   | 4   | 4                | 4   | 4   | 4   | 4 |
|   | NOT REMARKABLE:         | 4   | 3   | 4   | 4                | 4   | 4   | 2   | 4 |
| --INFLAMMATION, SUBACUTE  |                         | 0   | 1   | 0   | 0                | 0   | 0   | 2   | 0 |
| TESTIS (TE) .....   | NUMBER EXAMINED:        | 4   | 4   | 4   | 4                | 0   | 0   | 0   | 0 |
|   | NOT REMARKABLE:         | 0   | 0   | 0   | 1                | 0   | 0   | 0   | 0 |
| --JUVENILE TESTIS   |                         | 4   | 4   | 4   | 3                | 0   | 0   | 0   | 0 |

Toxicokinetics:

The sponsor's summary of TK parameters for DU-176b is shown in the table below. The Tmax values for DU-176B were 1 to 4 hours post-dosing in all groups. The plasma concentrations on both days show that exposures to DU-176b generally increased with dose, but were less than dose-proportional. No consistent changes in Cmax and AUC were observed after repeated dosing indicating no marked accumulation after the 13 weeks of daily dosing. No marked (>2-fold) gender differences were observed in Cmax and AUC values, except the 18 mg/kg/day dose level where males had a 2.3-fold higher mean Cmax than females.



|       |                        |        |      | Day 1                    |                       |                                | Day 91                   |                       |                                |
|-------|------------------------|--------|------|--------------------------|-----------------------|--------------------------------|--------------------------|-----------------------|--------------------------------|
| Group | Dose Level (mg/kg/day) | Gender |      | C <sub>max</sub> (ng/mL) | T <sub>max</sub> (hr) | AUC <sub>0-24</sub> (ng•hr/mL) | C <sub>max</sub> (ng/mL) | T <sub>max</sub> (hr) | AUC <sub>0-24</sub> (ng•hr/mL) |
| 2     | 6                      | M      | Mean | 582                      | 2.00                  | 5306                           | 375                      | 2.00                  | 3322                           |
|       |                        |        | SD   | 35                       | 1.41                  | 1376                           | 157                      | 1.41                  | 467                            |
|       |                        | F      | Mean | 542                      | 1.50                  | 4830                           | 366                      | 1.75                  | 3326                           |
|       |                        |        | SD   | 217                      | 0.58                  | 1372                           | 166                      | 0.50                  | 1026                           |
| 3     | 18                     | M      | Mean | 678                      | 1.50                  | 7242                           | 1070                     | 2.00                  | 10248                          |
|       |                        |        | SD   | 109                      | 0.58                  | 1518                           | 228                      | 0                     | 2809                           |
|       |                        | F      | Mean | 443                      | 1.50                  | 4992                           | 472                      | 1.00                  | 5540                           |
|       |                        |        | SD   | 151                      | 0.58                  | 1571                           | 77                       | 0                     | 1135                           |
| 4     | 54                     | M      | Mean | 924                      | 4.00                  | 13142                          | 658                      | 3.00                  | 10229                          |
|       |                        |        | SD   | 376                      | 0                     | 4220                           | 167                      | 1.15                  | 2062                           |
|       |                        | F      | Mean | 1090                     | 4.00                  | 16453                          | 761                      | 3.50                  | 10168                          |
|       |                        |        | SD   | 194                      | 0                     | 2931                           | 191                      | 1.00                  | 3324                           |
|       |                        |        | N    | 4                        | 4                     | 4                              | 4                        | 4                     | 4                              |

#### 6.6.6.4 Genetic toxicology

The sponsor previously submitted *in vitro* and *in vivo* genetic toxicology assays to IND 63, 266. Although the Ames assays were negative, the chromosomal aberration assay in Chinese hamster lung cells (CHL) and the human lymphocyte polyploidy assay were considered positive. DU-176b had no effect on the incidence of chromosomal aberrations, but increased the incidence of polyploidy CHL cells in the presence of metabolic activation. In human lymphocytes, DU-176b increased the incidence of polyploidy cells in the absence and presence metabolic activation at dose levels that produced precipitate formation. However, all *in vivo* genotoxicity assays were negative.

#### Sponsor's Summary of Genotoxicity Studies with DU-176b

| Assay Type                              | Results  | GLP | Report Number |
|---|----------|-----|---------------|
| <b>In vitro</b>                         |          |     |               |
| Reverse Mutation (Ames test)            | Negative | Y   | TOX 20020251  |
| Chromosomal Aberration (CHL/IU)         | Positive | Y   | TOX 20020321  |
| Human Lymphocyte Polyploidy Assay       | Positive | Y   | TOX 20030641  |
| Human Lymphocyte Micronucleus Assay     | Negative | Y   | TOX 20030528  |
| <b>In vivo</b>                          |          |     |               |
| Rat Bone Marrow Micronucleus Test (PO)  | Negative | Y   | TOX 20020578  |
| Rat Liver Micronucleus Test (PO)        | Negative | Y   | TOX 20030465  |
| Rat Unscheduled DNA Synthesis Test (PO) | Negative | Y   | TOX 20020498  |

PO=oral by gavage

#### 2.6.6.5 Carcinogenicity

No new carcinogenicity studies or protocol was submitted. The mouse and rat carcinogenicity protocols were reviewed by the Exec CAC in December 2006.

Based on an MTD in the 13-week oral gavage study in rats, the sponsor proposed doses (b) (4) for the carcinogenicity study in rats.

The Exec CAC concurred with the sponsor's proposed doses in males, but recommended doses of 0, 50, 100 and 200 mg/kg in females based on the sores and scabs observed in females at  $\geq 200$  mg/kg.

Based on an MTD in the 13-week oral gavage study in mice, the sponsor proposed doses of (b) (4) mg/kg/day for the carcinogenicity study in mice. The Exec CAC recommended doses of 0, 50, 150 and 500 mg/kg/day by oral

gavage for both males and females based on MTD (deaths at 1500 mg/kg/day). The recommended high dose of 500 mg/kg/day is one-third of lethal dose in mice.

Previously, the sponsor showed that DU-176b at 20 mg/kg did not enhance liver carcinogenesis in rats exposed to a single dose of N-nitrosodiethylamine (200 mg/kg ip).

| Sponsor's Summary of Medium-Term Liver Carcinogenicity Bioassay with Oral DU-176b |     |                  |             |                          |     |               |
|---|-----|------------------|-------------|--------------------------|-----|---------------|
| Species   | Sex | Duration (Weeks) | NOAEL       |                          | GLP | Report Number |
|   |     |                  | (mg/kg/day) | (mg/m <sup>2</sup> /day) |     |               |
| Rat   | M   | 6                | 20          | 120                      | Y   | TOX 20030708  |

### 2.6.6.6 Reproductive and developmental toxicology

No new reproductive and developmental study was submitted. Previously, DU-176b produced no effect on mating or fertility in male and female rats at oral doses of up to 1000 mg/kg/day. In rats, DU-176b produced no teratogenic effects, although slight embryotoxicity and maternal toxicity was observed at 300 mg/kg/day. In rabbits, DU-176b produced no teratogenic effects, although embryo-fetotoxicity was observed at doses (200 and 600 mg/kg/day) that produced maternal toxicity.

| Sponsor's Summary of Reproductive and Developmental Toxicity Studies with DU-176b |            |                        |             |                          |     |               |
|---|------------|------------------------|-------------|--------------------------|-----|---------------|
| Species   | Study Type | Dosing Duration (Days) | NOAEL       |                          | GLP | Report Number |
|   |            |                        | (mg/kg/day) | (mg/m <sup>2</sup> /day) |     |               |
| Rat   | Segment I  | <sup>a</sup>           | 1000        | 6000                     | Y   | TOX 20030552  |
| Rat   | Segment II | GD 7-17                | 100         | 600                      | Y   | TOX 20030532  |
| Rabbit  | Segment II | GD 7-20                | 60          | 720                      | Y   | TOX 20040022  |

a: 14 days prior to mating through Gestation Day (GD) 7

### 2.6.6.7 Local tolerance

No separate local tolerance study was submitted.

### 2.6.6.8 Special toxicology studies

Because DU-176b was retained in the eye and the skin of pigmented animals and absorbs light above 290 nm (APPENDIX 1), the sponsor conducted an *in vitro* phototoxicity assay. However, the phototoxicity of DU-176b was not evaluable in this assay. Therefore, the sponsor also conducted a photogenotoxicity assay using Chinese hamster lung cells (CHL/IU).

#### Study title: DU-176b: Phototoxicity test in BALB/3T3 cells

**Key findings:** The phototoxicity of DU-176b was judged as incapable of being evaluated, since the IC<sub>50</sub> value was greater than 1 mg/mL under both irradiation and non-irradiation conditions.

**Study no.:** 20060746 (F-06-090)

**Volume #, and page #:** N000, Vol. 1.5, p 6

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 8/29/06

**GLP compliance:** Indicated

**QA report:** yes ( X ) no ( )

**Drug, lot #, and % purity:** DU-176b, Lot BB202, purity 99.6%

### Methods

Using BALB/3T3 cells, DU-176b was evaluated in a neutral red uptake (NRU) phototoxicity test in comparison to dimethyl sulfoxide as a vehicle control and chlorpromazine hydrochloride (CPZ) as a positive control. A preliminary study showed no cytotoxic effect of a maximum dose of 1.0 mg/mL DU-176b both under irradiation and non-irradiation conditions. The final dose range for DU-176b was 0.0078 to 1.0 mg/mL, although DU-176b precipitated above 0.13 mg/mL.

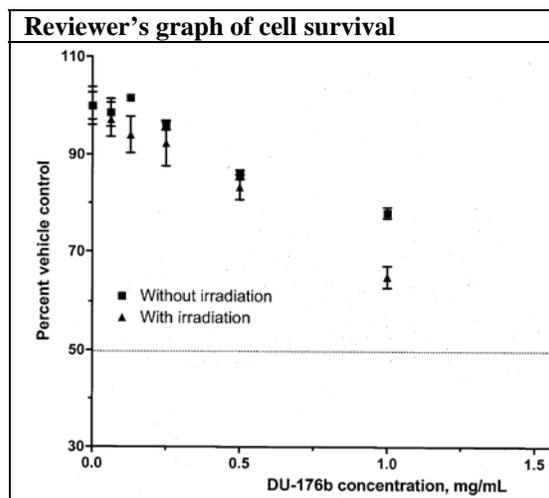
The cells were plated 22 hours prior to pretreatment with DU-176b for 60 minutes in an incubator. At room temperature outside an incubator, one set of plates was irradiated with UVA and UVB light at dosage levels of 7.3-7.7 and 0.41-0.42 J/cm<sup>2</sup>, respectively, while another set of plates was kept in the dark for 50 minutes. Cell survival was measured with the NRU assay on the following day.

### Results:

Since the optical density at 540 nm for the vehicle controls was above 0.5 with or without irradiation, the negative controls were acceptable. The positive control (CPZ) showed IC<sub>50</sub> values of 15.1 µg/mL without irradiation and 0.36 µg/mL with irradiation resulting in an acceptable photo-irritation factor (PIF) of 41.9.

DU-176b at 1.0 mg/mL showed a relative survival of 64.9% with irradiation and 78.0% without irradiation. The IC<sub>50</sub> values were greater than 1 mg/mL under both conditions. Therefore, the phototoxicity of DU-176b was judged as incapable of being evaluated. However, the sponsor concluded that DU-176b was not phototoxic.

When solubility and cytotoxicity are low at the maximum recommended concentration of test substance (1 mg/mL), the OECD guideline 431 recommends confirmatory testing using another assay system.



### Study title: DU-176b: Photochromosomal aberration test in Chinese hamster lung cells

**Key findings:** DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation.

**Study no.:** 20060731 (G-06-031)

**Volume #, and page #:** N000, Vol. 1.4, p 307

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** 8/4/06**GLP compliance:** Indicated**QA report:** yes ( X ) no ( )**Drug, lot #, and % purity:** DU-176b, Lot BB202, purity 99.6%**Methods**

The photo-cytogenetic effect of DU-176b was evaluated in Chinese hamster lung (CHL/IU) cells in comparison to dimethyl sulfoxide as a vehicle control and N-methyl-N'-nitro-N-nitrosoguanidine (MNG, 1 µg/mL) and 8-methoxypsoralen (8-MOP, 0.01 µg/mL) as positive controls. A preliminary study showed the IC<sub>50</sub> values for cell growth inhibition by DU-176b were 2600 and 3600 µg/mL in the absence and presence of irradiation, respectively. Precipitation was observed at the end treatment at 156 µg/mL and above.

The cells were plated 3 days prior to pretreatment with DU-176b in the absence of metabolic activation for 60 minutes in an incubator. At room temperature outside an incubator, one set of plates was irradiated with UVA and UVB light at dosage levels of 4.8-5.1 and 0.27-0.31 J/cm<sup>2</sup>, respectively, while another set of plates was kept in the dark for 50 minutes. Cell survival was measured with a Coulter counter on the following day. Chromosome preparations were made and evaluated for chromosomal aberrations and polyploidy on 100 and 400 metaphase cells per culture in a blinded manner by four examiners.

**Results:**

In the negative (vehicle and non-treatment) controls, incidences of cells with structural aberrations (0.5% without and 1.5-2.5% with irradiation) were within the range of historical control data. The incidences of polyploid cells (0.9-1.1%) in the negative controls without irradiation were higher than the maximum incidences in historical controls (0.63%). However, the incidences of polyploid cells (0.8-1.3%) in the negative controls with irradiation were within the range of historical control data. Since the current incidences of polyploidy cells were similar under both conditions, the sponsor concluded that the evaluation of the current study was not significantly affected. The positive controls significantly increased the incidence of cells with structural aberrations (MNG 50.5% and 8-MOP 72.0%) indicating the study was adequately conducted.

DU-176b dose-dependently inhibited the growth of CHL/IU cells with greater inhibition in the absence of irradiation than in the presence of irradiation. Severe cytotoxicity at 5000 µg/mL prevented analysis of these cultures. The incidences of cells with structural aberration in the DU-176b treatment groups (0.0-2.0%) were similar to the incidences in the negative controls (0.5-2.5%). At the highest dose of DU-176b tested (2500 µg/ml) the incidences of polyploid cells (1.6 and 1.9%) were higher than the incidences in the negative controls (1.1 or 1.3%) and the maximum incidences in the historical control data (0.63 and 1.38%). However, cell growth was inhibited more than 50% at this concentration of DU-176b. Furthermore, a Fisher's exact test indicated no statistical significance and no clear dose-dependency was observed. The reviewer notes that DU-176b previously produced polyploidy in CHL/IU cells and cultured human peripheral blood lymphocytes in association with excessive toxicity. Importantly, in the current

study the incidence of polyploidy was similar in the presence and absence of irradiation. The sponsor concluded DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation.

| Sponsor's tables  |  |               |                                    |                                       |                                |  |   |     |     |
|-------------------|--|---------------|------------------------------------|---------------------------------------|--------------------------------|--|---|-----|-----|
| Photo-irradiation | Treatment Time (min-min-hr) <sup>a</sup> | Test compound | Concentration (µg/mL) <sup>b</sup> | Relative Cell Growth (%) <sup>c</sup> | Mitotic Index (%) <sup>d</sup> | Structural Aberrant Cells (%) <sup>e</sup> | Numerical Aberrant Cells (%) <sup>f</sup> |     |     |
| Without           | 110-0-(22)                               | Non-treatment | —                                  | NA                                    | NA                             | 0.5  | 0.9                                       |     |     |
|                   |  | DMSO (2 vol%) | 0                                  | 100                                   | NA                             | 0.5  | 1.1                                       |     |     |
|                   |  | DU-176b       | 625 <sup>g</sup>                   | 90                                    | NA                             | 2.0  | 1.1                                       |     |     |
|                   |  |               | 1250 <sup>g</sup>                  | 68                                    | 8.6, 10.2                      | 0.0  | 0.4                                       |     |     |
|                   |  |               | 2500 <sup>g</sup>                  | 32                                    | 8.8, 1.8                       | 0.0 <sup>h</sup>                           | 0.0 <sup>i</sup>                          |     |     |
|                   |  |               | 5000 <sup>g</sup>                  | 24                                    | 7.4, 6.6                       | 1.5  | 1.6                                       |     |     |
|                   |  |               | 5000 <sup>g</sup>                  | 9                                     | NA                             | NA   | NA  |     |     |
|                   |  | MNNG          | 1                                  | NA                                    | NA                             | 50.5 <sup>*1</sup>                         | 0.1                                       |     |     |
|                   |  | With          | 60-50-(22)                         | Non-treatment                         | —                              | NA   | NA  | 2.5 | 0.8 |
|                   |  |               |                                    | DMSO (2 vol%)                         | 0                              | 100  | NA  | 1.5 | 1.3 |
| DU-176b           | 625 <sup>g</sup>                         |               |                                    | 85                                    | NA                             | 0.0  | 0.9                                       |     |     |
|                   | 1250 <sup>g</sup>                        |               |                                    | 69                                    | NA                             | 1.0  | 1.6                                       |     |     |
|                   | 2500 <sup>g</sup>                        |               |                                    | 40                                    | 5.8, 4.6                       | 1.5  | 1.9                                       |     |     |
|                   | 5000 <sup>g</sup>                        |               |                                    | 30                                    | 0.8, 3.6                       | NA   | NA  |     |     |
|                   | 5000 <sup>g</sup>                        |               |                                    | 0.01                                  | NA                             | NA   | 72.0 <sup>*1</sup>                        | 0.4 |     |
| 8-MOP             | 0.01                                     |               |                                    | NA                                    | NA                             | 72.0 <sup>*1</sup>                         | 0.4                                       |     |     |

| Historical control data using CHL/IU cells              |             |                   |                                 |         |         |
|---|-------------|-------------------|---------------------------------|---------|---------|
| Group   | No. of data | Light irradiation | Incidence (%) of aberrant cells |         |         |
|   |             |                   | Average                         | Maximum | Minimum |
| <Structural aberrations excluding cells with only gaps> |             |                   |                                 |         |         |
| Negative control <sup>1)</sup>                          | 23          | without           | 1.1                             | 3.5     | 0.0     |
| Negative control <sup>2)</sup>                          | 23          | with              | 5.0                             | 14      | 0.5     |
| <Polyploid cells>                                       |             |                   |                                 |         |         |
| Negative control <sup>1)</sup>                          | 23          | without           | 0.22                            | 0.63    | 0.00    |
| Negative control <sup>2)</sup>                          | 23          | with              | 0.63                            | 1.38    | 0.13    |

DMSO: Dimethyl sulfoxide, MNNG, N-Methyl-N-nitrosoguanidine, 8-MOP, Methoxsalen, NA: Not analyzed  
<sup>1)</sup> Two-tailed Fisher's exact probability test with Bonferroni adjustment (P<0.05).  
<sup>2)</sup> Treatment time was expressed as treatment time (min) without photo-irradiation, treatment time (min) with photo-irradiation, and recovery time (hr).  
<sup>3)</sup> Concentration levels of DU-176b are expressed as the amount of anhydrous free base.  
<sup>4)</sup> Based on cell counting with a Coulter Counter.  
<sup>5)</sup> The 500 cells per slide were analyzed.  
<sup>6)</sup> Excluding gaps. 100 metaphases per culture (200 metaphases per concentration) were analyzed.  
<sup>7)</sup> The 400 metaphases per culture (800 metaphases per concentration) were analyzed.  
<sup>8)</sup> Precipitation was observed at the beginning and the end of the treatment.  
<sup>9)</sup> Only one culture was analyzed (100 metaphases per concentration).  
<sup>10)</sup> Only one culture was analyzed (400 metaphases per concentration).

**2.6.6.9 Discussion and Conclusions**

In acute studies, doses of 2000 mg/kg to rats and 800 mg/kg to dogs did not result in mortality. These results indicate that the minimum lethal dose is greater than 2000 mg/kg in rats and 800 mg/kg in dogs.

The table below compares the findings in the longest repeated dose toxicology studies. In monkeys, the 10-20% prolongation of PT and aPTT values at 24 hours after dosing was an expected pharmacodynamic effect of DU-176B. In rats, PT and aPTT values were not prolonged in the 26-week study even at 54 mg/kg. However, in the 13-week study, slightly higher PT and aPTT times were observed in rats dosed at ≥200 mg/kg/day. The need for higher dosages in rats to observe a pharmacodynamic effect is consistent with DU-176b being less potent in rat plasma than in monkey or human plasma.

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| <b>Reviewer's comparison of longest repeat dose toxicology studies for DU-176B</b> |  |       |         |        |         |   |       |        |        |        |
|--|--|-------|---------|--------|---------|---|-------|--------|--------|--------|
| Species  | <b>Rat</b>   |       |         |        |         | <b>Monkey</b>   |       |        |        |        |
| Reviewer   | Tushar Kokate  |       |         |        |         | Patricia Harlow   |       |        |        |        |
| Study length   | 26 weeks   |       |         |        |         | 13 weeks  |       |        |        |        |
| Study code   | 20050334 (6630-159)  |       |         |        |         | 20050333 (6630-160)   |       |        |        |        |
| Administration   | Oral gavage  |       |         |        |         | Oral gavage   |       |        |        |        |
| Doses (mg/kg)  | 0, 6, 18 & 54 mg/kg/day  |       |         |        |         | 0, 6, 18, 54 mg/kg  |       |        |        |        |
| Number/sex/group   | Main study: 10<br>Recovery: 5 in control & high dose group                       |       |         |        |         | Main study: 4   |       |        |        |        |
| Treatment-related mortality  | None considered treatment-related<br>(One control male and one high dose female) |       |         |        |         | None  |       |        |        |        |
| Adverse clinical signs   | No treatment-related effect  |       |         |        |         | Incidence of black or red feces increased in high dose group                |       |        |        |        |
| Body weight gain   | No treatment-related effect  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| Food consumption   | No treatment-related effect  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| Ophthalmoscopy:  | No treatment-related effect  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| Electrocardiography  | Not monitored  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| PK/TK  | AUC  | Male  |         | Female |         | Cmax  | Male  |        | Female |        |
|  | Dose   | Day 1 | Day 178 | Day 1  | Day 178 | Dose  | Day 1 | Day 91 | Day 1  | Day 91 |
| AUC = ng hr/mL   | 6  | 768   | 1053    | 1080   | 2581    | 6   | 5306  | 3322   | 4830   | 3326   |
| Tmax 1 hr post dose  | 18   | 2968  | 2624    | 2514   | 2833    | 18  | 7242  | 10248  | 4992   | 5540   |
|  | 54   | 6706  | 5459    | 10243  | 9015    | 54  | 13142 | 10229  | 16453  | 10168  |
| Hematology   | No treatment-related effect  |       |         |        |         | Effects on RBC, Hb, Hct, reticulocytes in one mid- and one high dose female |       |        |        |        |
| Coagulation  | No treatment-related effect  |       |         |        |         | PT, APTT prolongation   |       |        |        |        |
| Blood chemistry  | No treatment-related effect  |       |         |        |         | No clear treatment-related effect   |       |        |        |        |
| Urinalysis   | Not monitored  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| Macroscopic pathology  | No treatment-related effect  |       |         |        |         | Findings related to hemorrhage in two control and four treated animals      |       |        |        |        |
| Relative organ weights   | No treatment-related effect  |       |         |        |         | No treatment-related effect   |       |        |        |        |
| Microscopic pathology  | No treatment-related effect  |       |         |        |         | No clear treatment-related effect   |       |        |        |        |
| Reviewer's NOEL  | 54 mg/kg   |       |         |        |         | 54 mg/kg  |       |        |        |        |
| Sponsor's NOAEL  | 54 mg/kg   |       |         |        |         | 54 mg/kg  |       |        |        |        |

In the previous 4 week study in monkeys, administration of 100 mg/kg/day resulted in two deaths that were attributed to drug-related hemorrhage in multiple tissues, especially the lungs and thymus. In addition, hemorrhage in stomach was observed in the 30 mg/kg/day group and hemorrhage in the adrenals and uterus was observed in the 100 mg/kg group. Slight reductions in RBC count, hemoglobin, and hematocrit in the 30 and 100 mg/kg/day groups were correlated with the incidence of hemorrhage in histopathologic examination. These hemorrhagic effects were attributed to exaggerated pharmacological activity. Other adverse effects in animals that survived to terminal sacrifice included focal vacuolization in the liver at 30 mg/kg and focal interstitial pneumonitis in the lung and perivascular mononuclear infiltrate in the spinal cord at 100 mg/kg. Increased urine protein was observed at 30 and 100 mg/kg/day in both males and females. The sponsor's NOAEL was 30 mg/kg, while the NOAEL of the previous reviewer was 10 mg/kg/day. In contrast, the NOAEL in the 13-week study was 54 mg/kg with no clear treatment related findings observed in the liver or kidney.

