CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208684Orig1s000
208685Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 208684 and 208685
Submission date: June 9, 2016
Drug: deflazacort
Applicant: Marathon Pharmaceuticals
Indication: Duchenne Muscular Dystrophy

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA.

The primary reviewer concluded that additional information was needed on major human metabolites to support approval. If the drug was approved without this information, the reviewer recommended that such information be collected as post-marketing requirements. The supervisor agreed that additional information was needed on the major human metabolites but concluded that the information could be collected post-marketing. Information needed includes an in vivo metabolic profile in humans and genotoxicity and carcinogenicity data for major human metabolites.

No developmental and reproductive toxicity studies were conducted with deflazacort because the patient population is almost entirely male. Potential effects on male fertility were assessed by histopathological evaluation of male reproductive organs in toxicity studies. No adverse effects were noted.

Carcinogenicity studies
A published rat carcinogenicity study of deflazacort was reviewed. Unusual bone neoplasms were observed. The division recommends describing these findings in labeling. The Division has also recommended that a mouse carcinogenicity study of deflazacort be conducted after approval. The division recommended that the feasibility of such a study should be determined initially.

Conclusions:
I agree that given the indication, the pharmacology/toxicology information is adequate to support approval of this application. The post-marketing requirements for additional information on major human metabolites and the additional carcinogenicity study are reasonable.

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/s/

PAUL C BROWN
02/08/2017

MEMORANDUM

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

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

Date: February 6, 2017

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

Subject: NDAs 208-684 and 208-685 (deflazacort; Emflaza)

NDAs 208-684 (oral tablet) and 208-685 (oral suspension) have been submitted by the sponsor (Marathon Pharmaceuticals) for use of deflazacort as a treatment for Duchenne muscular dystrophy (DMD). The NDAs were received on June 9, 2016, and filed on August 8, 2016; no potential review issues were identified in the August 8, 2016, filing letter. Although originally submitted as 505(b)(1) applications, both NDAs were changed to 505(b)(2) applications (November 16, 2016) based on use of published literature. Clinical development was conducted by Marathon under IND 119258.

The nonclinical studies conducted by the sponsor to support the NDAs consist of the following:

- In vitro pharmacology and ADME study of two deflazacort metabolites (includes in vitro hERG assay)
- Two in vitro metabolism studies
- 14-day dose-ranging toxicity studies of deflazacort in rat and monkey
- Chronic toxicity studies of deflazacort in rat (26-week) and monkey (39-week)
- A standard battery of genetic toxicology (Ames, in vitro chromosomal aberration in human lymphocytes, in vivo rat micronucleus) assays of deflazacort; in vitro genetic toxicology (Ames, chromosomal aberration in human lymphocytes) assays of metabolite, 21-desacetyl-deflazacort.
- Dose-ranging and pivotal 8-week juvenile animal toxicology studies in rat

The nonclinical data were reviewed in detail by Dr. Hawver (see *Pharmacology/Toxicology NDA Review and Evaluation, NDAs 208684 and 208685, David B. Hawver, Ph.D., January 30, 2017*). Based on this review, Dr. Hawver has concluded that the nonclinical data do not support approval of the NDAs, based on the lack of adequate information to determine whether or not two metabolite, identified as major circulating metabolites in humans (6β-OH-21-desacetyl-deflazacort and M-V) in a

published study, "have been adequately assessed for genotoxicity and carcinogenicity or if M-V has been adequately assessed for chronic toxicity." Dr. Hawver states that "If these NDAs are approved despite the insufficient assessment of major metabolites…, the deficiencies should be addressed as post-marketing requirements," specifically:

- In vitro genotoxicity (Ames, in vitro chromosomal aberration) and in vivo (micronucleus) assays and carcinogenicity studies for 6β-OH-21-desacetyl-deflazacort and M-V.
- The in vivo micronucleus assay would not be needed if the two in vitro genotoxicity assays are negative and the carcinogenic potential of M-V is adequately addressed.
- A chronic toxicity study of M-V in one species, unless an adequate carcinogenicity study, which includes an adequate evaluation of M-V, is conducted.

The nonclinical data will be briefly summarized in this memo, with a focus on the metabolite issue. A detailed description and evaluation of all the nonclinical data are in Dr. Hawver's review.

Pharmacology

Deflazacort is an oxazoline derivative of prednisolone. When administered orally, it is rapidly metabolized to the active metabolite, 21-desacetyl-deflazacort (21-desDFZ), which has immunosuppressive and anti-inflammatory effects. Deflazacort is not detectable in circulation in animals or humans.

The sponsor conducted only a single study to assess the primary pharmacology of 21-desDFZ and its metabolite, 6β-OH-21-desacetyl-deflazacort (6β-OH-21-desDFZ). That in vitro study, conducted in IM-9 cell (lymphoblastoid B cells from a patient with multiple myeloma) cytosol, identified binding to the human glucocorticoid receptor, with a K_i of 10 nM for 21-desDFZ; 6β-OH-21-desDFZ exhibited minimal binding (<15%) at concentrations up to 1 μM.

The sponsor cited published literature to illustrate the efficacy of deflazacort in vitro and in the mdx animal model of Duchenne muscular dystrophy (DMD). In vivo, deflazacort promoted myofiber repair, proliferation, and function (e.g., increased peak grip strength) when administered to mdx mice at daily doses of 1.2 mg/kg SC for 4 weeks (Anderson JE et al. Muscle Nerve 19(12):1576-1585, 1996; Anderson JE et al. Cell Transplant 9(4):551-564, 2000); deflazacort had no beneficial effects when administered daily for 4 weeks at 0.67 mg/kg SC. At a dose of 1 mg/kg IP Q2D for up to 3-4 weeks, deflazacort resulted in reduced degenerative changes in skeletal muscle, which was antagonized when administered in combination with cyclosporine, a calcineurin inhibitor (St-Pierre SJG et al. The FASEB J 18(15):1937-1939, Epub 2004 Sep 29). The authors hypothesize that deflazacort may exert therapeutic effects in the mdx mouse through activation of the phosphatase, calcineurin, resulting in retention of NF-ATc1 (a calcineurin-sensitive transcription factor) in the nucleus and, presumably, protection of myofiber integrity.

Deflazacort was also associated with a 2-fold increase in utrophin expression. What, if any, role these factors have in the therapeutic effects of deflazacort in DMD patients is unknown.

No secondary pharmacology studies were conducted by the sponsor.

In the only safety pharmacology study conducted by the sponsor, 21-desDFZ and 6β-OH-21-desDFZ were evaluated in an in vitro hERG assay in CHO-K1 cells. At the highest concentration tested (10 μM), the % inhibition of hERG current was ~8 and 14%, respectively. Therefore, no signal for QT_c prolongation was evident for either compound.

The sponsor cited several published studies of deflazacort in intact mouse or rat, but none provided sufficient information for evaluation.

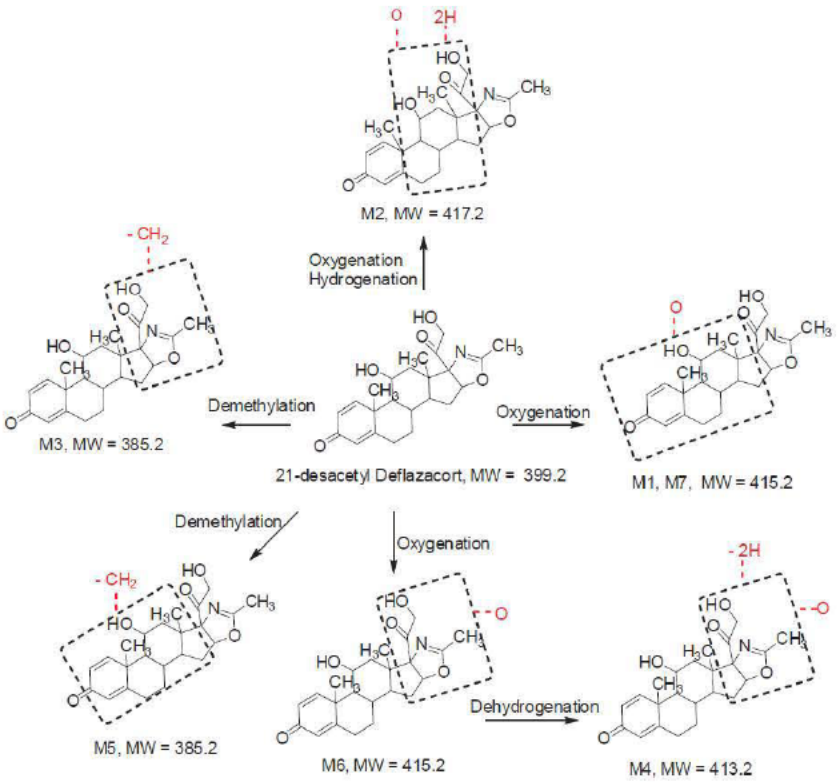
PK/ADME

The sponsor conducted no in vivo studies to evaluate the PK/ADME of deflazacort or 21-desDFZ. In vitro studies of 21-desDFZ were conducted to assess plasma protein binding and metabolic stability. In the in vitro protein binding assay, 21-desDFZ exhibited low binding to human serum albumin and α-1 acid glycoprotein (~55 and 20%, respectively).

The sponsor conducted two in vitro studies to assess the metabolic profile of 21-desDFZ. In Study MP-104-NC-011, 21-desDFZ was incubated with liver microsomes from rat (strain not specified), dog, and human. The extent of metabolism was greatest in human microsomes, with ~83, 85, and 40% 21-desDRZ remaining at the end of the 60-min incubation period.

In Study MP-104-NC-010, 21-desDFZ was incubated with liver microsomes from Sprague-Dawley rat, Beagle dog, cynomolgus monkey, and human. Seven metabolites were formed by human microsomes, with M1 and M7 being the most abundant. Of the seven, only 2 (M1 and M7) were formed in rat and 3 (M1, M2, and M6) were formed in dog. The data are summarized in the following sponsor's table (portions of Table 2) and figure (DMPK Study No NT-20140317-MID):

Species	Metabolites	Mass	Metabolism pathway	Area %
Human	M1	415.1995	Oxygenation	7.79
	M2	417.2151	Hydration	0.23
	M3	385.1889	Demethylation	0.5
	M4	413.1838	Hydroxylation + desaturation	1.42
	M5	385.1889	Demethylation	1.68
	M6	415.1995	Oxygenation	4.49
	Parent	399.2046		74.55
	M7	415.1995	Oxygenation	9.34
Monkey	M1	415.1995	Oxygenation	6.71
	M4	413.1838	Hydroxylation + desaturation	1.68
	M5	385.1889	Demethylation	0.32
	M6	415.1995	Oxygenation	11.79
	Parent	399.2046	Parent	74.71
	M7	415.1995	Oxygenation	4.79
Dog	M1	415.1995	Oxygenation	1.28
	M2	417.2151	Hydration	0.31
	M6	415.1995	Oxygenation	1.17
	Parent	399.2046		97.25
Rat	M1	415.1995	Oxygenation	2.23
	Parent	399.2046		97.45
	M7	415.1995	Oxygenation	0.22



The sponsor cited three published articles on the in vivo metabolic profile of deflazacort in various animal species and human, all from the same laboratory (Research Laboratories, Gruppo Lepetit S.p.A., Milan, Italy).

Martinelli et al. (Drug Metab Disp 7(5):335-339, 1979) administered ^{14}C-deflazacort at an oral dose of 5 mg/kg to male Wistar rats (gavage), male beagle dogs (powder in capsule), and humans (2 M, 1F; capsule). Radioactivity was quantitated in plasma, urine, and/or bile 1-8 hrs post dose. Deflazacort was not detected in any species tested. Five circulating metabolites were detected in rat plasma. Of these, all but M-IV were detected in dog. In humans, M-II (21-desDFZ), M-III (6β-OH-21-desDFZ), and M-V (structure unknown) were detected in plasma; M-IV was detected in urine but not plasma. M-II was the most abundant metabolite in all three species. In human plasma, M-III, and M-V were also major circulating metabolites, each accounting for ≥25% of total radioactivity. Both M-III and M-V were less abundant in rat and dog plasma compared to human plasma. The plasma data are summarized in the following table (portion of Table 1, taken from the publication):

Data represent means ± SE. *N* is shown in parentheses. *n.d.*, not detected.

The authors stated that M-V could not be definitively identified because of the presence of impurities; however, the analysis indicated that M-V (1) was desacetylated, (2) contained an unchanged 16α,17 α-2'-methyloxazoline ring and presence of the 11β-hydroxy function, (3) did not contain the Δ^{1-4} system, and (4) had oxidation "probably in the B-ring."

The proposed vivo metabolic profiles of deflazacort in rat, dog, and human are illustrated in the following figure (taken from the publication):

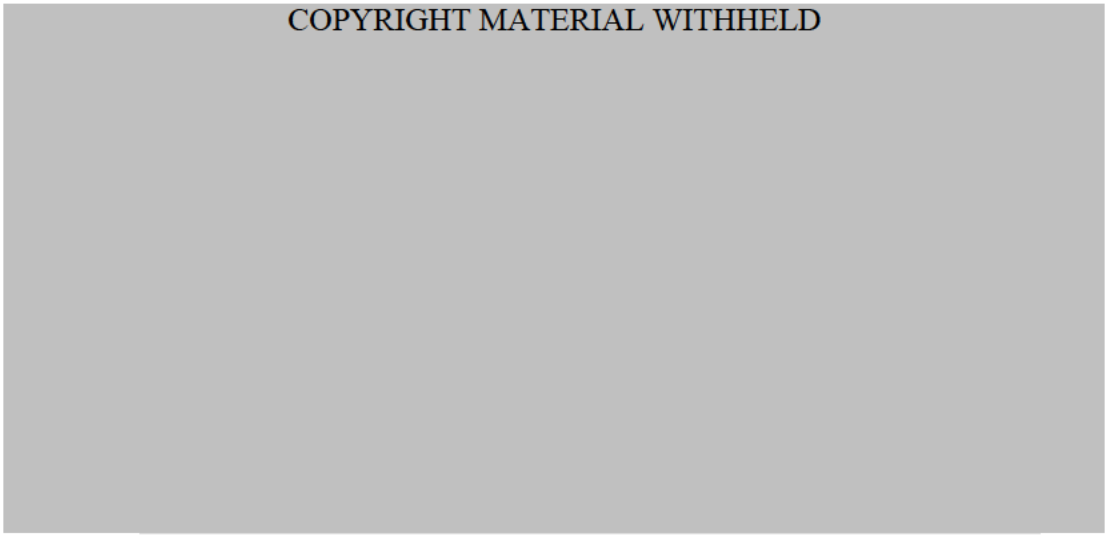
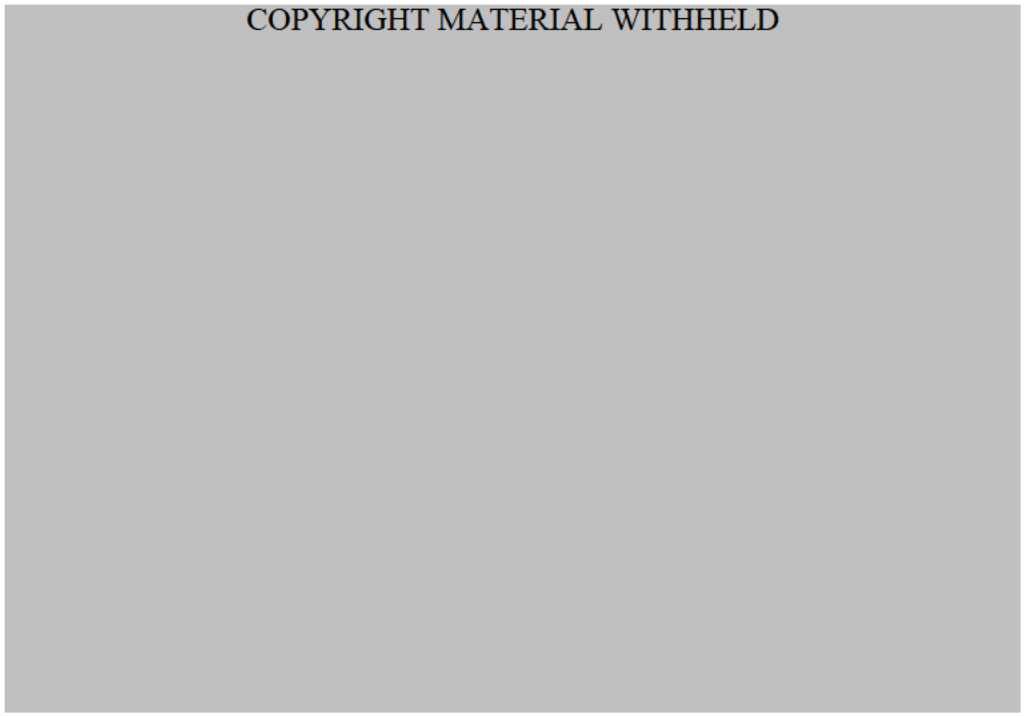
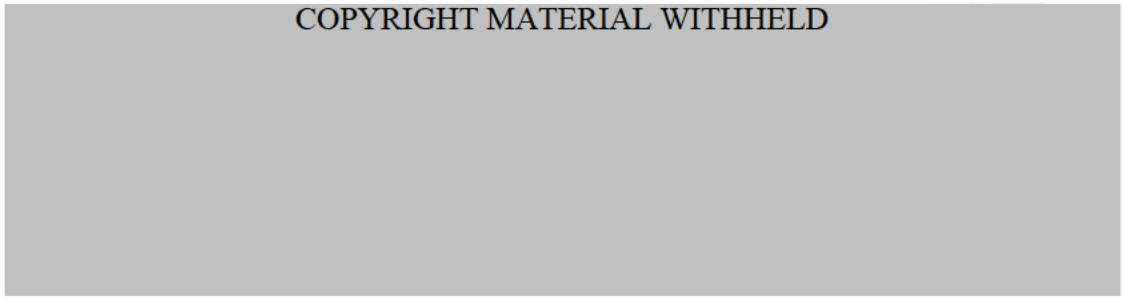


FIG. 1. *Deflazacort and its main unconjugated metabolites in the rat, dog, and man.*

Assandri et al. (Xenobiotica 13(3):185-196, 1983) investigated the in vivo metabolic profile of deflazacort in cynomolgus monkey (the nonrodent species used in the 39-week oral toxicity study conducted by the sponsor). In this study, ^{14}C-deflazacort was administered orally at a dose of 5 mg/kg PO and IV. Each animal received ^{14}C-deflazacort by both routes (one-month washout). Plasma samples were collected for up to 24 hrs post dose. (An additional 4 animals received unlabeled deflazacort [10 mg/kg] on 3 separate occasions and 24-hr urine samples were collected.) Deflazacort was not detected in plasma after PO or IV dosing. The main metabolites, M-II (21-desDFZ) and M-III (6β-OH-21-desDFZ), accounted for 46-32 and 24-27% of total plasma radioactivity, respectively. (Although, in the Discussion section, the authors stated that "(ii) deacetylate deflazacort, metabolite II, up to 24 h accounts for more than 80% of total ^{14}C and (iii) metabolite III, the 6β-hydroxy derivative of II, is the most abundant of the minor ones.") The proposed metabolic profile of deflazacort in monkey is illustrated in the following figure (taken from the publication):



In a subsequent publication, Assandri et al. (Adv Exp Med Biol 171:9-23, 1984) stated that ^{14}C-deflazacort was administered to Wistar rats, Beagle dogs, and cynomolgus monkeys at a dose of 5 mg/kg PO and IV and to healthy male humans at a dose of 50 mg PO. Some of the oral data (e.g., for M-II and M-III) and the oral and IV data for monkey appear to be exactly the same as those reported by Martinelli et al. (1979) or Assandri et al. (1983), although M-V levels in plasma were not provided. "Minor metabolites" were pooled. The only mention of M-V was a statement that in human urine M-V (unidentified) accounted for ~13% of administered dose. The plasma data are summarized in the following table (portion of Table 5, taken from publication):



The proposed in vivo metabolic profiles of deflazacort in rat, dog, monkey, and human are summarized in the following figure (taken from the publication):

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Dr. Hawver has raised concerns regarding M-V, which, according to the data reported by Martinelli et al. (1979), is a major circulating metabolite (as defined by ICH M3, January 2010) in humans but apparently not in the animal species used for the pivotal nonclinical studies. This issue is discussed further under Summary and Conclusions.

Toxicology

Chronic oral toxicity studies of deflazacort in Sprague-Dawley rat (26-week + 4-week recovery) and cynomolgus monkey (39-week) were conducted by the sponsor. Dose selection for these studies was based on 14-day dose-ranging studies in both species.

In rat, deflazacort was administered orally (gavage) at doses of 0, 0.05, 0.15, and 0.50 mg/kg to males and 0, 0.10, 0.30, and 1.0 mg/kg to females daily for 26 weeks. Additional animals were dosed to assess recovery (6/sex for C and HD; 4-week recovery period) and to collect TK data. Dose selection was based on a 14-day dose-ranging study, conducted at deflazacort doses of 0, 0.3, 1.0, and 3.0 mg/kg PO (gavage). The NOAELs in that study were 1.0 mg/kg in males and females (based on body weight loss or reduced body weight gain and reduced lymphocytes at the HD of 3.0 mg/kg).

In the 26-week study, the primary dose-related findings were clinical signs (including skin alopecia and discoloration at the MD and/or HD), reduced body weight gain (all doses), decreases in wbc and lymphocyte cts (all doses), and histopathological changes in

adrenal gland (atrophy), bone marrow (hypocellularity), esophagus and glandular stomach (epithelial atrophy), and skin (epidermal atrophy, follicular atrophy); lymphocyte depletion was evident in numerous tissues (thymus, GI tract, lymph nodes).

Most drug-related effects were partially or fully reversible; however, skin lesions persisted in some HD recovery animals. The lowest doses tested were identified as the NOAELs (0.05 and 0.10 mg/kg in males and females, respectively).

Plasma 21-desDFZ exposure data for Day 179 are summarized in the following table:

DOSE (mg/kg)	MALES		FEMALES	
	C_{max} (ng/mL)	$AUC_{(0\text{-}24\ hr)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0\text{-}24\ hr)}$ (ng*hr/mL)
0.05	21.5	94.7		
0.1			38.4	57.2
0.15	73.1	385		
0.3			101	189
0.5	255	978		
1.0			472	789

In monkey, deflazacort was administered orally (gavage) at doses of 0, 0.03/6.0, 1.0, and 3.0 mg/kg (4/sex/group) daily for 39 weeks. Additional animals were dosed to assess recovery (2/sex for C and MD [3.0 mg/kg]; 6-week recovery period). Dose selection was based on a 14-day dose-ranging study (1/sex/group), conducted at doses of 0, 0.3, 1.0, and 3.0 mg/kg PO (gavage); the NOAEL was 3.0 mg/kg.

In the 39-week study, the LD was increased to 6.0 mg/kg (the new HD) on Day 92 because of the lack of drug-related effects during the first 3 months of dosing. The primary drug-related findings were clinical signs (skin alopecia) and histopathological changes in skin (atrophy of hair follicles), mammary gland (hyperplasia), female reproductive organs (absence of corpora lutea, atrophy of uterus endometrium, vaginal epithelium), bone marrow (increased adipocytes), lymphoid tissue (lymphocyte depletion of lymph nodes), and adrenal gland (atrophy). Microscopic examination of testes in a stage-aware manner revealed no drug-related effects. Although there were no clear effects on circulating lymphocyte subsets, deflazacort decreased the TDAR (evaluated on Day 183 and on Day 15 of the recovery period) response (i.e., reduced levels of anti-KLH IgG and IgM) at 0.3/0.6 mg/kg, which persisted into the recovery period at 3.0 mg/kg (the only dose assessed during recovery).

Dr. Hawver agreed with the sponsor's identification of 6 and 3 mg/kg as NOAELs for 27 and 39 weeks of dosing, respectively. However, the persistent effect of deflazacort on TDAR and the microscopic evidence of lymphocyte depletion suggest a lower NOAEL (1.0 mg/kg) for 39 weeks.

The TK data for 21-desDFZ and 6β-OH-21-desDFZ on Day 271 are summarized in the following table:

DOSE* (mg/kg)	MALES		FEMALES	
	C_{max} (ng/mL)	$AUC_{(0\text{-}24\ hr)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0\text{-}24\ hr)}$ (ng*hr/mL)
21-desDFZ				
0.3/6	760 ± 338	2470 ± 793	1920 ± 270	3660 ± 468
1	168 ± 79.6	539 ± 93.8	145 ± 26.1	543 ± 15.8
3	554 ± 260	1460 ± 397	826 ± 502	1810 ± 769
6β-OH-21-desDFZ				
0.3/6	462 ± 134	2240 ± 676	862 ± 151	2430 ± 332
1	109 ± 33.6	485 ± 108	88.5 ± 17.8	419 ± 44.4
3	303 ± 102	1200 ± 291	436 ± 138	1270 ± 494

*In Group 2 (LD), the original dose (0.3 mg/kg) was increased to 6 mg/kg on Day 92.

Reproductive and Developmental Toxicology

Standard reproductive and development toxicology studies were not required or conducted for deflazacort because the DMD patient population is almost exclusively male. A juvenile animal toxicology study was conducted to assess the potential for adverse effects of deflazacort during postnatal development to support direct administration to DMD patients <12 years of age.

In the pivotal juvenile animal toxicology study, deflazacort was administered orally (gavage) to Sprague-Dawley rats at doses of 0, 0.1, 0.3, and 1.0 mg/kg QD from PND 21 to PND 80 (20/sex/group), followed by an 8-week recovery period (20/sex for C and HD groups). Dose selection was based on the results of a dose-ranging study in Sprague-Dawley rats administered deflazacort by oral gavage at doses of 0, 0.3, 1, and 3 mg/kg from PND 21 to PND 35. The primary drug-related effect was a decrease in body weight gain at all doses; the NOAEL was <0.3 mg/kg.

In the pivotal study, dose-related decreases in body weight (relative to C) and body weight gain were observed at all doses in males (≥22%) and females (≥9%), associated with dose-related decreases in food consumption. Hematological findings consisted mainly of dose-related decreases in platelets, wbc ct, and lymphocyte ct at all doses in males and at the MD and HD in females; reversibility was evident at the HD. Clinical signs and neurobehavioral effects were observed at all but the LD and consisted of:

- Stereotypy, abnormal respiration, and reduced grip strength and landing foot splay in MD and HD males and/or females, which persisted throughout the recovery period at the HD.
- Increases in motor activity in MD and HD males, which persisted into the recovery period.
- Decreases in acoustic startle response in HD males, which were not observed during recovery.
- Impaired performance on the Morris Water maze in HD males, which improved when tested during the recovery period; however, the same animals were tested

during the dosing and recovery periods, potentially confounding the results due to repeated testing.

There were no drug-related effects on sperm concentration or motility; sperm morphology was not assessed. Bone loss (decreases in bone mineral content and density), reduced bone length and diameter, and redistribution of bone from cortical to central regions were observed at all doses in males and in HD females. Although some reversibility was noted, bone effects generally persisted during the recovery period at the HD in both males and females. Histopathology findings consisted of adrenal gland atrophy and decreased cellularity of thymus, spleen (white pulp), and lymph nodes at all doses in males and in HD females. Mammary gland atrophy was detected only in males but at all doses. Examination of testes in a stage-aware manner and enhanced neurohistopathological evaluation revealed no adverse effects. No drug-related microscopic changes were detected in recovery animals, suggesting reversibility.

Toxicokinetic analysis indicated no detectable deflazacort in plasma. Plasma 21-desDFZ exposure data (PND 80) are summarized in the following table:

DOSE (mg/kg)	**MALES**		**FEMALES**	
	C_{max} (ng/mL)	**$AUC_{(0-t)}$ (ng*hr/mL)**	**C_{max} (ng/mL)**	**$AUC_{(0-t)}$ (ng*hr/mL)**
0.1	63.2 ± 8.38	167 ± 7.06	15.0 ± 2.21	23.2 ± 2.55
0.3	155 ± 7.16	448 ± 25.2	50.1 ± 11.0	68.7 ± 11.6
1	680 ± 47.0	1340 ± 72.1	243 ± 71.9	640 ± 64.7

The higher plasma exposures in males are generally consistent with the greater toxicity observed, compared to females.

According to Dr. Hawver, an NOAEL was not identified in males, based on adverse effects on body weight, hematology, and bone, and microscopic changes at all doses. In females, the NOAEL was the LD of 0.1 mg/kg, based on findings at higher doses similar, but less severe than those observed in males. The sponsor identified the HD as the NOAEL in both males and females, noting that "There were no unexpected toxicities noted across any study endpoint that were inconsistent with deflazacort's primary pharmacology and consistent with this class of corticosteroids." However, drug-related effects may be adverse, whether or not they represent a drug's primary pharmacology. The main developmental findings, as described by Dr. Hawver, should be included in labeling.

Genetic Toxicology

The sponsor conducted a standard battery of in vitro (Ames and chromosomal aberration in human lymphocytes, with and without metabolic activation [Sprague-Dawley rat S9]) and in vivo (Sprague-Dawley rat micronucleus) assays of deflazacort and in vitro (Ames, chromosomal aberration in human lymphocytes) assays of 21-desDFZ. Dr. Hawver concluded that these assays were adequate by design and conduct and negative for genotoxicity.

Carcinogenicity

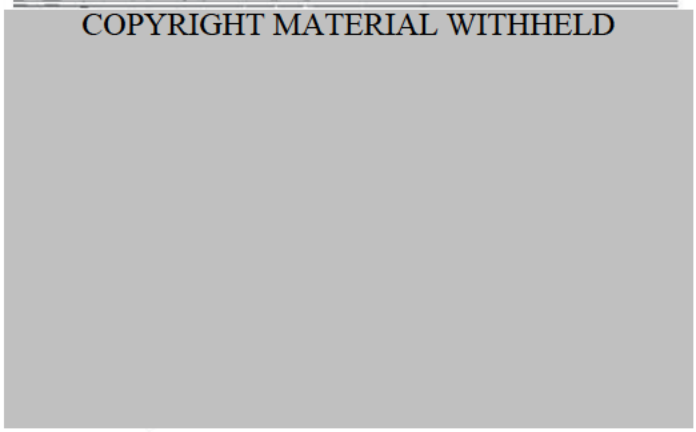
The sponsor has not conducted carcinogenicity studies of deflazacort. During clinical development, the Division agreed that these studies may be conducted post-approval, if deflazacort were to be approved. The sponsor provided the results of a 2-year carcinogenicity study in Sprague-Dawley rat (Zwicker GM, Eyster RC. Toxicol Pathol 24(2):246-250, 1996) and a request for waiver of additional carcinogenicity studies.

Zwicker and Eyster (1996) conducted a 1-year chronic toxicity study and a 2-year carcinogenicity study in rat, each at oral (dietary) doses of 0, 0.03, 0.06, 0.12, 0.25, 0.50, and 1.0 mg/kg; 50/sex/group were evaluated in each study. A complete necropsy was conducted, which included "collection of all gross bone lesions, a portion of sternum for bone marrow, a portion of femur, and cervical, thoracic, and lumbar portions of vertebral column." There is no statement that other tissues were examined microscopically. Also, no TK data were collected.

The authors reported no bone neoplasms in the 1-year study or during the first year of the 2-year study. However, a number of bone neoplasms were detected during the second year of the 2-year study, all in males at 0.25 mg/kg. Data were not available at higher doses because of a marked decrease in survival at 0.5 and 1.0 mg/kg.

The incidence of bone neoplasms and cause of death in males are presented in the following table (taken from the publication):

TABLE I.—Incidence of proliferative bone lesions found at necropsy—cause of morbidity in aged Sprague-Dawley rats



The osteoma of the head in one male at 0.06 mg/kg and the osteosarcoma of the head detected in one female at 1 mg/kg were considered incidental because of the lack of dose-response.

The mechanism underlying the bone tumors is unknown. Glucocorticoids are more often associated with increased bone resorption or decreased bone formation. However, bone tumors in animals are rare and the dose-related increase observed in the 2-year study cannot be dismissed. The authors stated that "…mice in a parallel 2-yr glucocorticoid

[presumed to be deflazacort] oncogenicity study were not affected; however, no other information was provided for that study.

Regarding the sponsor's request for waiver of carcinogenicity studies of deflazacort, Dr. Hawver agreed with the sponsor's conclusion that a new carcinogenicity study in rat would not provide additional meaningful data, based primarily on the inability to achieve doses (mg/m^2) or plasma exposures in rat >5-20% of that in humans because of excessive body weight effects in rat. However, the bone neoplasms, which Dr. Hawver recommends be (and the sponsor has) included in labeling, were observed at similarly low doses (i.e., the effect-dose was 4.4% of the recommended human dose of 0.9 mg/kg on a mg/m^2 basis). Therefore, it is difficult to conclude that testing of low drug exposures cannot provide useful information. However, considering the positive findings in the published rat carcinogenicity study, I agree that it is unnecessary to require another study.

Dr. Hawver has recommended that the requirement for a mouse carcinogenicity study be "deferred" until the results of a dose-ranging study to assess the feasibility of such a study are available. I would suggest that a mouse carcinogenicity study be a post-marketing requirement (PMR); if the sponsor submits dose-ranging data documenting that a carcinogenicity study (either a 26-week study in an appropriate transgenic animal model or a 2-year study) is infeasible, then the PMR can be released.

Summary and Conclusions

The sponsor has conducted a limited battery of nonclinical studies to support the NDAs for deflazacort. Based on his review, Dr. Hawver has concluded that the studies conducted by the sponsor, although consistent with the Division's recommendations provided during clinical development, are not adequate to support approval, from a nonclinical standpoint, because of the lack of safety data for two major metabolites, 6β-OH-21-desDFZ and M-V.

6β-OH-21-desDFZ: There are sufficient data to document that in all animal species tested and human, deflazacort is rapidly and completed metabolized to the active moiety, 21-desDFZ, and that 21-desDFZ is further metabolized to a number of other metabolites, with 6β-OH-21-desDFZ being a major circulating metabolite in humans and in cynomolgus monkey.

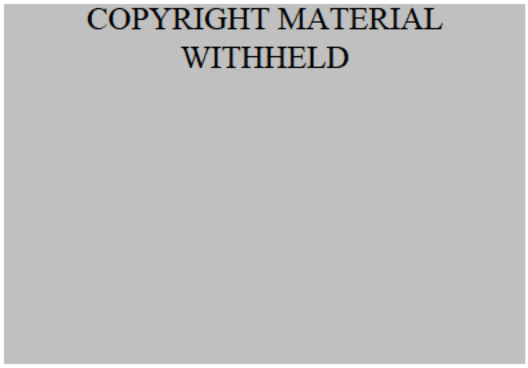
In three clinical studies (MP-104-CL-005, -026, and -058) in which 21-desDFZ and 6β-OH-21-desDFZ were both quantitated in plasma of healthy volunteers (36 mg) or pediatric patients (0.9 mg/kg QD for 8 days), levels of 6β-OH-21-desDFZ were ~70-130% those of 21-desDFZ. In pediatric patients, plasma AUC_{tau} for 21-desDFZ was 450 ± 254 ng*hr/mL in children and 622 ± 353 ng*hr/mL in adolescents; for 6β-OH-21-desDFZ, the values were 358 ± 166 ng*hr/mL in children and 417 ± 168 ng*hr/mL in adolescents. Plasma 6β-OH-21-desDFZ exposures achieved in the 39-week monkey study were up to almost 4 times the highest mean values in pediatric patients. Therefore, as Dr. Hawver notes, the 39-week monkey study provides an adequate evaluation of the chronic toxicity of metabolite 6β-OH-21-desDFZ.

In vitro genetic toxicology studies were conducted to directly assess the genotoxic potential of 21-desDFZ, as well as deflazacort itself. These studies were conducted using Sprague-Dawley rat S9 as the metabolic activation system. The sponsor has provided no data documenting sufficient formation of 6β-OH-21-desDFZ in these assays or in the in vivo micronucleus assay in Sprague-Dawley rat. The available data actually suggest minimal metabolism of 21-desDFZ to 6β-OH-21-desDFZ in either Wistar or Sprague-Dawley rat. Based on these data, it is also unlikely that 6β-OH-21-desDFZ was adequately assessed in the published 2-year carcinogenicity study in Sprague-Dawley rat. Therefore, I agree with Dr. Hawver that there are insufficient data to conclude that the genotoxic and carcinogenic potential of the known major human metabolite, 6β-OH-21-desDFZ, has been adequately assessed.

