

APPENDIX V

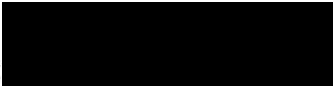
Diet Preparation Report

Experiment Number
2186, 2188


DIET PREPARATION
FINAL REPORT

Prepared by:

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Base Diet

The base diet used for this experiment was 5K96, Verified Casein 10 IF Irradiated Rodent Diet Meal, manufactured by Purina Mills International (PMI), P.O. Box 66812, St. Louis, MO 63166, see page 8 for more diet description and details. The 5K96 diet was received by ground shipment and immediately transferred to the Building 5 receiving dock for proper storage, see SOP 203.12, page 21 and 22. Prior to feeding, samples of the 5K96 were taken and transferred to the Chemistry Support Group, Division of Biochemical Toxicology (DBT) where a full analysis was performed, see pages 4 - 7 for results and refer to the Chemistry Support Group, Division of Biochemical Toxicology (DBT) summary report for additional details.

The 5K96 diet was stored at 10°- 21°C in Diet Preparation's 5D-112 walk-in cooler. The environmental temperature/humidity storage conditions were monitored by the Environment Monitoring Control Systems Unit (EMCS). Specific variation reports may be provided by the Division of Bioinformatics and Biostatistics in the Biometry Statistical Support Group.

Microbiological Surveillance

Microbiological evaluation was done to insure irradiation sterility was achieved. Samples of the 5K96 diet were taken for routine analyses in accordance with Diet Preparation SOP # 1006.09, pages 26 and 27. Microbiological samples were acceptable for animal study use by meeting NCTR's specifications of zero bacteria colony forming units per gram of feed tested and zero mold colony forming units per gram of feed tested. Refer to the Microbiology Surveillance/Diagnostic summary report for results. A detailed description is included on page 9 of this report. 5K96 diet feed samples not meeting the required specifications were resampled. If the resample did not meet the required specifications, the 5K96 diet was deemed unacceptable and not used.

Test and Control Article Receipt

The test article, Oxybenzone and the control article, 5K96, Verified Casein 10 IF Irradiated Rodent Diet Meal were used for Experiment 2186, 2188. The Oxybenzone was received to the Diet Preparation Support Group on 07/07/10 from Ivy Fine Chemicals, 1879 Old Cuthbert Road, Suite 23, Cherry Hill, NJ in one lot number, 1F100604. The 5K96, Verified Casein 10 IF Irradiated Rodent Diets were received on 05/04/12, lot number 120417-1, 08/01/12, lot number 12JUL11RTD1, 01/08/13, lot number 12DEC06RTD1, and 04/15/13, lot number 13MAR22RTD1. Inventory records of the Oxybenzone test article and 5K96, Verified Casein 10 IF Irradiated Rodent Diet are located in the Diet Preparation Support Group's Experiment 2186, 2188 raw data folders.

Test Article Storage Conditions

The bulk Oxybenzone was stored at room temperature in building 5D, room 161. The environmental temperature/humidity storage conditions were monitored by the Environment Monitoring Control Systems Unit (EMCS). Specific variation reports may be provided by the Division of Bioinformatics and Biostatistics in the Biometry Statistical Support Group.

Homogeneity

Homogeneity was established during the (Non-GLP) 2178 experiment on October 26, 2010 for the 1000 and 50,000 ppm dose levels. For detailed results, refer the Chemistry Support Group report.

Stability

Stability was established for Oxybenzone incorporated into 5K96, Verified Casein 10 IF Irradiated Rodent Diet Meal at the 1000 ppm dose level during the (Non-GLP) 2178 experiment. The DBT Chemistry Support Group used the 1000 ppm homogeneity sample (SCR # 2178 98 00002), stored at 2 - 8°C and in the dark for all the stability data. For detailed results, refer the Chemistry Support Group report.

Preparation

Mixing occurred on an as needed basis, however giving the Chemistry Support Group, Division of Biochemical Toxicology (DBT) enough time to obtain dose certification so that all mixes were within the required specifications before being shipped to the animal rooms for dosing. Refer to SOP 674.01 on pages 23 - 25 for a more detailed description of vehicle and test article mixing procedures.

Custody of the formulation dose groups were maintained by the Diet Preparation and Animal Care Support Groups by the use of delivery records, see example on page 19.

Dose Certification and Specifications

Samples of every batch mixed were submitted to the DBT Chemistry Support Group for dose certification. The samples were identified by using a NCTR generated "Sample Collection Report" with a specific experiment related number, for an example refer to page 17 and SOP 1312.02, page 28. Detailed information regarding the analysis results is provided in the DBT Chemistry Support Group's Summary Report.

The specifications for all dose groups were $\pm 10\%$ of the target dose and CV of 10% or less. All doses were delivered to the Animal Care facility once the official results were provided to the Diet Preparation work group by the Chemistry Support Group, Division of Biochemical Toxicology (DBT).