Toxicology studies of 6 months in rats and 3 months in monkeys support the proposed clinical trial of three months duration using doses of 30 to 120 mg per day. DU-176b has already been administered to humans at a maximum dose of 120 mg for 10 days. The table below shows that the human equivalent doses of the NOEL's in rats and monkeys are 9.7 and 17.5 mg/kg, respectively, or 4.9- and 8.8-fold higher than the highest

proposed human dose and provide a reasonable safety margin in the trial. Comparison based on exposure in the monkey with human exposure at 120 mg daily dose also provides a 3.6-4.5-fold safety margin.

| Calculation of NOAEL for DU-176B |         |                 |                  |        |                        | NOAEL HED compared to                     |  |                                 |
|----------------------------------|---------|-----------------|------------------|--------|------------------------|---|--|---------------------------------|
| Study length                     | Species | AUC µg.hr/mL    | NOEL Dose, mg/kg | Factor | HED <sup>†</sup> mg/kg | Proposed low dose, 30 mg qd (0.5 mg/kg/d) | Proposed high dose 60 mg bid (2 mg/kg/d) | Ratio AUC at NOEL vs human AUC* |
| 13-week                          | Rat     | 6073<br>10004   | 60               | 0.162  | 9.7                    | 19  | 4.9                                      | 1.6<br>2.7                      |
| 26-week                          | Rat     | 5459<br>9015    | 54               | 0.162  | 8.7                    | 17  | 4.4                                      | 1.5<br>2.4                      |
| 13-week                          | Monkey  | 13142,<br>16453 | 54               | 0.324  | 17.5                   | 35  | 8.8                                      | 3.6<br>4.5                      |

† From Guidance on a Harmonized approach to Safe Starting Dose Estimation for Clinical Trials of Therapeutics in Healthy Volunteers ‡ Human equivalent dose § Human proposed doses are 30 mg qd, 30 mg bid, 60 mg qd and 60 mg bid. \* Human AUC in repeated dose study = 3682 ng.hr/mL after 120 mg qd

No ophthalmology finding or histopathology finding in the eye was observed in the 13-week monkey study. However, the distribution studies in pigmented rats and monkeys showed prolonged retention of DU-176b in melanic tissues, especially the eye. The Division has previously consulted with the Division of Anti-Infective and Ophthalmology Products (DAIOP) concerning other products that bind to melanin, especially in the eye. DAIOP recommended that close ocular monitoring needs to be performed in human subjects receiving repeat doses of DU-176B in the absence of longer term animal studies demonstrating no ocular anatomic or functional pathology. The long term toxicology studies in pigmented animals should include electroretinograms performed at baseline and at regular intervals during the study

### 2.6.6.10 Tables and Figures

Relevant tables and figures were inserted at appropriate points in the text above.

### 2.6.7 TOXICOLOGY TABULATED SUMMARY

The following tables were provided by the sponsor.

| Species/Strain     | Route of Administration (Vehicle) | Dose (mg/kg)      | Sex and No. per group | Approximate Lethal Dose (mg/kg) | Noteworthy Finding  | Report No | Status of Submission        |
|--------------------|-----------------------------------|-------------------|-----------------------|---------------------------------|---|-----------|-----------------------------|
| Rat/Sprague Dawley | PO (0.5% MC <sup>a</sup> )        | 0<br>1500<br>2000 | 5M<br>5F              | >2000 for males and females     | No death and no toxic change  | 20020310  | Serial #000<br>May 27, 2004 |
| Cynomolgus Monkey  | PO (0.5% MC <sup>a</sup> )        | 0<br>200<br>400   | 2F                    | >400                            | ≥200 mg/kg: No death and no clinical sign, prolongation of PT and APTT (Day 2)<br>400 mg/kg: Decreased platelet count (Day 2) | 20020307  | Serial #000<br>May 27, 2004 |

<sup>a</sup> Methylcellulose  
PT: Prothrombin time, APTT: activated partial thrombin time

| Species/<br>Strain        | Route of<br>Admini-<br>tration<br>Vehicle) | Dose<br>(mg/kg/day) | Duration<br>of dose                            | Sex and<br>No. per<br>group    | Noteworthy Finding  | Report<br>No | Status<br>of<br>Submission         |
|---------------------------|--|---------------------|--|--------------------------------|---|--------------|------------------------------------|
| Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )              | 0, 20, 60,<br>200   | 4 weeks  | 10M<br>10F                     | ≥20 mg/kg/day, male: hemorrhage with inflammation in the pancreas<br>≥60 mg/kg/day, male: focal pneumonitis with hemoglobin crystal in the lung<br>200 mg/kg/day, female: focal pneumonitis with hemoglobin crystal in the lung, hemorrhage in the thymus<br>NOAEL: <20 mg/kg/day for males, 60 mg/kg/day for females | 20020612     | Serial #000<br>May 27, 2004        |
| Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )              | 0, 6, 12, 18        | 4 weeks  | 10M                            | No treatment-related change<br>NOAEL: 18 mg/kg/day for males  | 20030041     | Serial #000<br>May 27, 2004        |
| Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )              | 0, 6, 18, 54        | 26 weeks +<br>4 weeks<br>recovery <sup>b</sup> | 10M,10F<br>+5M,5F <sup>b</sup> | No treatment-related change<br>NOAEL: 54 mg/kg/day for males and females  | 20050334     | Serial #072<br>October 10,<br>2006 |

<sup>a</sup> Methylcellulose, <sup>b</sup> 0 and 54 mg/kg/day only

PT: Prothrombin time, APTT: activated partial thrombin time, RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit

| Species/<br>Strain    | Route of<br>Admini-<br>tration<br>Vehicle) | Dose<br>(mg/kg/day) | Duration<br>of dose                             | Sex and<br>No. per<br>group  | Noteworthy Finding   | Report<br>No | Status<br>of<br>Submission   |
|-----------------------|--|---------------------|---|------------------------------|--|--------------|------------------------------|
| Cynomolgus<br>Monkeys | PO<br>(0.5% MC <sup>a</sup> )              | 0, 10, 30,<br>100   | 4 weeks   | 4M<br>4F                     | ≥30 mg/kg/day: Prolongation of PT and APTT<br>100 mg/kg/day: Moribund sacrifice (Day 3) and death (Day 27) of one female each with hemorrhage in the gastrointestinal tract, hemorrhage in the adrenal cortex of one male<br>NOAEL: 30 mg/kg/day | 20020606     | Serial #000<br>May 27, 2004  |
| Cynomolgus<br>Monkeys | PO<br>(0.5% MC <sup>a</sup> )              | 0, 6, 18, 54        | 13 weeks  | 4M<br>4F                     | ≥18 mg/kg/day: Prolongation of PT and APTT<br>54 mg/kg/day: Decreased RBC, HGB and HCT and increased reticulocyte count in one male on only Day 28<br>NOAEL: 18 mg/kg/day for males and 54 mg/kg/day for females                                 | 20050333     | Serial #099<br>April 2, 2007 |
| Cynomolgus<br>Monkeys | PO<br>(0.5% MC <sup>a</sup> )              | 0, 5, 15, 45        | 52 weeks<br>+ 13 weeks<br>recovery <sup>b</sup> | 4M,4F<br>+2M,2F <sup>b</sup> | On-going   | In progress  | N/A                          |

<sup>a</sup> Methylcellulose, <sup>b</sup> 0 and 45 mg/kg/day only

| Test                      | Cell   | Concentration   | Metabolic<br>activation | Exposure<br>time        | Noteworthy Findings  | Report<br>No | Status<br>of<br>Submission   |
|---------------------------|--|---|-------------------------|-------------------------|--|--------------|------------------------------|
| Reverse<br>Mutation       | TA98<br>TA100<br>TA1535<br>TA1537<br>WP2uvrA | 156 – 5000 µg/plate<br>156 – 5000 µg/plate<br>78.1 – 5000 µg/plate<br>78.1 – 2500 µg/plate<br>156 – 5000 µg/plate | -S9<br>+S9              | -                       | Negative   | 20020251     | Serial #000<br>May 27, 2004  |
| Chromosomal<br>Aberration | Chinese hamster<br>lung<br>cell<br>(CHL/IU)  | 313 – 2500 µg/mL  | -S9                     | 6 h (18 h) <sup>a</sup> | ≥1250 µg/mL + S9: Increased frequency of numerical aberrations (polyploidy)<br>No structural aberrations | 20020321     | Serial #000<br>May 27, 2004, |
|                           |  | 313 – 2500 µg/mL  | +S9                     | 6 h (18 h) <sup>a</sup> |  |              |                              |
|                           |  | 19.5 – 78.1 µg/mL   | -S9                     | 24 h                    |  |              |                              |
| Polyploidy                | Human<br>peripheral<br>lymphocytes           | 157 – 1880 µg/mL  | -S9                     | 3 h (19 h) <sup>a</sup> | ≥625 µg/mL   | 20030641     | Serial #000<br>May 27, 2004, |
|                           |  | 157 – 1880 µg/mL  | +S9                     | 3 h (19 h) <sup>a</sup> | ≥313 µg/mL   |              |                              |
|                           |  | 157 – 2500 µg/mL  | -S9                     | 22 h                    | Negative   |              |                              |
|                           |  | 78.5 – 1250 µg/mL   | -S9                     | 46 h                    | ≥313 µg/mL   |              |                              |
|                           |  | 157 – 1250 µg/mL  | -S9                     | 3 h (43 h) <sup>a</sup> | ≥313 µg/mL   |              |                              |
| In vitro<br>Micronucleus  | Human<br>peripheral<br>lymphocytes           | 185.6 – 1113.6 µg/mL  | +S9                     | 3 h (21 h) <sup>a</sup> | No effect on frequency of micronuclei  | 20030528     | Serial #000<br>May 27, 2004, |
|                           |  | 185.6 – 1113.6 µg/mL  | +S9                     | 3 h (45 h) <sup>a</sup> |  |              |                              |
|                           |  | 46.4 – 1856 µg/mL   | -S9                     | 48 h                    |  |              |                              |

<sup>a</sup> ( ): Recovery period without DU-176b

| Test                                  | Species/<br>Strain        | Route of<br>Admini-<br>stration<br>(Vehicle) | Dose<br>(mg/kg)          | Duration<br>of dose | Sampling<br>time after<br>dosing                     | Sex, n/<br>Dose       | Noteworthy Findings | Report<br>No | Status<br>Submission         |
|---------------------------------------|---------------------------|--|--------------------------|---------------------|--|-----------------------|---------------------|--------------|------------------------------|
| Bone Marrow<br>Micronucleus           | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | 0, 500,<br>1000,<br>2000 | Single              | 24 and 48 h  | 5M                    | Negative            | 20020578     | Serial #000<br>May 27, 2004, |
|                                       | Cynomolgus<br>monkey      | PO<br>(0.5% MC <sup>a</sup> )                | 0, 10, 30,<br>100        | 4 weeks             | 24 h   | 4M<br>4F <sup>b</sup> | Negative            | 20020606     | Serial #000<br>May 27, 2004  |
| Liver<br>Micronucleus                 | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | 0, 2000                  | Single              | 3 or 5 days <sup>c</sup><br>5 or 7 days <sup>d</sup> | 5M                    | Negative            | 20030465     | Serial #000<br>May 27, 2004, |
| Liver<br>Unscheduled<br>DNA Synthesis | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | 0, 500,<br>1000,<br>2000 | Single              | 2-4 h<br>14-16 h                                     | 4M                    | Negative            | 20020498     | Serial #000<br>May 27, 2004, |

<sup>a</sup> Methylcellulose, <sup>b</sup> only two females at 100 mg/kg/day were analyzed, <sup>c</sup> partial hepatectomy on prior day of dosing, <sup>d</sup> partial hepatectomy on following day of dosing

| Test   | Species/<br>Strain        | Route of<br>Admini-<br>stration<br>(Vehicle) | Dose<br>(mg/kg/day)      | Duration<br>of dose  | Sex,<br>n/<br>Dose | Noteworthy Findings  | Report<br>No | Status<br>Submission               |
|--|---------------------------|--|--------------------------|----------------------|--------------------|--|--------------|------------------------------------|
| Medium-<br>Term Liver<br>Carcino-<br>genesis | Rat/<br>F344              | PO<br>(0.5% MC <sup>a</sup> )                | 0, 5, 10, 20             | 6 weeks <sup>b</sup> | 20M                | No effect on the number and area of GST-P positive foci in the liver   | 20030708     | Serial #000<br>May 27,<br>2004,    |
| Dose<br>Range-<br>Finding                    | Mouse/<br>CD1(ICR)        | PO<br>(0.5% MC <sup>a</sup> )                | 0, 60, 200,<br>600, 1500 | 13 weeks             | 10M<br>10F         | ≥60 mg/kg/day: Rough hair coat<br>≥200 mg/kg/day: Hunched posture, squinted eyes<br>1500 mg/kg/day: Death or moribund sacrifice, hypoactivity, red oral and nasal discharge, swollen ventral abdomen, tremor, audible, irregular or labored respiration, hypothermia, pale skin, decreased food consumption, body weight (male only), RBC, HGB, HCT, WBC and LYM<br>Recommended dose levels: 50, 150 and 500 mg/kg/day for males and females | 20050792     | Serial #072<br>October 10,<br>2006 |
|  | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | 0, 60, 200,<br>600, 1500 | 13 weeks             | 10M<br>10F         | ≤60 mg/kg/day: Rough hair coat<br>≤200 mg/kg/day: Alopecia and sores/scabs, prolongation of PT<br>600 mg/kg/day: Death (1 male)<br>1500 mg/kg/day: Death or moribund sacrifice, red nasal discharge, audible respiration, hunched posture, hypoactivity, thin appearance, alopecia, and sores/scabs, decreased body weight,<br>Recommended dose levels: 60, 200 and 600 mg/kg/day for males and 50, 150 and 500 mg/kg/day for females        | 20050791     | Serial #072<br>October 10,<br>2006 |

<sup>a</sup> Methylcellulose, <sup>b</sup> N-nitrosodiethylamine on Day 1 followed by DU-176b from Days 15 through 56 and partial hepatectomy on Day 22  
RBC: red blood cell count, HGB: hemoglobin, HCT: hematocrit, WBC: white blood cell count, LYM: lymphocyte count, PT: prothrombin time

| Test   | Species/<br>Strain        | Route of<br>Admini-<br>stration<br>(Vehicle) | Dosing<br>Period  | Dose<br>(mg/kg/<br>day)    | Sex, n/<br>Dose | Noteworthy Finding   | Report<br>No | Status<br>Submission           |
|--|---------------------------|--|---|----------------------------|-----------------|--|--------------|--------------------------------|
| Fertility and<br>Early<br>Embryonic<br>Development | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | 2 weeks<br>before<br>mating, and<br>up to the day<br>before<br>copulation<br>for males,<br>and until G7<br>for females. | 0,<br>100,<br>300,<br>1000 | 20M<br>20F      | No treatment-related change<br>NOAEL: 1000 mg/kg/day for male and female rats and their reproductive performance   | 20030552     | Serial #000<br>May 27,<br>2004 |
| Embryo-fetal<br>Development                        | Rat/<br>Sprague<br>Dawley | PO<br>(0.5% MC <sup>a</sup> )                | G7 - G17  | 0, 30,<br>100,<br>300      | 20F             | 300 mg/kg/day (dam): Vaginal hemorrhage<br>300 mg/kg/day (fetus): Increased post-implantation loss<br>NOAEL: 100 mg/kg/day for dams, embryo and fetus  | 20030532     | Serial #000<br>May 27          |
| Embryo-fetal<br>Development                        | Rabbit/<br>NZW            | PO<br>(0.5% MC <sup>a</sup> )                | G7 - G20  | 0, 60,<br>200,<br>600      | 26F-<br>30F     | 200 mg/kg/day (dam): 3 died and 2 aborted<br>600 mg/kg/day (dam): 4 died and 3 aborted<br>≥200 mg/kg/day (dam): Decreased food consumption, body weight and defecation, increased dark red contents of the uterus<br>≥200 mg/kg/day (fetus): Increased post-implantation loss, decreased number of live fetuses, decreased fetal weight, increased incidence of the absent or small gall bladder<br>600 mg/kg/day (fetus): Increased 13 <sup>th</sup> full ribs and 27 presacral vertebra<br>NOAEL: 60 mg/kg/day for dams, embryos and fetuses | 20040022     | Serial #000<br>May 27          |

<sup>a</sup> Methylcellulose  
G#: Day # of gestation

| Test                              | Cell                               | Route    | Dose (mg/mL) | Duration of Exposure | No. of culture | Special Features  | Noteworthy Findings | Report No | Status of Submission          |
|-----------------------------------|------------------------------------|----------|--------------|----------------------|----------------|---|---------------------|-----------|-------------------------------|
| Phototoxicity - 3T3 NRU Assay     | BALB/3T3 cells                     | In vitro | 0.0078-1.0   | 60 min               | 4 wells        | 60 minutes pretreatment with DU-176b and exposure of simulated sunlight (UVA, UVB and visible light) for 50 minutes followed by NRU assay   | Negative            | 20060746  | Serial # 099<br>April 2, 2007 |
| Photo-chromosomal Aberration Test | Chinese hamster lung cell (CHL/IU) | In vitro | 0.313 – 5.0  | 60 min               | 2 dishes       | 60 minutes pretreatment with DU-176b and exposure of simulated sunlight (UVA, UVB and visible light) for 50 minutes followed by 22 hours incubation for recover and chromosome analysis | Negative            | 20060731  | Serial # 099<br>April 2, 2007 |

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Conclusions:

Since DU-176B is a factor Xa inhibitor that has been demonstrated to be effective in animal models of thrombosis, the proposed use of DU-176B for prevention of thromboembolic events in patients with non-valvular atrial fibrillation is reasonable. The sponsor has previously evaluated a maximum daily dose of 120 mg of DU-176B in human subjects for a period of 10 weeks. The proposed trial will evaluate doses of 30 mg qd, 30 mg, bid, 60 mg, qd and 60 mg bid in patients with atrial fibrillation for three months. The NOEL's in the 6 month rat study and the 3 month monkey study provide a reasonable safety margin for the proposed trial to proceed.

DU-176b is retained by melanic tissues, especially the eye. Because DU-176b absorbs light above 290 nm, the sponsor conducted an *in vitro* phototoxicity assay. Since the phototoxicity of DU-176b was not evaluable in this assay, the sponsor also conducted a photogenotoxicity assay using Chinese hamster lung cells. DU-176b induced neither structural chromosomal aberrations nor polyploid cells in CHL/IU cells either in the presence or absence of photo-irradiation. Although no further phototoxicity testing is required, the reviewer notes that ophthalmology testing in animals did not include functional evaluation of the eye. The Division of Anti-Infective and Ophthalmology Products recommends that products that bind to melanin also be evaluated for retinal function using electroretinograms.

Unresolved toxicology issues (if any): None for the proposed protocol.

### Recommendations:

1. The sponsor has previously evaluated a maximum daily dose of 120 mg of DU-176B in human subjects for a period of 10 weeks. The proposed trial will evaluate doses of 30 mg qd, 30 mg, bid, 60 mg, qd and 60 mg bid in patients with atrial fibrillation for three months. The NOEL's in the 6 month rat study and the 3 month monkey study provide a reasonable safety margin for the proposed trial to proceed.

2. Given the retention of DU-176B in the melaniferous tissues of the eye, the sponsor should submit the absorption spectrum of DU-176B between 325 and 700 nm. Since the sponsor has conducted *in vitro* phototoxicity assays as specified in the Guidance for Photosafety Testing, CDER (May 2003), no further phototoxicity testing is required.

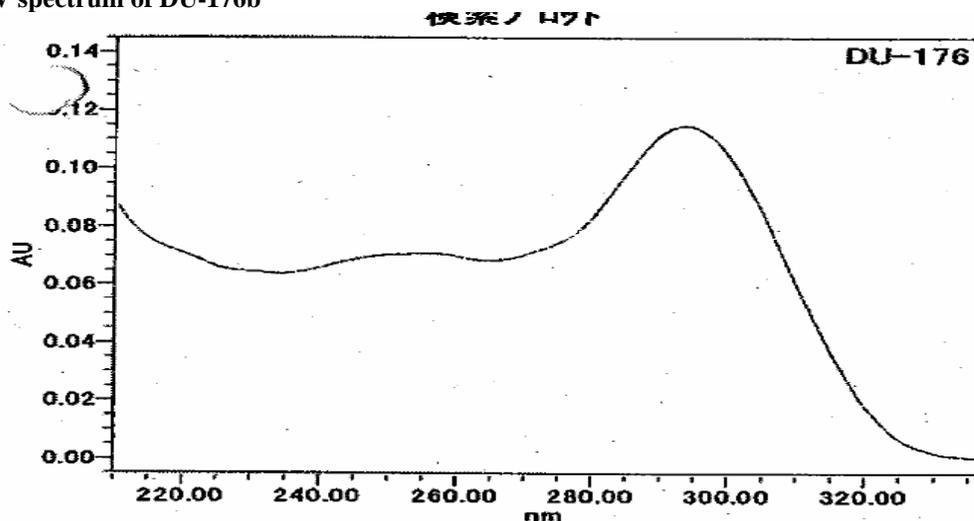
3. The sponsor should monitor intraocular pressure and obtain electroretinograms, in addition to standard slit-lamp biomicroscopy and funduscopy, before the treatment initiation and at multiple intervals in the longer toxicity studies in monkeys or pigmented animals. Careful histopathology of the eye should be performed on all animals. The Division has previously consulted with the Division of Anti-Infective and Ophthalmology Products concerning similar findings for other products. The Division of Anti-Infective and Ophthalmology Products recommended close ocular monitoring needs to be performed in human subjects receiving repeat doses of DU-176B in the absence of longer term animal studies demonstrating no ocular anatomic or functional pathology.