The potential for 6β-OH-21-desDFZ to have adverse effects on postnatal development in juvenile animals may also not have been adequately assessed. The completed juvenile animal study was conducted in Sprague-Dawley rat (dosing initiated on PND 21, equivalent to a 2 year old child), in which the extent of systemic exposure to 6β-OH-21-desDFZ was not documented. (b) (4)

M-V: Dr. Hawver's concerns regarding metabolite M-V are based on the data from Martinelli et al. (1979) indicating that M-V (structure not identified) accounts for ~25% of total plasma radioactivity in humans after a 50 mg oral dose, i.e., M-V is a major circulating metabolite. Subsequent publications from the same laboratory (Assandri et al. 1983; Assandri et al. 1984) provide no further evidence to support the original report. In fact, Assandri et al. (1984) presented no plasma data for M-V, even though the plasma data for M-II (21-desDFZ) and M-III (6β-OH-21-desDFZ) following oral administration were exactly the same as those reported by Martinelli et al. (1979). Instead, Assandri et al. (1984) combined the "minor metabolites," which were designated as "nX." The authors noted only that M-V in urine accounted for 13% of dose. In the in vitro metabolism study of 21-desDFZ conducted by the sponsor, 7 metabolites were detected but none accounted for >10% of total metabolites. The most abundant metabolite (M7) accounted for 9.34%. Based on the structures provided for the metabolites, none (including M7) was 6β-OH-21-desDFZ.

A more recent published study (Huber EW, Barbuch RJ Xenobiotica 25(2):175-183, 1995) isolated M-V from 24-hr urine samples collected from 24 "patients" administered a single oral dose of deflazacort (23 mg). Using NMR, mass, and infrared spectroscopy, the authors proposed M-V to be (1β,2β,3β,11β,16β)-1,2-epoxy-3,11,21-trihydroxy-2'-methyl-5H'-pregn-4-eno[17,16-d]oxazol-20-one, with a molecular weight of 417 (structure taken from the publication):



This does not match any of the structures previously identified as potential metabolites of 21-desDFZ. Also, formation of an epoxide metabolite, including 1,2-epoxides, would raise potential safety concerns (e.g., Ehrenberg L, Hussain S. Mutation Res/Review Genetic Tox 86(1):1-113, 1981). The authors identified M-V as a major deflazacort metabolite in humans, but only based on the data from Martinelli et al. (1979) and Assandri et al. (1984); plasma samples were not collected.

Overall, based on the available data, there appears to be a general lack of sufficient information on the in vivo metabolic profile of deflazacort in humans or animals. The data consistently demonstrate the lack of circulating intact deflazacort and the presence of two metabolites, 21-desDFZ and 6β-OH-21-desDFZ, in plasma of humans and animals. However, the structure of M-V, whether or not M-V represents one or multiple compounds, or whether or not there are additional major circulating metabolites in humans is uncertain. Without a clearer understanding of the in vivo metabolic profile in humans, it seems premature to make a decision on what, if any, additional nonclinical studies are needed to fully evaluate the safety of deflazacort. Therefore, I would suggest that the sponsor be required to provide additional data on the in vivo metabolic profile of deflazacort in humans, either by analyzing available plasma samples or conducting additional studies. When such data become available, a determination can be made on the need for additional nonclinical safety assessment.

Recommendations

Considering the severity of the indication and the lack of effective therapy, I believe the available nonclinical data are sufficient to support approval of deflazacort for DMD, with appropriate labeling. Studies to further evaluate the in vivo metabolic profile of deflazacort, as well as the genetic toxicology assays and carcinogenicity study previously discussed, may be conducted post-approval (as PMRs).

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/s/

LOIS M FREED
02/06/2017

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:	208684 (Deflazacort Oral Tablets)
	208685 (Deflazacort Oral Suspension)
Supporting document/s:	1, 19 (NDA 208684)
Applicant's letter date:	June 9, 2016
CDER stamp date:	June 9, 2016
Product:	Deflazacort
Indication:	Duchenne Muscular Dystrophy
Applicant:	Marathon Pharmaceuticals, LLC
Review Division:	Neurology Products
Reviewer:	David B. Hawver, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Laurie Kelley, PA-C

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1 Executive Summary

1.1 Introduction

Deflazacort, a derivative of the corticosteroid, prednisone, is a prodrug whose major active metabolite, 21-desacetyldeflazacort (21-desDFZ), is a glucocorticoid receptor agonist. Deflazacort has been marketed as an anti-inflammatory and immunosuppressive agent in countries outside the U.S. since 1982 for various conditions but not Duchenne Muscular Dystrophy (DMD). Deflazacort is not currently approved for any indication in the U.S. The recommended clinical dose of deflazacort for the treatment of DMD is 0.9 mg/kg/day via oral tablet or oral suspension.

1.2 Brief Discussion of Nonclinical Findings

Pharmacological studies have demonstrated that 21-desDFZ binds and activates cytosolic glucocorticoid receptors, triggering changes in gene transcription that lead to effects on muscle injury and repair, immunosuppressive and anti-inflammatory effects in multiple tissues, and effects on bone and cartilage.

In published studies, deflazacort was reported to reduce damage to skeletal muscles in the mdx mouse model of DMD, including reductions in myofiber calcification in the diaphragm, in degeneration in the TA, and in fibrosis in the myocardium; and increases in the weight of the gastrocnemius and quadriceps, and in regeneration in crushed areas of the TA. Functional improvements were reported in grip strength and in distance run during 24 hours of voluntary exercise in mice given deflazacort vs. controls.

In vitro studies showed that 21-desDFZ reduced proliferation of human lymphocytes and cytotoxic activity of human natural killer cells. Deflazacort reduced inflammation in several in vivo rat assays and in the tibialis anterior (TA) muscle, diaphragm, and myocardium in the mdx model of DMD.

Deflazacort showed mixed inhibitory and proliferative effects on bone and cartilage. 21-desDFZ inhibited proliferation and/or collagen synthesis in cultures of bone, osteoblasts, and chondrocytes; and femur length and mass were reduced in juvenile rats dosed with deflazacort. However, unlike prednisone, deflazacort enhanced rather than inhibited the anaerobic glycolysis necessary for growth and mineralization of epiphyseal cartilage in the tibia of rats, and osteomas and osteosarcomas were increased in a published rat carcinogenicity study of deflazacort.

Published studies showed that three major circulating human metabolites were formed after oral administration of a single dose of 50 mg [^{14}C]-deflazacort to humans: M-II (21-desDFZ), M-III (6β-OH-21-desDFZ), and M-V (structure unknown), accounting for 43.4%, 27.2%, and 25.2% of total radioactivity in plasma, respectively. In a similar study in rat, 21-desDFZ, 6β-OH-21-desDFZ, and M-V accounted for 68.6%, 5.8%, and 6.9% of drug-related material. In monkey, 21-desDFZ and 6β-OH-21-desDFZ accounted for 45.0% and 26.2% of drug-related material, respectively; M-V was not detected. 6β-OH-

21-desDFZ showed no pharmacologic activity at glucocorticoid receptors; M-V has not been tested.

Pivotal toxicity studies of deflazacort were conducted in monkeys (39-week study), adult rats (26-week study), and juvenile rats (8-week study). The primary effects in all three studies were immunosuppression (lymphocytic depletion and/or hypocellularity of lymphoid organs, reduced circulating lymphocytes, and/or impaired T-cell dependent antibody response) and atrophy of adrenal cortex and other organs. Additional effects observed in juvenile rats included adverse effects on neurological, functional, and neurobehavioral parameters and bone growth.

A published 2-year carcinogenicity study of deflazacort in rat demonstrated a combined incidence osteoma and osteosarcoma of 14% in males at the maximum tolerated dose compared to 0% in controls; a similar study in mice was reported to be negative, but no description of the study was provided.

Mean 21-desDFZ plasma exposures (AUC) at the NOAELs for male monkey, adult rat, and juvenile rat were 4, 0.15, and <0.27 times, respectively, that in adolescent DMD patients administered the recommended clinical dose of 0.9 mg/kg/day deflazacort. There is no safety margin for the adverse effects observed in adult or juvenile male rats.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted do not adequately support the approval of deflazacort for the treatment of DMD. Insufficient information has been provided to determine if major circulating human metabolites M-V or 6β-OH-21-desDFZ have been adequately assessed for genotoxicity and carcinogenicity or if M-V has been adequately assessed for chronic toxicity.

To support approval, the sponsor should submit information (including additional studies, if needed) to demonstrate that major human metabolites 6β-OH-21-desDFZ and M-V have been adequately assessed for carcinogenicity and genotoxicity (in vitro assays for mutagenicity and chromosomal aberrations, and an in vivo rodent micronucleus study, unless the two in vitro studies are negative and the carcinogenic potential of M-V is adequately assessed); and that M-V has been assessed for chronic toxicity in one species (an adequate carcinogenicity study may substitute for the chronic toxicity study).

If these NDAs are approved despite the insufficient assessment of major metabolites 6β-OH-21-desDFZ and M-V noted above, the deficiencies should be addressed as post-marketing requirements.

1.3.2 Additional Non Clinical Recommendations

The information provided support granting a waiver of the requirement for a carcinogenicity study in rat, based on the conclusion that a new 2-year study in rat would not likely yield meaningful additional information.

A dose range-finding study should be conducted in mouse, with toxicokinetic analysis of 21-desDFZ, 6β-OH-21-desDFZ, and M-V, to assess the feasibility of conducting an oral carcinogenicity study of deflazacort in mouse. Major metabolites may need to be directly assessed if their plasma exposures after administration of deflazacort are not ≥ 50% of those expected in humans at the recommended clinical dose. This study, and a subsequent carcinogenicity study in mouse, if warranted, should be post-marketing requirements.

1.3.3 Labeling

The following language (underlined) is recommended for the nonclinical sections of the deflazacort label:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Corticosteroids should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Infants born to mothers who have received substantial doses of corticosteroids during pregnancy should be carefully observed for signs of hypoadrenalism. There are no adequate and well-controlled studies with deflazacort in pregnant women to inform drug-associated risks. The estimated background risk of major birth defects and miscarriage for the indicated populations is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Animal reproduction studies have not been conducted with deflazacort. Animal reproduction studies conducted with other corticosteroids in pregnant mice, rats, hamsters, and rabbits using clinically relevant doses have shown an increased incidence of cleft palate. An increase in embryofetal death, intra-uterine growth retardation, and constriction of the ductus arteriosus were observed in some animal species.

(b) (4)

Data

Human Data
Multiple cohort and case controlled studies in humans suggest that maternal corticosteroid use during the first trimester increases the rate of cleft lip with or without cleft palate from about 1/1000 infants to 3-5/1000 infants. Two prospective case control studies showed decreased birth weight in infants exposed to maternal corticosteroids in utero.

8.2 Lactation

Risk Summary

Systemically administered corticosteroids appear in human milk and could suppress growth, interfere with endogenous corticosteroid production, or cause other untoward effects. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for EMFLAZA and any potential adverse effects on the breastfed child from EMFLAZA. There are no data on the effects on milk production.

8.4 Pediatric Use

The safety and effectiveness of EMFAZA have been established in patients (b) (4) 5 years and older. Use of EMFLAZA is supported by a multicenter, randomized, double-blind, placebo- and active-controlled study of 196 males (b) (4) (b) (4) *[see Clinical Studies (14)]*.

Safety and effectiveness of EMFLAZA have not been established in pediatric patients less than 5 years old.

EMFLAZA is not approved for use in (b) (4). Serious adverse reactions including fatal reactions and (b) (4) "gasping syndrome" occurred in premature neonates and low birth weight infants in the neonatal intensive care unit who received drugs containing benzyl alcohol as a preservative. In these cases, benzyl alcohol dosages of 99 to 234 mg/kg/day produced high levels of benzyl alcohol and its metabolites in the blood and urine (blood levels of benzyl alcohol were 0.61 to 1.378 mmol/L). Additional adverse reactions included gradual neurological deterioration, seizures, intracranial hemorrhage, hematologic abnormalities, skin breakdown, hepatic and renal failure, hypotension, bradycardia, and cardiovascular collapse. Preterm, low-birth weight infants may be more likely to develop these reactions because they may be less able to metabolize benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known (EMFLAZA Oral Suspension contains 10.45 mg of benzyl alcohol per mL) *[see Warnings and Precautions (5.13)]*.

Juvenile Animal Toxicity Data

Oral administration of deflazacort (0, 0.1, 0.3, and 1.0 mg/kg/day) to juvenile rats from postnatal day (PND) 21 to 80 resulted in decreased body weight gain and adverse effects on skeletal development (including decreased cellularity of growth plate and altered bone distribution) and on lymphoid tissue (decreased cellularity). A no-effect dose was not identified. In addition, neurological and neurobehavioral abnormalities were observed at the mid and/or high dose. Plasma 21-desDFZ exposure (AUC) at the lowest dose tested (0.1 mg/kg/day) was lower than that in humans at the recommended human dose of EMFLAZA (0.9 mg/kg/day).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Deflazacort is a corticosteroid prodrug, whose active metabolite, 21-desDFZ, acts through the glucocorticoid receptor to exert anti-inflammatory and immunosuppressive effects.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

In a published 2-year carcinogenicity study, oral administration of deflazacort (0, 0.03, 0.06, 0.12, 0.25, 0.50, or 1.0 mg/kg/day) resulted in bone tumors (osteosarcoma and osteoma) of the head at 0.25 mg/kg/day, the highest evaluable dose. Doses higher than 0.25 mg/kg/day could not be evaluated for tumors because of a marked decrease in survival.

Mutagenesis

Deflazacort and 21-desDFZ were negative in *in vitro* (bacterial reverse mutation and chromosomal aberration in isolated human lymphocytes) and deflazacort was negative in an *in vivo* (rat bone marrow micronucleus) assay.

Impairment of Fertility

Fertility studies in animals were not conducted with deflazacort. No effects on the male reproductive system were observed following oral administration of deflazacort to monkeys (0, 1.0, 3.0, or 6.0 mg/kg/day) for 39 weeks or rats (0, 0.05, 0.15, or 0.5 mg/kg/day) for 26 weeks. Plasma 21-desDFZ exposures (AUC) at the highest doses tested in monkey and rat were 4 and 2 times, respectively, that in humans at the recommended human dose of EMFLAZA (0.9 mg/kg/day).

2 Drug Information

2.1 Drug

CAS Registry Number
14484-47-0

Generic Name
Deflazacort

Code Name
None

Chemical Names
(11β,16β)-21-(acetyloxy)-11-hydroxy-2'-methyl-5'H-pregna-1,4-dieno(17,16-d)oxazole-3,20-dione

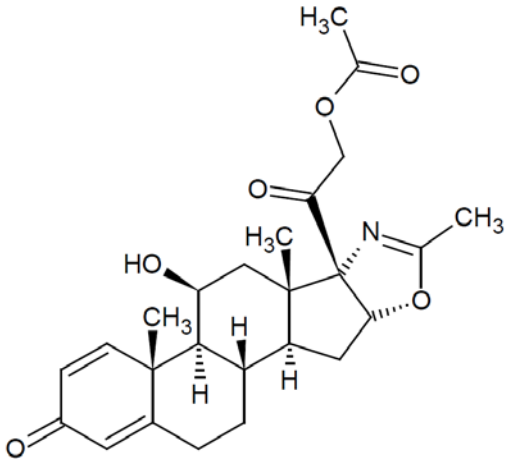
11β,21-dihydroxy-2'-methyl-5'H-pregna-1,4-dieno-[17,16d]-oxazole-3,20-dione-21-acetate

Proprietary Name
EMFLAZA

Molecular Formula
$C_{25}H_{31}NO_6$

Molecular Weight
441.52 g/mol

Structure or Biochemical Description



Pharmacologic Class
Corticosteroid prodrug

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 119258 Deflazacort for DMD, received October 3, 2014

2.3 Drug Formulation

NDA 208684: Immediate-release oral tablets, containing 6, 18, 30, or 36 mg deflazacort and the excipients shown in Table 1 below:

Table 1: Quantitative Composition of Deflazacort Tablets

Component	Quantity in mg per Tablet/Dosage Strength				Quantity (%)	Function	Quality Standard
	6 mg	18 mg	30 mg	36 mg			
Deflazacort, (b) (4)	6.00	18.0	30.0	36.0	(b) (4)	Active ingredient	In-house
Lactose Monohydrate						(b) (4)	NF
Pre-gelatinized Corn Starch							NF
Colloidal Silicon Dioxide							NF
Magnesium Stearate							NF
Total	**125.0**	**375.0**	**625.0**	**750.0**	**100.0**		

(page 4 of NDA 208684, Section 2.3.P Drug Product)

NDA 208685: Oral suspension containing 22.75 mg/mL deflazacort and the excipients shown in Table 1 below:

Table 1: Quantitative Composition of Deflazacort Oral Suspension

Component	Quantity in mg per mL of Suspension	Quantity (%w/v)	Function	Quality Standard
Deflazacort	22.75	(b) (4)	Active ingredient	In-house
Aluminum magnesium silicate			(b) (4)	NF/Ph. Eur.
Carboxymethylcellulose sodium				NF/Ph. Eur.
Benzyl alcohol				NF/Ph. Eur.
Sorbitol (b) (4)				USP/Ph. Eur.
Polysorbate 80				NF/Ph. Eur.
Acetic acid				USP/Ph. Eur.
Water, purified				USP/Ph. Eur.
Total	**1 mL**	**100.0**		

(page 1 of NDA 208685, Section 3.2.P.1 Description and Composition of the Drug Product)

2.4 Comments on Novel Excipients

There are no novel excipients in the drug products.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities or degradants of concern.

2.6 Proposed Clinical Population and Dosing Regimen

Deflazacort is proposed for the chronic (lifetime) treatment of patients with DMD at the recommended dose of 0.9 mg/kg/day administered orally once daily.

2.7 Regulatory Background

Nonclinical issues addressed prior to, and during, a Pre-IND Meeting held on November 21, 2013, are reflected in the following excerpt from the Minutes, dated December 20, 2013:

Question 2:
Does FDA concur that the existing and proposed preclinical study package is sufficient to support both the IND and NDA submissions?

FDA Preliminary Response to Question 2:
Based on the brief description provided, we have the following comments on your nonclinical plan:

- Considering the available previous human experience, safety pharmacology studies will not be needed.
- We recommend that you submit the final protocol for the juvenile animal toxicology study for review and comment prior to initiation of the study.
- Typically, carcinogenicity studies in two species (mouse and rat) would be needed to support approval of a drug for a chronic indication. Considering the seriousness of the indication, it may be possible to conduct these studies post-approval. If you believe sufficient data are available to warrant a waiver of this requirement, you should provide a scientific argument and all relevant supportive data in a waiver request to the IND.
- We agree that the standard battery of reproductive toxicology studies will not be needed for the DMD indication. However, you should conduct a focused histopathological evaluation of male reproductive organs (including sperm evaluation in a stage-aware manner) as part of the proposed chronic toxicology and juvenile animal studies.

***Meeting Discussion*:**
The sponsor stated that a published carcinogenicity study in rat will be submitted to fulfill the requirement for a second species for carcinogenicity testing. DNP responded that it is not likely that a published study would be adequate to fulfill the requirement for assessment of carcinogenicity in the rat because published studies generally do not provide sufficient information to adequately assess the results.

Original IND 119258 (Deflazacort for DMD) was received on October 3, 2014. No nonclinical issues were included in the May Proceed letter dated December 2, 2014.

Comments on a draft juvenile rat toxicology study protocol submitted on October 8, 2014, were provided to the sponsor on December 4, 2014.

Nonclinical issues addressed prior to a Pre-NDA Meeting held on August 4, 2015, are reflected in the following excerpt from the Minutes, dated September 3, 2015:

Question 24a:
Does the Agency concur that the nonclinical development program supports filing of an NDA for the treatment of patients with DMD?

FDA Response to Question 24a:

The nonclinical program, as described in the briefing package, appears sufficient to support NDA filing; the adequacy of the studies will be a matter of review. We acknowledge your intent to submit a request for waiver of the requirement to conduct studies to assess the carcinogenic potential of deflazacort. If, based on our evaluation of this request, we conclude that carcinogenicity studies are still needed, they may be conducted post approval, considering the seriousness of the indication and assuming the available nonclinical and clinical data support such a strategy.

Meeting Discussion: There was no meeting discussion.

Question 24b:
Would the agency be amenable to receiving an audited draft report (with a signed histopathology sub-report) from the chronic, 39-week monkey study at the time of NDA submission with the understanding that the final report be included within the 2-month period, prior to filing (NDA review)?

FDA Response to Question 24b:
The final study report for the 39-week monkey study should be included in the original NDA submission.

Meeting Discussion: There was no meeting discussion.

On December 21, 2016, the sponsor submitted an Information Amendment to the NDA to address M-V, an apparent major circulating human metabolite, which was identified as a substantive review issue during a Late-Cycle Review Meeting held on December 13, 2016. Review of this Information Amendment is included in the Metabolism section of this review.

3 Studies Submitted

3.1 Studies Reviewed

In Vitro Pharmacology and ADME Tox Study of Several Compounds
(Marathon Study MP-104-NC-056)

Metabolite Identification and Profiles of 21-desacetyl Deflazacort in Human, Monkey, Beagle Dog, and SD Rat Liver Microsomes
(Marathon Study MP-104-NC-010)

Information Amendment addressing Major Metabolite M-V
(Submitted December 21, 2016)

Metabolic Stability of 1 Compound in Human, Rat and Dog Liver Microsomes
(Marathon Study MP-104-NC-011)

14-Day Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of Deflazacort in Cynomolgus Monkeys
(Marathon Study MP-104-NC-032)

14-Day Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of Deflazacort in Sprague Dawley Rats
(Marathon Study MP-104-NC-035)

39-Week Oral Gavage Toxicity and Toxicokinetic Study of Deflazacort in Cynomolgus Monkeys with a 6-Week Recovery
(Marathon Study MP-104-NC-039)

26-Week Oral Gavage Toxicity and Toxicokinetic Study of Deflazacort in Sprague Dawley Rats with a 4 Week Recovery
(Marathon Study MP-104-NC-036)

Deflazacort: Bacterial Reverse Mutation Assay
(Marathon Study MP-104-NC-008)

21-Desacetyl-DFZ: Bacterial Reverse Mutation Assay
(Marathon Study MP-104-NC-009)

Deflazacort: In Vitro Chromosomal Aberration Assay in Human Lymphocytes
(Marathon Study MP-104-NC-006)

21-Desacetyl-DFZ: In Vitro Chromosomal Aberration Assay in Human Lymphocytes
(Marathon Study MP-104-NC-007)

Mammalian Erythrocyte Micronucleus Test of Deflazacort in Rat Bone Marrow

(Marathon Study MP-104-NC-041)

In Silico Mutagenicity Evaluation of the Deflazacort-Related Impurities (b) (4)
(Marathon Study MP-104-NC-027)

Potential Genotoxic Impurities in Deflazacort as Manufactured by (b) (4)
(Marathon Study MP-104-NC-059)

A Dose Range Finding Study of Deflazacort by Oral Gavage in Juvenile Rats
(Marathon Study MP-104-NC-028)

An 8-Week Study of Deflazacort by Oral Gavage in Juvenile Rats with an 8-Week Recovery Period
(Marathon Study MP-104-NC-030)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Because deflazacort was not measurable in humans or animals after oral administration due to rapid and complete metabolism to 21-desDFZ by plasma esterases, in vitro pharmacological evaluations focused on 21-desDFZ, and major metabolite, 6β-hydroxy-21-desDFZ (6β-OH-21-desDFZ).

***In Vitro* Pharmacology and ADME Tox Study of Several Compounds**
((b) (4) Study 100021109; Marathon Study MP-104-NC-056; (b) (4) Final Report dated June 25, 2015)
In an assay using cytosol isolated from IM-9 cells, 21-desDFZ inhibited binding of an agonist radioligand ([^{3}H]-dexamethasone) at the human glucocorticoid receptor (hGR) with IC_{50} = 21 nM and K_i = 10 nM. In a similar assay, prednisolone inhibited binding with IC_{50} = 4.9 nM and K_i = 2.4 nM. 6β-OH-21-desDFZ showed no inhibitory activity in this assay. The reference compound, dexamethasone inhibited binding of the radioligand with IC_{50} = 3.1 nM and K_i = 1.5 nM.

Summary of Relevant Pharmacology Data from Published Articles Cited by the Sponsor
Affinity for Glucocorticoid Receptors
In a luciferase transactivation assay in CV-1 (African green monkey kidney) cells, 21-desDFZ showed higher agonist activity for the hGR (EC_{50} = 4.37 nM) than for the human mineralocorticoid receptor (hMR; EC_{50} = 10.2 nM) (Grossman et al., *Eur J Endocrinol* 2004; 151(3):397-406). The relative binding affinity (percent of dexamethasone binding affinity) to cytosolic glucocorticoid receptors in rat kidney, thymus, and liver is shown for 21-desDFZ and other steroids in Table 2 below:

Table 2: Relative Binding Affinities for Cytosol GRs in Rat Kidney, Thymus and Liver

	Kidney		Thymus		Liver	
	2 hours	24 hours	2 hours	24 hours	2 hours	24 hours
Dexamethazone	100	100	100	100	100	100
Progresterone	44 ± 6	9 ± 1*	80 ± 11	15 ± 4*	26 ± 2	6 ± 2*
Aldosterone	14 ± 2	4 ± 1*	13 ± 2	5 ± 1*	17 ± 3	4 ± 1*
Deoxycorticosterone	56 ± 7	12 ± 5*	83 ± 4	23 ± 2*	19 ± 2	7 ± 3*
Deflazacort	3 ± 1	4 ± 1	2 ± 1	5 ± 1	11 ± 1	16 ± 2
21-desDFZ	22 ± 3	31 ± 6	32 ± 3	37 ± 6	17 ± 3	19 ± 3
Prednisolone	41 ± 2**	17 ± 2*	54 ± 3**	28 ± 7*	32 ± 1**	29 ± 4

Source: Luzzani F, et al. Eur J Pharmacol. 1981;76(4):427-30.[2]
21-desDFZ = 21-desacetyl deflazacort; GR = glucocorticoid receptor.
* $P < .01$ when compared to the corresponding values at 2 hours.
** $P < .01$ when compared to 2l-desDFZ at 2 hours.
Mean ± SE of either 3 or 4 experiments.

(page 6 of sponsor's Pharmacology Written Summary)

Oral administration of deflazacort to adrenalectomized male Sprague Dawley rats (N=4/group; 50-70 g) resulted in inhibition of specific binding of [^{3}H]-betamethasone (administered i.p., ± unlabeled betamethasone) to renal cytosolic glucocorticoid receptors (ED_{50} = 0.040 mg/kg) (Schiatti P, et al., *Arzneimittelforschung* 1980; 30(9):1543-49). Oral administration of deflazacort to adrenalectomized female Sprague Dawley rats (N=6-24/group; 50-75 g) resulted in depletion of glucocorticoid receptors in thymic cytosol (ED_{50} = 0.55 mg/kg; measured by specific binding of [^{3}H]-dexamethasone ex vivo) and thymolysis (reduced weight of the thymus relative to body weight; ED_{50} = ~0.30 mg/kg/day for 3 days) (Luzzani F et al., *Eur J Pharmacol* 1983; 87(1):61-66).

Administration of 400 µg/kg IV deflazacort to adrenalectomized male Sprague Dawley rats (N=4-11/group; ~250 g) resulted in displacement of [^{3}H]-Dexamethasone binding to cytosolic glucocorticoid receptors to a similar extent as the same dose of dexamethasone in hippocampus, pituitary, liver, and thymus; but to a lower extent in cerebral cortex (Coirini H et al., *J Steroid Biochem Molec Biol* 1994; 49(1):43-49). In the same publication, 21-desDFZ was shown to inhibit binding of [^{3}H]-dexamethasone to cytosolic glucocorticoid receptors in vitro with IC_{50} = 77.86 nM in liver and 73.53 nM in hippocampus, approximately four times greater than the IC_{50} values for dexamethasone in these tissues.

Effects on Muscle Injury and Muscle Repair

Administration of deflazacort (1 mg/kg/day IP every 2 days for 1-3 weeks) to mdx mice (N=3-5/group; 3 weeks old at start) resulted in reduced myofiber central nucleation in diaphragm (30% in controls, 15% in deflazacort-treated; indicative of reduced cumulative muscle regeneration, and, therefore, reduced injury); reduced myofiber size variability (sparing larger-diameter fibers); reduced myofiber degeneration and cellular infiltrates; increased activity of calcineurin; increased nuclear localization of nuclear factor of activated T-cells cytoplasmic-1 (NF-Atc1) in dystrophic skeletal muscle fibers; and up-regulation of NF-Atc1-dependent gene expression (e.g., utrophin, a dystrophin homologue) (St-Pierre SJG et al., *Faseb J* 2004; 18(15):1937-9). The effects of deflazacort were blocked by co-administration of cyclosporine-A, a calcineurin inhibitor, suggesting that activation of this calcium-dependent phosphatase is an important mediator of deflazacort's anti-dystrophic actions.

Similarly, administration of 1.2 mg/kg/day SC deflazacort to mdx mice (N=5-6/group; 3 weeks old at start) for 4 weeks prior to a crush injury of the right TA muscle resulted in the following changes compared to vehicle controls: increased diameter of all myofibers and of centrally nucleated fibers in the uncrushed TA muscle; promotion of myogenic repair (increased proportion of myotube nuclei) and reduced inflammation (infiltration of mononuclear cells) in crushed TA muscle; and decreased number of calcified muscle fibers in the diaphragm (Anderson JE et al., *Muscle Nerve* 1996; 19(12):1576-85).

Administration of deflazacort (1.2 mg/kg/day SC) to mdx mice (3.5 weeks old at start) for 4 weeks prior to a crush injury to the right TA muscle resulted in increased mean peak forelimb grip strength (15% within 10 days, maintained through Day 28;

normalized to body weight; N=10/group); increased number and earlier appearance of satellite cells (c-met$^+$) on centrally nucleated myofibers; increased (3-fold) proliferation of myogenin$^+$ (but not MyoD$^+$) myoblasts (N=22 deflazacort, 17 placebo); increased percentage of new myotube nuclei in regenerating areas of crushed muscle (60.1% vs. 41.5%); and increased laminin mRNA and protein expression (~1.3- to 5.5-fold, relative to mature CK expression) in crushed (regenerating) and non-crushed (dystrophic) TA muscle; compared to vehicle controls (Anderson JE et al., *Cell Transplant* 2000; 9(4):551-64). In a separate experiment, mdx mice (N=5/group) dosed for 2 weeks at 1.2 mg/kg/day SC deflazacort showed increased mean forelimb grip strength (normalized to body weight; compared to controls) that achieved statistical significance on Days 22 (~21%) and 57 (~16%); however, the deflazacort and control groups showed the same mean forelimb grip strength on Day 36.

Administration of deflazacort (1.2 mg/kg/day SC) to mdx mice (N=8-10 /group; 4 weeks old at start) for 3 weeks resulted in the following differences, compared to controls: increased weight of gastrocnemius and quadriceps muscles (relative to body weight); reduced myofiber damage (proportion of myofibers permeable to Evans blue dye) in the quadriceps after exercise; decreased nitrite levels in TA muscle (50-70%, indicative of reduced inflammation); reduced exercise-related increase in myogenic factor 5 expression (an indicator of myogenic regeneration in response to injury) in the quadriceps; and increased distance run (~2.5-fold, normalized to body weight) during a 24-hour period of voluntary exercise 3 months after the end of the dosing period (Archer JD et al., *Faseb J* 2006; 20(6):738-40).

Finally, male mdx mice given 1.3 mg/kg/day deflazacort in drinking water for 15 months showed reductions of 38% in the area of fibrosis and 79% in the density of inflammatory cells in myocardial sections compared to vehicle controls (N=12/group; 6 months old at start; Marques MJ et al., *Muscle Nerve* 2009; 40(3):466-8).

Immunosuppressive and Anti-inflammatory Responses

In in vitro studies, 21-desDFZ suppressed the mitogenic activity of phytohemagglutinin-stimulated human peripheral blood lymphocytes (~19-53% at 120 nM – 60 µM) and the cytotoxic activity of human natural killer cells (~30-45% at 670 nM – 17 µM), but showed comparatively little suppression of the antibody dependent cellular cytotoxic activity of human killer cells (~15% at 17 µM; Langhoff E et al., *Br J Clin Pharmac* 1986; 21(2):125-9).

Schiatti P et al. (1980) reported activity of deflazacort in various rat models of inflammation. Edema induced by subplantar injection of carageenan was reduced dose-dependently in rats (10 M/group; Sprague Dawley; 120-150 g) pretreated with 0.05 to 0.2 mg/kg IV or PO deflazacort. Edema induced by nystatin was reduced 30-40% in rats (10 F/group; Wistar; 130-180 g) dosed with deflazacort (0.3 mg/kg i.p.; 15 hours after nystatin) compared to vehicle controls. Mean net weight of dried cotton pellet granulomas was reduced 29-44% in rats (10 M/group; Sprague Dawley; 110-170 g) dosed with deflazacort (100-1000 µg/kg/day for 6 days, starting 6 hours after implantation of two 50 mg cotton pellets SC into the dorsal region) compared to vehicle

controls. Signs of adjuvant-induced arthritis were reduced dose-dependently in rats (N=8 F/group; Wistar; 120-150 g) dosed with deflazacort (0.05-0.5 mg/kg/day PO for 14 days, starting 14 days after adjuvant injection into the hind paws) compared to vehicle controls: 19.8-48.4% reduction in mean hind paw volume; 30.3-86.7% reduction in inflammatory units; 35.6-78.9% reduction in arthritis scores.

Effects on Bone and Cartilage

Deflazacort dose-dependently inhibited DNA synthesis (36-67% at 1-100 nM) and collagen synthesis (~82% at 100 nM) in cultures of fetal rat intact calvariae (skullcaps) when incubated for 72 hours; similar effects were observed at 100-1000 nM in cultures of calvarial-derived osteoblast-enriched cells (Canalis E and Avioli L, *J Bone and Miner Res* 1992; 7(9):1085-92). Similarly, collagen synthesis was inhibited 10-38% during 3-day incubations with 1-100 nM deflazacort or 21-desDFZ in cells isolated from 1-day old rat calvariae, but only in those obtained during the first digestion with collagenase, which likely originate from the outer layer (periosteum) (Guenther HL and Fleisch H, *Calcif Tissue Int* 1984; 36(2):145-52). This study also showed reduced collagen synthesis in secondary (but not primary) rabbit articular chondrocyte cultures incubated with deflazacort (25-39% at 1-10 µM) or 21-desDFZ (18-33% at 100 nM - 10 µM).

Administration of deflazacort (12.5 mg/kg/day SC) to male Sprague Dawley rats (N=5/group) for 20 days resulted in increased anaerobic glycolysis in epiphyseal cartilage isolated from tibial heads, possibly contributing to initiation of cartilage mineralization; in contrast, prednisone (10 mg/kg/day SC) inhibited anaerobic glycolysis needed for epiphyseal cartilage growth and mineralization (Russell JE et al. *Horm Metabol Res* 1985; 17(8):402-5).

4.2 Secondary Pharmacology

No secondary pharmacology studies were submitted or referenced.

4.3 Safety Pharmacology

***In Vitro* Pharmacology and ADME Tox Study of Several Compounds**

((b) (4) Study 100021109; Marathon Study MP-104-NC-056; (b) (4); Final Report dated June 25, 2015)

In an automated patch-clamp assay using CHO-K1 cells expressing hERG channels, 21-desDFZ showed mean inhibition of the hERG tail current of 2.8%, 5.1%, and 8.1%, and concentrations of 100 nM, 1 µM, and 10 µM, respectively. In a similar assay, 6β-OH-21-desDFZ showed mean inhibition of 5.7%, 9.6%, and 13.8% at 100 nM, 1 µM, and 10 µM, respectively. The positive control reference compound, E-4031, showed inhibition of the hERG tail current with IC_{50} = 26 nM. The effective free therapeutic plasma concentration of 21-desDFZ in children and adolescents administered 0.9 mg/kg/day deflazacort is ~0.86-1.05 µM (page 20 of the sponsor's Pharmacology Written Summary).