Raw Data

All raw data for the Diet Preparation of 2186, 2188 will be submitted to the study director for archival.

DBT CHEMISTRY SUPPORT PROGRAM INTRA-LABORATORY REPORT
5K96 DIET

SCR Number	Lot Number
21889900001	120417-1

Date Received: 7-MAY-2012
Date Completed: 16-OCT-12

ANALYSIS DATE	ANALYST INITIALS	ANALYSIS	RESULT
8-MAY-12	RMS	Total Fat (%)	4.8
21-MAY-12	RMS	Protein (%)	21.0
18-MAY-12	RMS	Vitamin A (ppm)	8.6
14-MAY-12	RMS	Vitamin B ₁ (ppm)	24.7
18-MAY-12	RMS	Vitamin E (ppm)	41.6
7-MAY-12	RMS	Volatiles/Moisture (%)	6.9

NUTRIENT	MDL	Minimum Acceptable Level	Maximum Acceptable Level	Rodent Diet Requirements ^a Rat
Fat	—	4.3%	—	5.00%
Protein	—	18%	—	15.00%
Vitamin A	—	10.3ppm	—	0.7ppm
Vitamin B ₁	—	—	—	4.00ppm
Vitamin E	—	50ppm	—	18.00ppm
Selenium	0.03 ppm	0.1ppm	See Below	0.150ppm

Calculated per M. Bryant e-mail from ARDL 10-15-12	}	Arsenic (ppm)	0.08
		Cadmium (ppm)	<MDL
		Lead (ppm)	0.37
		Mercury (ppm)	-----
		Selenium (ppm)	0.32
10-MAY-12	RMS	Aflatoxin B ₁ (ppb)	<MDL
10-MAY-12	RMS	Aflatoxin G ₁ (ppb)	<MDL
10-MAY-12	RMS	Aflatoxin B ₂ (ppb)	<MDL
10-MAY-12	RMS	Aflatoxin G ₂ (ppb)	<MDL
8-MAY-12	RMS	Total Fumonisin (ppb)	78
17-MAY-12	RMS	p,p'-DDT (ppb)	<MDL
17-MAY-12	RMS	Dieldrin (ppb)	<MDL
17-MAY-12	RMS	Heptachlor (ppb)	<MDL
17-MAY-12	RMS	Lindane (ppb)	<MDL
17-MAY-12	RMS	Malathion (ppb)	<MDL
17-MAY-12	RMS	PCBs (ppb)	<MDL

CONTAMINANT	MDL	MAXIMUM ACCEPTABLE LEVEL	
		Current DBT Chemistry Support Limits	May 2011 NTP Limits
Arsenic	0.03 ppm	1.0 ppm	0.50ppm
Cadmium	0.10 ppm	0.25 ppm	0.15ppm
Lead	0.20 ppm	1.50 ppm	1.00ppm
Mercury	0.09 ppm	0.10 ppm	0.05ppm
Selenium	0.03 ppm	0.65ppm	0.50ppm
Total Aflatoxins	0.1 ppb	5.0 ppb	5ppb
Total Fumonisin	20 ppb	750 ppb	None listed
Total DDT	5.0ppb	100.0ppb	30ppb
Dieldrin	5.0ppb	10.0ppb	20ppb
Heptachlor	5.0ppb	20.0ppb	20ppb
Lindane	1.0ppb	100.0ppb	20ppb
Malathion	50.0ppb	5000ppb	500ppb
PCBs	10.0ppb	50.0ppb	200ppb

- NOTES:
- 1) The MDLs for each analyte are estimated based on data available at this time.
 - 2) A designation of N.A. indicates that the sample was not analyzed for that particular analyte.
 - 3) Other analytical results that are required and NOT present in the above list are reported via an additional e-mail or other means of communication.
- a) NRC, 1995 requirements for growing rats

Daidzein Result: 0.07 ppm

Genistein Result: 0.33 ppm

DBT CHEMISTRY SUPPORT PROGRAM INTRA-LABORATORY REPORT
5K96 DIET

SCR Number	Lot Number
21889900012	12JUL11RTD1

Date Received: 6-AUG-2012
Date Completed: 16-OCT-12

ANALYSIS DATE	ANALYST INITIALS	ANALYSIS	RESULT
7-AUG-12	RMS	Total Fat (%)	5.3
10-AUG-12	RMS	Protein (%)	21.4
9-AUG-12	RMS	Vitamin A (ppm)	4.5
8-AUG-12	RMS	Vitamin B ₁ (ppm)	41.5
9-AUG-12	RMS	Vitamin E (ppm)	130.9
6-AUG-12	RMS	Volatiles/Moisture (%)	8.4