Suggested labeling: Not applicable at this time.

## APPENDIX/ATTACHMENTS

### APPENDIX 1: UV Spectrum of DU-176b

UV spectrum of DU-176b



**APPENDIX 2 Sponsor’s Summaries of Findings in Rat Toxicology Studies**

**Table 4.4.2.1.1. Summary of Toxicity Findings in Surviving Male and Female Rats Receiving DU-176b by Gavage for four Weeks**

| Daily Dose (mg/kg/day):                         | 0     |       | 20    |       | 60    |       | 200   |       |
|---|-------|-------|-------|-------|-------|-------|-------|-------|
| No. of Animals:                                 | M: 10 | F: 10 |
| <b>Toxicokinetics</b>                           |       |       |       |       |       |       |       |       |
| C <sub>max</sub> (ng/mL)                        |       |       |       |       |       |       |       |       |
| Day 1   | NE    | NE    | 691   | 820   | 1380  | 2410  | 1710  | 3330  |
| Day 28  | NE    | NE    | 1140  | 1290  | 2200  | 2910  | 2520  | 4520  |
| AUC <sub>0-24h</sub> (ng·h/mL)                  |       |       |       |       |       |       |       |       |
| Day 1   | NE    | NE    | 2418  | 2390  | 6636  | 7708  | 18243 | 21699 |
| Day 28  | NE    | NE    | 5702  | 6673  | 12123 | 12956 | 23745 | 44808 |
| <b>Noteworthy Findings</b>                      |       |       |       |       |       |       |       |       |
| Died or Moribund Sacrificed                     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| Clinical Observations                           | -     | -     | -     | -     | -     | -     | -     | -     |
| Body Weight                                     | -     | -     | -     | -     | -     | -     | -     | -     |
| Food Consumption                                | -     | -     | -     | -     | -     | -     | -     | -     |
| Ophthalmoscopy                                  | -     | -     | -     | -     | -     | -     | -     | -     |
| Urinalysis                                      | -     | -     | -     | -     | -     | -     | -     | -     |
| Hematology                                      | -     | -     | -     | -     | -     | -     | -     | -     |
| Bone Marrow                                     | -     | -     | -     | -     | -     | -     | -     | -     |
| Blood Biochemistry                              | -     | -     | -     | -     | -     | -     | -     | -     |
| Organ Weight                                    | -     | -     | -     | -     | -     | -     | -     | -     |
| Necropsy  | -     | -     | -     | -     | -     | -     | -     | -     |
| <b>Histopathology:</b>                          |       |       |       |       |       |       |       |       |
| Lung: Focal pneumonitis with hemoglobin crystal | 0     | 0     | 0     | 0     | 2     | 0     | 1     | 1     |
| Pancreas: Hemorrhage and inflammation           | 0     | 0     | 1     | 0     | 1     | 0     | 1     | 0     |
| Thymus: Hemorrhage                              | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     |

NE=not examined

-: No noteworthy finding or no change

**Table 4.4.2.1.2. Summary of Toxicity Findings in Surviving Male Rats Receiving DU-176b by Gavage for four Weeks**

| Daily Dose (mg/kg/day):        | 0     | 6     | 12    | 18    |
|--------------------------------|-------|-------|-------|-------|
| No. of Animals:                | M: 10 | M: 10 | M: 10 | M: 10 |
| <b>Toxicokinetics</b>          |       |       |       |       |
| C <sub>max</sub> (ng/mL)       |       |       |       |       |
| Day 1                          | NE    | 105   | 288   | 504   |
| Day 28                         | NE    | 279   | 717   | 1365  |
| AUC <sub>0-24h</sub> (ng·h/mL) |       |       |       |       |
| Day 1                          | NE    | 1063  | 1810  | 2563  |
| Day 28                         | NE    | 1051  | 3619  | 5786  |
| <b>Noteworthy Findings</b>     |       |       |       |       |
| Died or Sacrificed Moribund    | 0     | 0     | 0     | 0     |
| Clinical Observation           | -     | -     | -     | -     |
| Body Weight                    | -     | -     | -     | -     |
| Food Consumption               | -     | -     | -     | -     |
| Hematology                     | -     | -     | -     | -     |
| Blood Biochemistry             | -     | -     | -     | -     |
| Organ Weight                   | -     | -     | -     | -     |
| Necropsy                       | -     | -     | -     | -     |
| Histopathology                 | -     | -     | -     | -     |

NE=not examined

-: No noteworthy finding or no change

**Table 4.4.2.1.3. Summary of Findings in Surviving Male and Female Rats Receiving DU-176b by Gavage for 26 Weeks**

| Daily Dose (mg/kg/day):        | 0              |       | 6     |                   | 18    |                   | 54    |                   |
|--------------------------------|----------------|-------|-------|-------------------|-------|-------------------|-------|-------------------|
| No. of Animals:                | M: 15          | F: 15 | M: 10 | F: 10             | M: 10 | F: 10             | M: 15 | F: 15             |
| <b>Toxicokinetics</b>          |                |       |       |                   |       |                   |       |                   |
| $C_{max}$ (ng/mL)              |                |       |       |                   |       |                   |       |                   |
| Day 1                          | NE             | NE    | 121   | 189               | 888   | 795               | 1708  | 2060              |
| Day 178                        | NE             | NE    | 248   | 680               | 808   | 792               | 1222  | 2490              |
| AUC <sub>0-24h</sub> (ng·h/mL) |                |       |       |                   |       |                   |       |                   |
| Day 1                          | NE             | NE    | 768   | 1080              | 2968  | 2514              | 6706  | 10243             |
| Day 178                        | NE             | NE    | 1053  | 2581              | 2624  | 2833              | 5459  | 9015              |
| <b>Noteworthy Findings</b>     |                |       |       |                   |       |                   |       |                   |
| Died or Moribund Sacrificed    | 1 <sup>a</sup> | 0     | 0     | 0                 | 0     | 0                 | 0     | 1 <sup>b</sup>    |
| Clinical Observations          | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Body Weight                    | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Food Consumption               | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Water Consumption              | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Ophthalmoscopy                 | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Urinalysis                     | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Hematology                     | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| HCT (%) (Day 184)              | 44.4           | 44.0  | 45.2  | 42.1              | 45.0  | 41.3 <sup>c</sup> | 44.4  | 42.7              |
| MCV (fL) (Day 184)             | 51.4           | 56.0  | 50.7  | 54.4 <sup>c</sup> | 52.1  | 54.7              | 54.0  | 54.1 <sup>c</sup> |
| Bone Marrow                    | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Blood Biochemistry             | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| Organ Weight                   | -              | -     | -     | -                 | -     | -                 | -     | -                 |
| <b>Necropsy</b>                |                |       |       |                   |       |                   |       |                   |
| Trachea: perforated            | 1 <sup>a</sup> | 0     | 0     | 0                 | 0     | 0                 | 0     | 0                 |
| Mottled lung                   | 0              | 0     | 0     | 0                 | 0     | 0                 | 0     | 1 <sup>b</sup>    |
| <b>Histopathology:</b>         |                |       |       |                   |       |                   |       |                   |
|                                | -              | -     | -     | -                 | -     | -                 | -     | -                 |

a: Unscheduled death (Day 101) from a dosing error

b: Unscheduled death (Day 163) from a probable dosing error

c: P<0.05, compared to the control group (Dunnett's test)

-: No noteworthy findings

NE=not examined

## APPENDIX 3 Sponsor's Summaries of Findings in Monkey Toxicology Studies

Table 4.4.2.2.2. Summary of Toxicity Findings in Cynomolgus Monkeys Receiving DU-176b by Gavage for up to four Weeks

| Daily Dose (mg/kg/day) :              | 0    |      | 10   |      | 30                |                   | 100               |                    |
|---------------------------------------|------|------|------|------|-------------------|-------------------|-------------------|--------------------|
| No. of Animals:                       | M: 4 | F: 4 | M: 4 | F: 4 | M: 4              | F: 4              | M: 4              | F: 4               |
| <b>Toxicokinetics</b>                 |      |      |      |      |                   |                   |                   |                    |
| <i>C</i> <sub>max</sub> (ng/mL)       |      |      |      |      |                   |                   |                   |                    |
| Day 1                                 | NE   | NE   | 397  | 457  | 722               | 688               | 1630              | 1646               |
| Day 27                                | NE   | NE   | 358  | 328  | 495               | 553               | 701               | 691 <sup>a</sup>   |
| AUC <sub>0-24h</sub> (ng·h/mL)        |      |      |      |      |                   |                   |                   |                    |
| Day 1                                 | NE   | NE   | 4061 | 4187 | 10051             | 6860              | 21206             | 23365              |
| Day 27                                | NE   | NE   | 3273 | 3269 | 5963              | 6213              | 11307             | 10229 <sup>a</sup> |
| <b>Noteworthy Findings</b>            |      |      |      |      |                   |                   |                   |                    |
| Died or Sacrificed Moribund           | 0    | 0    | 0    | 0    | 0                 | 0                 | 0                 | 2 <sup>b</sup>     |
| Clinical Observations:                |      |      |      |      |                   |                   |                   |                    |
| Red nasal discharge                   | 0    | 0    | 0    | 0    | 0                 | 0                 | 0                 | 1 <sup>c</sup>     |
| Red oral discharge                    | 0    | 0    | 0    | 0    | 0                 | 0                 | 0                 | 2 <sup>c</sup>     |
| Body Weight                           | -    | -    | -    | -    | -                 | -                 | -                 | -                  |
| Food Consumption                      | -    | -    | -    | -    | -                 | -                 | -                 | -                  |
| Ophthalmic Examinations               | -    | -    | -    | -    | -                 | -                 | -                 | -                  |
| ECG                                   | -    | -    | -    | -    | -                 | -                 | -                 | -                  |
| Urinalysis                            | -    | -    | -    | -    | -                 | -                 | -                 | -                  |
| Hematology:                           |      |      |      |      |                   |                   |                   |                    |
| PT (s) Day 27                         | 10.1 | 10.3 | 11.0 | 10.9 | 11.6 <sup>d</sup> | 11.8 <sup>d</sup> | 12.9 <sup>d</sup> | 13.9 <sup>a</sup>  |
| APTT (s) Day 27                       | 17.8 | 17.9 | 20.5 | 20.1 | 21.7              | 21.8              | 22.5 <sup>d</sup> | 22.3 <sup>a</sup>  |
| Fecal occult blood (Day 2)            | 0    | 0    | 0    | 0    | 0                 | 0                 | 0                 | 1                  |
| Necropsy:                             |      |      |      |      |                   |                   |                   |                    |
| Gastrointestinal tract: Dark material | 0    | 0    | 0    | 0    | 0                 | 0                 | 0                 | 2 <sup>c</sup>     |

| Daily Dose (mg/kg/day) :        | 0    |      | 10   |      | 30   |      | 100  |                |
|---------------------------------|------|------|------|------|------|------|------|----------------|
| No. of Animals:                 | M: 4 | F: 4           |
| Lung: Dark area                 | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Thymus: Dark area               | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Organ Weight                    | -    | -    | -    | -    | -    | -    | -    | -              |
| Histopathology:                 |      |      |      |      |      |      |      |                |
| Thyroid: Hemorrhage             | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Lung: Hemorrhage                | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Paratracheal tissue: Hemorrhage | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Heart: Hemorrhage               | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Thymus: Hemorrhage              | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1 <sup>c</sup> |
| Adrenal: Hemorrhage in cortex   | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0              |

a: N=2

b: Found dead (Day 27) and killed in moribund condition (Day 3)

c: For the animals that died on test or were moribund killed

d: P&lt;0.05, compared to the control value (Dunnett's test)

NE: not examined

-: No noteworthy findings or no changes relative to the control

**Table 4.4.2.2.3. Summary of Findings in Cynomolgus Monkeys Receiving DU-176b by Gavage for 13 Weeks**

| Daily Dose (mg/kg/day):        | 0    |      | 6                 |      | 18                |                   | 54                |                   |
|--------------------------------|------|------|-------------------|------|-------------------|-------------------|-------------------|-------------------|
| No. of Animals:                | M: 4 | F: 4 | M: 4              | F: 4 | M: 4              | F: 4              | M: 4              | F: 4              |
| <b>Toxicokinetics</b>          |      |      |                   |      |                   |                   |                   |                   |
| $C_{max}$ (ng/mL)              |      |      |                   |      |                   |                   |                   |                   |
| Day 1                          | NE   | NE   | 582               | 542  | 678               | 443               | 924               | 1090              |
| Day 91                         | NE   | NE   | 375               | 366  | 1070              | 472               | 658               | 761               |
| AUC <sub>0-24h</sub> (ng·h/mL) |      |      |                   |      |                   |                   |                   |                   |
| Day 1                          | NE   | NE   | 5306              | 4830 | 7242              | 4992              | 13142             | 16453             |
| Day 91                         | NE   | NE   | 3322              | 3326 | 10248             | 5540              | 10229             | 10168             |
| <b>Noteworthy Findings</b>     |      |      |                   |      |                   |                   |                   |                   |
| Died or Sacrificed Moribund    | 0    | 0    | 0                 | 0    | 0                 | 0                 | 0                 | 0                 |
| Clinical Observations:         | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Body Weight                    | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Food Consumption               | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Ophthalmic Examinations        | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Electrocardiograph             | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Urinalysis                     | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| <b>Hematology:</b>             |      |      |                   |      |                   |                   |                   |                   |
| PT (s) Day -7                  | 10.1 | 10.4 | 10.6              | 10.3 | 10.2              | 10.5              | 10.3              | 10.1              |
| Day 28                         | 9.7  | 10.1 | 10.3 <sup>a</sup> | 10.0 | 10.4              | 10.7              | 11.2 <sup>a</sup> | 11.5 <sup>a</sup> |
| Day 90                         | 9.9  | 10.5 | 10.4              | 10.4 | 11.8 <sup>a</sup> | 11.1              | 11.3 <sup>a</sup> | 11.2              |
| APTT (s) Day -7                | 17.4 | 16.7 | 17.5              | 16.9 | 18.7              | 17.6              | 16.8              | 16.4              |
| Day 28                         | 16.2 | 17.1 | 18.3              | 17.7 | 20.0 <sup>a</sup> | 20.4              | 19.2 <sup>a</sup> | 19.9              |
| Day 90                         | 16.8 | 18.0 | 18.0              | 19.0 | 21.8 <sup>a</sup> | 20.7 <sup>a</sup> | 19.7              | 21.1 <sup>a</sup> |
| Clinical Biochemistry          | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Organ Weight                   | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Necropsy                       | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |
| Histopathology                 | -    | -    | -                 | -    | -                 | -                 | -                 | -                 |

a: p &lt; 0.05, compared to the control (Dunnett's test)

-: No noteworthy findings

NE=not examined

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/s/

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Patricia P. Harlow  
6/22/2007 03:38:54 PM  
PHARMACOLOGIST

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6/22/2007 04:10:58 PM  
PHARMACOLOGIST

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

**PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION**

Application Number: 77254  
DARRTS Supporting SD 1654  
Document Number(s) and Submit date: 04/19/2013  
Date(s): Received date: 04/22/2013  
Product: DU-176b  
Proposed Indication: Anti-thrombotic for prevention of stroke and thromboembolic events in patients with non-valvular atrial fibrillation  
Sponsor: Daiichi Medical Research, Inc.  
Review Division: Cardiovascular and Renal Products  
Reviewer: Patricia P. Harlow, Ph.D.  
Review Completion Date: 6/03/2013

*Template Version: September 1, 2010 (Modified by DCRP: November 8, 2012)*

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Studies Not Reviewed:

None.

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## 1. EXECUTIVE SUMMARY

### 1.1. Introduction (and Clinical Rationale)

Edoxaban (DU-176), an inhibitor of the coagulation Factor Xa (FXa), is being developed as an antithrombotic/anticoagulant agent for multiple indications. Under IND 77254, DU-176 is proposed for the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation.

### 1.2 Brief Discussion of Nonclinical Findings

In a ferric chloride model of thrombosis in rats, DU-176b decreased thrombus weights even when administered 1 hour after initiation of thrombus formation.

A tissue distribution study was conducted in male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old following a single oral administration of [<sup>14</sup>C]-DU-176b using quantitative whole-body autoradiography. Concentrations of radioactivity in blood and tissues in infant and juvenile rats were much higher than that in juvenile and adult rats. Radioactivity was retained in eyeball and skin even at 168 h post-dose in infant and juvenile rats as well as adult rats, suggesting that DU-176b and/or its metabolites show high affinity for melanin tissues in infant, juvenile and adult rats.

A tissue distribution study was conducted in male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old following an intravenous administration of [<sup>14</sup>C]-D21-2393, a metabolite of DU-176, using quantitative whole-body autoradiography. Adult rats exhibited rapid elimination of D21-2393 radioactivity in the blood and tissues. However, the concentrations of radioactivity in the blood and tissues in infant and juvenile rats were higher than those in adult rats. Radioactivity was still present in the eyeball at 24 h post-dose in infant and juvenile pigmented rats indicating affinity of D21-2393 to melanin-containing tissues.

Transport studies using oocytes indicated that DU-176 is not a substrate of OATP1B, but D21-2393 is a substrate of OATP1B1. Transport studies using cells expressing various transporters showed that DU-176 is not a substrate for OAT1, OCT3, and OCT2. Furthermore, DU-176 is unlikely to inhibit OAT1, OAT3, OCT, OCT2, OATP1B1 and OATP1B3 in vivo, because the in vitro IC<sub>50</sub> values of DU-176 were greater than 50 μM. Studies with various inhibitors and with purified recombinant human carboxylesterase 1 (CES1) indicated that DU-176 is metabolized to D21-2393 in human liver by CES1.

In a repeated dose toxicity study, male and female Crl:CD(SD) juvenile rats received 0, 2, 6, and 20 mg/kg/day of DU-176b in 0.5 w/v% methylcellulose by oral gavage once daily for 7 weeks from postnatal day (PND) 4 to PND 49. The sponsor concluded that DU-176b did not induce any toxicologically significant effects on postnatal development and growth, organ development, skeletal development, or sexual maturation in juvenile animals. The NOAEL of DU-176b was 20 mg/kg in male and female juvenile rats. At the

NOAEL, the mean AUC<sub>(0-24h)</sub> of DU-176 was 63100 ng·h/mL on PND 4, 41450 ng·h/mL on PND 14, 8470 ng·h/mL on PND 21, and 4510 ng·h/mL on PND 49.

In a repeated dose toxicity study, male and female CrI:CD(SD) juvenile rats received 0, 20, 60, and 200 mg/kg/day of D21-2393 in 0.5 w/v% methylcellulose by oral gavage once daily for 7 weeks from postnatal day (PND) 4 to PND 49. The sponsor concluded that D21-2393 did not induce any toxicologically significant effects on postnatal development and growth, organ development, skeletal development, or sexual maturation in juvenile animals. The NOAEL of D21-2393 was 200 mg/kg in male and female juvenile rats. At the NOAEL, the mean AUC<sub>(0-24h)</sub> of D21-2393 was 38650 ng·h/mL on PND 4, 52550 ng·h/mL on PND 14, 5065 ng·h/mL on PND 21, and 787 ng·h/mL on PND 49.

### **1.3. Recommendations**

#### **1.3.1. Clinical Study Safe to Proceed?**

Not applicable. (No protocol was included in the supporting documents reviewed.)

#### **1.3.2. Additional Recommendations**

*Internal:* The purpose of this document is to summarize the distribution and toxicology studies in juvenile animals. The medical officer needs to be aware of the high distribution and retention of DU-176 and its metabolite D21-2393 to the eye and skin of infant and juvenile rats at higher levels than in adult rats. In addition, exposure to DU-176 was 14-fold higher in the infant than in the adult. However, the juvenile toxicology studies did not identify any significant effects, particularly in the eye, of either DU-176 or D21-2393.

*To the sponsor:* None

## **2. DRUG INFORMATION**

### **2.1. Drug**

Trade Name: Not available

Generic Name: Edoxaban

Code Name: DU-176b (D11-4176b)

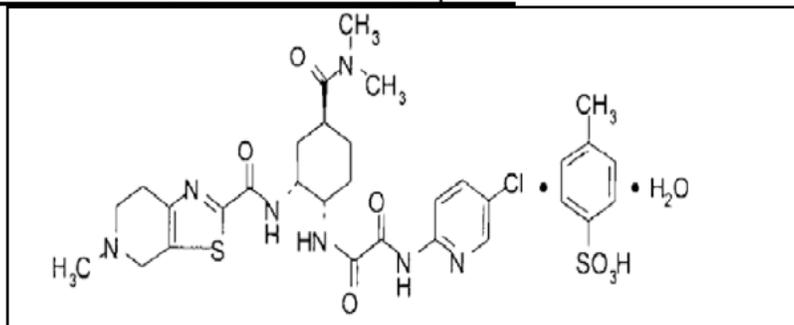
Chemical Name: N-(5-Chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(N,N-dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]ethanediamide p-toluenesulfonate monohydrate

CAS Registry Number: 480449-71-6

Molecular Formula: C<sub>24</sub>H<sub>30</sub>ClN<sub>7</sub>O<sub>4</sub>S, C<sub>7</sub>H<sub>8</sub>O<sub>3</sub>S•H<sub>2</sub>O

Molecular Weight: 738.27 (toluene sulfonate salt); 548.06 (anhydrous free base)

Structure or Biochemical Description:



Pharmacologic Class: Anti-thrombotic, FXa inhibitor

Route of Administration: Oral tablet

## 2.2. Relevant INDs, NDAs, BLAs and DMFs

IND 63, 266, DMF# (b) (4), DMF# (b) (4)

## 2.3. Drug Formulation

Clinical formulation: Tablets containing 40.4 mg DU-176b (30 mg DU-176). Excipients in the unlabeled drug product include (b) (4) mannitol, (b) (4) pregelatinized starch, crospovidone, magnesium stearate, hydroxypropylcellulose, (b) (4) (b) (4) hydroxypropylmethylcellulose (b) (4), talc, macrogol (b) (4), and titanium oxide, yellow ferric oxide, (b) (4)

## 2.4. Previous Clinical Experience

DU-176B has been administered to at least 1300 subjects in sixteen Phase 1 trials, three Phase 2 trials in patients undergoing orthopedic surgery and two Phase 2 trials in Japanese patients with atrial fibrillation. Doses up to a maximum of 120 mg qd or 60 mg bid were administered for 10 days in healthy subjects and patients undergoing hip-replacement surgery. The atrial fibrillation studies involved dose escalation (30 mg bid to 60 mg bid) over a period of ten weeks. In the Phase 2 trials the incidence of adverse effects on liver function was approximately 2%. Renal clearance was lower in elderly males than in young males resulting in a slightly higher exposure in the elderly (AUC of 3582 ng.hr/mL versus AUC of 2806 ng.hr/mL after 10 days of dosing).