Summary of Relevant Safety Pharmacology Data from Published Articles Cited by the Sponsor

Schiatti P et al. (1980) reported that deflazacort had no effects on the blood pressure or heart rate in conscious normotensive rats or on the central nervous system of mice (sleeping time or maximum electric shock assays); however, the procedural details of these studies were not provided. In the same publication, administration of single oral doses of deflazacort (0.5, 1.0, and 2.0 mg/kg) to adrenalectomized male Sprague Dawley rats (N=10/group; 140-170 g) resulted in increased urine volume (~2-fold) and increased potassium excretion (~3-fold) at all doses, compared to controls.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Nonclinical PK/ADME studies conducted by the sponsor were limited to two in vitro metabolism studies of 21-desDFZ (reviewed below) and several in vitro studies investigating the roles of specific human enzymes, transporters, and plasma proteins in the metabolism and distribution of 21-desDFZ, relevant to the potential for drug-drug interactions (reviewed by the Clinical Pharmacology team). The sponsor also provided information from the published literature relevant to the PK/ADME of deflazacort and 21-desDFZ, which is summarized below.

Absorption

Deflazacort was not detectable in plasma samples collected following oral administration in the pivotal studies in rat and monkey conducted by the sponsor, consistent with published findings that deflazacort was rapidly and completely converted to 21-desDFZ by plasma deacetylases in rat, dog, and human after oral or IV administration (Assandri et al. *Adv Exp Med Biol*. 1984;171:9-23). As shown in Table 1 below, peak levels of 21-des-DFZ were observed in plasma within 1-4 hours after oral administration of 5 mg/kg ^{14}C-deflazacort to rat, dog, monkey, and 0.6-0.7 mg/kg to human volunteers; oral bioavailability was ~92% in rat, 37% in dog, and 43% in monkey; and distribution into tissues was rapid and extensive.

Table 1: Pharmacokinetic Parameters in Rat, Dog, Monkey, and Human

Source: Assandri, et al. Adv Exp Med Biol. 1984;171:9-23[2]

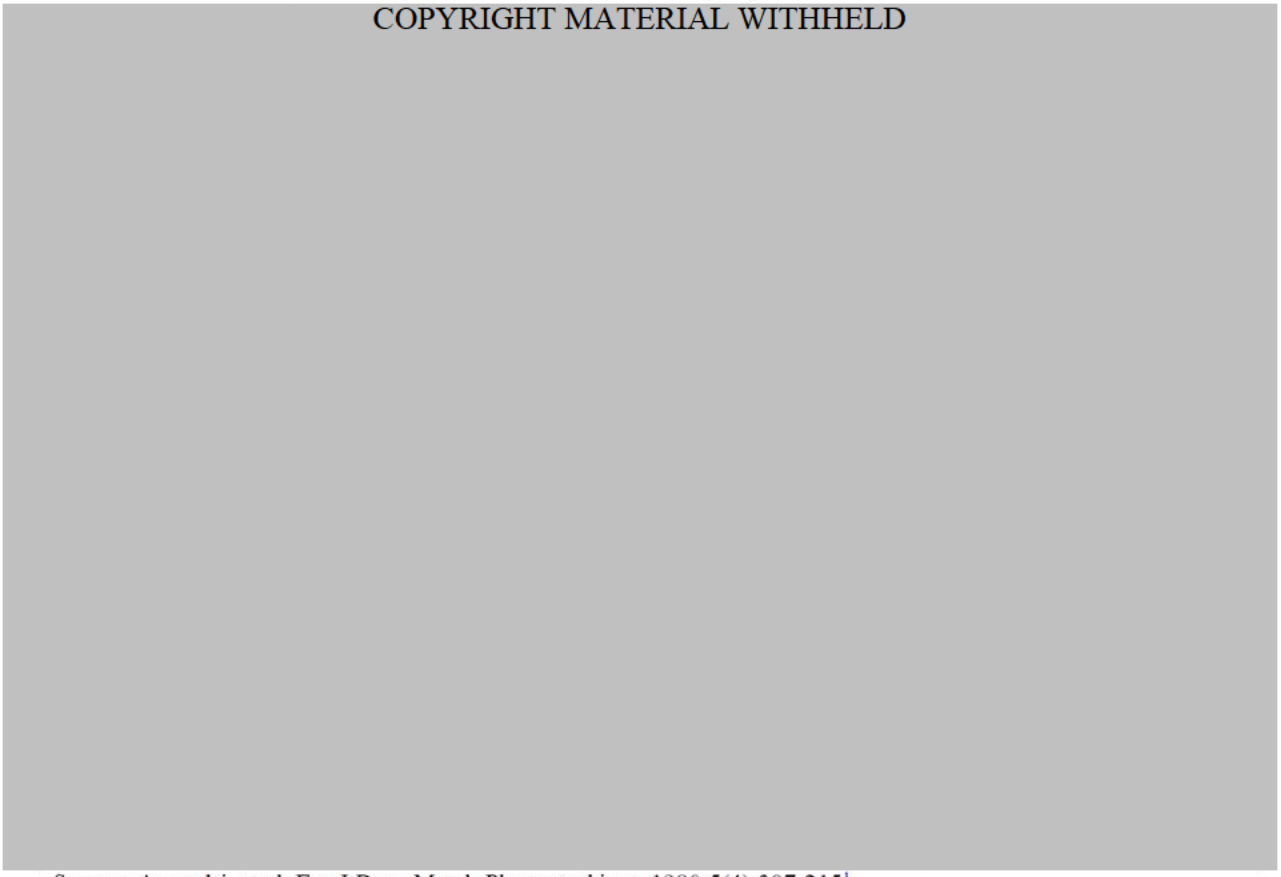
AUC_∞ = Area under the concentration-time curve from zero to infinity, CL_t = total plasma clearance, C_{max} = time to peak (maximum) plasma concentration, IV = intravenous, PO = oral, $t_{½}$ = half-life, t_{max} = time to peak (maximum) plasma concentration, V_d = volume of distribution.

(page 8 of sponsor's Pharmacokinetics Written Summary)

Distribution

Plasma protein binding of 21-desDFZ was reported to be 65.2 ± 1.02% in rat, 41.6 ± 2.09% in dog, and 39.8 ± 0.66% in human, based on in vitro equilibrium dialysis studies (Assandri A et al. *Eur J Drug Metab Pharmacokinet*. 1980;5(4):207-15). Quantitative tissue distribution and quantitative whole body autoradiography studies in male Wistar rats administered 5 mg/kg [^{14}C]-deflazacort via oral gavage or IV injection demonstrated high concentrations of radioactivity in liver and kidney; moderate concentrations in heart, pancreas, lungs, and submaxillary glands; and lower concentrations in cartilaginous tissues striated muscles, spleen, testes, and thymus; very little radioactivity was observed in the CNS (Assandri et al., 1980).

Table 3: Concentrations of Radioactive Material in the Tissues of Rat after a Single Oral Dose of 14C-Deflazacort at 5 mg/kg



Source: Assandri, et al. Eur J Drug Metab Pharmacokinet. 1980;5(4):207-215[1]

(pages 10-11 of sponsor's Pharmacokinetics Written Summary)

Metabolism

As described below, in vitro studies conducted by the sponsor showed that metabolism of 21-desDFZ by liver microsomes was more extensive in human than in rat or dog preparations, and that 7 metabolites were produced in human preparations: M1-M7.

Metabolite Identification and Profiles of 21-desacetyl Deflazacort in Human, Monkey, Beagle Dog, and SD Rat Liver Microsomes

(Marathon Study MP-104-NC-010; (b) (4) MRTP-20140401-MID; (b) (4); Study Initiation Date: 16 APR 2014)

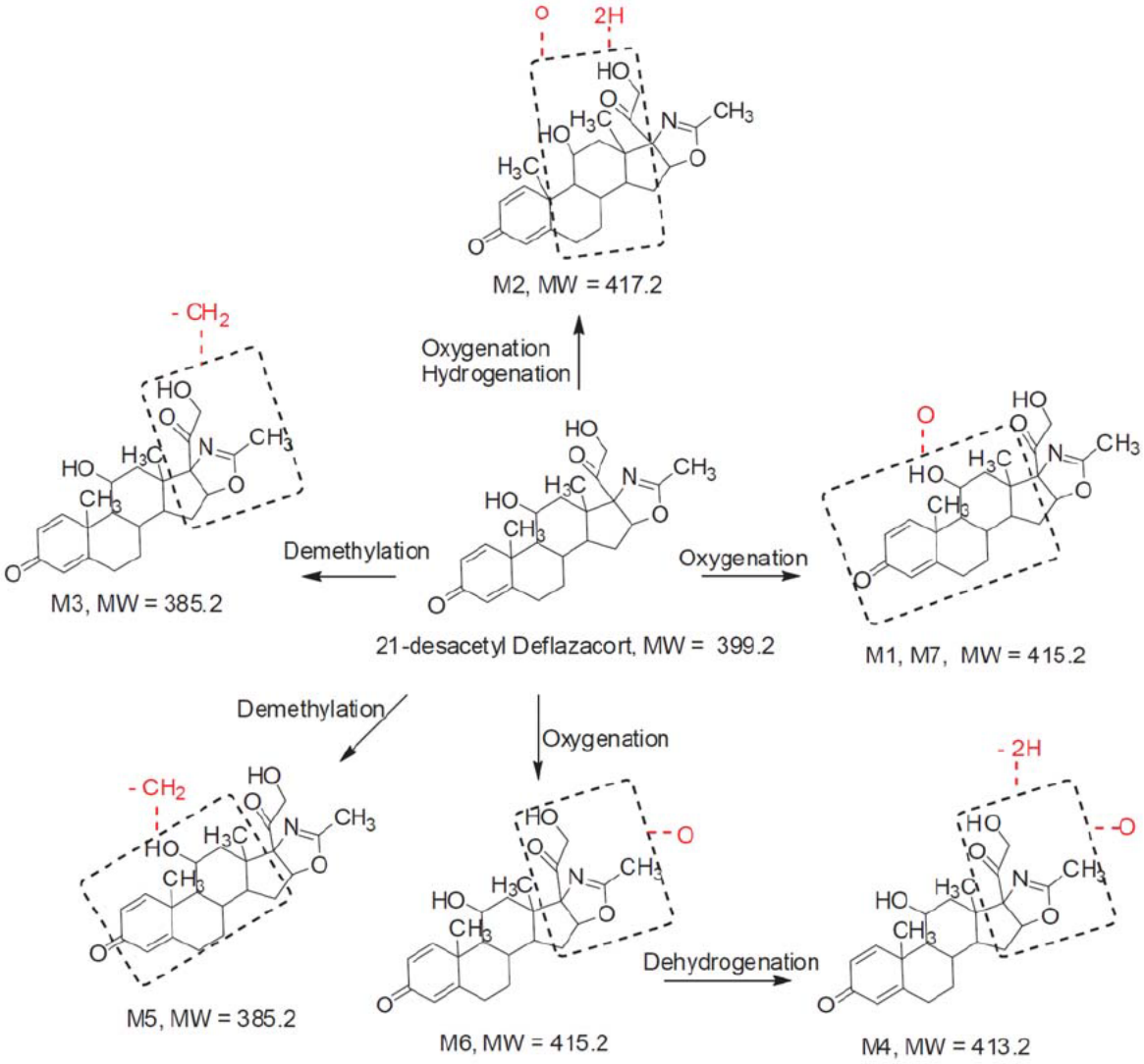
Metabolites were identified by LC-MS/MS following 60-minute incubations of 21-desDFZ with liver microsomes from human, Cynomolgus monkey, Beagle dog, and SD rat. 21-desDFZ was the most abundant drug-related compound present in all species. No unique human metabolites were observed, except M3, which accounted for only 0.5% of drug-related species in human microsomes.

Table 2. Observed metabolites of 21-desacetyl Deflazacort in human, monkey, dog and rat liver microsomes and related information on LC-MS^E scans

Species	Metabolites	Mass	Metabolism pathway	Formula	Mass Difference	*m/z* Found	Δ mDa	PPM	Time	Area Abs	Area %
Human	M1	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9954	416.2078	0.5	1.2	5.97	3423.3	7.79
	M2	417.2151	Hydration	$C_{23}H_{31}NO_6$	18.0104	418.2227	-0.2	-0.6	7.68	101.0	0.23
	M3	385.1889	Demethylation	$C_{22}H_{27}NO_5$	-14.0166	386.1958	-0.9	-2.4	7.82	218.9	0.5
	M4	413.1838	Hydroxylation + desaturation	$C_{23}H_{27}NO_6$	13.9802	414.1926	1	2.3	8.32	622.0	1.42
	M5	385.1889	Demethylation	$C_{22}H_{27}NO_5$	-14.0154	386.1970	0.3	0.7	9.51	736.8	1.68
	M6	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9943	416.2067	-0.6	-1.4	9.64	1975.2	4.49
	Parent	399.2046		$C_{23}H_{29}NO_5$	0	400.2123	-0.1	-0.2	10.35	32769.1	74.55
	M7	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.995	416.2074	0.1	0.3	10.8	4107.1	9.34
Monkey	M1	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9947	416.2071	-0.2	-0.5	5.93	1648.0	6.71
	M4	413.1838	Hydroxylation + desaturation	$C_{23}H_{27}NO_6$	13.9793	414.1917	0.1	0.2	8.24	411.4	1.68
	M5	385.1889	Demethylation	$C_{22}H_{27}NO_5$	-14.0138	386.1986	1.9	4.9	9.45	77.5	0.32
	M6	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9953	416.2076	0.3	0.8	9.59	2895.2	11.79
	Parent	399.2046	Parent	$C_{23}H_{29}NO_5$	0	400.2124	0	0.1	10.3	18340.5	74.71
	M7	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9951	416.2074	0.1	0.3	10.74	1177.0	4.79
Dog	M1	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9946	416.2070	-0.3	-0.7	5.97	716.3	1.28
	M2	417.2151	Hydration	$C_{23}H_{31}NO_6$	18.0108	418.2232	0.3	0.6	7.69	171.9	0.31
	M6	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9947	416.2071	-0.2	-0.5	9.64	656.2	1.17
	Parent	399.2046		$C_{23}H_{29}NO_5$	-0.0003	400.2121	-0.3	-0.7	10.35	54534.9	97.25
Rat	M1	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9947	416.2071	-0.2	-0.5	5.97	777.4	2.23
	Parent	399.2046		$C_{23}H_{29}NO_5$	0.0005	400.2128	0.4	1.1	10.35	33954.4	97.45
	M7	415.1995	Oxygenation	$C_{23}H_{29}NO_6$	15.9951	416.2075	0.2	0.5	10.79	75.6	0.22

(page 5 of Study Report)

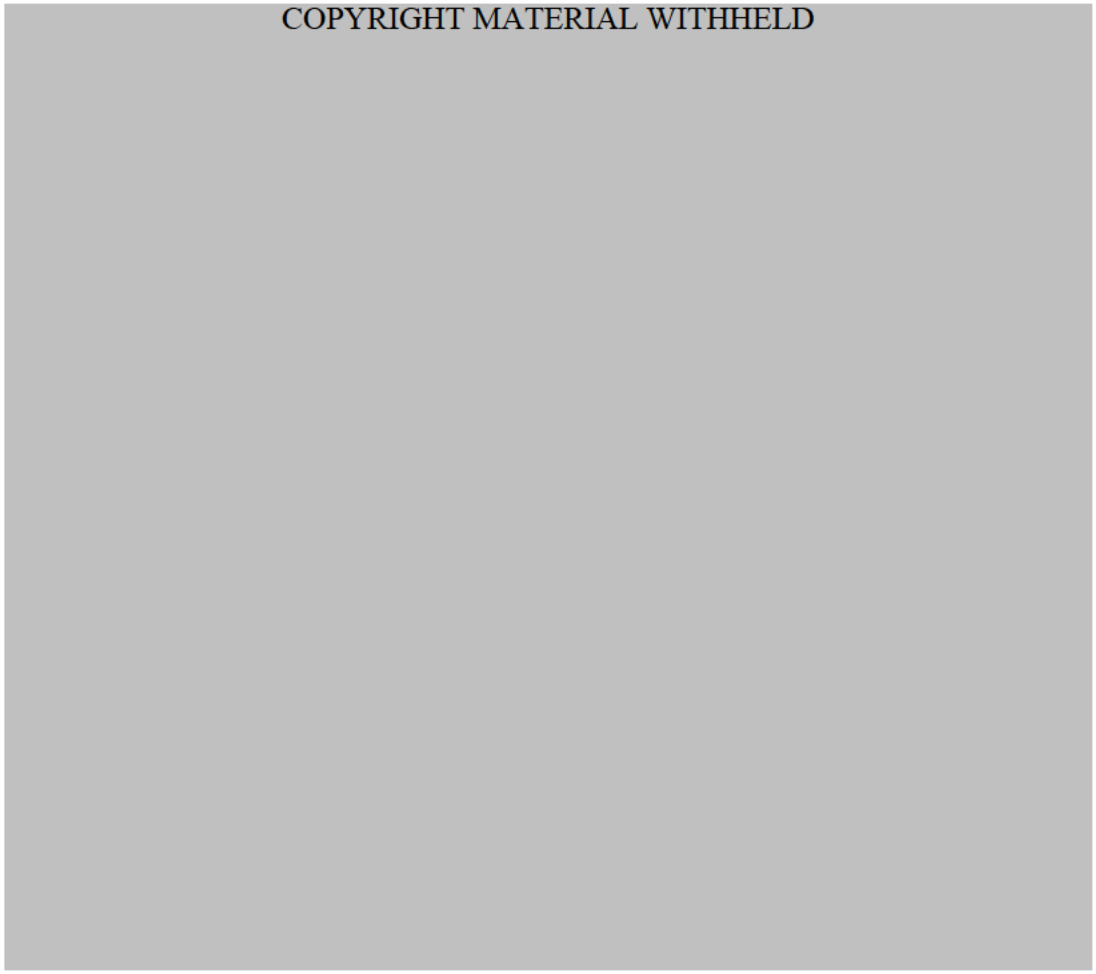
Scheme 1. Proposed metabolic pathways of 21-desacetyl Deflazacort in human, monkey, dog and/or rat liver microsomes



(page 6 of Study Report)

Metabolic Stability of 1 Compound in Human, Rat and Dog Liver Microsomes
(Marathon Study MP-104-NC-011; (b) (4) Study MRTP-20140220B V2-MMS; (b) (4); Study Initiation Date: (b) (4)
The metabolic stability of 21-desDFZ (1 µM final concentration) was tested during a 60-minute incubation at 37 °C with liver microsomes from human, dog, or rat (0.5 mg protein per mL final concentration), and an NADPH regenerating system (1 unit/mL final concentration), using LC/MS/MS. The amount of 21-des DFZ remaining after 60 minutes was 40.2% in human, 83.0% in rat, and 84.8% in dog preparations, compared to 93.3%, 94.3%, and 97.0%, respectively, in concurrent controls without liver microsomes. These results suggest that metabolism of 21-desDFZ may be significantly greater in human relative to rat and dog.

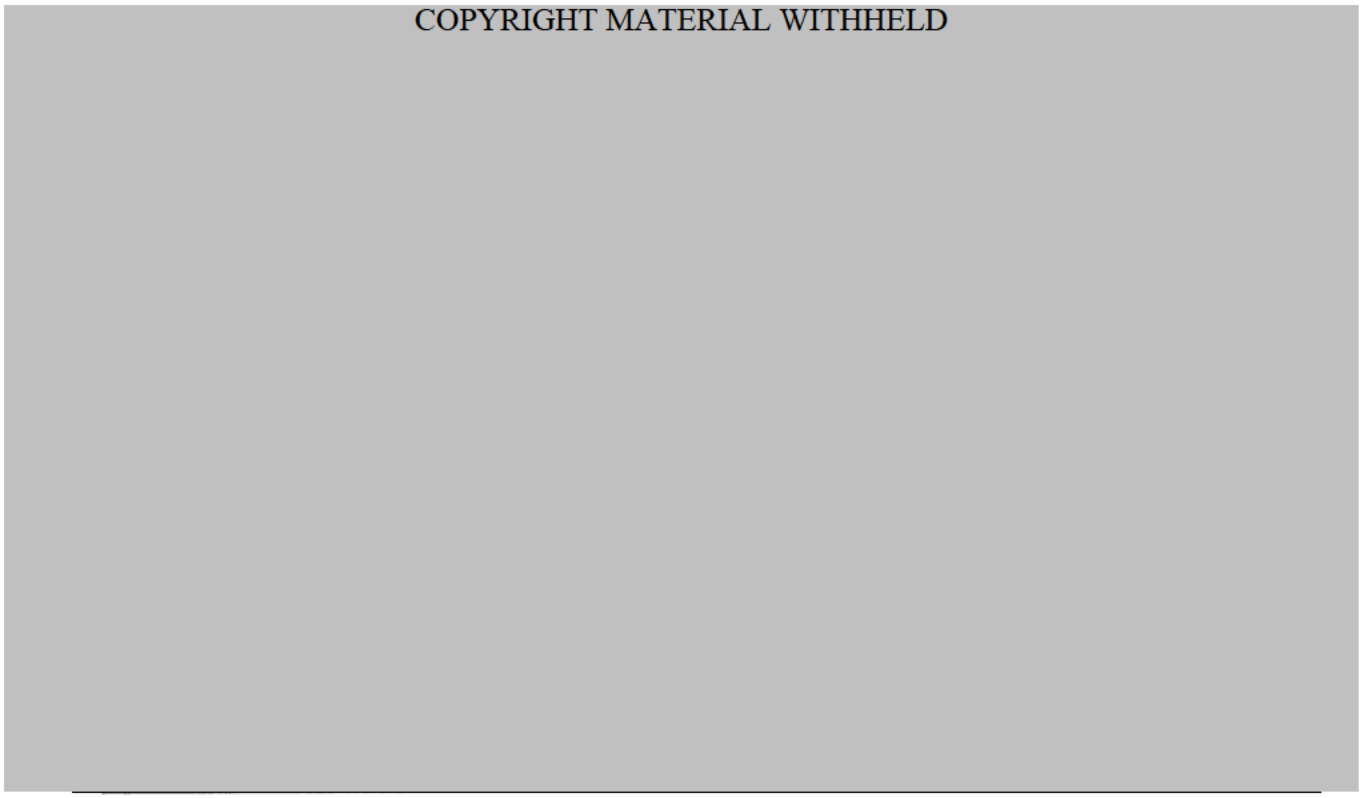
In vivo studies of [^{14}C]-deflazacort in overnight fasted male Wistar rats (N=5; 200-250 g; 5 mg/kg PO, gavage in saline + 10% ethanol), male Beagle dogs (N=4; 1015 kg; 5 mg/kg PO, oral capsule), and healthy volunteers (N=3; 2M, 1F; 25-27 years old; 50 mg PO, oral capsule) showed 5 metabolites in plasma; unchanged deflazacort was not detected (Martinelli et al. *Drug Metab Dispos*. 1979;7(5):335-9). Metabolite M-II, identified as 21-desDFZ, was the most abundant drug-related compound in plasma, accounting for 68.6% of total radioactivity in rat, 59.1% in dog, and 43.4% in human. As noted in Table 1 and Figure 1 below, two other major circulating metabolites were observed in human plasma, M-III (6β-OH-21-desDFZ; 27.2%) and M-V (unknown structure; 25.2%). A complete structure could not be determined for M-V "because of the impurities contained." The following information about the structure of M-V was derived from spectral analyses: "a) the steroid is desacetylated; b) the 16α,17α-2'-methyloxazoline ring and the side chain are unchanged; c) the 11β-hydroxy function is present; d) the $\Delta^{1,4}$ system is absent; e) there is oxidation, probably in the B-ring" (Martinelli et al., 1979). As shown in Table 1 below, M-V is a major human metabolite in urine as well as plasma. M-V in human urine accounted for ~13% of the administered dose (Assandri et al., 1984).



(page 336 of Martinelli et al., 1979)

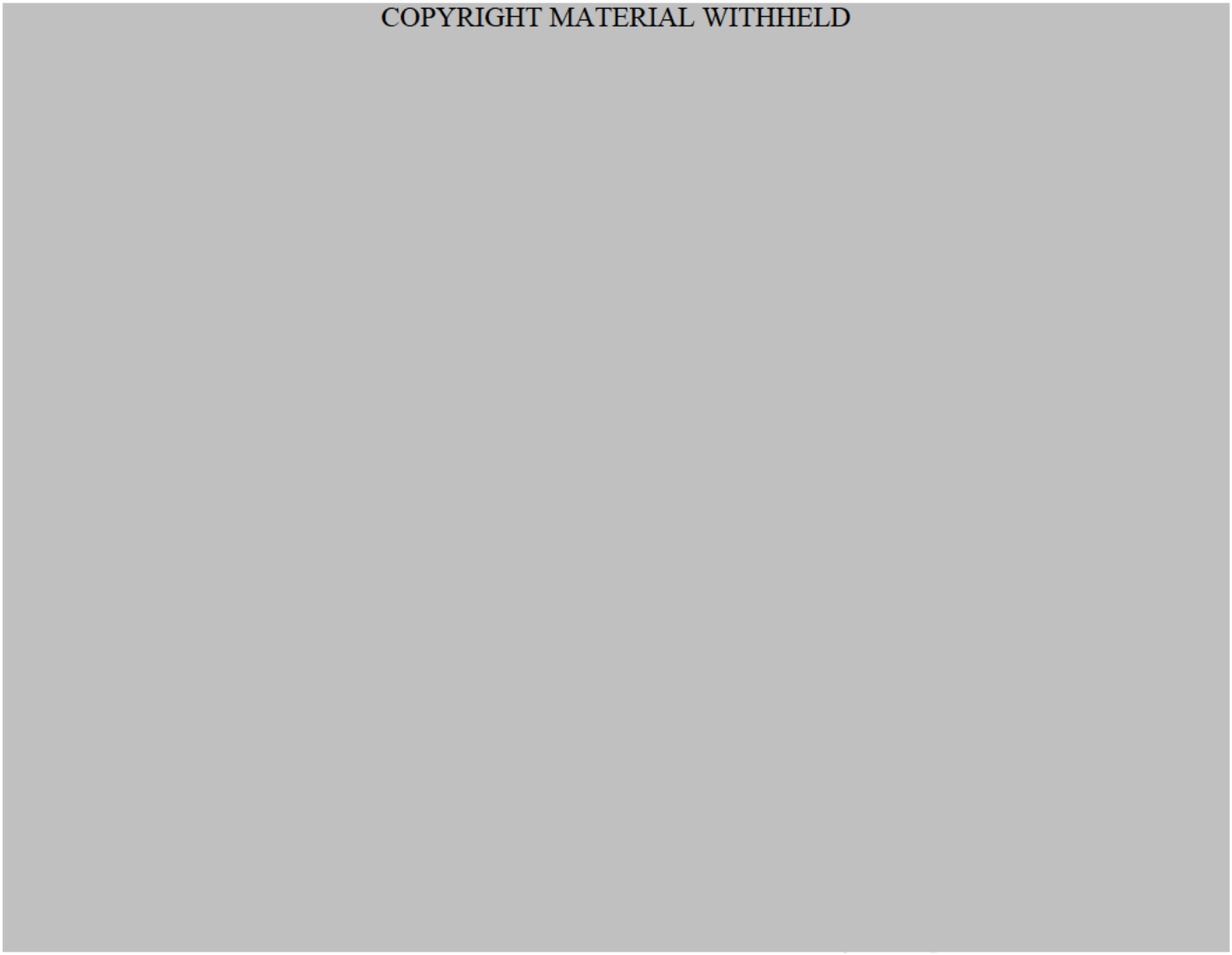
FIG. 1. *Deflazacort and its main unconjugated metabolites in the rat, dog, and man.*
(page 338 of Martinelli et al., 1979)

In a study of [^{14}C]-deflazacort administered PO (5 mg/kg via gastric gavage in 0.5% methylcellulose) or IV (5 mg/kg via bolus injection in saline + 20% ethanol) to fasted male Cynomolgus monkeys (N=3; 4.5-5.32 kg), two major metabolites were identified in plasma: M-II (21-desDFZ; 45.0% of total plasma radioactivity, PO) and M-III (6β-OH-21-desDFZ; 26.2%, PO) (Assandri A et al. *Xenobiotica*. 1983;13(3):185-96; Assandri et al., 1984); no deflazacort was detected. Minor metabolites observed in plasma included M-IV, M-VII, M-VIII, and M-IX. M-V was not detected.



(page 15 of Assandri et al., 1984)

Figure 4 below illustrates the proposed metabolic pathways for deflazacort in monkey.



(page 194 of Assandri et al., 1983)

Sponsor's Information Amendment Articulating Their Position on Metabolite V

The sponsor submitted an Information Amendment on December 21, 2016, in response to the Division's concern that M-V, an apparent major circulating human metabolite, may not have been adequately tested in the appropriate nonclinical studies. The sponsor stated that "While the results from *in vitro* and *in vivo* studies are complex, it is clear that metabolites with a molecular mass of 417.2 (the mass of Metabolite V) are well represented in all relevant toxicology species including rat, dog and monkey, although the exact concentrations relative to humans are unknown." However, the in vitro study showed only one metabolite with a MW of 417.2 (M2), which was present in human (0.23% of total area) and dog (0.31% of total area), but was not detected in the pivotal toxicology species, rat and monkey. Martinelli et al. (1979) showed that Metabolite V was the only metabolite in plasma with a MW of 417 in human, rat, and dog, accounting for 25.2%, 6.9%, and 8.0% of total plasma radioactivity, respectively. Assandri et al. (1983, 1984) reported that Metabolite VII was the only metabolite in

monkey plasma with a MW of 417, accounting for an uncertain percentage of the total plasma radioactivity ("minor metabolites" XI, VII, VIII, and IX together totaled 21.1% of total plasma radioactivity and ~10% of the administered dose in urine).

The sponsor stated that, "Metabolite V (reported in Martinelli et al, 1979) and Metabolite VII (reported in Assandri et al., 1983) represent the same mixture of metabolite isomers," based on their similar MW (~417) and MS/MS fragmentation patterns. While it is possible that the impurities in the M-V fraction that interfered with definitive determination of its structure represent a mixture of isomers of MW ~417, this was not suggested by the study's authors, and no data have been presented to support the idea. Furthermore, M-VII was identified by Assandri et al. (1983) as a minor metabolite in monkey plasma with a definitive structure (a 6α-hydroxylation, 5α-reduction derivative of 21-desDFZ), rather than as a mixture of isomers.

The ^{1}H-NMR data presented for M-V appear to be consistent with its being a 6α-hydroxylation, 5β-reduction derivative of 21-desDFZ, though this remains to be definitively established. The following δ chemical shifts for the $(CH_3)^{19}$ group were listed in the tables of ^{1}H-NMR data (in ppm): 1.41 for M-V, 1.70 for M-III [6β-hydroxy 21-desDFZ], 1.43 for M-II [21-desDFZ], and 1.46 for M-I [5β-reduced 21-desDFZ] (Martinelli et al., 1979); and at 1.25 for M-VII [6α-hydroxy, 5α-reduced 21-desDFZ], 1.43 for M-VI [6α-hydroxy 21-desDFZ], 1.66 for M-III, and 1.45 for 21-desDFZ (Assandri et al., 1983). Martinelli et al. (1979) noted that the hydrogen at C-5 in M-I was consistent with β-reduction, based on the $(CH_3)^{19}$ δ of 1.46—α-reduction would be calculated to have a $(CH_3)^{19}$ δ of 1.33 (based on the Zürcher tables); thus, β-reduction was associated with a $(CH_3)^{19}$ δ 0.03 ppm greater than, and α-reduction was associated with a $(CH_3)^{19}$ δ 0.10 lower than, that of its parent compound, 21-desDFZ (1.43 ppm). Applying these relationships to M-III as a parent compound ($(CH_3)^{19}$ δ 1.70, isolated from human urine in Table 4 of Martinelli et al., 1979), 6β-hydroxy, 5α-reduced 21-desDFZ should have a $(CH_3)^{19}$ δ of ~1.60, while 6β-hydroxy, 5β-reduced 21-desDFZ should have a $(CH_3)^{19}$ δ of ~1.73 ppm. 6α-hydroxylation of 21-desDFZ ($(CH_3)^{19}$ δ 1.45, isolated from monkey urine in Assandri et al., 1983) to produce M-VI ($(CH_3)^{19}$ δ 1.43) lowered $(CH_3)^{19}$ δ by 0.02 ppm, while 5α-reduction of M-VI ($(CH_3)^{19}$ δ 1.43) to produce M-VII ($(CH_3)^{19}$ δ 1.25) lowered $(CH_3)^{19}$ δ by 0.18 ppm. Therefore, starting with the value of $(CH_3)^{19}$ δ 1.43 for M-II isolated from human urine, 6α-hydroxy, 5α-reduced 21-desDFZ should have a $(CH_3)^{19}$ δ of ~1.23-1.33, while 6α-hydroxy, 5β-reduced 21-desDFZ should have a $(CH_3)^{19}$ δ of ~1.44 ppm; the latter is most consistent with the reported value of 1.41 ppm for M-V.

The sponsor proposed that the M-V/M-VII mixture of isomers at MW ~417 most likely represent predominantly 6β-hydroxy, 5α-reduced 21-desDFZ, "given the predominance of the 6β-hydroxylation preference across species" (6β-hydroxy 21-desDFZ was the primary metabolite of 21-desDFZ identified in plasma of human and monkey [but not dog or rat] and in urine of human, monkey, dog, and rat. However, as noted above, the ^{1}H-NMR data are not consistent with 6β-hydroxylation.

The sponsor then calculated that estimated plasma exposures ($AUC_{0\text{-}24\ hr}$) to M-V/M-VII achieved at the HD in the 39-week monkey study were 1.0- to 1.4-fold those observed in humans at the clinical dose of 0.9 mg/kg/day, assuming that M-V/M-VII accounted for ~30% of the minor metabolites (M-VI, VII, VIII, and IX) that together accounted for 21.2% of total plasma radioactivity in monkey (Table 5, Assandri et al., 1984). The basis for this assumption was said to be "the known metabolic pathways for 21-desDFZ," but was not adequately explained.

The sponsor provided the following concluding statements:

- "In conclusion, the various metabolites of DFZ have been qualified across species and are consistent with the metabolism of this class of corticosteroids."
- "In conclusion, a metabolite with a molecular mass of 417.2 is well represented in all relevant toxicology species including rat, dog and monkey."
- "Early reports suggest that these metabolites may different across species but this is likely due to misspecification of isomers given the known metabolic routes for deflazacort and other similar corticosteroids."

There is insufficient information available to conclude that all major circulating human metabolites have been adequately evaluated in nonclinical studies. M-V may be a major metabolite in human plasma (though this remains to be definitively established), but was not identified in monkey plasma and was present in rat plasma at levels that may be too low to provide an adequate assessment of its toxicity. Even if M-V were to be determined to be identical to M-VII, or a mixture of isomers, there is no reliable way to compare plasma exposures in humans to those in animals based on the available data.

Excretion

As shown in Table 7 below, the primary route of excretion after oral administration of [^{14}C]-deflazacort was urinary for rat (54% of the dose), monkey (50%), and human (68%); and fecal for dog (82%).

Table 7: Cumulative Values of the Urinary and Fecal Elimination (Percentage of the Administered Dose) in the Rat, Dog, Monkey, and Human Treated Orally with 14C-Deflazacort

Source: Assandri, et al. Adv Exp Med Biol. 1984;171:9-23[2]
nd = not determined.

(pages 18-19 of sponsor's Pharmacokinetics Written Summary)

5.2 Toxicokinetics

Toxicokinetic data for 21-desDFZ and 6β-OH-21-desDFZ were collected in the pivotal toxicity studies in monkey and rat.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted.

6.2 Repeat-Dose Toxicity

14-Day Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of Deflazacort in Cynomolgus Monkeys

(Sponsor Study MP-104-NC-032; (b) (4) Study 8310953; dosing initiated (b) (4) ; deflazacort Batch No. DFZγ14002, purity 99.53%; Non-GLP; Non-QA)

Cynomolgus monkeys (2-3 years old; 2.8-4.0 kg; 1/sex/group) were dosed via oral gavage at 10 mL/kg with vehicle (0.5% methylcellulose) or 0.3, 1.0, or 3.0 mg/kg/day deflazacort once daily for 14 days. Parameters evaluated included mortality, clinical observations, body weight, body weight change, food consumption, clinical pathology, toxicokinetics (Days 1 and 14), organ weights, and macroscopic changes. Histopathology was not performed.

