CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210867Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 210867
Agency receipt date: October 13, 2017
Drug: Moxidectin
Sponsor: Medicines Development for Global Health

Indication: Treatment of onchocerciasis

Reviewing Division: Division of Antiviral Products

The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of ibalizumab for the indication listed above.

Moxidectin is a semisynthetic derivative of a fermentation product of *Streptomyces cyanogriseus*. It is a broad spectrum endectocide that inhibits the development of embryos and sperm in adult filarial worms. The drug is a first-in-class new molecular entity and was granted both orphan drug status and breakthrough therapy designation. It is proposed to be administered as a single oral dose of 8 mg. The plasma half-life in patients (approximately 24 days) is longer than in rats (18-30 hours) and dogs (8-20 days).

The nonclinical program primarily consists of repeat-dose toxicity studies in rats (up to 3 months) and dogs (up to one year). The only general toxicities identified were transient CNS-related clinical signs and anorexia.

Moxidectin was negative in a battery of genotoxicity studies. A carcinogenicity assessment in rats and mice was conducted. While initial results appear to be negative, a comprehensive review requires submission of an electronic dataset for statistical evaluation. (b) (4)

Moxidectin was not associated with fertility or embryo-fetal developmental effects. However, a pre/postnatal development study identified reduced survival and body weight of first generation offspring during the lactation period at a dose slightly above the recommended clinical dose and reduced number of live fetuses at birth at a dose approximating 13 times the human dose. Since the study did not evaluate physical development and neurological function, a new pre/postnatal development study will be conducted as a post-marketing requirement.

Conclusion:
I agree with the Division pharmacology/toxicology conclusion that moxidectin can be approved from the nonclinical perspective. I have reviewed the proposed wording for the nonclinical sections of the product label and agree with the Division recommendations.

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/s/

TIMOTHY J MCGOVERN
06/08/2018

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	210867
Supporting document/s:	SD #001
Applicant's letter date:	10/13/2017
CDER stamp date:	10/13/2017
Product:	Moxidectin
Indication:	Treatment of Onchocerciasis (River Blindness)
Applicant:	Medicines Development for Global Health (MDGH)
Review Division:	Division of Anti-infective Products
Reviewer:	James S. Wild, Ph.D.
Supervisor/Team Leader:	Terry Miller, Ph.D.
Division Director:	Sumathi Nambiar, M.D., M.P.H.
Project Manager:	Kristine Parks, Ph.D.

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Onchocerciasis is a parasitic disease caused by the helminth *Onchocerca volvulus* which is transmitted to humans by the bite of the black fly of species *Simulium*. Infected black flies deposit *O. volvulus* larvae (microfilariae) with each bite, some of which develop into mature adult worms (macrofilariae). Adult female macrofilariae live for an average of 11 years (up to 18 years) in the human body, and produce millions of microfilariae that migrate through the skin, eyes and lymph nodes. A chronic host response to microfilariae causes the clinical manifestations of onchocerciasis, including severe dermatitis, depigmentation and atrophy of the skin, lymphadenitis, and most significantly, visual impairment leading to blindness. The current treatment for onchocerciasis is ivermectin in tablet form which is administered in a single annual dose of 150 to 200 mcg/kg body weight. Ivermectin does not kill the adult female macrofilariae, but suppresses the production of microfilariae larva for a few months following treatment.

Moxidectin is a semisynthetic derivative of a fermentation product of *Streptomyces cyanogriseus*. It is a macrocyclic lactone of the milbemycin class. Moxidectin is a broad-spectrum endectocide and its pharmacodynamics can be primarily described by activity at the glutamate-gated chloride channels (GluCls) present in nematodes and arthropods. GluCLs are present in the wall of the uterus and reproductive apparatus of female and male macrofilariae respectively, and moxidectin inhibits the development of embryos and sperm in adult filarial worms. Like ivermectin, moxidectin does not kill macrofilariae. The main action of moxidectin is to block reproduction of macrofilariae, leading to fewer microfilariae. Moxidectin is also active at the GABA-A receptor complex which may lead to somatic muscle paralysis in macrofilariae and microfilariae. The purported advantages of moxidectin compared to ivermectin is that moxidectin is more potent, minimally metabolized *in vivo*, more lipophilic than ivermectin, and has much longer plasma $t_{1/2}$ (20 to 40 days) compared to ivermectin (18 hours).

1.2 Brief Discussion of Nonclinical Findings

Moxidectin will be administered clinically in a single-oral dose of 8 mg (0.133 mg/kg for an average 60 kg human) suggesting toxicities that occurred with repeated dosing of much higher doses in test animals will not be a concern in patients. However, moxidectin has a very long plasma $t_{1/2}$ in humans, approximately 24 and 33 days in patients and normal subjects respectively, which is longer than in rats (18-30 hours) and dogs (8-20 days). Human toxicity is a possibility in cases where moxidectin is unintentionally overdosed or perhaps in a subpopulation of humans with increased sensitivity to moxidectin.

The primary moxidectin-related toxicity in test animals and the toxicity with the most potential for clinical relevance is dose-dependent CNS toxicity. Transient CNS toxicity occurred with single and repeated doses in mice, rats, and dogs where CNS-related clinical signs included piloerection, reduced arousal, tremors, abnormal gait, irregular or

slowed breathing, and impaired righting reflex in rodents and lacrimation, languid appearance, tremors, salivation and slight ataxia in dogs. In single- and repeated-dose studies, CNS-related clinical signs were observed at moxidectin threshold doses of ≥ 17 mg/kg in mice, ≥ 12.5 mg/kg in rats, and ≥ 1.5 mg/kg in dogs which are on the order of 6-10 fold higher than the 8 mg (0.133 mg/kg) dose in humans based on body surface area comparison. At doses below the threshold doses in animals, CNS-related clinical signs were not observed suggesting relative safety at the clinical dose. Also, in test animals, the effects were transient and not accompanied by correlating histopathology even with repeated moxidectin dosing and repeated episodes of CNS toxicity suggesting permanent structural or functional sequelae are not expected.

Moxidectin was assessed for toxicity in long-term studies with durations of 1 month (mice, rats, and dogs), 3 months (rats and dogs), and 1-year (dogs). In these studies, other than CNS-related clinical signs, moxidectin was associated with little toxicity. In all the test species at repeated moxidectin doses slightly lower than those associated with clinical signs, decreased food consumption, body-weight gain, and body weights were observed. However, animals resumed normal eating patterns and gained weight upon dosing cessation. Based on the results in nonclinical toxicology studies, the only general toxicities projected for doses of moxidectin in excess of the recommended clinical dose are transient CNS-related clinical signs, anorexia, and weight loss.

In a full battery of *in vitro* and *in vivo* genotoxicity studies, moxidectin was negative for mutagenicity and clastogenesis. Moxidectin was also tested in 2-year carcinogenicity studies in mice and rats, and a preliminary review of these studies suggests moxidectin did not stimulate tumor formation. A comprehensive review of the carcinogenicity studies awaits electronic submission of accurate tumor-tabulation tables necessary for a new statistical analysis performed within the FDA. (b) (4)

In a male and female fertility study in rats, moxidectin did not impair any fertility or pregnancy indices at doses approximately equal to the recommended human dose based on body surface area comparison. In embryo-fetal studies in rats and rabbits, moxidectin was associated with a moderate level of reduced food consumption and body weight gain at doses equivalent to approximately 12 times for both species the recommended human dose based on body surface area comparisons. In rats, one skeletal variation, wavy ribs was significantly increased for fetal and litter incidence and one malformation, cleft palate was significantly increased for fetal incidence but not litter incidence at a dose equivalent to approximately 15 times the recommended dose in humans based on body surface area comparison. In the rabbit embryo-fetal study, no evidence of impaired embryo-fetal development was observed at doses up to 24 times the recommended human dose based on body surface area comparison.

Range-finding and definitive pre-postnatal studies were conducted approximately 30 years ago with moxidectin. In these studies, parents and F_1 and F_2 offspring received dietary moxidectin. The results of these studies indicate that moxidectin at a dose approximately equivalent to the recommended clinical dose based on body surface area comparison did not inhibit survival, fertility or reduce body weights in the parental generation or in first and second generation offspring. However, at slightly higher moxidectin doses, approximately 1.3 times the recommended human dose based on body surface area comparison, the survival and body weights of first-generation offspring were significantly decreased during the lactation period, and the number of live fetuses at birth was significantly decreased with a moxidectin dose approximately equivalent to 13 times the recommended human dose based on body surface area comparison. The results of the current pre-postnatal studies are considered sufficient to support approval of NDA 210867. However, in these studies, physical development and neurological function were not assessed in first-generation offspring as recommended in the ICH S5a Guidance. Consequently a new pre-postnatal study which will include the assessments missing in the previously conducted studies will be conducted as a post-marketing requirement (PMR). Until the final study report for the new study has been received and evaluated, Section 8.1 of the product label for moxidectin will indicate: "…offspring were assessed for survival, body weights, and fertility, and developmental milestones were not assessed," in reference to pre-postnatal study results.

Moxidectin is considered acceptable for approval from a Pharmacology/Toxicology perspective. (b) (4)

1.3 Recommendations

1.3.1 Approvability

NDA 210867 is approvable from a Pharmacology/Toxicology perspective.

1.3.2 Additional Non Clinical Recommendations

Two deficiencies that are not considered to affect the approvability of NDA 210867 will be addressed post-marketing, one as a post-marketing requirement (PMR), (b) (4)

The first deficiency, the need to conduct a new pre-postnatal study, will be addressed as a PMR. The range-finding and definitive pre-postnatal studies that were submitted with the NDA application were conducted approximately 30 years ago and do not include currently recommended measurements in F_1 offspring, including assessments of physical development and neurological function. The new pre-postnatal study, conducted as a PMR should follow the recommendations included in the ICH Guidance S5a:" Detection of Toxicity to Reproduction for Medicinal Products," and include the assessments missing in the previously conducted studies. Until the final study report for the new study has been received and evaluated, Section 8.1 Pregnancy of the product label for moxidectin will indicate: "…offspring were assessed for survival, body weights,

and fertility, and developmental milestones were not assessed," in reference to the prior limited pre-postnatal study results.

1.3.3 Labeling

The labeling language proposed by the reviewer is shown below and the original draft labeling language from the Applicant that is recommended for deletion is crossed out.

8 USE IN SPECIFIC POPULATIONS
8.1 Pregnancy

Risk Summary

Limited available data on the use of Moxidectin Tablets in pregnant women are insufficient to establish whether there is a moxidectin-associated risk for major birth defects and miscarriage (b) (4) Moxidectin administered orally to pregnant rats during the period of organogenesis (Gestation Days (GD) 6 to 15), was not associated with significant embryo-fetal developmental effects at a dose approximately 15 times the recommended human dose based on body surface area. When moxidectin was dosed orally to pregnant rabbits during the period of organogenesis (GD 7-19), no embryo-fetal developmental effects were observed at maternally toxic oral doses of moxidectin up to 24 times the recommended human dose based on body surface area *[see Data]*.

Daily parental oral administration of dietary moxidectin to rats prior to mating, and through mating, gestation, and lactation was associated with decreased survival and body weights for first-generation offspring without maternal toxicity at moxidectin doses less than 2-times the recommended human dose based on body surface area comparison. Daily dietary moxidectin did not produce maternal toxicity or adverse effects for first- and second-generation offspring at doses approximately equivalent to the recommended human dose based on body surface area comparison. Offspring were assessed for survival, body weights, and fertility. Developmental milestones were not assessed in this study.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss or other adverse outcomes. In the U.S. general population, the estimated background risk

of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

(b) (4)

Data

Animal Data

In a rat embryo-fetal development study, daily oral administration of moxidectin at 12 mg/kg/day (approximately 15 times the recommended human dose of 8 mg based on body surface area comparison) during Gestation Days (GDs) 6 to 15 significantly increased the fetal incidence, but not the litter incidence of cleft palate and the fetal and litter incidence of a skeletal variation, wavy ribs, at a maternally toxic dose. Mean maternal food consumption, body weights, and body weight gain were significantly decreased at moxidectin doses of 10 and 12 mg/kg/day compared to control values. The no observed adverse effect level (NOAEL) value for maternal and fetal toxicity was considered to be 5 and 10 mg/kg/day respectively (approximately 6 and 12 times, respectively, the recommended human dose based on body surface area comparison). In the rabbit, daily oral administration of moxidectin at ≥ 5 mg/kg/day from GD7 to GD19 was not associated with fetal weight loss or malformations but resulted in significantly decreased maternal food consumption and body weight gains. The NOAEL value for maternal and fetal toxicity in the rabbit was 1 mg/kg/day and 10 mg/kg/day respectively (approximately 2 times and 24 times, respectively, the recommended human dose based on body surface area comparison).

In a pre-postnatal study in rats, parental oral administration of dietary moxidectin prior to mating, through mating, gestation, and lactation did not produce adverse effects in first-generation or second-generation offspring at a maternal NOAEL dose of 0.824

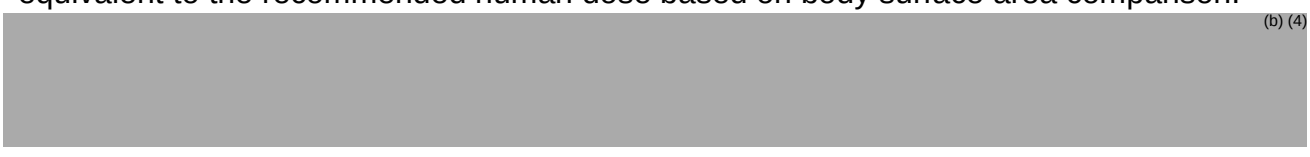
mg/kg/day (approximately equivalent to the recommended human dose based on body surface area comparison). However, at moxidectin doses ≥ 1.1 mg/kg/day (approximately equivalent to 1.3 times the recommended human dose based on body surface area comparison), the survival and body weights of first-generation offspring were significantly decreased during the lactation period, and the number of live fetuses at birth was significantly decreased with a maternal moxidectin dose of 11 mg/kg/day (approximately equivalent to 13 times the recommended human dose based on body surface area comparison). In this study, offspring were assessed for survival, body weights, and fertility, and developmental milestones were not assessed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Moxidectin was shown to be negative for genotoxicity in a battery of *in vitro* assays including a bacterial mutagenicity assay, mouse lymphoma cell mutagenicity assay, unscheduled DNA synthesis assay, and a chromosome aberration assay, as well as *in vivo* in a micronucleus assay in mice and a chromosome aberration assay in rats.

In fertility evaluations, male and female mating and fertility indices were not inhibited by oral-dietary moxidectin doses of approximately 0.86 mg/kg/day which is approximately equivalent to the recommended human dose based on body surface area comparison.



13.2 Animal Toxicology

Moxidectin was associated with transient CNS-related clinical signs. In rats, a single dose of 20 mg/kg (equivalent to approximately 24 times the recommended human dose based on body surface area comparison) moxidectin was associated with piloerection, reduced arousal and body tone, abnormal gait, slowed breathing, and impaired righting reflex. In dogs, repeated doses of 1.6 mg/kg/day moxidectin (equivalent to approximately 7 times the recommended human dose based on body surface area comparison) was associated with lacrimation, languid appearance, tremors, slight salivation, and slight ataxia.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 113507-06-5

Generic Name
Moxidectin

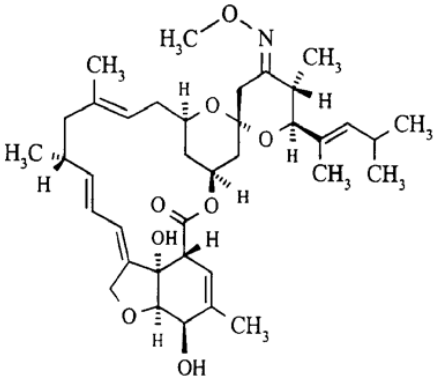
Code Names:
MX, WAY-204148, CL-301423, AC-301423, UK-84,709, PF-05208746, MOX, Mox

Chemical Name
(2a*E*,4*E*,5'*R*,6*R*,6'*S*,8*E*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6'-[(*E*)-1,3-dimethyl-1-butenyl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-

tetramethylspiro[11,15-methano-2*H,*13*H,*17*H*-furo[4,3,2-*pq*][2,6]benzodioxacyclooctadecin-13,2'-[2*H*]pyran]-4',17(3'*H*)-dione 4'-(*E*)-(*O*-methyloxime)

Molecular Formula/Molecular Weight: $C_{37}H_{53}NO_8$/639.82 Daltons

Structure or Biochemical Description



Pharmacologic Class

Antihelminthic

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 126876 for moxidectin; DMF (b) (4) for the moxidectin drug substance.

2.3 Drug Formulation

The drug product formulation for the 2 mg moxidectin tablets is shown in Table 1.

Table 1: Qualitative and Quantitative Composition of the Drug Product.
(Applicant's Table from Section 3.2.P.1 of the Electronic Submission)

Component	Quality Reference	Function	Quantity/Unit Dose (mg/tablet)
(b) (4) Moxidectin	USP	Active Ingredient	2.0
Microcrystalline Cellulose	NF	(b) (4)	
Anhydrous Lactose	NF		
Croscarmellose Sodium	NF		
Sodium Lauryl Sulfate	NF		
Colloidal Silicon Dioxide	NF		
Magnesium Stearate[a]	NF		
(b) (4)			
Total Tablet Weight	--	--	**100.0**

[a] (b) (4)

USP: United States Pharmacopeia; NF: National Formulary; NA: Not Applicable

2.4 Comments on Novel Excipients

There are no novel excipients. All of the commonly used excipients included in the moxidectin tablet are all qualified by their use in higher amounts in previously approved products (Table 2). All of the excipients have been used in amounts more than four times the amounts in each 2 mg tablet and therefore more than will be ingested with each daily 8 mg dose of moxidectin.

Table 2: The Moxidectin Product Excipients and Comparison to Amounts Used in Previously Approved Products.

Component	Unit Dose (mg/tablet)	Unit Dose in Previously Approved Oral Products
(b) (4) Moxidectin		
Microcrystalline Cellulose	(b) (4)	412.7 mg/oral tablet
Anhydrous Lactose		735.2 mg/oral tablet
Croscarmellose Sodium		180 mg/oral tablet
Sodium Lauryl Sulfate		51.69 mg/oral tablet
Colloidal Silicon Dioxide		24 mg/oral tablet
Magnesium Stearate		150 mg/oral tablet

2.5 Comments on Impurities/Degradants of Concern

Drug Substance - Organic Impurities

The moxidectin drug substance-related impurities and their specifications are shown in Table 3. (b) (4) In addition, each impurity has been qualified by its use in higher amounts than the specification in nonclinical studies with the exception of the product impurity, (b) (4) which was qualified by its use in higher amounts in healthy volunteers in a Phase 1 clinical study (Table 4).

Table 3: Specifications for the Moxidectin Drug Substance (Applicant's Table from Section 3.2.S.4.1 of the Electronic Submission)

Attribute	Test Method	Acceptance Criteria
Appearance	Visual Inspection	White to pale yellow solid
[redacted]	[redacted]	[redacted] (b) (4)

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Table 4 continued

Drug Substance - Residual Solvents

The specifications for all of the residual solvents shown in Table 3 are consistent with the limits recommended in the ICH Q3(R6) Guidance.

Drug Product

All of the impurities in the moxidectin drug product are degradants except one impurity, (b) (4) which is identified as a process-related impurity. In a 3/23/2018 Quality Response to Information Request, the Applicant agreed to (b) (4) the drug product specifications to (b) (4)% from the original (b) (4)% criteria. The final moxidectin drug product impurities/degradants and their specifications are shown in Table 5. For the recommended clinical dose of 8 mg moxidectin, at the current specification of (b) (4)%, the total daily intake of each of the drug product impurities/degradants is (b) (4) which falls within the recommended limit in the ICH Q3B(R2) Guidance.

Table 5: Moxidectin Drug Product Impurities. (Applicant's Table from Section 3.2.P.5.1 of the Electronic Submission)

Attribute		Test Type (Method Number)	Acceptance Criteria
Appearance		Visual Inspection (0100.02)	White to pale yellow, oval shaped tablet debossed with 'AKKA' on one side
Assay		HPLC (0092.00)	(b) (4) % of label claim
Identity by HPLC (Retention Time)[a]		HPLC (0092.00)	Retention time similar to the retention time of the standard
Identity by HPLC (DAD)[a]		HPLC (0092.00)	Consistent with reference standard
(b) (4)			
Degradants			(b) (4)
Uniformity of Dosage Units[a]			(b) (4)
Dissolution			
Microbial Limits	Total aerobic microbial count		
	Total combined yeasts and molds count		
	E. coli		

[a] Performed at release only
DAD: Diode array detection; HPLC: High-performance liquid chromatography; cfu: colony-forming unit; NMT: Not more than

Genotoxicity Qualification for the Drug Substance and Drug Product Impurities/Degradants.

The Sponsor conducted an *in silico* computational assessment of the potential bacterial genotoxicity of the moxidectin impurity, (b) (4) using CASE Ultra and DEREK/SARAH computational expert systems (Study No.: BM-CUDXSX-1273) as recommended by the ICH M7 Guidance. The remaining 11 moxidectin drug substance impurities (Impurities (b) (4) and the four drug product degradants/impurities (b) (4) were similarly assessed using Derek Nexus 6.0.1 (DX), Leadscope Model Applier 2.2.2-3 (LMA), and CASE Ultra 1.6.2.3 (CU) computational expert systems by the Computational Toxicology Consultation Service at the FDA (included in the Appendix of this document).

Based on the results of the *in silico* analyses, all but one of the drug substance impurities and drug product degradants/impurities was predicted to be negative for

bacterial mutagenicity. The single drug substance impurity, Impurity (b) (4) that was predicted to be positive for bacterial mutagenicity in the *in silico* assessments has a specification of (b) (4) % or (b) (4) in each 8 mg dose of moxidectin. According to Table 2 in the ICH M7 Guidance, the acceptable daily intake for an individual impurity that is administered for ≤ 1 month is 120 mcg/day. The maximum (b) (4) that will be administered in one dose of 8 mg moxidectin is considered acceptable because it is less than the 120 mcg/day limit.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical Population: Moxidectin is recommended for the treatment of onchocerciasis in adolescent patients 12 years and older and in adults.

Dosing Regimen: In adolescent and adult patients, the recommended dose is a single dose of 8 mg (four 2 mg tablets) taken orally with or without food.

Plasma Exposure Associated with Clinical Dosing: In onchocerciasis patients (n = 31), the mean plasma C_{max} and AUC values (± SD) were 63.1 ± 20.0 ng/ml and 2738 ± 1606 ng•hr/ml respectively following a single 8 mg oral dose of moxidectin. Following a single 8 mg oral dose, the mean terminal half-life ($t_{1/2}$) for moxidectin was 23.3 days in patients and 32.7 days in healthy volunteers.

2.7 Regulatory Background

Moxidectin was first approved for veterinary use in several products in the late 1980s. Subsequently, moxidectin was submitted to the FDA for human use in IND 126876 in 11/2016 followed by the NDA application for moxidectin (NDA 210867) submitted in 10/2017.

3 Studies Submitted

3.1 Studies Reviewed

Secondary Pharmacology

1. Moxidectin: Assessment of Binding to Human Biological Receptors Using Novascreen (Study Report No.: RPT-6443).

Safety Pharmacology

1. Safety Pharmacology – Neurofunctional and Pulmonary Assessment of PF-05208746 (Moxidectin) in Male Rats (Study No.: 10SN030).
2. Moxidectin: Effects on Cloned hERG Channels Expressed in Mammalian Cells (Study Report No.: RPT-73657).
3. Moxidectin: Single Oral (Capsule) Dose Cardiovascular Safety Pharmacology Study in Dogs (Study Report No.: RPT-74070).

Pharmacokinetics
Absorption

1. Pharmacokinetic Study of Moxidectin Following 14-Days Diet Administration in CD-1 Mice (Study No.: PH-DMPK-MDG-17-001).
2. Single Oral Dose Exploratory Study of PF-05208746 in Male Sprague Dawley Rats (Study No.: 10GR082).
3. Moxidectin Blood Profile at or Near Steady State in Sprague Dawley Rats Receiving a Diet Containing 100 ppm Moxidectin for 28 Consecutive Days (Study Report No.: RPT-62209).
4. Single Dose Oral (Capsule) Pharmacokinetic Study with a 12-Week Observation Period in Juvenile and Adult Dogs (Study Report No.: RPT-73592).
5. Moxidectin: Blood Profile in Dogs Receiving a Single Exposure to a Canine Diet Containing 45 ppm Moxidectin (Study Report No.: RPT-62210)
6. A Pharmacokinetic Study of Moxidectin Following Oral Administration in Pregnant Rabbits (Study No.: 70704-17-404).

Distribution and Excretion

1. Macrocyclic Lactones: Distribution in Plasma Lipoproteins of Several Animal Species including Humans. Bassissi MF, Alvinerie M, and Lespine A: Com Bioch Physiol, 138:437-444 (2004).
2. Moxidectin (CL 301423): Absorption, Distribution, Excretion, and Metabolism of Carbon-14 Labeled CL 301423 in the Rat – Report Amendment 01. (Study Report No.: RPT-77457).

Metabolism

1. *In Vitro* Metabolism of Moxidectin in Human Liver Microsomes, Cryopreserved Hepatocytes and Recombinant Human Cytochrome P450 and UDP-Glucuronosyltransferase Enzymes (Study No.: MDG-R5763).
2. Moxidectin: Metabolic Stability and Metabolism in Rat and Human Liver Microsomes (Study Report No.: RPT-51895).
3. Moxidectin: Potential Induction of Cytochrome P450 Genes and P-Glycoprotein (MDR1) by Moxidectin in Human Hepatocytes (Study Report No.: RPT-71215).
4. Evaluation of Time-Dependent Inhibition of CYP2D6 and CYP3A4/5 by Moxidectin (Study No. ADME-MOX-160510-TDI).
5. Moxidectin: IC_{50} Determination of the Inhibition of Cytochrome P450 Enzymes in Human Liver Microsomes (Study Report No.: RPT-51894).
6. Moxidectin: Evaluating the Potential for Induction of CYP3A45 by Moxidectin Using a CYP3A4 Reporter Gene Assay (Study Report No.: RPT-70409).

General Toxicology

Single-Dose Toxicology (The results of these studies were summarized, but not reviewed)

1. Moxidectin: Oral LD50 Study in the Albino Mouse with AC-301423 (Study Report No.: RPT-77273).

2. Moxidectin: Oral LD50 Study in the Female Albino Mouse with AC-301423 (Study Report No.: RPT-77293).
3. Moxidectin: Oral LD50 Study in the Female Albino Mouse with AC-301423 Technical (Study Report No.: RPT-77294).
4. Moxidectin: Single Dose Oral Toxicity Study in Albino (CD-1) Mice (Study Report No.: RPT-80141).
5. Moxidectin: Intraperitoneal LD50 Study in the Albino Mouse with AC-301423 (Study Report No.: RPT-77314).
6. Moxidectin: Subcutaneous LD50 Study in the Albino Mouse with AC-301423 (Study Report No.: RPT-77292).
7. Moxidectin: Oral LD50 Study in the Female Albino Rat with AC-301423 (Study Report No.: RPT-77275).
8. Single Oral Dose Exploratory Toxicity Study of PF-05208746 in Male Sprague-Dawley Rats (Study No.: 10GR082).
9. Moxidectin: Intraperitoneal LD50 Study in the Albino Rat with AC-301423 (Study Report No.: RPT-77276).
10. Moxidectin: Subcutaneous LD50 Study in the Albino Rat with AC-301423 (Study Report No.: RPT-77277).
11. Moxidectin: Dermal LD50 Study in Albino Rabbits with AC-301423 (Study Report No.: RPT-77274).
12. Single Dose Oral (Capsule) Pharmacokinetic Study with a 12-Week Observation Period in Juvenile and Adult Dogs (Study Report No.: RPT-73592).

Repeated-Dose Toxicology

1. AC-301423: A 28-Day Mouse Feeding Study. (b) (4) Report Number AX89-3 (Study Report No.: RPT-77313).
2. AC-301423: A 28-Day Rat Feeding Study. (b) (4). Report Number AX88-1 (Study Report No.: RPT-77295).
3. AC-301423: A 13-Week Rat Feeding Study. (b) (4) Report Number AX89-1 (Study Report No.: RPT-77312).
4. Moxidectin: 28-Day Range Finding Study in Purebred Beagle Dogs with AC-301423 (Study Report No.: RPT-77334).
5. Moxidectin: 91-Day Dietary Toxicity Study in Purebred Beagle Dogs with AC-301423 (Study Report No.: RPT-77335).
6. One-year Dietary Toxicity Study in Purebred Beagle Dogs with AC-301423 (Study Report No.: RPT-77336).

Genetic Toxicology

1. Moxidectin: Evaluation of CL-301423 in a Bacterial/Microsome Mutagenicity (Study Report No. RPT-77350).

2. Moxidectin: Evaluation of CL-301423 in the Mammalian Cell CHO/HGPRT Mutagenicity Test (Study Report No.: RPT-77351).
3. Moxidectin: Evaluation of AC-301423 in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with Colony Size Evaluation in the Presence and Absence of Induced Rat Liver S-9 with a Confirmatory Study (Study Report No.: RPT-77352).
4. Moxidectin: Unscheduled DNA Synthesis in Primary Rat Hepatocytes with AC-301,423 (Study Report No.: RPT-77441).
5. Moxidectin: Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation with AC 301423 (Study Report No.: RPT-77740).
6. Moxidectin: In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells with AC-301423 (Study Report No.: RPT-77442).
7. Moxidectin: Evaluation of Moxidectin in the In Vivo Chromosome Aberration Assay in Rat Bone Marrow Cells (Study Report No.: RPT-77443).

Carcinogenicity

1. Chronic Dietary toxicity and Oncogenicity Study with AC 30,423 in Mice (Study Report No: RPT-77458).
2. Chronic Dietary toxicity and Oncogenicity Study with AC 30,423 in Rats (Study Report No: RPT-77459).

Reproductive and Developmental Toxicity

1. Moxidectin: An Oral Developmental Toxicity Study with AC 301423 in Rats (Study Report No: RPT-77460).
2. Moxidectin: A Developmental Toxicity (Embryo-fetal Toxicity and Teratogenicity) Definitive Study with AC 301,423 in Rabbits (Study Report No.: RPT-77516).
3. Moxidectin: A Pilot One-generation (Two Litters) Reproduction Study with AC 301,423 to Rats (Study Report No.: RPT-77517).
4. Moxidectin: A Three Generation (Two Litters) Reperoductive Study with AC 301423 to Rats (Study Report No.: RPT-77518).

Special Toxicology Studies

1. Computational Assessment and Evaluation of Potential Genotoxicity of Moxidectin Impurity (b) (4) Impurity Using CASE Ultra and DEREK/SARAH. (Study No.: BM-CUDXSX-1273).

3.2 Studies Not Reviewed

1. Moxidectin: LC Method for Quantification of Moxidectin in Dog Plasma. (Study Report No.: RPT-73235).
2. Validation of an LC Method for Determination of Moxidectin in Rat Plasma. (Study Report No.: RPT-80785).

3. Moxidectin: Twenty-Four Hour Moxidectin Blood Profile at or Near Steady-State in Dogs Receiving a Diet Containing 45 ppm Moxidectin for 28 Consecutive Days (Study Report No.: RPT-62208).

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies were reviewed by the Clinical Microbiology Reviewer, Dr. Shukal Bala, Ph.D.

4.2 Secondary Pharmacology

1. **Moxidectin: Assessment of Binding to Human Biological Receptors Using Novascreen.** (Study Report No.: RPT-64443)

Methods
Moxidectin at a concentration of 10 ng/ml was tested for receptor binding to a panel of 64 biological receptors using appropriate radioligands for each receptor. Results were expressed as the percent inhibition of specific radioligands to each receptor. Moxidectin was tested both as neat compound and as a glyceryltristerate microsphere formulation. The test panel included receptors for neurotransmitters, steroids, ion channels, second messengers, prostaglandins, growth factors, hormones, brain and gut peptides, and enzymes.

Results
According to the data interpretation guidelines supplied by NOVASCREEN in the study report, the standard baseline for receptor binding ranges from -20% to +20% with compounds producing results in this range considered inactive for inhibition of receptor binding.

In the neat compound preparation, moxidectin generally exhibited little or no inhibition of any of the tested receptors. Moxidectin exhibited inhibition of receptor binding in excess of 20% to: vasopressin 1 (26.97% inhibition), H2 histamine receptor (24.46% inhibition), and acetylcholinesterase (22.50% inhibition). According to the data interpretation guidelines, compounds which demonstrate inhibition in the range of 20 to 49% show marginal activity at the receptor site.

In the microsphere formulation, moxidectin also generally exhibited minimal inhibition of receptor binding with the exception of binding to two ion channels, the ATP-sensitive potassium channel and site 2 of the sodium channel, both of which were inhibited by more than 97%. In addition to these receptors, the moxidectin microsphere formulation produced receptor binding inhibition in excess of 20% for the following targets: acetylcholinesterase (25.68% inhibition), LTB4 Leukotriene receptor (22.23% inhibition),

monoamine oxidase B (≈ 21%), and serotonin (20.62% inhibition). According to the interpretation guidelines, inhibition of receptor binding of ≥ 50% qualifies a compound as an active inhibitor of a particular receptor. Based on this interpretation, moxidectin is considered to actively inhibit the receptor binding of the two ion channels noted above.

4.3 Safety Pharmacology

1. **Study Title: Safety Pharmacology – Neurofunctional and Pulmonary Assessment of PF-05208746 (Moxidectin) in Male Rats.** (Study No.: 10SN030)

Methods

This GLP-compliant study included a quality assurance statement and was performed by Pfizer Global Research & Development in Kent UK in 2010. The purpose of this study was to determine the effects of PF-05208746 (moxidectin; Lot No.: MA07132-MC 186808002) administered in single oral doses (dose volume of 1 ml/kg) via oral gavage to male Sprague-Dawley rats (age of 55-70 days and weight of 247 to 324 g at dose initiation) in neurofunctional and pulmonary assessments. Animals were also assessed for clinical signs.

Four groups of 6 rats were orally administered single doses of vehicle (corn oil) or 1, 5, or 20 mg/kg moxidectin. Approximately 4 hours after dosing rats were assessed in a functional observational battery (FOB) and for body temperature. FOB measurements are summarized in below. In addition, upon completion of the FOB and body temperature assessments, rats were immediately placed in locomotor activity chambers and tested for both horizontal and vertical movement using an open-field photobeam monitoring system for a total of session length of 30 minutes.