Currently under IND 77254, >21,000 patients are being evaluated in a Phase 3, randomized, double-blind, double-dummy, parallel group, multi-center, multinational study for evaluation of efficacy and safety of DU-176b versus warfarin in subjects with atrial fibrillation (Study DU176b-C-U301, ENGAGE AF-TIMI 48). Under IND 63266, the target enrollment is 7500 patients in a Phase 3, randomized, double-blind, double-dummy, parallel-group, multi-center, multi-national study for evaluation of efficacy and safety of (LMW) heparin/edoxaban versus (LMW) heparin/warfarin in subjects with symptomatic deep-vein thrombosis and/or pulmonary embolism.

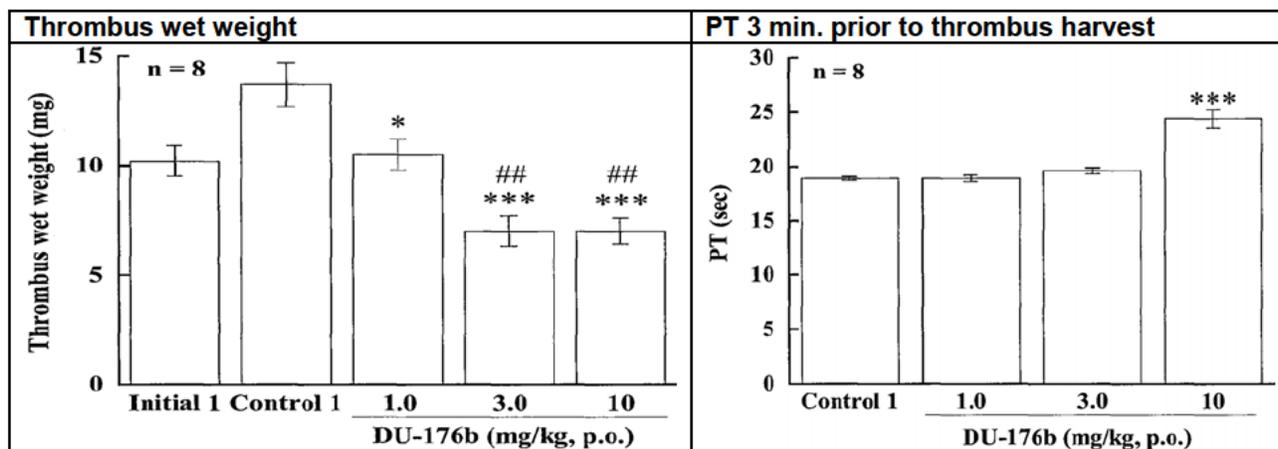
### 3. Pharmacology

#### 3.1. Primary Pharmacology

##### **3.1.1. Treatment of venous thrombosis with DU-176b, enoxaparin and fondaparinux in rats (Study BG11-H0012-R01)**

The effects of DU-176b, enoxaparin and fondaparinux on venous thrombosis were determined in three experiments using a ferric chloride model of thrombosis in rats. Venous thrombosis in the inferior vena cava was induced by partial stenosis plus topical application of 10% ferric chloride solution. After 1 h thrombus maturation, the thrombus wet weights of the initial group of rats (n = 8) were measured. The rats in the remaining four groups were treated with the appropriate vehicle (0.5 w/v% methyl cellulose 400 solution for DU-176b and saline for enoxaparin and fondaparinux) or DU-176b (1.0, 3.0 and 10 mg/kg, p.o.), enoxaparin (300, 1000 and 2000 IU/kg, s.c.), or fondaparinux (0.30, 1.0 and 3.0 mg/kg, s.c.) beginning 1 h after thrombus induction. The thrombi were harvested 3 h later and the wet weights were determined. Blood was collected from the jugular vein 3 min before the thrombus removal, and plasma was prepared for determination of prothrombin time (PT) and/or activated partial thromboplastin time (APTT).

Administration of DU-176b (1.0, 3.0 and 10 mg/kg) 1 h after thrombus formation statistically significantly reduced the thrombus wet weight compared with the control group in a dose-dependent manner. DU-176b (3.0 and 10 mg/kg) also statistically significantly reduced the thrombus weight compared with the initial group. DU-176b statistically significantly prolonged PT at 10 mg/kg.

**Figure 1: Sponsor's Figures for Edoxaban – Study BG11-H0012-R01**

Enoxaparin (300, 1000 and 2000 IU/kg, s.c.) statistically significantly and dose-dependently reduced the thrombus weight compared with the control group. Enoxaparin (1000 and 2000 IU/kg, s.c.) statistically significantly prolonged APTT. Fondaparinux (0.30, 1.0 and 3.0 mg/kg, s.c.) statistically significantly and dose-dependently reduced the thrombus weight compared with the control group and the initial group. However, fondaparinux (0:30, 1.0 and 3.0 mg/kg, s.c.) did not prolong either PT or APTT.

#### 4. Pharmacokinetics/ADME

##### 4.1. Distribution

##### 4.1.1 Quantitative Whole-body Autoradiography after a Single Oral Administration of <sup>14</sup>C-DU-176b to Juvenile and Adult Rats (AM10-C0055-R01)

Conducting laboratory and location: [REDACTED] (b) (4)

Study number(s): B100897 (AM10-C0055-R01)

Date of study initiation: 2/15/2011

Drug lot/batch number: [<sup>14</sup>C]-DU176b, CP-3679, 3.0 MBq/mg

GLP compliance: No

QA statement: Yes

##### Key Study Findings

This tissue distribution study was conducted in male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old following a single oral administration of [<sup>14</sup>C]-DU-176b (4 mg/kg) using quantitative whole-body autoradiography. Concentrations of radioactivity in blood and tissues in infant and juvenile rats were much higher than those in juvenile and adult rats. Radioactivity was retained in eyeball and skin even at 168 h post-dose in infant and

juvenile rats as well as in adult rats, suggesting that DU-176b and/or its metabolites show high affinity for melanin tissues in infant, juvenile and adult rats

### Methods

Male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old received a single oral administration of [ $^{14}\text{C}$ ]-DU-176b (4 mg/kg) in an unspecified formulation at a target dose of 4 mg/kg body weight. One infant, juvenile and adult Wistar rat per time point was euthanized at 1 h, 4 h, 24 hr, and 168 hr post-dose and one infant, juvenile and adult Brown Norway rat per time point was euthanized at 24 and 168 hr post-dose. The rats were embedded and sectioned for quantitative whole-body autoradiography. Selected sections were exposed to phosphorimaging screens, and the concentrations of radioactivity in selected tissues from the whole body autoradiograms were quantified using a validated image analysis system. Concentrations of radioactivity were interpolated from each standard curve as nanocurie per gram (nCi/g) and then converted to nanogram equivalent per gram (ng equiv/g) using the specific activity of [ $^{14}\text{C}$ ]-DU-176b. The LLOQ was 31.4 ng equiv/g.

### Results

In adult albino rats, at 1 h post-dose, the highest concentration of [ $^{14}\text{C}$ ]-DU-176b radioactivity was in the liver followed by the kidney, spleen, and adrenal gland (Table 1). The concentrations of radioactivity in brain, eyeball, testis, skin, skeletal muscle, white adipose tissue, and brown adipose tissue were lower than that in blood. At 4 h post-dose, the highest concentration of radioactivity was in the kidney, followed by the skin, liver, and adrenal gland. The concentration of radioactivity in the brain, eyeball, skeletal muscle, white adipose tissue, and the brown adipose tissue were lower than that in blood. At 24 h post-dose, radioactivity was detected in the skin, but not in other tissues. At 168 h post-dose, the radioactivity was not detected in any tissue.

In adult pigmented rats at 24 h post-dose, the highest concentration of [ $^{14}\text{C}$ ]-DU-176b radioactivity was detected in the eyeball, followed by the skin. The radioactivity was not detected in other tissues. At 168 h post-dose, the radioactivity concentration was still detected in the eyeball, but not in other tissues.

In juvenile albino rats at 1 h post-dose, the highest concentration of [ $^{14}\text{C}$ ]-DU-176b radioactivity was in the liver followed by the kidney, adrenal, and spleen. The concentrations of radioactivity in the brain, eyeball, testis, skin, skeletal muscle, white adipose tissue, and brown adipose tissue were lower than that in blood. At 4 h post-dose, the highest concentration of radioactivity was in the liver, followed by the kidney, adrenal gland, and spleen. The concentrations of radioactivity in the brain, eyeball, skin, skeletal muscle, white adipose tissue, and brown adipose tissue were lower than that in blood. At 24 and 168 h post-dose, radioactivity was not detected in any tissue.

In juvenile pigmented rats at 24 h post-dose, [ $^{14}\text{C}$ ]-DU-176b radioactivity was detected in the eyeball, but not in other tissues. At 168 h post-dose, radioactivity was still present in the eyeball and not in other tissues.

In infant albino rats at 1 h post-dose, the highest concentration of [ $^{14}\text{C}$ ]-DU-176b radioactivity was in the kidney followed by the liver, adrenal, and spleen. The concentrations of radioactivity in the brain, eyeball, testis, skin, skeletal muscle, and

white adipose tissue were lower than that in blood. At 4 h post-dose, the highest concentration of radioactivity was in the liver followed by adrenal gland, kidney, and spleen. The concentrations of radioactivity in the brain, eyeball, lung, and white adipose tissue were lower than that in blood. At 24 h post-dose, the highest concentrations of radioactivity were in the kidney, adrenal gland, liver, and spleen. The concentrations of radioactivity in the brain, eyeball, and white adipose tissue were lower than that in blood. At 168 h post-dose, radioactivity was detected in the kidney, but not in other tissues.

In infant pigmented rats at 24 h post-dose, the highest concentrations of [<sup>14</sup>C]-DU-176b radioactivity were in the eyeball and skin at levels 339-fold and 62-fold higher, respectively, than that in the blood. The concentrations of radioactivity in brain and white adipose tissue were lower than that in blood. At 168 h post-dose, radioactivity was detected only in the eyeball and skin, but not in the other tissues.

**Table 1: Sponsor's Summaries of [<sup>14</sup>C]-DU-176b Tissue Distribution**

| Adult                | Tissue      | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>b</sub> ) |             |             |             |                |             |
|----------------------|-------------|--|-------------|-------------|-------------|----------------|-------------|
|                      |             | Albino rats <sup>*1</sup>  |             |             |             | Pigmented rats |             |
|                      |             | 1 h  | 4 h         | 24 h        | 168 h       | 24 h           | 168 h       |
| Blood                | 275 (1.00)  | 19.9 (1.00)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Brain                | BLQ (N.C.)  | BLQ (N.C.)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Eyeball              | BLQ (N.C.)  | BLQ (N.C.)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | 889 (N.C.)     | 712 (N.C.)  |
| Heart                | 303 (1.10)  | 38.5 (1.93)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Lung                 | 353 (1.28)  | 52.4 (2.63)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Liver                | 922 (3.35)  | 129 (6.48)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Adrenal              | 569 (2.07)  | 80.6 (4.05)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Kidney               | 822 (2.99)  | 195 (9.80)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Spleen               | 628 (2.28)  | 76.7 (3.85)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Testis               | 107 (0.39)  | 45.1 (2.27)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Skin                 | 261 (0.95)  | 134 (6.73)   | 40.3 (N.C.) | N.A. (N.C.) | N.A. (N.C.) | 124 (N.C.)     | N.A. (N.C.) |
| Skeletal muscle      | 244 (0.89)  | N.A. (N.C.)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| White adipose tissue | 78.5 (0.29) | N.A. (N.C.)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Brown adipose tissue | 226 (0.82)  | N.A. (N.C.)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |

| Juvenile  | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, $K_b$ ) |                           |             |             |                |                              |             |             |             |  |
|---|---|---------------------------|-------------|-------------|----------------|------------------------------|-------------|-------------|-------------|--|
|   | Tissue  | Albino rats <sup>*1</sup> |             |             |                | Pigmented rats               |             |             |             |  |
|   |   | 1 h                       | 4 h         | 24 h        | 168 h          | 24 h                         | 168 h       |             |             |  |
| Blood   | 251 (1.00)  | 165 (1.00)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Brain   | BLQ (N.C.)  | BLQ (N.C.)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Eyeball   | 52.3 (0.21)   | 71.0 (0.43)               | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | 2760 (N.C.)                  | 974 (N.C.)  |             |             |  |
| Heart   | 257 (1.02)  | 165 (1.00)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Lung  | 327 (1.30)  | 212 (1.28)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Liver   | 724 (2.88)  | 515 (3.12)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Adrenal   | 576 (2.29)  | 273 (1.65)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Kidney  | 590 (2.35)  | 411 (2.49)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Spleen  | 473 (1.88)  | 259 (1.57)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Testis  | 139 (0.55)  | 199 (1.21)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Skin  | 176 (0.70)  | 134 (0.81)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Skeletal muscle   | 220 (0.88)  | 143 (0.87)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| White adipose tissue  | 106 (0.42)  | 39.5 (0.24)               | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Brown adipose tissue  | 216 (0.86)  | 132 (0.80)                | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) |  |
| Infant  | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, $K_b$ ) |                           |             |             |                |                              |             |             |             |  |
|   | Tissue  | Albino rats <sup>*1</sup> |             |             |                | Pigmented rats <sup>*2</sup> |             |             |             |  |
|   |   | 1 h                       | 4 h         | 24 h        | 168 h          | 24 h                         | 168 h       |             |             |  |
| Blood   | 2050 (1.00)   | 1850 (1.00)               | 166 (1.00)  | N.A. (N.C.) | 62.8 (1.00)    | N.A. (N.C.)                  |             |             |             |  |
| Brain   | 139 (0.07)  | 162 (0.09)                | BLQ (N.C.)  | N.A. (N.C.) | BLQ (N.C.)     | N.A. (N.C.)                  |             |             |             |  |
| Eyeball   | 471 (0.23)  | 951 (0.51)                | 136 (0.82)  | N.A. (N.C.) | 21300 (339.17) | 3030 (N.C.)                  |             |             |             |  |
| Heart   | 2290 (1.12)   | 2010 (1.09)               | 186 (1.12)  | N.A. (N.C.) | 110 (1.75)     | N.A. (N.C.)                  |             |             |             |  |
| Lung  | 2140 (1.04)   | 1580 (0.85)               | 166 (1.00)  | N.A. (N.C.) | 93.6 (1.49)    | N.A. (N.C.)                  |             |             |             |  |
| Liver   | 5850 (2.85)   | 4110 (2.22)               | 403 (2.43)  | N.A. (N.C.) | 197 (3.14)     | N.A. (N.C.)                  |             |             |             |  |
| Adrenal   | 3820 (1.86)   | 3500 (1.89)               | 431 (2.60)  | N.A. (N.C.) | 143 (2.28)     | N.A. (N.C.)                  |             |             |             |  |
| Kidney  | 6840 (3.34)   | 3180 (1.72)               | 451 (2.72)  | 16.5 (N.C.) | 262 (4.17)     | BLQ (N.C.)                   |             |             |             |  |
| Spleen  | 3240 (1.58)   | 2780 (1.50)               | 255 (1.54)  | N.A. (N.C.) | 157 (2.50)     | N.A. (N.C.)                  |             |             |             |  |
| Testis  | 1430 (0.70)   | 1970 (1.06)               | 204 (1.23)  | N.A. (N.C.) | 97.0 (1.54)    | BLQ (N.C.)                   |             |             |             |  |
| Skin  | 1470 (0.72)   | 1950 (1.05)               | 205 (1.23)  | BLQ (N.C.)  | 3880 (61.78)   | 1700 (N.C.)                  |             |             |             |  |
| Skeletal muscle   | 1790 (0.87)   | 1960 (1.06)               | 172 (1.04)  | N.A. (N.C.) | 97.3 (1.55)    | N.A. (N.C.)                  |             |             |             |  |
| White adipose tissue  | 604 (0.29)  | 678 (0.37)                | 49.9 (0.30) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.)                  |             |             |             |  |
| Brown adipose tissue  | 2240 (1.09)   | 2590 (1.40)               | 222 (1.34)  | N.A. (N.C.) | 117 (1.86)     | N.A. (N.C.)                  |             |             |             |  |
| <p>Values in parentheses are expressed as the ratio of the tissue concentration to the blood concentration.</p> <p>When the radioactivity was not detected by visual observation, the radioactivity was judged as not quantifiable and was not subjected to the calculation and was expressed as N.A. (not applicable).</p> <p>When the radioactivity concentrations in the blood and/or tissue were N.A. or AUQ or BLQ, the tissue/blood ratio were not calculated and are shown as N.C. (not calculated).</p> <p>*1: BLQ &lt; 31.4 ng eq./g</p> <p>*2: BLQ &lt; 30.9 ng eq./g</p> |   |                           |             |             |                |                              |             |             |             |  |

#### **4.1.2. Quantitative Whole-body Autoradiography after a Single Intravenous Administration of [<sup>14</sup>C]-D21-2393 to Juvenile and Adult Rats**

Conducting laboratory and location: [REDACTED]

(b) (4)

Study number(s): B100896 (AM10-C0056-R01)

Date of study initiation: 1/1/2011

Drug lot/batch number: [<sup>14</sup>C]-D21-2393, CP-3785, 4.1 MBq/mg

GLP compliance: No

QA statement: Yes

##### **Key Study Findings**

This tissue distribution study was conducted in male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old following a intravenous administration of [<sup>14</sup>C]-D21-2393 using quantitative whole-body autoradiography. Adult rats exhibited rapid elimination of radioactivity in the blood and tissues. However, the concentrations of radioactivity in the blood and tissues in infant and juvenile rats were higher than those in adult rats. The radioactivity was still present in the eyeball at 24 h post-dose in infant and juvenile pigmented rats indicating affinity of D21-2393 to melanin-containing tissues.

##### **Methods**

Male Brown Norway pigmented rats and male Wistar rats that were 4 days (infant), 3 weeks (juvenile), and 6 weeks (adult) old received a single intravenous administration of [<sup>14</sup>C]-D21-2393 in an unspecified formulation at a target dose of 1 mg/kg body weight. One infant, juvenile and adult Wistar rat per time point was euthanized at 0.25, h, 2 h, 24 h, and 168 hr post-dose and one infant, juvenile and adult Brown Norway rat per time point was euthanized at 2 h and 24 h post-dose. The rats were embedded and sectioned for quantitative whole-body autoradiography. Selected sections were exposed to phosphorimaging screens, and the concentrations of radioactivity in selected tissues from the whole body autoradiograms were quantified using a validated image analysis system. Concentrations of radioactivity were interpolated from each standard curve as nanocurie per gram (nCi/g) and then converted to nanogram equivalent per gram (ng equiv/g) using the specific activity of [<sup>14</sup>C]-D21-2393. The LLOQ was 9.9 ng equiv/g.

##### **Results**

In adult albino rats at 15 min post-dose, the highest concentrations of [<sup>14</sup>C]-D21-2393 radioactivity were detected in the liver followed by the kidney (Table 2). Both tissues had concentrations higher than that in the blood. The concentrations of radioactivity in other tissues were lower than that in the blood. Although radioactivity was detected in the gastrointestinal contents at 2 h post-dose, the radioactivity was not detected in blood and all tissues, indicating rapid elimination of [<sup>14</sup>C]-D21-2393.

In adult pigmented rats, a low level of [<sup>14</sup>C]-D21-2393 radioactivity was detected at 2 h post-dose only in the eyeball, and not in the other tissues. At 24 h post-dose, radioactivity was not detected in any tissue including skin and eyeball.

In juvenile albino rats at 15 min post-dose, the concentration of [<sup>14</sup>C]-D21-2393 radioactivity in blood was 3.5-fold higher than that of adult rats. Concentrations of radioactivity in tissues of juvenile albino rats were higher than those of adult rats. The highest concentrations of radioactivity were in the liver and kidney. The concentrations of radioactivity in other tissues were lower than that in the blood. Although radioactivity was detected in the gastrointestinal contents at 2 h post-dose, radioactivity was not detected in other tissues, indicating rapid elimination of [<sup>14</sup>C]-D21-2393 from body. At 24 h and 168 h post-dose, radioactivity was not detected in any tissue.

In juvenile pigmented rats at 2 h post-dose, the highest concentrations of [<sup>14</sup>C]-D21-2393 radioactivity were detected in the eyeball followed by the liver. Radioactivity was not detected in the blood and other tissues. At 24 h post-dose, the radioactivity was still present in the eyeball, but not in the other tissues.

In infant albino rats at 15 min post-dose, the concentration of [<sup>14</sup>C]-D21-2393 radioactivity in blood was 13-fold higher than that in adult rats. Concentrations of radioactivity in other tissues in infant rats were higher than those of adult rats. The highest concentrations of radioactivity were in the liver and kidney. The concentrations of radioactivity in other tissues were lower than that in the blood. At 2 h post-dose, the highest concentrations of radioactivity were in the kidney and the liver. The concentrations of radioactivity in the other tissues were lower than that in the blood. Although radioactivity was still observed in the gastrointestinal contents at 24 h post-dose, radioactivity was not detected in other tissues. At 168 h post-dose, radioactivity was not detected in any tissue.

In infant pigmented rats at 2 h post-dose, the highest concentrations of [<sup>14</sup>C]-D21-2393 radioactivity were detected in the liver and blood. At 24 h post-dose, radioactivity was still present in the eyeball, the kidney, and the skin, but not in the other tissues.