Drug-related changes were limited to small thymus in MDM and decreased relative weight of thymus in MDM and HDM. The NOAEL was the HD of 3.0 mg/kg/day, associated with mean Day 14 plasma 21-desDFZ C_{max} = 270 ng/mL (M), 422 ng/mL (F); and $AUC_{0\text{-}24\ hr}$ = 1630 ng*hr/mL (M), 969 ng*hr/mL (F). Deflazacort was not detected in plasma samples, indicating its rapid and extensive metabolism.

14-Day Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of Deflazacort in Sprague Dawley Rats

(Marathon Study MP-104-NC-035; (b) (4) Study 8310951; dosing initiated (b) (4) deflazacort Batch No. DFZγ14002, purity 99.53%; Non-GLP; Non-QA)

Sprague Dawley rats (8 weeks old; 256-291 g M, 173-201 g F; 5/sex/group + 3/sex control TK and 9/sex/group drug TK) were dosed via oral gavage at 10 mL/kg with vehicle (0.5% methylcellulose) or 0.3, 1.0, or 3.0 mg/kg/day deflazacort once daily for 14 days. Parameters evaluated included mortality, clinical observations, body weight, body weight change, food consumption, clinical pathology, toxicokinetics (Days 1 and 14), organ weights, and macroscopic changes. Histopathology was not performed.

Mean body weight loss (10% of baseline) and reduced mean final body weight (30% vs. control) observed in HDM were considered adverse. Reductions in body weight (7-16%) and body weight gain (41-75%) were observed in LDM and MDM, and in F at all doses, compared to controls. Dose-related reductions in food consumption (15-33%) were observed in M.

Absolute reticulocyte counts were reduced in LDM (38%), MDM (40%), HDM (79%), and HDF (28%), compared to controls. Absolute lymphocyte counts were reduced in LDM (35%), MDM 61%), MDF (44%), HDM (80%), and HDF (62%), compared to controls. Absolute monocyte counts were increased in MDM (143%), HDM (133%), and HDF (127%), compared to controls. RBC parameters were slightly increased at all doses in M (16-21%) and F (6-16%), suggesting mild dehydration.

Serum ALT and AST were increased (42-83%) in MDM and HDM. Triglycerides were increased (137%) in HDM. Total protein, albumin, and globulin were increased (12-30%) in M at all doses. Urea nitrogen was increased (17-33%) in M at all doses. Serum sodium was increased in HDM (2.1%) and HDF (2.9%). The increases in total protein, albumin, globulin, urea nitrogen, and sodium were consistent with dehydration.

Drug-related reductions were observed in the following organs (relative to body weight): adrenal glands (LDM: 30%; MDM: 61%; HDM: 44%; HDF: 31%); spleen (LDM: 29%; MDM: 31%; HDM: 41%; HDF: 30%); and thymus (LDM: 73%; LDF: 38%; MDM: 72%; MDF: 61%; HDM: 73%; HDF: 77%). The reductions in relative weight of adrenals and thymus correlated with macroscopic findings of small adrenals (in M at all doses) and small thymus (in M and F at all doses), respectively.

The NOAEL was 1 mg/kg/day for M, based on findings of body weight loss and reductions in reticulocyte count (marked) and lymphocytes (moderate) at the HD of 3 mg/kg/day. The NOAEL for F was the HD of 3 mg/kg/day. Mean Day 14 plasma exposures to 21-desDFZ at the NOAELs were: C_{max} = 323 ng/mL (M), 996 ng/mL (F); and $AUC_{0\text{-}24\ hr}$ = 2030 ng*hr/mL (M), 1830 ng*hr/mL (F). Deflazacort was not detected in plasma samples, indicating its rapid and extensive metabolism.

39-Week Oral Gavage Toxicity and Toxicokinetic Study of Deflazacort in Cynomolgus Monkeys with a 6-Week Recovery

Study no.:	Marathon Study MP-104-NC-039 (b) (4) Study 8310954
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of dosing initiation:	(b) (4)
GLP compliance:	Yes (exception: test article characterization was tested under GMP)
QA statement:	Yes
Separate pathology report:	Yes
Drug, lot #, and % purity:	Deflazacort batch DFZγ14007, 99.62% pure

Key Study Findings

- Deflazacort induced immunosuppression in monkeys after oral dosing at 6.0 mg/kg/day for 27 weeks and 3.0 mg/kg/day for 39 weeks, as indicated by impaired T-cell dependent antibody responses and minimal to severe lymphocytic depletion in thymus, spleen, and lymph nodes.

- Drug-related changes also included atrophy in the adrenal cortex, uterus, and vagina; hyperplasia in mammary glands (M); hyperplasia/hypertrophy in pituitary (M); increased adipocytes in bone marrow; and absence of corpus luteum in ovaries.

- Changes persisting through the 6-week recovery period following dosing at 3.0 mg/kg/day for 39 weeks included reduced alkaline phosphatase (ALP), alopecia, reduced weight of spleen, increased weight of pituitary, minimal hyperplasia/hypertrophy in pituitary, atrophy of the adrenal cortex, and reduced anti-KLH IgM and IgG responses to KLH immunization.

- The NOAEL for oral administration of deflazacort in monkey was 6.0 mg/kg/day for 27 weeks, and 3.0 mg/kg/day for 39-weeks; however, the immunosuppression observed at these doses may be adverse in a less sterile environment.

Methods

Doses:	1.0 (LD), 3.0 (MD), 0.3/6.0 (HD) mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Species/Strain:	Cynomolgus monkey, from (b) (4)
Number/Sex/Group:	4/sex/group main study; 2/sex/group 6-week recovery for control and MD
Age:	~4 years old
Weight:	3.2-4.1 kg (M); 2.6-3.5 kg (F)
Satellite groups:	NA
Unique study design:	HD group was dosed at 0.3 mg/kg/day on Days 1-91, and at 6.0 mg/kg/day on Days 92-274. KLH was injected on Day 183 for main study animals and Day 15 of the recovery period for recovery animals to evaluate T-cell dependent antibody responses. Immunophenotyping was performed to analyze lymphocyte subsets. A qualitative examination of spermatogenic stages was conducted using H&E stained sections of testis, epididymis, and seminal vesicle.
Deviation from study protocol:	Minor deviations were reported; these did not affect the overall interpretation or the validity of the study

Group[e]	No. of Animals[b,c] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)[a]	6	6	0	0
2 (High)	4	4	0.3/6.0[d]	0.03/0.60[d]
3 (Low)	4	4	1.0	0.10
4 (Mid)	6	6	3.0	0.30

a Group 1 animals were given vehicle control article only.
b The first four animals/sex/group designated for terminal sacrifice were sacrificed after a 39-week dosing phase, and remaining animals designated for recovery sacrifice were sacrificed after a 6-week recovery phase.
c Animals in Groups 1 through 4 designated for terminal sacrifices (the first four animals/sex/group) were given one dose of KLH (1 mg/mL, 1 mL/animal) via subcutaneous injection on Day 183 of the dosing phase. All remaining animals in Groups 1 and 4 were given KLH (1 mg/mL, 1 mL/animal) once via subcutaneous injection on Day 15 of the recovery phase.
d The dose level for Group 2 was increased from 0.3 to 6.0 mg/kg/day starting on Day 92 of the dosing phase.
e Dose groups were changed to high-dose for Group 2, low-dose for Group 3, and mid-dose for Group 4 starting from Week 14 of the dosing phase.

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Observations and Results

Dosing Solution Analysis

Two sets of duplicates samples from the top, middle, and bottom of formulations prepared at various time points were analyzed for homogeneity. Two sets of duplicates from the middle of formulations prepared during Weeks 1, 5, 14, 21, 29, and 39 and on Day 92 (M) or 91 (F) were analyzed for concentration using HPLC.

All homogeneity and concentration samples were within 10% of target concentrations (actual range: 93.8-109.1%). No significant levels of drug were found in control samples.

Mortality

All animals were observed twice daily for mortality.

All animals survived to scheduled termination.

Clinical Signs

All animals were evaluated by cageside observation once daily. Detailed observations were conducted twice predose, prior to dosing on Day 1, once weekly thereafter within 10 minutes of dosing, and on days of scheduled sacrifice.

No drug-related clinical signs were reported, except for an increased incidence of alopecia at ≥ 1 mg/kg/day, especially in females.

Body Weights

Body weights were recorded twice predose, prior to dosing on Day 1, weekly during Weeks 2-13, every 2 weeks during Weeks 15-39, prior to dosing on Day 273, weekly during the recovery period, and on Day 42 of the recovery period.

No drug-related effects on body weight were reported.

Food Consumption

Food consumption was recorded qualitatively once daily during predose, dosing, and recovery phases, except on days when animals were being fasted.

No drug-related effects on food consumption were observed.

Ophthalmoscopy

Ophthalmic examinations were performed using an indirect ophthalmoscope after dilation with a mydriatic agent once predose and once on Days 86 (M)/85 (F) and 267.

No drug-related ophthalmology findings were reported.

ECG

Eight-lead ECGs were recorded once predose, on Days 5 and 267 (~1 hour postdose), and once on Day 40 (M)/39 (F) of the recovery period.

No drug-related effects on ECG parameters were reported.

Hematology

Blood samples were collected via a femoral vein from all animals after overnight fast twice predose; on Days 83 (F)/84 (M), 181, and 274; and on Days 13 (F)/14 (M) and 43 of the recovery period. The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count, differential blood cell count, reticulocyte count, fibrinogen, prothrombin time, and activated partial thromboplastin time. Blood smears were prepared but not analyzed.

On Day 274, slight increases were observed in neutrophils (MDF: 84%; HDF: 64%), monocytes (MDM: 68%), and fibrinogen (MDM: 30%; MDF: 33%; HDM: 58%; HDF: 22%), compared to controls, suggesting mild inflammation. No changes were observed in MD recovery animals.

Clinical Chemistry

Blood samples were collected from all animals after overnight fast via a femoral vein twice predose; on Days 83 (F)/84 (M), 181, and 274; and on Days 13 (F)/14 (M) and 43 of the recovery period. The following parameters were analyzed: urea nitrogen, total protein, albumin, globulin, albumin:globulin ratio, ALT, ALP, AST, gamma glutamyltransferase (GGT), calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, and triglycerides.

On Day 274, reductions were observed in alkaline phosphatase (LDM: 41%; MDM: 50%; HDM: 69%; LDF: 20%; MDF: 44%; HDF: 53%) and phosphorous (LDM: 17%; MDM: 12%; HDM: 23%; LDF: 14%; MDF: 18%; HDF: 16%), compared to controls. Recovery animals showed reduced ALP (MDM: 33%; MDF: 60%), but no difference in phosphorus, compared to controls.

Urinalysis

Urine samples were collected overnight from animals fasted overnight (≥ 10 hours) prior to blood collections once predose; on Days 83 (F)/84 (M), 181, and 274; and on Day 43 of the recovery period. Parameters analyzed included clarity, color, volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, and microscopic examination of sediment.

No drug-related effects on urinalysis parameters were reported.

Gross Pathology

Necropsy was conducted after overnight fasting on Day 274 on 4/sex/group, and on Day 43 of the recovery period on 2/sex control and MD animals. Animals were terminated by exsanguination under sodium pentobarbital anesthesia.

Drug-related macroscopic observations included small adrenal glands (2/4 LDM; 1/4 LDF; 1/4 MDF; 1/4 HDF; correlated with adrenal cortical atrophy), small thymus (2/4 LDM; 1/4 LDF; 4/4 MDM; 2/4 MDF; 3/4 HDM; 1/4 HDF; correlated with microscopic findings of decreased lymphocytes); small spleen (1/4 HDM; correlated with microscopic findings of decreased lymphocytes), and alopecia of skin/subcutis (2/4 LDF; 1/4 MDM; 2/4 MDF; 1/4 HDM; 1/4 HDF; correlated with microscopic findings of follicular atrophy). Drug-related changes in recovery animals were limited to alopecia in the skin (2/2 MDF).

Organ Weights

Weights were recorded for the following organs: adrenal glands, brain, epididymides, heart, kidneys, liver with gallbladder (drained), lung (with large bronchi), ovaries, pituitary gland, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroid (with parathyroid), and uterus.

As shown in the tables below, drug-related changes observed in organ weights included increases in pituitary and reductions in adrenal glands, spleen, thymus, and uterus.

Changes persisting throughout the 6-week recovery period included reduced relative weight of spleen in MDF (31%) and increased relative weight of pituitary in MDM (89%), compared to controls.

Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Terminal Body Weight (g)	4450	93%	91%	92%	3950	77%	98%	84%
Adrenal								
Absolute Weight (g)	0.664	84%	65%	64%	0.670	73%	80%	100%
Body Weight Ratio (%)	0.0149	95%	72%	72%	0.0173	94%	87%	118%
Brain Weight Ratio (%)	0.8548	93%	69%	71%	1.0710	72%	80%	94%
Spleen								
Absolute Weight (g)	4.138	73%	53%*	60%*	3.747	59%*	64%*	50%*
Body Weight Ratio (%)	0.0931	77%	59%*	66%*	0.0941	77%	66%*	59%*
Brain Weight Ratio (%)	5.3729	77%	56%*	65%*	5.8381	59%*	66%*	47%*
Thymus								
Absolute Weight (g)	1.549	62%	40%	41%	2.123	64%	72%	50%
Body Weight Ratio (%)	0.0353	67%	44%	48%	0.0541	86%	80%	62%
Brain Weight Ratio (%)	2.0424	66%	41%	44%	3.3847	65%	72%	48%
Pituitary								
Absolute Weight (g)	0.052	106%	121%	129%	0.054	83%	109%	104%
Body Weight Ratio (%)	0.0012	108%	133%	142%	0.0014	107%	114%	121%
Brain Weight Ratio (%)	0.0664	113%	131%	144%	0.0862	83%	109%	97%
Uterus								
Absolute Weight (g)	NA	NA	NA	NA	4.276	75%	63%	57%
Body Weight Ratio (%)					0.1110	95%	66%	65%
Brain Weight Ratio (%)					6.8294	75%	61%	53%

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage control mean value.

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Text Table 3.2: Test Article-Related Changes in Organ Weight Parameters - Recovery Sacrifice

		Deflazacort			
	Sex	Males		Females	
	Dose Group	1	4	1	4
	Dose Level (mg/kg/day)	0	3.0	0	3.0
	Terminal body weights (g)	5200	88%	3550	87%
Adrenal					
	Absolute Weight (g)	0.588	79%	0.582	89%
	Body Weight Ratio (%)	0.0113	90%	0.0167	100%
	Brain Weight Ratio (%)	0.8602	73%	0.9450	91%
Spleen					
	Absolute Weight (g)	4.242	109%	4.831	60%
	Body Weight Ratio (%)	0.0814	125%	0.1360	69%
	Brain Weight Ratio (%)	6.1757	101%	7.7326	60%
Thymus					
	Absolute Weight (g)	1.508	152%	1.642	164%
	Body Weight Ratio (%)	0.0288	176%	0.0452	192%
	Brain Weight Ratio (%)	2.1680	145%	2.5880	170%
Pituitary					
	Absolute Weight (g)	0.050	150%	0.064	92%
	Body Weight Ratio (%)	0.0009	189%	0.0018	106%
	Brain Weight Ratio (%)	0.0722	141%	0.1024	96%

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage control mean value.

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Histopathology

The following tissues from all animals were examined microscopically: adrenal glands, aorta, bone and bone marrow (sternum, femur), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lung with large bronchi, lymph nodes (mandibular, mesenteric), mammary gland (F), muscle (biceps femoris), optic nerve, ovary, pancreas, pituitary gland, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicle, skin/subcutis, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus, vagina, gross lesions (if any).

The battery of tissues examined was adequate.

No Peer Review was conducted; a signed pathology report was provided.

As shown in the tables below, drug-related microscopic observations included adrenal cortical atrophy; lymphocytic depletion in thymus, spleen, and lymph nodes; hyperplasia in mammary glands (M); follicular atrophy in skin/subcutis; absence of corpus luteum in

ovary; hyperplasia/hypertrophy in pituitary (M); increased adipocytes in bone marrow (M, sternum); atrophy of uterus and vaginal epithelium; and increased secretion in the lumen of the cervix. Dose-dependent atrophy of the adrenal cortex was characterized by minimal to marked decreases in the cytoplasmic vacuolation and size of cells in the zona fasciculata. The hyperplasia observed in male mammary glands was characterized by increased prominence of mammary tissue and dilated ducts. The absence of corpora lutea in the ovary was associated with the presence of atretic and/or dilated follicles with pyknotic cells and with the findings in uterus, cervix, and vagina.

Changes observed in recovery animals were limited to slight atrophy of the adrenal cortex in 1/2 MDM, and minimal hyperplasia/hypertrophy of cells in pars distalis of the pituitary in 1/2 MDM.

Text Table 4.5: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Adrenal Cortex								
Number Examined	4	4	4	4	4	4	4	4
Atrophy, zona fasciculata								
Minimal	0	1	0	0	0	0	1	0
Slight	0	1	1	1	0	1	2	2
Moderate	0	0	3	0	0	0	1	2
Marked	0	0	0	3	0	0	0	0
Total Finding Incidence	0	2	4	4	0	1	4	4

Text Table 4.7: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Mammary Gland, Male								
Number Examined	4	4	4	4	NA	NA	NA	NA
Hyperplasia								
Minimal	0	1	0	0				
Slight	0	0	2	3				
Moderate	0	0	0	1				
Skin/Subcutis								
Number Examined	4	4	4	4	4	4	4	4
Atrophy, follicle								
Minimal	0	1	1	1	0	2	2	0
Slight	0	0	0	1	0	0	0	1

NA = Not applicable.

Text Table 4.6: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Spleen								
Number Examined	4	4	4	4	4	4	4	4
Lymphocytes, decreased								
Minimal	0	0	1	0	0	0	0	2
Slight	0	0	2	1	0	0	2	1
Moderate	0	0	1	3	0	0	1	1
Thymus								
Number Examined	4	4	3	2	4	4	2	3
Lymphocytes, decreased								
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	2	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	1
Marked	0	0	0	1	0	0	0	1
Severe	0	0	0	1	0	0	0	0
Lymph Node, Mandibular								
Number Examined	4	4	4	4	4	4	3	3
Lymphocytes, decreased								
Minimal	0	0	2	1	0	0	2	2
Slight	0	0	1	3	0	0	0	0
Lymph Node, Mesenteric								
Number Examined	4	4	4	4	4	4	4	4
Lymphocytes, decreased								
Minimal	0	0	0	0	0	0	0	2
Slight	0	0	3	4	0	0	3	2

Text Table 4.9: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Marrow, Sternum								
Number Examined	4	4	4	4	4	4	4	4
Adipocytes, increased								
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	0	1	0	0	0	0

Text Table 4.8: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	4	2	1	3	4	2
Dose Level (mg/kg/day)	0	1.0	3.0	0.3/6.0	0	1.0	3.0	0.3/6.0
Ovary								
Number Examined	NA	NA	NA	NA	4	4	4	4
Corpus luteum, absent								
Present					0	2	4	4
Uterus								
Number Examined	NA	NA	NA	NA	4	4	4	4
Atrophy, endometrium								
Minimal					0	1	1	0
Slight					0	0	1	3
Vagina								
Number Examined	NA	NA	NA	NA	4	4	4	4
Atrophy, epithelium								
Minimal					0	1	0	0
Slight					0	3	3	4
Cervix								
Number Examined	NA	NA	NA	NA	4	4	4	4
Secretion, lumen, increased								
Minimal					0	0	2	0
Slight					0	1	2	4
Pituitary								
Number Examined	4	4	4	4	4	4	4	4
Hyperplasia/hypertrophy								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	1	1	0	0	0	0

NA = Not available.

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Male Reproductive Tissue and Spermatogenesis Evaluations

A qualitative examination of H&E stained sections of testes, epididymides, prostate, and seminal vesicles was conducted to evaluate progression of the spermatogenic cycle.

No drug-related changes were observed.

T-Cell Dependent Antibody Analysis

KLH (1 mg/animal, in 0.9% saline) was injected subcutaneously on Day 183 for main study animals and on Day 15 of the recovery period for recovery animals. Blood samples were collected via a femoral vein prior to dosing on Days 1, 186, 190, 197, 204 and 211, and on Days 18, 22, 29, 36, and 43 of the recovery period. Serum samples were analyzed for anti-KLH IgM and IgG titers using an ECL assay, with 100 (1:100 dilution) as the lower limit of detection.

As shown in the sponsor's figures below, drug-related reductions (compared to controls) were observed in the mean titers of anti-KLH IgM during the dosing period in F dosed at 0.3/6.0 mg/kg/day, and during the recovery period in M and F dosed at 3.0 mg/kg/day.

Figure 6.1: IgM Response to KLH Challenge During the Dosing Phase – Males

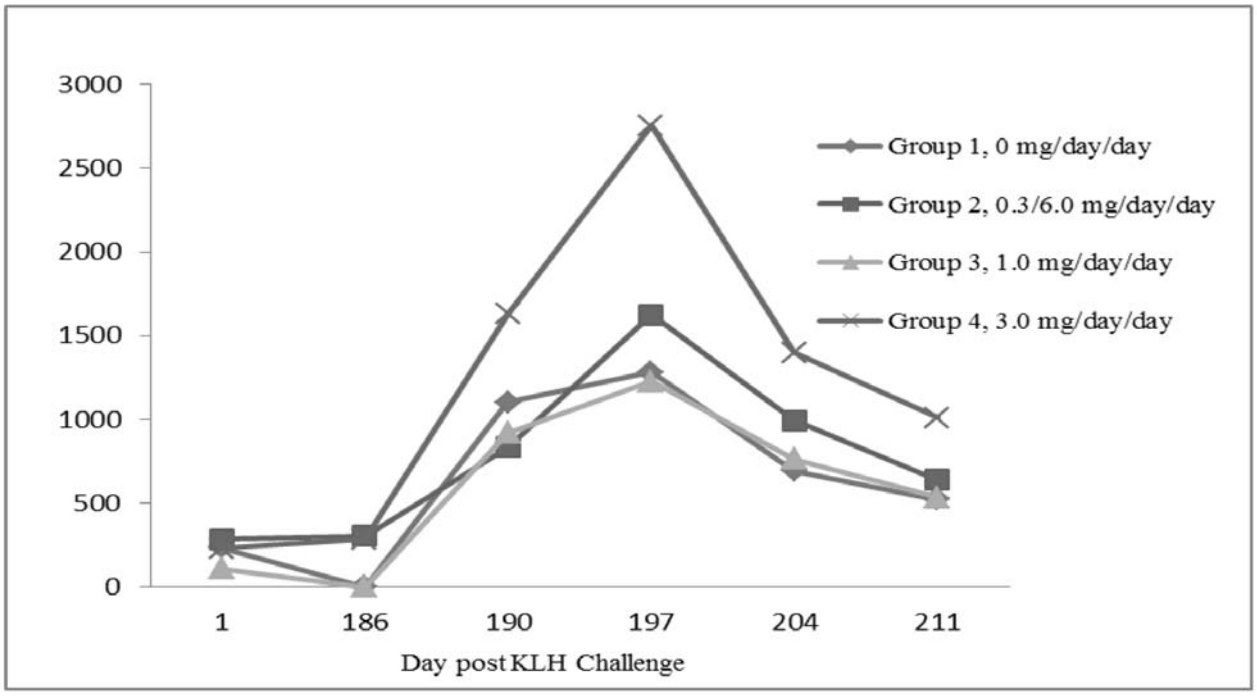

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Figure 6.2: IgM Response to KLH Challenge During the Dosing Phase – Females

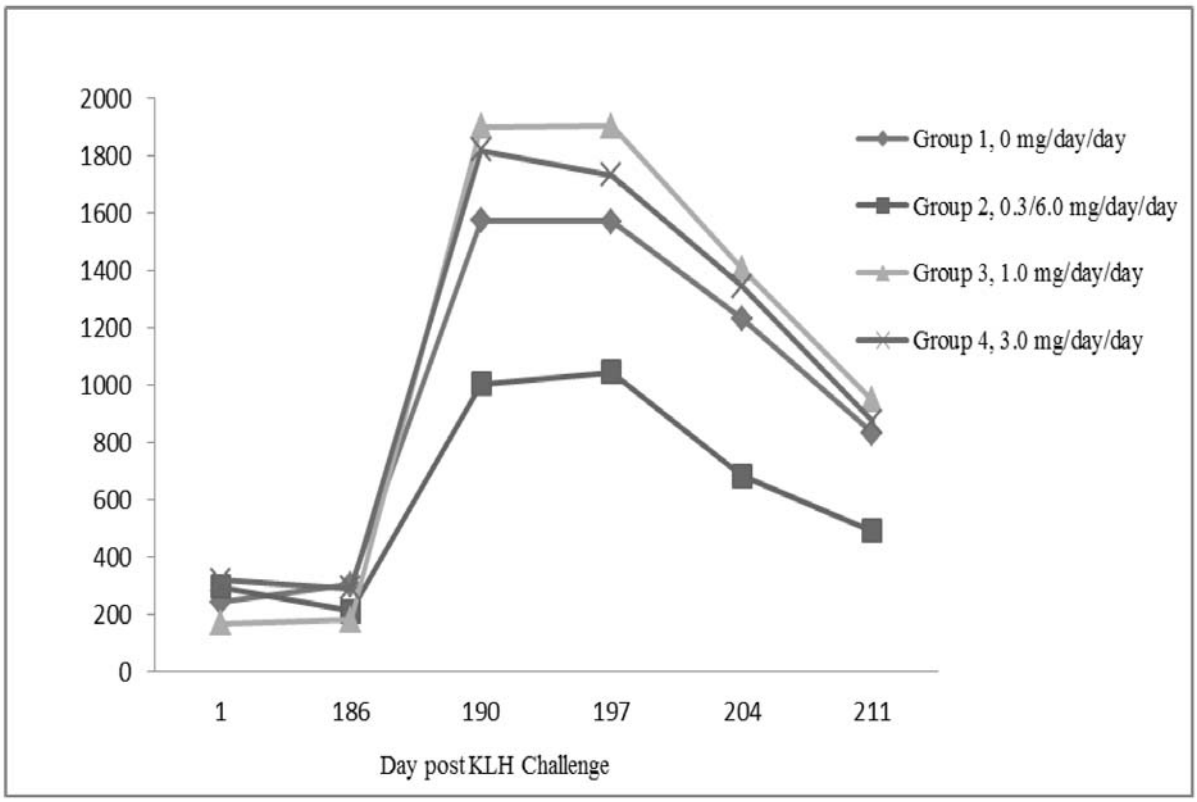

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Figure 6.3: IgM Response to KLH Challenge During the Recovery Phase – Males

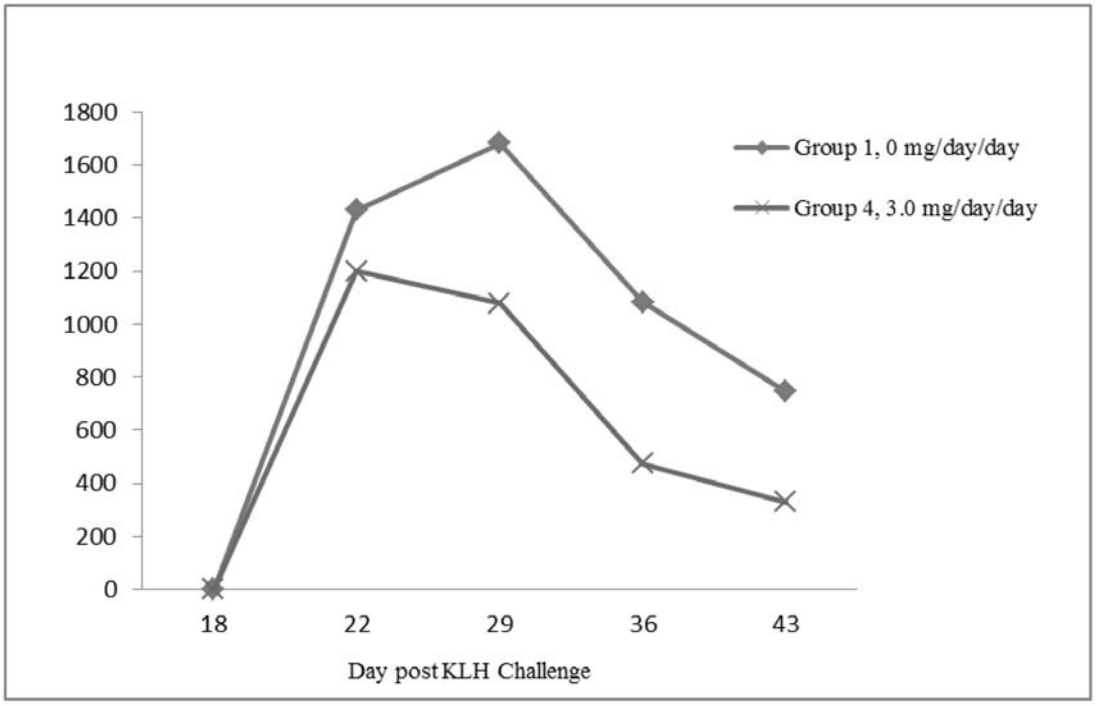

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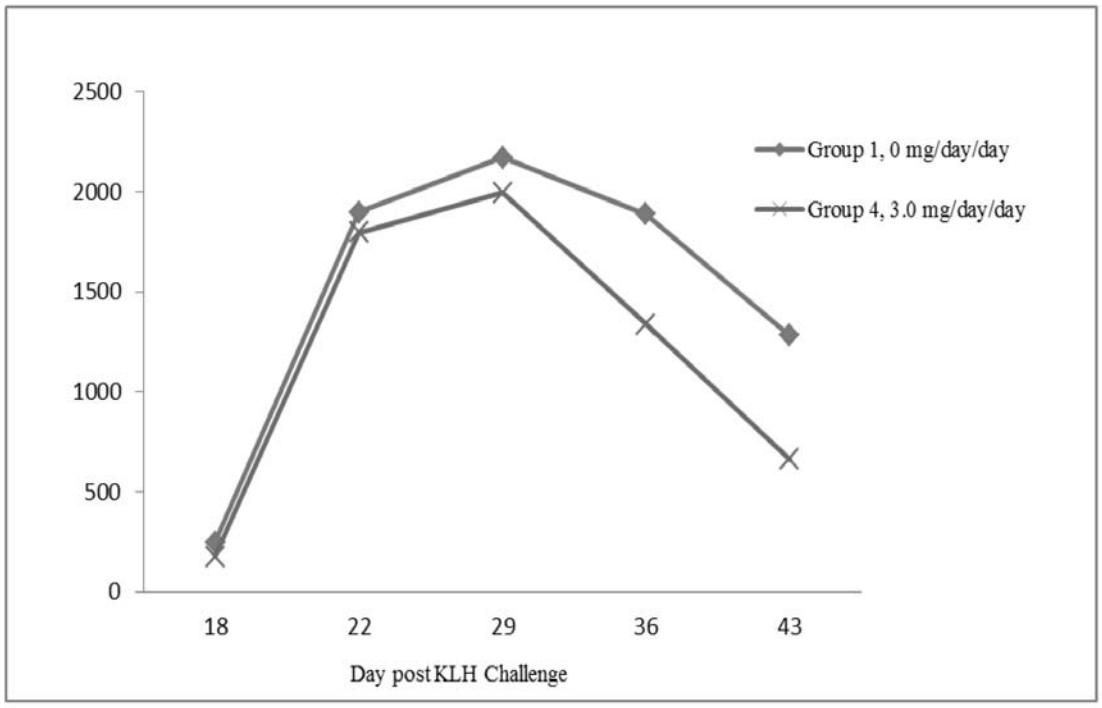
Figure 6.4: IgM Response to KLH Challenge During the Recovery Phase – Females



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Drug-related reductions (compared to controls) were observed in the mean titers of anti-KLH IgG during the dosing period in M and F dosed at 0.3/6.0 mg/kg/day, and during the recovery period in M and F dosed at 3.0 mg/kg/day.