Table 6: List of FOB Measurements for Study No.: 10SN030. (Table from the Study Report)

CNS Activity and Excitability	Autonomic Nervous System Function	Neuromuscular Function	Sensorimotor Function
Involuntary Motor Movements	Palpebral Closure	Gait	Click Response
Excessive Behaviors	Pupil Response	Extensor Thrust	Air Righting Reflex
Bizarre Behavior	Pupil Diameter	Forelimb Grip Strength	
Respiration	Palpebral Reflex	Gait Score	
Ease of Removal From Home Cage	Eye Observations	Body Tone	
Handling Reactivity	Salivation		
Arousal	Piloerection		
	Loose Stool		

An additional four groups of 6 rats orally administered single doses of vehicle or 1, 5, or 20 mg/kg moxidectin were assessed for pulmonary function (respiratory rate, tidal volume, and minute volume) immediately after dosing for approximately 300 minutes using whole body plethysmography.

A satellite group of 10 rats were orally dosed with vehicle or 1, 5, or 20 mg/kg moxidectin and blood samples were obtained via jugular vein catheters at 1, 2, 4, 8, 24, and 48 hours after dosing.

Results

Body temperature: Body temperature was significantly reduced by a mean value of 1.2°C in high-dose animals compared to vehicle control animals.

FOB measurements: All animals dosed with 20 mg/kg moxidectin had significantly increased ease of removal and significantly reduced handling reactivity, arousal and body tone, and piloerection. Other clinical signs in some or most animals in this group included: abnormal gait, reduced or no response to extensor thrust assessment, slowed breathing, reduced click response, and impaired air righting reflex (Table 7). In the 5 mg/kg group a one or two animals demonstrated similar clinical signs. Mean forelimb grip strength was also reduced by 17% in high-dose animals compared to control values.

Table 7: The Effect of Moxidectin on FOB Measurements in Study No.: 10SN030. (Table from the Study Report)

Assessment	Observation	Treatment			
		Vehicle	1 mg/kg	5 mg/kg	20 mg/kg
Ease of Removal	Easier than normal	0	0	1	5
	Normal	6	6	5	1
Handling Reactivity	Less than normal	0	1	1	6
	Normal	6	5	5	0
Arousal	Less than normal	0	1	0	6
	Normal	6	5	6	0
Piloerection	Not present	6	6	6	0
	Present	0	0	0	6
Body Tone	Decreased	0	2	1	6
	Normal	6	4	5	0
Gait	Normal	6	6	6	1
	Not normal	0	0	0	5
Gait Score	Normal	6	6	6	1
	Slight impairment	0	0	0	1
	Severe impairment	0	0	0	4
Extensor Thrust	No response	0	0	0	1
	Reduced response	0	2	1	4
	Normal response	6	4	5	1
Shaded cells indicate significantly different from vehicle ($p < 0.05$)					

Locomotor Activity: High-dose animals demonstrated a significant reduction in horizontal and vertical activity (Table 8).

Table 8: The Effect of Moxidectin on Locomotor Activity. (Table from the Study Report)

PF-05208746
Locomotor Activity Responses
Summary Statistics

	Treatment											
	Vehicle			1 mg/kg			5 mg/kg			20 mg/kg		
Assessment	n	Geo. Mean	IQR	n	Geo. Mean	IQR	n	Geo. Mean	IQR	n	Geo. Mean	IQR
Horizontal movements (beam breaks)	6	2451	623	6	2183	751	6	2023	308	6	415	437
Vertical movements (beam breaks)	5	758	397	6	595	313	6	590	241	4*	13	23

PF-05208746 was administered at 0, 1, 5, or 20 mg/kg by oral gavage to male rats 4 hours prior to testing in the neurofunctional assessment.
Shaded cells indicate significant differences from vehicle (corn oil) using the appropriate statistical analysis as described in the protocol ($p << 0.05$).
Geo. Mean = Geometric mean; IQR = Interquartile range.
* There were responses of zero for Vertical movements (beam breaks) in 20 mg/kg dose. The zero values have been excluded from the analysis. However, the true mean is the same as that reported.

Pulmonary Function: There was a significant increase in tidal volume at 60 and 240 minutes after dosing in the high dose group. Also respiratory rate was increased in animals receiving 1 mg/kg moxidectin at 100, 160, 180, and 200 minutes after dosing; in 5 mg/kg animals at 100, 160, and 200 minutes after dosing; and in high-dose animals at 60, 100, 120, 140, 160, 180, and 220 minutes after dosing. Minute volume was not affected by any of the moxidectin doses.
Toxicokinetic Measurements: Plasma C_{max} exposures increased in a roughly dose-proportional manner. Plasma $AUC_{(0\text{-inf})}$ values increased in a much greater than dose-proportional manner between the 1 and 5 mg/kg doses and in approximately a dose-proportional manner between the 5 and 20 mg/kg doses. Plasma $t_{1/2}$ values for moxidectin ranged from approximately 16 to 30 hours.

Table 9: Mean Toxicokientic Parameters for Plasma Moxidectin in Male Sprague-Dawley Rats after a Single Oral Administration of Moxidectin. (Table from the Study Report)

Dose (mg/kg/day)	Study Day	Gender	Cmax (ng/mL)			tmax (h)			T1/2 (h)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	1	Male	206.7	87.7	3	1.7	0.6	3	19.6	6.6	3
5	1	Male	1116.4	346.5	2	2.0	0	2	15.8	6.3	2
20	1	Male	3727.6	406.8	3	4.0	0	3	30.5	7.3	3

Table 9 continued

Dose (mg/kg/day)	Study Day	Gender	AUC(0-24) (ng*h/mL)			AUC(0-48) (ng*h/mL)			AUC(0-inf) (ng*h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	1	Male	1574	253	3	1894	298	3	2120	252	3
5	1	Male	14279	4889	2	18344	7166	2	20817	9642	2
20	1	Male	41202	7085	3	60720	9548	3	91995	28825	3

2. **Study Title: Moxidectin: Effects on Cloned hERG Channels Expressed in Mammalian Cells.** (Study Report No.: RPT-73657)

Methods

This GLP-compliant study including a quality assurance statement was conducted by (b) (4) in 2008. The objective of this study was to examine the potential *in vitro* effects of moxidectin (Lot No.: MB2632) on hERG (human ether-a-go-go-related gene) potassium channel currents. Moxidectin in concentrations of 1 mcM (0.6 mcg/ml), 3 mcM (1.9 mcg/ml) and 10 mcM (6.4 mcg/ml) was incubated with human embryonic kidney (HEK293) cells stably transfected with hERG cDNA. Higher concentrations of moxidectin were associated with precipitate and could not be assessed in the test system. The onset and steady-state inhibition of hERG postassium currents due to moxidectin, the positive control agent (60 nM terfenadine) and vehicle (HEPES-buffered physiological saline, HB-PS) were measured using voltage clamp procedures. Each recording ended with a final application of a supramaximal concentration for the reference substance (500 nM E-4031).

Results

Moxidectin produced a dose-dependent inhibition of hERG potassium channels with the greatest inhibition (19.7% inhibition) occurring with the highest concentration of 10 mcM. The IC_{50} concentration could not be determined but was estimated to exceed 10 mcM moxidectin. In contrast, the positive control agent, 60 nM terfenadine, produced an 82.3% inhibition.

Table 10: The Percent Inhibition of hERG Potassium Channels by Moxidectin. (Table from the Study Report)

Moxidectin Concentration (μM)	Mean	SD	SEM	N
0	0.5%	0.4%	0.2%	3
1	4.9%	0.8%	0.5%	3
3*	17.8%	5.0%	2.5%	4
10*	19.7%	3.5%	2.0%	3

*. Data significantly different from the vehicle control ($p \leq 0.05$)

Mean percent of hERG current block by Moxidectin (Mean), standard deviation (SD), standard error of the mean (SEM) and number of observations (N).

3. **Study Title: Moxidectin: Single Oral (Capsule) Dose Cardiovascular Safety Pharmacology Study in Dogs.** (Study No.: RPT-74070)

Methods
This GLP-compliant study which included a quality assurance statement was conducted by Wyeth Research in New York in 2008. The purpose of this study was to evaluate the potential cardiovascular effects of moxidectin administered in a single oral-capsule dose to Beagle dogs (approximately 6 months old, body weight range of 7.5 to 9.6 kg for males and 6.9 to 8.4 kg for females). Beagle dogs (3/sex/group) were administered single-oral doses of empty gelatin capsules or the same capsules containing 1.0 mg/kg moxidectin (Lot No.: MB2632). The dose of moxidectin was limited to 1 mg/kg because in a previous study, single oral doses of 3 mg/kg doses of moxidectin to adult dogs resulted in CNS-related clinical signs (ataxia, tremors, decreased motor activity). At least three weeks before dosing, dogs were surgically implanted with telemetry transmitters in subcutaneous space of the lower left abdomen. Measured parameters included: arterial blood pressure (systolic, diastolic, and mean), heart rate, and electrocardiogram parameters (PR, QRS and QT intervals). Telemetry data was collected for 60-second periods every 5 minutes for 25 hours prior to and 72 hours after dosing.

Results
Heart rate: Moxidectin (1 mg/kg) administration resulted in a decrease in the mean heart rate of 17 beats per minute (-14%) relative to vehicle control values in a 9-hour period extending from 6 to 14 hours after dosing.

Blood pressure: No consistent, significant changes in diastolic or mean blood pressure were observed. Intermittent changes in mean systolic blood pressure were observed, but because the changes were intermittent and inconsistent in direction, the meaning of the changes and their relationship to moxidectin is not clear. At 15 and 35 hours after dosing mean systolic blood pressure increased by an average of 15.6 mm Hg (12.3% relative to vehicle values) and 11.2 mm Hg (8.7% increase relative to control) respectively. In contrast, during a 10-hour period spanning from 56 to 65 hours after dosing, mean systolic blood pressure decreased by an average of 6 mm Hg (-5% relative to control).

ECG Analysis: No significant moxidectin-related changes in PR, QRS, QT and QTc (method of Spence) intervals were observed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical Methods

The study reports for the studies describing the validated analytical methods used to measure moxidectin in feed and animal plasma are not reviewed in this document. Validated methods for the measurement of moxidectin in mouse plasma (Study report

No.:PH-DMPK-MDG-17-001), rat serum (Study Report No.: RPT-62209), rat plasma (Method No.: D-116; Study Report No.: RPT-80785), dog serum (Method M-2710.01) dog plasma (Method No.: D-109, Study Report No.: RPT-73235) and dog food (Method M-2016) were developed and these methods were used in support of the moxidectin pharmacokinetic data.

Absorption

1. **Study Title: Pharmacokinetic Study of Moxidectin Following 14-Days Diet Administration in CD-1 Mice.** (Study No.: PH-DMPK-MDG-17-001)

Methods
This non-GLP study was conducted by (b) (4) in April, 2017. Male and female CD-1 mice (21/sex) received dietary moxidectin at a dose of 50 ppm for 14 days. Blood samples were collected from 3 mice/sex/timepoint pre-dose and at 4, 8, 12, 16, 20 and 24 hours after the last dose. Blood samples were processed to plasma and plasma concentrations of moxidectin were quantified using a qualified LC-MS/MS technique.

Results
The mean T_{max}, C_{max}, and AUC_{last} for moxidectin in the plasma of male and female mice is shown in Table 11. Plasma T_{max} values were 20 hours for both sexes, but mean values for plasma C_{max} and AUC were greater in males compared to females, possibly due to greater food intake in males. Plasma $t_{1/2}$ values were not reported.

Table 11: Mean Male and Female Pharmacokinetic Values for Moxidectin in Mouse Plasma Following 14-days of Dietary Administration of 50 ppm Moxidectin. (Table from the Study Report)

Animal	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (ng/mL*h)
Male	20.0	3150	62082
Female	20.0	2267	47414

2. **Single Oral Dose Exploratory Toxicity Study of PF-05208746 in Male Sprague Dawley Rats.** (Study No.: 10GR082)

Methods
This non-GLP study was conducted by Pfizer Global Research and Development in Connecticut in 2010. Male Sprague-Dawley rats (5/group, 8 weeks old at initiation of dosing) were administered moxidectin (Lot MA07132MC) in corn oil in single oral doses of 0 (corn oil), 3, 10, 20, and 30 mg/kg and animals were assessed for mortality, clinical signs, and body weights. Additional groups of animals (3/group) were treated in a similar manner for a toxicokinetic assessment. Blood samples were collected from

toxicokinetic animals on the day of dosing at 1, 2, 4, 8, 24, and 48 hours after dosing for determination of plasma moxidectin concentrations.

Results
The toxicity results for this study are summarized in in Section 6.1 (Single-Dose Toxicity) of this review. Toxicokinetic parameters for moxidectin following a single oral dose are shown in Table 12. Notably the range of plasma $t_{1/2}$ values in rats (18.0 to 25.5 hours) was much lower than that measured in humans (24 to 33 days).

Table 12: Male Toxicokinetic Parameters for Plasma Moxidectin Following a Single Oral Dose in Rats. (Table from the Study Report)

Dosage[a] (mg/kg)	t_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)	AUC_{0-inf} (ng*h/mL)
3	2 ± 0	20.5 ± 2.4	562 ± 172	5761 ± 2021	8022 ± 2731
10	2 ± 0	18.6 ± 2.0	2513 ± 221	29,397 ± 3846	42,114 ± 5205
20	3.3 ± 1.2	25.5 ± 1.7	3360 ± 1228	45,646 ± 11,554	88,787 ± 24,082
30	4 ± 0	18.0 ± 0.5	6027 ± 993	98,524 ± 4208	169,935 ± 4698

Abbreviations: AUC = area under concentration-time curve calculated to 24 hours or infinity, C_{max} = maximum exposure concentration, h = hour, inf = infinity, SD = standard deviation, t1/2 = half-life, t_{max} = time of maximum exposure.
[a] Moxidectin was administered by oral (gavage) to 3 male rats for TK evaluation (N = 3).

3. **Study Title: Moxidectin Blood Profile at or near Steady State in Sprague Dawley Rats Receiving a Diet Containing 100 ppm Moxidectin for 28 Consecutive Days.** (Study Report No.: RPT-62209)

Methods
This GLP-compliant study was conducted by Wyeth Research in Pennsylvania in June, 2006. Moxidectin was administered at a concentration of 100 ppm in feed for 28 days to male and female Sprague-Dawley rats (N = 20/sex). Blood samples for pharmacokinetic analysis were obtained on Day 28 at the start of the daily light cycle (0 hour), and 4, 8, 12, 16, 20, and 24 hours after the start of the light cycle. Samples were pooled from 5 rats at each timepoint. Additional samples were obtained before the start of dietary dosing and at the start of the daily light cycles on Days 21 and 24. Blood samples were processed to serum and serum moxidectin concentrations were determined using a validated HPLC/Fluorometic method with a lower limit of quantification of 1 ppb (1 ng/ml).

Results
No moxidectin was measureable in pre-dose serum samples. The calculated doses of moxidectin in animal feed for male and female rats, and the serum C_{max} and AUC values for moxidectin are shown in Table 13. The ingested dose was higher for females as were the associated serum C_{max} and AUC values which were approximately 2-fold higher in females compared to males. Based on similar moxidectin serum concentrations for each male or female pooled sample at time 0 for the Day 21, 24, and 28 samples, moxidectin serum concentrations were considered to be at or near steady state by Day 21.

Table 13: Calculated Doses and Serum Pharmacokinetic Values for Male and Female Rats Administered Dietary Moxidectin for 28 Days. (Table from the Study Report)

Sex	Dose[a] (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24hr} (ng·day/mL)	C_{max}/ Dose	AUC_{0-24hr}/ Dose
M	5.88	1137	0[b]	805	193	137
F	6.88	2213	0[b]	1703	322	248

a. Estimated ingested dosage
b. Coincides with start of light cycle

4. Single Dose Oral (Capsule) Pharmacokinetic Study with a 12-Week Observation Period in Juvenile and Adult Dogs. (Study No.: RPT-73592)

Methods

This non-GLP study was conducted by Wyeth Research in New York in 2008. Moxidectin (Batch No.: MB2632) was administered as a single dose by oral capsule to male and female (3/sex/group) juvenile (11 weeks old) and adult (10 months old) Beagle dogs at doses of 0, 0.3, 1, and 3 mg/kg then monitored for 12 weeks. Animals were observed for mortality, clinical signs, body weights, and clinical chemistry and pharmacokinetic measurements were performed. Blood was collected for pharmacokinetic analysis from adult and juvenile dogs predose and on the day of dosing at 1, 4, 8, 24, 72, and 168 hours after dosing and on Study Days 14, 28, 42, 56, 70, and 84. Moxidectin concentrations in plasma were measured using a validated HPLC-fluorescence method with a lower limit of quantification of 0.2 ng/ml.

Results

The toxicity results for this study are summarized in in Section 6.1 (Single-Dose Toxicity) of this review.

Plasma $t_{1/2}$ values, ranging from 13.6 to 18.4 days in adult dogs were much longer than the values reported in rats (approximately 20 hours) and more similar to human values (24 to 33 days). In adult and juvenile dogs, plasma C_{max} and AUC values were similar between sexes. In general the plasma $t_{1/2}$ values were slightly longer in adult rats compared to the juvenile rats (9.6 to 12.4 days), and plasma AUC values were higher in adult rats compared to juvenile rats, but plasma C_{max} values were more similar (Table 14).

Table 14: Mean (± SD) Moxidectin Pharmacokinetic Parameters in Adult and Juvenile Dogs after a Single, Oral (Capsule) Dose (0.3, 1, and 3 mg/kg) of Moxidectin. (Table from the Study Report)

Age	Dosage (mg/kg	Sex	C_{max} (ng/mL)	t_{max} (day)	$AUC_{0\text{-}83days}$ (ng•day/mL)	$AUC_{0\text{-}\infty}$ (ng•day/mL)	$t_{½}$ (day)	C_{max}/ Dose	$AUC_{0\text{-}\infty}$/ Dose
Adult	0.3	M	190 ± 105	0.17 ± 0.00	455 ± 255	474 ± 272	15.9 ± 2.3	632 ± 349	1581 ± 907
Adult	0.3	F	218 ± 62	0.17 ± 0.00	559 ± 303	588 ± 319	18.4 ± 4.8	725 ± 207	1959 ± 1064
Adult	1	M	841 ± 291	0.17 ± 0.00	2497 ± 924	2618 ± 1043	17.8 ± 1.4	841 ± 291	2618 ± 1043
Adult	1	F	1214 ± 621	0.13 ± 0.07	2948 ± 1527	3022 ± 1583	14.0 ± 3.2	1214 ± 621	3022 ± 1583
Adult	3	M[a]	1852 ± 903	0.17 ± 0.00	6091 ± 3445	6312 ± 3676	14.4 ± 3.1	617 ± 301	2104 ± 1225
Adult	3	F	1759 ± 633	0.17 ± 0.00	4927 ± 1380	5013 ± 1337	13.6 ± 3.6	586 ± 211	1671 ± 446
Juvenile	0.3	M	317 ± 143	0.08 ± 0.07	448 ± 104	450 ± 103	9.7 ± 1.7	1057 ± 477	1500 ± 344
Juvenile	0.3	F	162 ± 35	0.13 ± 0.07	357 ± 88	362 ± 91	12.4 ± 3.9	541 ± 118	1205 ± 302
Juvenile	1	M	545 ± 43	0.22 ± 0.10	1095 ± 161	1098 ± 162	9.7 ± 1.1	545 ± 43	1098 ± 162[c]
Juvenile	1	F	687 ± 373	0.18 ± 0.15	1486 ± 226	1502 ± 236	12.4 ± 0.9	687 ± 373	1502 ± 236[c]
Juvenile	3	M[b]	2761 ± 145	0.04 ± 0.00	3875 ± 145	3890 ± 142	10.1 ± 2.4	920 ± 48	1297 ± 47
Juvenile	3	F[b]	2282 ± 340	0.04 ± 0.00	3868 ± 1399	3878 ± 1408	9.6 ± 0.7	761 ± 113	1293 ± 469

a. Emesis observed in one animal.
b. Animals group housed, vomit observed in cage.
c. Significantly different from adult dogs, same dosage and sex ($p \leq 0.05$).
Note: n = 3 unless otherwise noted

5. **Study Title: Moxidectin: Blood Profile in Dogs Receiving a Single Exposure to a Canine Diet Containing 45 ppm Moxidectin.** (Study Report No.: RPT-62210)

Methods

This GLP-compliant study was conducted by Wyeth Research in Pennsylvania in June 2006. Male and female dogs (N=3/sex) were fed a single meal containing 45 ppm moxidectin. Blood samples for pharmacokinetic analysis were obtained pre-dose and 1, 2, 4, 6, 8, 12, and 24 hours after dosing and at 2, 3, 5, 7, 10, 14, 21, and 28 days after dosing. Blood samples were processed to serum and moxidectin concentrations in serum were determined using a validated HPLC/fluorometric method with a lower limit of quantification of 0.5 ppb (0.5 ng/ml).

Results

Low but quantifiable concentrations of moxidectin (0.50, 0.51, 0.52, and 1.32 ng/ml) were detected in pre-dose samples from 4 animals (2/sex). The reason for the apparent blood contamination was not reported, but given the low concentrations, the contamination was not considered to have altered the post-dose pharmacokinetic results.

All of the serum pharmacokinetic values were similar between sexes. The estimated serum $t_{1/2}$ value for moxidectin was 8.5 and 10.6 days in females and males respectively (Table 15).

Table 15: Mean (± SD) Pharmacokinetic Values for Moxidectin in the Serum of Male and Female Beagle Dogs Administered a Single Oral Dose of 45 ppm Dietary Moxidectin. (Table from the Study Report)

Sex	Dosage (mg/kg)	C_{max} (ng/mL)	t_{max} (day)	$AUC_{0\text{-}28days}$ (ng•day/mL)	$AUC_{0\text{-}\infty}$ (ng•·day/mL)	$t_{1/2}$ (day)	C_{max}/ Dose	$AUC_{0\text{-}\infty}$/ Dose
M	0.72 ± 0.10	253 ± 92	0.33 ± 0.14	631 ± 96	747 ± 70	10.6 ± 2.2	367 ± 161	1058 ± 194
F	0.99 ± 0.06	294 ± 42	0.39 ± 0.10	637 ± 226	693 ± 244	8.5 ± 1.3	297 ± 33	709 ± 267

6. **Study Title: A Pharmacokinetic Study of Moxidectin Following Oral Administration in Pregnant Rabbits.** (Study No.: 70704-17-404)

Methods

This non-GLP Study was conducted by (b) (4) in June 2017. Six pregnant rabbits (New Zealand White, age: approximately 4-6 months, approximate body weight: 3-5 kg) were administered 10 mg/kg moxidectin (Batch No.: MX-1606017 dissolved in corn oil), once daily by oral gavage on gestation days (GDs) 6 through 12. Blood samples (later processed to plasma) were collected predose and at 0.5, 1, 2, 4, 8, 24, and 48 hours after the last dose for pharmacokinetic analysis.

Results

The mean pharmacokinetic values for five pregnant rabbits is shown in Table 16. One female out the original six study animals was found to be not pregnant, and pharmacokinetic data from this animal was not included in the summary data.

Table 16: Mean Pharmacokinetic Values for Moxidectin Following 10 mg/kg Oral Administration to Female Pregnant Rabbits. (Table from the Study Report)

Animal	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng/mL*h)	AUC_{inf} (ng/mL*h)
Mean	4.00	21.7	324	9192	11905
SD	NA	8.7	130	5420	7204

Note: the data from non-pregnant was not used for the calculation of Mean and SD value.
NA=Not available

Distribution

1. **Macrocyclic Lactones: Distribution in Plasma Lipoproteins of Several Animal Species Including Humans.** (Bassissi MF, Alvinerie M, and Lespine A: Com Bioch Physiol, 138:437-444, 2004)

Methods

This study is reported in a literature manuscript. Plasma from goat, cow, sheep, rabbit, pig, and human was spiked and incubated with 10 ng/ml moxidectin for one hour at 37°C. Four plasma-lipoprotein fractions, very low density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and a lipoprotein deficient fraction were separated using KBr density gradient ultracentrifugation. The amount of lipoprotein binding to each of the plasma lipoprotein fractions was calculated.

Results
The overall finding is that moxidectin is highly bound by plasma lipoproteins. In plasma from all six of the species tested, greater than 95% of the spiked moxidectin bound plasma lipoproteins. However, the extent of binding to the HDL fraction varied between species with 70% of moxidectin binding HDL in humans and higher amounts binding HDL in the other species with the highest percent binding (92%) occurring in cow plasma.

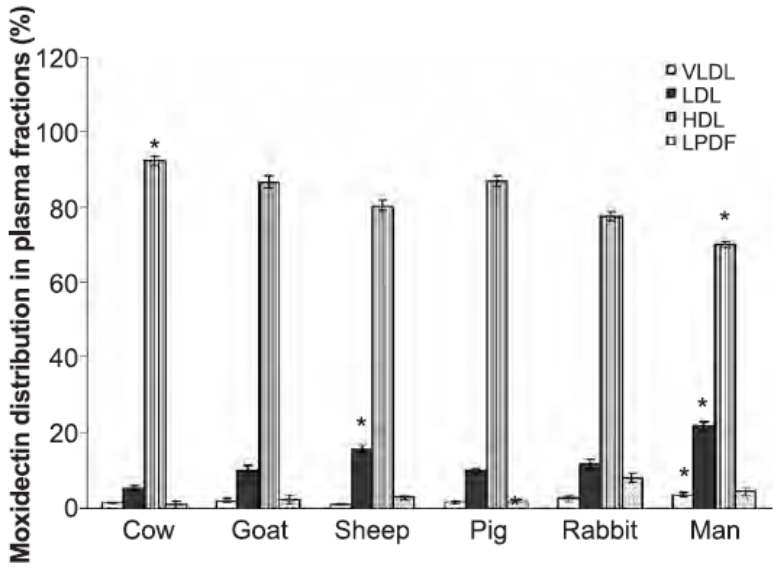


Figure 1: Distribution of Spiked Moxidectin in Lipoprotein Fractions from Plasma in Five Different Species. (Figure from Bassissi et al., 2004)

2. **Study Title: Moxidectin (CL 301423): Absorption, Distribution, Excretion, and Metabolism of Carbon-14 Labeled CL 301423 in the Rat - Report Amendment 01.** (Study Report No.: RPT-77457)

Methods
This GLP-compliant study was conducted by (b) (4) in 1991. The Study involved two parts, Part 1 addressing the mass balance and distribution of ^{14}C-moxidectin to tissues and Part 2 which entailed metabolite profiling.

In Phase I of Part 1 of the study, a single group of Sprague Dawley rats (5/sex/group) were administered single oral gavage doses of 1.5 mg/kg (Group A2) and 12 mg/kg (Group B) ^{14}C-moxidectin (CL 301,423) in a vehicle of corn oil. Urine and feces were collected at specific times after dosing and blood and select tissues (liver, kidney, muscle, fat, blood, brain, testis, ovary, and the residual carcass) were collected when animals were euthanized 168 hours after dosing. Urine and feces were collected at 0-6, 6-12, and 12-24, 24-48, 48-72, 72-120, and 120-168 hours after dosing. Cage rinses were also collected after animal euthanasia.

In Phase II of Part I of the study, a third group (Group C2) of 15 male and 15 female rats were administered daily doses of 1.5 mg/kg/day ^{14}C-moxidectin for seven consecutive days. Groups of 3/sex were euthanized at 6, 24, 72, 120, and 168 hours after the last dose. Urine and feces were collected at 6 and 24 hours after the last dose. Liver, kidney, muscle, and fat were collected at 6, 24, 72, 120, and 168 hours after the last dose.

Total radioactivity in collected samples was determined by liquid scintillation counting.

In Part II, samples collected from the study groups in Part I were analyzed for metabolite identification. Metabolites in the following samples were analyzed: urine samples from the 24 hour post-dose collections from Phase 1 (single dose administration); feces collected at 24 hours and 120-168 hours after Phase 1 dosing and liver, kidney, muscle, and fat collected at 168 hours after Phase 1 dosing and at 6, 24, and 168 hours after Phase 2 dosing. In a separate experiment, ^{14}C-moxidectin was incubated with a commercial S9 rat liver homogenate overnight in the presence of NADPH before analysis of any resulting metabolites. After extraction of ^{14}C-residues from the different sample matrixes, processed samples were analyzed using HPLC, MS, and nuclear magnetic resonance to separate and identify the structures of different moxidectin metabolites.

Results

Part 1/Phase 1

The total recovery of the radioactivity from urine, feces, tissues/organs, blood, carcass and cage rinse after 7 days, expressed as a percentage of the administered dose, averaged 90.0% (76.7-98.5%) for the low dose group (1.5 mg/kg body weight, Group A2) and 88.0% (72.5-94.2%) for the high dose group (12 mg/kg body weight, Group B2). Feces was the primary route of excretion and accounted for 59.7-91.3% of the administered radioactivity for all rats, with most of the activity excreted within 3 days after dosing. Urine excretion was minimal accounting for 0.4-1.3% of the dose. Distribution to liver, kidney, muscle, fat, and other organs (brain, ovary and testis) accounted for only a small fraction of the dosed radioactivity, for 0.7-3.5% of the dose, with the residual carcass accounting for an additional 3.9-16.7% in all rats. Blood contained less than 0.1 % of the dose. Among tissues, fat retained the most radioactivity, approximately 20 times more than liver, kidney, and muscle. Radioactivity content in ovaries was second only to fat in females. Testes contained less radioactivity than all other tissues except blood, and brain where radioactivity was below the measurable threshold. The residue levels in the tissue (liver, muscle, and fat) and in the carcass from female rats generally contained a higher percentage of the dose than did the corresponding tissues from male rats, perhaps because of a higher percent body fat in females.

Part 1/Phase II

In Phase II of the study, the total radioactivity in the examined tissues was greatest in fat, followed by liver, kidney and muscle. Radioactivity accumulated in tissues with

repeated dosing. Relative to the single-dose administrations, tissue radioactivity levels were on the order of 20 times higher after 7 days of dosing. As with the Phase I single-dose experiment, tissue radioactivity was higher in females compared to males at all the measurement timepoints. The average tissue $t_{1/2}$ for male and female rats was 2.4 days in liver and kidney, 3.9 days in muscle, and 11.5 days in fat.

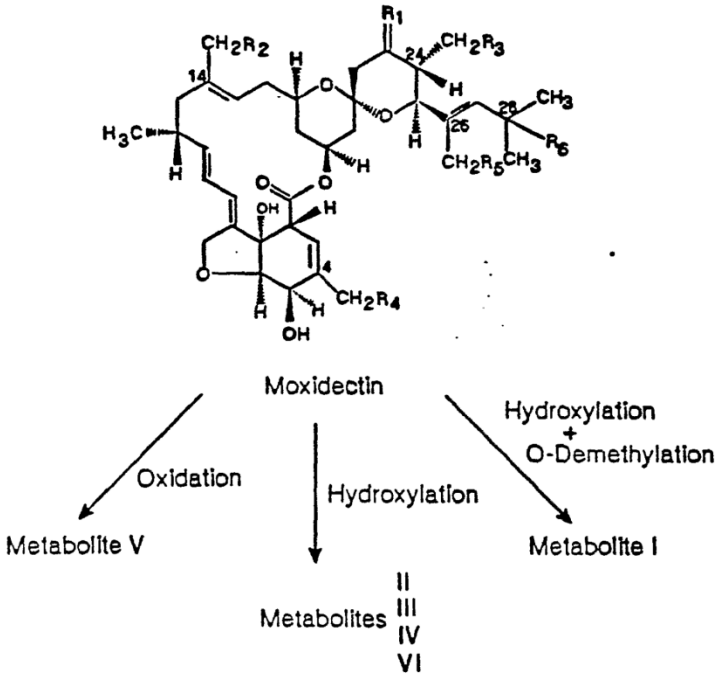
Part II

Tissues: Moxidectin parent was the primary component of the radioactive residues in all tissues (liver, kidney, muscle, and fat) accounting for 77-89% of the total radioactivity in fat where accumulation was greatest. A minimum of four minor components were detected in the radioactivity residues in liver. The same metabolic components were detected in feces and *in vitro* in liver extract and microsomal incubations.

Feces: Moxidectin parent was the major component and accounted for 60-82% of the total radioactivity in feces collected 24 hours after treatment. The six moxidectin metabolites detected in feces encompass the metabolic components detected in liver residues.

Urine: Unlike in tissues and feces, moxidectin parent was not the primary radioactive component excreted in urine accounting for approximately 5-15% of radioactivity collected 24 hours after treatment. The metabolites in urine were described as being generally more polar than those in tissue extracts, but the urine metabolites were not identified in the study report.

Metabolite Characterization: The chemical characterization of moxidectin metabolites I-VI are shown in Figure 2. Hydroxylation was identified as a principle mechanism of metabolism.



		R_1	R_2	R_3	R_4	R_5	R_6
Moxidectin	(CL 301,423)	N-OCH_3	H	H	H	H	H
Metabolite I	(CL 189,024)	N-OH	OH	H	H	H	H
Metabolite II	(CL 189,021)	N-OCH_3	OH	H	H	H	H
Metabolite III	(CL 189,022)	N-OCH_3	H	OH	H	H	H
Metabolite IV		N-OCH_3	H	H	H	OH or H	H or OH
Metabolite V	(CL 301,310)	O	H	H	H	H	H
Metabolite VI	(CL 189,023)	N-OCH_3	H	H	OH	H	H

Figure 2: Metabolic Pathway of Moxidectin in the Rat. (Figure from the Study Report)

Metabolism

1. **Study Title: *In Vitro* Metabolism of Moxidectin in Human Liver Microsomes, Cryopreserved Hepatocytes and Recombinant Human Cytochrome P450 and UDP-Glucuronosyltransferase Enzymes.** (Study No.: MDG-P5763).

Methods

This non-GLP Study was conducted by [redacted] (b) (4) in May 2016.

Incubations with Human Liver Microsomes, Cytosol, and Recombinant CYPs:
Incubations contained 10 mcM moxidectin, human liver microsomes or recombinant CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) as well as an NADPH generating system to initiate reactions. Incubations with human liver microsomes fortified with cytosol (2 mg/ml) in the absence of NADPH were also conducted to assess non-CYP450 mediated metabolic reactions. After 60-minute incubations, reactions were terminated with the addition of acetonitrile, and resulting mixtures were centrifuged and supernatants were analyzed for moxidectin metabolites using an LC/UV/MS technique. Midazolam (5 mcM) was included as a positive control to confirm the viability of the human liver microsome preparation.

Incubations with Cryopreserved Human Hepatocytes: Incubations containing 10 mcM moxidectin and cryopreserved hepatocyte suspensions that had been thawed, washed and checked for viability with Trypan Blue staining. Incubations continued for 4 hours at 37°C. Incubations were terminated with the addition of acetonitrile, and the resulting mixtures were centrifuged and supernatants were analyzed for moxidectin metabolites using an LC/UV/MS technique.

Results
Incubations with Human Liver Microsomes, Cytosol, and Recombinant CYPs:
Moxidectin was poorly metabolized in human liver microsome incubations yielding low levels of three hydroxylated metabolites, M655a, M655b, and M655c in relative abundances of 9.9%, 10.1% and 2.6% respectively relative to the parent compound (81.9% at the end of the incubation). No metabolites were detected following incubation in human liver cytosol indicating the lack of non-CYP450 mediated metabolism.

