



Chemical Repository Services for the EDSP

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1.0 TITLE AND APPROVAL

Chemistry Report for WA 2-17 17 β -Estradiol Extraction and Measurement from Dosed Game Bird Feed (Avian Dosing Study)

June 1, 2005

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2.0 QA REVIEWER AND CONTRIBUTOR PAGE

**Chemistry Report for WA 2-17
17 β -Estradiol Extraction and Measurement
from Dosed Game Bird Feed
(Avian Dosing Study)**

Reviewed by: _____
Mary Lynn, EDSP Quality Assurance Representative
Windward Company, Gig Harbor

Date: _____

Personnel participating in this study:

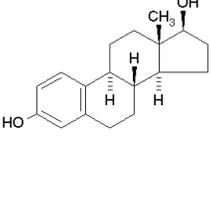
Analysts: Waverly Thorsen, Tim Fortman,

Chemical Repository Study Director: Michael Cobb

3.0 EXECUTIVE SUMMARY

Chemistry Report for WA 2-17 17 β -Estradiol Extraction and Measurement from Dosed Game Bird Feed (Avian Dosing Study)

Table 1. Key Materials Procurement Information

Parameter	Test Substance	Reference Substance	Test Substance Structure
Compound	17 β -Estradiol	17 β -Estradiol	17 β -Estradiol
Function	Spiked into Feed (& used for Ref Stnds.)	Calibration	
CAS #	50-28-2	50-28-2	
Central File No.	1932	2083	
Init. Receipt Date	02/27/2003	03/22/99	
Expiration Date	02/26/2005*	10/05	
Manufacturer	Sigma-Aldrich	Sigma-Aldrich	
Lot Number	052K1370	086H1138	
Battelle Study #	WA 2-17	WA 2-17	

*Certificate of analysis indicated a shelf life of 2 years with an end date of 01/2004. Additional document provided by supplier indicated proper storage should allow for 5 year expiration date – as a result expiration was reset by Chemical Repository to 02/26/05.

Executive Summary

Work Assignment (WA) 2-17 of the Environmental Protection Agency's Endocrine Disruptor Screening Program (EDSP) describes a reproductive and developmental study on Japanese quail exposed to 17 β -Estradiol (estradiol) via spiked feed. This supporting analytical study carried out by the EDSP Chemical Repository (CR) was to provide a data set to allow determination of the spiking accuracy and uniformity of the multiple preparations of dosed game bird feed prepared by the study team in Richland. In addition, several feed batches were submitted for testing stability and contamination of both spiked and control feed resulting from exposure in the trays in the game bird cages.

The test substance of interest for this portion of the study is estradiol. The test substance purity of estradiol, as determined by the manufacturer, was 100% (lot 052K1370). The analytical study required development of a method for both extraction and analysis of the estradiol from the game bird feed. The estradiol determinations were carried out using Gas Chromatography/Mass Spectrometry (GC/MS) (Standard Operating Procedure, MSL-O-018-00). To provide the required sensitivity on the GC/MS system, the estradiol was derivatized prior to analysis.

The first batch of feed samples was collected on October 30, 2003 and the final analysis of the last batch of feed samples was carried out on June 3rd and 4th, 2004. Samples from sixteen batches of dosed feed were submitted to and analyzed by the CR for testing one or more of the following parameters: feed dosing concentration verification, spiking uniformity, stability/contamination testing, and unspiked feed (analyzed as a control) for determination of endogenous estradiol.

For every analysis day, blank spikes (BS), Matrix Spikes (MS) and a Matrix Spike Duplicate were run, and continuing calibration verification (CCV) samples and initial calibration verification (ICV) samples were run¹. In several cases samples from more than one batch and/or stability/contamination set were run on the same day with shared QC samples. Table 2 below indicates the recovery ranges for each of the 15 batches tested for formulation accuracy.

¹ Batch 5 did not include ICV samples in the run

Table 2. Batch Feed Formulation Accuracy – Recovery Ranges

Batch #	Recovery Ranges
1	74% to 105%
2	83% to 132%
3	71% to 120%
4	66% to 110%
5	75% to 122%
6	72% to 121%
7	73% to 97%
8	54% to 102%
9 & 9A	74% to 142%
10	64% to 169%
11 ¹	22% to 77%
12	35% to 60%
13	64% to 102%
16	50% to 76%
19	68% to 80%

For the stability runs, for samples within the quantitation range of the calibration curve, only feed batch 10 showed any significant change between the initial measurement and the final measurement. For this batch, the 0.078 µg estradiol/g feed sample showed a reduction of about 83% in estradiol detected over the 3 day exposure. Also for batch 10, this same sample pair (target of 0.078 µg/g estradiol) quantitated at 154% of target at day zero. Though the day 3 value was below the MDL the stability calculation was still carried out but flagged. It should be noted that the samples were received at the CR at the end of the stated refrigerated stability term (received on 27th day, claim of 28 days stability). For three of those days, the samples were at ambient temperature in the brooder feed troughs. Following receipt of the samples at the CR, they were refrigerated, and extracted on day 32 from preparation – 4 days beyond the stated refrigerated stability period. The matrix blanks and spikes and ICV and CCV values for that day were normal save the CCV mentioned above in which was at 128% recovery for the estradiol and 148% for the surrogate recovery. The remaining batches tested for stability held within a range of 83% and 131% recovery over the stability test period.

Levels of feed cross-contamination, tested on batches 1, 4, 8, and 10 were all below the MDL indicating contamination for feed placed in the cages was undetectable.

A test for endogenous estradiol in blank feed showed levels below the MDL (nothing detected in 2 of 3 replicate samples).

There were two commercial feed formulations used during the study, Purina Game Bird Layena® (for reproductive birds) and Purina GameBird Startena® (for growing chicks).

¹ Recovery range for batch 11 includes 3 replicates of the 0.078 µg/g target sample that quantitated below the MDL. These data were included in the calculation since the expected value was above the MDL. Without inclusion of these values the quantitation range for batch 11 is 49% to 77%.

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5.0 INTRODUCTION

The goal of the Battelle-Sequim, Marine Sciences Laboratory (MSL) Chemical Repository (CR) for the Endocrine Disruptor Screening Program is to provide the participating laboratory with requested chemicals of documented quality at required concentrations and in a matrix appropriate for different toxicological tests. The CR provides the supplier's information regarding purity and stability, the material safety data sheet (MSDS) chemical information, and independent analysis of purity and stability in a matrix specified by the Study Protocol: *Purity and Stability of Test Substances for Work Assignment 2-17*, made in collaboration with the requestor Crystal Driver, the Principal Investigator for the study (this data is the subject of another report). In addition, the CR provides analytical services to the participating laboratories for evaluation of preparation accuracy for dosage and test media spiked with the test substance of interest and utilized in the particular EDSP study program. Under Work Assignment (WA) 2-17, Crystal Driver, (Principal Investigator for the study), of the Pacific Northwest National Laboratory requested analysis of a series of feed samples for determination of the accuracy of spiked estradiol concentrations and contamination of both spiked and control feed resulting from its exposure in the trays in the game bird cages. This analytical work on the spiked and control feed samples is the subject of this report.

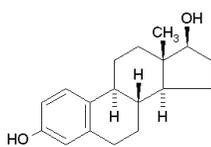
6.0 GENERAL METHODS

Methods of standard operation of the CR are currently addressed in MSL Standard Operating Procedures (SOPs) numbered R-001 through R-017. These SOPs address test substance procurement, ordering, handling, inventory, and storage requirements; test substance receipt and chain of custody, test substance log-in and labeling, inventory, test substance storage; stock solution preparation, documentation and archiving; test solution preparation, documentation and shipping; test substance disposal, and CR maintenance over time. The quality assurance (QA) requirements for procurement of test substances for use in the CR are addressed in the Quality Assurance Project Plan for the CR.