Figure 6.5: IgG Response to KLH During the Dosing Phase - Males

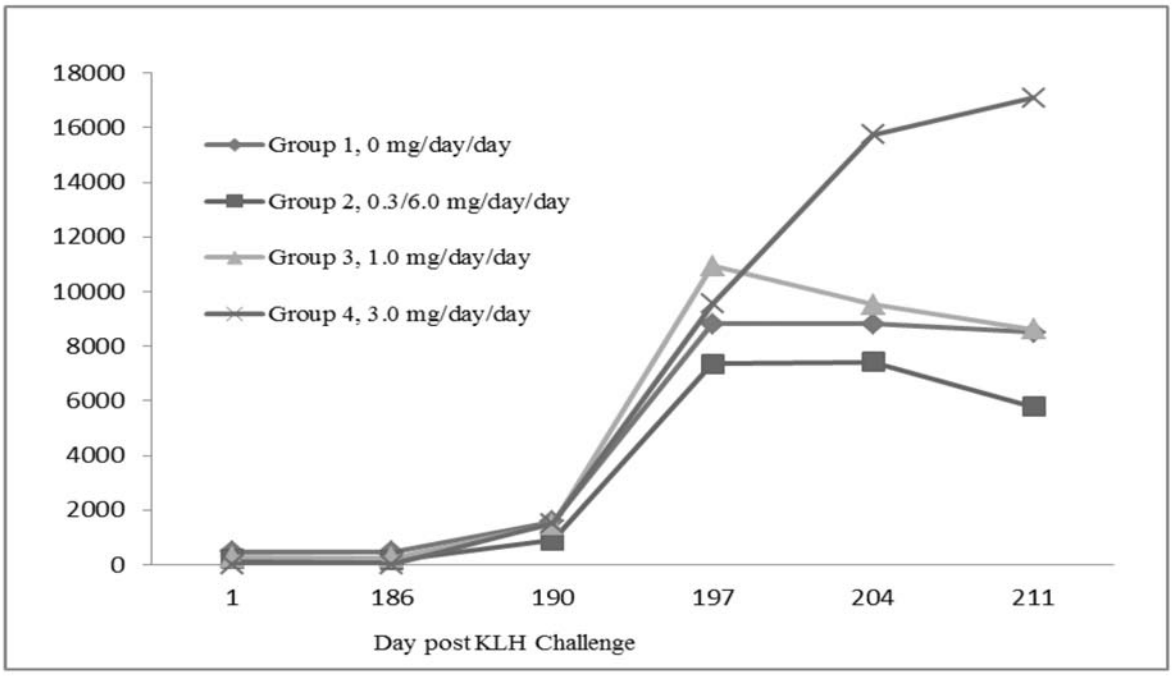

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Figure 6.6: IgG Response to KLH During the Dosing Phase – Females

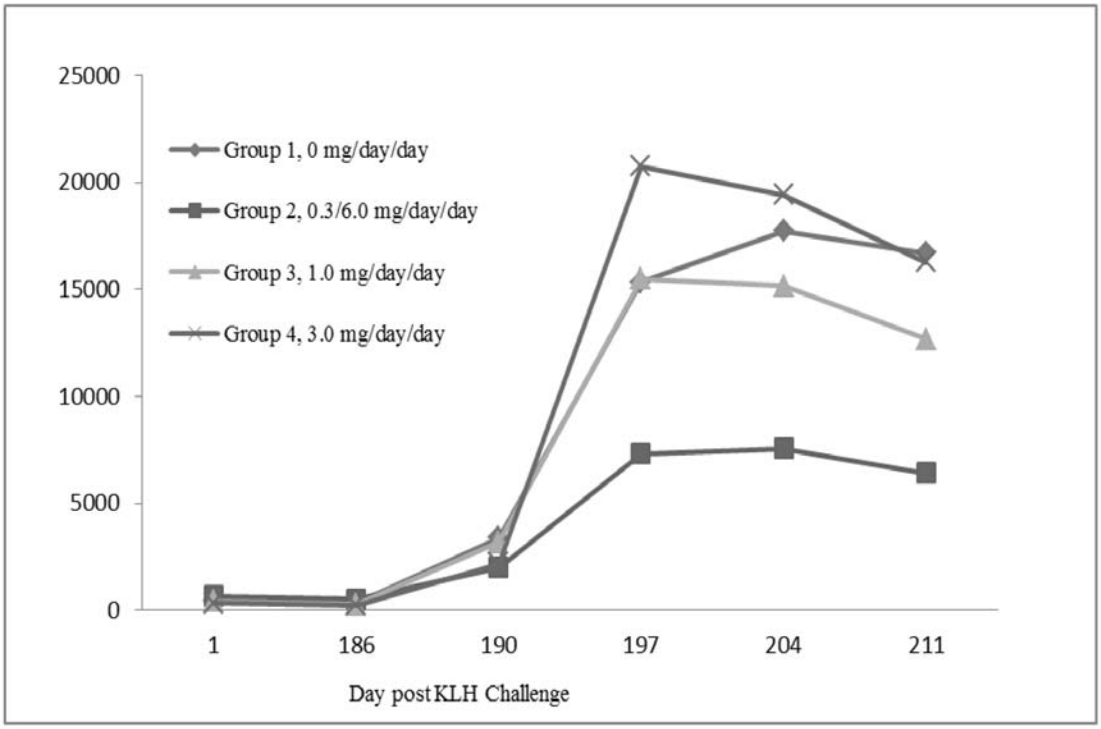

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Figure 6.7: IgG Response to KLH During the Recovery Phase – Males

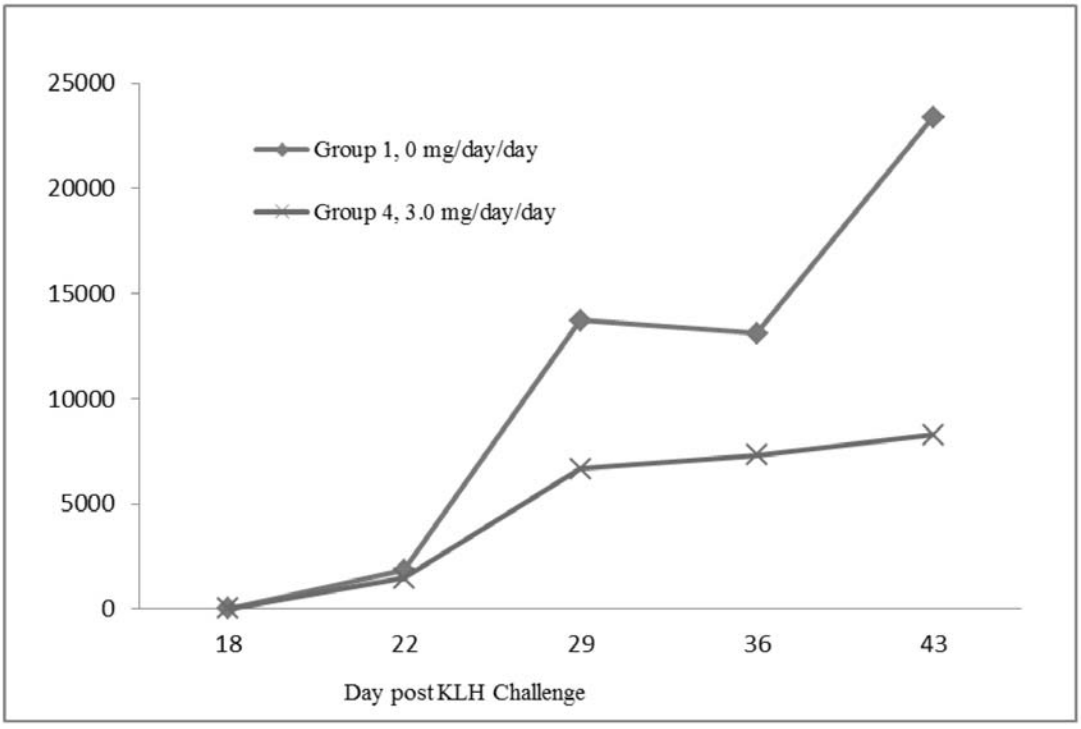

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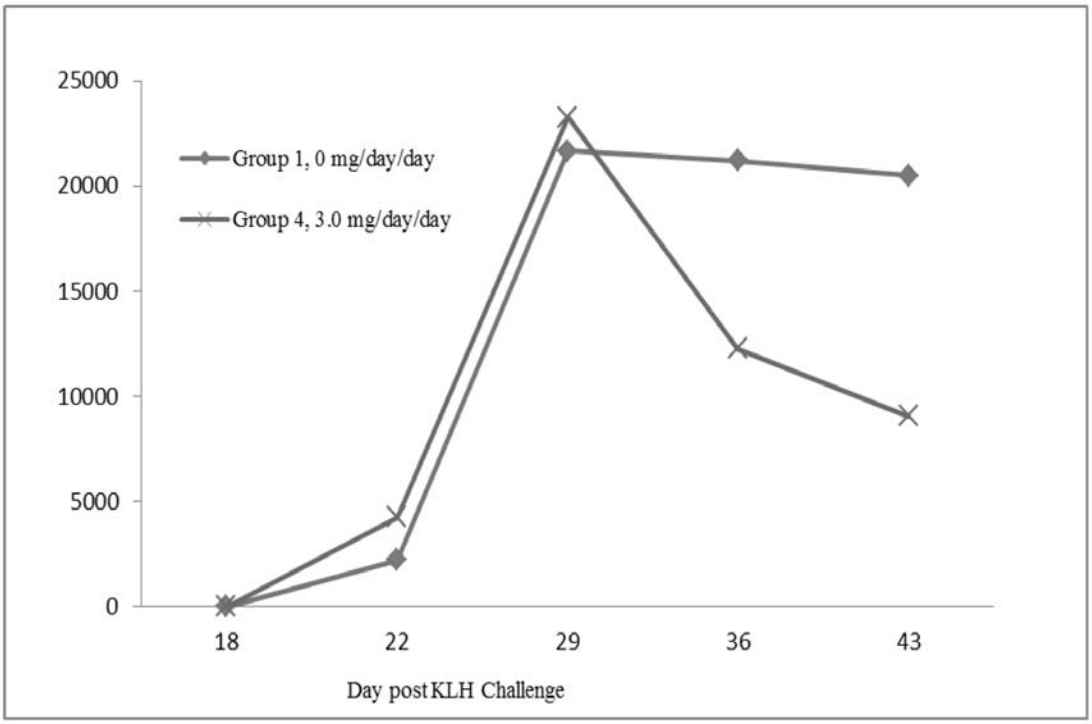
Figure 6.8: IgG Response to KLH During the Recovery Phase – Females



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Lymphocyte Subset Immunophenotyping Analysis

Blood samples were collected via a femoral vein on Day 17 predose, Days 188 and 274 of the dosing period, and Day 43 of the recovery period. Immunophenotyping was performed to assess the following parameters: CD3+CD4+ (helper T cells), CD3+CD8+ (cytotoxic T cells), CD3+CD4+/CD3+CD8+ (ratio of helper to cytotoxic T cells), and CD20+ (B cells).

No drug-related changes in lymphocyte subsets were observed.

Toxicokinetics

Blood samples were collected from nonfasted animals via a femoral vein on Days 1, 90, 92 (HD only, day of dose increase), 188, and 271 at the following timepoints: predose, and 0.5, 1, 2, 4, 8, and 24 hours postdose. Plasma was analyzed for levels of deflazacort and its active metabolites, 21-desDFZ and 6β-OH-21-desDFZ.

Plasma levels of deflazacort were below the limit of detection at all doses and time points, indicating rapid metabolism after oral administration.

Systemic exposures to 21-desDFZ generally increased in proportion to dose, though C_{max} increased less than dose-proportionally in F on Day 1, and more than dose-proportionally in F on Days 188 and 271. Exposures were generally slightly greater in F

than M, though C_{max} and $AUC_{0\text{-}24\ hr}$ were up to 2.5- and 1.5-fold greater, respectively, in F on Days 188 and 271. No consistent increase in exposure was observed with repeated dosing.

Systemic exposures to 6β-OH-21-desDFZ generally increased in proportion to dose, though C_{max} increased less than dose-proportionally in M and F on Day 1 (as did $AUC_{0\text{-}24\ hr}$ in M), and more than dose-proportionally in F on Day 90 and in M on Day 271. Sex differences were less than 2-fold and inconsistent. No accumulation with repeated dosing was observed. Day 271 6β-OH-21-desDFZ $AUC_{0\text{-}24\ hr}$ levels were 66-91% of Day 271 21-desDFZ levels.

Summary of 21-desacetyldeflazacort C_{max} and $AUC_{0\text{-}24}$ in Monkey Plasma

Interval (Day)	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	$AUC_{0\text{-}24}$ (hour*ng/mL)	AR* $AUC_{0\text{-}24}$ ((hour*ng/mL)/(hour*ng/mL))
1	2	0.3	M	32.2	174	-
			F	51.6	184	-
	3	1.0	M	94.4	637	-
			F	97.1	551	-
	4	3.0	M	282	1800	-
			F	285	1610	-
90	2	0.3	M	54.7	170	1.00
			F	70.8	184	1.00
	3	1.0	M	200	577	0.919
			F	194	573	1.06

	4	3.0	M	584	1530	0.853
			F	624	1730	1.09
92	2	6.0	M	1120	3380	-
			F	1770	3700	-
188	2	6.0	M	846	2810	0.824
			F	1980	3620	1.00
	3	1.0	M	177	587	0.921
			F	208	567	1.04
	4	3.0	M	648	1610	0.904
			F	800	1610	1.02
271	2	6.0	M	760	2470	0.720
			F	1920	3660	1.01
	3	1.0	M	168	539	0.869
			F	145	543	1.01
	4	3.0	M	554	1460	0.811
			F	826	1810	1.10

*AR: Accumulation ratio

(pages 388-389 of Study Report)

Summary of 6-β-OH-21-desacetyldeflazacort C_{max} and AUC_{0-24} in Monkey Plasma

Interval (Day)	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	AUC_{0-24} (hour*ng/mL)	AR* AUC_{0-24} ((hour*ng/mL)/(hour*ng/mL))
1	2	0.3	M	23.5	137	-
			F	32.8	151	-
	3	1.0	M	54.2	414	-
			F	66.5	462	-
	4	3.0	M	139	1030	-
			F	162	1040	-
90	2	0.3	M	29.5	125	0.905
			F	42.9	134	0.920
	3	1.0	M	138	460	1.14
			F	122	485	1.09
	4	3.0	M	401	1270	1.23
			F	329	1190	1.16
92	2	6.0	M	605	2420	-
			F	829	2400	-
188	2	6.0	M	480	2340	0.960
			F	792	2280	0.952
	3	1.0	M	107	432	1.05
			F	113	442	0.966
	4	3.0	M	362	1230	1.20
			F	407	1190	1.13
271	2	6.0	M	462	2240	0.915
			F	862	2430	1.01
	3	1.0	M	109	485	1.18
			F	88.5	419	0.929
	4	3.0	M	303	1200	1.16
			F	436	1270	1.19

*AR: Accumulation ratio

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26-Week Oral Gavage Toxicity and Toxicokinetic Study of Deflazacort in Sprague Dawley Rats with a 4 Week Recovery

Study no.:	Sponsor Study MP-104-NC-036 (b) (4) Study 8310952
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of dosing initiation:	(b) (4)
GLP compliance:	Yes (exception: test article characterization was tested under GMP)
QA statement:	Yes
Separate pathology report:	Yes
Drug, lot #, and % purity:	Deflazacort batch DFZγ14002, 99.53% pure

Key Study Findings

- Dose-dependent decreases were observed in body weight gain (34-85% M; 26-63% F) and final body weight (18-43% M; 10-27% F).
- Circulating lymphocytes were decreased (39-78% M; 30-58% F), neutrophils were increased (42-52% M), and triglycerides were increased (21-86% M).
- Minimal to severe lymphocytic depletion and hypocellularity were observed in lymphoid organs.
- Minimal to marked atrophy was observed in multiple organs: skin/subcutis, glandular stomach, esophagus, duodenum, rectum, and adrenal cortex.
- All changes showed full or partial recovery after a 4-week recovery period.
- The NOAELs were the LDs of 0.05 mg/kg/day for M and 0.10 mg/kg/day for F, based on the severity of the effects on the lymphoid system observed at the mid and high doses in both sexes (e.g., >50% reduction in circulating lymphocytes).

Methods

Doses:	M: 0, 0.05, 0.15, or 0.50 mg/kg/day deflazacort F: 0, 0.10, 0.30, or 1.00 mg/kg/day deflazacort
Frequency of dosing:	Once daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose
Species/Strain:	Sprague Dawley rat, (b) (4)
Number/Sex/Group:	Planned: 10/sex/group for main study; 6/sex control & HD for 4-week recovery. Actual: first 9/sex in control and HD groups were sacrificed after 26 weeks; remaining animals in these groups were sacrificed after the 4-week recovery period.
Age:	9-10 weeks old at dosing initiation
Weight:	306-368 g (M); 215-268 g (F)
Satellite groups:	4/sex TK control; 16/sex/group TK on drug
Unique study design:	Detailed spermatogenesis evaluation was conducted in control and HD males based on quantitative examination of spermatogenic stages using H&E-stained sections of testes and epididymides.
Deviation from study protocol:	Ophthalmic examinations were not conducted during Week 26. Other minor deviations were reported but did not affect the overall interpretation or the validity of the study

3.1.5 Study Design

Group	Subgroup	No. of Animals[b]		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
		Male	Female	Males	Female	Male	Female
1 (Control)[a]	1 (Toxicity)	16	-	0	-	0	-
	2 (Toxicokinetic)	4	-				
2 (Control)[a]	1 (Toxicity)	-	16	-	0	-	0
	2 (Toxicokinetic)	-	4				
3 (Low)	1 (Toxicity)	10	-	0.05	-	0.005	-
	2 (Toxicokinetic)	10	-				
4 (Low)	1 (Toxicity)	-	10	-	0.10	-	0.010
	2 (Toxicokinetic)	-	10				
5 (Mid)	1 (Toxicity)	10	-	0.15	-	0.015	-
	2 (Toxicokinetic)	10	-				
6 (Mid)	1 (Toxicity)	-	10	-	0.30	-	0.030
	2 (Toxicokinetic)	-	10				
7 (High)	1 (Toxicity)	16	-	0.50	-	0.050	-
	2 (Toxicokinetic)	10	-				
8 (High)	1 (Toxicity)	-	16	-	1.00	-	0.100
	2 (Toxicokinetic)	-	10				

- = Not applicable.

a Groups 1 and 2 animals received vehicle control article only.

b The first 9 toxicity animals from Group 1 and 10 toxicity animals/sex from Groups 2, 7, and 8 and all animals in Groups 3-6 designated for the terminal sacrifice were sacrificed after a 26-week dosing phase, and remaining animals designated for the recovery sacrifice were sacrificed after a 4-week recovery phase.

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Observations and Results

Dosing Solution Analysis

Homogeneity was evaluated in two sets of samples taken from the top, middle, and bottom of formulations at each concentration during Weeks 1 and 26. Concentration was evaluated in two sets of duplicate samples taken from the middle of the vehicle control and each drug formulation during Weeks 1, 5, 13, and 26.

Mean concentrations were within acceptable limits (±7% of the overall mean for homogeneity samples, and ±5% of target concentrations; range: 95.0-102.1%). No significant levels of drug were found in control samples.

Mortality

All animals were observed twice daily for mortality.

All animals survived to scheduled termination, with the exception of one control M, found dead on Day 165 after struggling during dosing.

Clinical Signs

All animals were evaluated by cageside observation once daily. Detailed observations were conducted twice predose, prior to dosing on Day 1, once weekly thereafter within 10 minutes of dosing, and on days of scheduled sacrifice.

As shown in the tables below, deflazacort induced dose-dependent increases in the incidence of skin lesions in both sexes, and in hyperactivity and reactivity to stimulus in males. Observations persisting through the 4-week recovery period were limited to skin lesions in a few HDM and HDF.

Text Table 4.2: Summary of Skin Lesions*

Group No. Sex	Dose Level (mg/kg/day)	Total No. Animals	No. Animals with Skin-observation	No. Skin-observation/ Animal	Total No. Skin-observation /Group
1 Male	0	16	2 (13%)	9.4	151
3 Male	0.05	10	3 (30%)	3.2	32
5 Male	0.15	10	8 (60%)	12.4	124
7 Male	0.5	16	14 (81%)	33.2	531
2 Female	0	16	6 (38%)	4.8	77
4 Female	0.1	10	7 (70%)	17.8	178
6 Female	0.3	10	8 (70%)	30.0	300
8 Female	1.0	16	16 (100%)	28.8	460

* = Skin lesions included discolored haircoat, skin alopecia, skin scab, and skin discoloration.

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Effect of deflazacort on clinical observations (% of animals affected)								
Dosage (mg/kg/day)	0	0	0.05	0.10	0.15	0.30	0.5	1.0
Sex	M	F	M	F	M	F	M	F
Hyperactive	0/16	0/16	0/10	0/10	0/10	0/10	3/16 (19%)	0/16
Increased reactivity to stimulus	0/16	0/16	0/10	0/10	3/10 (30%)	0/10	1/16 (6%)	0/16

(Reviewer's Table)

Body Weights

Body weights were recorded twice predose, prior to dosing on Day 1, weekly through Week 26, prior to dosing on Day 182, weekly during the recovery period, and on Day 28 of the recovery period.

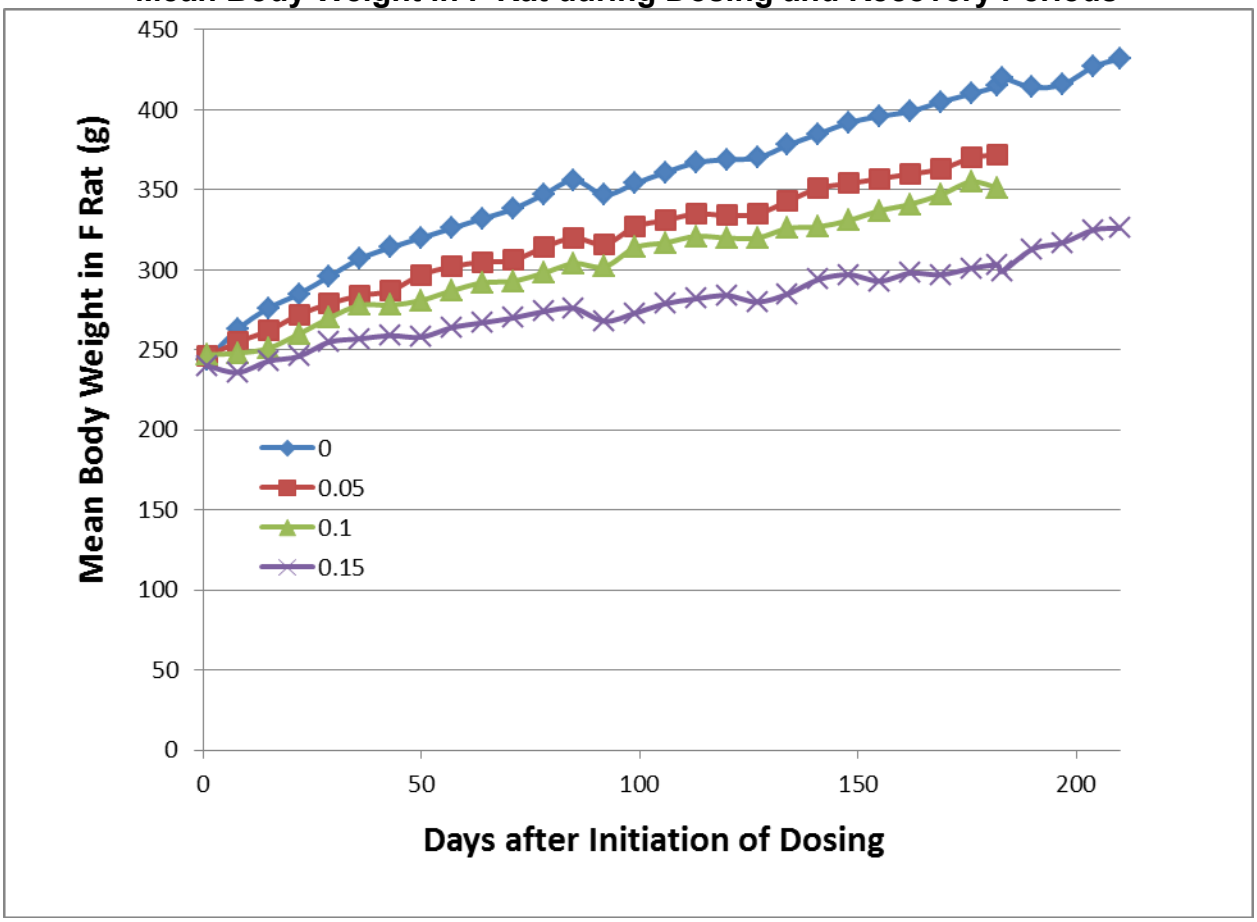
As shown in the table and figures below, oral administration of deflazacort resulted in dose-dependent reductions in mean body weight gain and final body weight.

Effect of deflazacort on body weight (% reduction vs. control)								
Dosage (mg/kg/day)	0	0	0.05	0.10	0.15	0.30	0.5	1.0
Sex	M	F	M	F	M	F	M	F
Body weight gain (Day 1-182)	0%	0%	34%	26%	62%	39%	85%	63%
Final body weight	0%	0%	18%	10%	33%	15%	43%	27%

(Reviewer's Table)

(Reviewer's Figure)

Mean Body Weight in F Rat during Dosing and Recovery Periods



(Reviewer's Figure)

Food Consumption

Food consumption was recorded qualitatively weekly during Weeks 1-25, daily on Days 176-182, weekly during Weeks 1-3 of the recovery period, and daily on Days 22-28 of the recovery period.

As shown in the table below, mean food consumption was reduced dose-dependently in males and females.

Text Table 4.4: Summary Food Consumption Data

Group/sex	Dose Level (mg/kg/day)	Dosing Phase (Days 1-182) g/animal/day	Dosing Phase (Days 1-182) %	Recovery Phase (Days 1-28) g/animal/day	Recovery Phase (Days 1-28) %
1M	0	31	NA	29	NA
3M	0.05	25	-19	NA	NA
5M	0.15	23*	-26	NA	NA
7M	0.5	21*	-32	26*	-10
2F	0	19	NA	20	NA
4F	0.1	19	0	NA	NA
6F	0.3	18	-5	NA	NA
8F	1	17*	-11	19	-5

- = Decreased; F = Female; M = Male; NA = Not applicable.
* = Statistically significant, $P \leq 0.05$.

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Ophthalmoscopy

Ophthalmic examinations were performed once predose using an indirect ophthalmoscope after dilation with a mydriatic agent. Examinations during Week 26 were required by the Protocol but were not conducted.

Hematology

Blood samples were collected from all non-TK animals after overnight fast via a jugular vein on Day 87 and on the day of scheduled sacrifice (Day 183 for main study, Day 212 for recovery animals). The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count, differential blood cell count, reticulocyte count, fibrinogen, prothrombin time, and activated partial thromboplastin time. Blood smears were prepared but not analyzed.

As shown in the tables below, oral administration of deflazacort resulted in increases in red cell mass (RBC counts, hemoglobin, and hematocrit), mean cell volume (MCV), mean cell hemoglobin (MCH), fibrinogen, and neutrophil counts; and reductions in reticulocyte counts, platelet counts, white blood cell counts, and lymphocyte counts. The most persistent changes were the decreases in WBC and lymphocyte counts, but all changes were at least partially reversed during the 4-week recovery period. Reticulocyte counts in HDM recovery animals showed a rebound increase in response to the suppression during the dosing period.

Day 183 Mean Hematology Parameters (% change vs. control)								
Dosage (mg/kg/day)	0	0	0.05	0.10	0.15	0.30	0.5	1.0
Sex	M	F	M	F	M	F	M	F
RBC count (E6/µL)	8.58	7.66	**9.15 (7%)**	7.92 (3%)	**9.26 (8%)**	**8.57 (12%)**	**9.28 (8%)**	**8.85 (16%)**
Hemoglobin (g/dL)	15.4	14.8	**16.7 (8%)**	15.2 (3%)	**17.3 (12%)**	**16.6 (12%)**	**17.7 (15%)**	**17.1 (16%)**
Hematocrit (%)	48.6	46.9	**51.7 (6%)**	48.0 (2%)	**53.1 (9%)**	**52.5 (12%)**	**54.8 (13%)**	**54.5 (16%)**
MCV (fL)	56.7	61.2	56.5 (0%)	60.7 (-1%)	57.4 (1%)	61.2 (0%)	**59.1 (4%)**	61.6 (1%)
MCH (pg)	18.0	19.3	18.2 (1%)	19.3 (0%)	18.7 (4%)	19.3 (0%)	**19.1 (6%)**	19.4 (1%)
Fibrinogen (mg/dL)	267	171	270 (1%)	172 (1%)	266 (0%)	**205 (20%)**	283 (6%)	**210 (23%)**
Neutrophils (E3/µL)	1.60	1.10	**2.43 (52%)**	1.05 (-5%)	**2.28 (42%)**	1.18 (7%)	**2.41 (51%)**	1.29 (17%)
Reticulocytes (E3/µL)	218.4	160.1	175.5 (-20%)	158.6 (-1%)	169.0 (-23%)	149.7 (-6%)	163.9 (-25%)	145.9 (-9%)
Platelets (E3/µL)	1187	937	**963 (-19%)**	920 (-2%)	**906 (-24%)**	777 (-17%)	**880 (-26%)**	869 (-7%)
WBC count (E3/µL)	7.76	5.07	**6.44 (-17%)**	**3.92 (-23%)**	**4.94 (-36%)**	**3.31 (-35%)**	**4.11 (-47%)**	**3.15 (-38%)**
Lymphocytes (E3/µL)	5.80	3.64	**3.56 (-39%)**	**2.55 (-30%)**	**2.27 (-61%)**	**1.80 (-51%)**	**1.26 (-78%)**	**1.54 (-58%)**

(Reviewer's Table; Bold = statistically significant, p≤0.05 ANOVA and Dunnett's)

Day 212 Mean Hematology Parameters (% change vs. control)				
Dosage (mg/kg/day)	0	0	0.5	1.0
Sex	M	F	M	F
MCV (fL)	54.8	58.9	**59.8 (9%)**	57.7 (-2%)
MCH (pg)	17.7	19.3	**19.4 (10%)**	19.1 (-1%)
Fibrinogen (mg/dL)	286	177	293 (2%)	184 (4%)
Reticulocytes (E3/µL)	201.4	183.7	**306.6 (52%)**	187.2 (2%)
Platelets (E3/µL)	1211	1070	1086 (-10%)	888 (-17%)
WBC count (E3/µL)	8.52	4.88	**6.16 (-28%)**	**3.08 (-37%)**
Lymphocytes (E3/µL)	6.41	3.90	**4.39 (-32%)**	**1.97 (-49%)**

(Reviewer's Table; Bold = statistically significant, p≤0.05 Two-sample t-test)

Clinical Chemistry

Blood samples were collected from all main study animals after overnight fast via a jugular vein on Day 87 and on the day of scheduled sacrifice (Day 183 for main study, Day 212 for recovery animals). The following parameters were analyzed: urea nitrogen, total protein, albumin, globulin, albumin:globulin ratio, alanine aminotransferase, ALP, AST, GGT, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, triglycerides.

As shown in the tables below, drug-related changes observed in LD animals were limited to decreased cholesterol and increased triglycerides in M; no statistically significant changes were reported in LDF. In addition to the changes seen in LDM, MDM and HDM showed increased albumin, decreased globulin, increased albumin:globulin (A:G) ratio, and increased ALP, compared to controls. MDF and HDF showed decreases in albumin, cholesterol, and calcium. ALT was slightly increased in HDM, and total protein was slightly decreased in HDF.

The only changes persisting through the 4-week recovery period were slight reductions in globulin, A:G ratio, and cholesterol in HDM.

Day 183 Mean Clinical Chemistry Parameters (% change vs. control)								
Dosage (mg/kg/day)	0	0	0.05	0.10	0.15	0.30	0.5	1.0
Sex	M	F	M	F	M	F	M	F
Total Protein (g/dL)	7.2	8.5	7.3 (1%)	8.1 (-5%)	7.2 (0%)	7.9 (-7%)	7.4 (3%)	**7.5 (-12%)**
Albumin (g/dL)	4.5	6.2	4.8 (7%)	5.9 (-5%)	**4.8 (7%)**	**5.6 (-10%)**	**5.3 (18%)**	**5.4 (-13%)**
Globulin (g/dL)	2.7	2.3	2.6 (-4%)	2.2 (-4%)	**2.3 (-15%)**	2.3 (0%)	**2.2 (-19%)**	2.1 (-9%)
A:G Ratio	1.7	2.7	1.9 (12%)	2.7 (0%)	**2.1 (24%)**	2.5 (-7%)	**2.5 (47%)**	2.6 (-4%)
Cholesterol (mg/dL)	93	126	**67 (-28%)**	107 (-15%)	**43 (-54%)**	**66 (-48%)**	**40 (-57%)**	**48 (-62%)**
Triglycerides (mg/dL)	58	73	**95 (64%)**	63 (-14%)	70 (21%)	68 (-7%)	**108 (86%)**	61 (-16%)
ALT (U/L)	38	39	43 (13%)	32 (-18%)	38 (0%)	34 (-13%)	**50 (32%)**	37 (-5%)
ALP (U/L)	69	23	89 (29%)	22 (-4%)	**102 (48%)**	31 (35%)	**99 (43%)**	34 (48%)
Calcium (mg/dL)	10.9	11.9	10.8 (-1%)	11.6 (-3%)	10.7 (-2%)	**11.4 (-4%)**	10.7 (-2%)	**11.2 (-6%)**

(Reviewer's Table; Bold = statistically significant, p≤0.05 ANOVA and Dunnett's)

Day 212 Mean Clinical Chemistry Parameters (% change vs. control)				
Dosage (mg/kg/day)	0	0	0.5	1.0
Sex	M	F	M	F
Total Protein (g/dL)	7.8	9.1	7.1 (-9%)	8.2 (-10%)
Albumin (g/dL)	4.8	6.7	4.7 (-2%)	6.1 (-9%)
Globulin (g/dL)	3.0	2.4	**2.4 (-20%)**	2.2 (-8%)
Albumin:Globulin Ratio	1.6	2.8	**1.9 (19%)**	2.9 (4%)
Cholesterol (mg/dL)	104	125	**80 (-23%)**	114 (-9%)
Triglycerides (mg/dL)	115	116	96 (-17%)	88 (-24%)
ALT (U/L)	34	34	35 (3%)	33 (-3%)
ALP (U/L)	68	19	77 (13%)	28 (47%)
Calcium (mg/dL)	11.4	12.0	11.6 (2%)	11.7 (-2%)

(Reviewer's Table; Bold = statistically significant, p≤0.05 Two-sample t-test)

Urinalysis

Urine samples were collected overnight from animals fasted overnight prior to blood collections on Day 87, and on the day of scheduled termination (Day 183 or 212). Parameters analyzed included clarity, color, volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, and microscopic examination of sediment.

No drug-related changes were observed.

Gross Pathology

Necropsy was conducted after overnight fasting on Day 183 on 10 animals/sex/group (9 control M, due to one accidental death on Day 165), and on Day 29 of the recovery period on 6 animals/sex control and HD animals. Animals were terminated by exsanguination under sodium pentobarbital anesthesia.

As shown in the tables below, drug-related macroscopic findings included alopecia, small adrenal glands, and small thymus. Changes persisting in recovery animals were limited to small adrenal in 1/5 HDM, indicating almost complete reversal.

Text Table 4.7: Incidence and Severity of Test Article-Related Macroscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	5	7	2	4	6	8
Dose Level (mg/kg/day)	0	0.05	0.15	0.50	0	0.10	0.30	1.00
Number Examined	9	10	10	10	10	10	10	10
Skin/Subcutis								
Alopecia	1	0	2	5	1	2	2	5
Mass	0	0	0	0	2	0	0	0
Scab	0	0	0	1	0	0	0	0
Adrenal								
Small	0	0	1	7	0	0	0	0
Thymus								
Small	0	0	0	0	0	2	1	5

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Organ Weights

Weights were recorded for the following organs: adrenal glands, brain, epididymides, heart, kidneys, liver, lung (with large bronchi), ovaries, pituitary gland, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroid (with parathyroid), and uterus.

Statistically significant drug-related changes in mean organ weights (relative to body weight) were limited to reductions of 15% and 27% in the spleen in MDM and HDM, respectively. These changes were completely reversed during the 4-week recovery period. Some individual animals (1 MDM, 4 HDM, and 1 HDM recovery) that showed small adrenals had corresponding substantial reductions (33-57%) in relative adrenal weight compared to controls. Similarly, 3 HDF that showed small thymus also had substantial reductions (29-65%) in relative weight of thymus compared to controls.

Histopathology

The following tissues from all control and HD animals were examined microscopically: adrenal glands*, aorta, bone and bone marrow (sternum*, femur*), brain, cecum, cervix, colon, duodenum*, epididymides, esophagus*, eyes, GALT*, Harderian gland, heart, ileum, jejunum, kidneys, liver*, lung* with large bronchi, lymph nodes (mandibular*, mesenteric*), mammary gland (F), muscle (biceps femoris), optic nerve, ovary, pancreas, pituitary gland, prostate, rectum*, salivary glands (mandibular), sciatic nerve, seminal vesicle, skin/subcutis*, spinal cord (cervical, thoracic, lumbar), spleen*, stomach*, testes, thymus*, thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus, vagina, gross lesions (if any). Suspected target tissues (indicated by an asterisk) were examined in all dose groups.

The battery of tissues examined was adequate.

No Peer Review was conducted. A signed pathology report was provided.

As shown in the tables below, drug-related microscopic observations included decreased lymphocytes in the cortex of the thymus, spleen, GALT/Peyer's patch, and lymph nodes; hypocellularity in the marrow of the femur and sternum; hepatocellular vacuolation in liver (cytoplasmic microvesicles in randomly distributed hepatocytes); clusters of vacuolated alveolar macrophage infiltrates in the lung; and atrophy in the skin/subcutis (multifocal thinning of epidermis, occasionally with orthokeratosis; decreased follicular size, fewer hair shaft cross sections, and/or absence of follicles), glandular stomach (diffusely decreased thickness of fundic epithelial mucosa), esophagus (diffusely decreased thickness of squamous epithelium), duodenum (focally decreased acinar size in submucosal glands), rectum (perineal epithelium and sebaceous glands), and adrenal cortex (relative thinning of the zona follicularis).

Changes in the following organs persisted during the 4-week recovery period, though generally at reduced incidence and/or severity: spleen, GALT/Peyer's patch, sternum, liver, lung, skin/subcutis, and glandular stomach.