NUTRIENT	MDL	Minimum Acceptable Level	Maximum Acceptable Level	Rodent Diet Requirements ^a Rat
Fat	—	4.3%	—	5.00%
Protein	—	18%	—	15.00%
Vitamin A	—	10.3ppm	—	0.7ppm
Vitamin B ₁	—	—	—	4.00ppm
Vitamin E	—	50ppm	—	18.00ppm
Selenium	0.03 ppm	0.1 ppm	See Below	0.150ppm

Calculated per M. Bryant e-mail from ARDL 10-15-12	}	Arsenic (ppm)	0.08
		Cadmium (ppm)	<MDL
		Lead (ppm)	0.32
		Mercury (ppm)	-----
		Selenium(ppm)	0.36
7-AUG-12	RMS	Aflatoxin B ₁ (ppb)	<MDL
7-AUG-12	RMS	Aflatoxin G ₁ (ppb)	<MDL
7-AUG-12	RMS	Aflatoxin B ₂ (ppb)	<MDL
7-AUG-12	RMS	Aflatoxin G ₂ (ppb)	<MDL
6-AUG-12	RMS	Total Fumonisin (ppb)	122

CONTAMINANT	MDL	Maximum Acceptable Level	
		Current DBT Chemistry Support Limits	May 2011 NTP Limits
Arsenic	0.03 ppm	1.0 ppm	0.50ppm
Cadmium	0.10 ppm	0.25 ppm	0.15ppm
Lead	0.20 ppm	1.50 ppm	1.00ppm
Mercury	0.09 ppm	0.10 ppm	0.05ppm
Selenium	0.03 ppm	0.65ppm	0.50ppm
Total Aflatoxins	0.1 ppb	5.0 ppb	5ppb
Total Fumonisin	20 ppb	750 ppb	None listed

- NOTES: 1) The MDLs for each analyte are estimated based on data available at this time.
2) A designation of N.A. indicates that the sample was not analyzed for that particular analyte.
3) Other analytical results that are required and NOT present in the above list are reported via an additional e-mail or other means of communication.
a) NRC, 1995 requirements for growing rats

Daidzein Result: 0.01 ppm

Genistein Result: 0.03 ppm

DBT CHEMISTRY SUPPORT INTRA-LABORATORY REPORT

5K96 DIET

SCR Number	Lot Number
21889900020	12DEC06RTD1

Date Received 10-Jan-13

Date Completed 1-Aug-13

ANALYSIS DATE	Analyst INITIALS	ANALYSIS	RESULT
23-Jan	RMS	Total Fat (%)	5.6
24-Jan	RMS	Protein (%)	20.4
25-Jan	RMS	Vitamin A (ppm)	6.8
22-Jan	RMS	Vitamin B1 (ppm)	44.2
25-Jan	RMS	Vitamin E (ppm)	65.1
22-Jan	RMS	Starch/Moisture (%)	8.8

Nutrient	MDL	Minimum Acceptable Level	Maximum Acceptable Level	Rodent Diet Requirements*
				Rat
Fat	-----	4.3%	-----	5.00%
Protein	-----	18%	-----	15.00%
Vitamin A	-----	10.3 ppm	-----	0.7 ppm
Vitamin B ₁	-----	-----	-----	4.00 ppm
Vitamin E	-----	50 ppm	-----	18.00 ppm
Selenium	0.03 ppm	0.1 ppm	See below	0.150 ppm

Calculated from ARDL report received from Matthew Bryant on 7/31/13.	Arsenic (ppm)	0.09	
	Cadmium (ppm)	<MDL	
	Lead (ppm)	0.72	
	Mercury (ppm)	-----	
	Selenium (ppm)	0.37	
18-Jan	RMS	Aflatoxin B1 (ppb)	<MDL
18-Jan	RMS	Aflatoxin G1 (ppb)	<MDL
18-Jan	RMS	Aflatoxin B2 (ppb)	<MDL
18-Jan	RMS	Aflatoxin G2 (ppb)	<MDL
15-Jan	RMS	Total Fumonisin (ppb)	234

CONTAMINANT	MDL	MAXIMUM ACCEPTABLE LIMIT	
		Current DBT Chemistry Support Limits	May 2011 NTP Limits
Arsenic	0.03 ppm	1.0 ppm	0.50 ppm
Cadmium	0.10 ppm	0.25 ppm	0.15 ppm
Lead	0.20 ppm	1.5 ppm	1.00 ppm
Mercury	0.09 ppm	0.10 ppm	0.05 ppm
Selenium	0.03 ppm	0.65 ppm	0.50 ppm
Total Aflatoxins	0.1 ppb	5.0 ppb	5 ppb
Total Fumonisins	20 ppb	750 ppb	None listed

- Notes: 1) The MDLs for each analyte are estimated based on data available at this time.
 2) A designation of N.A. indicates that the sample was not analyzed for that particular analyte.
 3) Other analytical results that are required and NOT present in the above list are reported via an additional e-mail or other means of communication.
 4) NRC, 1995 requirements for growing rats

Daidzein Result: 0.014 ppm

Genistein Result: 0.024 ppm

SCR Number: 2186 0000 24

DBT CHEMISTRY SUPPORT PROGRAM INTRA-LABORATORY REPORT, 5K96 DIET

No data available for the Routine Analysis feed report, refer to the Chemistry Support Group, Division of Biochemical Toxicology (DBT) summary report for additional details.