6.1 TEST SUBSTANCE PROCUREMENT

As requested (Figure 1) by Crystal Driver, 17β -estradiol, (estradiol), CAS No. 50-28-2, was purchased for purity and stability analysis and used as the test substance in a reproductive and developmental study on Japanese quail exposed to the test substance via spiked feed. The estradiol was purchased from Sigma-Aldrich, and the first shipment of lot number 052K1370 was received on 02/27/03 with an expiration date of 02/26/05 (Table 3). The test substance was logged in to the Chemical Management System (CMS) and given a CMS barcode and unique log-in (central file) number (CF-1932) as per the QA Project Plan (QAPP) for the CR. The test substance was shipped to Richland for use in the spiked feed preparation.

Table 3. Key Materials Procurement Information

Parameter	Test Substance	Reference Substance	Test Substance Structure
Compound	17β -Estradiol	17β -Estradiol	
Function	Spiked into Feed (& used for Ref Stnds.)	Calibration	
CAS #	50-28-2	50-28-2	
Central File No.	1932	2083	
Init. Receipt Date	02/27/2003	03/22/99	
Expiration Date	02/26/2005*	10/05	
Manufacturer	Sigma-Aldrich	Sigma-Aldrich	
Lot Number	052K1370	86H1138	
Battelle Study #	WA 2-17	WA 2-17	

*Certificate of analysis indicated a shelf life of 2 years with an end date of 01/2004. Additional document provided by supplier indicated proper storage should allow for 5 year expiration date – as a result expiration was reset by Chemical Repository to 02/26/05.

EDSP Chemical Request Form

For EPA WA:2-17

Study Director:

Name: Crystal Driver

Affiliation: PNNL/BNW

Location: PSL/1507 Richland, Washington

Telephone number: 509-375-2721

Bioassay Information:

Proposed Bioassay: Avian dosing study.

Test Chemical: 17beta-estradiol

Carrier(s): vegetable oil suspension (cottonseed oil has been used in the literature); or ethanol or other suitable volatile solvent

Concentrations/Dilution Series: To be determined by range finding. Assume a logarithmic dilutions series from a high concentration of a consumed dose of 1000 micrograms/bird/per day (assume a bird eats about 15 grams of food per day) to a low dose of 0.01 microgram/bird/day.

Test concentrations are likely to be in the 1 to 100 or 500 ug/bird/day range.

*Consider if analysis method detection limit which may be determined in Purity analysis is above or below desired test concentrations?

In vitro or *in vivo* tests? *in vivo*

Organism to be tested: Japanese quail

Method of test solution administration: In feed. The compound may have a short half-life under the light conditions in the animal room (10 lux up to 17 hours per day, 72- 76 F, 40-50% humidity). Also, there is some concern about particulate exposure for animal and feed handlers. Not sure which of the two feed incorporation methods will result in less fines or crystals of the compound that could become airborne.

Planned/proposed test duration: 40-44 weeks. However, nutrient stability in feed allows for a maximum of 120 days post mill date (i.e., 120 day shelf-life).

Chemical Information:

Chemical Name: 17beta-estradiol

CAS:

Any known purity information: may refer to attached documentation

Any known stability information: may refer to attached documentation

Desired purity (%) for test? As pure as possible, >99%

Is there a known calibration standard available? (I think you have this from fish studies??)

Name:

CAS:

Manufacturer:

Figure 1. EDSP Chemical Request Form for Estradiol

6.2 TESTING PLAN DESIGN AND DETAIL

A general plan for this phase of the Avian Dosing Study was outlined by the Principal Investigator (PI) and agreed to by the CR. The main purpose of the study was to provide analysis of the estradiol-spiked bird feed to determine accuracy and uniformity of the formulation. In addition, the study demonstrated the use stability of the feed by testing the estradiol levels in feed samples collected at the time of placement in the feed trays and again upon removal of the feed from the trays following the feeding period. The study also included testing the spiked levels of estradiol in both the Layena® and Startena® types of Purina GameBird Feed. All feed blending and spiking was carried out by the project team at the Pacific Northwest National Laboratory (PNNL) in Richland. The target levels of estradiol spiked into the feed were 0.078 µg/g, 0.31 µg/g, 1.25 µg/g and 5 µg/g, (µg estradiol/g feed), see Table 4. The spiked feed was shipped in coolers stored on blue ice from PNNL via overnight courier to the CR in Sequim for analysis. The spiked feed was stored refrigerated (2-8°C) upon receipt and for the duration of the study. These samples are currently maintained in refrigerated storage as archived samples by the CR.

Table 4. Target Levels of Estradiol Spiked in Feed

Spike Units	Dosage Level 1	Dosage Level 2	Dosage Level 3	Dosage Level 4
µg Estradiol/g feed	0.078	0.31	1.25	5

6.3 ANALYTICAL CHEMISTRY FOR FEED SPIKING ACCURACY TESTING

Game bird feed and acetone containing a specified concentration of estradiol were mixed in a large capacity mixer to formulate each test diet. Acetone was used as the solvent vehicle to transport the estradiol into the feed and was then evaporated off. Test substance spiking accuracy and uniformity was evaluated under storage conditions and matrix specifications as requested by the participating laboratory. Quantitation of estradiol was carried out by chromatographic analysis on each feed sample provided to the CR. Triplicate aliquots were tested (except for Batch 10).

The following text describes sample extraction and preparation for analysis – a detailed description of this procedure is provided in MSL SOP O-018-00. A measured quantity of estradiol spiked bird feed was extracted and dried with an acetone and sodium sulfate solution with ethynyl-estradiol, (EE2) added as an internal standard. The extracted sample was then centrifuged, filtered, dried, derivatized and analyzed by GC/MS (all per MSL SOP O-018-00), see Table 5. Data collected in method development indicated that sample analysis within 40 days of extraction was acceptable when extracted samples were stored at ≤ -6°C. A 6 point calibration curve (5 points acceptable), with target levels of 20, 70, 100, 200, 500, and 5000 ng/mL was generated with each sample set run. In addition to the calibration curve, initial calibration verification and continuing calibration verification (ICV/CCV) samples were analyzed to demonstrate on-going calibration accuracy.

Table 5. GC/MS Conditions

GC System	Agilent 5890 (Palo Alto, CA)
Column	J+W DB-5, 30 m x 0.25 mm (ID) x 2.5 µm film thickness (Bellefonte, PA)
Oven Temperature Program	120°C held for 2 minutes, increase 15°C/minute to 300°C, hold 5 minutes
Helium Carrier Gas/ Flow Rate	Helium at 1 mL/minute
Injection Volume/Mode	2 µL/ Splitless
Detector Type	Mass Spectrometer (MS)
Detector Mode	SIM (Selective Ion Monitoring)
Transfer Line Temperature	280°C
Ion Source Temperature	150°C
Detector Temperature	300°C
Injector Inlet Temperature	250°C
Run Time	19 minutes

7.0 RESULTS

7.1 ANALYTICAL CHEMISTRY FOR FEED SPIKING ACCURACY TESTING

Feed spiking accuracy testing was initiated on October 30, 2003 and the final analysis of the last batch of feed samples was carried out on June 4, 2004. Samples from sixteen batches of dosed feed were submitted to and analyzed by the CR for testing one or more of the following parameters: feed dosing concentration verification, spiking uniformity, stability/contamination testing, and unspiked feed (analyzed as a control) for determination of endogenous estradiol. Recovery statistics demonstrated an overall accurate spiking of the feed save two batches where recoveries came in at about half of nominal.

Table 6 below indicates the recovery ranges for the 15 batches tested for formulation accuracy.