**Table 2: Sponsor's Summaries of [<sup>14</sup>C]-D21-2393 Tissue Distribution**

| Adult                | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>b</sub> ) |             |             |             |             |                |             |  |
|----------------------|--|-------------|-------------|-------------|-------------|----------------|-------------|--|
|                      | Tissue   | Albino rats |             |             |             | Pigmented rats |             |  |
|                      |  | 15 min      | 2 h         | 24 h        | 168 h       | 2 h            | 24 h        |  |
| Blood                | 152 (1.00)   | N.A. (N.C.)    | N.A. (N.C.) |  |
| Brain                | BLQ (N.C.)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |  |
| Eyeball              | 20.5 (0.13)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | 13.3 (N.C.)    | BLQ (N.C.)  |  |
| Heart                | 56.0 (0.37)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Lung                 | 126 (0.83)   | N.A. (N.C.)    | N.A. (N.C.) |  |
| Liver                | 729 (4.80)   | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | BLQ (N.C.)     | N.A. (N.C.) |  |
| Adrenal              | 95.6 (0.63)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Kidney               | 369 (2.43)   | N.A. (N.C.)    | N.A. (N.C.) |  |
| Spleen               | 49.0 (0.32)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Testis               | 94.8 (0.62)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Skin                 | 85.7 (0.56)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Skeletal muscle      | 36.9 (0.24)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| White adipose tissue | 19.9 (0.13)  | N.A. (N.C.)    | N.A. (N.C.) |  |
| Brown adipose tissue | 70.3 (0.46)  | N.A. (N.C.)    | N.A. (N.C.) |  |

| Juvenile   | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>t</sub> ) |             |             |             |             |                |             |
|--|--|-------------|-------------|-------------|-------------|----------------|-------------|
|  | Tissue   | Albino rats |             |             |             | Pigmented rats |             |
|  |  | 15 min      | 2 h         | 24 h        | 168 h       | 2 h            | 24 h        |
| Blood  | 539 (1.00)   | N.A. (N.C.)    | N.A. (N.C.) |
| Brain  | 3.68 (N.C.)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    | N.A. (N.C.) |
| Eyeball  | 36.6 (0.07)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | 23.4 (N.C.)    | 10.4 (N.C.) |
| Heart  | 153 (0.28)   | N.A. (N.C.)    | N.A. (N.C.) |
| Lung   | 405 (0.75)   | N.A. (N.C.)    | N.A. (N.C.) |
| Liver  | 1840 (3.41)  | BLQ (N.C.)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | 8.23 (N.C.)    | N.A. (N.C.) |
| Adrenal  | 231 (0.43)   | N.A. (N.C.)    | N.A. (N.C.) |
| Kidney   | 603 (1.12)   | N.A. (N.C.)    | N.A. (N.C.) |
| Spleen   | 92.4 (0.17)  | N.A. (N.C.)    | N.A. (N.C.) |
| Testis   | 171 (0.32)   | N.A. (N.C.)    | N.A. (N.C.) |
| Skin   | 218 (0.40)   | N.A. (N.C.)    | N.A. (N.C.) |
| Skeletal muscle  | 135 (0.25)   | N.A. (N.C.)    | N.A. (N.C.) |
| White adipose tissue   | 39.8 (0.07)  | N.A. (N.C.)    | N.A. (N.C.) |
| Brown adipose tissue   | 140 (0.26)   | N.A. (N.C.)    | N.A. (N.C.) |
| Infant   | Radioactivity concentration (ng eq./g) (Tissue/blood ratio, K <sub>t</sub> ) |             |             |             |             |                |             |
|  | Tissue   | Albino rats |             |             |             | Pigmented rats |             |
|  |  | 15 min      | 2 h         | 24 h        | 168 h       | 2 h            | 24 h        |
| Blood  | 1990 (1.00)  | 567 (1.00)  | N.A. (N.C.) | N.A. (N.C.) | 874 (1.00)  | N.A. (N.C.)    |             |
| Brain  | 44.7 (0.02)  | 45.0 (0.08) | N.A. (N.C.) | N.A. (N.C.) | 72.1 (0.08) | N.A. (N.C.)    |             |
| Eyeball  | 479 (0.24)   | 280 (0.49)  | N.A. (N.C.) | N.A. (N.C.) | 405 (0.46)  | 120 (N.C.)     |             |
| Heart  | 651 (0.33)   | 189 (0.33)  | N.A. (N.C.) | N.A. (N.C.) | 352 (0.40)  | N.A. (N.C.)    |             |
| Lung   | 1450 (0.73)  | 281 (0.50)  | N.A. (N.C.) | N.A. (N.C.) | 502 (0.57)  | N.A. (N.C.)    |             |
| Liver  | 2570 (1.29)  | 765 (1.35)  | N.A. (N.C.) | N.A. (N.C.) | 1100 (1.26) | N.A. (N.C.)    |             |
| Adrenal  | 1110 (0.56)  | 222 (0.39)  | N.A. (N.C.) | N.A. (N.C.) | 364 (0.42)  | N.A. (N.C.)    |             |
| Kidney   | 2420 (1.22)  | 2920 (5.15) | N.A. (N.C.) | N.A. (N.C.) | 735 (0.84)  | 17.3 (N.C.)    |             |
| Spleen   | 402 (0.20)   | 128 (0.23)  | N.A. (N.C.) | N.A. (N.C.) | 171 (0.20)  | N.A. (N.C.)    |             |
| Testis   | 986 (0.50)   | 393 (0.69)  | N.A. (N.C.) | N.A. (N.C.) | 671 (0.77)  | N.A. (N.C.)    |             |
| Skin   | 1160 (0.58)  | 316 (0.56)  | N.A. (N.C.) | N.A. (N.C.) | 535 (0.61)  | 11.4 (N.C.)    |             |
| Skeletal muscle  | 1110 (0.56)  | 282 (0.50)  | N.A. (N.C.) | N.A. (N.C.) | 474 (0.54)  | N.A. (N.C.)    |             |
| White adipose tissue   | 561 (0.28)   | 131 (0.23)  | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.) | N.A. (N.C.)    |             |
| Brown adipose tissue   | 845 (0.42)   | 255 (0.45)  | N.A. (N.C.) | N.A. (N.C.) | 472 (0.54)  | N.A. (N.C.)    |             |
| <p>Values in parentheses are expressed as the ratio of the tissue concentration to the blood concentration.</p> <p>When the radioactivity was not detected by visual observation, the radioactivity was judged as not quantifiable and was not subjected to the calculation and was expressed as N.A. (not applicable).</p> <p>When the radioactivity concentrations in the blood and/or tissue were N.A. or BLQ, the tissue/blood ratio were not calculated and are shown as N.C. (not calculated).</p> <p>BLQ &lt; 9.92 ng eq./g</p> |  |             |             |             |             |                |             |

#### 4.2. Metabolism

#### **4.2.1. DU-176b: Transport study of DU-176 and D21-2393 using OATP1B1-expressing oocytes (AM10-C0061-R01)**

OATP1B1-mediated transport of DU-176 (free base of DU-176b) and D21-2393 (a metabolite of DU-176) was examined using OATP1B1-expressing *Xenopus laevis* oocytes (abbreviated to oocytes).

The uptake clearance of [<sup>14</sup>C] DU-176 into OATP1B1-expressing oocytes was similar to the uptake clearance into control oocytes. Uptake clearance of [<sup>14</sup>C] DU-176 into OATP1B1-expressing oocytes was not significantly altered in the presence of estrone sulfate (ES), an inhibitor of OATP1B1. These results indicate that DU-176 is not a substrate of OATP1B1.

In contrast to [<sup>14</sup>C]-DU-176, the uptake clearance of [<sup>14</sup>C]-D21-2393 into OATP1B1-expressing oocytes was significantly higher than that into control oocytes. In addition, the uptake transport of [<sup>14</sup>C]-D21-2393 into OATP1B1-expressing oocytes was significantly inhibited by the presence of ES. These results indicate that D21-2393 is a substrate of OATP1B1.

#### **4.2.2 Transport study of DU-176 via human OAT1, OAT3 and OCT2 using their expressing S2 cells (AM10-C0129- R01)**

The transport of DU-176 by human Organic Anion Transporters (OAT) OAT1 and OAT3 and human Organic Cation Transporter (OCT) OCT 2 was evaluated using uptake of [<sup>14</sup>C]-DU-176 into S2 cells expressing transporter genes for OAT1, OAT3 and OCT2. The transport of typical substrates ([<sup>3</sup>H]-aminohippuric acid for OAT1, [<sup>3</sup>H]-estrone 3-sulfate for OAT3 and [<sup>14</sup>C]-metformin for OCT2) and the inhibition of transport by typical inhibitors (probenecid for OAT1 and OAT3, quinidine for OCT2) confirmed the expression of each transporter in its transporter-expressing cell line. Comparison of the uptake clearance of [<sup>14</sup>C]-DU-176 into transporter-expressing cells with uptake clearance into control cells did not indicate OAT1-, OAT3- and OCT2- mediated transport of [<sup>14</sup>C]-DU-176. These results suggest that DU-176 is not a substrate for OAT1, OCT3, and OCT2.

#### **4.2.3. Inhibitory potential of DU-176 on OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 using their expressing cells (AM10-C0140-R01)**

Inhibitory effects of DU-176 (free form of DU-176b) on uptake transport activities of human Organic Anion Transporters (OAT) OAT1 and OAT3; Organic Cation Transporters (OCT) OCT1 and, OCT2; Organic Anion Transporting Polypeptide (OATP) OATP1B1 and OATP1B3 were evaluated using cells that express these transporters. The IC<sub>50</sub> values of DU-176 were >100 μM for OAT1, OAT3, OCT1 and OCT2. The IC<sub>50</sub> values of DU-176 were 62.7 μM and 50.8 μM for OATP1B1 and OATP1B3, respectively.

#### **4.2.4 DU-176 Hydrolase Activities to Produce D21-2393 in Human Liver Subcellular Fractions and Purified Human CES 1 Enzyme, and Effects of Inhibitors on the Metabolite Production**

The enzymes involved in the hydrolysis of DU-176 (free form of DU-176b) to D21-2393 were investigated using pooled human liver microsomes and cytosol in the absence and presence of inhibitors, and using purified recombinant human carboxylesterase 1 (CES1). The production of D21-2393 from DU-176 was quantified by an LC/MS/MS analytical method validated in this study.

The formation of D21-2393 from DU-176 was observed with both human liver microsomes and cytosol with the activity in human liver microsomes being higher than that of liver cytosol. The hydrolysis of DU-176 to D21-2393 in human liver microsomes was strongly inhibited by diisopropyl fluorophosphates (a serine protease inhibitor) and bis (4-nitrophenyl) phosphate (BNPP, a carboxylesterase inhibitor). However, only weak inhibition was observed in the presence of 5, 5'-dithiobis (2-nitrobenzoic acid) (an SH-modifying reagent) and eserine (a cholinesterase inhibitor). The hydrolysis of DU-176 to D21-2393 in human liver cytosol was also strongly inhibited by BNPP. In addition, purified CES1 catalyzed the formation of D21-2393 from DU-176. These results demonstrate that DU-176 is metabolized to D21-2393 in human liver by CES1.

## 5. General Toxicology

### 5.1. Repeat-Dose Toxicity

#### **5.1.1 Repeated Dose Toxicity Study in Juvenile Rats Treated Orally with DU-176b for 7 Weeks**

Conducting laboratory and location: [REDACTED] (b) (4)

Study number(s): SBL314-562 (AN11-C0004-R01)

Date of study initiation: 6/24/2011

Drug lot/batch number: DU-176b, MH409-U, purity 100.6%

GLP compliance: Yes

QA statement: Yes

#### Key Study Findings

In a repeated dose toxicity study, male and female Crl:CD(SD) juvenile rats received 0, 2, 6, and 20 mg/kg/day of DU-176b in 0.5 w/v% methylcellulose by oral gavage once daily for 7 weeks from postnatal day (PND) 4 to PND 49. The sponsor concluded that DU-176b did not induce any toxicologically significant effects on postnatal development and growth, organ development, skeletal development, or sexual maturation in juvenile animals. The NOAEL of DU-176b was 20 mg/kg in male and female juvenile rats. At the NOAEL, the mean AUC<sub>(0-24h)</sub> of DU-176 was 63100 ng·h/mL on PND 4, 41450 ng·h/mL on PND 14, 8470 ng·h/mL on PND 21, and 4510 ng·h/mL on PND 49.

#### Purpose

A repeated dose toxicity study was conducted in male and female Crl:CD(SD) juvenile rats to investigate the toxic effects of DU-176b administration on organ development after the completion of dosing and when animals reached maturity.

### Methods

A solution of DU-176b in 0.5 w/v% methylcellulose was administered orally by gavage once daily to Crl:CD(SD) rats (16 rats/sex/group) for 7 weeks from postnatal day (PND) 4 to PND 49 at dose levels of 0, 2, 6, and 20 mg/kg/day. Animals were sacrificed for examinations on PND 50 (10 rats/sex/group) and PND 91 (6 rats/sex/group). Blood samples were drawn from the satellite groups (3 rats/sex/group/sampling point) for analysis of plasma concentrations of DU-176.

### Results

#### Mortality/ clinical signs:

No animal died, and no abnormal changes were observed in males or females in any group during the study period.

#### Body weight:

In the high dose group, significantly lower body weight was observed in males on PND 17 and in females on PNDs 10, 14, 17, 21, 24, and 28 compared with the control group.

#### Food consumption:

Significantly lower food consumption was observed in the high dose females on PNDs 21 to 22 when compared with the control group.

#### Functional and Physical Development:

No significant differences were noted in functional developmental parameters (air righting reflex, pupillary reflex, Preyer's reflex, and pain response) or physical developmental parameters (incisor eruption and eyelid opening) in males or females between the control group and any test article group. The reviewer notes that extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted.

#### Morphological Differentiation of External Genitalia;

No significant differences were noted in the cleavage of the balano-preputial gland or vaginal opening between the control group and any test article group. The reviewer notes that the fertility, mating ability and reproductive performance were not specifically evaluated.

#### Hematology:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91. Although the sponsor noted high monocyte counts in females in the mid and high dose groups on PND Day 50, these values did not exhibit a dose relationship. Also, the sponsor noted low reticulocyte ratio in females in the 20 mg/kg group on PND 91, but maintained these changes were within the normal background range. The remaining significant changes did not exhibit a dose response and were considered incidental.

#### Clinical chemistry:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91. However, the sponsor noted high free fatty acids in males in the 6 and 20 mg/kg groups and high total bilirubin in females in the 20 mg/kg group on PND 50 and low total cholesterol in females in the 20 mg/kg group on PND 91. However, the sponsor maintained that these changes were incidental.

#### Necropsy findings:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91. However, the sponsor noted on PND 91 dilatation of the renal pelvis (bilateral or unilateral) in 1 male and 1 female in the 20 mg/kg group, and in 2 males and 2 females in the 6 mg/kg group.

#### Bone Length:

The sponsor concluded that no test article-related changes were noted in tibial length in males or females in any test article group on PND 50 or 91.

#### Organ Weight:

The sponsor concluded that no test article-related changes were noted in organ weight in males or females in any test article group on PND 50 or 91.

#### Histopathology

The sponsor concluded that no test article-related changes were noted in histopathology in males or females in the high dose group on PND 50 or 91 compared to the control group. No histopathology findings were noted as being present in the eyes and the skin of DU-176-treated animals.

#### Toxicokinetics:

The sponsor's summary of toxicokinetics is shown in Table 3. The reviewer notes a 14-fold higher exposure to DU-176 on PND 4 compared with the exposure on PND 49.

Table 3: Sponsor's Summary of DU-176 Toxicokinetics

| Days after birth | Dose (mg/kg/day) | Sex    | Plasma concentration (ng/mL) |       |      |      |      | T <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |       |
|------------------|------------------|--------|------------------------------|-------|------|------|------|----------------------|--------------------------|--------------------------------|-------|
|                  |                  |        | Pre                          | 1 h   | 2 h  | 4 h  | 24 h |                      |                          |                                |       |
| 4                | 2                | Male   | Mean (n=3)                   | -     | 257  | 531  | 472  | 70.1                 | 2.0                      | 531                            | 6950  |
|                  |                  |        | SD (n=3)                     | -     | 34.1 | 173  | 130  | 25.9                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | -     | 378  | 404  | 378  | 52.4                 | 2.0                      | 404                            | 5670  |
|                  |                  |        | SD (n=3)                     | -     | 115  | 86.5 | 68.1 | 7.87                 | -                        | -                              | -     |
|                  | 6                | Male   | Mean (n=3)                   | -     | 1500 | 1150 | 973  | 123                  | 1.0                      | 1500                           | 15200 |
|                  |                  |        | SD (n=3)                     | -     | 80.0 | 140  | 99.6 | 16.9                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | -     | 1290 | 999  | 1040 | 141                  | 1.0                      | 1290                           | 15600 |
|                  |                  |        | SD (n=3)                     | -     | 52.9 | 61.5 | 68.1 | 21.5                 | -                        | -                              | -     |
|                  | 20               | Male   | Mean (n=3)                   | -     | 4840 | 4350 | 4270 | 433                  | 1.0                      | 4840                           | 62700 |
|                  |                  |        | SD (n=3)                     | -     | 474  | 390  | 498  | 113                  | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | -     | 5380 | 4960 | 4270 | 375                  | 1.0                      | 5380                           | 63500 |
|                  |                  |        | SD (n=3)                     | -     | 401  | 311  | 161  | 86.9                 | -                        | -                              | -     |
| 14               | 2                | Male   | Mean (n=3)                   | 9.83  | 457  | 524  | 413  | 15.5                 | 2.0                      | 524                            | 5950  |
|                  |                  |        | SD (n=3)                     | 3.84  | 26.0 | 78.8 | 227  | 4.22                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 11.9  | 422  | 417  | 271  | 7.94                 | 1.0                      | 422                            | 4110  |
|                  |                  |        | SD (n=3)                     | 4.87  | 28.5 | 36.5 | 89.6 | 0.836                | -                        | -                              | -     |
|                  | 6                | Male   | Mean (n=3)                   | 35.9  | 1650 | 1240 | 852  | 23.7                 | 1.0                      | 1650                           | 13100 |
|                  |                  |        | SD (n=3)                     | 11.1  | 130  | 85.0 | 219  | 3.56                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 26.6  | 1650 | 1170 | 847  | 27.2                 | 1.0                      | 1650                           | 13000 |
|                  |                  |        | SD (n=3)                     | 3.68  | 146  | 17.3 | 144  | 4.76                 | -                        | -                              | -     |
|                  | 20               | Male   | Mean (n=3)                   | 99.7  | 4820 | 4710 | 2680 | 88.5                 | 1.0                      | 4820                           | 42300 |
|                  |                  |        | SD (n=3)                     | 28.3  | 644  | 90.7 | 15.3 | 22.6                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 121   | 4830 | 4160 | 2580 | 108                  | 1.0                      | 4830                           | 40600 |
|                  |                  |        | SD (n=3)                     | 28.0  | 537  | 136  | 451  | 3.61                 | -                        | -                              | -     |
| 21               | 2                | Male   | Mean (n=3)                   | 0.000 | 107  | 44.0 | 36.0 | 0.000                | 1.0                      | 107                            | 569   |
|                  |                  |        | SD (n=3)                     | 0.000 | 60.6 | 9.46 | 15.0 | 0.000                | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 0.000 | 90.1 | 59.1 | 39.6 | 0.000                | 1.0                      | 90.1                           | 614   |
|                  |                  |        | SD (n=3)                     | 0.000 | 22.0 | 13.8 | 6.51 | 0.000                | -                        | -                              | -     |
|                  | 6                | Male   | Mean (n=3)                   | 1.74  | 261  | 153  | 194  | 0.337                | 1.0                      | 261                            | 2630  |
|                  |                  |        | SD (n=3)                     | 1.72  | 66.2 | 47.1 | 48.5 | 0.583                | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 1.70  | 252  | 196  | 191  | 0.853                | 1.0                      | 252                            | 2660  |
|                  |                  |        | SD (n=3)                     | 0.432 | 27.0 | 30.5 | 29.5 | 0.754                | -                        | -                              | -     |
|                  | 20               | Male   | Mean (n=3)                   | 3.87  | 1060 | 798  | 567  | 1.00                 | 1.0                      | 1060                           | 8510  |
|                  |                  |        | SD (n=3)                     | 2.38  | 349  | 211  | 256  | 1.74                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 8.16  | 1660 | 891  | 489  | 5.32                 | 1.0                      | 1660                           | 8430  |
|                  |                  |        | SD (n=3)                     | 5.76  | 274  | 174  | 71.5 | 4.27                 | -                        | -                              | -     |
| 49               | 2                | Male   | Mean (n=3)                   | 0.000 | 51.6 | 30.5 | 25.0 | 0.000                | 1.0                      | 51.6                           | 372   |
|                  |                  |        | SD (n=3)                     | 0.000 | 16.6 | 13.4 | 9.19 | 0.000                | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 0.000 | 50.9 | 40.9 | 15.5 | 0.000                | 1.0                      | 50.9                           | 283   |
|                  |                  |        | SD (n=3)                     | 0.000 | 8.92 | 3.27 | 5.59 | 0.000                | -                        | -                              | -     |
|                  | 6                | Male   | Mean (n=3)                   | 1.81  | 193  | 121  | 60.7 | 1.65                 | 1.0                      | 193                            | 1060  |
|                  |                  |        | SD (n=3)                     | 0.278 | 58.3 | 34.1 | 10.6 | 2.86                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 1.12  | 341  | 153  | 59.9 | 0.917                | 1.0                      | 341                            | 1240  |
|                  |                  |        | SD (n=3)                     | 1.02  | 105  | 48.6 | 14.3 | 1.59                 | -                        | -                              | -     |
|                  | 20               | Male   | Mean (n=3)                   | 4.00  | 1230 | 297  | 176  | 5.39                 | 1.0                      | 1230                           | 3670  |
|                  |                  |        | SD (n=3)                     | 1.90  | 90.7 | 121  | 82.7 | 2.56                 | -                        | -                              | -     |
|                  |                  | Female | Mean (n=3)                   | 2.51  | 1520 | 660  | 254  | 4.17                 | 1.0                      | 1520                           | 5350  |
|                  |                  |        | SD (n=3)                     | 1.10  | 310  | 372  | 16.3 | 0.568                | -                        | -                              | -     |

### **5.1.1 Repeated Dose Toxicity Study in Juvenile Rats Treated Orally with D21-2393 for 7 Weeks**

Conducting laboratory and location: [REDACTED] (b) (4)

Study number(s): SBL314-563 (AN11-C0004-R01)

Date of study initiation: 6/24/2011

Drug lot/batch number: D21-2393, Lot D21-2393-09, purity 99.94%

GLP compliance: Yes

QA statement: Yes

#### **Key Study Findings**

In a repeated dose toxicity study, male and female Crl:CD(SD) juvenile rats received 0, 20, 60, and 200 mg/kg/day of D21-2393 in 0.5 w/v% methylcellulose by oral gavage once daily for 7 weeks from postnatal day (PND) 4 to PND 49. The sponsor concluded that D21-2393 did not induce any toxicologically significant effects on postnatal development and growth, organ development, skeletal development, or sexual maturation in juvenile animals. The NOAEL of D21-2393 was 200 mg/kg in male and female juvenile rats. At the NOAEL, the mean AUC<sub>(0-24h)</sub> of D21-2393 was 38650 ng·h/mL on PND 4, 52550 ng·h/mL on PND 14, 5065 ng·h/mL on PND 21, and 787 ng·h/mL on PND 49.