Text Table 4.8: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	5	7	2	4	6	8
Dose Level (mg/kg/day)	0	0.05	0.15	0.50	0	0.10	0.30	1.00
Adrenal Cortex								
Number Examined	9	10	10	10	10	10	10	10
Atrophy, zona follicularis								
Minimal	0	0	3	0	0	0	1	4
Slight	0	0	1	2	0	0	0	2
Moderate	0	0	0	3	0	0	0	0
Marked	0	0	0	4	0	0	0	0

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Text Table 4.9: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	5	7	2	4	6	8
Dose Level (mg/kg/day)	0	0.05	0.15	0.50	0	0.10	0.30	1.00
Spleen								
Number Examined	9	10	10	9	10	10	10	10
Lymphocytes, decreased								
Minimal	0	0	2	0	0	0	4	7
Moderate	0	0	1	1	0	0	0	0
Marked	0	0	0	9	0	0	0	0
Thymus								
Number Examined	9	10	9	10	10	10	10	9
Lymphocytes, decreased, cortex								
Minimal	0	5	2	0	0	3	5	3
Slight	0	1	0	0	0	0	1	1
Moderate	0	0	3	0	1	0	0	3
Marked	0	0	2	5	0	0	0	0
Severe	0	0	0	5	0	0	0	0
GALT/Peyer's Patch								
Number Examined	9	8	5	7	8	8	4	9
Lymphocytes, decreased								
Minimal	0	2	2	0	0	2	0	3
Slight	0	1	0	3	0	0	2	0
Moderate	0	4	0	3	0	0	1	0
Marked	0	0	0	1	0	0	0	0
Lymph Node, Mandibular								
Number Examined	9	10	10	9	10	10	10	9
Lymphocytes, decreased								
Minimal	0	4	3	1	0	3	3	0
Slight	0	4	6	6	0	3	3	4
Moderate	0	1	1	0	0	0	1	0
Lymph Node, Mesenteric								
Number Examined	9	10	9	9	10	10	10	10
Lymphocytes, decreased								
Minimal	0	0	1	0	0	0	1	1
Slight	0	0	5	1	0	0	0	0
Moderate	0	0	1	8	0	0	0	4

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Text Table 4.10: Incidence and Severity of Test Article-Related Microscopic Findings - Recovery Sacrifice

	Deflazacort			
Sex	Males		Females	
	1	7	2	8
Dose Level (mg/kg/day)	0	0.50	0	1.00
Spleen				
Number Examined	6	6	6	6
Lymphocytes, decreased				
Minimal	0	3	0	5
Slight	0	1	0	0
GALT/Peyer's Patch				
Number Examined	6	6	6	6
Lymphocytes, decreased				
Slight	0	1	0	0
Thymus				
Number Examined	6	6	6	6
Lymphocytes, increased, cortex				
Minimal	0	0	0	1
Slight	0	4	0	2
Moderate	0	2	0	1
Lymph Node, Mandibular				
Plasmacytes, increased				
Number Examined	6	6	6	6
Minimal	1	1	0	0
Slight	0	2	0	3

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Text Table 4.11: Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	5	7	2	4	6	8
Dose Level (mg/kg/day)	0	0.05	0.15	0.50	0	0.10	0.30	1.00
Marrow, femur								
Number Examined	9	10	10	10	10	10	10	10
Hypocellular								
Minimal	1	2	1	4	1	0	0	1
Slight	0	0	0	2	0	0	0	0
Marrow, sternum								
Number Examined	9	10	10	10	10	10	10	10
Hypocellular								
Minimal	0	1	3	5	1	1	6	7
Slight	0	0	0	4	0	0	0	2
Number Examined	9	10	10	10	10	10	10	10
Vacuolation, hepatocyte								
Minimal	2	3	8	6	0	2	6	7
Slight	0	1	0	2	0	0	0	1
Moderate	1	0	0	1	0	0	0	0
Lung								
Number Examined	9	10	10	10	10	10	10	10
Infiltrate, macrophages, alveolus								
Minimal	2	1	4	3	1	1	0	2
Slight	0	1	3	0	0	0	2	2
Moderate	0	0	0	6	0	0	0	0
Skin/Subcutis								
Number Examined	9	10	10	10	10	10	10	10
Atrophy, epidermis								
Minimal	0	2	5	10	0	1	1	1
Atrophy, follicle								
Slight	0	1	5	0	1	3	6	2
Moderate	0	0	0	10	0	1	0	2
Marked	0	0	0	0	0	1	1	0
Rectum								
Number Examined	9	10	10	10	9	10	10	10
Atrophy, epidermis, perineal								
Minimal	0	0	3	4	0	0	0	4
Atrophy, sebaceous glands, perineal								
Minimal	0	0	2	4	0	2	2	4
Stomach, Glandular								
Number Examined	9	10	10	10	10	10	10	10
Atrophy, epithelium, fundus								
Minimal	0	2	3	2	0	1	1	3
Slight	0	0	0	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Esophagus								
Number Examined	9	10	10	10	10	10	10	10
Atrophy, epithelium, fundus								
Minimal	3	0	1	8	0	0	0	6

	Deflazacort							
Sex	Males				Females			
Dose Group	1	3	5	7	2	4	6	8
Dose Level (mg/kg/day)	0	0.05	0.15	0.50	0	0.10	0.30	1.00
Duodenum								
Number Examined	9	10	10	10	10	NE	NE	10
Atrophy, submucosal gland								
Minimal	1	3	1	3	0	-	-	0
Slight	0	0	0	2	0	-	-	0

NE = Not examined.

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Text Table 4.12: Incidence and Severity of Test Article-Related Microscopic Findings - Recovery Sacrifice

	Deflazacort			
Sex	Males		Females	
	1	7	2	8
Dose Level (mg/kg/day)	0	0.50	0	1.00
Marrow, Sternum				
Number Examined	6	6	6	6
Hypocellular				
Minimal	0	0	0	1
Liver				
Number Examined	6	6	6	6
Vacuolation, hepatocyte				
Minimal	2	3	0	1
Lung				
Number Examined	6	6	6	6
Infiltrate, macrophages, alveolus				
Minimal	0	1	0	0
Slight	0	1	0	2
Moderate	0	2	0	0
Skin/subcutis				
Number Examined	6	6	6	6
Atrophy, epidermis				
Minimal	0	1	0	0
Atrophy, follicle				
Moderate	0	1	2	0
Stomach, glandular				
Number Examined	6	6	6	6
Atrophy, epithelium, fundus				
Minimal	0	0	0	1
Esophagus				
Number Examined	6	6	6	6
Atrophy, epithelium				
Minimal	2	2	0	0

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Male Reproductive Organ and Spermatogenesis Evaluations

A qualitative examination of H&E stained sections of testes, epididymides, prostate, and seminal vesicles was conducted to evaluate progression of the spermatogenic cycle in control and HDM.

No drug-related changes were observed.

Toxicokinetics

Blood samples were collected from nonfasted TK animals (3/sex/timepoint) via a jugular vein on Days 1, 86, and 179, at predose and 2 hours postdose for all groups, and at 0.5, 1, 4, and 24 hours postdose for drug-treated groups. Plasma was analyzed for levels of deflazacort and the major metabolite, 21-desDFZ, by HPLC with MS/MS detection, with a lower limit of quantitation = 0.200 ng/mL for each analyte.

Plasma levels of deflazacort were below the limit of detection at all doses and time points, indicating rapid metabolism after oral administration. Plasma levels of 21-desDFZ were below the limit of detection in control samples.

Systemic exposures to 21-desDFZ generally increased in proportion to dose. Mean 21-desDFZ $AUC_{0\text{-}24\ hr}$ values were up to 2-fold greater in M than F, despite the 2-fold higher dose levels administered to F. No consistent increase in exposure was observed with repeated dosing.

Text Table 4.1: Toxicokinetic Parameters for 21-desacetyldeflazacort in Rat Plasma

Interval (Day)	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	AUC_{0-24} (hour*ng/mL)	AR AUC_{0-24} ((hour*ng/mL)/(hour*ng/mL))
1	3	0.05	M	13.5	89.1	-
	4	0.1	F	17.0	60.7	
	5	0.15	M	36.5	280	-
	6	0.3	F	60.6	179	-
	7	0.5	M	107	866	-
	8	1.0	F	195	617	-
86	3	0.05	M	19.2	99.4	1.12
	4	0.1	F	33.9	58.2	0.960
	5	0.15	M	73.5	415	1.48
	6	0.3	F	89.8	201	1.12
	7	0.5	M	218	1320	1.53
	8	1.0	F	390	822	1.33
179	3	0.05	M	21.5	94.7	1.06
	4	0.1	F	38.4	57.2	0.943
	5	0.15	M	73.1	385	1.38
	6	0.3	F	101	189	1.05
	7	0.5	M	255	978	1.13
	8	1.0	F	472	789	1.28

AR = Accumulation ratio; F = Female; M = Male.

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7 Genetic Toxicology

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

Deflazacort: Bacterial Reverse Mutation Assay

Study no.:	(b) (4) Study 262-0007-GT Marathon Study MP-104-NC-008
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes; except for characterization of the test article, which was in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	Deflazacort Batch DFZγ13001, 99.82% pure

Key Study Findings

In the in vitro bacterial reverse mutation assay with the plate incorporation method, deflazacort was negative for genotoxicity in all strains tested, in the presence and absence of Aroclor-induced rat liver S9 metabolic activation.

Methods

Strains:	*S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537; *E. coli* tester strain WP2 *uvr*A
Concentrations in definitive study:	100, 250, 500, 1000, 2500, 5000 µg/plate deflazacort ± S9
Basis of concentration selection:	Absence of mutagenic responses, bacterial lawn toxicity, or heavy precipitates observed in an initial assay at up to 5000 µg/plate ± S9
Negative control:	Dimethylsulfoxide (DMSO)
Positive controls:	-S9: sodium azide (TA1535, TA100), 2-nitrofluorene (TA98), ICR-191 acridine (TA1537), and methyl methanesulfonate (WP2 *uvr*A) +S9: 2-aminoanthracene (all tester strains)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	~48 hours at 37°C
Metabolic activation:	Liver S9 from Aroclor 1254-induced male Sprague Dawley rats

Study Validity

Selection of bacterial tester strains was adequate based upon current guidelines. Positive and negative controls produced expected responses. Dose selection was adequate based upon use of the limit concentration 5000 µg/plate. The S9 concentration (10%) was within acceptable limits. 2-aminoanthracene was used as the positive control for all tester strains in the presence of metabolic activation, and the activity of the S9 preparation was adequately characterized by the sponsor, as recommended by OECD guidelines. Analysis samples of dosing solutions confirmed that concentrations of the test article were within ±10% of nominal concentrations.

Results

Criteria for a positive response were provided in the study report. Positive findings had to show increases in the mean number of revertants per plate that were dose-related over at least two increasing concentrations. For tester strains TA98, TA100, and WP2uvrA, the test article was considered positive if it produced at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. For tester strains TA1535 and TA1537, the test article was considered positive if it produced at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control.

No positive mutagenic responses were observed with any of the tester strains in the presence or absence of metabolic activation in the initial or confirmatory plate incorporation study. No cytotoxicity was observed. Slight precipitation was observed at 5000 µg/plate in the absence of S9 in all strains.

Conclusion

All criteria for a valid study were met. Deflazacort was negative for mutagenicity in the in vitro bacterial mutation assay in the presence and absence of metabolic activation.

21-Desacetyl-DFZ: Bacterial Reverse Mutation Assay

Study no.:	(b) (4) Study 262-0002-GT Marathon Study MP-104-NC-009
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes; except for characterization of the test article, which was in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	21-Desacetyl-Deflazacort (21-Desacetyl-DFZ) Batch ET1067-3, 99.16% pure

Key Study Findings

In the in vitro bacterial reverse mutation assay with the plate incorporation method, 21-Desacetyl-DFZ was negative for genotoxicity in all strains tested, in the presence and absence of Aroclor-induced rat liver S9 metabolic activation.

Methods

Strains:	*S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537; *E. coli* tester strain WP2 *uvr*A
Concentrations in definitive study:	100, 250, 500, 1000, 2500, 5000 µg/plate 21-Desacetyl-DFZ ± S9
Basis of concentration selection:	Absence of mutagenic responses, bacterial lawn toxicity, or heavy precipitates observed in an initial assay at up to 5000 µg/plate ± S9
Negative control:	DMSO
Positive controls:	-S9: sodium azide (TA1535, TA100), 2-nitrofluorene (TA98), ICR-191 acridine (TA1537), and methyl methanesulfonate (WP2 *uvr*A) +S9: 2-aminoanthracene (all tester strains)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	~48 hours at 37°C
Metabolic activation:	Liver S9 from Aroclor 1254-induced male Sprague-Dawley rats

Study Validity

Selection of bacterial tester strains was adequate based upon current guidelines. Positive and negative controls produced expected responses. Dose selection was adequate based upon use of the limit concentration 5000 µg/plate. The S9 concentration (10%) was within acceptable limits. 2-aminoanthracene was used as the positive control for all tester strains in the presence of metabolic activation, and the activity of the S9 preparation was adequately characterized by the sponsor, as recommended by OECD guidelines. Analysis of samples of dosing solutions confirmed that concentrations of the test article were within ±10% of nominal concentrations.

Results

Criteria for a positive response were provided in the study report. Positive findings had to show increases in the mean number of revertants per plate that were dose-related over at least two increasing concentrations. For tester strains TA98, TA100, and WP2uvrA, the test article was considered positive if it produced at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. For tester strains TA1535 and TA1537, the test article was considered positive if it produced at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control.

No positive mutagenic responses were observed with any of the tester strains in the presence or absence of metabolic activation in the initial or confirmatory plate incorporation study. No cytotoxicity or precipitate was observed.

Conclusion

All criteria for a valid study were met. 21-Desaceytl-DFZ was negative for mutagenicity in the in vitro bacterial mutation assay in the presence and absence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Deflazacort: In Vitro Chromosomal Aberration Assay in Human Lymphocytes

Study no.:	(b) (4) Study 262-0008-GT Marathon Study MP-104-NC-006
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes; except for characterization of the test article, which was in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	Deflazacort Batch DFZγ13001, 99.82% pure

Key Study Findings

In the in vitro chromosome aberration assay using human lymphocytes, deflazacort was negative in the presence and absence of rat liver S9 metabolic activation.

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	50, 100, 200, and 441.52 µg/mL deflazacort for all treatments
Basis of concentration selection:	Preliminary cytotoxicity assessment up to maximum dose of 1 mM (441.52 µg/mL)
Negative control:	DMSO
Positive control:	-S9: mitomycin C; +S9: cyclophosphamide
Formulation/Vehicle:	DMSO, 1% final concentration in medium
Incubation time:	-S9: 3 and 24 hours; +S9: 3 hours
Sampling time:	24 hours after initiation of incubation
Metabolic activation system:	Aroclor 1254-induced male S-D rat liver S9

Study Validity

The following criteria for a valid study were listed in the study report and were met: 1) "The percentage of cells with aberrations for the solvent control must be low and not exceed 2%"; 2) "There must be a minimum of three scorable concentrations, ie, concentrations that yield at least 200 scorable metaphases, and show no evidence of microbial contamination"; and 3) The positive control culture must yield a level of aberrant cells that is statistically significantly increased over the mean of the concurrent solvent controls ($p \leq 0.05$)."

Dose selection was adequate, based upon use of the limit dose for this assay, 1 mM. The S9 concentration used (1.5%) was within acceptable limits. The study was

performed using standard procedures. Analysis of samples of the dosing formulations confirmed that concentrations of deflazacort were within ±10% of nominal concentrations.

Results

A positive response was defined in the study report as one in which “the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant and clearly outside the historical vehicle control data ($p \leq 0.05$) without greatly exceeding 50% toxicity.” A negative response was defined as one in which the test article did not demonstrate a statistically significant increase in chromosome aberrations compared to negative or vehicle controls or when all of the test article responses are within the historical range for negative and/or vehicle control cultures.

No statistically significant increases in structural or numerical chromosomal aberrations (relative to vehicle control) were observed in human lymphocytes incubated with deflazacort at up to 441.52 µg/mL in the presence or absence of rat liver S9 metabolic activation. No cytotoxicity was observed up to the highest concentration tested, 441.52 µg/mL, in the 3-hour assay ±S9; in the 24-hour assay without S9, this concentration caused a reduction in the relative Mitotic Index of 43% compared to vehicle control. No precipitate was observed in any of the assays.

Conclusion

All criteria for a valid study were met. Deflazacort was negative in the in vitro chromosomal aberration assay in human lymphocytes in the presence and absence of metabolic activation.

21-Desacetyl-DFZ: In Vitro Chromosomal Aberration Assay in Human Lymphocytes

Study no.:	(b) (4) Study 262-0001-GT Marathon Study MP-104-NC-007
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes; except for characterization of the test article, which was in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	21-Desacetyl-Deflazacort (21-Desacetyl-DFZ) Batch ET1067-3, 99.16% pure

Key Study Findings

In the in vitro chromosome aberration assay using human lymphocytes, 21-Desacetyl-DFZ was negative in the presence and absence of rat liver S9 metabolic activation.

Methods

Cell line:	Human lymphocytes
Concentrations in definitive study:	40, 80, 160, and 399.48 µg/mL 21-Desacetyl-DFZ for all treatments
Basis of concentration selection:	Preliminary cytotoxicity assessment up to maximum dose of 1 mM (399.48 µg/mL)
Negative control:	DMSO
Positive control:	-S9: mitomycin C; +S9: cyclophosphamide
Formulation/Vehicle:	DMSO, 1% final concentration in medium
Incubation time:	-S9: 3 and 24 hours; +S9: 3 hours
Sampling time:	24 hours after initiation of incubation
Metabolic activation system:	Aroclor 1254-induced male S-D rat liver S9

Study Validity

The following criteria for a valid study were listed in the study report, and were met: 1) "The percentage of cells with aberrations for the solvent control must be low and not exceed 2%"; 2) "There must be a minimum of three scorable concentrations, ie, concentrations that yield at least 200 scorable metaphases, and show no evidence of microbial contamination"; and 3) The positive control culture must yield a level of aberrant cells that is statistically significantly increased over the mean of the concurrent solvent controls ($p \leq 0.05$)."

Dose selection was adequate, based upon use of the limit dose for this assay, 1 mM. The S9 concentration used (1.5%) was within acceptable limits. The study was performed using standard procedures. Analysis of samples of the dosing formulations

confirmed that concentrations of 21-Desacetyl-DFZ were within ±10% of nominal concentrations.

Results

A positive response was defined in the study report as one in which "the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant and clearly outside the historical vehicle control data ($p \leq 0.05$) without greatly exceeding 50% toxicity." A negative response was defined as one in which the test article did not demonstrate a statistically significant increase in chromosome aberrations compared to negative or vehicle controls or when all of the test article responses are within the historical range for negative and/or vehicle control cultures.

No statistically significant increases in structural or numerical chromosomal aberrations (relative to vehicle control) were observed in human lymphocytes incubated with 21-Desacetyl-DFZ at up to 339.48 µg/mL in the presence or absence of rat liver S9 metabolic activation. No cytotoxicity was observed up to the highest concentration tested, 339.48 µg/mL, in the 3-hour assay ±S9; in the 24-hour assay without S9, this concentration caused a reduction in the relative Mitotic Index of 29% compared to vehicle control. No precipitate was observed in any of the assays.

Conclusion

All criteria for a valid study were met. 21-Desacetyl-DFZ was negative in the in vitro chromosomal aberration assay in human lymphocytes in the presence and absence of metabolic activation.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Mammalian Erythrocyte Micronucleus Test of Deflazacort in Rat Bone Marrow

Study no:	(b) (4) Study 9800271 Marathon Study MP-104-NC-041
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes, with the exception of test article characterization and stability, which were conducted under GMP
QA statement:	Yes
Drug, lot #, and % purity:	Deflazacort Batch DFZγ14007, 99.62% pure

Key Study Findings

Deflazacort was negative in a valid in vivo rat bone marrow micronucleus assay.

Methods

Doses in definitive study:	0, 500, 1000, 2000 mg/kg/day deflazacort
Frequency of dosing:	Once daily for 2 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) Methylcellulose (4000 cP)
Species/Strain:	Sprague Dawley Crl:CD(SD) rats
Number/Sex/Group:	5 males per group
Satellite groups:	No
Basis of dose selection:	Dose range-finding study: eyes partly closed and decreased activity were observed at ≥ 1000 mg/kg/day in M; ≥ 1500 mg/kg/day in F; since effects were similar in M and F, only M were included in the definitive study.
Negative control:	Vehicle
Positive control:	Cyclophosphamide monohydrate, 20 mg/kg single dose, via oral gavage, 10 mL/kg, 24 hrs prior to sampling

Study Validity

The study is valid for the following reasons: 1) dose selection was adequate based on the use of the limit dose for this assay, 2000 mg/kg/day; 2) the proportion of micronucleated immature erythrocytes in vehicle controls was within or close to that observed in the laboratory historical vehicle/negative controls; 3) unequivocally positive responses were observed in positive controls; 4) the proportion of immature

erythrocytes in all groups was within or close to that observed in the laboratory historical vehicle/negative controls; 5) previous pharmacokinetic assessments demonstrated systemic exposure to the primary active metabolite, 21-desDFZ, in rats after oral gavage administration of deflazacort; 6) preparation and administration of the test substance was acceptable, based on analyses of the dosing solutions; 7) the species and number of animals/sex/group were acceptable; and 8) tissue sampling and analyses were acceptable.

Results

A positive response was defined in the study report as one in which the proportion of micronucleated immature erythrocytes in individual animals and/or the group mean exceeded the range of laboratory historical vehicle/negative controls and the group mean value exceeded by at least 2-fold the mean value of the concurrent vehicle controls. A negative result was defined as one in which individual and group mean values for the proportion of micronucleated immature erythrocytes were within or close to the range of laboratory historical vehicle/negative controls.

Dosing at up to 2000 mg/kg/day deflazacort resulted in clinical signs, including eyes partly closed, decreased activity, moderate dehydration, and red skin. No significant increases (compared to vehicle control) were observed in the incidence of micronucleated immature (polychromatic) erythrocytes in bone marrow of male Sprague Dawley rats. Reductions in mean ratios of immature to total erythrocytes were limited to ≤35% in drug-treated groups, suggesting that bone marrow was not excessively damaged by treatment with two doses of deflazacort.

Conclusion

All criteria for a valid study were met. Deflazacort was negative in the in vivo rat bone marrow micronucleus assay.

7.4 Other Genetic Toxicity Studies

In Silico Mutagenicity Evaluation of the Deflazacort-Related Impurities

(b) (4)

(Marathon Study MP-104-NC-027)
Evaluation of three impurities (b) (4) using quantitative structure-activity relationship (QSAR) analyses led to the conclusion that none of them posed a potential risk of mutagenicity, based on the following considerations:

1) A MultiCASE CASE Ultra program, employing four different modules (GT1_A7B, GT1_AT_ECOLI, SALM2013, and SALM2013PHARMA) did not identify any substructures positively associated with mutagenicity—however, some substructures were not covered by this analysis, so a definitive conclusion could not be made solely on this result.

2) Substructures not covered by the MultiCASE CASE Ultra analyses (except for the iodo moiety) were similar to those in deflazacort and 12-desacetyl-deflazacort, which have been demonstrated to be negative in a standard battery of genotoxicity assays.

3) A recent review article (b) (4) concluded that iodine and several iodine-containing compounds were negative for genotoxicity.

4) No structural alerts for genotoxicity were identified for any of the three impurities using the Derek Nexus expert rule-based SAR program.

Potential Genotoxic Impurities in Deflazacort as Manufactured by (b) (4)

(Marathon Study MP-104-NC-059)
Deflazacort and 14 impurities were analyzed for potential genotoxicity using in silico methods (b) (4) . Where necessary, an Ames test was conducted using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102, in the presence and absence of S9 metabolic activation. As indicated in Table 1 below, in some cases the in silico determination was made using a “read-across” strategy, where the results from a “similar” (usually structurally similar) reference or source compound were assumed to apply also to the “target” compound. A review of the criteria used in each case where the “read-across” strategy was employed confirmed that this approach was justified.

Table 1: Potential Impurities Studied

NAME	CHEMICAL NAME	STRUCTURE	METHOD OF ANALYSIS	RESULTS
(b) (4)			AMES TEST	Negative
			AMES TEST	Negative
			AMES TEST	Negative
			AMES TEST	Negative
			AMES TEST	Negative
			AMES TEST	Negative
			AMES TEST	Negative

(page 4 of Study Report)

(b) (4)	AMES TEST	Negative
	IN-SILICO STUDIES: READ ACROSS	Negative
	AMES TEST	Negative
	IN-SILICO STUDIES: READ ACROSS	Negative
	IN-SILICO STUDIES: READ ACROSS	Negative
	IN-SILICO STUDIES: (b) (4)	Negative
	IN-SILICO STUDIES: (b) (4)	Negative
	IN-SILICO STUDIES: (b) (4)	Negative

(page 5 of Study Report)

8 Carcinogenicity

No carcinogenicity studies of deflazacort were conducted by the sponsor; however, a published carcinogenicity study in rat was provided (Zwicker GM, Eyster RC *Toxicol Pathol* 1996; 24(2):246-250).

Zwicker GM, Eyster RC. Proliferative bone lesions in rats fed a diet containing glucocorticoid for up to two years. *Toxicol Pathol* 1996; 24(2):246-250

Zwicker and Eyster (1996) conducted concurrent 1- and 2-year studies of deflazacort in Sprague Dawley rats (Crl:CDBr; 50/sex/group) aged 4-6 weeks at initiation of dietary dosing at 0, 0.03, 0.06, 0.12, 0.25, 0.50, and 1.0 mg/kg/day. No proliferative bone lesions were observed in the 1-year study or in the first year of the 2-year study. A chordoma was observed in a control M sacrificed on day 617. Four M at 0.25 mg/kg/day were sacrificed in poor condition or died between days 474 (Week 68) and 575 (Week 82) because of osteosarcoma involving the head. Another M at 0.25 mg/kg/day with an osteosarcoma was sacrificed in poor condition on day 712 (Week 102) due to a C-cell thyroid carcinoma. Osteoma involving the head was observed in two M at 0.25 mg/kg/day sacrificed on days 661 (Week 94, due to "extremely severe suppurative inflammation of the prostate") and 481 (Week 69, due to pituitary neoplasm). Hyperostosis associated with an inflammatory lesion of the head was observed in a M at 0.12 mg/kg/day sacrificed in poor condition on day 712 (Week 102) due to a hepatocellular carcinoma. Osteoma of the head was observed in one M at 0.06 mg/kg/day that died on day 612 (Week 87) due to chronic progressive nephropathy. An osteosarcoma was observed in one F at 1.0 mg/kg/day that died of uncertain cause on day 661 (Week 94).

No osteomas or osteosarcomas were diagnosed during life. Osteosarcoma on the head was observed in 5/50 M at 0.25 mg/kg/day and in 1/50 F at 1.0 mg/kg/day. Osteoma on the head was observed in 2/50 M at 0.25 mg/kg/day and in 1/50 M at 0.06 mg/kg/day. Hyperostosis on the head was observed in 1/50 M 0.12 mg/kg/day. The incidence of osteosarcoma and osteoma combined was 7/50 (14%) in M at 0.25 mg/kg/day and 0/50 in control M. Osteosarcomas showed characteristics of osteoplastic type lesions, with "localized replacement of normal bone by proliferative cells resembling osteoblasts and producing osteoid matrix." "A few small neoplasms showed malignant characteristics based on peripheral cell anaplasia and invasive activity," and one metastasized to the lung. Osteomas "were expansile bone masses that appeared to arise from periosteal surfaces." Hyperostosis "was considered a reactive response to severe chronic inflammation of the skin and subcuticular tissues involving the head."

The following statements by the Zwicker and Eyster put these findings into context:

- "Spontaneous bone neoplasms at any site are uncommon (0-0.5%) in Sprague Dawley rats."

- "This is the first report we are aware of that suggests a relationship between chronic glucocorticoid exposure and an increased incidence of bone neoplasia in male rats."

- "The biological evidence for a treatment-related increase of bone neoplasms in 0.25-mg glucocorticoid/kg male rats is not complete. However, the statistically significant incidence in 0.25-mg males and morphologic evidence including both osteoma and osteosarcoma suggest a treatment relationship."

- "The third osteom [sic] occurred in a 0.06-mg/kg male rat (4462) and was probably not related to treatment because there was no dose response."

- "Because survival was greatly reduced at dose levels greater than 0.25 mg glucocorticoid/kg in both sexes, there was no graded response of increased incidence of proliferative bone lesions, earlier onset, or increased malignancy with increasing dose."

- "Also, mice in a parallel 2-yr glucocorticoid oncogenicity study were not affected."

- "There was no evidence that approximately equipotent doses of a second glucocorticoid given to both mice and rats in 2-yr oncogenicity studies in our laboratory caused bone lesions."

- "Ideally, therefore, a repeat study should be conducted using (b) (4) Sprague Dawley and other rat strains to help determine whether or not this glucocorticoid was responsible for the increased incidence of bone neoplasms."

The sponsor acknowledged that "based on the published results of this thoroughly conducted 2-year carcinogenicity study [Zwicker and Eyster, 1996], it is concluded that deflazacort is a male rat bone carcinogen." *(page 8 of Carcinogenicity Studies Waiver)* However, it is not clear from the description of the procedures in the published study that a full battery of tissues was evaluated microscopically.

Request for a Waiver: Carcinogenicity Studies

A request for a waiver of the standard requirement to assess the carcinogenicity of deflazacort in two rodent species was provided with the NDA submissions. The Agency had previously informed the sponsor that assessment of the carcinogenicity of deflazacort, if needed, could be conducted as a post-marketing requirement in view of the seriousness of the indication, provided that the available nonclinical and clinical data supported such a strategy (Pre-IND Meeting Minutes dated December 20, 2013; Pre-NDA Meeting Minutes dated September 3, 2015).

The sponsor provided the following justifications for the request for a waiver:

1) "The inherent sensitivity of rodents to the pharmacologic effects of glucocorticoids limits testing at higher doses and thus only outcomes related to the exaggerated effects of chronic glucocorticoid treatment have been observed"

2) “Results of the published and peer reviewed 2-year carcinogenicity study with deflazacort that demonstrated osteomas/osteosarcomas in male rats”

3) “…the mouse 2-year bioassay [with deflazacort] was negative…”

4) “..the bone and hepatic tumors described in rats administered chronic glucocorticoids are not representative of those seen in humans (i.e., skin cancer and lymphoma)…”

5) “Classical immunosuppressive glucocorticoid effects seen in chronic toxicity studies in rats and cynomolgus monkeys orally administered deflazacort”

6) “Rodent bioassays not predicting human tumor types associated with immunosuppressive drug classes, including glucocorticoids”

7) “…the immunosuppressive effects of glucocorticoids appear to be more predictive for carcinogenicity in humans”

8) “The above information indicates that deflazacort poses a likely risk for human carcinogenicity based on glucocorticoid-induced immunosuppression and that additional rodent carcinogenicity studies will not add value to this assessment”

Reviewer’s Comments

The findings of increased bone tumors in a two-year dietary carcinogenicity study of deflazacort in rat (Zwicker and Eyster, 1996) provide important safety information that should be included in the labeling, and suggest that the toxicity profile of deflazacort may differ from that of other glucocorticoids. A two-year carcinogenicity study of deflazacort in mice conducted in parallel with the rat study showed no effects, although the doses administered were not stated in the article. The level of detail provided is insufficient to support the conclusion that the carcinogenicity of deflazacort has been adequately assessed in mouse.

The sponsor’s statement that the increased sensitivity of rodents (compared to humans) to the toxic effects of glucocorticoids limits testing at higher doses is relevant to question of whether or not a new carcinogenicity assay in rat would provide meaningful information in addition to that provided by the published study. In the 26-week oral (gavage) toxicity study of deflazacort in rat, the lowest doses tested (0.05 mg/kg/day M; 0.10 mg/kg/day F) were associated with reductions in final body weight of 18% (M) and 10% in (F), and mean 21-desDFZ Day 179 $AUC_{0\text{-}24\ hr}$ = 94.7 ng*hr/mL (M) and 57.2 ng*hr/mL (F), approximately one fifth and one eighth, respectively, that expected in humans at the proposed dose of 0.9 mg/kg/day deflazacort (~450-510 ng*hr/mL). Moreover, the body weight reduction of 18% observed in M at 0.05 mg/kg/day exceeded the limit of 10% recommended for the high dose in two-year carcinogenicity studies, so that even one fifth of the expected human 21-desDFZ exposure may not be achievable

in M in an oral gavage study. Effects on body weight were not discussed in the published 2-year dietary carcinogenicity study of deflazacort in rat.

Plasma 21-desDFZ exposures achieved at the highest evaluable dose of 0.25 mg/kg/day in the dietary rat study were not provided, but the human equivalent dose (based on body surface area scaling using mg/m^2) is 0.04 mg/kg/day, 4.4% of the recommended human dose of 0.9 mg/kg/day.

Therefore, additional carcinogenicity studies of deflazacort in rat, via oral gavage or dietary administration, are not likely to provide meaningful additional information.

No pharmacokinetic or toxicokinetic data were submitted from studies of deflazacort in mice. Therefore, additional information is needed to determine whether or not a carcinogenicity study of deflazacort in mouse would provide meaningful information.

The sponsor should conduct a dose-ranging study in mouse, with toxicokinetic analysis, to assess the feasibility of achieving meaningful plasma exposures to 21-desDFZ via oral administration of deflazacort.

The sponsor's request for a waiver of the requirement to conduct carcinogenicity studies of deflazacort should be granted for rat, and deferred for mouse, pending submission of an MTD/TK feasibility study in mouse.

9 Reproductive and Developmental Toxicology

Assessments of the effects of deflazacort on female fertility, embryofetal development, and early postnatal development were not conducted because the DMD patient population is almost entirely male. No drug-related effects on the male reproductive system were observed in the 39-week monkey, 26-week rat, or 8-week juvenile rat studies of deflazacort, which included focused histopathologic evaluation of male reproductive organs, including sperm evaluation in a stage-aware manner. The age at sexual maturity (preputial separation in M; vaginal opening in F) was not assessed in the juvenile rat study. Effects of deflazacort on the growth and development of juvenile rats are described in the next section.

10 Special Toxicology Studies (Juvenile animal studies)

A Dose Range Finding Study of Deflazacort by Oral Gavage in Juvenile Rats

(Marathon Study MP-104-NC-028; (b) (4) Study 20061367; Deflazacort Batch DFZγ14002; 99.53% pure; Non-GLP; Non-QA)

Juvenile Sprague Dawley rats (8/sex/group) were dosed once daily via oral gavage with 0, 0.3, 0.1, or 3 mg/kg/day deflazacort in 0.5% methylcellulose at 10 mL/kg from PND 21 through PND 35. TK satellite groups (N=6/sex control and 9/sex/group deflazacort) were dosed similarly, and blood samples were collected on PND 21 at 1 hr postdose or on PND at 1, 2, 4, and 8 hours postdose. Main study animals were evaluated for viability, clinical signs, body weights, food consumption, gross necropsy, and organ weights.

No drug related changes were observed in clinical signs or mortality. Reductions in mean final body weight were observed at all doses compared to controls: LDM (16%), LDF (17%), MDM (22%), MDF (24%), HDM (29%), and HDF (31%). Reductions in mean body weight gain (PND 21-36) were observed at all doses compared to controls: LDM (22%), LDF (25%), MDM (32%), MDF (36%), HDM (41%), and HDF (46%), compared to controls. Reductions in mean food consumption were observed at all doses compared to controls: LDM (14%), LDF (13%), MDM (10%), MDF (18%), HDM (19%), and HDF (18%).

Drug-related changes in gross necropsy findings were limited to small thymus (5/8 MDM, 3/8 MDF, 8/8 HDM, and 8/8 HDF) and small adrenal glands (1/8 MDM, 2/8 HDM, and 4/8 HDF).

Drug-related reductions were observed in the absolute and relative weights of adrenal glands, spleen, lungs, thymus, and ovaries, as shown in the sponsor's table below:

Text Table 10
Organ Weight[(a)] Changes in Rats Administered Deflzacort - PND 36

Dose (mg/kg/day)		Males			Females		
		0.3	1	3	0.3	1	3
Organ							
Adrenals	Abs.	0.78	0.56	0.50	0.80	0.65	0.35
	Rel.	-	0.68	0.68	0.94	0.81	0.50
Spleen	Abs.	0.73	0.55	0.49	0.70	0.55	0.45
	Rel.	0.85	0.70	0.69	0.85	0.72	0.65
Lungs	Abs.	0.87	0.69	0.57	0.90	0.84	0.68
	Rel.	-	0.88	0.79	-	-	-
Thymus	Abs.	0.43	0.16	0.10	0.60	0.23	0.10
	Rel.	0.52	0.20	0.15	0.71	0.30	0.13
Ovaries	Abs.	NA	NA	NA	-	-	0.63
	Rel.	NA	NA	NA	-	-	0.90

[a] Changes are expressed as XFold from mean control value.
NA = not applicable.
'–': indicates results were not considered to be meaningfully different from mean control value.

(page 24 of Study Report)

As shown in the sponsor's summary table below, systemic exposures to the primary active metabolite, 21-desacetyl deflazacort, peaked at 1-2 hours postdose, increased generally in proportion to dose, and were up to 2.5 times greater in males than females.

Text Table 11
Summary Mean (±SE) 21-Desacetyl Deflazacort Toxicokinetic Parameters on PND 35

Dose (mg/kg/day)	Sex	Tmax (hr)	Cmax (ng/mL)	AUC(0-t) (nghr/mL)
0.3	Female	2.0	50.9 ± 18.3	145 ± 30.3
	Male	1.0	65.3 ± 1.73	158 ± 6.81
1	Female	1.0	215 ± 28.0	331 ± 43.5
	Male	1.0	350 ± 15.9	797 ± 33.3
3	Female	1.0	558 ± 124	1280 ± 206
	Male	1.0	933 ± 55.4	2340 ± 57.3

(page 25 of Study Report)

The NOAEL for oral administration of deflazacort to juvenile male and female rats was < 0.3 mg/kg/day, based on reductions in mean body weight gain and thymus weight observed at the LD of 0.3 mg/kg/day.