Table 6. Batch Feed Formulation Accuracy – Recovery Ranges

Batch #	Recovery Ranges	Generation
1	74% to 105%	P1
2	83% to 132%	P1A
3	71% to 120%	P1A
4	66% to 110%	P1A
5	75% to 122%	P1A & P1B
6	72% to 121%	P1A & P1B
7	73% to 97%	P1A & P1B
8	54% to 102%	P1A & P1B
9 & 9A	74% to 142%	F1A
10	64% to 169%	F1A
11 ¹	22% to 77%	F1A
12	35% to 60%	F1A
13	64% to 102%	F1A
16	50% to 76%	F1A
19	68% to 80%	F1A

The average measured concentration of estradiol in µg/g was close to the target doses and relatively consistent over time (Table 7). CVs ranged between 6 and 52%, however the elevated CVs were associated with concentrations below the method detection limit (MDL) of 36.7 ng/g (Figures 2 and 3).

¹Recovery range for batch 11 includes 3 replicates of the 0.078 µg/g target sample that quantitated below the MDL. These data were included in the calculation since the expected value was above the MDL. Without inclusion of these values the quantitation range for batch 11 is 49% to 77%.

Table 7. Estradiol Concentration in Quail Feed Over Time

Generation	Target Dose ug/g	Average E2 ug/g	CV over Time
P1	0	0.02 U	47%
P1	0.078	0.08	11%
P1	0.31	0.26	13%
P1	1.25	0.91	15%
P1	5	3.95	11%
F1	0	0.02	52%
F1	0.078	0.07	14%
F1	0.31	0.20	27%
F1	1.25	0.81	15%
F1	5	3.55	6%

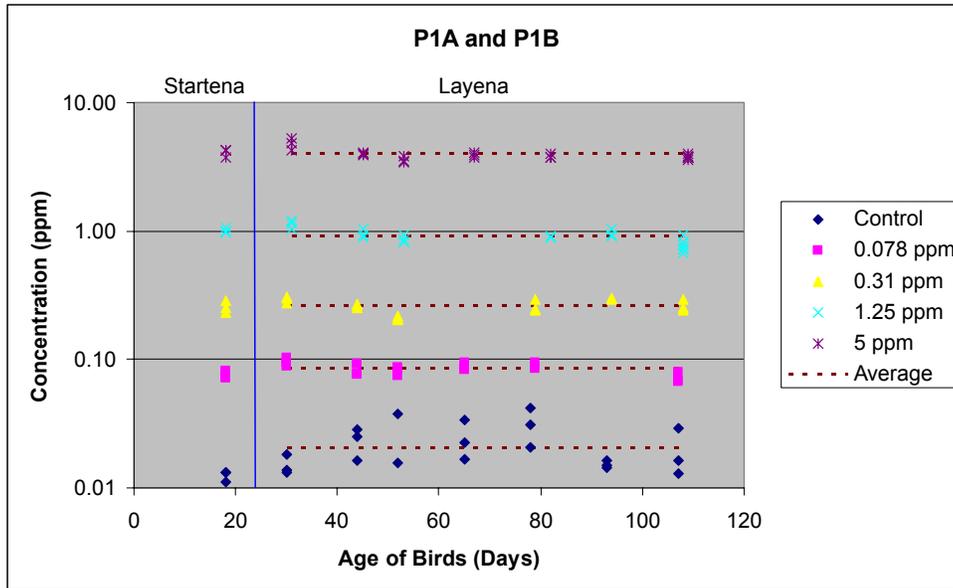


Figure 2. Overall and Observed Concentration of Quail Feed Over Time for the P1 Generation

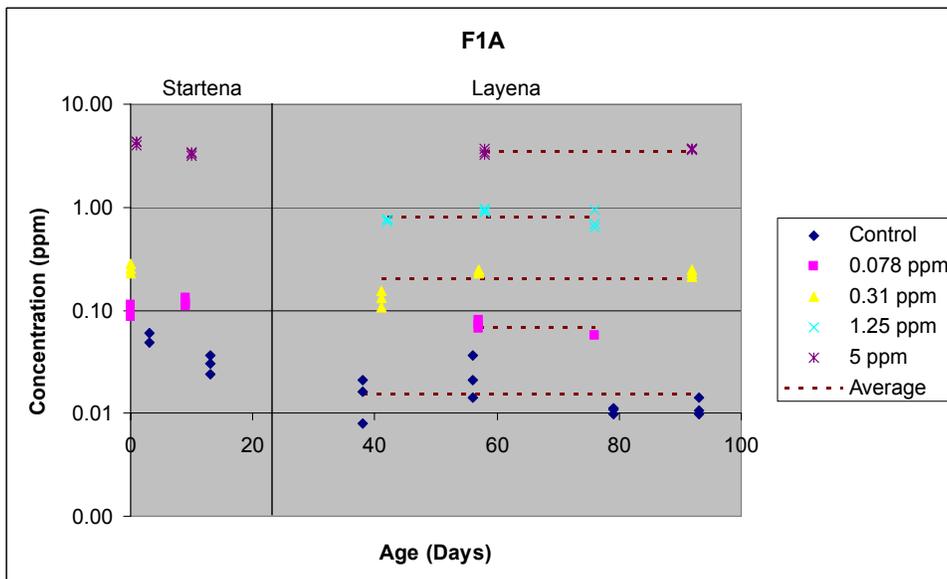


Figure 3. Overall and Observed Concentration of Quail Feed Over Time for the F1 Generation

For the stability runs, for samples within the quantitation range of the calibration curve, only feed batch 10 showed any significant change between the initial measurement and the final measurement (Table 8). For this batch, the 0.078 µg estradiol/g feed sample showed a major reduction of about 83% in estradiol detected over the 3¹ day exposure. Also for batch 10, this same sample pair (target of 0.078 µg/g estradiol) quantitated at 154% of target at day zero. Though the day 3 value was below the MDL the stability calculation was still carried out but flagged. It should be noted that the samples were received at the CR at the end of the stated refrigerated stability term (received on 27th day, claim of 28 days stability). For three of those days, the samples were at ambient temperature in the brooder feed troughs. Following receipt of the samples at the CR, they were refrigerated, and extracted on day 32 from preparation – 4 days beyond the stated refrigerated stability period. The matrix blanks and spikes and ICV and CCV values for day 3 were normal save the CCV mentioned above which was 128% recovery for the estradiol and 148% for the surrogate recovery. The remaining batches tested for stability held within a range of 83% and 131% recovery over the stability test period.

Table 8. Contamination and Stability of Feed During Storage and Exposure for P1 and F1 Generations

Stability Performance – Batch 1 (µg/g)			Contamination	Stability
Target Conc.	10/30/2003	11/7/2003	% Difference	% Difference
0	U	U	Below MDL	
0.078	0.08	0.08		0%
0.31	0.26	0.23		-11%
1.25	1.00	0.97		-3%
5	4.10	4.66		14%
Stability Performance – Batch 4 (µg/g)			Contamination	Stability
Target Conc.	12/3-12/4/03	12/16/2003	% Difference	% Difference
0	U	U	Below MDL	
0.078	0.081	0.076		-6%
0.31	0.209	0.263		26%
1.25	0.872	0.831		-5%
5	3.572	2.975		-17%
Stability Performance – Batch 8 (µg/g)			Contamination	Stability
Target Conc.	1/27-1/29/04	2/02,03/04	% Difference	% Difference
0	U	U	Below MDL	
0.078	0.072	0.071		-1.4%
0.31	0.263	0.264		1.5%
1.25	0.723	0.948		31.7%
5	3.760	4.318		14.8%
Stability Performance – Batch 10 (µg/g)			Contamination	Stability
Target Conc.	2/19,20,23/2004	3/10/2004	Target Conc.	% Difference
0	U	U	Below MDL	
0.078	0.12	0.02U		-83.3%*
0.31	Not analyzed	0.11		
1.25	Not analyzed	0.64		
5	3.30	2.50		-24.2%

*Day 3 result below MDL

A test for endogenous estradiol in blank feed (batch 15) showed levels below the MDL (nothing detected reported for 2 of 3 samples). Levels of feed cross-contamination, tested on batches 1, 4, 8, and 10 were all below the MDL indicating contamination for feed placed in the cages was

¹ Note that batch 10 was held in the refrigerator from date of preparation until placed in feed troughs for the three day exposure.

undetectable (Table 8). There were two commercial feed formulations used during the study, Purina Game Bird Layena® (for reproductive birds) and Purina GameBird Startena® (for growing chicks).