Several recombinant CYPs were able to metabolize moxidectin to hydroxylated analogues including CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5, but moxidectin was metabolized to the greatest extent by CYP3A4 and CYP3A5. As with the human liver microsomes, the primary metabolites were M655a and M655b. Because CYP3A4 occurs in a high abundance in human liver, most of the moxidectin metabolism after systemic administration may occur via this enzyme.

Incubations with Cryopreserved Human Hepatocytes: When incubated with reconstituted human hepatocytes, moxidectin was found to be metabolized to the same hydroxylated metabolites as in human liver microsomes and recombinant CYPs.

2. **Study Title: Moxidectin: Metabolic Stability and Metabolism in Rat and Human Liver Microsomes.** (Study Report No.: RPT-51895)

Methods
This non-GLP study was conducted by Wyeth Research in Pennsylvania in Nov., 2003. Moxidectin (Batch No.: MB2632, purity of 99%) was incubated with liver microsomes from Sprague-Dawley rats (pooled from three males), and human liver microsomes (pool from six subjects, 3/sex) to determine the *in vitro* metabolic $t_{1/2}$ for moxidectin

metabolism, and to characterize the moxidectin metobolites in rats and human liver microsomes.

Incubations to Determine Metabolic $t_{1/2}$: Moxidectin at a concentration of 1 mcM was incubated with rat or human liver microsomes in the presence of a NADPH generating system for up to 30 minutes. Control incubations were conducted under the same conditions without cofactors. Incubations containing midazolam (0.2 mcM) and 1 mcM diclofenac were also performed as positive controls for oxidative and glucuronidation activity respectively. At specified timepoints (0, 10, 20, and 30 minutes), protein was precipitated in individual incubation aliquots with the addition of acetonitrile, samples were centrifuged and reconstituted supernatants were analyzed using an LC/MS method.

Incubations for Metabolic Characterization: Moxidectin at a concentration of 100 mcM was incubated in the presence of a NADPH generating system with rat or human liver microsomes for 30 minutes at 37°C. The incubations were stopped with acetonitrile, centrifuged, and reconstituted supernatants were analyzed using a LC/MS system to characterize moxidectin metabolic products.

Results

Incubations to Determine Metabolic $t_{1/2}$: Moxidectin was minimally metabolized in rat and human liver microsomes in the presence of NADPH with an estimated *in vitro* $t_{1/2}$ > 60 min. After 30 minutes, approximately 21% and 7% of moxidectin was metabolized in incubations with rat and human microsomes respectively. Oxidative and glucuronidation activity in rat and human liver microsomes was confirmed by substrate depletion of the positive controls, but these processes appeared to minimally effect the metabolism of moxidectin. These results suggest low metabolic clearance of moxidectin in rats and humans.

Incubations for Metabolic Characterization: Four moxidectin-related metabolites, M1, M2, M3, and M4, all hydroxylation products, were detected by LC/MS. The metabolic profile was reportedly similar for rats and humans. The HPLC retention times and the putative structures for the four moxidectin metabolites detected in incubations with rat and human liver microsomes are shown in Figure 3.

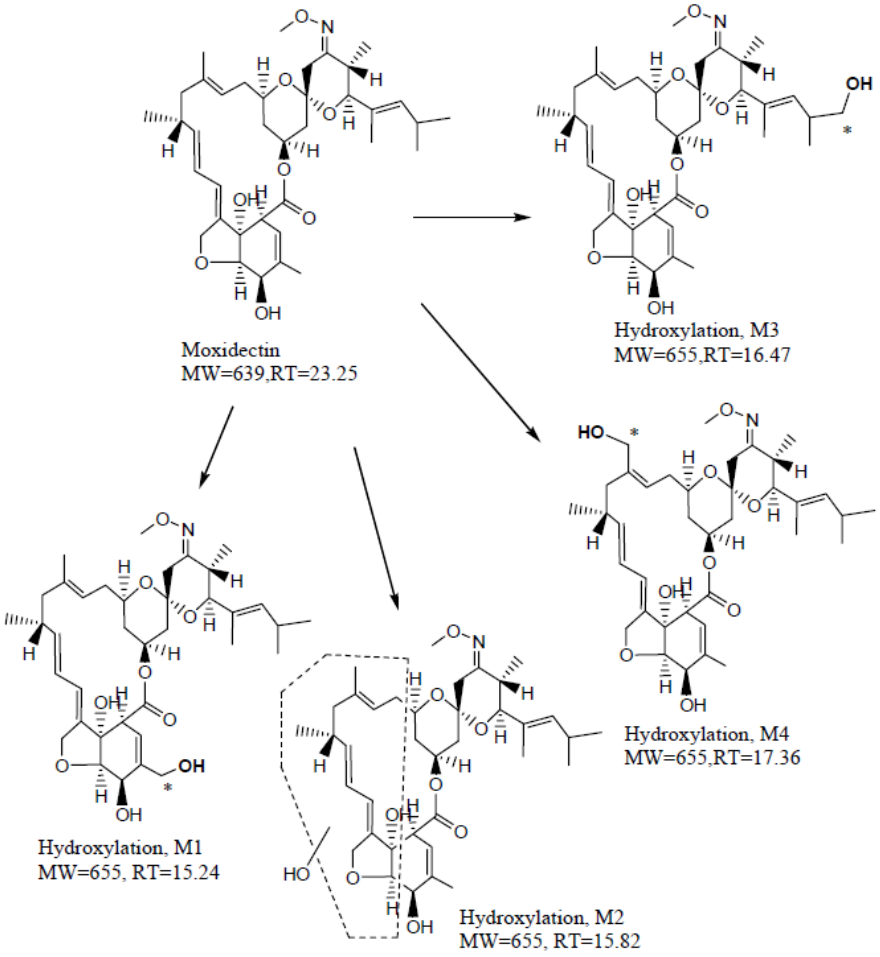


Figure 3: Proposed Metabolic Pathways for Moxidectin in Rat and Human Liver Microsomes. (Figure from the Study Report)

3. **Study Title: Moxidectin: Potential Induction of Cytochrome P450 Genes and P-Glycoprotein (MDR1) by Moxidectin in Human Hepatocytes.** (Study Report No.: RPT-71215)

Methods

This non-GLP study was conducted by Wyeth Research in Pennsylvania in 2006. Vehicle (0.1% DMSO), a positive control (10 mcM rifampicin), and moxidectin (Batch No.: MB2632) at concentrations of 0.05, 0.1, and 1.0 mcM was incubated with reconstituted cryopreserved human hepatocytes (0.7 x 10^6 cells per incubation) in triplicate cultures for 48 hours with repeated treatment after 24 hours.

For determination of CYP2B6 and CYP3A4 enzyme activity, after 48 hours of incubation, media was removed, cells rinsed with PBS, and then incubated for a further 15 minutes in media containing 200 mcM of bupropion (CYP2B6 substrate) and testosterone (CYP3A4 substrate). The media was harvested and stored for LC/MS analysis of the isozyme-specific products, hydroxybupropion for CYP2B6 and 6β

hydroxytestosterone for CYP3A4. The attached cells in each incubation well were subsequently lysed with 1 ml of TRIzol reagent, and the cell lysates were stored at -70°C until RNA isolation. Subsequently, CYP1A2, CYP2B6, CYP2C9, CYP3A4 and p-glycoprotein (MDR1) mRNA was quantified using Taqman.

Results
Moxidectin induced the activity of both CYP2B6 and CYP3A4 in a dose-dependent manner with maximal-induction values of approximately 2.5-fold and 4.7-fold respectively compared to vehicle control values. The positive control agent, rifampin also induced the activity of both CYP enzymes approximately 5.2-fold and 7.3-fold compared to vehicle control values for CYP2B6 and CYP3A4 respectively.

Moxidectin produced a substantial and concentration-dependent induction of human CYP3A4 expression with a mean induction of 23.7 fold compared to the mean vehicle control value at the highest test concentration of 1 mcM. CYP3A4 expression was also induced by rifampin by a mean value of approximately 43-fold compared to vehicle-control values. Moxidectin also produced weaker inductions, of CYP2B6 and CYP2C9 expression with respective mean inductions of 2.1- and 2.5-fold at the highest moxidectin concentration, and rifampin produced a similar effect with mean inductions of 4.07 and 2.98 respectively compared to mean control values. Both moxidectin and rifampin reduced the CYP1A2 message compared to the mean control value, consistent with previous Wyeth in-house results for rifampin.

4. **Study Title: Evaluation of Time-Dependent Inhibition of CYP2D6 and CYP3A4/5 by Moxidectin.** (Study Report No.: ADME-MOX-160510-TDI)

Methods
This non-GLP study was conducted in [redacted] (b) (4) in May 2016. The time-dependent inhibition of CYP2D6 and CYP3A4/5 activity in human liver microsomes by moxidectin (Lot No.: MX-1512046) in final concentrations of 0, 0.1, 0.5, 2, 10, and 50 mcM was evaluated in different pre-incubation conditions, 0 minute pre-incubation, 30 minute pre-incubation without NADPH, and 30 minute pre-incubation with NADPH. CYP2D6 and CYP3A4/5 activity in human liver microsomes was assessed by measuring the rate of formation of specific products derived from isozyme-specific substrates. The CYP2D6-specific substrate, bufuraral, and the CYP3A4/5-specific substrate, midazolam, were tested at concentrations of 25 and 15 mcM respectively. The positive control agents for inhibition of CYP2D6 and CYP3A4/5 were paroxetine and mifepristone respectively and both agent were tested in concentrations of 0.1, 0.5, 2, 10, and 50 mcM. Incubations were carried out at 37°C, but the duration of the incubations was not identified in the study report. Reaction products were quantified using an LC-MS/MS method.

Results
IC_{50} shifts between the 0-minute and 30-minute preincubations with NADPH were observed for the positive control inhibitors of CYP2D6 and CYP3A4/5 indictating the system was able to detect time-dependent inhibitory effects. Moxidectin did not produce

similar time-dependent inhibition, and the estimated IC_{50s} for moxidectin inhibition (> 50 mcM) remained the same under all conditions. The results indicate moxidectin did not produce time-dependent inhibition of CYP2D6 and CYP3A4/5 activity in human liver microsomes under the experimental conditions.

5. **Study Title: Moxidectin: IC_{50} Determination for the Inhibition of Cytochrome P450 Enzymes in Human Liver Microsomes.** (Study No.: RPT-51894)

Methods
This non-GLP study was conducted by Wyeth Research in 2003 in Pennsylvania. Different concentions of moxidectin (Batch No.: MB2632, purity of 99%) were incubated at different concentrations (0.1 to 100 mcM) with human liver microsomes (pool from six subjects) and CYP isozyme-specific substrates to determine if moxidectin inhibited any of the CYP isozymes. Isozyme-specific inhibitors (α-naphthoflavone, diethyldithiocarbamate, quercetin, sulfaphenzole, tranylcypromine, quinidine, and ketoconazole) were employed to confirm the sensitivity of the test system.

Results
Moxidectin did not inhibit CYP2A6, CYP2C8, CYP2C19, CYP2D6 and CYP3A4 activity in human liver microsomes at the highest test concentration of 100 mcM. Moxidectin weakly inhibited CYP1A2 and CYP2C9 activity with estimated IC_{50} values of 495 and 145 mcM respectively.

6. **Study Title: Moxidectin: Evaluating the Potential for Induction of CYP3A4 by Moxidectin Using a CYP3A4 Reporter Gene Assay.** (Study No.: RPT-70409).

Methods
This non-GLP study was conducted in Pennsylvania by Wyeth Research in 2007. In addition to positive (10 mcM rifampicin) and negative control (20 mcM pregnenolone 16α carbonitrile) compounds, moxidectin (Batch No.: MB2632) was tested at concentrations of 0 (0.1% DMSO), 0.05, 0.1, and 1 mcM in a CYP3A4/luciferous reporter gene assay in HepG2 cells transfected with CYP3A4 promoter/enhancer. Cells were incubated for 48 hours with repeat treatment after 24 hours. Cells were subsequently lysed and luciferase activity was measured and expressed as relative light units (RLU). Assays were conducted in triplicate.

Results
The results indicate that the 0.1 mcM and 1.0 mcM concentrations of moxidectin produced 3.1- and 16.1-fold increases in CYP3A4 induction compared to the mean vehicle-control value. The positive control inducer, rifampicin produced a larger, 47.7-fold increase in CYP3A4 induction compared to the mean value for the vehicle control. The results indicate that moxidectin induces CYP3A4 gene expression in a concentration-dependent manner. However, the moxidectin test concentration (1.0 mcM) associated with substantially induced expression is 10 times higher than the plasma C_{max} value (63.1 ng/ml ≈ 0.1 mcM) in humans administered a single oral dose of

8 mg moxidectin. Therefore at clinical concentrations, moxidectin is not expected to substantially induce CYP3A4 gene expression.

Excretion

The excretion results for ^{14}C-moxidectin in Study Report No.: RPT-77457 indicate that in rats, orally administered moxidectin and its metabolites are excreted primarily in feces (≥ 60%) with much less excretion in urine (approximately 1%).

5.2 Toxicokinetics

No toxicokinetic measurements were performed in any of the toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies were conducted in mice, rats, rabbits, and dogs using different routes of administration including oral, intraperitoneal, subcutaneous, and dermal routes of administration (Table 17). Extrapolated LD_{50} values were derived from mortality results for the moxidectin doses that were tested. Animals experienced dose-dependent mortality and clinical signs. The clinical signs, predominantly CNS related, generally resolved days after dosing.

In the studies in mice, when mortality occurred, it generally occurred between 8 and 24 hours after dosing. In three oral-dose mouse studies (Study Report Nos.: RPT-77273, 77293, and 77294), moxidectin doses ≥ 60 mg/kg were associated with clinical signs including decreased activity, tremors, and ataxia. In a 4th oral-dose study (Study No.: RPT-80141), clinical signs were observed at moxidectin doses of ≥ 25 mg/kg and included decreased motor activity, awkward gait with splayed hind limbs, tremors, ataxia, and irregular or labored respiration. Straub tail also occurred at moxidectin doses ≥ 75 mg/kg in this study. In an intraperitoneal study (Study Report No.: RPT-77314), using a moxidectin dose range of 40, 80, 160, and 320 mg/kg, mortality occurred in a dose-dependent manner with extrapolated LD_{50} values of 86 mg/kg in males and 87 mg/kg in females. Clinical signs included decreased activity and epistaxis at ≥ 80 mg/kg, tremors at ≥ 160 mg/kg, and loss of righting reflex at ≥ 320 mg/kg. In a subcutaneous study using a moxidectin-dose range of 80, 160, and 320 mg/kg, mortality occurred with the high dose of 320 mg/kg and clinical signs at this dose included decreased activity and tremors.

In rat oral dose studies, mortality occurred in a dose-dependent manner with extrapolated LD_{50} values of 122 mg/kg in males and 97 mg/kg in females. Clinical signs included ataxia at ≥ 20 mg/kg moxidectin, hypersensitivity to touch and sound, epistaxis, tremors and diarrhea at 75 mg/kg, decreased respiration, decreased activity, prostration, and tremors at ≥ 75 mg/kg, and red tears (chromodacryorrhea) at 300 mg/kg. In the intraperitoneal-dose study using higher doses of moxidectin (dose range of 160, 320, and 640 mg/kg), mortality occurred in a dose-dependent manner with extrapolated LD_{50} values of 453 mg/kg in males and 359 mg/kg in females. Clinical

signs included decreased activity, tremors, prostration, anorexia, and epistaxis at ≥ 160 mg/kg moxidectin and red tears occurring only in rats receiving the 160 mg/kg/dose. No mortality or clinical signs were observed in rats administered single subcutaneous doses of moxidectin up to 640 mg/kg.

In rabbits, no mortality or clinical signs were observed with single doses of dermal moxidectin up to 2000 mg/kg.

In juvenile (11 weeks old) Beagle dogs (3/sex) receiving single oral doses of 0, 0.3, 1, and 3 mg/kg moxidectin, clinical signs occurred as early as 2 hours after dosing and mostly resolved within 24 hours after dosing. The clinical signs included intermittent tremors, ataxia, abnormal posture, decreased motor activity, salivation, emesis, disorientation, and ptosis with the 3 mg/kg dose. In the same study with the same dose range, clinical signs in adult (10 months old) dogs (3/sex) occurred beginning 4 hours after dosing with the 3 mg/kg dose, and had not fully resolved 48 hours after dosing. Clinical signs included: intermittent tremors, ataxia, abnormal posture, decreased motor activity, salivation, vocalization, mydriasis, emesis, and/or retropulsion. No clinical signs were observed in juvenile or adult dogs receiving the lower doses and no mortality was observed at any dose.

Table 17: Single-dose Studies for Moxidectin. (Table Adapted from a Table in Section 2.6.6 Toxicology Written Summary in the NDA electronic submission)

Species (Report #)	Route	Animals (N/sex/group)	Doses (mg/kg)	Extrapolated LD_{50} (mg/kg)	
				Male	Female
Mouse (RPT-77273)	oral gavage	5	15, 30, 60, 120 (female only), 240	118	78
Mouse (RPT-77293)	oral gavage	5 females	30, 60, 120	NA	42
Mouse (RPT-77294)	oral gavage	5 females	30, 60, 120	NA	50
Mouse (RPT-80141)	oral gavage	5 females	5, 25, 75, 200	NA	75[a]
Mouse (RPT-77314)	IP	5	40, 80, 160, 320 (female only)	86	87
Mouse (RPT-77292)	SC	5	80, 160, 320	285	247
Rat (RPT-77275)	Oral gavage	5	75, 150, 300	122	97
Rat (10GR082)	Oral gavage	5 males	3, 10, 20, 30	>30	NA
Rat (RPT-77276)	IP	5	160, 320, 640	453	359
Rat (RPT-77277	SC	5	640	>640	>640
Rabbit (RPT-77274)	dermal	5	2000	>2000	>2000

Dog (RPT-73592)	Oral (capsule)	3 adults 3 juveniles	0.3, 1, 3	>3	>3
Abbreviations: IP = intraperitoneal; SC = subcutaneous, LD_{50} = median lethal dose in 50% of animals, NA = not applicable. **a** 75 mg/kg was the minimum lethal dose rather than the LD_{50}.					

6.2 Repeat-Dose Toxicity

Study title: AC-301423: A 28-Day Mouse Feeding Study.

Study no.: RPT-77313
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: July 26, 1989
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: Moxidectin (AC 301423), Lot No.: AC 6297-116, purity of 88.2%

Key Study Findings

- This study was adequately conducted, but some typical assessments including clinical chemistry were not included. Each study group included 5 animals/sex instead of the normal 10/sex/group. Also the panel of organs that were weighed and the panel of tissues and organs examined for histopathology were too limited to be considered adequate.
- All of the mice receiving 150 ppm dietary moxidectin and the majority of mice receiving 100 and 125 ppm died in the first 13 days of dosing.
- Clinical signs observed in the three highest dose groups (100, 125, and 150 ppm moxidectin) and also less frequently in the 75 ppm animals included: tremors, hypersensitivity to touch, urine stained fur, emaciation, and prostration.
- Body weights were reduced in a dose-dependent manner at moxidectin doses ≥ 75 ppm.
- Based on the clinical signs and body weight loss in mice receiving ≥75 ppm moxidectin (17.7 mg/kg/day), The NOAEL value was considered to be 33.7 ppm (average of 6.9 mg/kg/day).

Methods

Doses: 0, 50(33.7) 75, 100, 125, 150 ppm. Based on analysis of average food intake, the mg/kg values for moxidectin were estimated to be: 0, 6.9, 17.7, 23.2, and 24.1 mg/kg moxidectin for the 0, 50(33.7), 75, 100, and 125 ppm groups. The moxidectin mg/kg dose was not assessed

	for the 150 ppm group.
Frequency of dosing:	*Ad libitum* in animal food
Route of administration:	Oral; moxidectin was powdered and mixed in rodent chow (Purina Rodent Chow #5001).
Dose volume:	NA
Formulation/Vehicle:	none
Species/Strain:	CD®-1 [Crl:CD®-1(ICR)BR] albino mice
Number/Sex/Group:	5/sex/group
Age:	Approximately 6 weeks of age
Weight:	Males: 30 to 34 g; Females: 25-28 g
Satellite groups:	None
Unique study design:	Male and female CD-1 mice received dietary moxidectin in nominal concentrations of 0, 50, 75, 100, 175, and 150 ppm for 28 Days. Animals were euthanized on Day 29.
Deviation from study protocol:	Due to a mixing error during Weeks 2 and 3, the average dose for the 50 ppm dose group was 33.7 ppm. Other protocol deviations were noted. However, none of the deviations was considered to have altered the results of the study.

Observations and Results

Table 18: Observation Schedule for the 28-Day Mouse Feeding Study

Observations	Schedule
Mortality and Clinical Signs	All animals were observed daily for morbidity, death, and obvious indications of a toxic effect. At least once each week, each animal was removed from its cage and carefully examined for abnormalities and clinical signs of toxic effects.
Body Weights	Body weight data were collected once on Day 0 and weekly thereafter.
Food Consumption	Food consumption data were collected weekly.
Hematology	Blood samples were obtained from fasted animals for hematology analysis at necropsy.
Necropsy	Day 29

Mortality

All mice at the 150 ppm level died during the first 10 days of the study; 9/10 mice at the 125 ppm level died during the first 14 days of the study; 8/10 mice at the 100 ppm level died during the first 13 days of the study; and 1/10 mice at the 75 ppm level died on Day 8 of the study. All animals at the 33.7 ppm level survived the 28-day study period.

Clinical Signs

No clinical signs were observed in mice receiving 33.7 ppm moxidectin and the clinical signs described below were observed less frequently in the 75 ppm group compared to the groups receiving the higher doses of moxidectin (100, 125, 150 ppm). In the 75 ppm males including surviving animals, signs of toxicity included tremors, urine staining, and

hypersensitivity to touch mainly in males in Week 2 of the study and in 1/5 females on Day 28. Clinical signs were frequently observed in the higher dose moxidectin groups beginning on Day 6 including tremors, hypersensitivity to touch, hypersensitivity, urine stained fur, emaciation, and prostration.

Body Weights

Body weights were significantly decreased in female survivors at the 100 ppm and 125 ppm levels by 20% and 15% compared to vehicle control values during the first week of the study and continued to be depressed in the single remaining female in the 100 ppm group compared to control values for the remainder of the study period. Male body weights tended to be lower in the first week of the study in the 100 and 125 ppm groups, but not significantly so. Thereafter only single male animals remained in these groups and the body weights for these animals were similar to control values.

Body weight gains in females in the 100 and 125 ppm groups were significantly reduced in the first week compared to control values. In the single remaining animals in each of these groups after Week 1, body weight gains were similar to control values. In males in the 125 ppm group, body weight gains were significantly reduced in the first week. In later weeks, only single males remained in the 100 and 125 ppm groups and their body weight gains were similar to control values. Body weight gains for the 75 ppm males were slightly depressed during the first two weeks of the study and during the first week in corresponding females, but the differences from control values were not significant.

Body weights and weight gains for both sexes at the 33.7 ppm level were similar to those of the untreated controls at all measurement intervals during the 28-day study period.

Feed Consumption

Reportedly food intake was not altered in the surviving animals in the moxidectin-dose groups compared to control values.

Moxidectin Consumption in Rodent Chow

The moxidectin daily dose consumed for each dose group in surviving animals is shown in Table 19. Moxidectin intake was not calculated for the highest moxidectin dose (150 ppm) due to the rapid mortality of rats in this group. Females received a slightly greater mg/kg dose than males, but the average weekly dose for both sexes remained largely consistent for all four weeks of the study.

Table 19: Moxidectin Dietary Dose (mg/kg/day) in the 28-Day Mouse Study. (Table from the Study Report)

	AC 301,423 Dose in the Diet				
Time Interval	33.7 ppm	75 ppm	100 ppm	125 ppm	150 ppm
Males					
Week 1	6.1	13.9	18.2	22.8	----
Week 2	6.0	15.4	19.8	23.6	----
Week 3	7.2	18.3	25.9	22.1	----
Week 4	6.2	16.7	19.2	20.4	----
Average	6.4	16.1	20.8	22.2	----
Females					
Week 1	7.6	18.6	21.6	25.9	----
Week 2	6.8	17.3	25.0	----	----
Week 3	7.1	20.5	27.8	----	----
Week 4	7.6	20.9	27.9	----	----
Average	7.3	19.3	25.6	25.9	----
Study Average	6.9	17.7	23.2	24.1	

Ophthalmoscopy: Not performed

ECG: Not performed

Hematology

The following hematology parameters were assessed: hematocrit, hemoglobin, erythrocyte counts, platelet counts, and leucocyte counts (total and differential).

Significantly increased platelet counts were observed in female mice in the 33.7/50 and 75 ppm dose groups. Also neutrophil and monocyte counts were significantly reduced in females in the 33.7/50 ppm dose group. Corresponding male values were not similarly affected. The Sponsor stated that the altered female values were still within normal limits for mice.

Clinical Chemistry: Not performed

Urinalysis: Not performed

Gross Pathology

The only gross pathology findings related to moxidectin administration were those related to the severely reduced food intake of the animals that died in the groups receiving 100, 125, and 150 ppm moxidectin.

Organ Weights

The following organs were weighed at necropsy: liver, kidney (paired organs), heart, spleen, testes without epididymis (males, paired organs), uterus and ovaries (females).

No organ weights were changed in any of the moxidectin dose groups compared to control values.

Histopathology

Adequate Battery: No: the battery of tissues and organs that were examined microscopically for histopathology was not comprehensive and included: liver, kidney, heart, spleen, lungs, stomach, small intestine, adrenal glands, thyroid glands, testes, epididymis, ovaries, uterus, and any other tissues with gross lesions. Notably, despite the observed CNS-related clinical signs, brain and nervous tissues were not examined microscopically.

Peer Review: no peer review was conducted.

Histological Findings
Reportedly, no histopathology clearly indicative of the cause of death for the animals that died in the high dose groups was detected. In the surviving male and female animals in the 33.7/50 and 75 ppm dose groups, no moxidectin-related histopathology was observed.

Special Evaluation: No special evaluations were conducted

Toxicokinetics: Not performed

Dosing Solution Analysis

Each dose of moxidectin was prepared in weekly or biweekly batches and a representative 100 gram sample from each batch was frozen and retained for analysis. Duplicate 20 gram samples of each sample were assayed using a validated HPLC method with a detection range of 5-150 ppm moxidectin.

The acceptance criteria for actual concentrations of the moxidectin batches was considered to be 85 to 115% of the nominal values. The average actual concentrations of all the samples for all of the moxidectin dose batches were within ± 10% of the nominal concentrations except for samples for the lowest moxidectin dose (50 ppm). For this group, the mean concentration was determined to be 33.7 ppm largely due to the double batch fed to rats in Weeks 2 and 3 which was determined to have a concentration of 17.5 ppm or 34.9% of the nominal concentration.

Study title: AC-301423: A 28-Day Rat Feeding Study.

Study no.:	RPT-77295
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/16/1987
GLP compliance:	No

QA statement:	No
Drug, lot #, and % purity:	Moxidectin, Lot # AC 5561-82, purity of 91.4%

Key Study Findings

- This study was well executed, but some deficiencies limited the adequacy of the study. A better study design would have included clinical pathology measurements, where possible, in animals that died before the scheduled termination. Each study group included 5 animals/sex instead of the normal 10/sex/group. Also, despite the observed CNS-related clinical signs, brain weights were not obtained and brain and nervous tissues were not examined for histopathology.
- All the rats receiving 400 and 600 ppm dietary moxidectin were found dead or were sacrificed moribund during the first 8 days of the study. Also two female rats at the 200 ppm level were found dead on Study Days 19 and 22.
- Beginning on the first day of dosing, animals in the 200, 400, and 600 ppm groups exhibited clinical signs including: depression, ataxia, diuresis, dark material around the eyes and/or nose, hypersensitivity to touch, and piloerection.
- Body weights and food consumption was severely decreased (up to 70%) in the 400 and 600 ppm groups and to a lesser, but still significant degree in the 200 ppm animals.
- A multi-organ histopathology finding of diffuse atrophy in the animals that died prematurely in the 200, 400, and 600 ppm groups was considered related to severe weight loss and decreased food consumption in the afflicted groups.
- Based on mortality, clinical signs, decreased food intake, and body weight loss with doses ≥ 200 ppm moxidectin (22.8 mg/kg), the NOAEL was considered to be 100 ppm (average moxidectin dose of 12.2 mg/kg).

Methods

Doses:	0, 100, 200, 400, and 600 ppm moxidectin. Moxidectin intake was calculated on a mg/kg/ basis for the two highest dose groups. For the 100 and 200 ppm groups, the average daily intake of moxidectin calculated over all four weeks of the study was 12.2 mg/kg and 22.8 mg/kg respectively
Frequency of dosing:	Ad libitum; drug substance in animal food
Route of administration:	Oral, moxidectin was powdered and mixed in rodent chow.
Dose volume:	NA
Formulation/Vehicle:	Rodent chow
Species/Strain:	Crl:CD®(SD)BR Sprague Dawley rats
Number/Sex/Group:	5/sex/group

Age:	Approximately 4 weeks of age
Weight:	Males: 83-90 g; females: 76-83 g.
Satellite groups:	none
Unique study design:	Sprague-Dawley rats received 0, 100, 200, 400, and 600 ppm dietary moxidectin for 28 consecutive days before euthanasia on Day 29.
Deviation from study protocol:	Multiple study protocol deviations were reported. However, none was considered to have altered the study results.

Observations and Results

Observations	Schedule
Mortality and Clinical Signs	All animals were observed daily. At least once per week each animal was removed from its cage and carefully examined for abnormalities and clinical signs. Animals found dead were removed immediately, examined for gross pathology and tissues were preserved.
Body Weights	Body weight was collected once on Day 0 and weekly thereafter.
Food Consumption	Food consumption was determined weekly.
Clinical Pathology (hematology and clinical chemistry)	Blood samples were collected for hematology and clinical chemistry analysis at necropsy. Rats were fasted overnight prior to collection.
Necropsy	Animals were necropsied on Day 29

Mortality

All animals at the 100 ppm level survived the 28-day feeding period. All rats receiving 600 ppm and 400 ppm moxidectin either were found dead or were sacrificed moribund during the first 8 days of the study. Two female rats at the 200 ppm level were found dead on study days 19 and 22.

Clinical Signs

Clinical signs were observed beginning on Day 1 of the study in surviving animals in the 200, 400 and 600 ppm dose groups. Clinical signs included: depression, ataxia, diuresis, dark material around the eyes and/or nose, hypersensitivity to touch and piloerection. Beginning on Day 6 in the 400 and 600 ppm groups, additional clinical signs included tremors and salivation. Clinical signs were generally more prevalent and severe in female rats. In the 100 ppm moxidectin group, clinical signs were limited to two observations of hypersensitivity to touch on Days 2 and 3 of the study.

Body Weights

Body weights and weight gains were severely and significantly decreased by 40-60% in male and female survivors in the 400 ppm and 600 ppm groups during the first week of the study. Body weights and weight gains were significantly decreased by 10-20% at most measurement intervals in both sexes in the 200 ppm moxidectin dose group during the course of the study. The reduction in body weight gains correlated with the

decreased food consumption. Body weights and weight gains for both sexes at the 100 ppm level were comparable to those of the untreated controls at all measurement intervals during the 28-day study period.

Feed Consumption

Food intake for both sexes at the 600 ppm and 400 ppm levels was severely reduced up to 77% in the first week of the study. Food intake was significantly reduced by 5%-22% at all measurement intervals in male rats receiving 200 ppm compared to control values. In female rats in the 200 ppm group, food intake was significantly reduced during Week 1 and Week 3 by 35% and 42% respectively, with nonsignificant reductions of 8 -9% and in Weeks 2 and 4. Food intake for both sexes in the 100 ppm moxidectin dose group was generally similar to or in excess of those of the untreated controls during the 28-day study period.

Moxidectin Consumption in Rodent Chow

The amounts of moxidectin (mg/kg/day) consumed by the study rats in each dose group is shown in Table 20. Only limited data was collected for the highest two dose groups due to mortality in these groups. The average weekly amounts were largely consistent for all four weeks of dosing and average weekly values did not vary greatly between the sexes.

Table 20: Moxidectin Dietary Dose (mg/kg/day) in the 28-Day Rat Study. (Table from the Study Report)

	AC 301,423 Dose in the Diet			
Time Interval	100 ppm	200 ppm	400 ppm	600 ppm
Males				
Week 1	14.4	25.1	27.2	31.2
Week 2	13.5	25.9	----	----
Week 3	11.5	22.7	----	----
Week 4	9.8	20.2	----	----
Average	12.3	23.5	----	----
Females				
Week 1	14.3	22.2	25.5	----
Week 2	13.0	27.3	----	----
Week 3	11.1	16.2	----	----
Week 4	10.0	23.0	----	----
Average	12.3	22.2	----	----
Study Average	12.2	22.8	----	----

Ophthalmoscopy: Not performed

ECG: Not performed

Hematology

The following hematology parameters were measured with a Coulter counter: hemoglobin, hematocrit, RBC, WBC (total and differential) and platelets. Hematology parameters were only measured in samples from surviving animals in the 100 and 200 ppm moxidectin dose groups after overnight fasting.

No changes in any of the parameters relative to control values were observed.

Clinical Chemistry

The following serum chemistry parameters were measured using a Prisma autoanalyzer: gamma glutamyl transpeptidase (GGTP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), alkaline phosphatase, glucose, total protein, total bilirubin, albumin, creatinine, sodium, potassium, chloride. Serum chemistry was only measured in surviving animals in the 100 and 200 ppm groups after overnight fasting.

Serum SGOT levels were significantly elevated in the 100 and 200 ppm males, but females in the same groups were unaffected, and the male values were still within the normal range. Serum albumin levels were significantly decreased in both sexes in the 200 ppm group and total protein was significantly decreased in 200 ppm females. The decreased albumin and total protein may have been related to reduced food consumption.

Urinalysis: Not performed

Gross Pathology

At necropsy, animals were examined externally, then the body cavities were carefully opened and vital organs were examined *in situ*.

Other than the emaciated condition of several animals in the 400 and 600 ppm groups, no remarkable gross pathology findings related to moxidectin consumption were observed.

Organ Weights

The following organs were weighed: liver kidneys (paired organ weight), heart, spleen, adrenal glands (paired organ weight), testes without epididymis (paired organ weight; males), and uterus and ovaries (females). Organ weights were expressed as absolute weights and weights relative to body weight.

No organ weight changes were considered related to moxidectin administration.

Histopathology

Adequate Battery: The battery of examined tissues was not comprehensive. The organs and tissues examined microscopically for histopathology were: liver, kidney, heart, spleen, lungs, adrenal glands, thyroid glands with trachea, testes, epididymis, ovaries, uterus and other tissues with gross lesions.