#### **Purpose**

A repeated dose toxicity study was conducted in male and female Crl:CD(SD) juvenile rats to investigate the toxic effects of D21-2393 administration on organ development after the completion of dosing and when animals reached maturity.

#### **Methods**

A solution of D21-2393 in 0.5 w/v% methylcellulose was administered orally by gavage once daily to Crl:CD(SD) rats (16 rats/sex/group) for 7 weeks from postnatal day (PND) 4 to PND 49 at dose levels of 0, 20, 60, and 200 mg/kg/day. Animals were sacrificed for examinations on PND 50 (10 rats/sex/group) and PND 91 after birth (6 rats/sex/group). Blood samples were drawn from the satellite groups (3 rats/sex/group/sampling point) for analysis of plasma concentrations of D21-2393.

#### **Results**

Mortality/ clinical signs:

No animal died, and no abnormal changes were observed in males or females in any group during the study period.

Body weight:

No test article related changes were noted in body weight in males or females in any group during the study period.

Food consumption:

No test article related changes were noted in body weight in males or females in any group during the study period.

#### Functional and Physical Development:

No significant differences were noted in functional developmental parameters (air righting reflex, pupillary reflex, Preyer's reflex, and pain response) or physical developmental parameters (incisor eruption and eyelid opening) in males or females between the control group and any test article group. The reviewer notes that extensive behavioral assessments for acoustic startle, locomotor activity, learning and memory were not conducted.

#### Morphological Differentiation of External Genitalia;

No significant differences were noted in the cleavage of the balano-preputial gland or vaginal opening between the control group and any test article group. The reviewer notes that the fertility, mating ability and reproductive performance were not specifically evaluated.

#### Hematology:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91. The observed significant changes did not exhibit a dose response and were considered incidental.

#### Clinical chemistry:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91. Although the sponsor noted higher a2-globulin ratio in females in the 200 mg/kg group on PND 91, the sponsor maintained that these changes were incidental.

#### Necropsy findings:

The sponsor concluded that no test article-related changes were noted in males or females in any group on PNDs 50 or 91.

#### Bone Length:

The sponsor concluded that no test article-related changes were noted in tibial length in males or females in any test article group on PND 50 or 91.

#### Organ Weight:

The sponsor concluded that no test article-related changes were noted in organ weight in males or females in any test article group on PND 50 or 91. Although the sponsor noted higher relative kidney weight in males in the 200 mg/kg group on PND 91, the sponsor maintained that these changes were incidental because the values were similar to the normal control range.

#### Histopathology

The sponsor concluded that no test article-related changes were noted in histopathology in males or females in the high dose group on PND 50 or 91 compared

to the control group. No histopathology findings were noted as being present in the eyes and the skin of D21-2393-treated animals.

Toxicokinetics:

The sponsor's summary of toxicokinetics is shown in Table 4. The reviewer notes a 49 to 67-fold higher exposure to D21-2393 on PND 4 and PND 14 compared with the exposure on PND 49.

Table 4: Sponsor's Summary of D21-2393 Toxicokinetics

| Days after birth | Dose (mg/kg/day) | Sex    |            | Plasma concentration (ng/mL) |      |       |       |        | T <sub>max</sub> (h) | C <sub>max</sub> (ng/mL) | AUC <sub>0-24h</sub> (ng·h/mL) |
|------------------|------------------|--------|------------|------------------------------|------|-------|-------|--------|----------------------|--------------------------|--------------------------------|
|                  |                  |        |            | Pre                          | 1 h  | 2 h   | 4 h   | 24 h   |                      |                          |                                |
| 4                | 20               | Male   | Mean (n=3) | -                            | 203  | 260   | 399   | 19.1   | 4.0                  | 399                      | 5170                           |
|                  |                  |        | SD (n=3)   | -                            | 16.9 | 23.8  | 71.5  | 8.57   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | -                            | 223  | 309   | 343   | 21.3   | 4.0                  | 343                      | 4670                           |
|                  |                  |        | SD (n=3)   | -                            | 28.2 | 79.2  | 61.0  | 16.0   | -                    | -                        | -                              |
|                  | 60               | Male   | Mean (n=3) | -                            | 781  | 844   | 1320  | 69.7   | 4.0                  | 1320                     | 17300                          |
|                  |                  |        | SD (n=3)   | -                            | 206  | 128   | 535   | 15.2   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | -                            | 872  | 767   | 1270  | 58.6   | 4.0                  | 1270                     | 16600                          |
|                  |                  |        | SD (n=3)   | -                            | 238  | 245   | 173   | 4.34   | -                    | -                        | -                              |
|                  | 200              | Male   | Mean (n=3) | -                            | 1160 | 3750  | 2780  | 135    | 2.0                  | 3750                     | 38700                          |
|                  |                  |        | SD (n=3)   | -                            | 365  | 670   | 780   | 23.9   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | -                            | 1380 | 3090  | 2750  | 235    | 2.0                  | 3090                     | 38600                          |
|                  |                  |        | SD (n=3)   | -                            | 276  | 672   | 773   | 160    | -                    | -                        | -                              |
| 14               | 20               | Male   | Mean (n=3) | 192                          | 549  | 633   | 268   | 155    | 2.0                  | 633                      | 6090                           |
|                  |                  |        | SD (n=3)   | 74.6                         | 89.2 | 148   | 52.8  | 44.5   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 95.5                         | 650  | 584   | 343   | 175    | 1.0                  | 650                      | 7100                           |
|                  |                  |        | SD (n=3)   | 71.0                         | 158  | 62.1  | 54.0  | 34.4   | -                    | -                        | -                              |
|                  | 60               | Male   | Mean (n=3) | 224                          | 1330 | 1350  | 954   | 195    | 2.0                  | 1350                     | 15900                          |
|                  |                  |        | SD (n=3)   | 25.2                         | 5.77 | 271   | 267   | 56.0   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 249                          | 1100 | 1100  | 1240  | 198    | 4.0                  | 1240                     | 18500                          |
|                  |                  |        | SD (n=3)   | 27.2                         | 185  | 52.9  | 195   | 29.5   | -                    | -                        | -                              |
|                  | 200              | Male   | Mean (n=3) | 202                          | 5230 | 3530  | 3970  | 214    | 1.0                  | 5230                     | 56400                          |
|                  |                  |        | SD (n=3)   | 48.8                         | 303  | 369   | 1270  | 18.5   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 203                          | 5820 | 3330  | 3270  | 183    | 1.0                  | 5820                     | 48700                          |
|                  |                  |        | SD (n=3)   | 49.5                         | 1230 | 558   | 465   | 25.5   | -                    | -                        | -                              |
| 21               | 20               | Male   | Mean (n=3) | 1.76                         | 96.0 | 49.7  | 52.8  | 2.79   | 1.0                  | 96.0                     | 780                            |
|                  |                  |        | SD (n=3)   | 0.350                        | 17.3 | 2.29  | 16.4  | 2.12   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 1.04                         | 75.4 | 51.1  | 48.2  | 1.50   | 1.0                  | 75.4                     | 698                            |
|                  |                  |        | SD (n=3)   | 0.545                        | 14.0 | 1.97  | 6.13  | 0.958  | -                    | -                        | -                              |
|                  | 60               | Male   | Mean (n=3) | 2.60                         | 182  | 112   | 54.7  | 4.11   | 1.0                  | 182                      | 994                            |
|                  |                  |        | SD (n=3)   | 1.85                         | 14.6 | 21.5  | 4.73  | 3.18   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 3.51                         | 163  | 86.9  | 126   | 1.38   | 1.0                  | 163                      | 1690                           |
|                  |                  |        | SD (n=3)   | 2.74                         | 16.7 | 28.3  | 50.6  | 0.361  | -                    | -                        | -                              |
|                  | 200              | Male   | Mean (n=3) | 24.2                         | 661  | 322   | 412   | 1.83   | 1.0                  | 661                      | 5710                           |
|                  |                  |        | SD (n=3)   | 8.64                         | 240  | 111   | 92.3  | 1.00   | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 21.4                         | 763  | 418   | 266   | 8.92   | 1.0                  | 763                      | 4420                           |
|                  |                  |        | SD (n=3)   | 3.57                         | 169  | 78.3  | 101   | 5.91   | -                    | -                        | -                              |
| 49               | 20               | Male   | Mean (n=3) | 0.355                        | 19.2 | 6.74  | 7.51  | 0.281  | 1.0                  | 19.2                     | 115                            |
|                  |                  |        | SD (n=3)   | 0.259                        | 7.92 | 3.37  | 5.94  | 0.203  | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 0.321                        | 17.0 | 8.38  | 2.99  | 0.201  | 1.0                  | 17.0                     | 64.6                           |
|                  |                  |        | SD (n=3)   | 0.162                        | 11.4 | 8.38  | 0.406 | 0.0791 | -                    | -                        | -                              |
|                  | 60               | Male   | Mean (n=3) | 0.919                        | 41.8 | 38.9  | 24.4  | 1.15   | 1.0                  | 41.8                     | 381                            |
|                  |                  |        | SD (n=3)   | 0.758                        | 6.42 | 35.5  | 8.27  | 0.982  | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 0.799                        | 35.7 | 30.3  | 11.0  | 0.715  | 1.0                  | 35.7                     | 210                            |
|                  |                  |        | SD (n=3)   | 0.791                        | 26.3 | 7.62  | 4.37  | 0.241  | -                    | -                        | -                              |
|                  | 200              | Male   | Mean (n=3) | 4.10                         | 163  | 101   | 62.7  | 1.24   | 1.0                  | 163                      | 1020                           |
|                  |                  |        | SD (n=3)   | 3.92                         | 26.0 | 77.1  | 13.7  | 0.672  | -                    | -                        | -                              |
|                  |                  | Female | Mean (n=3) | 1.78                         | 85.8 | 41.9  | 34.5  | 2.45   | 1.0                  | 85.8                     | 554                            |
|                  |                  |        | SD (n=3)   | 0.519                        | 43.3 | 0.929 | 23.7  | 0.631  | -                    | -                        | -                              |

## 6. Summary and Evaluation

The purpose of this review is to summarize the distribution and toxicology studies in juvenile animals. The medical officer needs to be aware of the high distribution and retention of DU-176 and its metabolite D21-2393 to the eye and skin of infant and juvenile rats at higher levels than in adult rats. In addition, exposure to DU-176 was 14-fold higher in the infant than in the adult. However, the juvenile toxicology studies did not identify any significant toxicities, particularly in the eye, of either DU-176 or D21-2393.

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/s/  
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PATRICIA P HARLOW  
06/03/2013

THOMAS PAPOIAN  
06/03/2013  
Concur.

IND 63,266

**PHARMACOLOGY AND TOXICOLOGY REVIEW**

(Amendments Serial # 098 dated March 22, 2007; Serial # 103 dated May 02, 2007;  
Serial # 201 dated August 19, 2008; and Serial # 203 dated September 02, 2008)

**Sponsor and Address:** Daiichi Sankyo, Inc.  
Edison, NJ

**Reviewer:** Ronald Honchel, Ph.D.  
Toxicologist, HFD-160

**Date of Review:** March 23, 2009

**Drug:** DU-176b

**Category:** antithrombotic agent, Factor Xa inhibitor

**Submission Contents:**

DU-176b: Effects of DU-176b on Human Platelet Aggregation (Study # 20060958 submitted in amendment serial # 098)

DU-176b: Metabolism of DU176b in Male Rats after a Single Oral Administration of <sup>14</sup>C-DU-176b (Study # 20061350 submitted in amendment serial # 103)

DU-176b: Metabolism of DU176b in Male Monkeys after a Single Oral Administration of <sup>14</sup>C-DU-176b (Study # 20061351 submitted in amendment serial # 103)

Micronucleus Study of D21-2393 in Rats (Study #SBL314-148 submitted in amendment serial # 201)

Repeated Oral Dose Micronucleus Study of D21-2393 for 14 Days in Rats (Study # SBL314-152 submitted in amendment serial # 201)

Polyploidy Test of D21-2393 in Human Lymphocytes (Study # SBL314-151 submitted in amendment serial # 201)

Sponsor's Risk Assessment for Genotoxic Potential of D21-2393 (Submitted in amendment serial # 203).

## PHARMACOLOGY

### DU-176b: Effects of DU-176b on Human Platelet Aggregation (Study # 20060958 submitted in amendment serial # 098)

The IC<sub>50</sub> value of DU-176b was determined in human platelet-rich plasma (PRP) and washed platelet (WP) suspensions isolated from 12 healthy, medication-free, volunteers. Collagen, U46619, ADP, and thrombin were used to induce platelet aggregation (the concentration for these agonists were not provided, the sponsor stated only that the concentration of each agonist was optimized for each assay to induce >60% platelet aggregation). Abciximab (anti-glycoprotein IIb/IIIa antibody that inhibits platelet aggregation) was used as a positive control. For the PRP study, 200 µL of PRP (n =5/group) was stimulated by the addition of collagen, U46619, or ADP in the presence of vehicle (DMSO), DU-176b (0.01, 1, and 100 µM), or Abciximab (0.2 or 20 µg/mL). For the WP study, 200 µL of WP in 1mM CaCl<sub>2</sub> was stimulated by the addition of thrombin in the presence of vehicle (DMSO for DU-176b, buffer for Abciximab), DU-176b (stated as 0.1, 1, and 10 µM in a study design table and as 0.01, 1, and 100 µM in Appendix 4), or Abciximab (0.2 or 20 µg/mL). The extent of aggregation for both studies was then estimated by the percentage of maximum increase in light transmission. In the PRP study, there was no evidence of significant inhibition of platelet aggregation by DU-176b (IC<sub>50</sub> > 100 µM) whereas 92-100% inhibition of platelet aggregation was observed in 20 mg/mL Abciximab groups (platelet aggregation was not noticeably affected by treatment with 0.2 mg/mL Abciximab). In the WP study, 78-92% inhibition of platelet aggregation was observed in the high dose DU-176b group (platelet aggregation was not noticeably affected by treatment with 1 µM DU-176b) whereas 63-93% inhibition of platelet aggregation was observed in 20 mg/mL Abciximab group (platelet aggregation was not noticeably affected by treatment with 0.2 mg/mL Abciximab). The sponsor stated the mean IC<sub>50</sub> for DU-176b in the WP study was 2.90 µM (95% CI 1.99-3.81 µM).

There were a number of problems with this study such as failure to provide the concentration of agonist used in each assay and conflicting information provided for the DU-176b concentrations used in the WP study (0.1, 1, and 10 µM appear to be the likely concentrations used since the stated IC<sub>50</sub> was between 1 and 10 µM). However, even with these problems, the study suggested that under assay conditions, DU-176b at high enough concentrations can inhibit platelet aggregation via the direct inhibition of thrombin activity.

## PHARMACOKINETICS – METABOLISM

### DU-176b: Metabolism of DU176b in Male Rats after a Single Oral Administration of <sup>14</sup>C-DU-176b (Study # 20061350 submitted in amendment serial # 103)

**Methods:** Fasted male Wistar rats were administered via gavage 3 mg/kg <sup>14</sup>C-DU-176b (Lot # CP-3113, specific activity 2.96 MBq/mL, 98% radiochemical purity). Plasma, urine, feces, bile (animals in this group had a cannula surgically inserted into the bile duct

followed by drug administration after recovery from anesthesia), liver, and kidney were then collected as indicated in the Table below (provided by the sponsor). Radioactivity levels were determined via liquid scintillation counting. Plasma, bile and urine were directly mixed with scintillation fluid. Liver, kidney and fecal homogenates were first solubilized then mixed with scintillation fluid. Metabolite analyses were performed using LC/MS.

**Experimental group:**

| Group            | Test substance          | Dose                  | Volume  | Frequency | Route | Number of animals | Biological sample         | Sampling time <sup>3)</sup> |
|------------------|-------------------------|-----------------------|---------|-----------|-------|-------------------|---------------------------|-----------------------------|
| 1                | <sup>14</sup> C-DU-176b | 3 mg/kg <sup>1)</sup> | 6 mL/kg | Single    | po    | 3                 | Plasma, liver, and kidney | 1 h                         |
| 2                |                         |                       |         |           |       | 3                 |                           | 4 h                         |
| 3                |                         |                       |         |           |       | 3                 | Urine and feces           | 0-24 h                      |
| 4                |                         |                       |         |           |       | 3                 | Bile                      | 0-24 h                      |
| 4a <sup>2)</sup> |                         |                       |         |           |       | 2                 |                           |                             |
| 5                |                         |                       |         |           |       | 3                 | Plasma                    | 1 h                         |
| 6                | 3                       | 4 h                   |         |           |       |                   |                           |                             |

1): The dose is expressed as equivalents of DU-176.

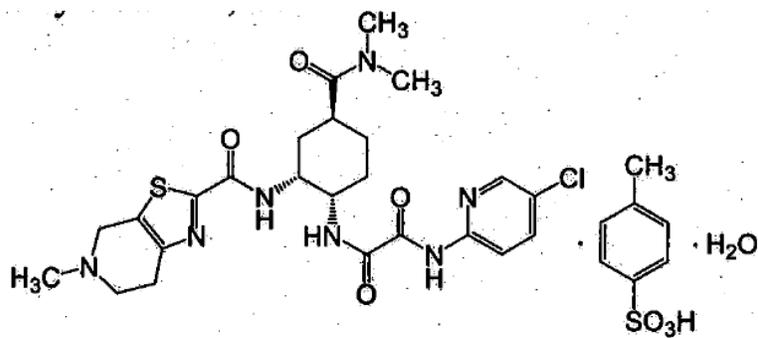
2): The animals in Group 4a received a dose, and samples were collected as spares for animals in Group 4. However, no samples were used.

3): After administration

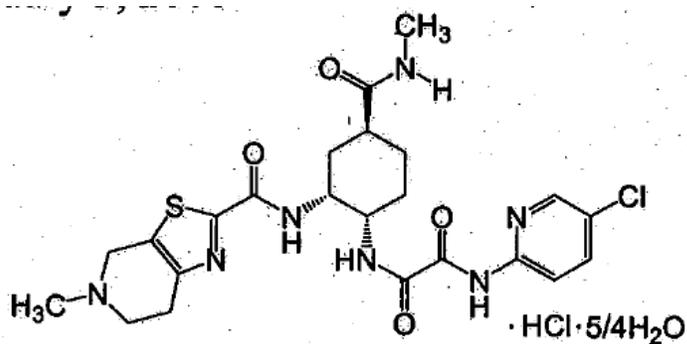
Details of actual administered doses are shown in Appendix 7.

**Results:** Metabolites were assigned alphanumeric names bases on species (R for rat), biological sample type (P, L, K, U, F, and B for plasma, liver, kidney, urine, feces, and bile, respectively), and the order of retention times on radio-chromatograms. A total of 5 metabolites (identified as D21-3231, D21-3221, D21-2393, D21-1402, and D21-2135) and the parent compound were identified on LC/MS chromatograms and the structures were confirmed using reference standards. There were a number of peaks that were detected but not identified. Usually these peaks were of relatively low magnitude. Peak RB7 detected in bile samples was the one exception (see Table 11 below). In all samples, the parent drug was responsible for highest percentage of recovered radioactivity. The structures of DU-176b and the identified rat metabolites are shown in the Figures below (provided by the sponsor).

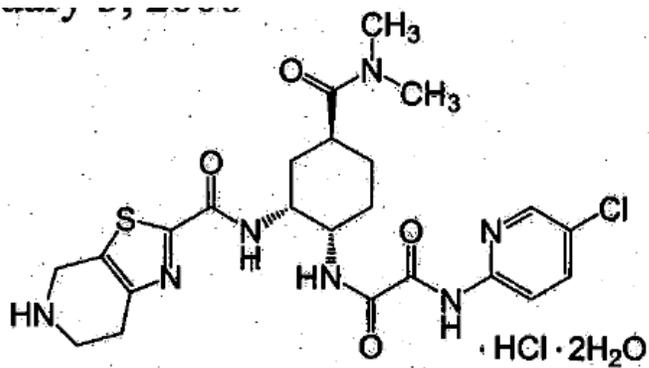
**DU-176b**



D21-2135

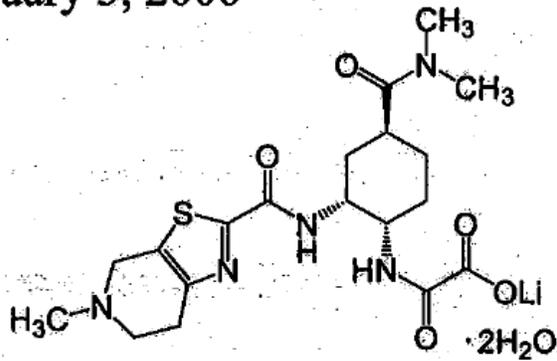


D21-1402



D21-3231

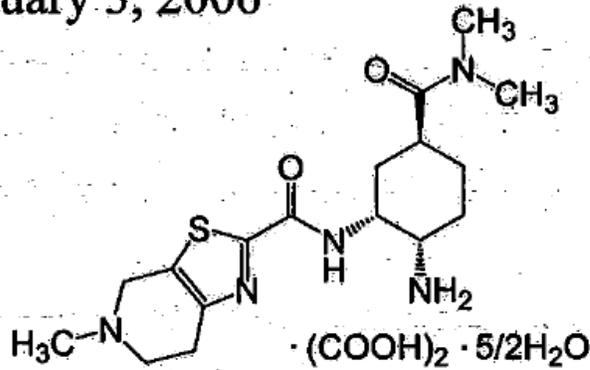
July 3, 2000



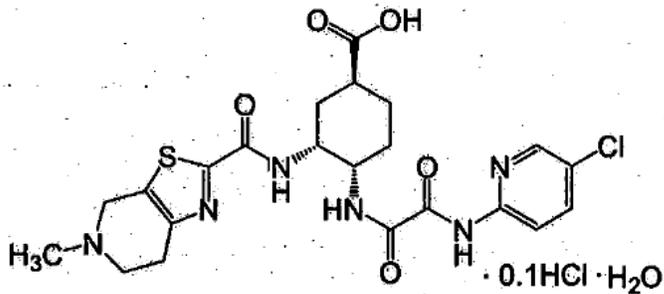
D21-3221

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ON ORIGINAL

July 3, 2000



D21-2393



Plasma results are summarized below in Table 1 (provided by the sponsor). D21-3231 was the major metabolite observed in rat plasma at 1 and 4 hours after dose administration.