8.0 CONCLUSIONS

Levels of estradiol spiked into game bird feed under WA 2-17 compared favorably, over a majority of the batches submitted and tested, with the analytical results generated by the EDSP Chemical Repository at the Marine Sciences Laboratory in Sequim, Washington. Cross-contamination of feed occurring in the cages in the trays was deemed insignificant. Data (from batch 15) suggests that the endogenous estradiol level in feed was insignificant.

APPENDIX A

MANUFACTURER'S CERTIFICATE OF ANALYSIS/PURITY



SIGMA-ALDRICH

Certificate of Analysis

Product Name	β -Estradiol, $\geq 98\%$
Product Number	E8875
Product Brand	
CAS Number	50-28-2
Molecular Formula	$C_{18}H_{24}O_2$
Molecular Weight	272.38

TEST**APPEARANCE****SOLUBILITY****PURITY BY HPLC****SHELF LIFE SOP QC-12-006****QC ACCEPTANCE DATE****SPECIFICATION**

WHITE TO OFF-WHITE POWDER

CLEAR COLORLESS SOLUTION AT 200 MG PLUS 4 ML OF ETHANOL

98% MINIMUM

2 YEARS

LOT 052K1370 RESULTS

WHITE POWDER

CONFORMS

100%

JANUARY 2004

JANUARY 2002

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

APPENDIX B

ANALYTICAL RESULTS OF TESTING

Qualifier Key
D=Derivatization Issue
U = Analyte Detected Below MDL
Q = Questionable Sample
A = Anomalous

Feed batch #1

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-7	2122-13	10/31/2003	0.01	U	--	0.01	Feed Type – Startena® Bird Generation – P1 (Chicks) Collection Date – 10/30/2003 Extraction Date – 10/31/2003 Feed Lot 03AUG12SPK2 Analysis Date – 11/18/2003 <i>*sample did not have aluminum foil liner upon receipt</i>
0	ADTD-8	2122-14	10/31/2003	0.01	U	--		
0	ADTD-9	2122-15	10/31/2003	0.01	U	--		
0.078	ADTD-1	2122-7	10/31/2003	0.08		105%	0.076	
0.078	ADTD-2	2122-8	10/31/2003	0.07		95%		
0.078	ADTD-3	2122-9	10/31/2003	0.07		93%		
0.31	ADTD-4	2122-10	10/31/2003*	0.25		81%	0.26	
0.31	ADTD-5	2122-11	10/31/2003	0.29		94%		
0.31	ADTD-6	2122-12	10/31/2003	0.23		74%		
1.25	ADTD-13	2122-4	10/31/2003	1.05		84%	1.00	
1.25	ADTD-14	2122-5	10/31/2003	0.99		79%		
1.25	ADTD-15	2122-6	10/31/2003	0.97		78%		
5	ADTD-10	2122-1	10/31/2003	4.28		86%	4.10	
5	ADTD-11	2122-2	10/31/2003	4.26		85%		
5	ADTD-12	2122-3	10/31/2003	3.75		75%		

Stability samples from Feed Batch #1

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-16	2122-16	11/8/2003	0.01	U	--	0.01	Feed Type – Startena® Bird Generation – P1 (Chicks) Collection Date – 11/07/2003 Extraction Date – 11/10/2003 Feed Lot 03AUG12SPK2 Analysis Date – 11/18,19/2003 <i>*sample was broken upon receipt and PI asked that it not be analyzed</i>
0	ADTD-17	2122-17	11/8/2003*	0.01	Q U	--		
0	ADTD-18	2122-18	11/8/2003	0.01	U	--		
0.078	ADTD-19	2122-19	11/8/2003	0.08		97%	0.076	
0.078	ADTD-20	2122-20	11/8/2003	0.08		103%		
0.078	ADTD-21	2122-21	11/8/2003	0.07		90%		
0.31	ADTD-22	2122-22	11/8/2003	0.23		73%	0.23	
0.31	ADTD-23	2122-23	11/8/2003	0.25		79%		
0.31	ADTD-24	2122-24	11/8/2003	0.21		68%		
1.25	ADTD-25	2122-25	11/8/2003	1.01		81%	0.97	
1.25	ADTD-26	2122-26	11/8/2003	0.96		77%		
1.25	ADTD-27	2122-27	11/8/2003	0.94		76%		
5	ADTD-28	2122-28	11/8/2003	4.25		85%	4.66	
5	ADTD-29	2122-29	11/8/2003	5.65		113%		
5	ADTD-30	2122-30	11/8/2003	4.08		82%		

Feed Batch #1 Stability/Contamination Performance^a

Stability Performance – Batch 1 (µg/g)			Contamination	Stability
Target Conc.	10/30/2003	11/7/2003	% Difference	% Difference
0	U	U	0%	
0.078	0.08	0.08		0%
0.31	0.26	0.23		-11%
1.25	1.00	0.97		-3%
5	4.10	4.66		14%

^aFeed was refrigerated from preparation (10/30/03) until placed in feeders (11/05/03). Samples collected from feeders on 11/07/03.

Feed Batch #4

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-61	2122-61	12/9/2003	0.04		--	0.02	Feed Type – Layena® Bird Generation – P1A Collection Date – 12/3&4/2003 Extraction Date – 12/09/2003 Feed Lot 03OCT09 Analysis Date – 12/15/2003
0	ADTD-62	2122-62	12/9/2003	0.00	U	--		
0	ADTD-63	2122-63	12/9/2003	0.02	U	--		
0.078	ADTD-64	2122-64	12/9/2003	0.09		110%	0.08	
0.078	ADTD-65	2122-65	12/9/2003	0.07		96%		
0.078	ADTD-66	2122-66	12/9/2003	0.08		107%		
0.31	ADTD-67	2122-67	12/9/2003	0.22		70%	0.21	
0.31	ADTD-68	2122-68	12/9/2003	0.21		66%		
0.31	ADTD-69	2122-69	12/9/2003	0.21		67%		
1.25	ADTD-70	2122-70	12/9/2003	0.93		75%	0.87	
1.25	ADTD-71	2122-71	12/9/2003	0.86		69%		
1.25	ADTD-72	2122-72	12/9/2003	0.82		66%		
5	ADTD-73	2122-73	12/9/2003	3.82		76%	3.57	
5	ADTD-74	2122-74	12/9/2003	3.47		69%		
5	ADTD-75	2122-75	12/9/2003	3.43		69%		