Daidzein Result: 0.038 ppm

Genistein Result: 0.047 ppm

5K96 Diet Description

**Advanced Protocol®
Verified Casein Diet 10 IF**

5K96*

DESCRIPTION

Advanced Protocol® Verified Casein Diet 10 IF is a natural ingredient diet, formulated to be used in experimental protocols where dietary estrogenic activity is a concern. Recommended for rats, mice and hamsters, it meets the nutrient specifications as shown for NIH-31 in the 1996 update. NIH-31 is usually autoclaved, however 5K96 had some adjustments made to the vitamin content in order to compensate for the different levels in vitamin destruction when comparing irradiation and autoclaving.

Features and Benefits

- 5K96 consistently analyzes at less than 10.0 ppm total isoflavones (aglycone equivalents of genistein, daidzein and glycitein), while other natural ingredient laboratory rodent diets contain higher levels.
- Complete life-cycle diet designed to be fed free-choice.
- Available in irradiated or non-irradiated.

Product Forms Available

- Round pellets, 1 1/2" round x 3/4" long
- Meal (ground pellets)

Catalog #

55909
1810461

GUARANTEED ANALYSIS

Crude protein not less than19.0%
Crude fat not less than4.0%
Crude fiber not more than5.0%

INGREDIENTS

Ground wheat, ground corn, wheat middlings, ground oats, fish meal, casein, corn gluten meal, dicalcium phosphate, monocalcium phosphate, soybean oil, brewers dried yeast, calcium carbonate, salt, choline chloride, magnesium oxide, chromium potassium sulfate, dl-alpha tocopheryl acetate (vitamin E), manganese oxide, nicotinic acid, vitamin A acetate, calcium pantothenate, thiamin mononitrate, menadione sodium bisulfite (vitamin K), pyridoxine hydrochloride, riboflavin, cholecalciferol (vitamin D₃), cyanocobalamin (vitamin B₁₂), folic acid, biotin, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate.

FEEDING DIRECTIONS

Feed ad libitum to rodents. Plenty of fresh, clean water should be available at all times.

Verified lots have password protected isoflavone levels posted at www.labdiet.com. Contact info@labdiet.com for further information.

CHEMICAL COMPOSITION¹

Nutrients²

Protein, %	19.0
Arginine, %	0.93
Cystine, %	0.23
Glycine, %	0.82
Histidine, %	0.45
Isoleucine, %	0.91
Leucine, %	1.70
Lysine, %	0.99
Methionine, %	0.45
Phenylalanine, %	0.90
Tyrosine, %	0.60
Threonine, %	0.71
Tryptophan, %	0.22
Valine, %	1.04
Serine, %	0.94
Aspartic Acid, %	1.60
Glutamic Acid, %	4.61
Alanine, %	1.14
Proline, %	1.71
Taurine, %	0.03
Fat (ether extract), %	4.3
Fat (acid hydrolysis), %	5.3
Cholesterol, ppm	243
Linoleic Acid, %	2.00
Linolenic Acid, %	0.17
Arachidonic Acid, %	0.01
Omega-3 Fatty Acids, %	0.26
Total Saturated Fatty Acids, %	0.99
Total Monounsaturated Fatty Acids, %	1.09
Fiber (Crude), %	3.5
Neutral Detergent Fiber ³ , %	14.3
Acid Detergent Fiber ⁴ , %	4.7
Nitrogen-Free Extract (by difference), %	57.3
Starch, %	44.0
Glucose, %	0.15
Fructose, %	0.15
Sucrose, %	0.36
Lactose, %	0.00
Total Digestible Nutrients, %	75.8
Gross Energy, kcal/gm	4.06
Physiological Fuel Value⁵, kcal/gm	3.44
Metabolizable Energy, kcal/gm	3.15
Minerals	
Ash, %	5.7
Calcium, %	1.15
Phosphorus, %	0.89
Phosphorus (non-phytate), %	0.68
Potassium, %	0.43
Magnesium, %	0.19

Sulfur, %	0.17
Sodium, %	0.28
Chlorine, %	0.47
Fluorine, ppm	14
Iron, ppm	170
Zinc, ppm	86
Manganese, ppm	130
Copper, ppm	10
Cobalt, ppm	0.31
Iodine, ppm	0.88
Chromium, ppm	0.78
Selenium, ppm	0.29