**Table 1. Proportions of DU-176 and its Metabolites in the Rat Plasma Samples Collected at 1 h and 4 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |                   |
|----------------------------|--|-------------------|
|                            | 1 h  | 4 h               |
| RP1 (D21-3231)             | 32.4 ± 5.9                                       | 26.7 ± 2.8        |
| RP2 (D21-1402)             | 0.9 ± 0.1  | 1.2 <sup>2)</sup> |
| RP3                        | 0.8 ± 0.3  | 1.0 <sup>2)</sup> |
| RP4 (DU-176)               | 50.9 ± 7.1                                       | 52.7 ± 6.3        |
| D21-3221                   | ND   | ND                |
| D21-2393                   | ND   | ND                |
| D21-2135                   | ND   | ND                |
| Others                     | 8.1 ± 1.4  | 14.4 ± 3.4        |
| Recovery (%) <sup>1)</sup> | 93.1 ± 1.4                                       | 95.3 ± 0.8        |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the plasma.

2): The mean for two animals.

ND: Not detected.

Liver results are summarized below in Table 3 (provided by the sponsor). D21-1402 was the major metabolite observed in rat liver at 1 and 4 hours after dose administration.

**Table 3. Proportions of DU-176 and its Metabolites in the Rat Liver Samples Collected at 1 h and 4 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |            |
|----------------------------|--|------------|
|                            | 1 h  | 4 h        |
| RL1 (D21-3231)             | 4.5 ± 0.8  | 8.2 ± 0.2  |
| RL2                        | 0.9 ± 0.3  | 2.7 ± 0.7  |
| RL3 (D21-1402)             | 11.1 ± 1.1                                       | 11.5 ± 0.5 |
| RL4                        | 7.4 ± 1.2  | 4.1 ± 0.7  |
| RL5 (DU-176)               | 63.3 ± 2.5                                       | 55.5 ± 0.5 |
| D21-3221                   | ND   | ND         |
| D21-2393                   | ND   | ND         |
| D21-2135                   | ND   | ND         |
| Others                     | 7.1 ± 0.5  | 9.4 ± 1.0  |
| Recovery (%) <sup>1)</sup> | 94.3 ± 0.6                                       | 91.4 ± 0.7 |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the liver homogenate.

ND: Not detected.

Kidney results are summarized below in Table 5 (provided by the sponsor). D21-3231 was the major metabolite observed in rat kidney at 1 and 4 hours after dose administration.

**Table 5. Proportions of DU-176 and its Metabolites in the Rat Kidney Samples Collected at 1 h and 4 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |                   |
|----------------------------|--|-------------------|
|                            | 1 h  | 4 h               |
| RK1 (D21-3231)             | 12.8 ± 1.4                                       | 15.0 ± 2.5        |
| RK2                        | ND   | 1.6 <sup>2)</sup> |
| RK3 (D21-1402)             | 2.6 ± 0.5  | 3.6 ± 1.8         |
| RK4                        | 0.7 ± 0.2  | ND                |
| RK5 (DU-176)               | 71.8 ± 1.3                                       | 56.4 ± 5.1        |
| D21-3221                   | ND   | ND                |
| D21-2393                   | ND   | ND                |
| D21-2135                   | ND   | ND                |
| Others                     | 5.1 ± 0.4  | 13.8 ± 2.1        |
| Recovery (%) <sup>1)</sup> | 93.0 ± 0.6                                       | 89.9 ± 1.1        |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the kidney homogenate.

2): The mean for two animals.

ND: Not detected.

Urine results are summarized below in Table 7 (provided by the sponsor). D21-3231 was the major metabolite observed in rat urine over the 0-24 hour interval after dose administration. Approximately 25% of all excreted radioactivity was recovered in the urine.

**Table 7. Proportions of DU-176 and its Metabolites in the Rat Urine Samples Collected over 0-24 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite (% in sample) |
|----------------------------|---|
| RU1 (D21-3231)             | 19.7 ± 1.0                                    |
| RU2 (D21-1402)             | 1.4 ± 0.1                                     |
| RU3                        | 0.6 ± 0.1                                     |
| RU4 (DU-176)               | 70.7 ± 3.4                                    |
| D21-3221                   | ND  |
| D21-2393                   | ND  |
| D21-2135                   | ND  |
| Others                     | 6.6 ± 4.6                                     |
| Recovery (%) <sup>1)</sup> | 99.0 ± 0.5                                    |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the urine.

ND: Not detected.

Fecal results are summarized below in Table 9 (provided by the sponsor). There were no metabolites observed in rat feces over the 0-24 hour interval after dose administration that contributed to at least 10% of the recovered radioactivity (approximately 70% of the recover radioactivity was in the form of parent compound). Approximately 64% of all excreted radioactivity was recovered in the feces.

**Table 9. Proportions of DU-176 and its Metabolites in the Rat Feces Samples Collected over 0-24 h after a Single oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite (% in sample) |
|----------------------------|---|
| RF1 (D21-3231)             | 5.8 ± 0.2                                     |
| RF2 (D21-3221)             | 0.5 ± 0.1                                     |
| RF3 (D21-1402)             | 3.9 ± 0.4                                     |
| RF4                        | 5.6 ± 0.1                                     |
| RF5 (D21-2135)             | 0.9 ± 0.1                                     |
| RF6 (DU-176)               | 69.7 ± 1.0                                    |
| D21-2393                   | ND  |
| Others                     | 6.7 ± 0.5                                     |
| Recovery (%) <sup>1)</sup> | 93.2 ± 1.1                                    |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the fecal homogenate.

ND: Not detected.

Bile results are summarized below in Table 11 (provided by the sponsor). D21-3231 and RB7 (an unidentified metabolite) were the major metabolites observed in rat bile over the 0-24 hour interval after dose administration. Approximately 25% of all excreted radioactivity was recovered in the bile.

**Table 11. Proportions of DU-176 and its Metabolites in the Rat Bile Samples Collected over 0-24 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 3 mg as DU-176/kg to Male Rats**

| Metabolite                 | Proportion of DU-176 metabolite (% in sample) |
|----------------------------|---|
| RB1 (D21-3231)             | 17.5 ± 1.8                                    |
| RB2                        | 1.2 ± 0.2                                     |
| RB3                        | 4.2 ± 0.4                                     |
| RB4                        | 1.3 ± 0.5                                     |
| RB5 (D21-1402)             | 6.8 ± 2.1                                     |
| RB6                        | 1.2 ± 0.2                                     |
| RB7                        | 17.1 ± 3.5                                    |
| RB8 (D21-2135)             | 1.3 ± 0.2                                     |
| RB9 (DU-176)               | 35.5 ± 1.7                                    |
| RB10                       | 1.8 ± 0.3                                     |
| RB11                       | 1.5 ± 0.5                                     |
| D21-3221                   | ND  |
| D21-2393                   | ND  |
| Others                     | 7.2 ± 0.6                                     |
| Recovery (%) <sup>1)</sup> | 96.5 ± 1.0                                    |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the bile.

ND: Not detected.

DU-176b: Metabolism of DU176b in Male Monkeys after a Single Oral Administration of <sup>14</sup>C-DU-176b (Study # 20061351 submitted in amendment serial # 103)

**Methods:** Fasted cynomolgus monkeys (n = 3) were administered via gavage 1 mg/kg <sup>14</sup>C-DU-176b (Lot # CP-3113, specific activity 2.96 MBq/mL, 98% radiochemical purity). Plasma, urine, and feces were collected at 2 and 4 hours, 0-8 and 8-48 hours, and 0-24 and 24-72 hours, respectively, after dose administration. Radioactivity levels were determined via liquid scintillation counting. Plasma and urine were directly mixed with scintillation fluid. Fecal homogenates were first solubilized then mixed with scintillation fluid. Metabolite analyses were performed using LC/MS.

**Results:** Metabolites were assigned alphanumeric names based on species (M for monkey), biological sample type (P, U, and F for plasma, urine, and feces, respectively), and the order of retention times on radio-chromatograms. Like the rat study above, a total of 5 metabolites (identified as D21-3231, D21-3221, D21-2393, D21-1402, and D21-2135) and the parent compound were identified on LC/MS chromatograms and the structures were confirmed using reference standards. There were a number of peaks that were detected but not identified. Although most unidentified peaks were of relatively low magnitude, peaks MU1 and MF5 were unidentified peaks that contributed to at least 10% of the recovered radioactivity in urine and fecal samples (see Table 3 & 5 below).

Plasma, urine and fecal results are summarized below in Tables 1, 3, and 5, respectively (provided by the sponsor). In all samples, the parent drug was responsible for highest percentage of recovered radioactivity. There were metabolites observed in the plasma that contributed at least 10% to the recovered radioactivity. MU1 (an unidentified metabolite) at the 0-8 hour after dose administration interval was the metabolite observed in urine that contributed at least 10% to the recovered radioactivity. D21-2135 and MF5 (an unidentified metabolite) were major metabolites observed in feces at both the 0-24 hour and 24-72 hour intervals. Approximately 31% and 11% of the total administered radioactivity was recovered from the urine at the 0-8 hour and 8-48 hour intervals, respectively. Approximately 18% and 26% of the total administered radioactivity was recovered from the feces at the 0-24 hour and 24-72 hour intervals, respectively.

**Table 1. Proportions of DU-176 and its Metabolites in the Monkey Plasma Samples Collected at 2 h and 4 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 1 mg as DU-176/kg to Male Monkeys**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |                   |
|----------------------------|--|-------------------|
|                            | 2 h  | 4 h               |
| MP1                        | 7.4 <sup>2)</sup>                                | 5.5 <sup>2)</sup> |
| MP2 (D21-3231)             | 5.0 ± 2.5  | 5.4 ± 2.1         |
| MP3                        | 0.9 ± 0.4  | 1.6 <sup>2)</sup> |
| MP4                        | 2.2 ± 0.6  | 1.4 ± 0.4         |
| MP5 (D21-1402)             | 3.9 ± 0.9  | 4.6 ± 0.9         |
| MP6                        | 7.2 ± 1.1  | 8.3 ± 1.4         |
| MP7 (DU-176)               | 60.7 ± 6.9                                       | 56.0 ± 3.4        |
| D21-3221                   | ND   | ND                |
| D21-2393                   | ND   | ND                |
| D21-2135                   | ND   | ND                |
| Others                     | 11.7 ± 3.1                                       | 16.3 ± 1.8        |
| Recovery (%) <sup>1)</sup> | 96.5 ± 1.5                                       | 96.8 ± 0.8        |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the plasma.

2): The mean for two animals.

ND: Not detected.

**Table 3. Proportions of DU-176 and its Metabolites in the Monkey Urine Samples Collected at 0-8 h and 8-48 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 1 mg as DU-176/kg to Male Monkeys**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |                   |
|----------------------------|--|-------------------|
|                            | 0-8 h  | 8-48 h            |
| MU1                        | 17.7 ± 15.0                                      | 8.0 ± 6.1         |
| MU2 (D21-3231)             | 5.5 ± 1.8  | 5.6 ± 0.9         |
| MU3                        | 0.6 ± 0.2  | 0.8 ± 0.3         |
| MU4                        | 1.1 ± 0.3  | 1.2 ± 0.2         |
| MU5                        | 0.6 ± 0.2  | 0.8 <sup>2)</sup> |
| MU6 (D21-1402)             | 5.5 ± 2.0  | 4.5 ± 0.5         |
| MU7                        | 9.1 ± 1.7  | 6.5 ± 0.7         |
| MU8 (D21-2135)             | 1.0 ± 0.3  | 1.7 ± 0.7         |
| MU9 (DU-176)               | 52.7 ± 10.9                                      | 58.5 ± 4.5        |
| D21-3221                   | ND   | ND                |
| D21-2393                   | ND   | ND                |
| Others                     | 5.4 ± 1.7  | 11.9 ± 3.9        |
| Recovery (%) <sup>1)</sup> | 99.2 ± 0.4                                       | 99.1 ± 0.3        |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the urine.

2): The mean for two animals.

ND: Not detected.

**Table 5. Proportions of DU-176 and its Metabolites in the Monkey Feces Samples Collected at 0-24 h and 24-72 h after a Single Oral Administration of <sup>14</sup>C-DU-176b at a Dose of 1 mg as DU-176/kg to Male Monkeys**

| Metabolite                 | Proportion of DU-176 metabolite<br>(% in sample) |            |
|----------------------------|--|------------|
|                            | 0-24 h   | 24-72 h    |
| MF1                        | 1.2 ± 0.4  | 1.2 ± 0.2  |
| MF2 (D21-3231)             | 4.7 ± 0.8  | 4.0 ± 0.8  |
| MF3                        | 3.7 ± 1.1  | 3.8 ± 0.9  |
| MF4 (D21-1402)             | 12.1 ± 2.8                                       | 13.5 ± 1.3 |
| MF5                        | 19.7 ± 4.5                                       | 20.6 ± 3.4 |
| MF6 (D21-2135)             | 5.0 ± 1.3  | 7.2 ± 1.1  |
| MF7 (DU-176)               | 34.6 ± 11.6                                      | 27.8 ± 8.5 |
| D21-3221                   | ND   | ND         |
| D21-2393                   | ND   | ND         |
| Others                     | 9.8 ± 1.1  | 11.3 ± 2.1 |
| Recovery (%) <sup>1)</sup> | 90.7 ± 1.1                                       | 89.3 ± 0.3 |

Data are expressed as the mean ± SD for three animals.

1): Recovery of radioactivity extracted from the fecal homogenate.

ND: Not detected.

## GENETIC TOXICOLOGY

**Study title:** Micronucleus Study of D21-2393 in Rats (submitted in amendment serial # 201)

**Key findings:** D21-2393 is a major human metabolite of DU-176b. D21-2393 was not genotoxic in the rat micronucleus assay.

**Study no.:** SBL314-148

**Volume #, and page #:** Volume 1, page 4

**Conducting laboratory and location:**

(b) (4)

(b) (4)

**Date of study initiation:** April 14, 2008

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** D21-2393, Lot # D21-2393-07, 99.8% purity

### Methods

**Strains/species/cell line:** Rat/Crl:CD(SD)

Doses used in definitive study: 0 (vehicle), 500, 1000, and 2000 mg/kg D21-2393.

Basis of dose selection: Doses greater than 2000 mg/kg are generally considered unnecessary for toxicology studies.

Negative controls: Vehicle (0.5% methylcellulose)

Positive controls: Cyclophosphamide monohydrate (20 mg/kg)

Incubation and sampling times: Male Sprague-Dawley rats (n = 6/group/timepoint; approximately 8 weeks old at dosing) were administered a single dose via gavage of vehicle, positive control, or drug. Animals were observed for clinical signs 3 times on the day of dosing and once daily afterwards. Groups (2/dose level) were sacrificed at 24 or 48 hour after dosing except for positive control which had only 1 group that was sacrificed at 24 hours after dosing. The sponsor did not determine plasma drug levels in this study. However, the sponsor cited TK results from a 14-day rat oral toxicity study (Study # SBL314-112) as verification of exposure of target rat bone marrow cells.

## **Results**

Study validity: A bone marrow smear was made from the right femur of each rat. Smears were then fixed, stained with acridine orange, and the number of micronucleated polychromatic erythrocytes or micronucleated immature erythrocytes (MNIIEs) as the sponsor calls them from a population of 2000 IEs were counted manually under a fluorescent microscope. The proportion of IEs per 500 erythrocytes was also determined for each rat. The criterion for a positive response was a statistically significant increase in the incidence of MNIE in the test article groups compared to the negative control group.

Study outcome: There were no clinical signs observed in this study. Results are summarized below in Table 4 (provided by the sponsor). Mean negative control values were within historical negative control ranges. There were no significantly significant increases in the mean incidence of MNIIEs at 24 or 48 hours after dosing in treated animals compared to vehicle controls. A dose related decrease in the mean incidence of IEs was observed at both the 24 and 48 hour timepoints that was statistical significant at the 2000 mg/kg D21-2393 dose. The mean incidence of MNIIEs was significantly increased in the positive control group.

Table 4. Group results of micronucleus test

| Group            | Dose level<br>(mg/kg) | Sex  | Sampling<br>time (h) | IE%            |                         | Total | MNIE%         |                         |
|------------------|-----------------------|------|----------------------|----------------|-------------------------|-------|---------------|-------------------------|
|                  |                       |      |                      | Mean ± S.D.    | Minimum - Maximum value |       | Mean ± S.D.   | Minimum - Maximum value |
| Negative control | -                     |      |                      | 48.97 ± 1.19   | 47.0 - 50.4             | 13    | 0.11 ± 0.04   | 0.05 - 0.15             |
| D21-2393         | 500                   |      |                      | 48.47 ± 1.06   | 47.0 - 49.8             | 16    | 0.13 ± 0.05   | 0.10 - 0.20             |
| D21-2393         | 1000                  | male | 24                   | 47.03 ± 1.71   | 44.8 - 48.8             | 15    | 0.13 ± 0.05   | 0.05 - 0.20             |
| D21-2393         | 2000                  |      |                      | 46.50 ± 0.67 * | 45.8 - 47.4             | 18    | 0.15 ± 0.05   | 0.10 - 0.25             |
| Positive control | 20                    |      |                      | 38.13 ± 1.70 * | 36.4 - 40.4             | 288   | 2.40 ± 0.21 # | 2.00 - 2.60             |
| Negative control | -                     |      |                      | 48.63 ± 1.58   | 47.4 - 51.2             | 13    | 0.11 ± 0.04   | 0.05 - 0.15             |
| D21-2393         | 500                   | male | 48                   | 47.80 ± 1.60   | 45.8 - 50.2             | 11    | 0.09 ± 0.05   | 0.05 - 0.15             |
| D21-2393         | 1000                  |      |                      | 46.57 ± 0.83   | 45.6 - 47.6             | 13    | 0.11 ± 0.06   | 0.05 - 0.20             |
| D21-2393         | 2000                  |      |                      | 45.10 ± 1.22 * | 44.0 - 47.2             | 16    | 0.13 ± 0.07   | 0.05 - 0.20             |

Notes)

IE%: Ratio of immature erythrocytes

MNIE%: Incidence of micronucleated immature erythrocytes

Negative control: 0.5 w/v% Methylcellulose

Positive control: Cyclophosphamide monohydrate

Sampling time: Approximately sampling time of bone marrow after dosing

Total: Total number of MNIE per group

\*: Significantly different from negative control (p&lt;0.01, Student's t test)

# : Significantly increase from negative control (p&lt;0.05, Kastenbaum and Bowman's method)

**Study title:** Repeat Oral Dose Micronucleus Study of D21-2393 for 14 Days in Rats (submitted in amendment serial # 201)

**Key findings:** D21-2393 is a major human metabolite of DU-176b. D21-2393 was not genotoxic in the 14-day rat micronucleus assay.

**Study no.:** SBL314-152

**Volume #, and page #:** Volume 1, page 45

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** April 30, 2008

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** D21-2393, Lot # D21-2393-07, 99.8% purity

## Methods

Strains/species/cell line: Rat/Crl:CD(SD)

Doses used in definitive study: 0 (vehicle), 500, 1000, and 2000 mg/kg D21-2393.

Basis of dose selection: Doses greater than 2000 mg/kg are generally considered unnecessary for toxicology studies.

Negative controls: Vehicle (0.5% methylcellulose)

Positive controls: Cyclophosphamide monohydrate (20 mg/kg)

Incubation and sampling times: Male Sprague-Dawley rats (n = 6/group; approximately 8 weeks old at dosing) were administered a once-daily dose via gavage of vehicle or drug for 14 consecutive days. Positive control was administered once via oral gavage on Day 13. Animals were observed for clinical signs twice daily. Body weights were recorded on Days 0, 2, 6, 9, 13, and 14. All groups were sacrificed 24 hours after the last dose (only dose for the positive controls). The sponsor did not report plasma drug levels in this study. However, the sponsor stated that TK results from this study are reported in the toxicology study report (Study # SBL314-112). The sponsor stated that the TK results from that study as verify exposure of target rat bone marrow cells.

## Results

Study validity: A bone marrow smear was made from the right femur of each rat. Smears were then fixed, stained with acridine orange, and the number of micronucleated polychromatic erythrocytes or micronucleated immature erythrocytes (MNIIEs) from a population of 2000 IEs were counted manually under a fluorescent microscope. The

proportion of IEs per 500 erythrocytes was also determined for each rat. The criterion for a positive response was a statistically significant increase in the incidence of MNIE in the test article groups compared to the negative control group.

**Study outcome:** There were no clinical signs or drug-related effects on body weights observed in this study. Mean negative control values (0.08% and 45.27% for MNIE% and IE%, respectively) were within historical negative control ranges. There were no significant increases in the mean incidence of MNIEs (0.08%-0.13%) or IEs (43.40%-46.03%) in treated animals compared to vehicle controls. The mean incidence of MNIEs (2.12%) was significantly increased and the mean incidence of IEs was significantly decreased (37.23%) in the positive control group compared to the vehicle control group.

**Study title:** Polyploidy Test of D21-2393 in Human Lymphocytes (submitted in amendment serial # 201)

**Key findings:** D21-2393 was negative in the human peripheral lymphocyte polyploidy assay. However, D21-2393 was suspended in water, not in solution, when added to the cultures. Test article precipitation was observed at D21-2393 dose levels of 156 µg/mL and greater when drug suspension was first added to cultures. The sponsor stated that precipitation was noted at dose levels of 625 and 2500 µg/mL for short-term incubations and continuous incubations, respectively, by the end of treatment. It appears a positive control was not used for short term incubations suggesting a positive control was not used with S9 mix.

**Study no.:** SBL314-151

**Volume #, and page #:** Volume 1, page 84

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** May 09, 2008

**GLP compliance:** Yes

**QA reports:** yes ( X ) no ( )

**Drug, lot #, and % purity:** D21-2393, Lot # D21-2393-07, 99.8% purity

## **Methods**

**Strains/species/cell line:** Peripheral lymphocytes collected from two healthy adult volunteer donors.

**Doses used in definitive study:** See “Incubation and sampling times” below.

**Basis of dose selection:** Dose levels above 5000 µg/mL are generally not required for these types of genetic toxicology studies.

**Negative controls:** Vehicle (distilled water )

Positive controls: Noscapine hydrochloride hydrate (NOS) (used in 24 and 48 hour continuous treatment cultures only).

Incubation and sampling times: Dose levels for short-term treatments (3-hour treatment followed by 21- or 45-hour recovery period) in the presence or absence of S9 mix were 625, 1250, 2500, and 5000 µg/mL D21-2393. Dose levels for continuous treatment for 24 hours were 313, 625, 1250, 2500, and 5000 µg/mL D21-2393. Dose levels for continuous treatment for 48 hours were 78.1, 156, 313, 625, 1250, 2500, and 5000 µg/mL D21-2393. Cells were then harvested (0.2 µg/mL colcemid was added to all cultures 3 hours before harvesting) 24 or 48 hours after initiation of treatment.