Peer Review: No

Histological Findings
A histopathological finding of diffuse atrophy was observed in multiple organs (liver, kidneys, spleen, heart, adrenal glands, thyroid glands, testes, epididymides, uterus and ovaries) in animals that died prematurely in the 200, 400, and 600 ppm moxidectin groups. Surviving animals in the 200 ppm group did not demonstrate the same finding. The finding was attributed to severely reduced food intake and body weight loss in these animals.

Special Evaluation: Not performed

Toxicokinetics: Not performed

Dosing Solution Analysis

For each weekly batch of moxidectin in animal feed, a representative 100 gram sample was assayed on the same day of receipt or held frozen until analysis. Duplicate 20 g portions of each sample were assayed using a validated HPLC-UV method.

The range of actual concentrations for all preparation weeks ranged from 101 to 112 ppm, 205 to 209 ppm, 408 to 412 ppm, and 607 to 645 ppm for the 100, 200, 400, and 600 ppm moxidectin dose preparations. All the actual concentrations were within a ± 15% acceptance criteria.

Study title: AC-301423: A 13-Week Rat Feeding Study.

Study no.:	RPT-77312
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/29/1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin, Lot # AC5819-147, purity of 84.7%

Key Study Findings

- This study was appeared to be adequate for execution and largely so for design with a few exceptions. Clinical pathology measurements were only performed on surviving animals. However, gross pathology and histopathology were assessed in surviving animals and animals that died moribund.
- Three high-dose females (150 ppm, 12.23 mg/kg/day) were sacrificed in moribund condition before study termination. All other study animals survived.
- Clinical signs including lethargy, hypersensitivity to touch, aggressive behavior, tremors, ataxia, and urine stained coats occurred in high-dose animals. In the 100 ppm dose group hypersensitivity to touch was observed in both sexes.

Clinical signs diminished partially (females) or completely (males) over the course of the experiment.

- Food consumption and body weights were significantly decreased in high-dose animals.
- Based on the limited mortality, clinical signs, and decreased food consumption and body weights with the 150 ppm (12.23 mg/kg/day) moxidectin dose, the NOAEL was considered to be 100 ppm (7.99 mg/kg/day) moxidectin.

Methods

Doses:	0, 25, 50, 100, 150 ppm moxidectin. Based on food consumption analysis the daily average doses were estimated to correspond to 0, 1.95, 3.90, 7.99, and 12.23 mg/kg moxidectin for females and 0, 1.86, 3.79, 7.61, and 11.24 for males in the respective groups.
Frequency of dosing:	*Ad libitum*. Drug substance was powdered and mixed in the animal food (Purina 5002 certified laboratory rodent meal).
Route of administration:	oral
Dose volume:	NA
Formulation/Vehicle:	Animal food
Species/Strain:	Crl:CD® (SD)BR Sprague-Dawley rats
Number/Sex/Group:	20/sex/group
Age:	Approximately 4 weeks of age
Weight:	Males: 90 to 120 g; females: 90 to 110 g.
Satellite groups:	None
Unique study design:	Male and female albino Sprague Dawley rats were administered doses of 25, 50, 100, and 150 ppm dietary moxidectin for 13 weeks. Animals were euthanized the day after the end of dosing.
Deviation from study protocol:	Study deviations were not included in the study report.

Observations and Results

Table 21: Observation Schedule

Observations	Schedule
Mortality and Clinical Signs	All animals were observed daily. At least once per week each animal was removed from its cage and carefully examined for abnormalities and clinical signs. Animals found dead were removed immediately, examined for gross pathology, and tissues were preserved.
Body Weights	Body weight was collected once on Day 0 and weekly thereafter.
Food Consumption	Food consumption was determined weekly.

Clinical Pathology	Blood samples were collected for hematology and clinical chemistry analysis at necropsy. Rats were fasted overnight prior to collection.
Necropsy	Animals were necropsied on Day 29

Mortality

All of the male and female animals in the 25 ppm, 50 ppm, and 100 ppm dose groups as well as all of the males and 17/20 females in the 150 ppm group survived throughout the study. Three high-dose females died or were sacrificed in moribund condition before study termination. One high-dose female was sacrificed in moribund condition on Day 15, one female was found dead on Day 20 and one female was found dead on Day 55.

Clinical Signs

No clinical signs were observed in the 25 and 50 ppm dose groups. In the 100 ppm dose group hypersensitivity to touch was observed in both sexes beginning on Day 5 and continuing to Day 14. In the 150 ppm dose group, clinical signs were observed in both sexes beginning on Day 3 of the study including: lethargy; hypersensitivity to touch, aggressive behavior, tremors, ataxia, and urine stained coats. Clinical signs were more prevalent and severe in female rats than in corresponding male rats and tended to diminish during the later stages of dosing. By Week 3, clinical signs in male rats had disappeared in the majority of the test animals. Female rats continued to exhibit signs of toxicity throughout the 13-week study period although signs were diminished in severity.

Body Weights

Weekly body weights at the 150 ppm level were significantly decreased by 6 -13% in males and 8 -16% in females during the first six weeks of the study. Body weights tended to be depressed in both sexes for the remainder of the study in the 150 ppm group. Corresponding weekly weight gains were significantly decreased by 35% and 58% in males and females respectively during the first week of the study and were non-significantly reduced at most measurement intervals for the remainder of the study period. Cumulative body weight gains over the course of the experiment were non-significantly reduced approximately 5% in male rats in the 150 ppm group and significantly reduced 12% in corresponding females. Body weights for male rats at the 100 ppm level were in general somewhat lower than those of the untreated controls and body weights of corresponding female rats were comparable to those of the untreated controls. Weight gains for 100 ppm males were significantly reduced by 13% during the first week of the study and in general somewhat lower than corresponding controls at most measurement intervals while corresponding female weight gains were similar to control values. Total gains for 100 ppm male rats were reduced approximately 6% in comparison to the untreated controls. Total gains for 100 ppm females were comparable to those of the untreated controls. Body weights and weight gains for both sexes at the 50 ppm and 25 ppm levels were comparable to or in excess of those of the untreated controls.

Feed Consumption

Food intakes for both sexes at the 150 ppm dosage level were significantly reduced by 12-25% in males and 14-29% in females during the first two weeks of the study and

then generally comparable to the untreated controls for the remainder of the study period. Food intakes for both sexes at the 100 ppm, 50 ppm and 25 ppm levels were generally comparable to or in excess of those of the untreated controls during the 13-week study period.

Dietary Moxidectin Consumption

The average daily intake of moxidectin in rodent chow is shown in Table 22. Food intake reduced over time such that for all groups moxidectin intake in Week 13 was approximately one half to one third of the moxidectin intake in Week 1.

Table 22: Average Daily Intake (mg/kg/day) of Moxidectin in the 13-Week Toxicology Study in Rats. (Table from the Study Report)

	AC 301,423 Dose in the Diet			
Study Period	25 ppm	50 ppm	100 ppm	150 ppm
		Males		
WEEK 1	3.18	6.30	12.29	15.92
WEEK 2	2.78	5.61	11.10	16.67
WEEK 3	2.40	4.87	9.30	15.56
WEEK 4	2.09	4.39	8.30	13.12
WEEK 5	1.84	3.81	7.94	11.98
WEEK 6	1.82	3.62	7.14	10.72
WEEK 7	0.71	3.39	6.68	9.86
WEEK 8	2.04	3.31	7.03	9.83
WEEK 9	1.73	2.97	6.23	8.96
WEEK 10	1.51	2.87	5.71	8.88
WEEK 11	1.43	2.87	6.05	8.31
WEEK 12	1.32	2.65	5.82	8.00
WEEK 13	1.30	2.67	5.32	8.34
Average	1.86	3.79	7.61	11.24
		Females		
WEEK 1	3.20	6.34	12.34	14.38
WEEK 2	2.73	5.42	11.00	16.34
WEEK 3	2.50	4.79	10.70	18.79
WEEK 4	2.40	4.44	8.72	15.11
WEEK 5	2.20	4.18	8.77	15.08
WEEK 6	2.07	3.97	7.90	13.12
WEEK 7	0.82	3.60	7.76	12.46
WEEK 8	2.14	3.45	7.26	12.15
WEEK 9	1.81	3.47	7.36	11.94
WEEK 10	1.77	3.30	6.94	11.09
WEEK 11	1.61	3.15	6.63	10.06
WEEK 12	1.56	2.88	5.92	9.69
WEEK 13	1.64	3.15	7.44	11.46
Average	2.03	4.01	8.36	13.21
Study Average	1.95	3.90	7.99	12.23

* - Formula used to calculate AC 301,423 intake: $\frac{\text{Food Intake (kg/day)} \times \text{Dose (ppm)}}{\text{Average body weight for Study Period (kg)}}$

Ophthalmoscopy: Not performed

ECG: Not performed

Hematology

The following hematology parameters were measured with a coulter counter: hemoglobin, hematocrit, RBC, WBC (total and differential), and platelets. Blood samples for hematology assessments were acquired only from surviving animals at the termination of the study.

Platelets were significantly decreased in male rats at the 50 ppm and 150 ppm dose groups. In the absence of any changes in the 100 ppm males or in females in any of the moxidectin groups, the changes were not considered to be related to moxidectin administration. No other significant changes were observed in any of the other hematological parameters measured during the study.

Clinical Chemistry

The following serum chemistry parameters were measured using a Prisma autoanalyzer: gamma glutamyl transpeptidase (GGTP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), alkaline phosphatase, glucose, total protein, total bilirubin, albumin, creatinine, sodium, potassium, chloride. Blood samples for serum chemistry assessments were acquired only from surviving animals at the termination of the study.

Values for albumin were significantly elevated by 10% in males in the 100 ppm moxidectin group, but not in any other moxidectin groups including the high-dose group. Because albumin values were not altered in any moxidectin group for females and due to the lack of dose-dependency in males, the albumin results were not considered related to moxidectin administration. No other serum chemistry parameters were significantly altered in a dose-dependent manner.

Urinalysis

The following urinalysis parameters were assessed in surviving animals: appearance, specific gravity, yeast, bilirubin, microscopic examination of sediment, color, glucose, occult blood, urobilinogen, pH, protein, ketones, and nitrite.

No moxidectin-related changes in any uninalysis parameter were observed.

Gross Pathology

Surviving animals and as well as animals that died prematurely were examined externally and internally for gross pathology.

No gross pathology findings attributable to moxidectin administration were observed.

Organ Weights

The following organs were weighed at necropsy in surviving animals: liver, kidney (paired organs), heart, spleen, brain, adrenal glands (paired organs), thyroid glands (paired organs), testes without epididymis (males, paired organs), uterus and ovaries (females). Both absolute weights and weights relative to body weight were determined.

Significant increases in absolute kidney and adrenal weights was observed in females in the 150 ppm dose group and the relative weights of liver, kidney, heart, and adrenal

gland weights were also significantly increased. Also, in females in the 100 ppm group, absolute and relative adrenal gland weights were significantly increased. Changes observed in the liver and heart were considered to be secondary to the lower terminal body weights observed in females in the 150 ppm group. No correlating histopathology was observed in the affected organs in any group.

Relative testes weights were significantly increased in male rats at the 100 ppm level, but no correlating histopathology was observed, and similar increases did not occur in high-dose males.

There were no statistically significant changes in any of the absolute or relative organ weights in male rats at the 150 ppm level or in either sex in the 25 and 50 ppm moxidectin groups.

Histopathology

Adequate Battery: Yes. The following large battery of organs were examined microscopically for histopathology: adrenal glands, aorta, bone with marrow, brain (3 levels), cecum, colon, duodenum, epididymis, esophagus, eye with optic nerve, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph nodes, mandibular lymph nodes, mammary gland, muscle, ovaries (females), pancreas, parathyroid, pituitary, prostate, salivary gland, seminal vesicles, sciatic nerve, skin, sternum, spinal cord, spleen, stomach, testes (males), thymus, thyroid glands, tongue, trachea, urinary bladder, uterus (females), vagina (females), and any other tissues with gross lesions. Histopathology assessments were performed for surviving animals and also in animals that died prematurely.

Peer Review: A peer review of the histopathology findings was not performed.

Histological Findings

No histopathology findings considered related to moxidectin administration were reported.

Special Evaluation: Not performed

Toxicokinetics: Not performed

Dosing Solution Analysis

Concentration Analysis: Each dose of moxidectin in feed was prepared in weekly batches. For each batch of feed, a representative 100 gram sample was sealed in a plastic bag and analyzed for concentration. Duplicate 20 gram portions of each rodent sample were assayed by a HPLC method validated for a moxidectin range of 5 to 160 ppm.

Moxidectin was not detected in the control diet samples. For the 25 ppm dose samples, the actual concentration values ranged from 87.9% to 110.6% of the nominal concentration with an average of 100.0%. For the 50 ppm dose samples, actual moxidectin concentrations ranged from 99.2 to 109.6% of nominal with an average of

103%. At the 100 ppm concentration, actual concentrations ranged from 97.4 to 108.7% of the nominal concentration with an average of 102.6%, and for the 150 ppm concentration actual concentrations ranged from 90.9 to 109.5% of the nominal concentration with an average of 97.3%.

Homogeneity and Stability Analysis: Triplicate 100 gram samples of medicated diet batches for the 25 and 150 ppm doses were scooped from each of six places in the mixing blender: top left, top right, middle left, middle right, bottom left and bottom right, and analyzed for moxidectin concentration. For determination of stability, the remaining samples were placed in typical rat feeder jars located in the animal room and exposed to ambient light, humidity and temperature. After 7 and 14 days of exposure one sample from each of the blender locations was sealed in a plastic bag and submitted for analysis.

The mixing of the samples was found to be acceptable with 100% mean homogeneity for the 25 ppm samples and 109.8% mean homogeneity for the 150 ppm samples. After 7 and 14 days the concentration of the 25 and 150 ppm samples was at least 97% of nominal indicating adequate stability.

Study title: Moxidectin: 28-Day Range Finding Study in Purebred Beagle Dogs with AC-301423

Study no.:	RPT-77334
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 17, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AC 301423 (moxidectin), Lot # AC-6297-47, purity of 85.3%

Key Study Findings

- This study was not adequate in its design in that it did not include serum chemistry measurements and the panels of organs and/or tissues that were examined for weights and histopathology were too small to be adequate.
- In male and female dogs in the 80 and 160(50) ppm groups, moxidectin-related clinical signs included tremors, languid appearance, thinness, mydriasis, ataxia, emesis, prostration, dehydration, sensitivity to touch, slight salivation, few or no feces, and inability to hold the head up.
- High-dose animals receiving 160 ppm moxidectin lost weight in the first week of dosing, but gained weight after the dosing interruption and resumption with a 50 ppm dose.
- Moxidectin treatment resulted in a dose-dependent reduction in mean absolute testes weights as well as reduced testes weights relative to body weight and

brain weight compared to control values. Because only two male animals were present in each group, significant differences were not determined.

- Males in the 80 and 160(50) ppm moxidectin groups exhibited fewer spermatocytes and spermatogonia in their testes compared to the two control male dogs. It was not clear if the testicular atrophy and reduced spermatogenesis was a direct effect of moxidectin, or an indirect effect of delayed sexual maturation due to reduced food intake in the two highest dose groups.
- Based on clinical signs in the 80 and 160(50) ppm dose groups and reduced testicular weights and spermatogenic activity in the same groups, the NOAEL was considered to be the low moxidectin dose (20 ppm).

Methods

Doses:	0, 20, 80, and 160(50) ppm (the high dose group received 160 ppm for Days 1-5, 0 ppm for Days 6-7 and 50 ppm for Days 8-29. The moxidectin doses were not calculated on a mg/kg/day basis
Frequency of dosing:	Ad libitum in feed provided for 1 hour per day.
Route of administration:	Oral
Dose volume:	NA
Formulation/Vehicle:	Moxidectin was premixed in dog chow in a blender until a homogeneous mixture was achieved which was then blended in a larger blender with more dog chow (Purina certified canine diet meal #5007).
Species/Strain:	Beagle dogs
Number/Sex/Group:	2/sex/group
Age:	18-21 weeks old at treatment initiation
Weight:	Males: 6.2 to 7.8 kg for the males and 4.2 to 5.7 kg for the females.
Satellite groups:	None
Unique study design:	Due to the severity of the findings in the high-dose group (Group 4, 160 ppm moxidectin), treatment with 160 ppm moxidectin was stopped on Day 5 and dogs were fed control diet for two days (Days 6 and 7) before resuming treatment with 50 ppm moxidectin on Day 8.
Deviation from study protocol:	Multiple study deviations were identified, but the deviations were not considered to have altered the study results.

Observations and Results

Observations	Schedule
Mortality and Clinical Signs	All animals were observed twice daily for mortality and morbundity. Beginning during Week 2, each dog underwent careful cageside observations once

	daily approximately 3 hours after feeding.
Body Weights	Individual body weights were recorded three times prior to treatment and weekly thereafter.
Food Consumption	Food consumption was recorded prior to treatment (three times) and weekly thereafter.
Ophthalmoscopy	Ophthalmoscopic examinations were performed on all animals prior to initiation and at termination using indirect and slit lamp (if necessary) ophthalmoscopes.
Clinical Pathology	Blood samples were collected for hematology analysis prior to treatment and at necropsy. Urine was collected according to the same schedule. All animal were fasted for food and water overnight prior to collection. Serum chemistry analysis was not performed.
Necropsy	Animals were necropsied on Day 29

Mortality

No dogs died prior to the terminal sacrifice during the study.

Clinical Signs

In Group 3 and 4 animals, moxidectin-related clinical signs included tremors, languid appearance, thinness, mydriasis, ataxia, emesis, prostration, dehydration, sensitivity to touch, slight salivation, few or no feces, and inability to hold the head up. Clinical signs were first noted on Day 4. Due to the severity of the findings in the high-dose group (Group 4, 160 ppm moxidectin), treatment with 160 ppm moxidectin was stopped on Day 5 and dogs were fed control diet for two days (Days 6 and 7) before resuming treatment with 50 ppm moxidectin on Day 8. Despite exhibiting clinical signs, animals in Group 3 receiving 80 ppm moxidectin continued with uninterrupted dosing.

Body Weights

Group 4 animals experienced approximately 6% mean body weight loss in conjunction with reduced food consumption during Week 1. After the dosing interruption and resumption of dietary dosing at the lower dose of 50 ppm moxidectin, Group 4 animals exhibited an increase in weekly mean body weights for the duration of the study. Group 3 animals also lost weight in the first two weeks of the study followed by increased mean weekly body weights thereafter. Groups 1 and 2 animals gained weight at all the weekly measurements.

Feed Consumption

Food consumption mirrored the pattern of weight loss. In the first week, Group 4 males and females consumed approximately 66% and 76% less food than the respective control animals. Group 3 males and females consumed approximately 36% and 31% less food than control animals in the same period. By Week 3 both groups of animals consumed food in amounts similar to control animals.

Moxidectin Consumption in Dog Chow: Not included in the study report

Ophthalmoscopy

Opthalmoscopic examinations were performed on all animals prior to initiation and at termination using indirect opthalmoscope and slit lamp (if necessary). Examinations were performed by a veterinary ophthalmologist.

No moxidectin-related ocular changes were observed.

ECG: Not performed

Hematology

Blood was collected on all dogs at baseline prior to initiation of dosing and during Week 4. The following hematology parameters were measured: mean cell volume, mean cell hemoglobin (MCH), mean cell hemoglobin concentration, platelet count, reticulocyte count, total leukocyte count, corrected leukocyte count, differential leukocyte count, absolute reticulocyte count, erythrocyte count, cell morphology, hematocrit, and hemoglobin.

Hematology parameter measurements in the moxidectin-treatment groups were similar to control values.

Clinical Chemistry: Not performed

Urinalysis

The following urinalysis parameters were measured: appearance and color, specific gravity, pH, bilirubin, glucose, reducing substances, ketones, occult blood, protein, urobilinogen, and microscopic examination of sediment. Urine was collected on all dogs at baseline prior to initiation of dosing and during Week 4.

Urinalysis parameters were not significantly altered by treatment with moxidectin.

Gross Pathology

On Day 29, all animals were euthanized and necropsied.

No moxidectin-related gross pathology findings were observed.

Organ Weights

The following organs were weighed: liver with drained gallbladder, kidneys, adrenals, testes, thyroid with parthyroids, brain, and ovaries.

Moxidectin treatment resulted in a dose-dependent reduction in mean absolute testes weights as well as reduced testes weights relative to body weight and brain weight compared to control values. In the high-dose males, absolute and relative testes weights were reduced more than 50% compared to control values. Because only two male animals were present in each group, significant differences were not determined.

Table 23: Mean Absolute and Relative Testes Weights in Dogs Administered Dietary Moxidectin for 28 Days.

Groups	Absolute Testis Weight	Testis Weight Relative to Body Weight	Testis Weight Relative to Brain Weight

Group 1	5.31	0.68	0.71
Group 2	3.94	0.50	0.55
Group 3	2.42	0.33	0.35
Group 4	2.28	0.34	0.34

Histopathology

Adequate Battery: No; a small panel of tissues was examined for histopathology including: adrenals, brain, heart, kidneys, liver, ovaries, testes, thyroid/parathyroids, gross lesions, and lung with mainstem bronchi.

Peer Review: no peer review was conducted

Histological Findings

The testes in Group 3 and 4 males exhibited decreased relative numbers of spermatocytes and precursor cells, when compared to the two control male dogs (Table 24). One male dog each in Groups 3 and 4 had seminiferous tubules that were essentially free of spermatocytes and precursor cells, while the remaining animal in each group had reduced production of spermatogonia and spermatocytes. One confounding factor in the interpretation of these findings is that dogs achieve sexual maturity at varying ages, and the microscopic differences could be explained as normal variability in the onset of sexual maturity. The results may also indicate a delay in onset of sexual maturation due to reduced food intake related to moxidectin administration in the males in Groups 3 and 4 or the results may represent a direct toxicological effect of moxidectin. The testes of the 20 ppm dogs were comparable in spermatogenic activity to the control animals.

Table 24: Histopathology Findings in Testes of Dogs Administered Dietary Moxidectin for 28 Days. (Table from the Study Report)

TESTIS (TE) ..... NUMBER EXAMINED:	2	2	2	2
NOT REMARKABLE:	0	0	0	0
--SPERMATOGENIC ACTIVITY				
GRADE "1" : ESSENTIALLY CONTAINS ONLY SERTOLI CELLS 1>	0	0	1	1
GRADE "2" : SOME SPERMATOGONIA / SPERMATOCYTES 2>	0	0	1	1
GRADE "3" : NUMEROUS SPERMATOGONIA / SPERMATOCYTES 3>	2	2	0	0
GRADE "4" : SPERMATIDS WITH SOME MATURE SPERMATOZOA TL>	2	2	2	2
GRADE "5" : NUMEROUS MATURE SPERMATOZOA MN>	3.0	3.0	1.5	1.5

Special Evaluation: Not performed

Toxicokinetics: Not performed

Dosing Solution Analysis

Moxidectin was powdered with a mortar and mixed in animal feed and stored refrigerated on a weekly basis. Samples of feed containing moxidectin were taken prior to initiation of treatment and weekly thereafter then shipped for analysis. All diet samples were stored at -8°C and assayed within a month of receipt. For homogeneity and stability tests, 100 gram samples were taken from the right and left sides of the top, middle, and bottom of the low- and high-dose diet preparations. For the mixing study, two representative samples of each weekly batch of each concentration of moxidectin in chow was collected and frozen, with later analysis of one of the samples. Duplicate 20

gram portions of rodent meal samples were assayed for homogeneity and stability (low and high doses) and concentration (all doses) by two HPLC methods with lower limits of detection of 1 and 5 ppm respectively.

The homogeneity results indicated actual concentration for all layers that were ±10% of the nominal concentration. The average actual concentrations for the stability samples were all within the acceptance criteria of ±10% of the nominal concentrations. For all of the weekly samples, the actual moxidectin concentrations were within the acceptance criteria of ±10% of the nominal concentrations.

Study title: Moxidectin: 91-Day Dietary Toxicity Study in Purebred Beagle Dogs with AC-301423.

Study no.:	RPT-77335
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 14, 1988
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC-301423)

Key Study Findings

- The study was adequate in design and execution.
- Clinical signs that were observed in high-dose (60 ppm) males and females included: lacrimation, thin appearance, tremors, slight salivation, and slight ataxia.
- Mean body weight gains were significantly lower in high-dose males by approximately 44% and non-significantly lower in high-dose females by approximately 40% compared to control values. Nonsignificant reductions in body weight of 17% in high-dose males and by 20% in high-dose females compared to control values were observed.
- Mean cumulative food consumption was significantly reduced by approximately 29% and 28% in high-dose males and females respectively.
- The NOAEL value was considered to be 30 ppm moxidectin (1.59 mg/kg/day in males and 1.66 mg/kg/day in females).

Methods

Doses:	0, 10, 30, and 60 ppm. Upon analysis of food intake, the ppm doses were estimated to correspond to approximate moxidectin doses of 0, 0.3, 0.9, and 1.6 mg/kg/day.
Frequency of dosing:	*Ad libitum* in food provided 1 hour per day.
Route of administration:	Oral; moxidectin was ground to a fine powder then homogeneously mixed into food.

Dose volume:	NA
Formulation/Vehicle:	none
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group
Age:	Approximately 4-6 weeks old at study initiation
Weight:	Males: 4.9 to 7.2 kg; Females: 4.9 to 6.9 kg at dosing initiation.
Satellite groups:	None
Unique study design:	Male and female Beagle dogs were administered dietary moxidectin in dog chow for 91 days. Dogs were allowed access to food for 1 hour per day. On Day 92, the dogs were euthanized then necropsied.
Deviation from study protocol:	Three minor protocol deviations were reported. However, none was considered to have altered the results.

Observations and Results

Observations	Schedule
Mortality and Clinical Signs	All dogs were observed twice daily for mortality and morbundity. Each dog was observed cageside once daily approximately 4-6 hours after feeding. Physical exams were performed once a week
Body Weights	Individual body weights were recorded pretreatment, at the beginning of dosing, and weekly thereafter.
Food Consumption	Food consumption was recorded daily during the week before the start of dosing and weekly thereafter.
Clinical Pathology (hematology and clinical chemistry)	Blood was collected from all surviving dogs for hematology and serum chemistry measurements in Weeks 2, 7, and 13. Urine was collected according to the same schedule
Ophthalmoscopic Examinations	Ophthalmoscopic examinations were performed on all animals prior to initiation and prior to terminal sacrifice using an indirect ophthalmoscope and slit lamp.
Necropsy	Animals were sacrificed after at least 90 days of treatment.

Mortality

No animals died prematurely in this study.

Clinical Signs

Clinical signs that were observed in both males and females in the high-dose group (60 ppm) included: lacrimation, thin appearance, tremors, slight salivation, and slight ataxia.

Body Weights

Mean body weight were reduced by approximately 17% in high-dose males and by 20% in high-dose females at the end of dosing compared to control values. The differences were not significant, but a trend toward moxidectin dose-dependent weight reduction was evident throughout the experiment. At the end of the study, mean body weight

gains were significantly lower in high-dose males by approximately 44% and non-significantly lower in high-dose females by approximately 40% compared to control values.

Feed Consumption

In both males and females, a moxidectin dose-dependent trend toward reduced food consumption was apparent in all the weekly measurements throughout the study. Over the course of the study, mean cumulative food consumption was significantly reduced by approximately 29% and 28% in high-dose males and females respectively.

Mean Moxidectin Consumption

Mean consumption of moxidectin in units of mg/kg/day for all the moxidectin dose groups is shown below in Table 25. Female consumption was slightly higher than in males.

Table 25: Mean Moxidectin Consumption (mg/kg/day) in Male and Female Dogs in the 91-Day Dietary Toxicity Study in Dogs. (Table from the Study Report)

GROUP AND DOSE LEVEL (PPM)	WEEK:	13	MEANS[a] WKS 1-13
		MALES	
1 .000	MEAN	.00	.00
	S.D.	.00	.00
	N	4	13
2 10.000	MEAN	.27	.29
	S.D.	.03	.02
	N	4	13
3 30.000	MEAN	.81	.87
	S.D.	.09	.06
	N	4	13
4 60.000	MEAN	1.52	1.59
	S.D.	.04	.09
	N	4	13
		FEMALES	
1 .000	MEAN	.00	.00
	S.D.	.00	.00
	N	4	13
2 10.000	MEAN	.28	.30
	S.D.	.03	.02
	N	4	13
3 30.000	MEAN	.78	.90
	S.D.	.10	.08
	N	4	13
4 60.000	MEAN	1.72	1.66
	S.D.	.23	.09
	N	4	13

[a] Taken from average weekly compound consumption.

Ophthalmoscopy

No moxidectin-related ophthalmoscopy findings were observed.

ECG: Not performed

Hematology

The following hematology parameters were measured: mean cell volume, mean cell hemoglobin (MCH), mean cell hemoglobin concentration, platelet count, reticulocyte count, total leukocyte count, corrected leukocyte count, differential leukocyte count, absolute reticulocyte count, erythrocyte count, cell morphology, hematocrit, and hemoglobin.

No changes in hematology parameters were considered to be related to moxidectin administration.

Clinical Chemistry

The following serum chemistry parameters were measured: albumin, creatinine, carbon dioxide, gamma glutamyltransferase, glucose, sodium, potassium, chloride, alanine aminotransferase, total bilirubin, total cholesterol, albumin/globulin ratio, blood urea nitrogen, creatine kinase, globulin, serum alkaline phosphatase, calcium, inorganic phosphorus, aspartate aminotransferase, serum lactate dehydrogenase, direct bilirubin, total protein.

No mean values for any of the measured serum chemistry parameters were significantly altered in the moxidectin groups compared to control values. However, two high-dose dogs, one male and one female exhibited increased serum alanine aminotransferase levels in Weeks 7 and 13 that were 2-3 times as high as the pretreatment and mean concurrent control values. Also 3/4 males and 3/4 females in the high-dose group exhibited increased serum alkaline phosphatase, approximately 1.5-fold higher than mean concurrent control levels particularly in Week 7. The moderate elevations in alkaline phosphatase may have been a result of reduced food consumption in the affected animals. Alternatively, the results in high-dose animals may represent mild, incidental increases in liver enzymes or possibly that some dogs are more sensitive to minimal liver toxicity associated with chronic exposure to high doses of moxidectin.

Urinalysis

The following urinalysis parameters were measured: appearance and color, specific gravity, pH, bilirubin, glucose, reducing substances, ketones, occult blood, protein, urobilinogen, and microscopic examination of sediment.

Urinalysis parameters were not significantly altered by treatment with moxidectin.

Gross Pathology

No moxidectin-related gross pathology findings were observed.

Organ Weights

Organ weights were determined on an absolute and relative (to body weight; to brain weight) basis. The following organs were weighed: adrenals, brain with brainstem, testes/epididymides, heart, kidneys, ovaries, liver, lungs, parathyroid and thyroid (combined), pituitary, and spleen.

In Group 4 animals, significantly decreased absolute heart weights (females) and absolute pituitary weights (males) were observed. The relevance of these findings is not clear, and the findings did not correlate with specific histopathology findings.

Absolute and relative testicular weights tended to decrease in a moxidectin dose-dependent manner, but not to a significant degree (Table 26). The histopathology examination did not examine spermatogenesis but the trend toward lower testicular weights may correlate with histopathology findings of testis that were considered juvenile (immature) in 2/4, 3/4, 3/4, and 4/4 males in the control, 10 ppm, 30 ppm, and 60 ppm moxidectin groups respectively. The trend toward lower testicular weights may have been influenced by significantly reduced food consumption resulting in delayed sexual maturation in high-dose males.

Table 26: Absolute and Relative Testicular Weights in the 91-Day Toxicology Study in Dogs.

Group	Mean Testicular Weights (Mean ± SD)		
	Absolute Weights (g)	Relative to Terminal Body Weight	Relative to Brain Weight
Group 1: 0 ppm moxidectin	12.1 ± 5.6	0.12 ± 0.06	0.15 ± 0.07
Group 2: 10 ppm modidectin	10.8 ± 4.3	0.12 ± 0.04	0.14 ± 0.04
Group 3: 30 ppm moxidectin	9.5 ± 4.1	0.11 ± 0.03	0.13 ± 0.04
Group 4: 60 ppm moxidectin	8.4 ± 3.9	0.10 ± 0.04	0.11 ± 0.05

Histopathology

Adequate Battery: Yes, The following organs and tissues were examined for histopathology: Adrenals, aorta, bone and marrow (sternum), brain with brainstem, cecum, cervix, colon, epididymides, esophagus, eyes (both), gallbladder, heart, duodenum, ileum, jejunum, kidney (both), liver, lungs with mainstem bronchi (left and right), lymph.nodes (mediastinal and mesenteric), mammary gland, vagina, optic nerve, ovaries (both), pancreas, parathyroid, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin (mammary area), spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes (both), thymus, thyroids (both), tongue, trachea, urinary bladder, uterus, and any-gross lesions

Peer Review: A peer review was not conducted.

Histological Findings
No histopathology findings that were considered clearly related to moxidectin administration were identified. In microscopic analysis of testes, a greater incidence of juvenile (immature) testes occurred in the high-dose group compared to control values with incidences of 2/4, 3/4, 3/4, and 4/4 males in the control, 10 ppm, 30 ppm, and 60 ppm moxidectin groups respectively.

Special Evaluation: Not performed

Toxicokinetics: Not performed

Dosing Solution Analysis

Moxidectin was powdered with a mortar and mixed in animal feed and stored refrigerated on a weekly basis. Samples of feed containing moxidectin were taken prior to initiation of treatment and weekly thereafter then shipped for analysis. All diet samples were stored at -8°C and assayed within a month of receipt. For homogeneity and stability tests, 100 gram samples were taken from the right and left sides of the top, middle, and bottom of the low- and high-dose diet preparations. For the mixing study, two representative samples of each weekly batch of each concentration of moxidectin in chow was collected and frozen, with later analysis of one of the samples. Duplicate 20 gram portions of rodent meal samples were assayed for homogeneity and stability (low and high doses) and concentration (all doses) by two HPLC methods with a lower limits of detection of 1 and 5 ppm respectively.

The homogeneity results indicated actual concentration for all layers that were ±10% of the nominal concentration. The average actual concentrations for the stability samples were all within the acceptance criteria of ±10% of the nominal concentrations. For all of the weekly samples, the actual moxidectin concentrations were within the acceptance criteria of ±10% of the nominal concentrations.

Study title: One year Dietary Toxicity Study in Purebred Beagle Dogs with AC-301423

Study no.:	(b) (4) Study No.: 362-200; RPT-77336
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 15, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot No.: AC 6736-16, purity of 88.5%

Key Study Findings

- This study was adequate in design and execution.
- Unlike in other rat and dog toxicology studies, clinical signs were not observed with the high dose of moxidectin (45 ppm; approximately 1 mg/kg moxidectin in the last week of dosing).
- Also body weights, body weight gains, and cumulative food consumption were not changed in any of the moxidectin dose groups compared to control values.
- The NOAEL value was considered to be the high dietary dose of 45 ppm (approximately 1 mg/kg/day) moxidectin.