Stability Samples from Feed Batch #4

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD76	2122-76	12/18/2003	0.049		--	0.04	Feed Type – Layena® Bird Generation – P1A Collection Date – 12/16/2003 Extraction Date – 12/18/2003 Feed Lot 03OCT09 Analysis Date – 01/08/2004
0	ADTD77	2122-77	12/18/2003	0.033	U	--		
0	ADTD78	2122-78	12/18/2003	0.030	U	--		
0.078	ADTD79	2122-79	12/18/2003	0.083		106%	0.08	
0.078	ADTD80	2122-80	12/18/2003	0.068		87%		
0.078	ADTD81	2122-81	12/18/2003	0.078		100%		
0.31	ADTD82	2122-82	12/18/2003	0.307		99%	0.26	
0.31	ADTD83	2122-83	12/18/2003	0.252		81%		
0.31	ADTD84	2122-84	12/18/2003	0.230		74%		
1.25	ADTD85	2122-85	12/18/2003	0.806		65%	0.83	
1.25	ADTD86	2122-86	12/18/2003	0.847		68%		
1.25	ADTD87	2122-87	12/18/2003	0.841		67%		
5	ADTD88	2122-88	12/18/2003	2.844		57%	2.97	
5	ADTD89	2122-89	12/18/2003	3.004		60%		
5	ADTD90	2122-90	12/18/2003	3.076		62%		

Feed Batch #4 Stability/Contamination Performance^a

Target Conc.	Stability Performance – Batch 4 (µg/g)		Contamination	Stability
	12/3-12/4/03	12/16/2003	% Difference	% Difference
0	0.018	0.037	106%*	
0.078	0.081	0.076		-6%
0.31	0.209	0.263		26%
1.25	0.872	0.831		-5%
5	3.572	2.975		-17%

^aFeed stored in refrigerator from preparation (12/3,4/03) until placed in feeder (12/12/03). Feeder samples collected 12/16/03

*both at or below detection limit

Feed Batch #8¹

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-136	2122-136	1/30/2004	0.03	U	--	0.02	Feed Type – Layena® Bird Generation – P1A & P1B Collection Date – 01/27,28,29/2004 Extraction Date – 02/02/2004 Feed Lot 03NOV10 Analysis Date – 02/05/2004
0	ADTD-137	2122-137	1/30/2004	0.01	U	--		
0	ADTD-138	2122-138	1/30/2004	0.02	U	--		
0.078	ADTD-139	2122-139	1/30/2004	0.07		88%	0.072	
0.078	ADTD-140	2122-140	1/30/2004	0.08		102%		
0.078	ADTD-141	2122-141	1/30/2004	0.07		87%		
0.31	ADTD-142	2122-142	1/30/2004	0.25		81%	0.26	
0.31	ADTD-143	2122-143	1/30/2004	0.24		79%		
0.31	ADTD-144	2122-144	1/30/2004	0.29		94%		
1.25	ADTD-145	2122-145	1/30/2004	0.77		61%	0.72	
1.25	ADTD-146	2122-146	1/30/2004	0.67		54%		
1.25	ADTD-147	2122-147	1/30/2004	0.73		58%		
5	ADTD-148	2122-148	1/30/2004	3.80		76%	3.76	
5	ADTD-149	2122-149	1/30/2004	3.77		75%		
5	ADTD-150	2122-150	1/30/2004	3.71		74%		

Stability Samples from Feed Batch #8

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD166	2122-166	2/6/2004	0.015	U	--	0.015	Feed Type – Layena® Bird Generation – P1A & P1B Collection Date – 02/02,03/2004 Extraction Date – 2/10&11/2004 Feed Lot 03NOV10 Analysis Date – 02/18,19/2004
0	ADTD167	2122-167	2/6/2004	0.010	U	--		
0	ADTD168	2122-168	2/6/2004	0.008	U	--		
0*	ADTD-181	2122-181	2/6/2004	0.021	U	--		
0*	ADTD-182	2122-182	2/6/2004	0.016	U	--		
0*	ADTD-183	2122-183	2/6/2004	0.020	U	--		
0.078	ADTD169	2122-169	2/6/2004	0.072		92%	0.071	
0.078	ADTD170	2122-170	2/6/2004	0.068		87%		
0.078	ADTD171	2122-171	2/6/2004	0.073		94%		
0.31	ADTD172	2122-172	2/6/2004	0.229		74%	0.264	
0.31	ADTD173	2122-173	2/6/2004	0.275		89%		
0.31	ADTD174	2122-174	2/6/2004	0.287		93%		
1.25	ADTD175	2122-175	2/6/2004	0.896		72%	0.948	
1.25	ADTD176	2122-176	2/6/2004	1.060		85%		
1.25	ADTD177	2122-177	2/6/2004	0.888		71%		
5	ADTD178	2122-178	2/6/2004	4.345		87%	4.318	
5	ADTD179	2122-179	2/6/2004	4.771		95%		
5	ADTD180	2122-180	2/6/2004	4.141		83%		
5*	ADTD-193	2122-192	2/6/2004	4.976		100%		
5*	ADTD-194	2122-193	2/6/2004	3.817		76%		
5*	ADTD-195	2122-194	2/6/2004	3.858		77%		

*CR provided with two sets of identical samples, both sets were analyzed with data for Stability/Contamination performance calculations.

Feed Batch #8 Stability/Contamination Performance^a

Target Conc.	Stability Performance – Batch 8 (µg/g)		Contamination	Stability
	1/27-1/29/04	2/02,03/04	% Difference	% Difference
0	U	U	U	
0.078	0.072	0.071		-1.4%
0.31	0.26	0.264		1.5%
1.25	0.72	0.948		31.7%
5	3.760	4.318		14.8%

^aFeed stored refrigerated until placed in feeders (1/29,30/04).
Feeder samples collected 2/2,3/04.

¹ Samples were received at a temperature above the acceptable range; backup samples sent for analysis (*Resent Feed Batch #8* next page). Based on data, used initial Batch #8 results (above) for Stability/Contamination analysis.

Resent Feed Batch #8

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
1.25	ADTD160	2122-160	2/6/2004	0.72		58%	0.82	Feed Type – Layena® Bird Generation – P1A & P1B Collection Date – 01/27-29/2004 Extraction Date – 02/09/2004 Feed Lot 03NOV10 Analysis Date – 02/18/2004
1.25	ADTD-161	2122-161	2/6/2004	0.81		65%		
1.25	ADTD-162	2122-162	2/6/2004	0.92		74%		
5	ADTD-163	2122-163	2/6/2004	3.72		74%	3.76	
5	ADTD-164	2122-164	2/6/2004	3.61		72%		
5	ADTD-165	2122-165	2/6/2004	3.95		79%		

Feed Batch #10

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0.078	ADTD217	2122-216	2/25/2004	0.11		139%	0.12	Feed Type – Startena® Bird Generation – F1A Collection Date – 02/19,20,23/2004 Extraction Date – 02/26/2004 Feed Lot - 03OCT07, 03NOV24, 03DEC04 Analysis Date – 03/03/2004
0.078	ADTD218	2122-217	2/25/2004	0.12		148%		
0.078	ADTD219	2122-218	2/25/2004	0.13		169%		
5	ADTD226	2122-225	2/25/2004	3.30		66%	3.30	
5	ADTD227	2122-226	2/25/2004	3.18		64%		
5	ADTD228	2122-227	2/25/2004	3.42		68%		
0	ADTD214	2122-213	2/25/2004	0.03	U	--	0.03	
0	ADTD215	2122-214	2/25/2004	0.02	U	--		
0	ADTD216	2122-215	2/25/2004	0.04		--		

Stability Brooder Feeder Samples from Batch #10

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-244	2122-243	3/17/2004	0.024	U	--	0.017	Feed Type – Startena® Bird Generation – F1A Collection Date – 03/10/2004 Extraction Date – 03/22/2004 Feed Lot – 03OCT07 03NOV24, 03DEC04 Analysis Date – 04/01/2004
0	ADTD-245	2122-244	3/17/2004	0.009	U	--		
0	ADTD-246	2122-245	3/17/2004	0.019	U	--		
0	ADTD-254	2122-253	3/17/2004	0.015	U	--	0.015	
0.078	ADTD-255	2122-254	3/17/2004	0.016	U	21%	0.016	
0.31	ADTD-256	2122-255	3/17/2004	0.113		36%	0.113	
1.25	ADTD-257	2122-256	3/17/2004	0.637		51%	0.637	
5	ADTD-253	2122-252	3/17/2004	2.364		47%	2.500	
5	ADTD-258	2122-257	3/17/2004	2.636		53%		

Feed Batch #10 Stability/Contamination Performance*

Target Conc.	Stability Performance – Batch 10 (µg/g)		Contamination	Stability
	2/19,20,23/2004	3/10/2004	% Difference	% Difference
0	U	U	U	
0.078	0.12	0.02		-83%**
0.31	Not analyzed	0.11		-
1.25	Not analyzed	0.64		-
5	3.30	2.50		-24%

*Feed stored refrigerated from preparation (2/19,20,23/04) until placed in brooder feeders (3/7/04). Samples collected 3/10/04.