Vitamins

Carotene, ppm	1.8
Vitamin K (as menadione), ppm	7.1
Thiamin Hydrochloride, ppm	24
Riboflavin, ppm	8.6
Niacin, ppm	91
Pantothenic Acid, ppm	29
Choline Chloride, ppm	1800
Folic Acid, ppm	2.7
Pyridoxine, ppm	11
Biotin, ppm	0.3
B ₁₂ , mcg/kg	43
Vitamin A, IU/gm	25
Vitamin D ₃ (added), IU/gm	2.0
Vitamin E, IU/kg	95
Ascorbic Acid, mg/gm	—

Calories provided by:

Protein, %	22.092
Fat (ether extract), %	11.339
Carbohydrates, %	66.569

***Product Code**

1. Formulation based on calculated values from the latest ingredient analysis information. Since nutrient composition of natural ingredients varies and some nutrient loss will occur due to manufacturing processes, analysis will differ accordingly.
2. Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.
3. NDF = approximately cellulose, hemicellulose and lignin.
4. ADF = approximately cellulose and lignin.
5. Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbohydrate (use Nitrogen Free Extract) x 4,9,4 kcal/gm respectively.

For ordering information, contact TestDiet® at 765-966-1885.



NIH-41 Irradiated Base Diet Microbiology Analyses

Lot #: 120417-1	
SCR #: 2188 99 00002	
Date Sampled: 05/07/12	
Results	
Feed-Bacteria Count	0 cfu/gram
Spec Bacterial Path.	Negative
Feed-Mold Count	0 cfu/gram
Spec Fungal Path.	Negative

Lot #: 12DEC06RTD1	
SCR #: 2188 99 000 21	
Date Sampled: 001/08/13	
Results	
Feed-Bacteria Count	0 cfu/gram
Spec Bacterial Path.	Negative
Feed-Mold Count	0 cfu/gram
Spec Fungal Path.	Negative

Lot #: 12JUL11RTD1	
SCR #: 2188 99 000 13	
Date Sampled: 08/03/12	
Results	
Feed-Bacteria Count	0 cfu/gram
Spec Bacterial Path.	Negative
Feed-Mold Count	0 cfu/gram
Spec Fungal Path.	Negative

Lot #: 13MAR22RTD1	
SCR #: 2186 99 000 25	
Date Sampled: 04/17/13	
Results	
Feed-Bacteria Count	0 cfu/gram
Spec Bacterial Path.	Negative
Feed-Mold Count	0 cfu/gram
Spec Fungal Path.	Negative

Oxybenzone in 5K96 meal, IR, Homogeneity

DOSE LEVEL	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
1000	OX1-10001	10/26/10	12/15/10	2178 98 0000 2	Homogeneity

DOSE LEVEL	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
50000	OX5-10001	10/26/10	12/15/10	2178 98 0000 3	Homogeneity

Data used from Experiment 2178.

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Oxybenzone in 5K96 meal, IR

DOSE LEVEL (PPM)	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
0	IRM-12001	06/06/12	10/14/13	2188 99 0000 4	Dose Cert.
0	IRM-12002	07/17/12	10/14/13	2188 99 0000 8	Dose Cert
0	IRM-12003	08/23/12	10/14/13	2188 99 000 14	Dose Cert
0	IRM-13001	01/22/13	06/04/13	2188 99 000 23	Dose Cert
0	IRM-13002	03/06/13	06/04/13	2186 98 000 15	Dose Cert
0	IRM-13003	04/01/13	06/04/13	2186 98 000 19	Dose Cert
0	IRM-13004	05/06/13	10/19/13	2186 98 000 26	Dose Cert

Diet Preparation Services Support
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Oxybenzone in 5K96 meal, IR

DOSE LEVEL (PPM)	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
3000	OX2-12001	06/06/12	07/25/12	2188 99 0000 5	Dose Cert.
3000	OX2-12002	07/18/12	09/05/13	2188 99 0000 9	Dose Cert
3000	OX2-12003	08/23/12	10/11/13	2188 99 000 15	Dose Cert
3000	OX2-13001	01/22/13	03/12/13	2186 98 0000 2	Dose Cert
3000	OX2-13002	03/06/13	04/24/13	2186 98 000 16	Dose Cert
3000	OX2-13003	04/01/13	05/20/13	2186 98 000 20	Dose Cert
3000	OX2-13004	05/06/13	06/24/13	2186 98 000 27	Dose Cert

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Oxybenzone in 5K96 meal, IR

DOSE LEVEL (PPM)	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
10000	OX3-12001	06/06/12	07/25/12	2188 99 0000 6	Dose Cert.
10000	OX3-12002	07/18/12	09/05/13	2188 99 000 10	Dose Cert
10000	OX3-12003	08/23/12	10/11/13	2188 99 000 16	Dose Cert
10000	OX3-13001	01/22/13	03/12/13	2186 98 0000 3	Dose Cert
10000	OX3-13002	03/06/13	04/24/13	2186 98 000 17	Dose Cert
10000	OX3-13003	04/01/13	05/20/13	2186 98 000 21	Dose Cert
10000	OX3-13004	05/06/13	06/24/13	2186 98 000 28	Dose Cert