Methods

Doses:	0, 10, 20, and 45 ppm. Based on analysis of food intake, these doses corresponded to approximate moxidectin doses of 0.3, 0.6, and 1.20 mg/kg/day on the first day of dosing.

Frequency of dosing:	*Ad libitum* in food provided one hour per day.
Route of administration:	Oral; the drug substance was ground to a fine powder then dispersed in a homogeneous fashion in dry food.
Dose volume:	Determined by food intake
Formulation/Vehicle:	Dog food
Species/Strain:	Beagle dogs
Number/Sex/Group:	6/sex/group
Age:	23-24 weeks of age at initiation of dosing
Weight:	Males: 5.5 to 8.8 kg and Females: 4.7 to 7.4 kg at the initiation of dosing.
Satellite groups:	none
Unique study design:	Male and female Beagle dogs were administered dietary moxidectin for 52 weeks before the animals were euthanized.
Deviation from study protocol:	Multiple deviations in the study protocol were observed. However, none was considered to have altered the study report.

Observations and Results

Table 27: Observation Scheduled or the 1-Year Toxicology Study in Dogs

Observations	Schedule
Mortality and Clinical Signs	All dogs were observed twice daily for mortality and morbundity. Each dog was observed cageside three times daily for the first two weeks and at least once daily therafter. approximately 4 hours after feeding. Physical exams were performed once a week.
Body Weights	Individual body weights were recorded three times pretreatment, and weekly thereafter.
Food Consumption	Food consumption was recorded daily and reported weekly.
Clinical Pathology (hematology, urinalysis, and clinical chemistry)	Blood was collected from all surviving dogs for hematology and serum chemistry measurements in Weeks 1, 6, 13, 26, and 52. Urine was collected according to the same schedule. Animals were deprived of food and water overnight prior to collection.
Ophthalmoscopic Examinations	Ophthalmoscopic examinations were performed on all animals prior to initiation and during Weeks 13, 26, and 52 using an indirect ophthalmoscope.
Necropsy	Animals were sacrificed after at least 90 days of treatment.

Observations and Results

Mortality

No dogs died prematurely during the study.

Clinical Signs

No moxidectin-related clinical signs were observed in the study even with the high dose of moxidectin.

Body Weights

No significant changes in body weights or body weight gain were observed. Mean body weights expressed as the percent of gain over pretreatment values are summarized in Table 28. The data indicate a moxidectin dose-dependent trend toward moderately reduced body weight gain for both sexes throughout the study.

Table 28: Percent Body Weight Gain Over Pretreatment Values in the 1-Year Toxicology Study in Dogs. (Table from the Study Report)

Group	Males				Females			
	Weeks				Weeks			
	13	26	39	52	13	26	39	52
1	128	129	136	138	124	135	140	144
2	118	125	129	129	121	121	123	123
3	120	127	132	133	121	128	135	139
4	117	125	129	132	117	121	124	127

Feed Consumption

Unlike in the 28-day and 91-day studies in dogs with higher doses of moxidectin, food consumption was not significantly decreased in high-dose males in this study (Table 29). Cumulative food consumption was significantly decreased in the low- and mid-dose females compared to control values, but not in the high-dose females.

Table 29: Mean Total Food Consumption and Standard Deviations in Grams in the 1-year toxicology Study in Dogs. (Table from the Study Report)

GROUP AND DOSE LEVEL (PPM)		WEEK 1 - 52
		MALES
1	MEAN	78966.8
	S.D.	13085.18
.000	N	4
2	MEAN	85072.6
	S.D.	9956.77
10.000	N	5
3	MEAN	83027.0
	S.D.	6588.98
20.000	N	6
4	MEAN	75495.5
	S.D.	7535.24
45.000	N	6
		FEMALES
1	MEAN	80389.8
	S.D.	7428.79
.000	N	6
2	MEAN	60779.8*
	S.D.	9046.72
10.000	N	6
3	MEAN	65993.3*
	S.D.	10001.34
20.000	N	6
4	MEAN	69083.7
	S.D.	10014.48
45.000	N	6

* Significantly different from control value, $p \leq 0.05$.

Moxidectin Consumption in Food

Moxidectin consumption tended to decrease with the duration of dosing in the study (Table 30). Male and female values were similar throughout the study. The study report included the average weekly compound consumption data for the entire 52 weeks of the study: 0.25, 0.49, and 1.12 mg/kg/day and 0.25, 0.51, and 1.15 mg/kg/day for Group 2, 3, and 4 males and females respectively.

Table 30: Mean Moxidectin Consumption (mg/kg/day) on Specific Dates in Male and Female Dogs in the 1-Year Dietary Toxicity Study in Dogs. (Table Derived from Summary Data in the Study Report)

Group	Mean Moxidectin Consumption in Food (mg/kg/day)					
	Week 1		Week 26		Week 52	
	Male	Female	Male	Female	Male	Female
Group 1: 0 ppm Moxidectin	0	0	0	0	0	0
Group 2: 10 ppm Moxidectin	0.28	0.30	0.25	0.24	0.23	0.23
Group 3: 20 ppm Moxidectin	0.56	0.61	0.47	0.50	0.44	0.46
Group 4: 45 ppm Moxidectin	1.16	1.30	1.13	1.09	0.92	0.97

Ophthalmoscopy

No moxidectin-related ophthalmology findings were observed.

ECG: Not performed

Hematology

The following hematology parameters were measured: mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, reticulocyte count, total leukocyte count, corrected leukocyte count, differential leukocyte count, absolute reticulocyte count, erythrocyte count, cell morphology, hematocrit, and hemoglobin.

No hematology parameters were significantly altered in a moxidectin-related manner.

Clinical Chemistry

The following serum chemistry parameters were measured: albumin, creatinine, carbon dioxide, gamma glutamyltransferase, glucose, sodium, potassium, chloride, alanine aminotransferase, total bilirubin, total cholesterol, albumin/globulin ratio, blood urea nitrogen, creatine kinase, globulin, alkaline phosphatase, calcium, inorganic phosphorus, aspartate aminotransferase, lactate dehydrogenase, direct bilirubin, and total protein.

No mean values for any of the serum chemistry values were significantly altered between groups. However, in two high-dose animals, one male and one female, serum alkaline phosphatase was increased as much as 100% over the course of experiment. Given the low incidence of this effect, it may be incidental, a result of reduced food intake, or possibly indicative of a low level of moxidectin-related liver toxicity in a subset of sensitive animals.

Urinalysis

The following urinalysis parameters were measured: appearance and color, specific gravity, pH, bilirubin, glucose, reducing substances, ketones, occult blood, protein, urobilinogen, and microscopic examination of sediment.

Urinalysis parameters were not altered by dosing with moxidectin.

Gross Pathology

No gross pathology findings were attributed to moxidectin.

Organ Weights

Organ weights were determined on an absolute and relative (to body weight; to brain weight) basis. The following organs were weighed: adrenals, brain with brainstem, testes/epididymides, heart, kidneys, ovaries, liver, lungs, parathyroid and thyroid (combined), pituitary, thymus, and spleen.

Absolute ovary weights were significantly decreased in high-dose females and ovary weights relative to body weight and brain weight were decreased in a moxidectin dose-dependent manner (Table 31). However, the absolute ovary weights in high-dose females were within the historical control range (0.68 to 2.63 grams) and ovary weights are known to change in conjunction with different stages of the estrous cycle. The number of high-dose females with estrous activity (4/6) was slightly decreased relative to control values (6/6) and low- (5/6) and mid- (6/6) dose moxidectin values. Based on the nonsignificant differences in relative ovary weight comparisons and the possible influence of the estrous cycle, the relationship of the ovary weight differences to moxidectin is not clear.

Table 31: Absolute and Relative Ovary Weights in Female Dogs Administered Dietary Moxidectin for 1 year.

Group	Mean Ovary Weights (Mean ± SD)		
	Absolute Weights (g)	Relative to Terminal Body Weight (%)	Relative to Brain Weight
Group 1: 0 ppm moxidectin	1.18 ± 0.32	0.013 ± 0.004	0.017 ± 0.004
Group 2: 10 ppm modidectin	0.89 ± 0.18	0.013 ± 0.005	0.013 ± 0.004
Group 3: 20 ppm moxidectin	0.97 ± 0.28	0.012 ± 0.003	0.013 ± 0.003
Group 4: 45 ppm moxidectin	0.73 ± 0.13*	0.009 ± 0.001	0.010 ± 0.002
* Significantly different from control values, p ≤ 0.05			

Unlike data from the 28-day and 91-day toxicology studies in dogs, absolute and relative testicular weights did not decrease in a moxidectin dose-dependent manner (Table 32). Also, the histopathology examination did not report that any males in any of the groups had testes that were considered juvenile (immature).

Table 32: Absolute and Relative Testicular Weights in Male Dogs Administered Dietary Moxidectin for 1 year.

Group	Mean Testicular Weights (Mean ± SD)		
	Absolute Weights (g)	Relative to Terminal Body Weight (%)	Relative to Brain Weight

Group 1: 0 ppm moxidectin	14.9 ± 1.6	0.16 ± 0.03	0.20 ± 0.03
Group 2: 10 ppm modidectin	17.1 ± 2.5	0.18 ± 0.02	0.23 ± 0.03
Group 3: 20 ppm moxidectin	17.7 ± 2.8	0.18 ± 0.04	0.23 ± 0.04
Group 4: 45 ppm moxidectin	16.2 ± 3.2	0.19 ± 0.03	0.23 ± 0.04

Histopathology

Adequate Battery: Yes, The following organs and tissues were examined for histopathology: Adrenals, aorta, bone and marrow (sternum), brain with brainstem, cecum, cervix, colon, epididymides, esophagus, eyes (both), gallbladder, heart, duodenum, ileum, jejunum, kidney (both), liver, lungs with mainstem bronchi (left and right), lymph.nodes (mediastinal and mesenteric), mammary gland, vagina, optic nerve, ovaries (both), pancreas, parathyroid, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, skeletal muscle, skin (mammary area), spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes (both), thymus, thyroids (both), tongue, trachea, urinary bladder, uterus, and any-gross lesions.

Peer Review: No

Histological Findings
No histopathology findings clearly related to moxidectin administration were observed.

The incidence of minimal inflammation in liver was slightly increased in the moxidectin-dose groups but not in a dose-dependent manner. The incidence was 4/12 in the control group, and 7/12, 8/12, and 6/12 in the 10, 20, and 45 ppm moxidectin groups respectively.

Two apparently incidental histopathology findings included a malignant lymphoma in the renal cortex of one low dose male and lymphocytic infiltration of the thyroid of one mid-dose male.

Special Evaluation: Not performed

Toxicokinetics: Not performed

Dosing Solution Analysis

Moxidectin was powdered with a mortar and mixed in animal feed and stored refrigerated on a weekly basis. Samples of feed containing moxidectin were taken prior to initiation of treatment and weekly thereafter then shipped for analysis. All diet samples were stored at -8°C and assayed within a month of receipt. For homogeneity and stability tests, 100 gram samples were taken from the right and left sides of the top, middle, and bottom of the low- and high-dose diet preparations. For the mixing study, two representative samples of each weekly batch of each concentration of moxidectin in chow was collected and frozen, with later analysis of one of the samples. Duplicate 20 gram portions of rodent meal samples were assayed for homogeneity and stability (low and high doses) and concentration (all doses) by two HPLC methods with a lower limits of detection of 1 and 5 ppm respectively.

The homogeneity results indicated actual concentration for all layers that were ±10% of the nominal concentration. The average actual concentrations for the stability samples were all within the acceptance criteria of ±10% of the nominal concentrations. For all of the weekly samples, the actual moxidectin concentrations were within the acceptance criteria of ±10% of the nominal concentrations.

7 Genetic Toxicology

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

Study title: Evaluation of CL-301423 in a Bacterial/Microsome Mutagenicity Test

Study no.:	RPT-77350
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 5, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (CL 301423), Batch No.: AC-6736-16a, purity of 88.5%

Key Study Findings

- Despite incomplete data supporting typical study validation criteria, the results of this study were considered to be valid. Background revertant values in the vehicle control groups were repeatable and low, and revertant values for the positive control groups were consistently significantly higher than vehicle control values.
- Moxidectin was negative for mutagenicity in an Ames assay using *Escherichia coli* and multiple test strains of *Salmonella typhimurium*.

Methods

Strains:	TA 100, TA 1535, TA 1537, TA 1538, and WP-2 uvrA
Concentrations in definitive study:	See Table 33.
Basis of concentration selection:	The study report notes: “The concentrations of CL 301.423 selected for this study are based on OECD Guidelines for testing of chemicals, on EPA Toxic Substances Control Act Test Guidelines; Final Rules and on Guidelines established by the Japan Ministry of Agriculture, Forestry and Fisheries(l-3).” According to the study report, concentrations of moxidectin above 2000 mcg/plate caused precipitate to form in the study system. According to current FDA Guidance, S2(R1), precipitation is a

	suitable reason to limit the highest concentration.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The four positive control agents that were used were: N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2-NF), 9-aminoacridine hydrochloride (9-AA) and 2-aminoantracene (2-AA). For information regarding the concentrations and use of each positive control agent, see Table 33.
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	Plates were incubated for 48 hours at 37°C.

Table 33: Study Design for the Ames Assay with Moxidectin. (Sponsor's Table)

TESTER STRAIN	TREATMENT	CONCENTRATION µg/plate Trial 1	Trial 2	NO.OF REPLICATES +S9	-S9
All	Vehicle	-	-	3	3
All	CL 301,423	3000	2000	3	3
All	CL 301,423	2000	1000	3	3
All	CL 301,423	900	540	3	3
All	CL 301,423	450	108	3	3
All	CL 301,423	90	54	3	3
TA98	2-AA	5	5	3	0
	2-NF	20	20	0	3
TA1537	2-AA	5	5	3	0
	9-AA	50	50	0	3
TA1535\ TA100 /	2-AA	5	5	3	0
	MNNG	10	10	0	3
TA1538	2-NF	20	20	0	3
	2-AA	5	5	3	0
WP2 uvrA-	MNNG	10	10	0	3
	2-AA	5	5	3	0

2-AA = 2-Aminoanthracene, 2-NF = 2-Nitrofluorene, 9-AA = 9-Amino-acridine, MNNG = N-methyl-N'-nitro-N-nitrosoguanidine

2-AA which requires metabolic activation was used to confirm the activity of the S-9 homogenate. 2-NF, 9-AA and MNNG are activation independent mutagens.

The vehicle was dimethylsulfoxide, dose volume was 0.1 ml/plate

Study Validity

The normal study validity criteria, namely that revertant values for each strain in the vehicle control plates and positive control plates fall within historical control ranges, were not addressed in the study report. However, revertant values in vehicle control groups were similar to those reported for the moxidectin treatment plates suggesting repeatability, and positive control plates demonstrated many fold increases in revertants compared to the vehicle control plates.

Results

In the first trial, plates incubated with 3000 mcg/plate moxidectin with and without S9 activation exhibited precipitate that reportedly rendered the plates unreadable. For all the readable plates treated with moxidectin, no increase in revertants was observed for any tester strain with and without S9 activation. These results indicate that moxidectin was negative for mutagenicity in this study for concentrations < 3000 mcg/plate. In contrast, the positive control agents produced substantial increases in mean revertant numbers, on the order of 20- to 50- fold above revertant values for vehicle control plates with and without S9 activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Moxidectin: Evaluation of CL-301423 in the Mammalian Cell CHO/HGPRT Mutagenicity Test.

(b) (4)

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(b) (4)

Reviewer Comment: *The study report is incomplete in that the study protocol and historical control data for the negative and positive control conditions were not included. Appendixes that may contain some or all of the missing information were not included with the study report. The study was reported to be conducted according to published procedures,* (b) (4) *However, in the absence of a study protocol, the study is not considered valid.*

Study title: Moxidectin: Evaluation of AC-301423 in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with Colony Size Evaluation in the Presence and Absence of Induced Rat Liver S-9 with a Confirmatory Study.

Study no.:	RPT-77352
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 23, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AC 301423, Lot No.: GMP-98-8, purity of 90.84%.

Key Study Findings

- This study was considered to be valid.

- In primary and confirmatory assays, moxidectin did not increase mutation frequencies more than 2-fold above solvent control levels in L5178Y TK+/- cells in a mouse lymphoma mutagenicity assay.

Methods

Cell line:	L5178Y TK+/- mouse lymphoma cells, clone 3.7.2C.
Concentrations in definitive study:	B1 mutagenicity assay: Without S9 activation: 10, 12.5, 15, 17.5, and 20 mcg/ml. With S9 activation: 10, 25, 40, and 55 mcg/ml. B2 confirmatory assay: Without S9 activation: 5, 10, 12.5, 15, and 17.5 mcg/ml. B3 confirmatory assay: Without S9 activation: 17.5, 18.5, 19.5, and 20.5 mcg/ml.
Basis of concentration selection:	A range-finding test was performed using moxidectin concentrations of 0.005, 0/01, 0/05, 0.1, 0.5, 1.0, 5.0, 25, and 50 mcg/ml with and without S9 activation. The cytotoxicity results of this assay informed the moxidectin concentrations that were used in the B1 mutation assay (B1) and the confirmatory mutation assays (B2 and B3).
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Without S9 activation: 5 and 10 mcg/ml Hycanthone methanesulfonate (HYC) With S9 activation: 5.0 and 7.5 mcg/ml 7,12-Dimethylbenz(α)anthracene (DMBA)
Formulation/Vehicle:	Dimethylsulfoxide (DMSO)
Incubation & sampling time:	Incubation periods of 4 hours were used for the mutation assay and periods of 24 hours in confirmatory mutation assays.

Study Validity

The results for the solvent control cultures in the different experiments in the study were consistent with the following study-validity criteria.

1. The average cloning efficiency of the solvent control cultures was 50% or higher.
2. The average mutation frequencies of the solvent control cultures were less than 100 per 1 million viable cells.

The results for the positive control cultures in the different experiments in the study were consistent with the following study validity criteria.

1. The treated cultures had mutation frequencies that were three times or greater than the average of their solvent control cultures.
2. Their solvent controls had an average cloning efficiency of 50% or greater.

Results

Range-finding Assay: In incubations in the absence of S9 activation, 100% cytototoxicity occurred with the two highest moxidectin concentrations (25 and 50 mcg/ml). In the incubations including S9 activation, 17% and 60% cytotoxicity occurred with moxidectin concentrations of 25 and 50 mcg/ml respectively.

Mutation Assay B1: All of the moxidectin cultures with and without S9 activation had mutation frequencies (MFs) that were less than 2-fold the mean MFs for solvent controls (mean MF values of 74 and 91 mutants/10^6 surviving cells without and with S9 activation respectively) which were within the historical control range for DMSO (0-113; mean of 53 mutants/10^6 surviving cells without S9 activation and 31-115; mean of 65 mutants/10^6 cells with S9 activation). The mean MF values for the moxidectin cultures not associated with ≥ 60% cytotoxicity ranged from 78–120 and 9 –127 mutants/10^6 cells in the absence and presence of S9 activation respectively. In contrast, the positive control agents, HYC (mean MF value of 1035 mutations/10^6 cells) and DMBA (mean MF of 297 mutations/10^6 cells) produced significant increases in MF responses.

Confirmatory Assay B2: S9 activation was not included in any of the B2 confirmatory assays. All of the moxidectin cultures without S9 activation had mutation frequencies (MFs) that were less than 2-fold (range of the mean MFs of 39-56 mutations/10^6 cells) for solvent controls (mean MF value of 53 mutations/10^6 cells). In contrast, the positive control agent, HYC (589 mutations/10^6 cells) produced significant increases in MF responses. The MF values for the solvent control cultures were within the historical solvent-control range.

Confirmatory Assay B3: All of the moxidectin cultures without S9 activation had mutation frequencies (MFs) that were less than 2-fold (range of 38-60 mutations/10^6 cells) the mean MFs for solvent control (mean MF of 38 mutations/10^6 cells). In contrast, the positive control agents, HYC produced significant increases in MF responses (mean MF of 1179 mutations/10^6 cells). The MF values for the solvent control cultures were within the historical solvent control range for DMSO.

Study title: Moxidectin: Unscheduled DNA Synthesis in Primary Rat Hepatocytes with AC-301423.

Study no.:	T9090.380025, RPT-77441
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 19, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot No.: AC 6736-16, purity of 88.5%

Key Study Findings

- This study was considered to be valid.

- Under the conditions of the assay, moxidectin did not produce a significant increase in unscheduled DNA synthesis in rat hepatocytes.

Methods

Cell line:	Primary rat hepatocytes from the livers of male Fischer 344 rats.
Concentrations in definitive study:	0.1, 0.3, 1.0, 3.0, 5.0, 10, 20, and 30 mcg/ml
Basis of concentration selection:	A preliminary cytotoxicity test was performed and the results of this assay informed the moxidectin concentrations that were used in the definitive study. The moxidectin concentrations tested in the preliminary assay were: 0.13, 0.43, 1.3, 4.3, 13, 43, 129, 431, 1290, and 4310 mcg/ml.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	Dimethylbenz(α)anthracene (DMBA)
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation & sampling time:	Cells were treated for 18-20 hours before sampling.

Study Validity

The assay was considered valid if the positive control compound induced a significant increase in the net nuclear grain count, the proportion of cells in repair in the negative control was less than 15%, and the net nuclear grain count of the vehicle control was less than one. These criteria were fulfilled for this study.

Results

Preliminary Cytotoxicity Assay: Precipitate formed as soon as the moxidectin solutions were added to the cell incubations at moxidectin concentrations of 129, 431, 1290, and 4310 mcg/ml. At termination of the incubation after 18-20 hours, precipitate was also noted in the incubation treated with 43 mcg/ml moxidectin. LDH activity in the incubation supernatants as a measure of cytotoxicity indicated percent cytotoxicity measurements of 16%, 82%, 93%, 84%, 80%, and 52% at moxidectin concentrations of 4310, 1290, 431, 129, 43, and 13 mcg/ml respectively. Normal cell morphology and background LDH activity occurred with moxidectin concentrations less than 4.3 mcg/ml. Based on these results, the highest concentration chosen for the definitive assay was 30 mcg/ml.

Definitive Assay: LDH activity in the culture supernatants of the definitive study indicated cytotoxicities of 99%, 78%, 57%, and 25% at concentrations of 30, 20, 10, and 5 mcg/ml respectively. Due to high levels of cytotoxicity, cells exposed to 30, 20, and 10 mcg/ml moxidectin could not be evaluated for unscheduled DNA synthesis (UDS). Despite some cytotoxicity, cells treated with 5.0 mcg/ml moxidectin could be evaluated for UDS, as well as cells treated with the lower moxidectin concentrations. The UDS results indicate that none of the moxidectin concentrations that could be evaluated for UDS produced a mean net increase in nuclear silver grain counts compared to vehicle control values. The moxidectin concentrations produced 0% of cells with 5 or more

nuclear grains compared to 0-1% for the vehicle control treatments. In contrast, the positive control agent, DMBA at both the test doses of 1.0 and 3.0 mcg/ml produced 73% and 81% of cells with 5 or more nuclear grains indicating significant increases in the average net nuclear count of silver grains compared to vehicle control values.

Study title: Moxidectin: Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells with and without Metabolic Activation with AC 301423.

Study no.:	971-98-131, RPT 77740
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 13, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot No.: GMP-98-8, purity of 90.84%

Key Study Findings

- This study was considered to be valid.
- Under the conditions of the study, moxidectin did not increase the percentage of CHO cells with chromosome aberrations in definitive and confirmatory assays with and/or without S9 activation.

Methods

Cell line:	CHO-W-B1 cells
Concentrations in definitive study:	Definitive Assay (B1): 0, 1.0, 5.0, 10, 20, and 30 mcg/ml moxidectin for both activated and non-activated conditions. Confirmatory Assay (B2): 0, 0.1, 0.5, 1.0, 5.0, 10, and 15 mcg/ml moxidectin (non-activated conditions only). Second Confirmatory Assay (B3): 0, 0.005, 0.01, 0.1, 0.5, 1.0, 5.0, 10, 15, and 20 mcg/ml moxidectin (non-activated conditions only).
Basis of concentration selection:	A range-finding test (A1) was conducted to determine moxidectin-concentration related cytotoxicity, and the results of this test informed the moxidectin concentrations that were used in the definitive study. The concentrations tested in the range-finding test were: 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, and 10 mcg/ml moxidectin without S9 activation and 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 mcg/ml moxidectin.

Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	Mitomycin C (MMC) in the absence of S9 activation and cyclophosphamide (CP) in the presence of S9 activation.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Cells were exposed to moxidectin or the positive or negative control conditions for 3 hours, and then the cells were washed and incubated for an additional 15 hours with 0.1 mcg/ml colcemid added for the last 2 hours. All the cultures were harvested 18 hours after initiation of treatment.

Study Validity

The following criteria for a valid assay were fulfilled:

1. In the solvent control, the percentage of cells with aberrations should not have exceeded 4%.
2. At least 25% of the cells scored in the positive control should have shown one or more chromosome aberrations.
3. At least one of the test concentrations scored should have shown greater than 50% reduction in the relative cell growth (RCG) and/or relative mitotic index (RMI). This requirement should not be applied to test articles where no apparent toxicity could be achieved at the maximum soluble concentration or the highest allowable concentration.

Results

Range-finding Assay (A1): None of the tested moxidectin concentrations produced more than a 50% reduction in RCG except for the 20 mcg/ml moxidectin concentration with S9 activation which reduced RCG to 47% of control values.

Definitive Chromosome Aberration Assay (B1): In the definitive assay without S9 activation, moxidectin concentrations of 20 and 30 mcg/ml reduced RCG by 86 and 87% respectively and RMI at these concentrations was 0%. RCG was reduced 74% by 30 mcg/ml moxidectin in the cultures with S9 activation and the RMI at this concentration was 0%. The RMI for the 10 mcg/ml moxidectin concentration without S9 activation and the 20 mcg/ml moxidectin concentration with S9 activation were 43% and 25% respectively. Based on this data, chromosome aberrations were scored for moxidectin concentrations of 1.0, 5.0, and 10.0 mcg/ml in the non-activated system and 5.0, 10.0, and 20.0 mcg/ml in the activated system. With and without S9 activation, none of the scored moxidectin concentrations increased the percentage of cells with chromosome aberrations above the solvent control values. In contrast, the positive control treatment significantly increased the percentage of cells with chromosome aberrations more than 50-fold above solvent control values.

Confirmatory Chromosome Aberration Assay (B2): None of the moxidectin concentrations produced reductions in RCG above 50%, but the RMI values ranging from 0-40% indicated cell toxicity at all concentrations. Chromosome aberrations were scored for the three lowest moxidectin concentrations, 0.1, 0.5, and 1.0 mcg/ml. In non-activated conditions, none of the scored moxidectin concentrations significantly increased the percentage of cells with chromosome aberrations above the control values. In contrast, the positive control agent, MMC, stimulated a greater than 50-fold increase above control levels.

Second Confirmatory Chromosome Aberration Assay (B3): The highest tested concentration of moxidectin, 20 mcg/ml, produced a greater than 50% reduction in RCG, but the highest 3 concentrations, 10, 15, and 20 mcg/ml produced RMIs of 0% indicating complete toxicity and the RMI for the 5.0 mcg/ml moxidectin concentration was 45%. Chromosome aberrations were scored for the 0.1, 0.5 and 5.0 mcg/ml concentrations. In non-activated conditions, none of the scored moxidectin concentrations significantly increased the percentage of cells with chromosome aberrations above control levels and the positive control (MMC) stimulated a significant increase.

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

Study title: Moxidectin: In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells with AC-301423.

Study no:	RPT-77442
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 13, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot No.: GMP-98-8, purity of 90.84%

Key Study Findings

- This study was considered to be valid.
- Under the conditions of the assay, a single dose of moxidectin at doses up to 30 mg/kg did not increase micronucleated polychromatic erythrocytes in bone marrow cells in male and female CD-1 mice.

Methods

Doses in definitive study:	0, 7.5, 15, and 30 mg/kg moxidectin
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Corn oil

Species/Strain:	CD-1 Mice
Number/Sex/Group:	5/sex/group
Satellite groups:	none
Basis of dose selection:	The doses in the definitive study were based on mortality results from two range-finding studies. Animal death occurred in the majority of animals administered ≥ 50 mg/kg moxidectin.
Negative control:	Corn oil
Positive control:	80 mg/kg cyclophosphamide

Study Validity

The following study validity criteria were fulfilled in the study.

1. In the vehicle control group the average number of micronucleated polychromatic erythrocytes (MPCE) per 2000 polychromatic erythrocytes (PCE) should not exceed 10.
2. In the positive control, the increase in the average number of MPCE per 2000 PCE over the average number of MPCE for the vehicle control should be statistically significant.

Results

The percentage of PCE and MPCE were determined in bone marrow cells from treated animals euthanized 24, 48, and 72 hours after dose administration. The percentage of PCE was not observed to decrease more than 20% compared to vehicle control values in any treatment group at any sacrifice timepoint except at the 48 hour timepoint in high-dose females (-23.8%).

The mean numbers of MPCE in 2000 PCE in male and female mice were not significantly increased in any of the moxidectin-dose groups above concurrent control levels. In contrast, mean number of MPCE in 2000 PCE in the positive control group were significantly increased 72.6% compared to vehicle-control values.

Study title: Moxidectin: Evaluation of Moxidectin in the In Vivo Chromosome Aberration Assay in Rat Bone Marrow Cells.

Study no:	RPT-77443, 89-14-002
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 27, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin, Lot No.: AC 6736-16, purity of 88.5%

Key Study Findings

- This study was considered valid.

- Under the conditions of the assay, a single dose of moxidectin at doses up to 60 mg/kg did not induce chromosome aberrations in bone marrow cells in male and female Sprague-Dawley rats.

Methods

Doses in definitive study:	0, 15, 30, 60 mg/kg moxidectin
Frequency of dosing:	Single doses
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Corn oil
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	15/sex/group; 5/sex/group/sacrifice timepoint
Satellite groups:	None
Basis of dose selection:	The doses in the definitive study were based on the results of a range-finding study using single doses of 50, 100, 125, and 150 mg/kg moxidectin. In the range-finding study, all of the animals receiving 150 mg/kg moxidectin died and moxidectin produced a dose-dependent decrease in the mitoxic index of bone marrow cells indicating bone marrow cell toxicity at doses above 50 mg/kg/day.
Negative control:	Corn oil
Positive control:	Cyclophosphamide monohydrate (CP), 40 mg/kg

Table 35: Study Design for the Rat Chromosome Aberration Study. (Table from the Study Report)

	Number of Animals Treated and Sacrificed Post Treatment at						Total
	12 Hrs.		24 Hrs.		48 Hrs.		
Treatment	M	F	M	F	M	F	
60 mg/kg	5	5	5	5	5	5	30
30 mg/kg	5	5	5	5	5	5	30
15 mg/kg	5	5	5	5	5	5	30
Vehicle Control (corn oil 10 ml/kg)	5	5	5	5	5	5	30
Positive Control (cyclophosphamide 40 mg/kg)			5	5			10
Total	20	20	25	25	20	20	130

Study Validity

The study was considered valid because the positive controls showed a significant response and the vehicle controls were within the expected range of historical values.

Results

In the definitive study, many animals exhibited clinical signs including tremors, low activity, and diarrhea at all the moxidectin doses either after dosing or at the time of colchicine administration.

The analysis of chromosome aberrations indicated that none of the moxidectin doses produced significantly more chromosome aberrations per cell (mean group values ranged from 0.000 to 0.008) or a significant increase in the percent aberrant cells (mean group values ranged from 0.0% to 0.8%) at any of the harvest timepoints in either males or females. The range of mean control values for chromosome aberrations per cell and percent aberrant cells were 0.000 to 0.016 and 0.0% to 1.2% respectively. In contrast, at the 24 hour harvest, the positive control agent, 40 mg/kg cyclophosphamide, induced a significant increase in chromosome aberrations per cell (0.976 for females and 1.06 for males) and the percent aberrant cells (29.2% for females and 23.7% for males) compared to control values which were within the historical control ranges.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Reviewer Comment: *Final evaluation of the results of the 2-year carcinogenicity studies in mice (Study Report No.: PRT-77458) and rats (Study Report No.: PRT-77459) have not been completed due to mistakes detected in the electronic tumor data (tumor.xpt) submitted by the Applicant. Until the mistakes are corrected, an internal CDER statistical review of the tumor data from each study, and comprehensive reviews of the studies cannot be completed. The reviews shown below are based on the study reports for the two studies. For the reasons stated, the reviews below should be considered preliminary. Because moxidectin, if approved, will only be approved for administration as a single dose, the lack of a final review of the carcinogenicity data is not considered to be an approval issue.* (b) (4)

(b) (4)

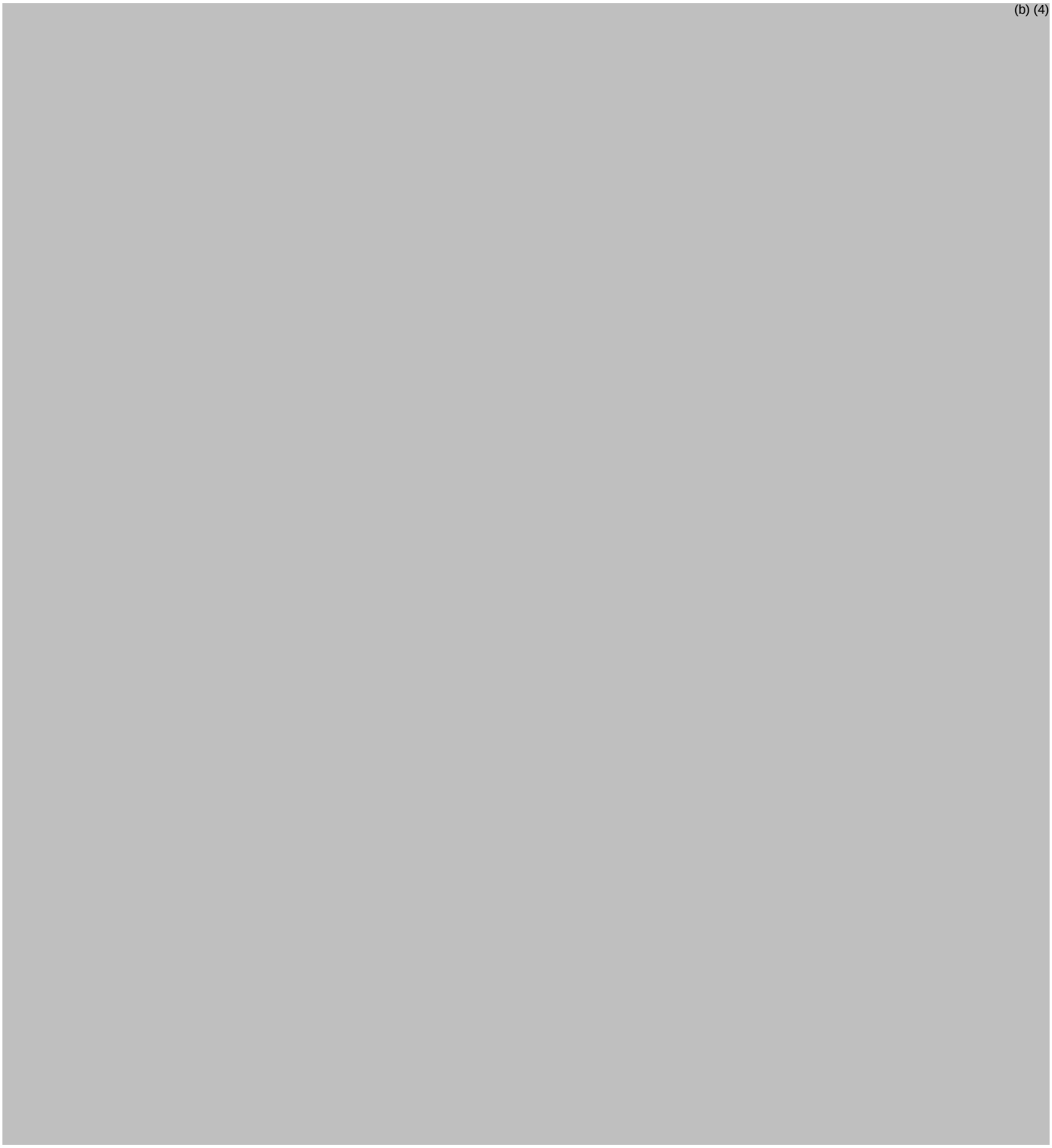
Study title: Moxidectin: Chronic Dietary toxicity and Oncogenicity Study with AC 301423 in Mice.