**calculation carried out in spite of exposed sample result <MDL – therefore this result is an estimate only.

Feed Batch 2 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-31	2122-31	11/13/2003	0.01	U	--	0.02	Feed Type – Layena® Bird Generation – P1A Collection Date – 11/11&12/2003 Extraction Date – 11/18/2003 Feed Lot 03AUG08 Analysis Date – 11/19&20/2003
0	ADTD-32	2122-32	11/13/2003	0.01	U	--		
0	ADTD-33	2122-33	11/13/2003	0.02	U	--		
0.078	ADTD-34	2122-34	11/13/2003	0.09		121%	0.10	
0.078	ADTD-35	2122-35	11/13/2003	0.10		132%		
0.078	ADTD-36	2122-36	11/13/2003	0.09		115%		
0.31	ADTD-37	2122-37	11/13/2003	0.28		89%	0.29	
0.31	ADTD-38	2122-38	11/13/2003	0.31		99%		
0.31	ADTD-39	2122-39	11/13/2003	0.30		96%		
1.25	ADTD-40	2122-40	11/13/2003	1.04		83%	1.13	
1.25	ADTD-41	2122-41	11/13/2003	1.19		95%		
1.25	ADTD-42	2122-42	11/13/2003	1.14		92%		
5	ADTD-43	2122-43	11/13/2003	5.28		106%	4.81	
5	ADTD-44	2122-44	11/13/2003	4.88		98%		
5	ADTD-45	2122-45	11/13/2003	4.27		85%		

Feed Batch 3 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD46	2122-46	12/2/2003	0.03	U	--	0.02	Feed Type – Layena® Bird Generation – P1A Collection Date – 11/25,26/2003 Extraction Date – 12/02/2003 Feed Lot 03OCT09 Analysis Date – 01/21/2004*
0	ADTD47	2122-47	12/2/2003	0.02	U	--		
0	ADTD48	2122-48	12/2/2003	0.02	U	--		
0.078	ADTD49	2122-49	12/2/2003	0.08		100%	0.08	
0.078	ADTD50	2122-50	12/2/2003	0.09		120%		
0.078	ADTD51	2122-51	12/2/2003	0.08		99%		
0.31	ADTD52	2122-52	12/2/2003	0.25		81%	0.26	
0.31	ADTD53	2122-53	12/2/2003	0.27		88%		
0.31	ADTD54	2122-54	12/2/2003	0.26		85%		
1.25	ADTD55	2122-55	12/2/2003	0.94		75%	0.95	
1.25	ADTD56	2122-56	12/2/2003	1.04		83%		
1.25	ADTD57	2122-57	12/2/2003	0.89		71%		
5	ADTD58	2122-58	12/2/2003	4.02		80%	4.00	
5	ADTD59	2122-59	12/2/2003	3.89		78%		
5	ADTD60	2122-60	12/2/2003	4.10		82%		

*Analysis date exceeds method specified holding time documented as an SOP deviation.

Feed Batch 5 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD91	2122-91	12/19/2003	0.02	U	--	0.02	Feed Type – Layena® Bird Generation – P1A&P1B Collection Date – 12/16, 17, 18/2003 Extraction Date – 12/22/2003 Feed Lot - 03NOV10 Analysis Date – 01/29/2004
0	ADTD92	2122-92	12/19/2003	0.02	U	--		
0	ADTD93	2122-93	12/19/2003	0.03	U	--		
0.078	ADTD94	2122-94	12/19/2003	0.08		108%	0.09	
0.078	ADTD95	2122-95	12/19/2003	0.10		122%		
0.078	ADTD96	2122-96	12/19/2003	0.08		106%		
0.31	ADTD97	2122-97	12/19/2003	Not Analyzed		--	--	
0.31	ADTD98	2122-98	12/19/2003	Not Analyzed		--		
0.31	ADTD99	2122-99	12/19/2003	Not Analyzed		--		
1.25	ADTD100	2122-100	12/19/2003	Not Analyzed		--	--	
1.25	ADTD101	2122-101	12/19/2003	Not Analyzed		--		
1.25	ADTD102	2122-102	12/19/2003	Not Analyzed		--		
5	ADTD103	2122-103	12/19/2003	3.75		75%	3.92	
5	ADTD104	2122-104	12/19/2003	3.89		78%		
5	ADTD105	2122-105	12/19/2003	4.10		82%		

Feed Batch 6 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-106	2122-106	01/06/2004	0.03	U	--	0.03	Feed Type – Layena® Bird Generation – P1A&P1B Collection Date – 12/29&30/2003 & 01/02/2004 Extraction Date – 01/06/2004 Feed Lot - 03NOV10 Analysis Date – 01/08/2004
0	ADTD-107	2122-107	01/06/2004	0.04		--		
0	ADTD-108	2122-108	01/06/2004	0.02	U	--		
0.078	ADTD-109	2122-109	01/06/2004	0.09		114%	0.09	
0.078	ADTD-110	2122-110	01/06/2004	0.09		109%		
0.078	ADTD-111	2122-111	01/06/2004	0.09		121%		
0.31	ADTD-112	2122-112	01/06/2004	0.25		79%	0.26	
0.31	ADTD-113	2122-113	01/06/2004	0.24		78%		
0.31	ADTD-114	2122-114	01/06/2004	0.29		95%		
1.25	ADTD-115	2122-115	01/06/2004	0.90		72%	0.90	
1.25	ADTD-116	2122-116	01/06/2004	0.90		72%		
1.25	ADTD-117	2122-117	01/06/2004	0.91		73%		
5	ADTD-118	2122-118	01/06/2004	3.77		75%	3.83	
5	ADTD-119	2122-119	01/06/2004	3.71		74%		
5	ADTD-120	2122-120	01/06/2004	3.99		80%		

Feed Batch 7 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-121	2122-121	1/20/2004	0.02	U	--	0.02	Feed Type – Layena® Bird Generation – P1A&P1B Collection Date – 01/13,14,15/2004 Extraction Date – 01/21/2004 Feed Lot - 03NOV10 Analysis Date – 02/05/2004
0	ADTD-122	2122-122	1/20/2004	0.01	U	--		
0	ADTD-123	2122-123	1/20/2004	0.02	U	--		
0.078	ADTD-124	2122-124	1/20/2004	Not Analyzed		--	--	
0.078	ADTD-125	2122-125	1/20/2004	Not Analyzed		--		
0.078	ADTD-126	2122-126	1/20/2004	Not Analyzed		--		
0.31	ADTD-127	2122-127	1/20/2004	0.29		94%	0.30	
0.31	ADTD-128	2122-128	1/20/2004	0.30		97%		
0.31	ADTD-129	2122-129	1/20/2004	0.30		97%		
1.25	ADTD-130	2122-130	1/20/2004	1.04		83%	0.96	
1.25	ADTD-131	2122-131	1/20/2004	0.93		74%		
1.25	ADTD-132	2122-132	1/20/2004	0.91		73%		
5	ADTD-133	2122-133	1/20/2004	Not Analyzed		--	--	
5	ADTD-134	2122-134	1/20/2004	Not Analyzed		--		
5	ADTD-135	2122-135	1/20/2004	Not Analyzed		--		