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Oxybenzone in 5K96 meal, IR

DOSE LEVEL (PPM)	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
30000	OX6-12001	06/06/12	07/25/12	2188 99 0000 7	Dose Cert.
30000	OX6-12002	07/18/12	09/05/13	2188 99 000 11	Dose Cert
30000	OX6-12003	08/23/12	10/11/13	2188 99 000 17	Dose Cert
30000	OX6-13001	01/22/13	03/12/13	2186 98 0000 4	Dose Cert
30000	OX6-13002	03/06/13	04/24/13	2186 98 000 18	Dose Cert
30000	OX6-13003	04/01/13	05/20/13	2186 98 000 22	Dose Cert
30000	OX6-13004	05/06/13	06/24/13	2186 98 000 29	Dose Cert

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Ethynl Estradiol in 5K96 meal, IR

DOSE LEVEL (PPM)	BATCH #	MIX DATE	EXPIRATION DATE	CHEMISTRY SCR #	COMMENTS
0.05	EE8-12001	06/01/12	10/21/13	2188 99 0000 3	Dose Cert
0.05	EE8-12002	10/04/12	03/21/13	2188 99 000 18	Dose Cert
0.05	EE8-13001	01/17/13	07/04/13	2186 98 0000 6	Dose Cert
0.05	EE8-13002	04/01/13	09/17/13	2186 98 000 23	Dose Cert

Dosed Diet Mixing Record

Mixing Record

Experiment #: 2186-2188
 Chemical: Oxybenzone
 Batch #: OX6-13004
 Feed Type: 5K96, IR Meal
 Lot #(s): 430322-13MAR22RTD1
 Room #: 5D-159

Final Premix N/A
 Dose Level: 30,000 PPM
 Grind #: NA
 Premix #: NA
 Date Mixed: 05/06/13
 Expiration Date:

Feed 10/19/13	Compound 06/24/13
------------------	----------------------

 Mixer Type: 2.5 cuft. PK

INGREDIENTS (Attach Labels)

5K96 - IR
 Lot #: 13MAR22RTD1 20 kg
 Exp. Date: 10/19/13
 Exp. #: 2186

5K96 - IR
 Lot #: 13MAR22RTD1 20 kg
 Exp. Date: 10/19/13
 Exp. #: 2186

30,000 ppm in 5K96 - IR
 1200.0 grams Oxybenzone
 Lot #: 1F100604
 Experiment 2186-2188
 Date: 05/06/13

05-06-13

PROCESS INFORMATION

Process	Start	Stop	Mixer Type
Dry Mix	<u>7:42am</u>	<u>8:02am</u>	PK <input checked="" type="checkbox"/>
Wet Mix	NA	NA	Hobart <input type="checkbox"/>
Stock Solution	NA	NA	Marion <input type="checkbox"/>
Drying	NA	NA	Other <input type="checkbox"/>

Activate	Yes	No	Activate	Yes	No
Intensifier Bar	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Chiller	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Heater	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Blow Back	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Circulator	<input type="checkbox"/>	<input checked="" type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

SAMPLE INFORMATION


Micro	Chem	Date	SCR #	Clear	Rpt	Date	Init
<input type="checkbox"/>	<input checked="" type="checkbox"/>	05/06/13	<u>218698 00029</u>	<u>clean</u>	<u>99.0</u>	<u>05-08-13</u>	<input checked="" type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>						
<input type="checkbox"/>	<input type="checkbox"/>						
<input type="checkbox"/>	<input type="checkbox"/>						
<input type="checkbox"/>	<input type="checkbox"/>						

Additional Info. Mix for 20 minutes.

1 top, 1 middle, 1 bottom

	DATE
Technician <u>NA</u>	<u>NA</u>
Technician <u>[Redacted]</u>	<u>05-03-13</u>
Technician <u>[Redacted]</u>	<u>5-3-13</u>
Supervisor <u>[Redacted]</u>	<u>05-03-13</u>
Quality Control <u>[Redacted]</u>	<u>1-26-15</u>