Study no.:	RPT-77458
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 22, 1989
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot # AC6736-72B, purity of 81.5%
CAC concurrence:	No CAC concurrence for the protocol

Key Study Findings

Based on a preliminary review of the data, CD-1® mice administered moxidectin in dietary doses of up to 50 ppm (corresponding to approximate doses of 7.7 and 9.7 mg/kg/day in male and female rats respectively) for two years, did not exhibit an increased incidence of neoplastic lesions. This preliminary conclusion will need to be confirmed following the new CDER statistical analysis before the conclusion can be finalized.

(b) (4)



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Study title: Moxidectin: Chronic Dietary toxicity and Oncogenicity Study with AC 301423 in Rats.

Study no.:	HWI Study No.: 362-202; Study Report No.: RPT-77459
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 8, 1989
GLP compliance:	Yes, 1978 Standards
QA statement:	Yes
Drug, lot #, and % purity:	Moxidectin (AC 301423), Lot # AC6736-72B, purity of 81.5%
CAC concurrence:	No CAC concurrence for the protocol

Reviewer Comment: *Final evaluation of the results of the 2-year carcinogenicity study in rats (Study Report No.: PRT-77459) have not been completed due to mistakes detected in the electronic tumor data (tumor.xpt) submitted by the Applicant. Until the mistakes are corrected, an internal CDER statistical review of the tumor data from the study and a comprehensive review of the study cannot be completed. The review shown below is based on the study report and for the reasons stated, should be considered preliminary. Because moxidectin, if approved, will only be approved for administration as a single dose, the lack of a final review of the rat carcinogenicity data is not considered to be an approval issue.* (b) (4)

(b) (4)

Key Study Findings

Sprague Dawley rats administered moxidectin in dietary doses of up to 100 ppm moxidectin (corresponding to approximate doses of 5.11 and 7.06 mg/kg/day in male and female rats respectively) for two years, did not exhibit an increased incidence of neoplastic lesions.

(b) (4)

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Moxidectin: A Pilot One-Generation (Two Litters) Reproduction Study with AC 301,423 to Rats.

Study no.:	RPT-77517
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not identified, the Study ended on September 4, 1991.
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Moxidectin (AC 301423), Batch # AC 6297-119, purity of 88.6%

Reviewer Comment: *This pilot reproduction study included fertility assessments for male and female rats as well as survival and body weight data for first generation offspring.*

Key Study Findings

- Dietary administration of up to 125 ppm moxidectin (approximately 9.77 mg/kg/day in male rats and 11.99 mg/kg in female rats) did not inhibit male or female mating rates, female pregnancy rates, or male fertility rates compared to control values.
- However, the majority of fetuses died at birth in the group receiving the moxidectin dose of 125 ppm and significantly more fetuses died during the lactation period for moxidectin doses of 10, 15, 25, 50, and 125 ppm compared to control values.
- Spermatogenesis in males and corpora lutea counts, preimplantation loss, and early and late resorptions in females were not assessed.

Methods

Doses:	For the initial mating interval (F_{1a}): 25, 50, and 125 ppm). For the follow-up mating interval (F_{1b}): 5, 10, and 15 ppm
Frequency of dosing:	*Ad libitum.* Test compound was finely ground and dispersed in food.
Dose volume:	Determined by food intake
Route of administration:	Oral in diet
Formulation/Vehicle:	Animal feed
Species/Strain:	Albino rat, COBS®, CD® (Sprague-Dawley derived)
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Study design:	Adult male and female rats (approximately 6-7 weeks old) were orally administered moxidectin in feed in dietary concentrations of 0, 25, 50, and 125 ppm) for a 9-week premating period and throughout the ensuing mating, gestation, and lactation intervals to produce the F_{1a} litter. Because of a high rate of death in offspring for the higher dose groups, animals were rested for two weeks and administered 0, 5, 10, and 15 ppm moxidectin in animal chow with administration continuing through a new interval of mating, gestation, and lactation with the production of F_{1b} litters (Table 46). The mating period was Weeks 10-12 for the F_{1a} mating interval and Weeks 18-20 for the F_{1b} mating interval.
Deviation from study protocol:	Multiple study deviations were noted. However, none was considered to have altered the study results.

Table 46: Study Design for the Pilot Fertility Study with Moxidectin. (Table from the Study Report)

Group	Dose Level[a] ppm	Dose Level[b] ppm	Number of Animals P_1 Generation Males	Number of Animals P_1 Generation Females	No. of Matings Per Litter Interval F_{1a},F_{1b}
I	0[c]	0[c]	25	25	2
II	25	5	25	25	2
III	50	10	25	26[d]	2
IV	125	15	25	25	2

[a] P_1 generation animals were treated at these dietary levels during the nine week pre-mating treatment period and ensuing, mating, gestation and lactation periods of the F_{1a} litters through to when the decision was made by the sponsor to terminate the F_{1a} litters due to excessive pup mortality among the treated groups.

[b] P_1 generation animals were treated at these dietary levels during a two week between litter rest period and during the ensuing mating, gestation and lactation periods of the F_{1b} litters. P_1 parental animals continued to consume diets at these concentration levels until killed following weaning of the F_{1b} litters.

[c] Untreated diet.

[d] One female was sacrificed moribund after nine days of treatment and one additional female was sorted into the group.

Observations and Results

Mortality

Parental study rats were evaluated for mortality and clinical signs twice daily. Once per week, animals also received a detailed physical examination.

In the F_{1a} mating interval, one female receiving 50 ppm moxidectin was sacrificed in moribund condition due to an eye lesion after 9 days of dosing. No other parent males or females died or were sacrificed prematurely during either of the mating intervals in the study.

Clinical Signs

No clinical signs were reported.

Body Weight

For the parental generation, male body weights were assessed weekly throughout the study except during mating and female body weights were assessed weekly during the pre-mating treatment period and during the rest period, after mating on GDs 0, 7, 14, and 20 and in lactating females on LDs 0, 4, 7, 14, and 21.

F_{1a} Mating Interval

Premating Period (Weeks 1-9): Mean weekly body weights during the pre-mating period and mean weight gains over the entire nine week interval were comparable for all groups of males and for the females in the control, 25 and 50 ppm groups. In high-dose females, all measurements were similar to control values except at week 8 of the premating period when mean body weights for high-dose females were significantly lower (-8.1%) compared to control values. Also the overall body weight change for high-dose females was significantly reduced compared to control values by 12.4%.

Mating Period (males only): No significant changes in male body weight occurred during the mating period.

Gestation Period (females only): Female body weights were similar between groups during the gestation period.

Lactation Period (females only): Body weights during the lactation period were not performed because the lactation period was prematurely terminated due to excessive pup mortality among the moxidectin groups.

F_{1b} Mating Interval
Note: no premating period was included for the F_{1b} Mating Interval.

Mating Period (males only): No significant changes in male body weight occurred during the mating period.

Gestation Period (females only): Mean maternal gestation weight for the moxidectin treatment groups was comparable or higher than control values.

Lactation Period (females only): Mean body weights and mean body weight gain over the entire lactation period was comparable between groups. However, fetal death occurred in the high-dose (15 ppm) group during the lactation period and only five females in this group retained their litters for the whole lactation period.

Feed Consumption

Male food consumption for the parental generation was assessed weekly throughout the study except during mating, and female food consumption was determined weekly during the pre-mating period and on GDs 0-7, 7-14, and 14-20.

F_{1a} Mating Interval
Pre-Mating Period (Weeks 1-9): Mean weekly food consumption was comparable between the control, 25, and 50 ppm groups for males and for all the female groups throughout the pre-mating period. In high-dose (125 ppm) males, a slight (-4.2%), but significant reduction in mean food consumption was seen only for the first week of dosing.

The average weekly intake of moxidectin in animal feed for the male and female parents during the pre-mating period in Experiment F_{1a} is shown in Table 47.

Table 47: Mean Moxidectin Intake During the Premating Period in Male and Female Rats in the F_{1a} Experiment. (Table from the Study Report)

Group (ppm)	Mean Test Substance Intake (mg/kg/day)[a] Pre-Mating Treatment Period	
	Males	Females
II (25)	1.82	2.26
III (50)	3.86	4.46
IV (125)	9.77	11.99

[a]Derived as a mean of nine weekly values (Weeks 1-9).

Gestation Period: Female food consumption was similar between all groups except the high-dose (125 ppm) females during the gestation period. In the high-dose females, food consumption was higher than in control females for part of the gestation period (Days 0-7) and similar for the rest of the gestation period until Day 20.

During the F_{1a} gestation period, mean moxidectin ingestion for females in the 25, 50, and 125 ppm dose groups was 2.13, 4.10, and 10.90 mg/kg/day respectively.

F_{1b} Mating Interval

Gestation Period: Female food consumption was similar between groups during the gestation period.

During the F_{1b} gestation period, mean moxidectin ingestion for females in the 5, 10, and 15 ppm dose groups was 0.37, 0.77, and 1.13 mg/kg/day respectively.

Toxicokinetics: Not performed

Dosing Solution Analysis

Moxidectin was powdered with a mortar and mixed in animal feed and stored refrigerated on a weekly basis. Samples of feed containing moxidectin were taken prior to initiation of treatment and weekly thereafter then shipped for concentration analysis. All diet samples were stored at -8°C and assayed within a month of receipt. For homogeneity and stability tests, 100 gram samples were taken from the right and left sides of the top, middle, and bottom of the low- and high-dose diet preparations. Duplicate 20 gram portions of rodent meal samples were assayed for homogeneity and stability (low and high doses) and concentration (all doses) by two HPLC methods with a lower limits of detection of 1 and 5 ppm respectively.

The homogeneity results for all layers indicated average concentrations within ±10% of the nominal concentrations. The average actual concentrations for the stability samples were all within the acceptance criteria of ±10% of the nominal concentrations. For all of the weekly samples, the actual moxidectin concentrations were within the acceptance criteria of ±10% of the nominal concentrations.

Necropsy

Parental males and females were sacrificed and necropsied after weaning of the last F_{1b} litters. Animals were examined for gross pathology but no specimens were collected for histopathology.

In parental animals, no gross pathology findings considered related to moxidectin administration were observed.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

F_{1a} Mating Interval

Male and female mating and fertility rates were not affected by moxidectin up to a dietary dose of 125 ppm. Female pregnancy rates were also unaffected (Table 48).

Table 48: Mating, Pregancy, and Fertility Rates for the F_{1a} Mating Interval. (Table from the Study Report)

Mating, Pregnancy and Fertility Rates

Group (ppm)	No. Animals at Initiation of Mating		Mating				Pregnancy		Fertility	
			Females		Males		Females		Males	
	Females	Males	No. Mated[a]/Total No.	%	No. Mated[b]/Total No.	%	No. Pregnant[c]/No. Mated No.	%	No. Impregnating[d]/No. Mated No.	%
P_1 Generation (for F_{1a} Litters)										
STAT SYMBOL:			NS		NS		NS		NS	
I (0)	25	25	25/25	100.0	22/25	88.0	24/25	96.0	22/22	100.0
II (25)	25	25	24/25	96.0	23/25	92.0	23/24	95.8	22/23	95.7
III (50)	25	25	25/25	100.0	24/25	96.0	23/25	92.0	22/24	91.7
IV (125)	25	25	24/25	96.0	23/25	92.0	22/24	91.7	21/23	91.3

[a]Number of females showing evidence of mating (plug and/or sperm and/or pregnancy).

[b]Number of males for which mating was confirmed in at least one female.

[c]Number of females for which parturition was evident.

[d]Number of males mated with at least one female for which parturition was evident.

Gestation Length: Gestation length was not affected by moxidectin treatment with values of 22.0, 22.2, 22.1, and 22.4 for the control, 25, 50 and 125 ppm groups respectively.

Pup Survival Indices: The mean number of live, dead, and total pups at birth was comparable for the control, 25 and 50 ppm groups. In the high-dose (125 ppm) group,

the mean number of live pups at birth (2.3) was significantly lower than the control value (13.3). The mean number of dead pups at birth was significantly higher for the 125 ppm group (8.7) compared to the control value (0.2). Total pups/litter for the 125 ppm group (11.0) was not significantly lower than the control value (13.5). The pup viability index at birth was similar for the control, 25, and 50 ppm groups but the value for the 125 ppm group (21.0%) was significantly lower than the control index (98.5%). Pup survival was significantly reduced in all three moxidectin-treatment groups compared to control values in the LD 0-4 period. None of the high-dose pups survived after LD 4. Due to the high rate of mortality in all of the moxidectin groups, all pups in the F_{1a} litter group were euthanized on Day 14; consequently survival indices were not measured for the LD 4-21 period (Table 49).

Table 49: Pup and Litter Survival Data for the F_{1a} Litter Group. (Table from the Study Report)

Group (ppm)	Pup Viability Index No.[a]	Pup Viability Index %	Pup Survival Indices Lactation Days Day 0-4 No.[b]	Pup Survival Indices Lactation Days Day 0-4 %	Pup Survival Indices Lactation Days Day 4-21 No.[c]	Pup Survival Indices Lactation Days Day 4-21 %	Litter Survival[e] Index No.[d]	Litter Survival[e] Index %
			P_1 Generation - F_{1a} Litters[e]					
STAT SYMBOL:	C+A+F+		C+A+F+					
I (0)	320/325	98.5	309/320	96.6	—	—	—	—
II (25)	284/296	95.9	198/284 **	69.7	—	—	—	—
III (50)	279/290	96.2	69/279 **	24.7	—	—	—	—
IV (125)	51/243 **	21.0	0/50[f] **	0	—	—	—	—

[a] Total number of live pups at day 0/total number of pups born (live plus dead).

[b] Total number of live pups at Day 4 (pre-cull)/total number of live pups at Day 0.

[c] Total number of live pups at Day 21/total number of live pups at Day 4 (post-cull).

[d] Total number of litters at weaning (Day 21)/total number of litters with live pups at Day 0.

[e] Due to high pup mortality encountered among the treated groups at all dose levels early in lactation of the F_{1a} litter interval, the decision was made to terminate all surviving litters mid-way during the lactation period. Thus, no data are presented for pup survival Days 4-21 and litter survival Day 21.

[f] Excludes data for female No. 4523 which had one viable pup in the litter at Day 1 when the decision was made to terminate the surviving F_{1a} litters.

Pup Body Weights: The pup body weights at birth were similar for all groups (Table 50). However, as the lactation period progressed, the pups in the moxidectin groups experienced dose-dependent weight loss.

Table 50: Pup Weight Data During the Lactation Period in the F_{1a} Litter Group.
(Table Adapted from Information in the Study Report)

Group		LD 0	LD 4	LD 7	LD 14
Group 1- 0 ppm	Mean	6.0	9.0	14.2	26.8
	SD	0.5	1.2	2.0	2.5
	N	24	24	22	19
Group 2 – 25 ppm	Mean	5.9	6.3	7.1	----
	SD	0.8	1.1	1.0	----
	N	23	22	13	----
Group 2 – 50 ppm	Mean	5.7	4.7	4.3	----
	SD	0.6	0.7	0.3	----
	N	23	11	2	----
Group 2 – 125 ppm	Mean	5.6	----	----	----
	SD	0.6	----	----	----
	N	16	----	----	----

Mean Litter Sizes: By Day 4, mean litter sizes for the 25 (9.0 pups) and 50 (6.3 pups) ppm groups were significantly lower than control values (12.9 pups). In the 125 ppm group, no pups survived until Day 4.

Sex Distribution: Pup sex distribution indices at birth (Day 0), were similar for the control (ratio of males to females of 1.0), 25 (0.9), and 50 (1.2) ppm groups. In the 125 ppm moxidectin group, male pups were slightly increased (1.4).

F_{1b} Mating Interval

Male and female mating and fertility rates and female pregnancy rates were not significantly affected by moxidectin up to a dietary dose of 15 ppm (Table 51).

Table 51: Mating, Pregnancy, and Fertility Rates for the F_{1b} Mating Interval. (Table from the Study Report)

Group (ppm)	No. Animals at Initiation of Mating		Mating				Pregnancy		Fertility	
			Females		Males		Females		Males	
	Females	Males	No. Mated[a]/Total No.	%	No. Mated[b]/Total No.	%	No. Pregnant[c]/No. Mated No.	%	No. Impregnating[d]/No. Mated No.	%
			P_1 Generation (for F_{1b} Litters)							
STAT SYMBOL:			NS		NS		NS		NS	
I (0)	25	25	25/25	100.0	24/25	96.0	23/25	92.0	22/24	91.7
II (5)	25	25	23/25	92.0	23/25	92.0	21/23	91.3	21/23	91.3
III (10)	25	25	23/25	92.0	23/25	92.0	23/23	100.0	23/23	100.0
IV (15)	25	25	23/25	92.0	22/25	88.0	23/23	100.0	22/22	100.0

Mating Assignments, Mating Performance and Pregnancy Status for individual animals are presented in Appendix C.

[a]Number of females showing evidence of mating (plug and/or sperm and/or pregnancy).

[b]Number of males for which mating was confirmed in at least one female.

[c]Number of females for which parturition was evident.

[d]Number of males mated with at least one female for which parturition was evident.

Gestation Length: Gestation length was not affected by moxidectin treatment with values of 21.9, 22.0, 22.1, and 22.0 for the control, 5, 10 and 15 ppm groups respectively.

Pup Survival Indices: One control and one high-dose female had litters containing only dead pups at birth. The mean number of live, dead, and total pups at birth was comparable for the control and moxidectin-treatment groups including the high-dose group receiving 15 ppm moxidectin. However, as the lactation period progressed, fewer pups in the moxidectin-treatment groups survived. Consequently, survival in pre-cull pups was significantly reduced in all three moxidectin-treatment groups compared to control values in the LD 0-4 period. In post-cull pups, the survival index for the period of LD 4 to LD 21 in the mid- (81.1%) and high-dose (18.2%) groups was significantly lower than the control value (100%). The litter survival index for the entire lactation period was significantly lower in the high-dose group (22.7%) compared to the control value (100%) (Table 52).

Table 52: Pup and Litter Survival Data for the F_{2a} Litter Group. (Table from the Study Report)

Group (ppm)	Pup Viability Index No. [a]	Pup Viability Index %	Pup Survival Indices Lactation Days Day 0-4 No. [b]	Day 0-4 %	Day 4-21 No. [c]	Day 4-21 %	Litter Survival [e] Index No. [d]	Litter Survival [e] Index %
			P_1 Generation - F_{1a} Litters[e]					
STAT SYMBOL:	C+A+F+		C+A+F+					
STAT SYMBOL:	C+F+		C+A+F+		C+A+F+		C+A+F+	
I (0)	292/312	93.6	290/292	99.3	173/173	100.0	22/22	100.0
	**		**					
II (5)	293/294	99.7	260/293	88.7	153/158	96.8	20/21	95.2
	**		**		**			
III (10)	281/286	98.3	265/281	94.3	137/169	81.1	20/23	87.0
			**		**		**	
IV (15)	270/288	93.8	157/270	58.1	24/132	18.2	5/22	22.7

[a]Total number of live pups at day 0/total number of pups born (live plus dead).

[b]Total number of live pups at Day 4 (pre-cull)/total number of live pups at Day 0.

[c]Total number of live pups at Day 21/total number of live pups at Day 4 (post-cull).

[d]Total number of litters at weaning (Day 21)/total number of litters with live pups at Day 0.

[e]Due to high pup mortality encountered among the treated groups at all dose levels early in lactation of the F_{1a} litter interval, the decision was made to terminate all surviving litters mid-way during the lactation period. Thus, no data are presented for pup survival Days 4-21 and litter survival Day 21.

[f]Excludes data for female No. 4523 which had one viable pup in the litter at Day 1 when the decision was made to terminate the surviving F_{1a} litters.

Pup Body Weights: Mean pup weights were similar in all groups at birth, and mean pup weights on subsequent recording days during lactation were similar for Groups 1, 2, and 3. The mean body weights of high-dose pups were significantly reduced compared to control values on LDs 4, 7, 14, and 21 (Table 53).

Table 53: Pup Weight Data During the Lactation Period in the F_{1b} Litter Group.
(Table adapted from Information in the Study Report)

Group		LD 0	LD 4	LD 7	LD 14	LD 21
Group 1- 0 ppm	Mean	6.0	9.4	15.6	32.0	51.6
	SD	0.5	1.1	1.6	2.6	4.5
	N	22	22	22	22	22
Group 2 – 5 ppm	Mean	6.2	9.5	15.3	32.3	52.7
	SD	0.4	1.1	1.8	3.2	4.9
	N	21	20	20	20	20
Group 2 – 10 ppm	Mean	6.2	9.3	13.6	27.8	46.6
	SD	0.6	1.9	3.6	6.6	9.3
	N	23	22	21	20	20
Group 2 – 15 ppm	Mean	6.0	6.1**	7.3**	17.1**	31.5**
	SD	0.6	1.0	1.9	5.2	7.0
	N	22	20	12	5	5

Mean Litter Sizes: Mean litter sizes were similar for all groups at birth (ranging from 11.7 to 14.0 pups per litter). By Lactation Day (LD) 4, mean litter sizes for the 15 ppm group (7.9 surviving pups per litter) was significantly lower than control values (13.2) and litter sizes for the 15 ppm group remained significantly reduced on LDs 7, 14, and 21. The litter size in the 10 ppm moxidectin group (12.0) was not significantly reduced compared to the control value (13.2) on LD 4, but on LDs 14 and 21 mean litter sizes for this group were significantly reduced compared to control values.

Sex Distribution: Pup sex distribution indices at birth (Day 0), were similar for the control (ratio of males to females of 1.2), 5 (0.9), and 10 (1.0) ppm groups. In the 15 ppm moxidectin group, the sex ratio was lower (0.7), but not to a significant degree.

Study title: Moxidectin: A Three-Generation (Two Litters) Reproduction Study with AC 301,423 to Rats.

Study no.:	RPT-77518
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 18, 1989
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Moxidectin (AC 301,423), Batch # AC 6297-119, purity of 88.6%.

Reviewer Comment: *This multigenerational reproduction study was a definitive study and included pre-postnatal measurements as well as fertility assessments for male and female rats. The following study review pertains only to the fertility measurements in the study.*

Key Study Findings

- Dietary administration of up to 10 ppm moxidectin (approximately 0.795 mg/kg/day in male rats and 0.922 mg/kg/day in female rats), did not inhibit male or female mating rates, female pregnancy rates, or male fertility rates compared to control values.
- The survival of first (F_1) generation offspring during lactation was not changed in the moxidectin treatment groups compared to control values. Also moxidectin did not change the mean litter size or sex ratio for F_1 offspring, and F_1 body weights were not altered.
- Spermatogenesis in males and corpora lutea counts, preimplantation loss, and early and late resorptions in females were not assessed.
- The NOAEL value was considered to be the highest dose of dietary moxidectin, 10 ppm.

Methods

Doses:	0, 1, 2, 5, 10 ppm moxidectin
Frequency of dosing:	*Ad libitum* in food. Moxidectin was finely ground, then homogenously mixed into the animal food
Dose volume:	NA
Route of administration:	Oral, dietary
Formulation/Vehicle:	Animal food
Species/Strain:	Albino rat, COBS®, CD® (Sprague-Dawley derived)
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Study design:	Beginning at 43 days of age, male and female rats received control feed or feed containing different doses (1, 2, 5, or 10 ppm) of moxidectin for 10 weeks prior to initiation of mating and through gestation, birth, lactation, and rest periods in conjunction with two mating intervals until sacrifice. Males received the treatment diets for 197 or 198 days and females received treatment diets for 212 or 213 days. Each male and female underwent two rounds of mating (two mating intervals) separated by gestation and lactation periods, and two week rest periods. Two litter groups (F_{1a} and F_{1b}) were born to parental females. Males were sacrificed as a group approximately four weeks after completion of the mating period associated with production of the F_{1b} litter group. Females were sacrificed after the end of the lactation period for the second group of litters (F_{1b}).
Deviation from study protocol:	Multiple deviations in the study protocol were reported, but none of the deviations was

considered to have altered the study results.

Observations and Results

Mortality

In the parental generation no mortality was considered related to moxidectin administration. One male and one female died in the control group. Two 2 ppm females died during parturition of F_{1b} litters but the deaths were not considered related to treatment because of the absence of deaths at higher doses. One 10 ppm male was sacrificed moribund and another male died after mating of F_{1a} litters. The mortality rate in this group (8%) was not statistically different from the control (4%).

Clinical Signs

Animals were observed twice/day for clinical signs.

No drug-related clinical signs were observed.

Body Weight

Body weight of males and females was recorded weekly during premating and rest periods. Mated females: on Days 0, 7, 14, and 20 of gestation. Lactating females: on days 0, 4, 7, 14, and 21 of lactation.

Mean body weights and mean weight gains were significantly higher than the controls in females of the 5 and 10 ppm groups from Weeks 5 to 10 (premating period). In Weeks 9 and 10, the 1 ppm group also had significantly higher values than the control. The increase in weight was not considered an adverse effect related to the treatment.

Feed Consumption

For males, food consumption was assessed weekly throughout the study except during mating. For females, food consumption was assessed weekly during the premating periods and during the rest periods. Food consumption for females was not recorded during the mating or lactation periods.

Mean weekly food consumption during the premating periods for both males and females was comparable between the control and moxidectin-treatment groups.

Moxidectin Consumption in Rodent Chow

Moxidectin Consumption in the Premating Period

The daily doses of moxidectin expressed as mg/kg/day for each moxidectin dose group during the 10 week pre-mating periods are shown in Table 54.

Table 54: Mean Moxidectin Intake (mg/kg/day) for Males and Females During the Premating Period. (Adapted from Information in the Study Report)

Dose Group	P1 Generation	
	Males	**Females**
Group 2: 1 ppm moxidectin	0.069	0.082
Group 3: 2 ppm moxidectin	0.147	0.172

Group 4: 5 ppm moxidectin	0.385	0.455
Group 5: 10 ppm moxidectin	0.795	0.922
Represents the mean of weekly mean values for Weeks 1-10.		

Moxidectin Consumption During Gestation

During the F_{1a} gestation period, mean moxidectin ingestion for females in the 1, 2, 5, and 10 ppm dose groups was 0.087, 0.170, 0.423, and 0.897 mg/kg/day respectively. During the F_{1b} gestation period, mean moxidectin ingestion for females in the 1, 2, 5, and 10 ppm dose groups was 0.070, 0.150, 0.373, and 0.750 mg/kg/day respectively. For both litter groups combined during gestation, mean moxidectin ingestion for females in the 1, 2, 5, and 10 ppm dose groups was 0.079, 0.160, 0.398, and 0.824 mg/kg/day respectively.

Toxicokinetics: Not performed

Dosing Solution Analysis

Moxidectin was powdered with a mortar and mixed in animal feed and stored refrigerated on a weekly basis. Samples of feed containing moxidectin were taken prior to initiation of treatment and weekly thereafter then shipped for analysis. All diet samples were stored at -8°C and assayed within a month of receipt. For homogeneity and stability tests, 100 gram samples were taken from the right and left sides of the top, middle, and bottom of the low- and high-dose diet preparations. For the mixing study, two representative samples of each weekly batch of each concentration of moxidectin in chow was collected and frozen, with later analysis of one of the samples. Duplicate 20 gram portions of rodent meal samples were assayed for homogeneity and stability (low and high doses) and concentration (all doses) by two HPLC methods with a lower limits of detection of 1 and 5 ppm respectively.

The homogeneity results indicated actual concentration for all layers that were ±10% of the nominal concentration. The average actual concentrations for the stability samples were all within the acceptance criteria of ±10% of the nominal concentrations. For all of the weekly samples, the actual moxidectin concentrations were within the acceptance criteria of ±10% of the nominal concentrations.

Necropsy

Necropsy examinations were performed for all parental animals including those found dead or sacrificed in moribund condition. Postmortem examinations of females reportedly included a count of uterine implantation scars when present.

Males: Males were sacrificed and necropsied as a group upon completion of the mating period associated with the second litter group (F_{1b}). In addition, the following tissues from in the control and high-dose males were preserved and examined for histopathology: testes and epididymides (both), seminal vesicles (both), prostate, pituitary, and gross lesions.

No gross pathology associated with moxidectin was reported for the parental male rats. Also no moxidectin-related histopathology in the testes and epididymus, seminal vesicles, prostate or the pituitary glands of high-dose males was observed. Spermatogenesis was not assessed. Bilateral germinal epithelial degeneration in testes was reported for 1/25 males in both the control and high-dose groups.

Females: Following the end of the lactation period for the second F_1 litter (F_{1b}), dams were necropsied and examined for gross pathology. The following tissues from the dams in the control and high-dose groups were preserved and examined for histopathology: ovaries (both), pituitary, uterus, vagina, and gross lesions. No information on the uterine implantation counts was included in the pathology report.

No maternal gross pathology associated with moxidectin was reported. Also no moxidectin-related histopathology in the ovaries, uterus, or the pituitary glands of the dams in the high-dose group was observed.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Gestation Length: Mean gestation lengths were comparable between the control and moxidectin-treatment groups for both litter groups (F_{1a} and F_{1b}) with all values ranging from 21.9 days to 22.1 days.

Mating, Pregnancy, and Fertility Rates: The rates of male and female mating, female pregnancy, and male fertility for moxidectin doses up to 10 ppm were not significantly changed from control values for both litter groups (Table 55).

Table 55: Mating, Pregnancy and Fertility Rates for Rats Administered Dietary Moxidectin. (Table Adapted from Information in the Study Report)

Group	Mating		Pregnancy	Fertility
	Male	Female	Female	Male
Litter F_{1a}				
0 ppm	25/25 (100%)	25/25 (100%)	22/25 (88%)	25/25 (88%)
1 ppm	25/25 (100%)	25/25 (100%)	25/25 (100%)	25/25 (100%)
2 ppm	25/25 (100%)	24/25 (96%)	23/25 (92%)	22/24 (91.7%)
5 ppm	24/25 (96%)	22/25 (88%)	21/25 (87.5%)	19/22 (86.4%)
10 ppm	25/25 (100%)	24/25 (96%)	22/25 (88%)	21/25 (87.5%)
Litter F_{1b}				
0 ppm	25/25 (100%)	23/25 (92%)	22/25 (88%)	20/23 (87%)
1 ppm	25/25 (100%)	20/25 (80%)	25/25 (100%)	20/20 (100%)
2 ppm	24/25 (96%)	23/25 (92%)	22/24 (91.7%)	21/23 (91.3%)
5 ppm	24/25 (96%)	20/25 (80%)	22/24 (91.7%)	19/20 (95%)
10 ppm	25/25 (100%)	19/23 (82.6%)	22/25 (88%)	19/19 (100%)

Pup Survival Indices: The mean number of live, dead, and total pups were similar for all groups in both litter groups (F_{1a} and F_{1b}) at birth, and on Lactation Days (LDs) 4, 7, 14, and 21. In the F_{1a} litter, the percent of surviving pups in the 2 ppm group was signifinatly

lower on LD 4 before culling (94.1%) compared to the control value (98.3%). Also the percent of surviving pups in the 10 ppm groups was slightly but significantly lower on LD 4 before culling (91.7%) and on LD 21 after culling (91.6%) compared to the respective control values of 98.3% and 99.4%. In each case, the reductions in percent survival mainly occurred in one litter in the respective treatment groups in the respective measurement periods (pre- and post-cull) suggesting the effect was not related to moxidectin. Also, the percent of surviving pups in all the moxidectin treatment groups including the 10 ppm group were not significantly lower in the F_{1b} litter group at the same timepoints compared to control values.

Table 56: Survival of F_1 Pups and the Number of Litters with Pup Mortality. (Table Adapted from Information in the Study Report)

F_1 Pup Survival and Litters with Pup Mortality								
Group	**Day 0 - 4**				**Day 4 - 21**			
	Pup Survival		Lit. w/ Pup Mort.		Pup Survival		Lit. w/ Pup Mort.	
	No.	%	No.	%	No.	%	No.	%
F_{1a} Litters								
0 ppm	286/291	98.3	3/21	14.3	167/168	99.4	1/21	4.8
1 ppm	320/327	97.9	5/25	20.0	194/196	99.0	2/25	8.0
2 ppm	286/304	94.1*	6/23	26.1	174/178	97.8	3/23	13.0
5 ppm	255/260	98.1	4/21	19.0	157/158	99.4	1/20	5.0
10 ppm	264/288	91.7**	6/22	27.3	153/167	91.6**	6/21	28.6
F_{1b} Litters								
0 ppm	282/285	98.9	2/22	9.1	176/176	100.0	0/22	0.0
1 ppm	314/320	98.1	6/25	24.0	194/194	100.0	0/25	0.0
2 ppm	267/272	98.2	4/20	20.0	160/160	100.0	0/20	0.0
5 ppm	279/287	97.2	5/21	23.8	168/168	100.0	0/21	0.0
10 ppm	282/288	97.9	4/22	18.2	172/175	98.3	2/22	9.1
* significantly reduced compared to vehicle control values ($p \leq 0.05$)								
** significantly reduced compared to vehicle control values ($p \leq 0.01$)								

Pup Body Weights: Mean pup body weights were similar for all groups in both litters at birth, and on Days 4, 7, 14, and 21 for both litters (Data for the F_{1a} litter group is shown in Table 57).