An 8-Week Study of Deflazacort by Oral Gavage in Juvenile Rats with an 8-Week Recovery Period

Study no.:	Marathon Study MP-104-NC-030 20061369 (b) (4))
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes (exceptions: test article characterizations and stability performed by sponsor; no PI coverage for bioanalytical phase for 51 days; deviations did not affect integrity of study)
QA statement:	Yes
Drug, lot #, and % purity:	Lot #DFZγ14007, purity 99.62%

Key Study Findings

- Drug-related changes were generally seen at lower doses and at greater incidence and/or severity in M vs. F, consistent with the 2- to 7-fold greater systemic exposures to the active metabolite, 21-desDFZ, observed in M.
- Dose-dependent reductions were observed in body weight, body weight gain, and food consumption.
- Drug-related changes in functional and neurobehavioral parameters included increases in stereotyped behavior, abnormal respiration, and motor activity, decreased acoustic startle response, decreased grip strength and landing foot splay, and impaired Morris water maze performance.
- Drug-related changes in femur included reduced size and mass, reduced diaphyseal cortical BMC and BMD, and increased metaphyseal trabecular BMC and BMD.
- Drug-related microscopic changes included decreased cellularity of lymphoid organs and the physis of the femur, and atrophy of the adrenal cortex and male mammary glands.
- The NOAEL in M was < 0.1 mg/kg/day, based on adverse findings of reduced body weight and body weight gain; redistribution of bone in femur from peripheral to central regions; adrenal cortical atrophy; and decreased cellularity of lymphoid organs associated with reductions in relative organ weight and in circulating lymphocytes.
- The NOAEL in F was 0.1 mg/kg/day, based on effects less severe than those observed for M at this dose, becoming adverse at the MD of 0.3 mg/kg/day.
- HDM recovery animals showed persistent differences from controls in abnormal respiration, reduced grip strength, and landing foot splay. Reductions in body weight, impairment in Morris water maze performance, and changes in femur size and mass were partially reversed in HD animals after 8 weeks of recovery.

Methods

Doses:	0, 0.1, 0.3, and 1.0 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	0.5% methylcellulose in reverse osmosis deionized water
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	20/sex/group for main study, and for control and HD recovery groups
Satellite groups:	Toxicokinetic (TK) groups
Study design:	Sprague Dawley rat pups were treated with either vehicle or test article from postnatal day (PND) 21 to PND 80 at the specified doses. Main study animals were euthanized on PND 81 at the end of the dosing period. Gross pathology and histopathology evaluations were conducted at the end of the dosing period. Recovery animals were evaluated after an eight-week recovery period, euthanized on PND 138 ± 2. All main study and recovery animals were evaluated for viability, clinical observations, body weights, food consumption, clinical pathology, ophthalmology, and sexual maturation during the dosing period. Subset 1 (10/sex/group main study and recovery) was evaluated for standard histopathology, bone measurement and densitometry, and male reproductive parameters at the time of necropsy. Subset 2 (10/sex/group main study and recovery) was evaluated for neurohisto-pathology and brain morphometry. Neuro-behavioral testing (functional observational battery, motor activity, acoustic startle habituation, and Morris water maze) was also conducted during the dosing period for main study groups and at the end of the recovery period for recovery groups.
Deviation from study protocol:	No important deviations were reported.

Observations and Results

Mortality

Methods
Animals were evaluated for viability at least twice daily.

Results
No drug-related mortality was observed. One MDM was sacrificed on PND 69 due to a fractured snout; microscopic findings in this animal were similar to other animals in this group that survived to scheduled termination.

Clinical Signs

Methods
Animals were observed daily predose and between 1 and 2 hours after dosing.

Results
HDM showed increased piloerection (14/20; intermittent, starting PND 58) and mild dehydration (8/20; based on skin turgor; intermittent, starting PND 51) compared to controls. These signs were limited to 1-2 HDM during the recovery period, indicating recovery in most animals.

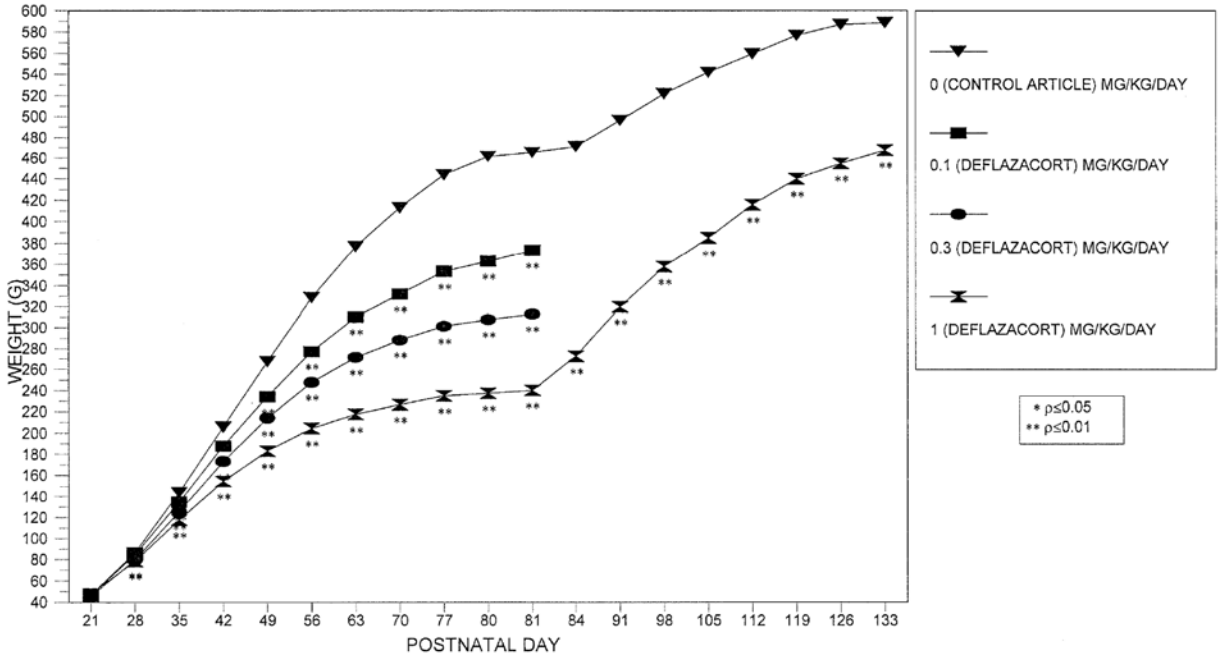
Body Weight

Methods
Body weights were recorded daily during the dosing period, twice weekly during the recovery period, and on the day of scheduled euthanasia.

Results
Mean body weight gain (PND 21 to PND 81) was dose-dependently reduced in LDM (22%), LDF (9%), MDM (36%), MDF (18%), HDM (54%), and HDF (26%) compared to controls. Mean final body weight (PND 81) was dose-dependently reduced in LDM (20%), LDF (7%), MDM (33%), MDF (16%), HDM (48%), and HDF (22%) compared to controls. At the end of the 8-week recovery period, mean body weight remained below controls in HDM (22%) and HDF (8%).

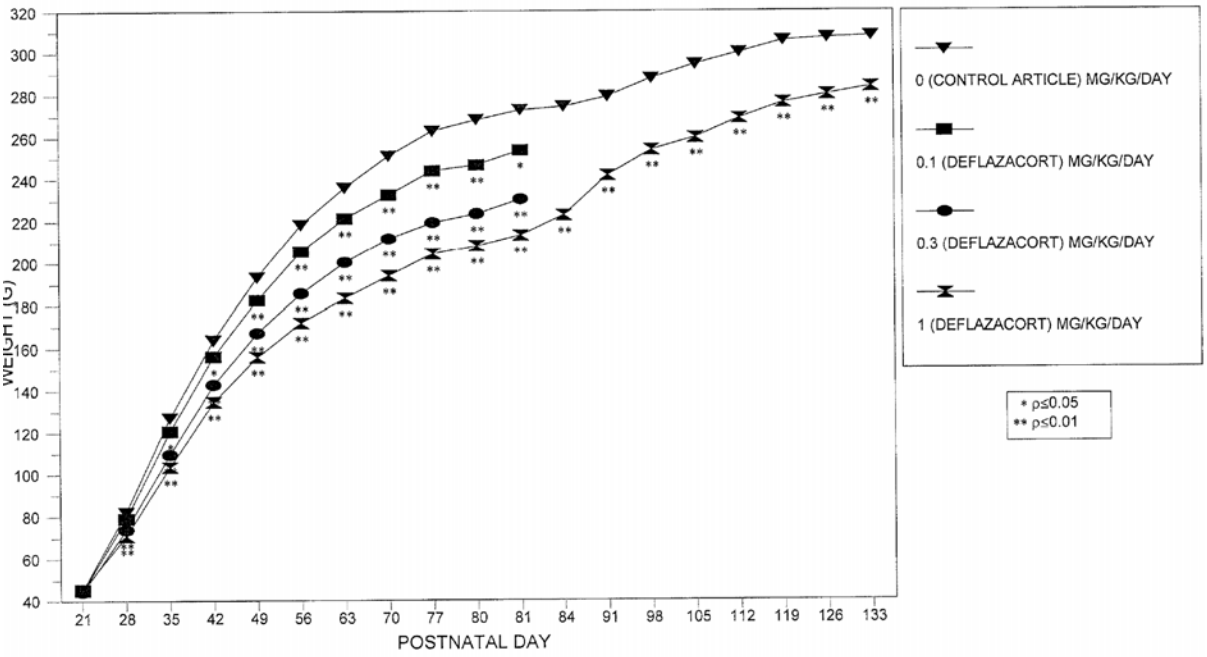
BODY WEIGHTS: MALE RATS

Figure 1



BODY WEIGHTS: FEMALE RATS

Figure 2



(pages 76 and 78 of Study Report)

Food Consumption

Methods

Food consumption was recorded weekly starting on PND 21.

Results
Mean daily food consumption (PND 21 to PND 80) was dose-dependently reduced in LDM (14%), LDF (4%), MDM (20%), MDF (12%), HDM (33%), and HDF (17%) compared to controls.

Hematology and Clinical Chemistry

Methods
Whole blood samples were collected from all surviving (fasted) Subset 1 main study (PND 81) and recovery animals (PND 138 ± 2). Blood was collected via the vena cava after euthanasia.

Results
Dose-related reductions were observed in platelets, leukocytes, and lymphocytes in all M and MDF and HDF compared to controls. Dose-related increases were observed in segmented neutrophils (all M), RBC parameters (all M and HDF), and reticulocytes (all M) compared to controls. The decreases in leukocytes and lymphocytes and the increase in neutrophils were attributed to the expected pharmacological activity of deflazacort. The slight increases in RBC parameters are consistent with hemoconcentration due to mild dehydration. Additional changes that achieved statistical significance are included in the table below. All hematology changes showed reversibility after 8 weeks of recovery.

Effect of deflazacort on hematology parameters (% change from control)								
Dosage (mg/kg/day)	0		0.1		0.3		1.0	
Sex	M	F	M	F	M	F	M	F
Erythrocytes (10^6/cm^2)	7.85	7.86	8.41[a] **(7.1%)**	8.02 (2.0%)	8.60[a] **(9.5%)**	8.04 (2.3%)	8.75[a] **(11.4%)**	8.19 (4.1%)
Hemoglobin (g/dL)	14.6	14.6	15.5[a] **(5.6%)**	15.0 (2.7%)	15.6[a] **(6.4%)**	15.1 (3.2%)	16.3[a] **(10.9%)**	15.4[a] **(5.4%)**
Hematocrit (%)	43.3	43.1	46.0[a] **(6.2%)**	44.0 (2.2%)	46.3[a] **(7.1%)**	44.5 (3.4%)	47.9[a] **(10.7%)**	5.2[a] **(5.2%)**
Reticulocytes (10^9/L)	164.1	154.0	145.0 (-12%)	146.2 (-5.1%)	128.5[a] **(-22%)**	153.7 (-0.2%)	128.9[a] **(-22%)**	160.5 (4.2%)
Red cell distribution width (%)	11.3	10.7	11.4 (1%)	10.7 (-0.6%)	11.6 (2.8%)	10.8 (0.7%)	12.1[a] **(7.3%)**	10.9 (2.1%)
Platelets (10^3/cm^2)	1010	1008	841[a] **(-17%)**	955 (-5.3%)	856[a] **(-15%)**	893 (-12%)	765[a] **(-24%)**	784[a] **(-22%)**
Leukocytes (10^3/cm^2)	14.27	12.79	9.34[a] **(-34%)**	13.27 (3.8%)	7.64[a] **(-46%)**	7.93[b] **(-38%)**	6.44[a] **(-55%)**	6.38[b] **(-50%)**
Lymphocytes (10^3/cm^2)	11.80	10.82	6.26 (-47%)	11.04 (2.1%)	3.01[b] **(-74%)**	5.93[b] **(-45%)**	2.11[b] **(-82%)**	4.38[b] **(-60%)**
Segmented neutrophils (10^3/cm^2)	1.65	1.36	2.31[b] **(40%)**	1.52 (12%)	3.94[b] **(138%)**	1.40 (3.2%)	3.58[b] **(117%)**	1.52 (12%)
Eosinophils (10^3/cm^2)	0.12	0.10	0.13 (11%)	0.12 (16%)	0.10 (16%)	0.09 (-9%)	0.07[b] **(-43%)**	0.08 (-20%)
Basophils (10^3/cm^2)	0.10	0.09	0.05 (-48%)	0.11 (24%)	0.03[b] **(-71%)**	0.04[b] **(-60%)**	0.02[b] **(-77%)**	0.03[b] **(-68%)**

Large unstained cells ($10^3/cm^2$)	0.085	0.082	0.036 (-58%)	0.075 (-8.5%)	0.020 (-76%)	0.053 (-35%)	0.018[b] **(-79%)**	0.024[b] **(-71%)**
Effects after 8-week recovery period								
Erythrocytes ($10^6/cm^2$)	8.63	7.86					8.57 (-0.7%)	7.99 (1.7%)
Hemoglobin (g/dL)	14.6	14.0					15.1[a] **(3.4%)**	14.3 (1.8%)
Hematocrit (%)	44.3	41.8					45.1 (1.9%)	42.6 (1.9%)
Reticulocytes ($10^9/L$)	142.4	108.0					130.5 (-8.4%)	106.8 (-1.1%)
Red cell distribution width (%)	12.5	11.2					11.4[a] **(-9.2%)**	11.1 (-0.1%)
Platelets ($10^3/cm^2$)	864	891					786 (-9.0%)	838 (-6.0%)
Leukocytes ($10^3/cm^2$)	11.07	6.46					15.46[a] **(40%)**	9.81[a] **(52%)**
Lymphocytes ($10^3/cm^2$)	8.70	4.86					12.56[a] **(44%)**	8.15[a] **(68%)**
Segmented neutrophils ($10^3/cm^2$)	1.56	0.457					1.88 (21%)	0.310 (-7.1%)
Eosinophils ($10^3/cm^2$)	0.19	0.15					0.20 (3.6%)	0.16 (9%)
Basophils ($10^3/cm^2$)	0.06	0.02					0.11[a] **(98%)**	0.05[a] **(138%)**
Large unstained cells ($10^3/cm^2$)	0.059	0.025					0.091 (54%)	0.065 (160%)

[a]P≤0.05 (Dunnett); [b]P≤0.05 (Kruskal-Wallis/Dunn's). Reviewer's table. **Bold** = statistically significant.

Drug-related changes observed in clinical chemistry parameters included reductions in cholesterol (LDM: 24%, LDF: 31%, MDM: 34%, MDF: 46%, HDM: 39%, HDF: 53%), total protein (HDM: 8.1%; HDF: 14%, and globulin (HDM: 25%; HDF: 17%). These changes were reversed during the 8-week recovery period, except that globulin was still 8% lower than controls in HDM.

Urinalysis

Methods
Urine samples were collected from surviving Subset 1 main study and recovery animals overnight on PND 80 and again the night before scheduled euthanasia in Subset 1 recovery animals, with animals fasted during the collection period.

Results
There were no drug-related findings.

Ophthalmological evaluation

Methods
Ophthalmological examinations were conducted during PND 73-77 and PND 129-132.

Results
There were no drug-related findings.

Sexual maturation: Not assessed.

Neurobehavioral testing

Functional observational battery (FOB)

Methods
Main study and recovery animals were tested prior to daily dosing on PND 59 ± 2; recovery animals were also tested on PND 118 ± 2.

Results
The incidence of urination in an open field was reduced in HDM (11/40) compared to controls (22/40), consistent with findings of mild dehydration in this group. Stereotyped behavior in an open field was observed in 30/40 HDM and 4/40 HDF, but not in controls or any other group. The following clinical signs were noted in drug-treated rats, but not in controls: piloerection (8/20 MDM, 38/40 HDM); abnormal respiration (14/20 MDM, 7/20 MDF, 35/40 HDM, 24/40 HDF); and unkempt appearance (4/20 LDM, 10/20 MDM, 37/40 HDM, 5/40 HDF). Slight reductions were observed in grip strength in forelimbs (13% maximum; 17% average) and hind limbs (7% maximum; 11% average) and landing foot splay (9%) in HDM compared to controls.

HDM recovery groups showed the following differences from controls: reduced urination (13/20 vs. 18/20); abnormal respiration (17/20 vs. 0/20); unkempt appearance (4/20 vs. 0/20); reduced grip strength for forelimbs (12% maximum; 13% average) and hindlimbs (15% maximum; 12% average); and reduced landing foot splay (12%). Full reversibility was observed for stereotyped behavior and piloerection, while partial reversibility was showed for unkempt appearance. Abnormal respiration, reduced grip strength, and reduced landing foot splay did not reverse during the 8-week recovery period. Abnormal respiration was observed in HDF recovery animals (7/20).

Motor activity

Methods
Main study and recovery animals were tested on PND 61 ± 2; recovery animals were also tested on PND 120 ± 2. The movements of each animal were monitored by an automated system using infrared sensors.

Results
Increases (compared to controls) were observed during the first two 10-minute trials in the mean numbers of ambulations (MDM 1st: 72%, 2nd: 84%; HDM 1st: 83%, 2nd: 92%) and fine movements (MDM 1st: 32%, 2nd: 40%; HDM 1st: 29%, 2nd: 35%). Increases were maintained the mean number of ambulations in HDM recovery animals compared to controls (1st: 40%, 2nd: 87%).

Acoustic startle habituation

Methods
Main study and recovery animals were tested prior to daily dosing on PND 63 ± 2; recovery animals were also tested on PND 122 ± 2.

Results
HDM showed decreases in the average and maximum amplitude of the acoustic startle response at each trial block compared to controls (average: 34-43%; maximum: 37-46%). No significant differences were observed between HDM and control recovery groups.

Morris Water Maze testing

Methods
Main study and recovery animals were tested prior to daily dosing between PND 65 and PND 80; recovery animals were also tested between PND 125 and PND 135.

Results
As shown in the sponsor's tables below, the average time to reach the platform was increased in HDM compared to controls overall (Trials 1-9) in Session 1 (28%) and Session 2 (38%). HDM recovery animals showed increased average time to reach the platform during Trials 1-3 of Session 1 (51%), and Trials 1-9 of Session 1 (33%), compared to controls, but no differences were seen in Session 2.

The proportion of time spent in the goal quadrant during probe trials was slightly reduced in HDM compared to controls (39.10% vs. 44.15%, respectively), and in HDM recovery animals compared to controls (41.65% vs. 46.45%, respectively), but these differences did not reach statistical significance ($p > 0.05$).

The lack of significant differences between HDM and control groups after the 8-week recovery period in the average time to reach the platform overall in Session 2 suggests partial reversibility of the drug-related impairment in learning and memory. However, repeated testing of the same animals at the end of the dosing period and at the end of the recovery period may have confounded these results due to sustained learning—the apparent partial reversibility may not be reliable. No meaningful differences in performance were observed in LDM or MDM, or in females at any dose, compared to controls.

TABLE 6 (PAGE 1): SUMMARY OF MORRIS WATERMAZE PERFORMANCE: MALE RATS

COHORTS 1 & 2 (BETWEEN POSTNATAL DAY 65 AND POSTNATAL DAY 80)a					
GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	DEFLAZACORT	DEFLAZACORT	DEFLAZACORT
DOSE LEVEL (MG/KG/DAY)b		0 (CONTROL)	0.1	0.3	1
SESSION 1					
NUMBER OF RATS	N	40	20	20	40
AVERAGE TRIALS 1-3	MEAN ± S.D.	43.82 ± 10.37	44.15 ± 14.14	45.50 ± 12.08	50.81 ± 11.19
AVERAGE TRIALS 4-6	MEAN ± S.D.	22.63 ± 13.48	16.44 ± 8.37	20.22 ± 13.94	28.42 ± 15.98
AVERAGE TRIALS 7-9	MEAN ± S.D.	12.85 ± 8.41	12.23 ± 7.62	13.03 ± 8.03	21.82 ± 16.67
AVERAGE TRIALS 1-9	MEAN ± S.D.	26.42 ± 7.69	24.29 ± 5.83	26.26 ± 8.79	33.69 ± 11.67**
SESSION 2					
NUMBER OF RATS	N	40	20	19c	40
AVERAGE TRIALS 1-3	MEAN ± S.D.	13.53 ± 6.82	12.57 ± 8.66	14.51 ± 10.27	19.74 ± 11.75
AVERAGE TRIALS 4-6	MEAN ± S.D.	8.03 ± 3.86	6.80 ± 3.73	8.58 ± 6.80	10.19 ± 10.49
AVERAGE TRIALS 7-9	MEAN ± S.D.	6.73 ± 3.47	5.07 ± 1.75	8.28 ± 6.22	9.10 ± 9.90
AVERAGE TRIALS 1-9	MEAN ± S.D.	9.42 ± 3.54	8.14 ± 3.01	10.45 ± 6.52	13.02 ± 9.15*
SESSION 3					
NUMBER OF RATS	N	40	20	19c	40
PROBE TRIAL (%)d	MEAN ± S.D.	44.15 ± 13.35	44.80 ± 13.31	40.26 ± 15.49	39.10 ± 16.22

AVERAGE TRIALS = AVERAGE TIME (SECONDS) TO REACH THE PLATFORM FOR ALL RATS IN A GROUP FOR THE SPECIFIED TRIALS
a. Testing occurred over a three day period for each rat.
b. Dose administration occurred on Postnatal Days 21 through 80.
c. Excludes values for rat 7266, which was euthanized on Postnatal Day 69 due to adverse clinical observations.
d. Probe trial was recorded in seconds and reported as a percentage of time the rat spent in the goal quadrant.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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TABLE 6 (PAGE 2): SUMMARY OF MORRIS WATERMAZE PERFORMANCE: MALE RATS

COHORT 2 (BETWEEN POSTNATAL DAY 125 AND POSTNATAL DAY 135)a					
GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	DEFLAZACORT	DEFLAZACORT	DEFLAZACORT
DOSE LEVEL (MG/KG/DAY)b		0 (CONTROL)	0.1	0.3	1
SESSION 1					
NUMBER OF RATS	N	20	0	0	20
AVERAGE TRIALS 1-3	MEAN ± S.D.	12.90 ± 8.35			19.42 ± 9.96*
AVERAGE TRIALS 4-6	MEAN ± S.D.	6.59 ± 2.24			7.92 ± 5.55
AVERAGE TRIALS 7-9	MEAN ± S.D.	5.47 ± 1.11			5.91 ± 2.06
AVERAGE TRIALS 1-9	MEAN ± S.D.	8.32 ± 3.25			11.08 ± 4.53*
SESSION 2					
NUMBER OF RATS	N	20	0	0	20
AVERAGE TRIALS 1-3	MEAN ± S.D.	6.87 ± 4.41			6.91 ± 2.97
AVERAGE TRIALS 4-6	MEAN ± S.D.	5.78 ± 1.95			4.48 ± 1.03
AVERAGE TRIALS 7-9	MEAN ± S.D.	4.43 ± 1.25			4.93 ± 2.30
AVERAGE TRIALS 1-9	MEAN ± S.D.	5.70 ± 2.04			5.45 ± 1.51
SESSION 3					
NUMBER OF RATS	N	20	0	0	20
PROBE TRIAL (%)c	MEAN ± S.D.	46.45 ± 10.17			41.65 ± 13.58

AVERAGE TRIALS = AVERAGE TIME (SECONDS) TO REACH THE PLATFORM FOR ALL RATS IN A GROUP FOR THE SPECIFIED TRIALS
a. Testing occurred over a three day period for each rat.
b. Dose administration occurred on Postnatal Days 21 through 80.
c. Probe trial was recorded in seconds and reported as a percentage of time the rat spent in the goal quadrant.
* Significantly different from the control group value ($p \leq 0.05$).

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Estrous cycling: Not assessed.

Sperm analysis (Subset 1)

Methods
A sample was collected from right or left vas deferens for the evaluation of sperm motility. A homogenate was prepared from the left cauda epididymis for the evaluation of sperm concentration (sperm per gram of tissue weight). Concentration and motility were evaluated using computer-assisted sperm analysis. Sperm morphology was not assessed.

Results
No drug-related changes in sperm motility or concentration were observed.

Femur evaluation (Subset 1; length and density)

Methods
At the time of necropsy, the right femur was excised and cleaned and the length was recorded. Bone densitometry analysis was performed on the right femur using ex vivo peripheral quantitative computed tomography (pQCT) at the level of the distal femur metaphysis and in the mid-diaphysis. The following parameters were recorded:

Scan Site	Reporting
Metaphysis site: Distal Femur	Total area (mm^2), BMC (mg/mm) and BMD (mg/cm^3) Trabecular area (mm^2), BMC (mg/mm) and BMD (mg/cm^3) Cortical/subcortical area (mm^2), BMC (mg/mm) and BMD (mg/cm^3)
Diaphysis site: Femur	Total area (mm^2) Cortical area (mm^2), BMC (mg/mm) and BMD (mg/cm^3) Cross-sectional moment of inertia in the plane of bending (mm^4) Cortical thickness (mm) Periosteal circumference (mm) Endosteal circumference (mm)

Results
Dose-related changes were observed in femur size and mass in M at all doses, and in F primarily at the HD. Drug-related decreases in femur length and diameter were consistent with decreases in diaphyseal total area and periosteal circumference. Decreases in femur diaphysis cortical area and thickness were associated with decreases in cortical BMC and BMD. In the femur metaphysis, increases were observed in trabecular BMC and BMD without important changes in cortical/subcortical bone parameters. The findings are consistent with those reported for other glucocorticoids: bone loss and growth retardation associated with redistribution of bone from the peripheral (cortical) region to the central (trabecular) region.

Partial reversibility of these bone changes was observed following an 8-week recovery period. Reductions were observed in HD animals compared to controls in the following parameters: bone length; femur diaphysis total area, cortical area, and cortical thickness; distal femur metaphysis cortical/subcortical BMC, BMD; and diaphysis cortical BMC and BMD. The sponsor considered the changes observed at the HD in bone growth (primarily in M) and in the cortical bone compartment (M and F) to be adverse based on their magnitude and persistence following 8 weeks of recovery.

Effect of deflazacort on right femur parameters (% change from control)								
Dosage (mg/kg/day)	0		0.1		0.3		1.0	
Sex	M	F	M	F	M	F	M	F
Length (mm)	36.35	31.94	34.85[B] **(-4%)**	32.03 (0%)	33.54[C] **(-8%)**	31.63 (-1%)	31.48[C] **(-13%)**	30.71[C] **(-4%)**
Diaphysis total area (mm^2)	12.19	9.379	11.70 (-4%)	9.704 (3%)	11.53 (-5%)	9.793 (4%)	10.52[C] **(-14%)**	9.358 (0%)
Diaphysis cortical periosteal circumference (mm)	12.37	10.85	12.12 (-2%)	11.03 (2%)	12.03 (-3%)	11.09 (2%)	11.49[C] **(-7%)**	10.83 (0%)
Diaphysis cortical area (mm^2)	7.163	5.471	6.343[C] **(-11%)**	5.343 (-2%)	5.977[C] **(-17%)**	5.333 (-3%)	5.217[C] **(-27%)**	4.890[B] **(-11%)**
Diaphysis cortical thickness (mm)	0.706	0.613	0.625[C] **(-11%)**	0.580[A] **(-5%)**	0.586[C] **(-17%)**	0.575[B] **(-6%)**	0.531[C] **(-25%)**	0.532[C] **(-13%)**
Diaphysis cortical BMC (mg/mm)	8.969	6.803	7.806[C] **(-13%)**	6.582 (-3%)	7.266[C] **(-19%)**	6.516 (-4%)	6.227[C] **(-31%)**	5.896[C] **(-13%)**
Diaphysis cortical BMD	1252	1243	1230[C] **(-2%)**	1232 (-1%)	1215[C] **(-3%)**	1222[B] **(-2%)**	1194[C] **(-5%)**	1206[C] **(-3%)**
Metaphysis trabecular BMC (mg/mm)	1.784	1.663	2.817[A] **(58%)**	2.048 (23%)	3.742[C] **(110%)**	2.053 (23%)	5.419[C] **(204%)**	2.093 (26%)
Metaphysis trabecular BMD (mg/cm^3)	193.16	287.6	296.5[A] **(54%)**	333.8 (16%)	395.8[C] **(105%)**	339.0 (18%)	583.4[C] **(202%)**	344.8 (20%)
Metaphysis cortical/subcortical BMC (mg/mm)	10.28	7.661	10.07 (-2%)	8.137 (6%)	10.49 (2%)	7.928 (3%)	11.30 (10%)	7.534 (-2%)
Metaphysis cortical/subcortical BMD (mg/cm^3)	742.4	884.8	705.6 (-5%)	883.7 (0%)	739.7 (0%)	868.0 (-2%)	806.3 (9%)	831.2 (-6%)
Effects after 8-week recovery period								
Length (mm)	39.81	34.27					38.66[a] **(-3%)**	33.13 (-3%)
Diaphysis total area (mm^2)	14.72	10.01					13.77[a] **(-6%)**	10.19 (2%)
Diaphysis periosteal circumference (mm)	13.59	11.21					13.14[a] **(-3%)**	11.30 (1%)
Diaphysis cortical area (mm^2)	9.194	6.425					7.401[c] **(-20%)**	5.973 **(-7%)**
Diaphysis cortical thickness (mm)	0.839	0.717					0.672[c] **(-20%)**	0.643[c] **(-10%)**
Diaphysis cortical BMC (mg/mm)	12.19	8.409					9.337[c] **(-23%)**	7.624[b] **(-9%)**
Diaphysis cortical BMD (mg/cm^3)	1325	13.09					1262[c] **(-5%)**	1277[c] **(-2%)**
Metaphysis trabecular BMC (mg/mm)	1.418	1.319					1.163 (-18%)	1.226 (-7%)

Metaphysis trabecular BMD (mg/cm^3)	152.3	241.4					133.8 (-12%)	227.01 (-6%)
Metaphysis cortical/subcortical BMC (mg/mm)	11.89	8.367					9.880[C] **(-17%)**	7.854[a] **(-6%)**
Metaphysis cortical/subcortical BMD (mg/cm^3)	857.9	1025					761.3[C] **(-11%)**	974.6[a] **(-5%)**

[A]P≤0.05 (Dunnett);[B]P≤0.01 (Dunnett); [C]P≤0.001 (Dunnett); [a]P≤0.05 (*t*-test); Reviewer's table.
Bold = statistically significant.

Toxicokinetics

Methods

On PND 21 and PND 80, blood samples were collected via the vena cava (or jugular vein for in-life samples on PND 80) from 3/sex/group/time point. Samples were collected at 1 hour post dose from group 1 and at 1, 2, 4, and 8 hours post dose from groups 2 through 4. Samples were analyzed for the concentration of DFZ and 21-desDFZ.

Results

Plasma levels of DFZ were below the lower limit of quantitation (LOQ < 0.2 ng/mL) in all placebo and drug-treated groups. Plasma levels of 21-desDFZ were below the LOQ in all animals in the placebo group. Peak plasma levels of 21-DFZ in drug-treated animals were observed at 1-2 hours postdose on PND 21 and at 1 hour postdose on PND 80. 21-DFZ systemic exposure (Cmax and AUC_{0-t}) increased approximately in proportion with dose, except in F on PND 80 from 0.3 to 1 mg/kg/day, in which exposure was greater than dose proportional. Exposure was 2.5- to 5.3-fold greater on PND 80 than PND 21, except in LDF and MDF, in which there was no consistent difference. Exposure was 2- to 7-fold greater in M vs. F on PND 80, but no consistent difference was observed on PND 21. Mean concentrations of 21-desDFZ were consistently ≤ 10% of peak values by the 8-hr time point, so mean AUC_{0-t} values (t=8 hr) are approximately equal to $AUC_{0-24\ hr}$ values. Estimates of $t_{½}$ elimination were not provided.

Summary Mean (± SE) 21-desacetyldeflazacort Toxicokinetic Parameters in Female and Male Sprague-Dawley Rat Plasma Following Oral Administration of Deflazacort at 0.1, 0.3, and 1 mg/kg/day on PND 21 and PND 80

Dose (mg/kg/day)	Day	Sex	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)	R_{AUC}[a]
0.1	PND 21	Female	1.00	7.67 ± 0.454	27.5 ± 2.51	NA
		Male	2.00	10.9 ± 1.47	35.0 ± 5.36	NA
0.3	PND 21	Female	2.00	26.9 ± 1.19	89.3 ± 3.81	NA
		Male	1.00	28.0 ± 7.06	94.0 ± 10.6	NA
1	PND 21	Female	2.00	73.7 ± 10.9	261 ± 18.2	NA
		Male	1.00	68.9 ± 11.8	253 ± 20.9	NA
0.1	PND 80	Female	1.00	15.0 ± 2.21	23.2 ± 2.55	0.844
		Male	1.00	63.2 ± 8.38	167 ± 7.06	4.77
0.3	PND 80	Female	1.00	50.1 ± 11.0	69.7 ± 11.6	0.781
		Male	1.00	155 ± 7.16	448 ± 25.2	4.77
1	PND 80	Female	1.00	243 ± 71.9	640 ± 64.7	2.45
		Male	1.00	680 ± 47.0	1340 ± 72.1	5.3

NA = not applicable

a. R_{AUC} = PND 80 $AUC_{(0-t)}$/PND 21 $AUC_{(0-t)}$

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Necropsy

Methods

All surviving main study animals were euthanized after the last day of dosing (PND 81) and a gross necropsy was performed, including examination of the carcass and musculoskeletal system; external surfaces and orifices; cranial cavity and surfaces of the brain; and thoracic, abdominal and pelvic viscera. Recovery animals were euthanized on PND 138 ± 2 and a gross necropsy was performed. Euthanasia was by exsanguination after blood collection under isofluorane/oxygen anesthesia (Subset 1, histopathology, male reproductive assessment, and bone densitometry) or by infusion with heparin/sodium pentobarbital followed by perfusion with neutral buffered 10% formalin (Subset 2, neurohistopathology and brain morphometry).

The following tissues were collected: adrenals, aorta, bone marrow smear (Subset 1), bone marrow (femur, left), bone (femur, right), bone (femur, left; distal end including femoral tibial joint), bone (sternum), brain, cecum, colon, duodenum, epididymides, esophagus, eyes (Subset 1; with optic nerves), GALT, harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, ovaries, pancreas, pituitary (Subset 1), prostate, rectum, sciatic nerve, salivary gland (mandibular and sublingual), seminal vesicles (with coagulating glands), skeletal muscle (left thigh), skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (glandular and nonglandular), testes, thymus, thyroid (and parathyroids), tongue, trachea, ureter, urinary bladder, uterus (with oviducts and cervix), and vagina. Gross lesions from all rats were retained for histological evaluation.

Tissues from Subset 1 (10/sex/group) in the control and the HD group, including gross lesions, were processed, embedded in paraffin, sectioned at 5 microns, stained with H&E, and evaluated histopathologically.

The following tissues were processed and evaluated in Subset 1 main study LD and MD groups: gross lesions, adrenal glands, mandibular lymph nodes, spleen, thymus, mesenteric lymph nodes, GALT, bone marrow, mammary glands (M only), and femur (M only).

The following organs from Subset 1 rats were weighed at scheduled euthanasia: adrenals, brain, epididymides, femur (right), heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles (with coagulating gland; with fluid), spleen, testes, thymus, thyroid lobes and parathyroids, uterus (with cervix). Paired organs were weighed together, with the exception of the following organs that were collected from males to assess the potential toxicity of the test article on the male reproductive system: right testis, left testis, left epididymis (whole and cauda), and right epididymis.
The same organs were weighed in Subset 2 rats, except for those in eyes and pituitary.