NCTR Sample Collection Report

SAMPLE COLLECTION REPORT		<p>NCTR Sample Number Exp / Test 2186 98 Sequence No 000 29</p> 
Initial: _____	Date: <u>05/06/13</u>	
Date Sampled: _____	<u>05/06/13</u>	
Building: <u>5D</u> Room: <u>159</u>		
Sample Type: _____	<u>Feed</u>	
Sub-Type: <u>Dosed</u>		
<input type="checkbox"/> Microbiology	<input checked="" type="checkbox"/> Chemistry	<input type="checkbox"/> Pathology
Species _____ Animal AIN, UIN, or Tattoo: _____		
Strain Code: _____ Sex: _____ Date of Birth ____/____/____		
Treatment: _____ Ear Clip: _____ MM DD YY		
ISOL # _____ Rack # _____ # of Cages _____ # Samples Composited _____		
Cage # (s) _____		
# of Indicators _____ Autoclave # _____ Temp. F _____ # of Swabs _____		
Material Type: <u>5K96</u>	# Samples Composited: <u>03</u>	
Dose Code: <u>OX6</u>	Dose Level: <u>30,000 PPM</u>	
Pasteurization Run #: <u>NA</u>		
Bedding Shipment Lot #: <u>NA</u>		
Feed Shipment Lot # <u>13MAR22RTD1</u>		
Process ID #: <u>OX6-13004</u>		
Additional Information: 5K96 Meal Irradiated, 30,000 PPM, Oxybenzone, Experiment #: 2186 Dose Cert.		

Packaging Record

PACKAGING RECORD - EXPERIMENT #: 2186																																																																											
Date Packaged: 05-03-13								OXE-13011																																																																			
CARRYING CASE LABELS ORDERED					PACKAGING TECHNICIANS																																																																						
Exp. #: 2186	DOSE CODES	EXP. DATE	LOT #		<div style="display: flex; justify-content: space-between;"> <div style="width: 60%; border-bottom: 1px solid black; margin-bottom: 5px;"></div> <div style="width: 35%; text-align: right;">05-03-13</div> </div> <div style="display: flex; justify-content: space-between; margin-bottom: 5px;"> <div style="width: 60%; border-bottom: 1px solid black;"></div> <div style="width: 35%; text-align: right;">5-3-13</div> </div> <div style="display: flex; justify-content: space-between; margin-bottom: 5px;"> <div style="width: 60%; border-bottom: 1px solid black;"></div> <div style="width: 35%; text-align: right;">5-3-13</div> </div> <div style="display: flex; justify-content: space-between;"> <div style="width: 60%; text-align: center;">Signature</div> <div style="width: 35%; text-align: center;">Date</div> </div>																																																																						
	IRM	10/19/13	13MAR22RTD1																																																																								
	OX2	06/24/13	13MAR22RTD1																																																																								
	OX3	06/24/13	13MAR22RTD1																																																																								
	OX6	06/24/13	13MAR22RTD1																																																																								
	EE8	06/24/13	13MAR22RTD1																																																																								
COMMENTS					SOURCES																																																																						
Diet Type: Dosed 5K96 Irradiated Clean-up Solution: Hot Water/190 proof Ethyl Alcohol Case Type: Metal Case (RAT) Chemical: Oxybenzone / EE2 <div style="text-align: right; margin-top: 20px;">05-03-13</div>					FINAL DIET(S)	AMOUNT (KG)	ACTUAL (KG)	RETURN AMOUNT (KG)																																																																			
					IRM-13004	12.00	27.76	27.54																																																																			
					OX2-13004	12.00	27.96	27.86																																																																			
					OX3-13004	12.00	28.20	28.08																																																																			
					OX6-13004	12.00	29.24	29.01																																																																			
		EE8-13002	12.00	48.03	47.94																																																																						
					Total (KG) 60.00																																																																						
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th colspan="6">Experiment #: 2186-2488 - Rats</th> <th colspan="4">5K96 Irradiated Meal</th> </tr> <tr> <th>DOSE LEVEL</th> <th># OF CASES</th> <th># OF FEEDERS</th> <th>FEEDER TYPE</th> <th>PACKAGED AMOUNT (g)</th> <th>DATE DELIVERED</th> <th>BLDG./ RM #</th> <th>DOSE LEVEL</th> <th># OF CASES</th> <th># OF FEEDERS</th> <th>FEEDER TYPE</th> <th>PACKAGED AMOUNT (g)</th> <th>DATE DELIVERED</th> <th>BLDG./ RM #</th> </tr> </thead> <tbody> <tr> <td>0 PPM</td> <td>4</td> <td>48</td> <td>RAT</td> <td>250</td> <td>05-03-13</td> <td>53A-116</td> <td colspan="7" rowspan="5" style="text-align: center; vertical-align: middle; font-size: 4em;">/</td> </tr> <tr> <td>3,000 PPM</td> <td>4</td> <td>48</td> <td>RAT</td> <td>250</td> <td>05-03-13</td> <td>53A-116</td> </tr> <tr> <td>10,000 PPM</td> <td>4</td> <td>48</td> <td>RAT</td> <td>250</td> <td>05-03-13</td> <td>53A-116</td> </tr> <tr> <td>30,000 PPM</td> <td>4</td> <td>48</td> <td>RAT</td> <td>250</td> <td>05-03-13</td> <td>53A-116</td> </tr> <tr> <td>0.05 PPM</td> <td>4</td> <td>48</td> <td>RAT</td> <td>250</td> <td>05-03-13</td> <td>53A-116</td> </tr> </tbody> </table>										Experiment #: 2186-2488 - Rats						5K96 Irradiated Meal				DOSE LEVEL	# OF CASES	# OF FEEDERS	FEEDER TYPE	PACKAGED AMOUNT (g)	DATE DELIVERED	BLDG./ RM #	DOSE LEVEL	# OF CASES	# OF FEEDERS	FEEDER TYPE	PACKAGED AMOUNT (g)	DATE DELIVERED	BLDG./ RM #	0 PPM	4	48	RAT	250	05-03-13	53A-116	/							3,000 PPM	4	48	RAT	250	05-03-13	53A-116	10,000 PPM	4	48	RAT	250	05-03-13	53A-116	30,000 PPM	4	48	RAT	250	05-03-13	53A-116	0.05 PPM	4	48	RAT	250	05-03-13	53A-116
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										QC Signature		Date																																																															
												05-03-13																																																															