Table 57: Mean F_1 Pup Weights (Mean ± SD) During Lactation for the F_{1a} Litter Groups. (Table adapted from Information in the Study Report)

Group	Day 0	Day 4 (pre-cull)	Day 7	Day 14	Day 21
0 ppm	5.9 ± 0.4	8.9 ± 0.8	14.7 ± 1.3	30.8 ± 3.1	47.2 ± 3.7
1 ppm	6.2 ± 0.5	9.2 ± 1.1	14.8 ± 1.8	31.0 ± 3.2	47.9 ± 5.6
2 ppm	6.0 ± 0.6	9.0 ± 1.6	15.0 ± 1.6	31.3 ± 2.6	48.8 ± 4.3
5 ppm	6.1 ± 0.7	9.4 ± 1.6	15.2 ± 2.6	31.6 ± 4.4	49.3 ± 6.7
10 ppm	6.0 ± 0.4	9.3 ± 1.3	14.2 ± 2.6	29.5 ± 5.3	44.9 ± 7.5

Mean Litter Sizes: Mean litter sizes on LD 0 and LD 4 and on later dates, LDs 7, 14, and 21, were similar for all groups in both litter groups.

Sex Distribution: Sex distribution data in both litter groups was similar for all treatment groups with male/female ratio values ranging from 0.8 to 1.2.

9.2 Embryonic Fetal Development

Study title: Moxidectin: An Oral Development Toxicity Study with AC 301423 in Rats

Study no.:	RPT-77460
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 24, 1989
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Moxidectin (AC 301,423, Lot No.: 6736-16, purity of 88.5%.

Key Study Findings

- Over the course of the dosing period (GD6 - GD16), maternal body-weight gains were significantly reduced by 19.3% and 35.2% in the 10 and 12 mg/kg/day groups respectively compared to control values. Corresponding reductions in food intake over the dosing period were a nonsignificant 4.0% reduction in the 10 mg/kg/day group and a significant 13.4% reduction in the 12 mg/kg/day group.
- Maternal body weights were significantly reduced on GD17 (the day after the end of dosing) by 3.9% and 5.7% in the 10 and 12 mg/kg/day groups respectively.
- None of the Caesarian section parameters nor fetal body weights were significantly changed by moxidectin administration.
- The number of fetuses with cleft palate was significantly increased in the high-dose group (12 mg/kg/day) compared to control fetuses. However, in the same group, the litter incidence of cleft palate was not significantly increased.
- Also in high-dose fetuses, the fetal and litter incidence of one skeletal variation, wavy ribs, was significantly increased compared to control values.
- The NOAEL values for maternal and fetal toxicity were considered to be 5 and 10 mg/kg/day respectively.

Methods

Doses:	0, 2.5, 5.0, 10.0, 12.0 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Corn oil, (Mazola®, Best Foods, CPC International)
Species/Strain:	Crl:CD®(SD)BR: females were 71 days old at receipt (July 18, 1989) and weighed 167 to 217

	grams; Males were 73 days old upon receipt and weighed 213 to 336 grams.
Number/Sex/Group:	25 females
Satellite groups:	None
Study design:	Male and female rats were cohabitated for 4 days. Moxidectin was administered by oral gavage once per day to pregnant female rats from Gestation Day (GD) 6 to GD 15. All female rats were sacrificed and underwent Caesarian section on GD 20.
Deviation from study protocol:	Multiple deviations in the study protocol were observed. However none was considered to have altered the study results.

Observations and Results

Mortality

Female rats were examined for viability twice per day throughout the study.

No deaths occurred in the study.

Clinical Signs

Female rats were observed for clinical signs and/or general appearance several times during the acclimation period, and on GD 0. Observations for clinical signs of the test substance effect, abortions, premature deliveries and/or viability were also conducted several times per day during the period of dosing. After GD 15, on GD 16 to GD 20, observations were made once per day.

A significant incidence of high-dose females receiving 12 mg/kg/day exhibited urine-stained abdominal fur (4/25 dams throughout dosing) and red substance on fur (3/25 dams for a total of 16 days) as well as red tears (2/25 dams for 5 days). Similar clinical signs were not observed in control dams or dams in the other moxidectin dose groups, and no other clinical signs were considered to be related to moxidectin administration.

Body Weight

Body weights of rats were recorded at least once per week prior to mating and in female rats on GDs 0, 6, and 20.

Dams administered 10 and 12 mg/kg/day moxidectin demonstrated significant reductions in mean maternal body weight gains during the dosage period (GDs 6 to 16). Body weight gains were significantly reduced for the 10 mg/kg/day group during GDs 9-12 and for the 12 mg/kg/day group during GDs 9-12 and GDs 12-16. For the entire dosage period, mean body weight gains for the 10 and 12 mg/kg/day groups were reduced by 19.3% and 35.2% respectively compared to control value. During the post-dose period from GD 16 to 20, body weights in the 10 and 12 mg/kg/day groups tended to rebound. However, for the entire gestation period (GD 0 to GD 20) average maternal

body weight gains were significantly reduced in the 10 and 12 mg/kg/day groups by 7.7% and 8.5% respectively compared to control values.

In conjunction with the reduced body weight gains in these groups, maternal body weights were also significantly reduced in the 10 mg/kg/day group on GDs 15 and 16 and in the 12 mg/kg/day group on GDs 10, 12, 13, 15, and 16. Mean body weights were significantly reduced by 3.9% in the 10 mg/kg/day group on Day 17 and by 5.7% and 4.1% in the 12 mg/kg/day group on GDs 17 and 18 respectively compared to control values.

Feed Consumption

Food consumption was recorded in female rats on GDs 0, 6, and 20.

Significantly decreased mean values for absolute (g/day; 13.4% decrease) and relative (g/kg/day; 10.4% decrease) food consumption was observed in dams in the 12 mg/kg/day moxidectin group during dosing (GDs 6-16) compared to vehicle control values. Animals in the 10 mg/kg/day group demonstrated smaller decrements in absolute (-4.0%) and relative (-1.2%) food consumption. Relative maternal food consumption significantly increased after dosing (GDs 16-20), but despite the rebound, mean values for absolute food consumption were still significantly reduced for the GD 6 to GD 20 period in high-dose animals.

Toxicokinetics: Not performed

Dosing Solution Analysis

One 10 ml sample of each dosing concentration was retained on the first and last days of dosing. Samples were sent to the Sponsor [redacted] (b) (4) for analysis.

Necropsy

The abdomen of each pregnant rat was opened, and the intact uterus was excised and weighed. The thoracic and abdominal cavities were examined for gross lesions.

None of the gross pathology findings occurred in a moxidectin-dependent manner.

Gravid uterine weights were not changed in any of the moxidectin treatment groups compared to concurrent control values.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnant females underwent Cesarian section on GD 20. Corpora lutea in each ovary were counted. The number and placement of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not evident. A late resorption was defined as one in which the occurrence of organogenesis was evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Administration of the test substance to pregnant rats at dosages as high as 12 mg/kg/day did not affect any of the parameters examined on GD 20 at Cesarean-sectioning of the dams. There were 23, 24, 25, 24 and 24 pregnant rats in the 0(vehicle), 2.5, 5, 10 and 12 mg/kg/day dosage groups, respectively. There were no dosage-dependent or significant differences among the five groups in the average numbers of corpora lutea, implantations, live litter sizes or resorptions, or the numbers of dams with resorptions (Table 58).

Table 58: Summary of Cesarean Section Data in the Rat Embryo-Fetal Study.
(Table from the Study Report)

DOSAGE GROUP DOSAGE (MG/KG/DAY)		I 0	II 2.5	III 5	IV 10	V 12
RATS - TESTED	N	25	25	25	25	25
PREGNANT	N(%)	23(92.0)	24(96.0)	25(100.0)	24(96.0)	24(96.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 20	N	23	24	25	24	24
CORPORA LUTEA	MEAN±S.D.	15.3 ± 1.4	15.4 ± 2.0	15.4 ± 1.8	14.8 ± 1.9	15.2 ± 2.0
IMPLANTATIONS	MEAN±S.D.	14.6 ± 1.6	14.2 ± 1.4	13.8 ± 1.8	13.8 ± 1.4	14.0 ± 2.4
LITTER SIZE	MEAN±S.D.	13.9 ± 2.1	13.5 ± 1.6	13.1 ± 2.0	12.7 ± 1.8	13.4 ± 2.2
LIVE FETUSES	N	319	324	328	305	322
	MEAN±S.D.	13.9 ± 2.1	13.5 ± 1.6	13.1 ± 2.0	12.7 ± 1.8	13.4 ± 2.2
DEAD FETUSES	N	0	0	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.7 ± 1.1	0.8 ± 0.7	0.7 ± 0.6	1.1 ± 1.5	0.5 ± 0.7
EARLY RESORPTIONS	N	16	18	18	25	13
	MEAN±S.D.	0.7 ± 1.1	0.8 ± 0.7	0.7 ± 0.6	1.0 ± 1.5	0.5 ± 0.7
LATE RESORPTIONS	N	0	0	0	1	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	11(47.8)	15(62.5)	16(64.0)	16(66.7)	11(45.8)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	24(100.0)	25(100.0)	24(100.0)	24(100.0)

DAY = DAY OF PRESUMED GESTATION

Offspring (Malformations, Variations, etc.)

Each fetus was removed from the uterus, placed in an individual container and individually identified with a tag. Each fetus was subsequently weighed and examined to identify sex and gross external alterations. Live fetuses were then sacrificed by carbon dioxide asphyxiation. Approximately one-half of the fetuses in each litter were fixed and examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated, and their skeletons were stained with alizarin red S and examined for skeletal alterations. Late resorptions were examined to the extent possible.

Mean fetal body weights were reduced in the 5, 10, and 12 mg/kg/day moxidectin dose groups compared to vehicle control value (Table 59). However, the reductions were not

statistically significant, did not progress in a dose-dependent manner, and were within the historical control range. All the other fetal parameters, including live fetuses, percent male fetuses, and percent resorbed fetuses per litter were not significantly changed in any of the moxidectin treatment groups compared to control values.

Table 59: Summary of Litter Data for Caesarean-Delivered Fetuses in the Rat Embryo-Fetal Study. (Table from the Study Report)

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)		0	2.5	5	10	12
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	24	25	24	24
IMPLANTATIONS	MEAN±S.D.	14.6 ± 1.6	14.2 ± 1.4	13.8 ± 1.8	13.8 ± 1.4	14.0 ± 2.4
LIVE FETUSES	N	319	324	328	305	322
	MEAN±S.D.	13.9 ± 2.1	13.5 ± 1.6	13.1 ± 2.0	12.7 ± 1.8	13.4 ± 2.2
LIVE MALE FETUSES	N	157	161	162	149	152
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.5 ± 13.7	49.4 ± 12.9	49.4 ± 12.2	48.6 ± 12.9	47.2 ± 10.5
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	3.40 ± 0.22	3.39 ± 0.20	3.24 ± 0.27	3.29 ± 0.21	3.23 ± 0.18
MALE FETUSES	MEAN±S.D.	3.45 ± 0.24	3.48 ± 0.22	3.33 ± 0.27	3.38 ± 0.23 2%	3.33 ± 0.18 3%
FEMALE FETUSES	MEAN±S.D.	3.34 ± 0.23	3.30 ± 0.19	3.16 ± 0.26 5%	3.20 ± 0.19	3.14 ± 0.18 6%
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.0 ± 8.4	5.4 ± 4.8	5.3 ± 4.6	7.8 ± 10.0	3.6 ± 4.4

Fetal Alterations (Malformations and Variations)

The number of fetuses with fetal alterations, defined as malformations (irreversible changes which occur at low incidences in this species and strain) or variations (common findings in this species and strain with reversible delays or accelerations in development), were significantly increased in the two highest dose groups receiving maternal doses of 10 and 12 mg/kg/day (Table 60). However, the number of litters including fetuses with alterations was not significantly increased. Both the 10 and 12 mg/kg/day groups experienced significant reductions in maternal body weight gain during dosing and on the day after the end of dosing, and the increased number of fetal alterations in these groups may have been influenced by these reductions in the affected groups.

Table 60: Summary of Fetal Alterations in the Rat Embryo-fetal Study. (Table from the Study Report)

		Dosage Group (mg/kg/days 6-15 of Gestation)				
		0(Vehicle)	2.5	5	10	12
Litters Evaluated	N	23	24	25	24	24
Fetuses Evaluated	N	319	324	328	305	322
Live	N	319	324	328	305	322
Dead	N	0	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(%)	6(26.1)	10(41.7)	10(40.0)	13(54.2)	13(54.2)
Fetuses with any Alteration Observed	N(%)	10(3.1)	14(4.3)	18(5.5)	24(7.9)**	27(8.4)**
% Fetuses with any Alteration/Litter	$\bar{X}$±S.D.	3.90±10.64	4.15±6.06	6.27±9.79	7.84±9.44	8.09±9.98

** Significantly different from the vehicle control group value ($P \leq 0.01$).

External Malformations

All of the Caesarean-delivered fetuses were examined for fetal malformations. Most of the malformations did not significantly increase for incidence in the moxidectin dose groups or increase in a moxidectin-dose related manner (Table 58). However, one external malformation, cleft palate was significantly increased for fetal incidence in the high-dose group, and the litter incidence of this malformation was increased, but not to a significant degree. Because maternal body weights and food consumption were significantly reduced for dams in the 12 mg/kg/day group, and clinical signs were also noted for these animals, it is not clear how much maternal toxicity influenced the increased incidence of this malformation.

Table 61: Summary of Fetal External Malformations in the Rat Embryo-fetal Study.

Parameters	Dose Group (mg/kg/day) on GD6-GD15)				
	0 (Vehicle)	2.5	5	10	12
Litters Evaluated: N	23	24	25	24	24
Fetuses Evaluated: N	319	324	328	305	322
Live: N	319	324	328	305	322
Dead: N	0	0	0	0	0
Bulging, Depressed Eye					
Litter Incidence: N(%)	1(4.3)	0	0	0	0
Fetal Incidence: N(%)	1(0.3)	0	0	0	0
Short Snout					
Litter Incidence: N(%)	0	0	1(4.0)	0	0
Fetal Incidence: N(%)	0	0	1(0.3)	0	0
Jaw (Micrognathia)					
Litter Incidence: N(%)	0	0	1(4.0)	0	0

Fetal Incidence: N(%)	0	0	1(0.3)	0	0
Protruding Tongue					
Litter Incidence: N(%)	0	0	1(4.0)	0	0
Fetal Incidence: N(%)	0	0	1(0.3)	0	0
Cleft Palate [a]					
Litter Incidence: N(%)	0	0	1(4.0)	1(4.2)	4(16.7)
Fetal Incidence: N(%)	0	0	1(0.3)	2(0.6)	5(1.6)**
Body – Umbilical Hernia					
Litter Incidence: N(%)	0	1(4.2)	1(4.0)	1(4.2)	0
Fetal Incidence: N(%)	0	1(0.3)	1(0.3)	1(0.3)	0
Short Tail					
Litter Incidence: N(%)	0	1(4.2)	0	0	0
Fetal Incidence: N(%)	0	1(0.3)	0	0	0
Constricting Ring Around Tail					
Litter Incidence: N(%)	0	0	1(4.0)	0	0
Fetal Incidence: N(%)	0	0	1(0.3)	0	0
Anus: Agenesis					
Litter Incidence: N(%)	0	1(4.2)	0	0	0
Fetal Incidence: N(%)	0	1(0.3)	0	0	0

[a] The incidence of cleft palate includes the incidence identified by soft tissue and skeletal examinations.

** Significantly different from the vehicle control group value ($p \leq 0.01$).

Soft Tissue Malformations

A number of soft tissue malformations were observed (Table 62). However, none was significantly increased for fetal or litter incidence in the moxidectin dose groups compared to control values, and none of the malformations increased in a moxidectin dose-related manner. These results suggest none of the observed soft tissue malformations were related to moxidectin administration.

Table 62: Summary of Fetal Soft Tissue Malformations[a] in the Rat Embryo-fetal Study.

Parameters	Dose Group (mg/kg/day) on GD6-GD15)				
	0 (Vehicle)	2.5	5	10	12
Litters Evaluated: N	23	24	25	24	24
Fetuses Evaluated: N	154	155	158	147	153
Live: N	154	155	158	147	153
Dead: N	0	0	0	0	0
Eye - Microphthalmia					
Litter Incidence: N(%)	1(4.3)	0	0	0	0
Fetal Incidence: N(%)	1(0.6)	0	0	0	0
Eye - Folded Retina					
Litter Incidence: N(%)	0			1(4.2)	
Fetal Incidence: N(%)	0			1 (0.7)	
Brain - Lateral and/or Third Ventricles, Slightly or Moderately Dilated					
Litter Incidence: N(%)	0	1(4.2)	1(4.0)	0	0
Fetal Incidence: N(%)	0	1 (0.6)	1 (0.6)	0	0

Heart - Ventricular Septal Defect					
Litter Incidence: N(%)	0	1(4.2)		0	0
Fetal Incidence: N(%)	0	1 (0.6)		0	0
Kidney – Slight or Moderated Dilation of the Pelvis					
Litter Incidence: N(%)	0	1(4.2)	0	1(4.2)	0
Fetal Incidence: N(%)	0	2 (1.3)	0	1 (0.7)	0
Intestines – Absent Meconium					
Litter Incidence: N(%)	0	1(4.2)	0	0	0
Fetal Incidence: N(%)	0	1 (0.6)	0	0	0

[a] Soft tissue malformations that were also identified as external malformations, including cleft palate, micrognathia, and umbilical hernia, are omitted from this table.

Skeletal Variations

A higher percentage of high-dose fetuses and litters exhibited wavy ribs (Table 63). This skeletal variation correlates with maternal weight loss and reduced food consumption for high-dose dams and may be a result of moxidectin-related maternal toxicity. Other significant skeletal variation findings did not occur in a moxidectin-dose related manner, and were not considered to be related to moxidectin administration.

Table 63: Summary of Skeletal Variations in the Rat Embryo-fetal Study.

Parameters	Dose Group (mg/kg/day) on GD6-GD15				
	0 (Vehicle)	2.5	5	10	12
Litters Evaluated: N	23	24	25	24	24
Fetuses Evaluated: N	165	168	170	158	169
Live: N	165	168	170	158	169
Dead: N	0	0	0	0	0
Skull: Incompletely Ossified Palate					
Litter Incidence: N(%)		0	0	1(4.2)	1(4.2)
Fetal Incidence: N(%)		0	0	1 (0.6)	1 (0.6)
Thoracic Vertebrae – Centrum, Bifid					
Litter Incidence: N(%)	2(8.7)	2(8.3)	2(8.0)	4(16.7)	3(12.5)
Fetal Incidence: N(%)	3(1.8)	1 (1.2)	2(1.2)	4(2.5)	3(1.8)
Incompletely Ossified Lumbar Arches					
Litter Incidence: N(%)	0	0		2(8.3)	1(4.2)
Fetal Incidence: N(%)	0	0		2(1.3)	1(0.6)
Ribs – Cervical Rib Present					
Litter Incidence: N(%)	2(8.7)	1(4.2)	0	1(4.2)	1(4.2)
Fetal Incidence: N(%)	2(1.2)	1(0.6)	0	1(0.6)	1(0.6)
Ribs – Incompletely Ossified					
Litter Incidence: N(%)	0	1(4.2)	2(8.0)	1(4.2)	4(16.7)
Fetal Incidence: N(%)	0	1(0.6)	2(1.2)	1(0.6)	5(3.0)
Ribs - Wavy					

Litter Incidence: N(%)	0	1(4.2)	3(12.0)	2(8.3)	7(29.2)**
Fetal Incidence: N(%)	0	1(0.6)	3(1.8)	4(2.5)	13(7.7)**
Sternebrae – Incompletely Ossified					
Litter Incidence: N(%)	1(4.3)	4(16.7)	6(24.0)	2(8.3)	5(20.8)
Fetal Incidence: N(%)	1(0.6)	6(3.6)	8(4.7)	5(3.2)	7(4.1)
Sternebrae – Not Ossified					
Litter Incidence: N(%)	0	1(4.2)	5(20.0)**	1(4.2)	0
Fetal Incidence: N(%)	0	1(0.6)	6(3.5)**	2(1.3)	0
Sternebrae – Asymetric					
Litter Incidence: N(%)	0	0	1(4.0)	0	0
Fetal Incidence: N(%)	0	0	1(0.6)	0	0
Pelvis – Incompletely Ossified Pubis					
Litter Incidence: N(%)	2(8.7)	1(4.2)	5(20.0)	5(20.8)	3(12.5)
Fetal Incidence: N(%)	1(1.2)	3(1.8)	9(5.3)**	10(6.3)**	5(3.0)
Pelvis – Incompletely Ossified Ischia					
Litter Incidence: N(%)	1(4.3)	1(4.2)	2(8.0)	4(16.7)	3(12.5)
Fetal Incidence: N(%)	1(0.6)	2(1.2)	2(1.2)	4(2.5)	5(3.0)
Pelvis – Unossified Pubis					
Litter Incidence: N(%)	1(4.3)	0	0	1(4.2)	0
Fetal Incidence: N(%)	3(1.8)	0	0	1(0.6)	0
** Significantly different from the vehicle control group value ($p \leq 0.01$).					

Study title: Moxidectin: A Developmental Toxicity (Embryo-fetal Toxicity and Teratogenicity) Definitive Study with AC 301,423 in Rabbits.

Study no.: RPT-77516
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: December 7, 1988
GLP compliance: Yes, EPA Good Laboratory Practice Standards.
QA statement: Not included with the study report.
Drug, lot #, and % purity: Moxidectin (AC 301,423), Lot # AC 6297-47, purity of 81.9%.

Key Study Findings

- Mean maternal body weight gain and food consumption was significantly reduced in the mid- and high-dose groups throughout dosing.
- Caesarian-section parameters and fetal indices were not altered by treatment with moxidectin.
- Moxidectin did not increase fetal malformations or variations.
- The NOAEL values for maternal and fetal toxicity were considered to be 1.0 and 10 mg/kg/day respectively.

Methods

Doses:	0(vehicle), 1.0, 5.0, and 10.0 mg/ml.
Frequency of dosing:	Once per day from gestation Day (GD) 7 to GD 19
Dose volume:	1.0 ml/kg/day
Route of administration:	Oral via stomach tube
Formulation/Vehicle:	Corn oil (Mazola™ Best foods, CPC International)
Species/Strain:	HRa (NZW)SPF rabbits (approximately 5 months old upon receipt).
Number/Sex/Group:	18 pregnant females per group
Satellite groups:	none
Study design:	Corn oil and corn oil solutions of AC 301,423 (moxidectin) were given orally via a stomach tube once daily to artificially inseminated pregnant rabbits on GDs 7-19 at doses of 0(vehicle), 1, 5, and 10 mg/kg/day in dosage volumes of 1.0 ml/kg/day). All surviving mothers were euthanized and Caesarean-sectioned on GD 29.
Deviation from study protocol:	Multiple protocol deviations were noted. However, none of the deviations was considered to have altered the study results.

Observations and Results

Mortality

Viability of the rabbits was noted twice daily throughout the study (at the beginning and end of the workday).

No deaths occurred that were considered related to moxidectin administration. One vehicle control dam was found dead on GD 14. One high-dose dam was found dead on GD 7 just after attempted administration of the first dose.

Abortions

Two low-dose dams and one high-dose dam aborted on GDs 21, 25, and 22 respectively. In the absence of a dose-dependent response, the abortions were not considered related to moxidectin administration.

Clinical Signs

Dams were observed for clinical signs and/or general appearance several times during the acclimation period and on GD 0. Each rabbit was also observed for clinical signs of a moxidectin effect, abortion, premature delivery and viability immediately prior to dosing each day beginning on GD 7 and continuing through GD 19 and approximately one-half hour and one hour after dosing. Clinical signs were assessed once daily from GD 20 to GD 29.

Clinical signs prior to death for the vehicle control dam that was found dead on GD 14 consisted of labored breathing, decreased motor activity, loss of righting reflex, and red substance around the mouth. The clinical signs and death were considered to be related to a dosing accident. The high-dose dam found dead on GD 7 had clinical signs that included: clonic convulsions and red substance around its mouth prior to death. The diaphragm was perforated and the death was considered to be related to a dosing accident.

Body Weight

Body weights of the rabbits were recorded at least once weekly prior to insemination and on GD 0 and daily from GD 7 to GD 29.

Administration of 5 and 10 mg/kg/day dosages of moxidectin significantly inhibited average maternal body weight gains compared to control values (Table 64). The inhibitory effects of these dosages were significant between GDs 7 and 10 (high dose animals), and GDs 7 and 14 and 7 and 17 of gestation (middle- and high-dose animals) compared to control group values. Weight loss occurred for the mid-dose group between GDs 7 and 14 and for the high-dose group between GDs 7 and 17. Body weight gains were not significantly different between GD 7 and 20 although values tended to be lower in the 5 and 10 mg/kg/day moxidectin groups. In the days after dosing, from GD 20 to GD 29, maternal weight gains rebounded in the 5 and 10 mg/kg/day moxidectin groups with higher body weight gains (approximately 19% and 10% respectively) compared to control values but the differences between groups were not significant.

Table 64: Mean Changes in Maternal Body Weight at Progressive Intervals of the Dosing Period.

Group	Mean Maternal Body Weight Changes in kg in Different Dosing Intervals During Gestation		
	GD7 – GD10	GD7 – GD14	GD7 – GD17
Vehicle Control	+0.04	+0.12	+0.14
1 mg/kg moxidectin	+0.01	+0.11	+0.09
5 mg/kg moxidectin	-0.01	-0.01* (-108%)	+0.01* (-92.9%)
10 mg/kg moxidectin	-0.06* (-250%)	0.00* (-100%)	-0.02* (-114%)
Values are shown in kg. The percentile values shown in parentheses represent the percent change in body weight gain compared to control values. GD = Gestation Day. * Significantly different ($p \leq 0.05$) than control weight gain values.			

Mean body weights tended to be lower in the mid- and high-dose animals, but differences were not significantly lower than vehicle-control values (Figure 7). Body weights were non-significantly reduced by 5.1% and 5.4% on Day 17 in the mid- and high-dose females compared to control values.

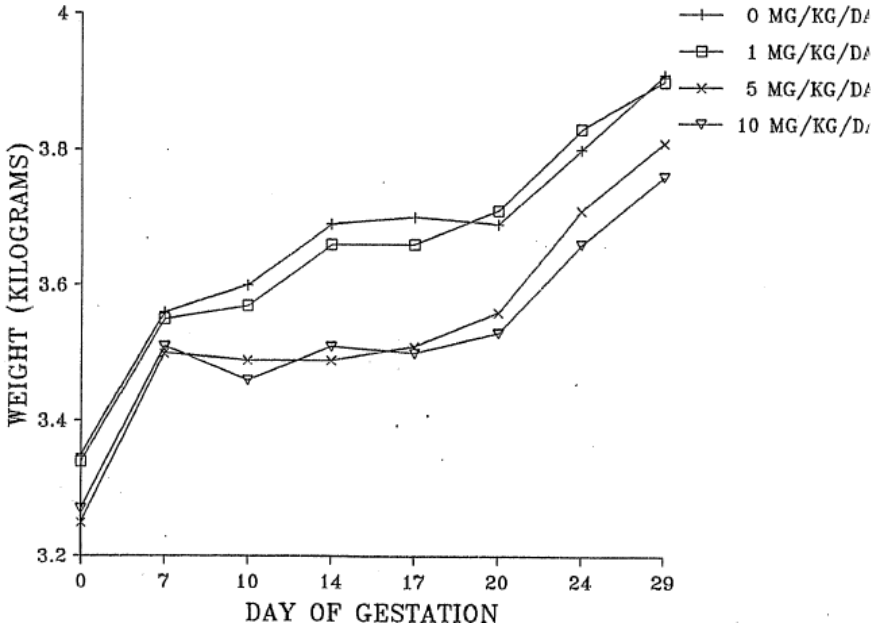

Figure 7: Mean Maternal Body Weights in the Rabbit Embryo-fetal Study. (Figure from the Study Report)

Feed Consumption

Food Consumption was recorded daily during the acclimation period and from GD 0 to GD 29.

Administration of the 5 and 10 mg/kg/day dosages of moxidectin significantly decreased mean maternal feed consumption between GD 7 and GD 10 for the high-dose group and between GD 10 and GD 14, GD 7 and GD 14, and GD 7 and GD 17 of for the mid- and high-dose groups compared to control values (Table 65). Food consumption was similar in all groups during the post-dosage period (GD 20 to GD 29).

Table 65: Mean Changes in Maternal Food Consumption in Successive Intervals of the Dosing Period.

Group	Mean Grams of Maternal Food Consumption in Different Dosing Intervals During Gestation			
	GD7 – GD10	GD10 – GD14	GD7 – GD14	GD7 – GD17
Vehicle Control	168.6	153.8	160.1	148.0
1 mg/kg moxidectin	155.3	150.1	151.6	146.8
5 mg/kg moxidectin	148.2	111.9**	127.8*	114.8*
10 mg/kg moxidectin	124.2**	112.5**	117.5**	112.7*
Values are shown in kg. GD = Gestation Day. * Significantly different ($p \leq 0.05$) than control weight gain values. ** Significantly different ($p \leq 0.01$) than control weight gain values.				

Toxicokinetics: Not performed

Dosing Solution Analysis

Samples of the dosing solutions were reportedly frozen for possible analysis by the Sponsor, but the analysis results were not reported.

Necropsy

All surviving dam were necropsied on GD 29, and the thoracic and abdominal cavities were examined for gross pathology. Tissues with gross pathology were fixed for possible future evaluation.

All of the necropsy findings were related to dosing accidents (lung perforation and hemorrhage) or occurred incidentally without relationship to moxidectin administration (Table 66).

Table 66: Summary Necropsy Observations in the Rabbit Embryo-Fetal Study.
(Table from the Study Report)

	DOSAGE GROUP	I	II	III	IV
RABBITS - TESTED	N	18	18	18	18
RABBITS - FOUND DEAD	N	1a	0	0	1b
RABBITS - ABORTED AND SACRIFICED	N	0	2	0	1
NECROPSY OBSERVATIONS d					
LUNGS:					
HEMORRHAGIC AREAS THROUGHOUT	N	1a	0	0	0
RIGHT DIAPHRAGMATIC LOBE;					
PERFORATION PRESENT	N	1a	0	0	1b
DISCOLORED	N	0	0	1c	0
THORACIC CAVITY:					
MASS PRESENT	N	0	0	1c	0
ABDOMEN:					
HERNIA	N	1	0	0	0
PAROVARIAN CYST(S)	N	11	7	8	6

DOSAGE GROUP:
I - 0 MG/KG/DAY II - 1 MG/KG/DAY III - 5 MG/KG/DAY IV - 10 MG/KG/DAY
Dosage occurred on days 7-19 of presumed gestation.
a. Occurred in rabbit 14171 that was found dead on day 14 of gestation.
b. Occurred in rabbit 14232 that was found dead on day 7 of gestation.
c. Occurred in rabbit 14210 that survived an intubation accident.
d. Observations cited by exception.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Following necropsy on GD 29, the intact uterus of each dam was excised and weighed. Corpora lutea in each ovary were counted. The number and placement of implantations, early and late resorptions, and live and dead fetuses were noted. An early resorption was defined as one in which organogenesis was not evident. A late resorption was

defined as one in which the occurrence of organogenesis was evident. A live fetus was defined as a fully developed fetus that responded to mechanical stimuli. Nonresponding fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Oral administration of moxidectin up to 10 mg/kg/day did not significantly alter female fertility or litter size, or the incidence of implantation, corpora lutea, live and dead fetuses, or early and late resorptions compared to the vehicle control or historical control values (Table 67).

Table 67: Summary of the Caesarean-Section Data in Rabbits. (Table from the Study Report)

	DOSAGE GROUP	0 MG/KG/DAY	1 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
RABBITS - TESTED	N	18	18	18	18
RABBITS - PREGNANT	N(%)	14(77.8)	16(88.9)	16(88.9)	18(100.0)
ABORTED	N	0	2	0	1
FOUND DEAD	N	1	0	0	1
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29	N	13	14	15a,b	15c
CORPORA LUTEA	MEAN±S.D.	10.9 ± 2.1	10.6 ± 1.6	11.1 ± 4.4	10.5 ± 1.9
IMPLANTATIONS	MEAN±S.D.	7.7 ± 2.5	7.6 ± 2.4	7.8 ± 2.2	7.4 ± 2.0
LITTER SIZE	MEAN±S.D.	7.1 ± 2.2	7.4 ± 2.4	7.5 ± 1.9	6.2 ± 2.5
LIVE FETUSES	N	92	103	112	93
	MEAN±S.D.	7.1 ± 2.2	7.4 ± 2.4	7.5 ± 1.9	6.2 ± 2.5
DEAD FETUSES	N	0	0	0	0
...SORPTIONS	MEAN±S.D.	.6 ± .8	.2 ± .4	.3 ± .7	1.2 ± 1.7
EARLY RESORPTIONS	N	2	2	4	18
	MEAN±S.D.	.2 ± .4	.1 ± .4	.3 ± .6	1.2 ± 1.7
LATE RESORPTIONS	N	6	1	1	0
	MEAN±S.D.	.5 ± .7	.1 ± .3	.1 ± .2	.0 ± .0
DOES WITH ANY RESORPTIONS	N(%)	6(46.2)	3(21.4)	3(20.0)	8(53.3)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0	0	0	0
DOES WITH VIABLE FETUSES	N(%)	13(100.0)	14(100.0)	15(100.0)	15(100.0)

DAY refers to the day of gestation.
Dosage occurred on days 7-19 of gestation.
a. Excludes values for rabbit 14207 that had a litter consisting of only one fetus.
b. Includes values for rabbit 14210 that survived an intubation accident.
c. Excludes values for rabbit 14217 that had a litter consisting of only one fetus.

First generation litter data was similarly unaffected by moxidectin doses up to 10 mg/kg/day (Table 68). No significant changes between values for the moxidectin treatment groups and control values were noted for the number of litters with one or more live fetuses, implantations, live fetuses, or the percent live male fetuses/litter, body

weights for live male and female fetuses, and percent dead or resorbed conceptuses/litter.