Results
Drug-related changes were observed in the adrenal glands, lymphoid organs, bone, and mammary glands. All findings were reversible during the 8-week recovery period.

Dose-dependent reductions were observed at all doses compared to controls in the relative weight of the adrenal glands (LDM: 24%, MDM: 47%, HDM: 65%, HDF: 18%). These changes were correlated with gross findings of small adrenal glands (LDM: 8/20, MDM: 19/20, HDM: 20/20, HDF: 5/20) and microscopic findings of minimal (min) to moderate (mod) adrenal cortical atrophy (LDM: 5/10 [2 min, 3 mild], MDM: 8/8 [2 mild, 6 mod], HDM: 10/10 [1 mild, 9 mod]; HDF: 5/10 [3 min, 2 mild]. The atrophy was characterized by an overall thinning of the cortex, especially in the zona reticularis and zona fasciculata, with reductions in cell size in the latter.

Dose-dependent reductions were observed at all doses compared to controls in the relative weight of the thymus (LDM: 64%, LDF: 19%, MDM: 73%, MDF: 40%, HDM: 78%, HDF: 57%). These changes were correlated with gross findings of small thymus (LDM: 14/20, LDF: 1/20, MDM: 18/20, MDF: 4/20, HDM: 19/20, HDF: 10/20) and microscopic findings of minimal to marked decreased cellularity (LDM: 5/10 [mild], MDM: 9/9 [3 mild, 6 mod], MDF: 3/10 [1 min, 2 mild], HDM: 10/10 [marked], HDF: 8/10 [mild]).

Dose-dependent reductions were observed at all doses compared to controls in the relative weight of the spleen (LDM: 24%, MDM: 47%, HDM: 65%, HDF: 18%). These changes were correlated with gross findings of small spleen (LDM: 3/20, MDM: 10/20, MDF: 3/20, HDM: 18/20, HDF: 6/20) and microscopic findings of minimal to marked decreased cellularity of white pulp (LDM: 4/10 [3 min, 1 mild], MDM: 9/9 [4 mild, 5 mod], HDM: 10/10 [5 mod, 5 marked], HDF: 7/10 [mild]).

Small mandibular lymph node was observed in 2/20 HDM, correlating with microscopic findings of minimal to marked decreased cellularity (LDM: 2/10 [mild], MDM: 7/8 [1 min, 4 mild, 1 mod, 1 marked], HDM: 9/10 [1 mod, 8 marked], HDF: 5/10 [mild]). Mild to marked cellularity was also observed in the mesenteric lymph node (LDM: 4/10 [mild], MDM: 9/9 [4 mild, 5 mod], HDM: 10/10 [5 mod, 5 marked], HDF: 7/10 [mild]).

Mild to marked decreased cellularity was observed in the bone marrow (MDM: 2/9 [mild], HDM: 10/10 [6 mild, 3 mod, 1 marked], HDF: 6/10 [5 mild, 1 mod]). In addition, minimal decreased cellularity was observed in the physis of the femur in all HDM.

Minimal to marked decreased cellularity was observed in the GALT (LDM: 2/9 [min], MDM: 6/9 [2 min, 3 mild, 1 mod], HDM: 7/8 [marked], HDF: 6/9 [mild]).

Minimal to marked atrophy was observed in the mammary gland in M only (LDM: 6/10 [4 min, 2 mild], MDM: 8/8 [7 min, 1 mod], HDM: 10/10 [4 mild, 5 mod, 1 marked]). Atrophic mammary epithelium showed a columnar to cuboidal appearance and reduced amount of eosinophilic cytoplasm compared to controls.

Microscopic examination of the testes in HDM revealed no abnormalities and confirmed that all stages of spermatogenesis were present.

No drug-related organ weight, gross, or microscopic changes were observed in HD recovery animals.

Neurohistopathology (Subset 2)

Methods
The following tissues were collected, processed, and (for HD and controls) examined microscopically: brain, including olfactory bulbs; eyes; gasserian ganglia (5^{th} cranial nerves and ganglia); cervical, thoracic, and lumbar regions of spinal cord; dorsal root ganglia and associated dorsal and ventral spinal nerve roots from the cervical, thoracic, and lumbar regions of the spinal cord; sciatic, tibial, peroneal (fibular), and sural nerves; and skeletal muscle (gastrocnemius and soleus).

Results
No drug-related changes were observed.

Whole Brain Weights and Morphometric Measurements (Subset 2)

Methods
Brain weights were measured after a similar fixation time, and a Vernier caliper was used to measure two Anterior to Posterior (AP) distances for each brain: AP length of the cerebrum, from the anterior pole to the posterior pole, excluding the olfactory bulbs; and AP length of the cerebellum, from the anterior edge of the cortex to the posterior pole, on a diagonal corresponding to the slope of the posterior portion of the cerebellar cortex. Brain morphometry performed on sections stained with luxol fast blue/cresyl

violet included: thickness of frontal cortex; thickness of parietal cortex; diagonal width of the caudate-putamen and underlying globus pallidus; thickness of the corpus callosum; thickness of all hippocampal layers combined; and the maximum height of cerebellum at the level of the seventh cranial nerve bundles.

Results
No drug-related changes were observed.

11 Integrated Summary and Safety Evaluation

Deflazacort is a corticosteroid prodrug that is rapidly and completely deacetylated by plasma esterases to 21-desDFZ following oral administration. Like other drugs in this class (e.g., dexamethasone and prednisolone), 21-desDFZ binds to glucocorticoid receptors in cells, triggering translocation of the ligand-receptor complex into the nucleus, where interactions with DNA result in altered transcription of genes that produce anti-inflammatory, immunosuppressive, and many other effects. The proposed therapeutic dosing regimen in patients with DMD is 0.9 mg/kg once daily via oral tablet or oral suspension.

The sponsor submitted an in vitro pharmacology study demonstrating affinity of 21-desDFZ for cytosolic human glucocorticoid receptors (IC_{50} = 21 nM, K_i = 10 nM); binding affinity of prednisolone was approximately 4 times higher (IC_{50} = 4.9 nM, K_i = 2.4 nM). All other pharmacology information was provided by reference to published articles, including in vitro and ex vivo binding and functional activity studies, and in vivo studies evaluating the effects of deflazacort on immunosuppressive and inflammatory responses, bone and cartilage, and muscle injury and repair.

In vitro studies of 21-desDFZ demonstrated specific binding to rat cytosolic glucocorticoid receptors from kidney, thymus, and liver with relative affinity approximately 4, 3, and 6 times lower, respectively, than dexamethasone; and with IC_{50} in liver and hippocampus approximately 4 times greater than dexamethasone. In an in vitro luciferase gene reporter transactivation assay, 21-desDFZ showed approximately 2.3 times greater agonist activity for the human glucocorticoid receptor than for the human mineralocorticoid receptor (EC_{50} = 4.37 and 10.2 nM, respectively). In ex vivo studies of deflazacort administered orally or intravenously to adrenalectomized rats, evidence of binding to cytosolic glucocorticoids was demonstrated in kidney, thymus, liver, pituitary, hippocampus, and cerebral cortex.

Several published studies of deflazacort in mdx mice have demonstrated prevention of injury, enhanced repair, and/or improvement in function of skeletal muscles after damage due to the mdx mutation alone or experimental injury. Compared to controls, deflazacort-treated mdx mice showed reduced inflammation, central nucleation, and calcification of myofibers in the diaphragm; reduced inflammation, myofiber degeneration and size variability, and increased myofiber diameter and proliferation of myogenic$^+$ myoblasts the in TA; increased weight of gastrocnemius and quadriceps muscles; and decreased area of fibrosis and inflammation in the myocardium. Additional differences in deflazacort-dosed mdx mice included increased percentage of new myotube nuclei in regenerating areas of crushed TA muscle; and reduced myofiber damage and increased regeneration-related myogenic factor 5 expression in quadriceps after injury due to exercise. Functional improvements associated with deflazacort administration to mdx mice included increased mean normalized peak forelimb grip strength Day 10-28); increased mean normalized (non-peak) forelimb grip strength Days 22 and 57, but not Day 36); and increased distance run during a 24-hour period of voluntary exercise 3 months after the end of the dosing period. The increase in grip

strength reported in mdx mice contradicts the reduction in grip strength reported in the juvenile rat study of deflazacort.

Immunosuppressive effects of 21-desDFZ demonstrated in vitro included suppression of phytohemagglutinin-induced mitogenesis of human lymphocytes and of the cytotoxic activity of human natural killer cells. Anti-inflammatory effects of deflazacort demonstrated in rats after IV, IP and/or PO administration included reduced carageenan-induced edema; reduced nystatin-induced edema; reduced weight of dried cotton-pellet granulomas; and reduced adjuvant-induced arthritis.

In vitro effects of deflazacort and/or 21-desDFZ on bone and cartilage were somewhat contradictory, showing inhibitory effects in some studies and proliferative effects in others. Inhibition of proliferation and collagen synthesis was observed in cultures of fetal rat skullcaps and osteoblasts and of collagen synthesis in secondary cultures of rabbit articular chondrocytes. These effects are in agreement with the reduction in femur size and mass observed in the juvenile rat toxicity study. In vivo administration of deflazacort to male rats for 20 days, however, increased anaerobic glycolysis in epiphyseal cartilage from the tibia, in contrast to prednisone, which decreased the anaerobic glycolysis necessary for epiphyseal cartilage growth and mineralization. This growth-stimulating effect is in agreement with the findings of increased osteoma and osteosarcoma in a rat carcinogenicity study of deflazacort.

Safety pharmacology information on deflazacort provided by the sponsor was limited to one hERG study, which demonstrated no appreciable inhibition of hERG current in CHO-K1 cells at up to 10 μM 21-desDFZ or 6β-OH-21-desDFZ, and a published study. In the latter, deflazacort was reported to lack effects on blood pressure or heart rate in rats, or on CNS parameters (including sleeping time and maximum electroshock assays) in mice, but no details were provided to assess the adequacy of these studies; urine volume and potassium excretion were increased in adrenalectomized male rats given 0.5-2.0 mg/kg deflazacort orally.

With the exception of several in vitro studies related to the potential for drug-drug interactions (reviewed by the Clinical Pharmacology Team) and two in vitro metabolism studies, the information on the pharmacokinetics (PK), absorption, metabolism, and excretion of deflazacort provided by the sponsor consisted of references to articles in the published literature. Published reports of rapid and complete conversion of deflazacort to its major active metabolite, 21-desDFZ, by plasma esterases after oral or IV administration to rat, dog, or human were consistent with the absence of detectable levels of deflazacort in plasma samples in the repeat dose oral toxicity studies of deflazacort in rat and monkey. In a published PK study, peak levels of 21-desDFZ were observed within 1-4 hours after oral dosing with deflazacort in rat, dog, monkey, and human, oral bioavailability was ~92% in rat, 37% in dog, and 43% in monkey, and 21-desDFZ was rapidly cleared from plasma into tissues ($t_{½}$ = 2-4 hours).

In vitro plasma protein binding of 21-desDFZ was 65%, 42%, and 40% in rat, dog, and human, respectively (no data were provided for monkey). Distribution studies in rat

showed the highest concentrations of radioactivity in liver and kidney, with moderate levels in heart, pancreas, lungs, and submaxillary glands, after oral administration of [^{14}C]-deflazacort.

In vitro metabolism studies conducted by the sponsor showed extensive metabolism of 21-desDFZ in human liver microsomal preparations, with only 40% of the initial amount remaining after 60 minutes, compared to 83% and 85% in preparations from rat and dog, respectively. Unchanged 21-desDFZ accounted for 75% of drug-related material in human and monkey preparations, and 97% in rat and dog preparations. Seven human metabolites were identified: oxygenated derivatives M7, M1, and M6, present at 9, 8, and 4.5%, respectively; and M2, M3, M4, and M5, each present at < 2%. M3 was the only unique human metabolite, and accounted for only 0.5% of total drug-related species in vitro.

A published report of in vivo metabolism studies of oral [^{14}C]-deflazacort conducted in male Wistar rat, male beagle dog, and healthy human volunteers (2 male, 1 female) confirmed the absence of measurable deflazacort in plasma in all species, and demonstrated the presence of three major human plasma metabolites: M-II (21-desDFZ), M-III (6β-OH-21-desDFZ), and M-V (structure unknown). 21-desDFZ accounted for 43.4, 59.1, and 68.6% of total plasma radioactivity in human, dog, and rat, respectively; 6β-OH-21-desDFZ accounted for 27.2, 7.5, and 5.8%, respectively; and M-V accounted for 25.2, 8.0, and 6.9%, respectively. M-V accounted for 19.8, 2.2, and 12.7% of radioactivity in urine of human, dog, and rat, respectively, and urinary M-V accounted for approximately 13% of the total administered dose in human. Limited information provided on the structure of M-V, included that the steroid is deacetylated, the 16α,17α-2'-methyloxazoline ring and the side chain are unchanged, the 11β-hydroxy function is present, the $\Delta^{1,4}$ system is absent, and there is oxidation, probably in the B-ring. Major metabolite 6β-OH-21-desDFZ showed no binding activity at human glucocorticoid receptors in an in vitro binding assay; the pharmacologic activity of M-V has not been evaluated. In another published study, oral administration of [^{14}C]-deflazacort to male Cynomolgus monkeys resulted in the formation of two major plasma metabolites: 21-desDFZ (45.0% of total plasma radioactivity) and 6β-OH-21-desDFZ (26.2%). Minor metabolites identified in monkey plasma included M-IV, M-VII, M-VIII, and M-IX; M-V was not detected. The sponsor proposed that M-V and M-VII may be similar mixtures of isomers that "have been qualified across species," but the available data do not support this conclusion.

The primary route of excretion after oral administration of [^{14}C]-deflazacort was urinary for human (68% of the dose), monkey (50%), and rat (54%); and fecal for dog (82%).

The toxicology of deflazacort was assessed in oral (gavage) studies in Cynomolgus monkeys (14-day and 39-week), adult Sprague Dawley rats (14-day and 26-week), and juvenile Sprague Dawley rats (15-day and 8-week). Across species, primary target organs were lymphoid organs and adrenal cortex.

In the 14-day monkey study, doses of 1.0 and 3.0 mg/kg/day resulted in small thymus and/or reduced relative weight of thymus, compared to controls; no effects were observed at the LD of 0.3 mg/kg/day.

In the 39-week monkey study, deflazacort was administered once daily via oral gavage at 0, 0.3, 1.0, and 3.0 mg/kg/day for 91 days, at which time the dose of 0.3 mg/kg/day was changed to 6.0 mg/kg/day based on the absence of dose-limiting toxicities at the original HD of 3.0 mg/kg/day. Key toxicities observed at doses of 3.0 and 0.3/6.0 mg/kg/day included immunosuppression (impaired T-cell dependent antibody responses and minimal to severe lymphocytic depletion in thymus, spleen, and lymph nodes); atrophy in adrenal cortex, uterus, and vagina; hyperplasia in mammary glands (M); hyperplasia/hypertrophy in pituitary (M); increased adipocytes in bone marrow; and absence of corpus luteum in ovaries. The LD of 1.0 mg/kg/day was associated with similar findings at lower incidence and/or severity in adrenal glands, thymus, spleen, mammary gland, ovaries, uterus, and vagina. Incomplete recovery was demonstrated by the following findings in groups allowed to recover for 6 weeks following 39 weeks of dosing at 3.0 mg/kg/day: reduced weight of spleen, increased weight of pituitary, minimal hyperplasia/hypertrophy in pituitary, atrophy of the adrenal cortex, and reduced anti-KLH IgM and IgG responses to KLH immunization. The NOAEL for oral administration of deflazacort in monkey was 6.0 mg/kg/day for 27 weeks and 3.0 mg/kg/day for 39-weeks; however, the immunosuppression observed at these doses may be adverse in a less sterile environment.

In the 14-day rat study, adverse findings observed in HDM at 3 mg/kg/day included body weight loss, marked reduction in reticulocytes, and moderate reduction in lymphocytes. Other findings in this group included increased monocytes, RBC parameters (mild dehydration), serum transaminases, triglycerides, total protein, globulin, urea nitrogen, sodium; and reduced relative weight of adrenals, spleen, and thymus (histopathology was not performed). Similar, but less severe, findings were observed dose-dependently in males at 0.3 and 1 mg/kg/day. Effects in females included reductions in body weight, reticulocytes (HD), lymphocytes (MD, HD), and relative weights of adrenals (HD), spleen (HD), and thymus; and increases in RBC parameters, monocytes (HD), and serum sodium (HD); none were considered adverse.

In the 26-week oral toxicity study of deflazacort in rat, dose-dependent findings included decreased body weight gain, final body weight, and circulating lymphocytes (up to 78% in M and 58% in F); increased neutrophils (M) and triglycerides (M); minimal to severe lymphocytic depletion and/or hypocellularity in lymphoid organs; and minimal to marked atrophy in skin/subcutis, glandular stomach, esophagus, duodenum, rectum, and adrenal cortex. All changes showed at least partial reversibility during the 4-week recovery period. The NOAELs in this study were the LDs of 0.05 mg/kg/day in M and 0.10 mg/kg/day in F, based on the excessive lymphoid depletion observed at the mid dose (0.15 mg/kg/day in M, 0.30 mg/kg/day in F) and high dose (0.50 mg/kg/day in M, 1.00 mg/kg/day in F). The marked reduction in circulating lymphocytes observed in this study was not observed in the 39-week study in monkey.

In the 15-day oral (gavage) dose range-finding study in juvenile rats given deflazacort at 0, 0.3, 1, and 3 mg/kg/day from PND 21 to PND 35, dose-dependent reductions were observed in body weight, body weight gain, food consumption, size and weight of adrenal glands and thymus, and weight of spleen, lungs, and ovaries (histopathology was not performed). The NOAEL was not established, based on excessive reductions in body weight gain and thymus weight at the LD.

In the pivotal 8-week oral (gavage) study in juvenile rats given deflazacort at 0, 0.1, 0.3, and 1.0 mg/kg/day from PND 21 to PND 80, dose-dependent effects observed included reductions in body weight, body weight gain, food consumption; adverse effects on functional and neurobehavioral parameters (increased stereotypy, abnormal respiration, and motor activity; decreased grip strength, landing foot splay, and acoustic startle response; and impaired performance in the Morris water maze); bone changes in femur (reductions in size, mass, and diaphyseal cortical BMC and BMD; increases in metaphyseal trabecular BMC and BMD); decrease cellularity of lymphoid organs and the physis of the femur; and atrophy of the adrenal cortex and male mammary glands. After 8 weeks of recovery, HDM still showed the following differences from controls: increased abnormal respiration; reduced body weight, grip strength, landing foot splay, femur size and mass; and impaired Morris water maze performance. The NOAEL in M was not established, based on the following adverse findings at the LD of 0.1 mg/kg/day: reduced body weight and body weight gain; redistribution of bone in femur from peripheral to central regions; adrenal cortical atrophy; and decreased cellularity of lymphoid organs associated with reductions in relative organ weight and in circulating lymphocytes. The NOAEL in F was 0.1 mg/kg/day, based on the lower severity of effects at this dose compared to LDM and MDF. The sponsor's speculation that the observed effects of deflazacort on the functional observation battery and neurobehavioral assessments in this study "were likely secondary to the pharmacological effects on growth and stature" was not adequately justified by the data provided.

Deflazacort and 21-desDFZ were negative in standard valid in vitro bacterial reverse mutation and human lymphocyte chromosomal aberration assays in the presence and absence of S9 rat liver metabolic activation, and in a valid in vivo rat bone marrow micronucleus assay.

Various potentially genotoxic impurities were evaluated by acceptable methods using in silico and "read-across" strategies, supplemented by in vitro bacterial reverse mutation (Ames) assays where appropriate.

Carcinogenicity studies of deflazacort were not submitted by the sponsor, based on prior communications from FDA that such studies could be conducted, if needed, as post-marketing requirements in view of the seriousness of the indication, as long as the available data support that strategy. The sponsor submitted a request for a waiver of the requirement to conduct carcinogenicity studies of deflazacort based on the argument that additional rodent carcinogenicity studies would not add meaningful knowledge regarding the risk of human carcinogenicity. The sponsor provided the following

rationale for this argument: a published 2-year dietary carcinogenicity study in rat showed drug-related increases in osteosarcomas and osteomas in males at the maximum tolerated dose of 0.25 mg/kg/day; a similar 2-year study in mouse mentioned in the same publication was negative; the increased risk of skin cancers and lymphoma in humans treated with glucocorticoids is already well-known, and not typically seen in 2-year rodent studies; and the greater sensitivity of rodents (compared to humans) to the pharmacologic effects of glucocorticoids limits the doses that can be tolerated in a 2-year study.

While the level of detail available in the published study provided was less than that generally needed to document an adequate assessment of carcinogenic risk, the reduction in final body weight observed in the 26-week rat study conducted by the sponsor even at the lowest doses tested (18% in LDM; 10% in LDF) suggests that 21-desDFZ plasma AUC exposures achievable in a 2-year carcinogenicity study in rat may be less than one fifth of those in humans at the recommended clinical dose of 0.9 mg/kg/day. The highest tolerated dose of 0.25 mg/kg/day in the published dietary rat carcinogenicity study is equivalent to a human dose of ~0.04 mg/kg/day (on a mg/m^2 basis), only 4.4% of the recommended human dose of 0.9 mg/kg/day. Therefore, the sponsor's argument that a new carcinogenicity study in rat is not likely to provide meaningful information in addition to the published study is reasonable. However, insufficient data have been provided to determine whether or not a carcinogenicity study in mouse would provide meaningful information.

Because the DMD patient population is almost entirely male, assessments of the effects of deflazacort on female fertility, embryofetal development, and pre- and postnatal development were neither required nor conducted. The pivotal 39-week monkey, 26-week rat, and 8-week juvenile rat studies of deflazacort showed no drug-related effects on the male reproductive system.

Summary of Key Toxicities and Plasma Exposures in Pivotal Toxicity Studies

Toxicity	Duration and Species	NOAEL (mg/kg/kg)		Mean 21-desDFZ $AUC_{0\text{-}24\ hr}$ [#] (µg•hr/mL)		Safety Margin Based on AUC*	
		Male	Female	Male	Female	Male	Female
Immuno-suppression	39-Week Cynomolgus Monkey	0.3/6.0	0.3/6.0	2470	3660	4.0	5.9
	26-Week Rat	0.05	0.10	94.7	57.2	0.15	0.09
	8-Week Juvenile Rat	<0.1	0.3	<167	23.2	<0.27	0.04
Atrophy in adrenal cortex and other organs	39-Week Cynomolgus Monkey	0.3/6.0	0.3/6.0	2470	3660	4.0	5.9
	26-Week Rat	0.15	1.0	385	789	0.62	1.3
	8-Week Juvenile Rat	<0.1	1.0	<167	640	<0.27	1.0
Reduced body weight, bone effects	8-Week Juvenile Rat	<0.1	0.3	<167	23.2	<0.27	0.04

(Reviewer's Table)

*Day 8 AUC in adolescent DMD patients at 0.9 mg/kg/day: 622 ng*hr/ml; Study MP-104-CL-005

[#]$AUC_{0\text{-}24\ hr}$: Day 271 for monkey, Day 179 for adult rat, PND 80 for juvenile rat

As shown in the summary table above, there is a 4-fold safety factor for the AUC at the NOAEL in male monkey relative to the AUC observed in adolescent DMD patients at the recommended clinical dose of 0.9 mg/kg/day deflazacort. However, as noted previously, that exposure level was associated with significant immunosuppression that may be adverse in a less controlled environment. There is no safety margin for the adverse effects observed in adult or juvenile male rats.

Comparison of Animal and Human Plasma Exposures after Oral Deflazacort

Study	Dose	21-des-DFZ C_{max} (ng/mL)	21-des-DFZ *$AUC_{0\text{-}tau}$ (ng*hr/mL)	6β-OH-21-desDFZ C_{max} (ng/mL)	6β-OH-21-desDFZ $AUC_{0\text{-}t}$ (ng*hr/mL)	Major Human Metabolite V C_{max} (ng/mL)	Major Human Metabolite V $AUC_{0\text{-}t}$ (ng*hr/mL)
#MP-104-CL-005 Children Day 8	0.9 mg/kg/day	256	450	125	358	&*149*	&*261*
#MP-104-CL-005 Adolescents Day 8	0.9 mg/kg/day	370	622	159	413	&*215*	&*361*
39-Week Monkey HDM Day 271	0.3/6.0 mg/kg/day	760	2470	462	2240	Not identified in monkey	Not identified in monkey
26-Week Rat HDM Day 179	0.5 mg/kg/day	255	978	$*22*	$*83*	&*26*	&*98*
8-Week Juvenile Rat HDM PND80	1.0 mg/kg/day	680	1340	$*58*	$*114*	&*68*	&*134*

(Reviewer's Table)

*$AUC_{0\text{-}tau}$ = Area under the concentration-time curve during a dosing interval at steady state (tau); concentrations were near zero by the last collection timepoint (8 hours postdose in humans, 24 hours postdose in animals)

#MP-104-CL-005: N=16 male children with DMD, 4-11 years old, mean weight = 37.5 kg;
N=8 male adolescents with DMD, 12-16 years old, mean weight = 49.5 kg

$Estimates of mean 6β-OH-21-desDFZ C_{max} and AUC were calculated based on values of 5.8% and 68.6% of Total Plasma Radioactivity for 6β-OH-21-desDFZ and 21-desDFZ, respectively, in pooled plasma samples collected 1, 2, 4, and 8 hours after dosing 5 M Wistar rats with 5 mg/kg [^{14}C]-deflazacort PO: 5.8%/68.6% = 0.085, multiplied by the mean 21-desDFZ C_{max} and AUC values reported in the pivotal rat toxicity studies (Martinelli et al., 1979; and Table 1 on page 26 of this review).

&Estimates of mean Major Human Metabolite V C_{max} and AUC were calculated based on values of 25.2% and 43.4% of Total Plasma Radioactivity for Major Human Metabolite V and 21-desDFZ, respectively, in pooled plasma samples collected 1, 2, 4, and 8 hours after dosing 3 healthy volunteers (2M, 1F) with 50 mg/kg [^{14}C]-deflazacort PO (25.2%/43.4% = 0.581, multiplied by the mean 21-desDFZ C_{max} and AUC values reported in clinical study MP-104-CL-005); and on values of 6.9% and 68.6% of Total Plasma Radioactivity for Major Human Metabolite V and 21-desDFZ, respectively, in pooled plasma samples collected 1, 2, 4, and 8 hours after dosing 5 M Wistar rats with 5 mg/kg [^{14}C]-deflazacort PO: 6.9%/68.6% = 0.10, multiplied by the mean 21-desDFZ C_{max} and AUC values reported in the pivotal rat toxicity studies (Martinelli et al., 1979; and Table 1 on page 26 of this review).

As shown in the table above, major metabolites 21-desDFZ and 6β-OH-21-desDFZ have been adequately assessed for general toxicity in at least one species. Estimates

have been calculated for the C_{max} and AUC of major human metabolite M-V in human and rat based on the ratios of M-V:21-desDFZ presented in Table 1 of Martinelli et al. (1979; page 26 of this review). Based on the values for adolescents, M-V may not have been adequately assessed for general toxicity in at least one species, as recommended for major human plasma metabolites, which need to be present at plasma exposures ≥ 50% of those expected in humans at the recommended clinical dose. It is not clear if sufficient levels of 6β-OH-21-desDFZ or M-V were present in the Sprague-Dawley rats administered deflazacort in the published 2-year dietary carcinogenicity study to constitute adequate assessments—therefore, these metabolites may need to be assessed in the carcinogenicity study in mouse, with direct administration of 6β-OH-21-desDFZ and/or M-V if adequate exposures cannot be achieved otherwise. Such a study may obviate the need for a separate chronic toxicity study of M-V. In addition, 6β-OH-21-desDFZ and M-V may need to be evaluated in an in vitro study that detects point mutations, an in vitro study that detects chromosomal aberrations, and in an in vivo rodent micronucleus study (if either of the two in vitro studies is equivocal or positive, or if their carcinogenic potential is not able to be assessed) *(Safety Testing of Drug Metabolites, Guidance for Industry, CDER, November 2016, Revision 1; Guidance for Industry, M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals Q&A (R2), ICH, February 2013, Revision 1).*

Based on the differences in strain of the rats used for the in vivo metabolite study (Wistar) and those used for the pivotal toxicity studies (Sprague Dawley), the small number of animals (5) and humans (3) studied, and the absence of information on M-V plasma exposures in DMD patients or in the pivotal toxicity studies, there is insufficient information available to determine whether or not the general toxicity, genotoxicity and carcinogenicity of major metabolite M-V have been adequately assessed. The genotoxicity and carcinogenicity of major metabolite 6β-OH-21-desDFZ may also not have been adequately assessed.

Recommendations

The nonclinical data submitted do not adequately support the approval of deflazacort for the treatment of DMD. Insufficient information has been provided to determine if major circulating human metabolites M-V or 6β-OH-21-desDFZ have been adequately assessed for genotoxicity and carcinogenicity or if M-V has been adequately assessed for chronic toxicity.

To support approval, the sponsor should submit information (including additional studies, if needed) to demonstrate that major human metabolites 6β-OH-21-desDFZ and M-V have been adequately assessed for carcinogenicity and genotoxicity (in vitro assays for mutagenicity and chromosomal aberrations, and an in vivo rodent micronucleus study, unless the two in vitro studies are negative and the carcinogenic potential of M-V is adequately assessed); and that M-V has been assessed for chronic toxicity in one species (an adequate carcinogenicity study may substitute for the chronic toxicity study).

If these NDAs are approved despite the insufficient assessment of major metabolites 6β-OH-21-desDFZ and M-V noted above, the deficiencies should be addressed as post-marketing requirements.

The information provided support granting a waiver of the requirement for a carcinogenicity study in rat, based the conclusion that a new 2-year study in rat would not likely yield meaningful additional information.

A dose range-finding study should be conducted in mouse, with toxicokinetic analysis of 21-desDFZ, 6β-OH-21-desDFZ, and M-V, to assess the feasibility of conducting an oral carcinogenicity study of deflazacort in mouse. Major metabolites may need to be directly administered if their plasma exposures after administration of deflazacort are not ≥ 50% of those in humans at the recommended clinical dose. This study, and a subsequent carcinogenicity study in mouse, if warranted, should be post-marketing requirements.

12 Appendix

Clinical Study MP-104-CL-005: "A Multi-center Study to Evaluate the Pharmacokinetics of 21-Desacetyldeflazacort and the Safety of Deflazacort after Oral Administration of Deflazacort Tablets to Children and Adolescent Subjects with Duchenne Muscular Dystrophy"

Deflazacort was administered via oral tablet at a fixed dose of 0.9 mg/kg/day. t ≤ 24 hr.

Table 5-3. Summary of Key Pharmacokinetic Parameters for Plasma 21-desDFZ by Study Population on Day 8

Study Population	Statistic	Cmax (ng/mL)	Tmax (h)	AUClast (h*ng/mL)	AUC0-8 (h*ng/mL)	AUCtau (h*ng/mL)	CLss/F (L/h)	Vzss/F (L)	Cmax/Dose (ng/mL/mg)	AUClast/Dose (h*ng/mL)/mg	AUCtau/Dose (h*ng/mL)/mg	AR Cmax	AR AUC
Children	N	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	256	NC	444	444	450	67.5	111	8.15	14.0	14.3	1.11	0.953
	SD	153	NC	250	249	254	18.7	27.7	2.44	3.48	3.54	0.503	0.207
	CV%	59.7	NC	56.3	56.1	56.4	27.7	25.0	30.0	24.8	24.9	45.4	21.7
	Geometric Mean	214	NC	372	374	378	65.4	108	7.84	13.6	13.8	1.03	0.934
	Geometric CV%	71.2	NC	72.7	71.8	72.4	26.2	25.4	28.9	26.3	26.2	39.2	20.4
	Min	63.4	0.50	95.1	98.5	98.5	39.4	69.7	5.28	7.92	8.21	0.495	0.723
	Median	218	1.00	479	479	490	60.6	110	7.00	14.8	14.9	0.949	0.878
	Max	571	2.00	946	946	964	110	175	13.5	22.5	22.9	2.50	1.48
Adolescent	N	8	8	8	8	7	7	7	8	8	7	8	8
	Mean	370	NC	630	630	622	58.7	107	9.54	15.7	16.7	0.941	0.908
	SD	187	NC	324	324	353	19.3	33.0	4.48	4.87	4.82	0.427	0.233
	CV%	50.5	NC	51.3	51.3	56.8	32.9	30.8	47.0	31.0	28.9	45.3	25.7
	Geometric Mean	329	NC	567	567	552	56.3	103	8.73	15.1	16.1	0.864	0.884
	Geometric CV%	57.5	NC	50.8	50.8	54.1	31.2	30.1	46.1	32.5	31.2	46.0	25.0
	Min	143	0.50	333	333	335	36.8	66.0	5.22	9.25	9.31	0.486	0.643
	Median	342	1.00	479	479	440	57.6	99.7	8.52	15.1	15.7	0.874	0.784
	Max	697	1.50	1190	1190	1220	97.2	171	16.6	24.3	24.6	1.63	1.30

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Table 5-5. Summary of Key Pharmacokinetic Parameters for Plasma 6β-OH-21-desDFZ by Study Population on Day 8

Study Population	Statistic	Cmax (ng/mL)	Tmax (h)	AUClast (h*ng/mL)	AUC0-8 (h*ng/mL)	AUCtau (h*ng/mL)	Cmax/Dose (ng/mL)/mg	AUClast/Dose (h*ng/mL)/mg	AUCtau/Dose (h*ng/mL)/mg	AR Cmax	AR AUC
Children	N	15	15	15	15	15	15	15	15	15	15
	Mean	125	NC	338	338	358	4.29	11.5	12.1	1.28	1.10
	SD	56.7	NC	148	147	166	1.16	2.39	2.54	0.663	0.299
	CV%	45.3	NC	43.6	43.5	46.3	27.1	20.7	21.0	51.8	27.0
	Geometric Mean	113	NC	309	309	324	4.15	11.3	11.9	1.15	1.07
	Geometric CV%	49.9	NC	47.3	47.3	49.3	26.8	21.3	21.4	48.3	28.1
	Min	49.6	1.00	147	147	154	2.70	7.74	8.04	0.615	0.696
	Median	110	1.50	301	301	320	4.13	12.1	12.6	1.13	1.13
	Max	259	2.00	610	610	698	6.50	16.1	16.6	2.99	1.58
Adolescent	N	8	8	8	8	7	8	8	7	8	8
	Mean	159	NC	413	413	417	4.01	10.6	11.6	1.25	1.10
	SD	59.3	NC	149	149	168	0.755	2.26	2.04	0.458	0.223
	CV%	37.4	NC	36.1	36.1	40.3	18.8	21.3	17.6	36.5	20.3
	Geometric Mean	149	NC	392	392	393	3.95	10.4	11.4	1.19	1.08
	Geometric CV%	40.2	NC	34.9	34.9	36.5	18.6	21.7	17.2	36.1	19.0
	Min	81.9	1.00	228	228	239	3.11	7.24	9.54	0.701	0.918
	Median	148	1.00	361	361	364	3.84	9.95	10.7	1.16	1.01
	Max	242	1.50	718	718	769	5.27	14.5	15.0	2.11	1.51

(page 35 of Pharmacokinetics Report from Study Report MP-104-CL-005)

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/s/

DAVID B HAWVER
01/30/2017

LOIS M FREED
01/30/2017

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Numbers: 208684 and 208685

Applicant: Marathon Pharmaceuticals, LLC

Stamp Date: June 9, 2016

Drug Name: Deflazacort

NDA Type: Original NDA

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?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required and requested IND studies (in accord with 505 (b)(1) and (b)(2) 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)?		X	A carcinogenicity waiver was requested; studies in two species, if needed, may be conducted as PMRs, per prior agreement. Reproductive and developmental studies were not conducted; however, male reproductive organs were evaluated in the chronic toxicity and juvenile animal toxicity studies; since DMD occurs almost exclusively in males, this is sufficient. Based on the extensive human experience with deflazacort, safety pharmacology studies were not required.
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 submitted a rationale to justify the alternative route?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Proposed nonclinical labeling sections will be revised as needed during the review process.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			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.

n/a

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

There are no nonclinical review issues at this time.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DAVID B HAWVER
07/08/2016

LOIS M FREED
07/08/2016