## **Results**

Study validity: The number of replicates used was 2 for treatment and positive controls and 4 for negative controls. Air-dried slides were prepared from each culture and stained with 2.5% Giemsa. Five-hundred cells from each dish were observed to determine the ratio of metaphases per 1000 cells (mitotic index). One-thousand metaphase spreads from each culture were scored microscopically for polyploidy and endoreduplication. A cell with 69 chromosomes was classified as polyploidy as to distinguish from endoreduplication. The criteria for a positive response included: 1) a statistically significant increase in the incidence of cells bearing aberration in chromosomal number compared to negative control values; and 2) the increase is dose dependent.

Study outcome: Test article precipitation was observed at D21-2393 dose levels of 156 µg/mL and greater when drug suspension was first added to cultures. The sponsor stated that precipitation was noted at dose levels of 625 and 2500 µg/mL for short-term incubations and continuous incubations, respectively, by the end of treatment. The dose levels selected for chromosomal analyses were based on the lowest dose to reduce mitotic index by at least 50% or high dose if mitotic index was not reduced by at least 50% at any dose level.

Results are summarized in the Tables below (provided by the sponsor). The incidence of cells having numerical aberration in the negative control was within the historical range. There were no significant increases in numerical chromosomal aberrations observed at any D21-2393 dose level with either short term (with or with S9 mix) or continuous treatment groups. A statistically significant decrease in numerical chromosomal aberrations was observed in Volunteer A at the 5000 and 2500 µg/mL dose levels after continuous treatment for 24 and 48 hours, respectively, but such a finding would not be considered toxicologically significant. NOS induced a marked increase in the incidence of numerical aberrations after continuous treatment for 24 or 48 hours. It appears a positive control was not used for short term incubations suggesting a positive control was not used with S9 mix.

**Table 3-1 Results of chromosomal aberration test (short-term treatment, 21h recovery, Volunteer A)**

Test article: D21-2393

| Treatment time (h) | S9 mix | Dose (µg/mL)                            | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |            |
|--------------------|--------|---|--------------------|--|------------|--------------------|------------|
|                    |        |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total      |
| 3 - 21             | -      | Negative control<br>Water for injection | 100                | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 1000   | 9          | 0                  | 9          |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |
| 3 - 21             | -      | 1250                                    | 92.8               | 1000   | 8          | 0                  | 8          |
|                    |        |   |                    | 1000   | 13         | 0                  | 13         |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |
| 3 - 21             | -      | 2500                                    | 87.6               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 2000   | 22         | 0                  | 22 ( 1.1 ) |
| 3 - 21             | -      | 5000                                    | 72.2               | 1000   | 7          | 0                  | 7          |
|                    |        |   |                    | 1000   | 6          | 0                  | 6          |
|                    |        |   |                    | 2000   | 13         | 0                  | 13 ( 0.7 ) |
| 3 - 21             | +      | Negative control<br>Water for injection | 100                | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 1000   | 3          | 0                  | 3          |
|                    |        |   |                    | 2000   | 14         | 0                  | 14 ( 0.7 ) |
| 3 - 21             | +      | 1250                                    | 82.1               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |
| 3 - 21             | +      | 2500                                    | 76.4               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 8          | 0                  | 8          |
|                    |        |   |                    | 2000   | 18         | 0                  | 18 ( 0.9 ) |
| 3 - 21             | +      | 5000                                    | 58.5               | 1000   | 7          | 0                  | 7          |
|                    |        |   |                    | 1000   | 8          | 0                  | 8          |
|                    |        |   |                    | 2000   | 15         | 0                  | 15 ( 0.8 ) |

Remarks) Final concentration of S9 was 5%, Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 & 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100%  
-S9 mix: Without metabolic activation, +S9 mix: With metabolic activation,

Test article precipitation in the culture medium was observed at 625 µg/mL and greater at the start and end of test article treatment.

No significant difference was noted when compared with the negative control group using the chi-square test (p&lt;0.05).

**Table 3-2 Results of chromosomal aberration test (short-term treatment, 45h recovery, Volunteer A)**

Test article: D21-2393

| Treatment time (h) | S9 mix | Dose (µg/mL)                            | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |            |
|--------------------|--------|---|--------------------|--|------------|--------------------|------------|
|                    |        |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total      |
| 3 - 45             | -      | Negative control<br>Water for injection | 100                | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 16         | 0                  | 16         |
|                    |        |   |                    | 2000   | 26         | 0                  | 26 ( 1.3 ) |
| 3 - 45             | -      | 1250                                    | 89.5               | 1000   | 15         | 0                  | 15         |
|                    |        |   |                    | 1000   | 9          | 0                  | 9          |
|                    |        |   |                    | 2000   | 24         | 0                  | 24 ( 1.2 ) |
| 3 - 45             | -      | 2500                                    | 78.1               | 1000   | 16         | 0                  | 16         |
|                    |        |   |                    | 1000   | 13         | 0                  | 13         |
|                    |        |   |                    | 2000   | 29         | 0                  | 29 ( 1.5 ) |
| 3 - 45             | -      | 5000                                    | 70.5               | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 2000   | 22         | 0                  | 22 ( 1.1 ) |
| 3 - 45             | +      | Negative control<br>Water for injection | 100                | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 2000   | 24         | 0                  | 24 ( 1.2 ) |
| 3 - 45             | +      | 1250                                    | 82.7               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 2000   | 22         | 0                  | 22 ( 1.1 ) |
| 3 - 45             | +      | 2500                                    | 76.5               | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 1000   | 13         | 0                  | 13         |
|                    |        |   |                    | 2000   | 25         | 0                  | 25 ( 1.3 ) |
| 3 - 45             | +      | 5000                                    | 63.3               | 1000   | 15         | 0                  | 15         |
|                    |        |   |                    | 1000   | 6          | 0                  | 6          |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |

Remarks) Final concentration of S9 was 5%, Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 &amp; 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100%

-S9 mix: Without metabolic activation, +S9 mix: With metabolic activation,

Test article precipitation in the culture medium was observed at 625 µg/mL and greater at the start and end of test article treatment.

No significant difference was noted when compared with the negative control group using the chi-square test (p&lt;0.05).

**Table 3-3 Results of chromosomal aberration test (short-term treatment, 21h recovery, Volunteer B)**

Test article: D21-2393

| Treatment time (h) | S9 mix | Dose ( $\mu\text{g/mL}$ )               | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |            |
|--------------------|--------|---|--------------------|--|------------|--------------------|------------|
|                    |        |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total      |
| 3 - 21             | -      | Negative control<br>Water for injection | 100                | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |
| 3 - 21             | -      | 1250                                    | 90.6               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 26         | 0                  | 26         |
|                    |        |   |                    | 2000   | 36         | 0                  | 36 ( 1.8 ) |
| 3 - 21             | -      | 2500                                    | 86.6               | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 2000   | 20         | 0                  | 20 ( 1.0 ) |
| 3 - 21             | -      | 5000                                    | 82.7               | 1000   | 8          | 0                  | 8          |
|                    |        |   |                    | 1000   | 20         | 0                  | 20         |
|                    |        |   |                    | 2000   | 28         | 0                  | 28 ( 1.4 ) |
| 3 - 21             | +      | Negative control<br>Water for injection | 100                | 1000   | 14         | 0                  | 14         |
|                    |        |   |                    | 1000   | 7          | 0                  | 7          |
|                    |        |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 ) |
| 3 - 21             | +      | 1250                                    | 91.4               | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 2000   | 22         | 0                  | 22 ( 1.1 ) |
| 3 - 21             | +      | 2500                                    | 77.6               | 1000   | 17         | 0                  | 17         |
|                    |        |   |                    | 1000   | 18         | 0                  | 18         |
|                    |        |   |                    | 2000   | 35         | 0                  | 35 ( 1.8 ) |
| 3 - 21             | +      | 5000                                    | 67.2               | 1000   | 15         | 0                  | 15         |
|                    |        |   |                    | 1000   | 4          | 0                  | 4          |
|                    |        |   |                    | 2000   | 19         | 0                  | 19 ( 1.0 ) |

Remarks) Final concentration of S9 was 5%, Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 &amp; 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100%

-S9 mix: Without metabolic activation, +S9 mix: With metabolic activation,

Test article precipitation in the culture medium was observed at 625  $\mu\text{g/mL}$  and greater at the start and end of test article treatment.No significant difference was noted when compared with the negative control group using the chi-square test ( $p < 0.05$ ).

**Table 3-4 Results of chromosomal aberration test (short-term treatment, 45h recovery, Volunteer B)**

Test article: D21-2393

| Treatment time (h) | S9 mix | Dose (µg/mL)                            | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |            |
|--------------------|--------|---|--------------------|--|------------|--------------------|------------|
|                    |        |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total      |
| 3 - 45             | -      | Negative control<br>Water for injection | 100                | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 1000   | 17         | 0                  | 17         |
|                    |        |   |                    | 2000   | 28         | 0                  | 28 ( 1.4 ) |
| 3 - 45             | -      | 1250                                    | 92.9               | 1000   | 12         | 0                  | 12         |
|                    |        |   |                    | 1000   | 23         | 0                  | 23         |
|                    |        |   |                    | 2000   | 35         | 0                  | 35 ( 1.8 ) |
| 3 - 45             | -      | 2500                                    | 77.0               | 1000   | 9          | 0                  | 9          |
|                    |        |   |                    | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 2000   | 19         | 0                  | 19 ( 1.0 ) |
| 3 - 45             | -      | 5000                                    | 72.6               | 1000   | 7          | 0                  | 7          |
|                    |        |   |                    | 1000   | 9          | 0                  | 9          |
|                    |        |   |                    | 2000   | 16         | 0                  | 16 ( 0.8 ) |
| 3 - 45             | +      | Negative control<br>Water for injection | 100                | 1000   | 13         | 0                  | 13         |
|                    |        |   |                    | 1000   | 4          | 0                  | 4          |
|                    |        |   |                    | 2000   | 17         | 0                  | 17 ( 0.9 ) |
| 3 - 45             | +      | 1250                                    | 92.7               | 1000   | 11         | 0                  | 11         |
|                    |        |   |                    | 1000   | 9          | 0                  | 9          |
|                    |        |   |                    | 2000   | 20         | 0                  | 20 ( 1.0 ) |
| 3 - 45             | +      | 2500                                    | 80.7               | 1000   | 13         | 0                  | 13         |
|                    |        |   |                    | 1000   | 15         | 0                  | 15         |
|                    |        |   |                    | 2000   | 28         | 0                  | 28 ( 1.4 ) |
| 3 - 45             | +      | 5000                                    | 72.5               | 1000   | 16         | 0                  | 16         |
|                    |        |   |                    | 1000   | 10         | 0                  | 10         |
|                    |        |   |                    | 2000   | 26         | 0                  | 26 ( 1.3 ) |

Remarks) Final concentration of S9 was 5%, Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 &amp; 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100%

-S9 mix: Without metabolic activation, +S9 mix: With metabolic activation,

Test article precipitation in the culture medium was observed at 625 µg/mL and greater at the start and end of test article treatment.

No significant difference was noted when compared with the negative control group using the chi-square test (p&lt;0.05).

**Table 4-1 Results of chromosomal aberration test (continuous treatment, Volunteer A)**

Study No. SBL314-151

Test article: D21-2393

| Treatment time (h) | Dose (µg/mL)                            | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |              |
|--------------------|---|--------------------|--|------------|--------------------|--------------|
|                    |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total        |
| 24 - 0             | Negative control<br>Water for injection | 100                | 1000   | 6          | 0                  | 6            |
|                    |   |                    | 1000   | 20         | 0                  | 20           |
|                    |   |                    | 2000   | 26         | 0                  | 26 ( 1.3 )   |
| 24 - 0             | 1250                                    | 90.1               | 1000   | 5          | 0                  | 5            |
|                    |   |                    | 1000   | 12         | 0                  | 12           |
|                    |   |                    | 2000   | 17         | 0                  | 17 ( 0.9 )   |
| 24 - 0             | 2500                                    | 70.3               | 1000   | 6          | 0                  | 6            |
|                    |   |                    | 1000   | 6          | 0                  | 6            |
|                    |   |                    | 2000   | 12         | 0                  | 12 ( 0.6 )   |
| 24 - 0             | 5000                                    | 57.1               | 1000   | 7          | 0                  | 7            |
|                    |   |                    | 1000   | 0          | 0                  | 0            |
|                    |   |                    | 2000   | 7          | 0                  | 7 ( 0.4 ) *  |
| 24 - 0             | Positive control<br>NOS<br>120          | 56.0               | 1000   | 312        | 0                  | 312          |
|                    |   |                    | 1000   | 243        | 1                  | 244          |
|                    |   |                    | 2000   | 555        | 1                  | 556 ( 27.8 ) |
| 48 - 0             | Negative control<br>Water for injection | 100                | 1000   | 11         | 0                  | 11           |
|                    |   |                    | 1000   | 15         | 0                  | 15           |
|                    |   |                    | 2000   | 26         | 0                  | 26 ( 1.3 )   |
| 48 - 0             | 625                                     | 77.8               | 1000   | 14         | 0                  | 14           |
|                    |   |                    | 1000   | 13         | 0                  | 13           |
|                    |   |                    | 2000   | 27         | 0                  | 27 ( 1.4 )   |
| 48 - 0             | 1250                                    | 69.8               | 1000   | 7          | 0                  | 7            |
|                    |   |                    | 1000   | 10         | 0                  | 10           |
|                    |   |                    | 2000   | 17         | 0                  | 17 ( 0.9 )   |
| 48 - 0             | 2500                                    | 39.7               | 1000   | 6          | 0                  | 6            |
|                    |   |                    | 1000   | 2          | 0                  | 2            |
|                    |   |                    | 2000   | 8          | 0                  | 8 ( 0.4 ) *  |
| 48 - 0             | Positive control<br>NOS<br>120          | 56.3               | 1000   | 335        | 0                  | 335          |
|                    |   |                    | 1000   | 282        | 0                  | 282          |
|                    |   |                    | 2000   | 617        | 0                  | 617 ( 30.9 ) |

Remarks) Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 &amp; 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100% (mean of 2 dishes)

NOS: Noscapine hydrochloride hydrate

Test article precipitation in the culture medium was observed at 156 µg/mL and greater at the start and 2500 µg/mL and greater at the end of test article treatment.

\*: Significant decrease was noted when compared with the negative control group by chi-square test (p&lt;0.05).

Dose-dependent decrease was noted in Cochran-Armitage trend test.

**Table 4-2 Results of chromosomal aberration test (continuous treatment, Volunteer B)**

Study No. SBL314-151

Test article: D21-2393

| Treatment time (h) | Dose ( $\mu\text{g/mL}$ )               | Mitotic index (%)# | No. of cells with numerical aberrations (incidence%) |            |                    |              |
|--------------------|---|--------------------|--|------------|--------------------|--------------|
|                    |   |                    | No. of cells observed                                | Polyploidy | Endo-reduplication | Total        |
| 24 - 0             | Negative control<br>Water for injection | 100                | 1000   | 6          | 0                  | 6            |
|                    |   |                    | 1000   | 15         | 0                  | 15           |
|                    |   |                    | 2000   | 21         | 0                  | 21 ( 1.1 )   |
| 24 - 0             | 1250                                    | 82.1               | 1000   | 9          | 0                  | 9            |
|                    |   |                    | 1000   | 13         | 0                  | 13           |
|                    |   |                    | 2000   | 22         | 0                  | 22 ( 1.1 )   |
| 24 - 0             | 2500                                    | 70.8               | 1000   | 8          | 0                  | 8            |
|                    |   |                    | 1000   | 9          | 0                  | 9            |
|                    |   |                    | 2000   | 17         | 0                  | 17 ( 0.9 )   |
| 24 - 0             | 5000                                    | 64.2               | 1000   | 7          | 0                  | 7            |
|                    |   |                    | 1000   | 4          | 0                  | 4            |
|                    |   |                    | 2000   | 11         | 0                  | 11 ( 0.6 )   |
| 24 - 0             | Positive control<br>NOS<br>120          | 58.5               | 1000   | 406        | 0                  | 406          |
|                    |   |                    | 1000   | 412        | 0                  | 412          |
|                    |   |                    | 2000   | 818        | 0                  | 818 ( 40.9 ) |
| 48 - 0             | Negative control<br>Water for injection | 100                | 1000   | 11         | 0                  | 11           |
|                    |   |                    | 1000   | 17         | 0                  | 17           |
|                    |   |                    | 2000   | 28         | 0                  | 28 ( 1.4 )   |
| 48 - 0             | 625                                     | 62.9               | 1000   | 9          | 0                  | 9            |
|                    |   |                    | 1000   | 18         | 0                  | 18           |
|                    |   |                    | 2000   | 27         | 0                  | 27 ( 1.4 )   |
| 48 - 0             | 1250                                    | 47.0               | 1000   | 10         | 0                  | 10           |
|                    |   |                    | 1000   | 3          | 0                  | 3            |
|                    |   |                    | 2000   | 13         | 0                  | 13 ( 0.7 )   |
| 48 - 0             | 2500                                    | 39.4               | 1000   | 8          | 0                  | 8            |
|                    |   |                    | 1000   | 8          | 0                  | 8            |
|                    |   |                    | 2000   | 16         | 0                  | 16 ( 0.8 )   |
| 48 - 0             | Positive control<br>NOS<br>120          | 58.3               | 1000   | 370        | 0                  | 370          |
|                    |   |                    | 1000   | 259        | 0                  | 259          |
|                    |   |                    | 2000   | 629        | 0                  | 629 ( 31.5 ) |

Remarks) Treatment time: Treatment time - recovery time

The data of each plate per dose fill in line 1 &amp; 2, and the total in line 3. #: Relative values when mitotic index in the negative control group was taken as 100% (mean of 2 dishes)

NOS: Noscaphine hydrochloride hydrate

Test article precipitation in the culture medium was observed at 156  $\mu\text{g/mL}$  and greater at the start and 2500  $\mu\text{g/mL}$  and greater at the end of test article treatment.No significant difference was noted when compared with the negative control group using the chi-square test ( $p < 0.05$ ).

(b) (4) **AND EVALUATION**

DU-176b had no effect on collagen-, U46619-, and ADP-induced platelet aggregation, but inhibited thrombin-induced platelet aggregation (the mean IC<sub>50</sub> was 2.90 μM) in a pharmacology study submitted in amendment serial # 098 (dated March 22, 2007).

In amendment serial # 103, the sponsor submitted metabolism studies in male rats and monkeys after single oral administration of <sup>14</sup>C-DU-176b. D21-2393 was the major metabolite observed in rat plasma at 1 and 4 hours post dose. D21-2393 was not detected and there were no major metabolites (contributing at least 10% of the total detected radioactivity) observed in monkey plasma at 2 and 4 hours post dose. D21-2393 was not detected in monkey urine and feces or rat urine, feces bile, liver, or kidney samples. Approximately 25% and 64% of the total administered radioactivity was collected in rat urine and feces, respectively. In bile cannulated rats, approximately 25% of the administered radioactivity was excreted into the bile. Approximately 42% and 54% of the total administered radioactivity was collected in monkey urine and feces, respectively. DU-176b accounted for the majority of radioactivity in all samples (plasma, urine, feces, or bile) for both rat and monkey.

In amendment serial # 201, the sponsor submitted genotoxicity studies performed on D21-2393 (the major human metabolite of DU-176b). D21-2393 was not genotoxic in the rat single-dose or 14-day repeat dose micronucleus assay, or the polyploidy human lymphocyte assay. (b) (4)

In amendment serial # 203 (dated September 02, 2008) the sponsor submitted an attachment summarizing their interpretation (risk assessment) of the data obtained from the genetic toxicology studies performed on D21-2393. In their risk assessment, the sponsor concluded that D21-2393 did not show any mutagenic potential in the Ames test or in single-dose and 14-day repeat-dose rat micronucleus assays. The sponsor stated that D21-2393 induced numerical chromosomal aberrations in Chinese Hamster Lung (CHL) cells at concentrations of 1250 μg/mL and greater. (b) (4)

However, there are potential problems with the Sponsor's risk assessment. Although we agree that D21-2393 was not genotoxic in the Ames test (previously reviewed) or in single-dose and 14-day repeat-dose rat micronucleus assays, the CHL cell genotoxicity study has not yet been submitted and, as mentioned in this review, there are potential problems with the human lymphocyte polyploidy assay. (b) (4)

## RECOMMENDATIONS

1. [REDACTED] <sup>(b) (4)</sup>  
[REDACTED] The Sponsor should evaluate D21-2393 in an in vitro cytogenetic assay using a vehicle where D21-2393 is in solution, [REDACTED] <sup>(b) (4)</sup> (solubility is an acceptable limiting factor for high dose as long as the Sponsor demonstrates that they are using the vehicle that provides the maximum feasible dose), when added to cultures. That assay should include positive controls for both the presence and absence of S9-mix. The Sponsor should also submit the D21-2393 CHL cell genotoxicity study report (a risk assessment for D21-2393 cannot be completed until the above studies are completed, submitted, and reviewed by FDA).

Linked Applications

Sponsor Name

Drug Name / Subject

-----  
IND 63266

-----  
DAIICHI SANKYO INC

-----  
DU-176B

-----  
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/s/  
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RONALD HONCHEL

03/23/2009

ADEBAYO A LANIYONU

03/24/2009

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/s/  
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BAICHUN YANG  
08/11/2014

THOMAS PAPOIAN  
08/12/2014  
Concur.

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA or Supplement

**NDA Number: 206316**

**Applicant: Daiichi Sankyo Inc.**

**Stamp Date: 1/8/2014**

**Drug Name: Savaysa™  
(edoxaban) tablets**

**NDA Type: 505(b)(1)**

On **initial** overview of the NDA application for filing:

|   | Content Parameter  | Yes | No | Comment |
|---|--|-----|----|---------|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?  | Yes |    |         |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?   | Yes |    |         |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin?   | Yes |    |         |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?        | Yes |    |         |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). |     |    | N/A     |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?   | Yes |    |         |
| 7 | Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?  | Yes |    |         |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?  | Yes |    |         |

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA or Supplement**

|    | <b>Content Parameter</b>  | <b>Yes</b> | <b>No</b> | <b>Comment</b> |
|----|---|------------|-----------|----------------|
| 9  | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57? | Yes        |           |                |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)   | Yes        |           |                |
| 11 | Has the applicant addressed any abuse potential issues in the submission?   |            |           | N/A            |
| 12 | If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?   |            |           | N/A            |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Baichun Yang 1/22/2014  
 Reviewing Pharmacologist (DCRP) Date

Shwu-Luan Lee 1/22/2014  
 Reviewing Pharmacologist (DHOT) Date

Thomas Papoian 1/22/2014  
 Team Leader/Supervisor (DCRP) Date

Haleh Saber 1/22/2014  
 Team Leader/Supervisor (DHOT) Date

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/s/  
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BAICHUN YANG  
01/22/2014

SHWU LUAN LEE  
01/24/2014

HALEH SABER  
01/24/2014

THOMAS PAPOIAN  
01/24/2014  
Concur.