Table 68: Summary of the Litter Data for F_1 Offspring in the Rabbit Embryo-fetal Study (Table from the Study Report)

	DOSAGE GROUP	0 MG/KG/DAY	1 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY
LITTERS WITH ONE OR MORE LIVE FETUSES EXAMINED ON DAY 29	N	13	14	15a,b	15c
IMPLANTATIONS	MEAN±S.D.	7.7 ± 2.5	7.6 ± 2.4	7.8 ± 2.2	7.4 ± 2.0
LIVE FETUSES	N	92	103	112	93
	MEAN±S.D.	7.1 ± 2.2	7.4 ± 2.4	7.5 ± 1.9	6.2 ± 2.5
LIVE MALE FETUSES	N	50	52	56	52
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	54.5 ± 19.2	46.5 ± 23.5	47.4 ± 22.7	58.2 ± 21.6
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	45.42 ± 5.38	45.08 ± 6.40	43.64 ± 5.04	45.29 ± 5.92
MALE FETUSES	MEAN±S.D.	47.15 ± 6.52	44.23 ± 5.85 [12]d	42.79 ± 5.84 [14]e	45.80 ± 6.24
FEMALE FETUSES	MEAN±S.D.	44.38 ± 5.86	44.71 ± 7.21	44.60 ± 4.60	44.65 ± 5.93 [13]f
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	7.3 ± 8.5	2.5 ± 4.9	3.3 ± 7.2	16.0 ± 22.6

DAY refers to the day of gestation.
Dosage occurred on days 7-19 of gestation.
[ ] = Number of values averaged.
a. Excludes values for rabbit 14207 that had a litter consisting of only one fetus.
b. Includes values for rabbit 14210 that survived an intubation accident.
c. Excludes values for rabbit 14217 that had a litter consisting of only one fetus.
d. Excludes litters 14182 and 14187 that had no male fetuses.
e. Excludes litter 14199 that had no male fetuses.
f. Excludes litters 14218 and 14222 that had no female fetuses.

Offspring (Malformations, Variations, etc.)

Each Caesarean-delivered fetus was placed in an individual container, weighed, examined for gross external alterations and individually identified with a tag. Live fetuses were sacrificed by an intraperitoneal injection of pentobarbital sodium. All fetuses were examined to identify sex and external and visceral alterations; the brain was free-hand cross-sectioned (a single cross-section made between the parietals and the frontals) and examined. Abnormal fetal tissues considered appropriate for retention were preserved in neutral buffered 10% formalin. The fetuses were then eviscerated, and their skeletons stained with alizarin red and evaluated for skeletal alterations. All skeletal preparations were stored in 80% glycerin with thymol crystals added to retard fungal growth. Aborted and dead or resorbed fetuses were examined to the extent possible.

Fetal alterations were defined as 1) malformations (irreversible changes normally occurring at a low incidence in the experimental species and strain), and 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

The total alterations, the combined incidences of malformations and variations identified in fetal external, soft tissue, and skeletal examinations did not demonstrate any moxidectin dose-dependent or statistically-significant differences among the four groups (Table 69).

Table 69: Summary of Fetal Alterations in the Rabbit Embryo-Fetal Study. (Table from the Study Report)

		Dosage Group (mg/kg/days 7-19 of Gestation)			
		0(Vehicle)	1	5	10
Litters Evaluated	N	13	14	16[a]	16[a]
Fetuses Evaluated	N	92	103	113[a]	94[a]
Live	N	92	103	113[a]	94[a]
Dead	N	0	0	0	0
Litters with Fetuses with any Alteration Observed	N(%)	13(100.0)	14(100.0)	16(100.0)	15(93.8)
Fetuses with any Alteration Observed	N(%)	46(50.0)	53(51.4)	62(54.9)	44(46.8)
% Fetuses with any Alteration/Litter	X̄±S.D.	49.85±15.80	51.31±13.23	58.83±19.41	48.19±24.32

a. Includes observations for litters 14207 and 14217 that consisted of only one fetus.

Malformations

There were no significant or dose-dependent increases in any external, visceral or skeletal malformation in the moxidectin dose groups compared to control values (Table 70, Table 71 and Table 72). Malformations that occurred in the high-dose group were never present in more than one fetus except for one skeletal malformation (skull frontals contain holes) where two high-dose fetuses were affected.

Table 70: External Malformations in the Rabbit Embryo-Fetal Study.

	Moxidectin Doses (mg/kg/day)			
	0 (vehicle)	1	5	10
Litters Evaluated	13	14	16[a]	16[a]
Fetuses Evaluated	92	103	113	94
Malformation				
Domed Head	0(0)	0(0)	0(0)	1(1)
Protruding Tongue	0(0)	0(0)	0(0)	1(1)
Umbilical Hernia	1(1)	0(0)	0(0)	0(0)
Abdomen: bloated with dark fluid	0(0)	1(1)	0(0)	0(0)
Skin: reddish-purple	0(0)	1(1)	0(0)	0(0)

Front Limbs: rotated inward	0(0)	0(0)	0(0)	1(1)
Hind Limbs: rotated inward	0(0)	0(0)	0(0)	1(1)
Data expressed as the number of litters (number of fetuses). [a] Includes one litter that contained only one fetus.				

Table 71: Visceral Malformations in the Rabbit Embryo-Fetal Study.

	Moxidectin Doses (mg/kg/day)			
	0 (vehicle)	1	5	10
Litters Evaluated	13	14	16[a]	16[a]
Fetuses Evaluated	92	103	113	94
Malformation				
Brain: hydrocephalus	0(0)	0(0)	0(0)	1(1)
Enlarged Heart	0(0)	1(1)	0(0)	0(0)
Malformed aorta, pulmonary artery	0(0)	1(1)	0(0)	0(0)
Abdomen: contained clear liquid	0(0)	1(1)	0(0)	0(0)
Data expressed as the number of liters (number of fetuses). [a] Includes one litter that contained only one fetus.				

Table 72: Skeletal Malformations in the Rabbit Embryo-Fetal Study.

	Moxidectin Doses (mg/kg/day)			
	0 (vehicle)	1	5	10
Litters Evaluated	13	14	16[a]	16[a]
Fetuses Evaluated	92	103	113	94
Malformation				
Skull				
Enlarged anterior and posterior fontanelles	0(0)	1(1)	0(0)	1(1)
Frontals contain holes	0(0)	1(1)	2(3)	1(2)
Vertebrae				
Unilateral ossification of cervical centrum	0(0)	1(1)	0(0)	0(0)
Asymmetric cervical centrum	0(0)	1(1)	0(0)	0(0)
hemivertebra thoracic vertebrae	0(0)	1(2)	0(0)	1(1)
Fused thoracic centra/arches	0(0)	1(2)	2(2)	0(0)
Thorascic, centrum, unilateral ossification	0(0)	1(1)	1(1)	0(0)
Thoracic, centrum, asymmetric	0(0)	0(0)	0(0)	1(1)
Thoracic, centrum, bifid	0(0)	1(1)	0(0)	0(0)
Caudal misaligned	1(1)	0(0)	0(0)	1(1)
Caudal bifid	0(0)	0(0)	0(0)	1(1)
Ribs				
Two or more fused	0(0)	1(1)	1(1)	0(0)

One or more split	0(0)	1(2)	0(0)	1(1)
Not ossified	0(0)	0(0)	1(1)	0(0)
Claviculae				
wavy	0(0)	0(0)	0(0)	1(1)
Scapulae				
Body and wing, bent	0(0)	0(0)	0(0)	1(1)
Forelimbs and Hindlimbs				
Humerus, radius, and ulna, bent	0(0)	0(0)	0(0)	1(1)
Femur, fibula, and tibia, bent	0(0)	0(0)	0(0)	1(1)
Pelvis				
Ilia, bent	0(0)	0(0)	0(0)	1(1)
Metatarsals				
bent	0(0)	0(0)	0(0)	1(1)
Data expressed as the number of liters (number of fetuses). **a** Includes one litter that contained only one fetus.				

Variations

Visceral and skeletal variations were not significantly increased or did not increase in a dose-dependent manner in the moxidectin-dose groups compared to the vehicle control group (Table 73 and Table 74).

Table 73: Visceral Variations in the Rabbit Embryo-Fetal Study.

	Moxidectin Doses (mg/kg/day)			
	0 (vehicle)	1	5	10
Litters Evaluated	13	14	16[a]	16[a]
Fetuses Evaluated	92	103	113	94
Malformation				
Eye: circumcorneal hemorrhage	0(0)	2(2)	1(1)	0(0)
Lung: intermediate lobe, agenesis	0(0)	1(1)	2(2)	1(2)
Stomach and intestines; Contained dark green fluid	0(0)	1(1)b	0(0)	0(0)
Gall bladder: agenesis	0(0)	1(1)b	0(0)	0(0)
Ectopic kidney:	0(0)	0(0)	0(0)	1(1)
Data expressed as the number of liters (number of fetuses). **a** Includes one litter that contained only one fetus.				

Table 74: Skeletal Variations in the Rabbit Embryo-Fetal Study.

	Moxidectin Doses (mg/kg/day)			
	0 (vehicle)	1	5	10
Litters Evaluated	13	14	16[a]	16[a]
Fetuses Evaluated	92	103	113	94
Malformation				

Skull				
Summary of all Irregular Ossification of the Skull	12(37)	14(44)	15(52)	14(37)
Hyoid: Ala, angulated	6(8)	3(5)	5(8)	7(9)
Vertebrae				
Cervical rib present	0(0)	1(1)	1(1)	0(0)
Ribs				
Thickened areas of ossification	0(0)	1(1)	0(0)	1(1)
One or more flat ribs	0(0)	1(1)	0(0)	0(0)
One or more wavy ribs	0(0)	0(0)	0(0)	1(1)
Sternebrae				
Fused sternebrae	1(2)	3(3)	1(1)	0(0)
Asymetric sternebrae	1(2)	1(1)	1(1)	0(0)
Scapulae				
Irregular shape	0(0)	0(0)	1(1)	0(0)
Pelvis				
Pubes, incompletely ossified	0(0)	1(1)	0(0)	0(0)
Data expressed as the number of liters (number of fetuses). **a** Includes one litter that contained only one fetus.				

9.3 Prenatal and Postnatal Development

Study title: Moxidectin: A Three Generation (Two Litters) Reproduction Study with AC 301,423 to Rats.

Study no.:	RPT-77518
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September, 18, 1989
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	Moxidectin (AC 301,423), Batch # AC 6297-119, purity of 88.6%.

Reviewer Comment: *This multigenerational reproduction study included pre-postnatal measurements as well as fertility assessments for male and female rats. The following study review pertains only to the pre-postnatal measurements in the study.*

Key Study Findings

- In a pre-postnatal study in rats, moxidectin administration prior to mating, and through mating, gestation, and lactation did not produce adverse effects, inhibit survival or inhibit reproduction in F_1 or F_2 offspring at maternal doses of 0.897 mg/kg/day.
- The study design was unusual in that: in all the moxidectin treatment groups all generations of animals (parents, F_1 and F_2 offspring were administered dietary

moxidectin; each generation underwent intervals of breeding resulting in two litter groups, and physical development and neurological assessments were not performed in F_1 offspring.

- The NOAEL was considered to be the highest maternal dose of 10 ppm.

Methods

Doses:	0 (Group 1), 1 (Group 2), 2 (Group 3), 5 (Group 4), and 10 (Group 5) ppm. In Groups 2-5 respectively, the moxidectin dietary doses were equivalent to approximately 0.087, 0.170, 0.423, and 0.897 mg/kg/day moxidectin intake during the gestation period (GDs 0-20) in parental female animals.
Frequency of dosing:	*Ad libitum* in food.
Dose volume:	NA
Route of administration:	Oral, dietary: Moxidectin was finely ground, and then homogenously mixed into the animal food (Purina Cerifie4d rodent Chow® Brand Animal Diet #5002).
Formulation/Vehicle:	Animal food
Species/Strain:	Albino rat, COBS®, CD® (Sprague-Dawley derived)
Number/Sex/Group:	25/sex/group
Satellite groups:	none
Study design:	The parental generation, male and female animals, received the control or moxidectin diets for 10 weeks prior to the initiation of mating and treatment continued until sacrifice. Parents and F_1 and F_2 offspring were mated twice and reproduction was assessed for every generation. This study was of an atypical design particularly with regard to the following elements: in the moxidectin treatment groups, all generations of animals (parents, F_1, and F_2 offspring) were fed diets containing moxidectin; each generation underwent two intervals of breeding resulting in two litter groups; and developmental milestones were not assessed in any of the offspring.
Deviation from study protocol:	Multiple protocol deviations were observed. However none was considered to have altered the study results.

Observations and Results (Optional Table)

F_0 Dams

Survival:	No mortality occurred in animals receiving 1 ppm, 5 ppm, or 10 ppm moxidectin. One control female died following the mating interval and two Group 3 (2 ppm moxidectin) females died during the

parturition.

Clinical signs: No maternal clinical signs related to moxidectin were reported.

Body weight: Body weights were assessed weekly and on GDs 0, 7, 14, and 20 and on lactating days (LDs) 0, 4, 7, 14, and 21.

No maternal weight loss or decreased weight gain was observed in females receiving any of the moxidectin doses compared to control values during the mating, gestation, or lactation periods.

Feed consumption: Food consumption was assessed weekly and on the following gestation intervals: GDs 0-7, 7-14, and 14-20.

Maternal food consumption for the high-dose group was not significantly different than control values during the mating periods associated with the two litter groups, F_{1a} and F_{1b}, or in the associated gestation and lactation periods. In the GD 1-7, GD 7-14, and GD 14-20 intervals for the first pregnancy, food consumption in the low and mid-dose moxidectin groups (Groups 2, 3, and 4) was at times significantly lower than control values. However in the absence of a dose-dependent effect, this result was not considered to be related to moxidectin administration. In the second pregnancy, food consumption during mating, gestation, and in the lactation period was similar for all groups.

Uterine content: The mean number of live, dead, and total pups was similar for control and moxidectin treatment groups for each litter. Uterine content parameters including the number of implantations, corpora lutea, and early and late resorptions were not assessed.

Necropsy observation: Following the end of the lactation period for the second F_1 litter (F_{1b}), dams were necropsied and examined for gross pathology. Also the following tissues from the dams in the control and high-dose groups were preserved and examined for histopathology: ovaries (both), pituitary, uterus, vagina, and gross lesions.

No maternal gross pathology associated with moxidectin was reported. Also no moxidectin-related histopathology in the ovaries, uterus, or the pituitary glands of the dams in the high-dose group

was observed.

Toxicokinetics: Not performed

Dosing Solution Analysis: Moxidectin doses in animal feed were assessed weekly for concentration and dose batches were also assessed for homogeneity and stability.

All of the actual concentrations of the dosing preparations (1, 5, and 10 ppm moxidectin) were on average within ± 10% of the nominal concentrations. The dosing preparations for the 1 and 10 ppm doses were shown to be homogeneous and stable for 2 weeks in feeding jars at room temperature and for 2 weeks in frozen bulk storage.

Other: Mean maternal gestation lengths were comparable between the control and treatment groups for both F_1 litters.

F_1 Generation

Survival: Survival for the F_1 offspring was similar for all groups at birth and through LD 21. Mean pup percent survival was significantly decreased for the high-dose group (10 ppm moxidectin) from LDs 0-4 and LDs 4-21 for the F_{1a} litters compared to concurrent control levels. However, the reductions in pup survival mainly occurred in one litter. Survival was not significantly decreased during any post-birth interval for the F_{1b} litters.

Clinical signs: No clinical signs in the F_1 offspring were observed.

Body weight: Body weights for the F_1 offspring were not significantly reduced at birth or on lactation days (LDs) 4 (pre and post cull), Day 7, and Day 21.

Feed consumption: The mean weekly food consumption was similar for all groups.

Physical development: Physical development of F_1 offspring was not assessed for the typical parameters including pinna unfolding, coat growth, incisor eruption, reflexes, surface righting, auditory startle response, air righting, pupil constriction, response to light, vaginal opening in females and day of testes descent and cleavage of balonoprepuputial gland in males.

Following the end of the lactation period for the second F_2 litter (F_{2b}), F_1 dams were necropsied and examined for gross pathology. In addition, F_{1a} and F_{1b} pups (male and female) that were not used for breeding were euthanized on Days 21 and 28 of

lactation respectively and examined for gross pathology. Also, the following tissues from the F_1 dams in the control and high-dose groups were preserved and examined for histopathology: ovaries (both), pituitary, uterus, vagina, and gross lesions.

No gross pathology associated with moxidectin was reported for F_1 dams or pups. Also no moxidectin-related histopathology in the ovaries, uterus, or the pituitary glands of the dams in the high-dose group was observed.

Neurological assessment: Neurological assessments were not performed other than an assessment of excitation which did not differ between control and moxidectin-treatment groups.

Reproduction: In both litter groups (F_{2a} and F_{2b}) born to F_1 offspring, the mean number of live, dead, and total pups were comparable between the control and moxidectin-treatment groups. The male/female sex ratio was also similar for all groups.

Other: Mean gestation length was comparable between the control and moxidectin-treatment groups.

F_2 Generation

Survival: Pup survival in F_{2a} generation offspring was significantly reduced in high-dose pups in the LD_{0-4} interval but tended to be non-significantly decreased in other post-birth intervals. Survival for the second litter group born to F_1 dams, the F_{2b} offspring, was not significantly lower than control values for any of the post-birth intervals.

Body weight: Body weights for the F_2 offspring (litter groups F_{2a} and F_{2b}) were not significantly reduced at birth or in lactation days (LDs) 4 (pre and post cull), 7, and 21 compared to concurrent control values.

External evaluation: Following the end of the lactation period for the second F_3 litter (F_{3b}), F_2 dams were necropsied and examined for gross pathology. In addition F_{2a} and F_{2b} pups (male and female) that were not used for breeding were euthanized on Days 21 and 28 of lactation respectively and examined for gross pathology. Also the following tissues from the dams in the control and high-dose groups were preserved and examined for histopathology: ovaries (both), pituitary, uterus, vagina, and gross lesions.

No maternal gross pathology associated with

	moxidectin was reported for F_2 dams or pups. Also no moxidectin-related histopathology in the ovaries, uterus, or the pituitary glands of the dams in the high-dose group was observed.
Male/Female ratio:	The male/female ratio was similar in all F_2-generation pups.
Other:	No clinical signs in F_2 offspring were observed. Reproduction for F_2 offspring was not impaired in the moxidectin groups. For the two litter groups (F_{3a} and F_{3b}) born to F_2 offspring, litter size, litter survival, sex distribution, pup weights and pup survival indices were not significantly reduced compared to concurrent control values, or when significant differences were noted, the values in the moxidectin groups were within the historical control range.

10 Special Toxicology Studies

1. **Computational Assessment and Evaluation of Potential Genotoxicity of Moxidectin Impurity (b) (4) Impurity Using CASE Ultra and DEREK/SARAH.** (Study No.: BM-CUDXSX-1273)

Methods

This non-GLP study was conducted by (b) (4) in 2016. Computational screening of the moxidectin drug substance impurity, (b) (4) was performed using four sets of *in silico* systems, CASE Ultra 1.6.0.3, Consolidator Database version 1.4, Derek Nexus 5.0.1, and Sarah Nexus 2.0.1 to predict the mutagenicity potential of the impurity.

Results

Based on the resulting QSAR evidence and expert opinion review, the moxidectin impurity, (b) (4) was predicted to be negative for mutagenicity in humans.

11 Integrated Summary and Safety Evaluation

Moxidectin has a long history of oral and topical use in animals as a veterinary product. For the treatment of onchocerciasis in humans, moxidectin will be administered clinically in a single-oral dose of 8 mg (0.133 mg/kg for an average 60 kg human) suggesting toxicities that occurred with repeated dosing of much higher doses in test animals will not be a concern in patients. However, moxidectin has a very long plasma $t_{1/2}$ in humans, approximately 24 and 33 days in patients and normal subjects respectively. Also, off-label, repeated clinical doses at an interval of 6 months or 1 year are expected in the treatment of onchocerciasis at locations outside the United States. Due to these factors, human toxicity is a possibility in cases where moxidectin is unintentionally overdosed or perhaps in a subpopulation of humans with increased sensitivity to moxidectin.

(b) (4) Also the specification for each impurity was qualified by its use in nonclinical and clinical studies with moxidectin batches containing impurity levels higher than the specified levels. The specifications for the residual solvents in the drug substance are consistent with the limits recommended in the ICH Q3C(R6) Guidance[1]. The moxidectin drug product contains three degradants and one impurity and the specifications for all four are lower than the thresholds designated in the ICH Q3B(R2) Guidance[2] for drug product impurities. All of the drug substance and drug product impurities and degradants were evaluated with *in silico* computational analyses to assess the potential for bacterial mutagenicity. Based on the results of the *in silico* analyses all but one of the impurities/degradants was predicted to be negative for bacterial mutagenicity. The single drug-substance impurity (Impurity (b) (4) predicted to be positive for bacterial mutagenicity in the *in silico* assessments is specified at an acceptance level of (b) (4) % or (b) (4) in each 8 mg dose of moxidectin drug product. This specification is below the 120 mcg/day acceptable daily intake specified in the ICH M7 Guidance for an individual impurity that is administered for ≤ 1 month.

Pharmacokinetic parameters for single- or repeated-oral doses of moxidectin were measured in mice, rats, and dogs. Clear findings from these studies include evidence that the plasma half-life ($t_{1/2}$) is relatively long in rats (18-30 hours) but much longer in dogs (8-20 days). In a literature report moxidectin was shown to be almost completely bound by lipoproteins in plasma from cows, goats, sheep, pigs, rabbits, and humans. In a mass-balance study in rats, ^{14}C-labeled moxidectin was shown to distribute well to tissues and organs with the highest concentrations in fat and the lowest in brain and testes. In addition to parent moxidectin, up to six moxidectin metabolites were detected in liver and feces, and hydroxylation was a common mechanism for moxidectin metabolism. In rats, moxidectin was primarily excreted as the parent compound in feces (70-90%) with a much lower percentage (approximately 1%) excreted in urine. In human hepatocytes, moxidectin was shown to induce CYP2B6 and CYP3A4 gene expression in a dose-dependent manner and in human liver microsomes, moxidectin weakly inhibited the activity of CYP1A2 and CYP2C9. However, both CYP induction and inhibition occurred at moxidectin concentrations approximately 10 times higher than clinical concentrations suggesting a lack of clinical relevance.

1 http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3C/Q3C__R6___Step_4.pdf

2 http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3B_R2/Step4/Q3B_R2__Guideline.pdf

In a safety pharmacology study assessing hERG potassium-channel activity, moxidectin produced a dose-dependent inhibition of hERG channels up to approximately 20% at the highest test concentration of 10 mcM (6.4 mcg/ml). In a cardiovascular study in conscious dogs, 1 mg/kg moxidectin was associated with a 14% decrease in heart rate, but produced no changes in ECG parameters including QTc values. Because the IC_{50} for moxidectin inhibition of hERG channels, considered to be in excess of 6.4 mcg/ml, is at least 100 times greater than the plasma C_{max} value (63.1 ng/ml) associated with the recommended clinical dose of moxidectin, it is considered unlikely that moxidectin will cause adverse ECG effects in patients.

The primary moxidectin-related toxicity in test animals and the toxicity with the most potential for clinical relevance is dose-dependent CNS toxicity. Moxidectin-related CNS toxicity is unexpected because in a mass-balance study in rats, radio-labelled moxidectin was shown to distribute poorly to the brain. However, moxidectin is reported to be active at the γ-aminobutyric acid (GABA)-A receptor complex and this interaction may mediate some of the observed CNS toxicity. Transient CNS toxicity occurred with single and repeated doses in mice, rats, and dogs where CNS-related clinical signs included piloerection, reduced arousal, tremors, abnormal gait, irregular or slowed breathing, and impaired righting reflex in rodents and lacrimation, languid appearance, tremors, salivation, and slight ataxia in dogs. Some of the clinical signs such as tremors and irregular or slowed breathing would be troubling adverse events if observed in humans. However in test animals, the effects were transient and not accompanied by correlating histopathology even with repeated moxidectin dosing and repeated episodes of CNS toxicity suggesting permanent structural or functional sequelae are not expected.

In test animals, a defining characteristic of the CNS toxicity is its dose dependency. In single and repeated-dose studies, CNS-related clinical signs were observed at moxidectin threshold doses of ≥ 17 mg/kg in mice, ≥ 12.0 mg/kg in rats, and ≥ 1.5 mg/kg in dogs which are approximately 6-14 fold higher than the 8 mg (0.133 mg/kg) dose in humans based on body surface area comparison (Table 75). The safety margins are larger if the moxidectin-related CNS toxicity is correlated with plasma C_{max} values. The plasma C_{max} values associated with the threshold doses of moxidectin range from an estimated 1028 ng/ml in dogs to 2513 ng/ml in rats. At the recommended human dose of 8 mg, the plasma C_{max} was measured as 63 ng/ml in patients with onchocerciasis which is approximately 16- to 40-fold less than the plasma C_{max} values associated with threshold doses of moxidectin in rats and dogs (Table 76). At doses below the threshold doses in animals, CNS-related clinical signs were not observed suggesting relative safety at the clinical dose. The theoretical safety of the recommended clinical dose of moxidectin for CNS toxicity is supported by current safety data in clinical trials where CNS-related adverse events other than headache have not been reported.

Table 75: Human Equivalent Dose (HED) and Safety Margin Calculations for CNS-related Clinical Signs in General Toxicology Studies with Moxidectin in Mice, Rats, and Dogs.

Study Type/ Study Report No.	Threshold for CNS-Clinical Signs (mg/kg)	HED (mg/kg)	Safety Margin Based on Body Surface Area Comparison
28-Day Study in Mice/ Study Report No.: RPT-77313	≈ 17.0	1.4	11
13-week Toxicology Study in rats/ Study Report No.: RPT-77312	≈ 12.0	1.9	14
91-Day Toxicology Study in Dogs/ Study Report No.: RPT-77335	≈ 1.5	0.8	6
The recommended human dose of moxidectin is a single dose of 8 mg which for an average 60 kg human is equal to 0.133 mg/kg.			

Table 76: Plasma C_{max} and AUC Values and Safety Margin Calculations for CNS-related Clinical Signs in General Toxicology Studies with Moxidectin in Mice, Rats, and Dogs.

Study Type/ Study Report No.	Threshold for CNS-Clinical Signs (mg/kg)	Plasma C_{max} (ng/ml)	Plasma AUC (ng•hr/ml	Safety Margin	
				Based on C_{max}	Based on AUC
28-Day Study in Mice/ Study Report No.: RPT-77313	≈ 17.0	NA	NA	-----	-----
13-week Toxicology Study in rats/ Study Report No.: RPT-77312	≈ 12.0	2513[a]	42114[a]	40	15
91-Day Toxicology Study in Dogs/ Study Report No.: RPT-77335	≈ 1.5	1028[b]	32676[b]	16	12
In onchocerciasis patients (n = 31), the mean plasma C_{max} and AUC_{inf} values (± SD) were 63.1 ± 20.0 ng/ml and 2738 ± 1606 ng•hr/ml respectively following a single 8 mg oral dose of moxidectin. [a] The plasma C_{max} and AUC values are for a 10 mg/kg single-oral dose in male Sprague Dawley rats in Study No.: 10GR082. [b] The plasma C_{max} and AUC values are the average values for a 1 mg/kg single-oral dose in male and female Beagle dogs in Study Report No.: RPT-73592.					

Moxidectin was assessed for toxicity in long-term studies with durations of 1-month (mice, rats, and dogs), 3 months (rats and dogs), and 1-year (dogs). In these studies, other than CNS-related clinical signs, moxidectin was associated with little toxicity. In all the test species at repeated-moxidectin doses slightly lower than those associated with clinical signs, decreased food consumption, body weight gain, and body weights were observed. However, animals resumed normal eating patterns and gained weight upon dosing cessation. In dog studies, a moderate elevation of some liver enzymes occurred in a few dogs receiving high doses of moxidectin, but group mean values were not significantly elevated, and correlating liver histopathology was not observed. Based on the results in nonclinical toxicology studies, the only toxicities projected for doses in excess of the recommended clinical dose of moxidectin are dose-related clinical signs, anorexia, and reduced body weights.

In a full battery of *in vitro* and *in vivo* genotoxicity studies, moxidectin was negative for mutagenicity and clastogenesis. Moxidectin was also tested in 2-year carcinogenicity studies in mice and rats, and a preliminary review of these studies suggests moxidectin did not stimulate tumor formation at doses in excess of the recommended human dose (Table 77). A comprehensive review of the carcinogenicity studies awaits electronic submission of accurate tumor-tabulation tables necessary for a new statistical analysis performed within the FDA. (b) (4)

(b) (4)

Table 77: Human Equivalent Dose (HED) and Safety Margin Calculations for the Mouse and Rat Carcinogenicity Studies.

(b) (4)

In a male and female fertility study in rats, moxidectin did not impair any fertility or pregnancy indices at doses approximately equivalent to the recommended human dose based on body surface area comparison (Table 78).

Table 78: Human Equivalent Dose (HED) and Safety Margin Calculations for the Fertility Study in Male and Female Rats.

Study Type/ Study Report #	NOAEL[a] (mg/kg/day)	HED[b] (mg/kg/day)	Safety Margin[c]
Rat Fertility Study/ RPT-77518	Male NOAEL = 0.795	0.128	0.96
	Female NOAEL = 0.922	0.149	1.12
	Mean NOAEL = 0.859	0.138	1.04

[a] The NOAEL value of 10 ppm dietary moxidectin was associated with calculated moxidectin consumption of 0.795 and 0.922 mg/kg/day for males and female rats respectively.

[b] The conversion factors for determining human equivalent dose (HED) values based on body surface area comparison are divide by 6.2 for rats.

[c] The recommended human dose of moxidectin is a single dose of 8 mg which for an average 60 kg human is equal to 0.133 mg/kg.

In embryo-fetal studies in rats and rabbits, moxidectin was associated with a moderate level of reduced maternal food consumption and body weight gain at doses equivalent to approximately 12 times for both species the recommended human dose based on body surface area comparisons (Table 79). In rats, one skeletal variation, wavy ribs, was significantly increased for fetal and litter incidence and one malformation, cleft palate, was significantly increased for fetal incidence but not litter incidence at a high dose of 12 mg/kg/day which is equivalent to approximately 15 times the recommended dose in humans based on body surface area comparison. In the rabbit embryo-fetal study, no evidence of impaired embryo-fetal development was observed at a high dose of 10 mg/kg/day which is equivalent to 24 times the recommended human dose based on body surface area comparison.

Table 79: Human Equivalent Dose (HED) and Safety Margin Calculations for the Embryo-fetal and Pre-postnatal Studies.

Study Type/Study Report #	NOAEL or Toxic Dose (mg/kg/day)	HED[a] (mg/kg/day)	Safety Margin[b]
Rat Embryo-Fetal Study/	Maternal toxic dose = 10	1.61	12.1
	Maternal NOAEL = 5	0.81	6.1
	Maternal high dose = 12	1.94	14.6
	Fetal NOAEL = 10	1.61	12.1
Rabbit Embryo-Fetal Study/	Maternal toxic dose = 5	1.61	12.1
	Maternal NOAEL = 1	0.32	2.4
	Fetal NOAEL = 10	3.23	24.3
Rat Pre-Postnatal Study	F_1 decreased survival = 1.1	0.177	1.3
	F_1 increased death at birth = 11	1.77	13.3
	NOAEL = 0.824[c]	0.133	1.0

[a] The conversion factors for determining human equivalent dose (HED) values based on body surface area comparison are divide by 6.2 and 3.1 for rats and rabbits respectively.
[b] The recommended human dose of moxidectin is a single dose of 8 mg which for an average 60 kg human is equal to 0.133 mg/kg.
[c] The NOAEL value of 10 ppm dietary moxidectin was associated with a calculated maternal consumption of 0.824 mg/kg/day moxidectin during the gestation period.

Pilot and definitive pre-postnatal studies were conducted approximately 30 years ago with moxidectin. The results of these studies indicate that moxidectin at a dose approximately equivalent to the recommended clinical dose based on body surface area comparison did not inhibit survival or fertility or reduce body weights in the parental generation or second-generation offspring (Table 79). However, at a slightly higher dose (approximately 1.3 times the recommended clinical dose based on body surface area comparison) and higher doses, the survival and body weights of first generation offspring were significantly decreased in a moxidectin dose-dependent manner. Increased deaths at birth for first generation offspring occurred at a moxidectin dose of 11 mg/kg/day which is equivalent to approximately 13 times the recommended clinical dose based on body surface area comparison. The mechanisms underlying the adverse effects on first generation offspring are difficult to ascertain. The designs of the pre-postnatal studies were atypical in that all generations, parents and first and second generation offspring, were administered dietary moxidectin over the entire course of

study. Consequently the adverse effects on first generation offspring may have resulted from parental and/or embryo exposure to moxidectin during gestation, or exposure in first generation offspring to moxidectin in breast milk or in feed. A new pre-postnatal study limiting moxidectin administration to mothers during gestation and lactation may provide further clarity.

The results of the current pre-postnatal studies are considered sufficient to support approval of NDA 210867. However, in these studies, physical development and neurological function were not assessed in first generation offspring as recommended in the ICH S5a Guidance. Consequently a new pre-postnatal study which will include the assessments missing in the previously conducted studies will be conducted as a post-marketing requirement (PMR). Until the final study report for the new study has been received and evaluated, Section 8.1 of the product label for moxidectin will indicate: "offspring were (b) (4) assessed for survival, body weights, and fertility; developmental milestones were not assessed in this study," in reference to pre-postnatal study results.

Moxidectin is considered acceptable for approval from a Pharmacology/Toxicology perspective (b) (4)

12 Appendix/Attachments

To: James Wild
cc: Terry Miller
From: CDER/OTS/OCP/DARS: The Chemical Informatics Program
Re: NDA 210867
Date: April 11, 2018

Fifteen compounds for the drug substance moxidectin were evaluated by the CDER/OTS/OCP/DARS Chemical Informatics Program for bacterial mutagenicity using (Q)SAR models. The (Q)SAR models used in this analysis do not differentiate pairs of stereoisomers, leading to identical predictions. As such, the results for **Impurity** (b) (4) and **Degradant** (b) (4) are combined in the table below. Three software programs were used: *Derek Nexus* 6.0.1 (*DX*), *Leadscope Model Applier* 2.2.2-3 (*LMA*), and *CASE Ultra* 1.6.2.3 (*CU*). To maximize sensitivity and negative predictivity, a positive prediction from any one software program was used to justify a positive overall prediction. All (Q)SAR model outputs were reviewed with the use of expert knowledge in order to provide additional supportive evidence on the relevance of any positive, negative, conflicting or inconclusive prediction and provide a rationale to support the final conclusion.

The (Q)SAR assessment of mutagenic potential for the compounds is consistent with recommendations described in the final ICH M7 guideline (i.e., prediction of bacterial mutagenicity using multiple complementary methodologies). The following summary table reports the overall prediction for each compound.

Chemical Number	Chemical Name	*Salmonella* Mutagenicity Expert Prediction[1]	*E. coli*/ TA102 Mutagenicity Expert Prediction[1]
(b) (4)			

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[1] + = positive; – = negative; Eqv = equivocal; NC = test chemical features are not adequately represented in the model training data set, leading to a no call.

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

/s/

JAMES S WILD
05/29/2018

TERRY J MILLER
05/31/2018