Feed Batch 9 & 9A – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0.078	ADTD199	2122-198	2/13/2004	0.11		142%	0.10	Feed Type – Startena® Bird Generation – F1A Collection Date – 02/10,11,13/2004 Extraction Date – 02/16&24/2004 Feed Lot - 03NOV25 03DEC04 Analysis Date – 03/03/2004
0.078	ADTD200	2122-199	2/13/2004	0.09		110%		
0.078	ADTD201	2122-200	2/13/2004	0.09		120%		
0.31	SDTD202	2122-201	2/13/2004	0.23		74%	0.26	
0.31	ADTD203	2122-202	2/13/2004	0.29		92%		
0.31	ADTD204	2122-203	2/13/2004	0.25		82%		
5	ADTD208	2122-207	2/13/2004	4.01		80%	4.20	
5	ADTD209	2122-208	2/13/2004	4.30		86%		
5	ADTD210	2122-209	2/13/2004	4.30		86%		
0	ADTD211	2122-210	2/17/2004	0.26	A	--	0.12	
0	ADTD212	2122-211	2/17/2004	0.06		--		
0	ADTD213	2122-212	2/17/2004	0.05		--		

Feed Batch 11 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0.078	ADTD-232	2122-231	3/9/2004	0.019	U	25%	0.02	Feed Type – Startena®/Layena® mix Bird Generation – F1A Collection Date – 03/05&06/2004 Extraction Date – 03/10/2004 Feed Lot 03DEC07 03DEC09 Analysis Date – 04/01/2004
0.078	ADTD-233	2122-232	3/9/2004	0.017	U	22%		
0.078	ADTD-234	2122-233	3/9/2004	0.019	U	25%		
1.25	ADTD-238	2122-237	3/9/2004	0.607		49%	0.72	
1.25	ADTD-239	2122-238	3/9/2004	0.762		61%		
1.25	ADTD-240	2122-239	3/9/2004	0.785		63%		
5	ADTD-241	2122-240	3/9/2004	3.773		75%	3.75	
5	ADTD-242	2122-241	3/9/2004	3.632		73%		
5	ADTD-243	2122-242	3/9/2004	3.831		77%		

Feed Batch 12 – Formulation Accuracy Testing*

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-264	2122-263	3/25/2004	0.01	U	--	0.02	Feed Type – Layena® Bird Generation – F1A Collection Date – 03/19,22,23/2004 Extraction Date – 03/25/2004 Feed Lot – 03DEC09 Analysis Date – 04/01/2004
0	ADTD-265	2122-264	3/25/2004	0.02	U	--		
0	ADTD-266	2122-265	3/25/2004	0.02	U	--		
0.31	ADTD-270	2122-269	3/25/2004	0.11		35%	0.13	
0.31	ADTD-217	2122-270	3/25/2004	0.15		49%		
0.31	ADTD-272	2122-271	3/25/2004	0.13		42%		
1.25	ADTD-273	2122-272	3/25/2004	0.72		58%	0.74	
1.25	ADTD-274	2122-273	3/25/2004	0.76		60%		
1.25	ADTD-275	2122-274	3/25/2004	0.76		60%		

Batch received at 13C where specification was 4±2C. While results are low they are comparable to Batch 11 where receipt temperature specifications were met.

Feed Batch 13 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-279	2122-278	4/13/2004	0.02	U	--	0.02	Feed Type – Layena® Bird Generation – F1A Collection Date – 04/6,7,8/2004 Extraction Date – 04/14/2004 Feed Lot – 03DEC09 Analysis Date – 04/19/2004
0	ADTD-280	2122-279	4/13/2004	0.04*	U	--		
0	ADTD-281	2122-280	4/13/2004	0.01	U	--		
0.078	ADTD-282	2122-281	4/13/2004	0.08		102%	0.07	
0.078	ADTD-283	2122-282	4/13/2004	0.07		92%		
0.078	ADTD-284	2122-283	4/13/2004	0.07		86%		
0.31	ADTD-285	2122-284	4/13/2004	0.23		75%	0.24	
0.31	ADTD-286	2122-285	4/13/2004	0.25		80%		
0.31	ADTD-287	2122-286	4/13/2004	0.23		74%		
1.25	ADTD-288	2122-287	4/13/2004	0.98		78%	0.92	
1.25	ADTD-289	2122-288	4/13/2004	0.91		73%		
1.25	ADTD-290	2122-289	4/13/2004	0.89		71%		
5	ADTD-291	2122-290	4/13/2004	3.21		64%	3.42	
5	ADTD-292	2122-291	4/13/2004	3.38		68%		
5	ADTD-293	2122-292	4/13/2004	3.66		73%		

* defined as MDL because value was not detected

Feed Batch 16 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (µg/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0.078	ADTD-300	2122-299	4/30/2004	0.00	D	--	0.06	Feed Type – Layena® Bird Generation – F1A Collection Date – 04/25,26,29/2004 Extraction Date – 05/14/2004 Feed Lot – 04FEB26 Analysis Date – 06/04/2004
0.078	ADTD-301	2122-300	4/30/2004	45.13	D	--		
0.078	ADTD-302	2122-301	4/30/2004	0.06		73%		
1.25	ADTD-306	2122-305	4/30/2004	0.63		50%	0.75	
1.25	ADTD-307	2122-306	4/30/2004	0.69		55%		
1.25	ADTD-308	2122-307	4/30/2004	0.95		76%		
0	ADTD-297	2122-296	4/30/2004	0.01	U	--	0.01	
0	ADTD-298	2122-297	4/30/2004	0.01	U	--		
0	ADTD-299	2122-298	4/30/2004	0.01	U	--		

Feed Batch 19 – Formulation Accuracy Testing

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0.31	ADTD-318	2122-317	5/18/2004	0.21		68%	0.23	Feed Type – Layena® Bird Generation – F1A Collection Date – 05/12,13/2004 Extraction Date – 05/26/2004 Feed Lot – 04APR01 Analysis Date – 06/04/2004
0.31	ADTD-319	2122-318	5/18/2004	0.23		73%		
0.31	ADTD-320	2122-319	5/18/2004	0.25		80%		
5	ADTD-324	2122-323	5/18/2004	3.72		74%	3.68	
5	ADTD-325	2122-324	5/18/2004	3.71		74%		
5	ADTD-326	2122-325	5/18/2004	3.60		72%		
0	ADTD-312	2122-311	5/18/2004	0.01	U	--	0.01	
0	ADTD-313	2122-312	5/18/2004	0.01	U	--		
0	ADTD-314	2122-313	5/18/2004	0.01	U	--		

Feed Batch 15 – Undosed, Endogenous Estrogen Determination

Target Conc. (µg/g)	Sponsor Code	Sample Name	Receipt Date	E2 (ug/g)	Qualifier	% Recovery	Average Batch (µg/g)	Logistics
0	ADTD-295	2122-294	4/23/2004	0.04*	U	--	0.03	Feed Type – Startena® Bird Generation – F2 Collection Date – 04/16/04 Extraction Date – 04/26/2004 Feed Lot – 04FEB19 Analysis Date – 04/28/2004
0	ADTD-296	2122-295	4/23/2004	0.04*	U	--		
0	ADTD-294	2122-293	4/23/2004	0.03	U	--		

*Assigned MDL since not detected

Procedural Blanks (PB) And Matrix Spikes (MS)/Matrix Spike Duplicates (MSD)

Target Conc.	Sample Name	Extraction Date	Analysis Date	E2 (ng/mL)	% Recovery	RSD	Surr. % Recovery
PB	2122-PB1	10/31/2003	11/18/2003	<5x MDL			120.0
1000 ng/g MS	2122-MS1	10/31/2003	11/18/2003	943.2	93.2	4.89%	109.6
1000 ng/g MSD	2122-MSD1	10/31/2003	11/18/2003	880.2	87.0		113.9
PB	2122-PB2	11/18/2003	11/20/2003*	<5x MDL			69.0
100* ng/g MS	2122-MS2	11/18/2003	11/20/2003*	95.7	94.5	1.40%	83.8
100* ng/g MSD	2122-MSD2	11/18/2003	11/20/2003*	93.8	92.7		72.3
PB	2122-pb4	12/9/2003	12/15/2003	<5x MDL			113.7
1000 ng/mL MS	2122-ms4	12/9/2003	12/15/2003	939.7	92.9	4.33%	93.3
1000 ng/mL MSD	2122-msd4	12/9/2003	12/15/2003	883.8	87.3		90.0
PB	2122-pb5	12/18/2003	1/8/2004	<5x MDL			93.4
PB	2122-pb7	1/6/2004	1/8/2004	<5x MDL			89.0
1000 ng/g MS	2122-ms5	12/18/2003	1/8/2004	937.6	92.6	8.72%	89.3
1000 ng/g MSD	2122-msd5	12/18/2003	1/8/2004	1060.8	104.8		103.5
1000 ng/g MS	2122-ms7	1/6/2004	1/8/2004	914.2	90.3	1.53%	85.5
1000 ng/g MSD	2122-msd7	1/6/2004	1/8/2004	894.6	88.4		90.1
PB	2122-pb3	12/2/2003	1/21/2004	<5x MDL			107.1
1000 ng/mL MS	2122-ms3	12/2/2003	1/21/2004	895.2	88.5	1.31%	101.0
1000 ng/mL MSD	2122-msd3	12/2/2003	1/21/2004	911.9	90.1		107.6
PB	2122-pb6	12/22/2003	1/29/2004	<5x MDL			117.1
1000 ng/mL MS	2122-ms6	12/22/2003	1/29/2004	853.9	84.4	0.17%	115.8
1000 ng/mL MSD	2122-ms6	12/22/2003	1/29/2004	851.9	84.2		116.7
PB	2122-pb9	2/2/2004	2/5/2004	<5x MDL			133.8
1000 ng/g MS	2122-ms9	2/2/2004	2/5/2004	890.4	88.0	1.00%	100.8
1000 ng/g MSD	2122-msd9	2/2/2004	2/5/2004	903.1	89.2		107.0
PB	2122-pb12	2/9/2004	2/18/2004	<5x MDL			126.8
1000 ng/g MS	2122-ms12	2/9/2004	2/18/2004	994.2	98.2	6.83%	117.6
1000 ng/g MSD	2122-msd12	2/9/2004	2/18/2004	906.4	89.6		105.7
PB	2122-pb11	2/10/2004	2/19/2004	<5x MDL			97.0
1000 ng/g MS	2122-ms11	2/10/2004	2/19/2004	900.2	89.0	1.30%	95.2
1000 ng/g MSD	2122-msd11	2/10/2004	2/19/2004	881.6	87.1		96.3
PB	2122-pb13	2/16/2004	3/3/2004	<5x MDL			105.1
1000 ng/g MS	2122-ms13	2/16/2004	3/3/2004	896.8	88.6	4.34%	109.7
1000 ng/g MSD	2122-msd13	2/16/2004	3/3/2004	843.4	83.3		105.8
PB	2122-pb16	3/10/2004	4/1/2004	<5x MDL			97.7
1000 ng/g MS	2122-ms16	3/10/2004	4/1/2004	817.2	80.8	1.10%	104.6
1000 ng/g MSD	2122-msd16	3/10/2004	4/1/2004	801.6	79.2		118.8
PB	2122-pb18	4/14/2004	4/19/2004	<5x MDL			111.1
1000 ng/g MS	2122-ms18	4/14/2004	4/19/2004	914.7	90.4	0.4%	102.3
1000 ng/g MSD	2122-msd18	4/14/2004	4/19/2004	909.5	89.9		101
PB	2122-pb19	5/14/2004	6/4/2004	<5x MDL			106.2
1000 ng/g MS	2122-ms19	5/14/2004	6/4/2004	925.1	91.4	10.62%	104.5
1000 ng/g MSD	2122-msd19	5/14/2004	6/4/2004	1075.4	106.3		112.3
PB	2122-pb20	5/26/2004	6/4/2004	<5x MDL			111.8
1000 ng/g MS	2122-ms20	5/26/2004	6/4/2004	952.1	94.1	3.05%	104.4
1000 ng/g MSD	2122-msd20	5/26/2004	6/4/2004	994.0	98.2		107.9

* accidentally spiked 100ug/mL matrix spike (E2) into samples, so resultant expected concentration is 100 ng/mL

Initial Calibration Verification & Continuing Calibration Verification

Calibration Date	Standard Type	Raw E2 ng/mL	RIS Corr E2 ng/mL	% Recovery E2	% Recovery (EE2)
11/18/03	icv	198.7	209.0	103.3	91.1
11/18/03	ccv	261.9	200.8	99.2	92.0
11/18/03	ccv	282.7	197.5	97.6	86.9
11/19/03	ccv	296.5	201.9	99.8	98.7
11/19/03	ccv	284.2	198.7	98.2	92.2
11/19/03	ccv	152.7	173.3	85.6	87.7
11/19/03	ccv	235.6	236.1	116.7	110.6
11/19/03	ccv	243.6	209.2	103.4	94.1
11/20/03	ccv	204.2	253.0	125.0	112.4
12/15/03	icv	230.3	200.4	96.5	85.7
12/15/03	ccv	317.9	207.2	102.4	98.4
12/15/03	ccv	269.8	202.7	100.2	95.5
01/08/04	icv	185.2	164.5	79.2	65.2
01/08/04	ccv	208.3	213.9	105.7	96.3
01/08/04	ccv	270.3	217.1	107.3	97.8
01/08/04	ccv	275.7	163.8	80.9	74.2
01/21/04	ccv	283.2	211.4	104.5	117.2
01/21/04	ccv	312.5	208.1	102.8	111.6
02/05/04	ccv	255.6	213.6	105.5	110.8
02/05/04	ccv	246.9	232.3	114.8	112.9
02/05/04	ccv	298.7	207.6	102.6	105.1
02/18/04	ccv	288.3	210.5	104.0	115.8
02/18/04	ccv	319.9	214.6	106.0	113.5
02/19/04	ccv	240.2	219.7	108.5	164.3
02/19/04	ccv	277.1	215.2	106.3	175.5
03/03/04	ccv	309.2	214.9	106.2	138.1
03/03/04	ccv	344.1	199.5	98.6	130.1
03/03/04	ccv	339.4	211.2	104.3	137.3
04/01/04	ccv	145.1	259.6	128.2	148.4
04/01/04	ccv	143.4	212.1	104.8	113.8
04/01/04	ccv	158.8	212.0	104.8	116.0
04/19/04	ccv	233.4	215.2	106.3	112.9
04/19/04	ccv	240.0	212.3	104.9	110.0
04/28/04	ccv	187.3	200.5	99.1	127.9
06/03/04	icv	209.5	200.4	99.0	90.8
06/03/04	ccv	162.9	218.1	107.7	91.9
06/03/04	ccv	285.8	201.2	99.4	98.6
06/04/04	ccv	303.8	202.3	100.0	99.0
06/04/04	ccv	306	203.7	100.6	101.8