Delivery Record

Diet Preparation Delivery Record
NCTR Jefferson, Arkansas

Experiment #: 2186
Diet Type: 5K96 IRRADIATED / Oxybenzone / EE₂

Delivery

Diet Prep:
Recipient:

Delivery Date: 2-8-13

BLDG/ROOM #	Lot #	Quantity and Description	Total Packages
53A-116	12DEC06RTD1	0 PPM OXYBENZONE in 5K96-IR OXE-13002 Expires: 06/04/13 2 cases packaged @ 250 grams IRM	2
		3,000 PPM OXYBENZONE in 5K96-IR OXE-13002 Expires: 03/12/13 2 cases packaged @ 250 grams OX2	2
		10,000 PPM OXYBENZONE in 5K96-IR OXE-13002 Expires: 03/12/13 2 cases packaged @ 250 grams OX3	2
		30,000 PPM OXYBENZONE in 5K96-IR OXE-13002 Expires: 03/12/13 2 cases packaged @ 250 grams OX6	2
		0.05 PPM EE ₂ in 5K96-IR OXE-13002 Expires: 06/04/13 2 cases packaged @ 250 grams EE8	2

TS: Patrick Clayton [REDACTED]

Store at 2° - 8°C

QC Signature: [REDACTED]

DATE: 02-07-13

Verified By: [REDACTED]

DATE: 02-08-13

Delivered By: [REDACTED]

DATE: 2-8-13

Received By: [REDACTED]

DATE: 2-8-13

Appendix Table of Contents

SOP # 203.12	21, 22
SOP # 674.01	23-25
SOP # 1006.09	26, 27
SOP # 1312.02	28

Diet Preparation Services Support
NCTR Experiment NCTR Experiment 2186, 2188

SOP # 203.12
REPLACES SOP # 203.11
EFFECTIVE DATE: 2-24-12
PAGE 1 OF 2

APPROVED BY:

	2-24-12
QC/Safety	Date:
	02/23/12
Diet Preparation, Manager	Date Approved:

**THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
DIET PREPARATION
STANDARD OPERATING PROCEDURE**

TITLE: RECEIPT AND STORAGE OF INCOMING FEED SHIPMENTS MANUALLY
OR BY USING FORK LIFT.

DIRECTIONS:

Manual System:

1. Upon receipt of feed from the manufacturer, notify the Diet Preparation Management, or responsible personnel.
2. Obtain empty pallets for feed storage. Follow these steps when unloading truck manually:
 - A. Place feed bags on pallets, with the manufacture date or tag visible.
 - B. Stack on pallets, in an overlapping and crosswise manner, a maximum of 75 bags and 27 boxes per pallet.
 - C. Reject torn, wet, moldy or otherwise unacceptable bags.
3. Stack the feed in a manner in which the oldest dated feed is rotated forward and utilized first.
4. After feed bags are stacked and inventory is complete, place placards on all newly stacked pallets with the following information:
 - A. Feed type.
 - B. Lot number.
 - C. Manufacture Date.
 - D. Expiration Date.

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP # 203.12
REPLACES SOP # 203.11
EFFECTIVE DATE: 2-24-12
PAGE 2 OF 2

Fork Lift System:

1. Upon receipt of feed from the manufacturer, notify the Diet Preparation Management, or responsible personnel.
2. Make sure fork lift is properly charged before attempting to unload feed, if fork lift is not charged, use pallet jack instead, it is very important the fork lift does not run out of charge while inside the feed truck trailer.
3. Once the feed truck is safely backed up to loading dock with wheels chocked and the fork lift slip is in place, use the fork lift to retrieve pallets of feed from the truck and transfer them to the feed storage room (5D-112) and place on shelving system. Feed pallets maybe placed in room 5D-102 temporarily for sampling or organizational purposes.
4. After feed pallets are neatly stacked and inventory is complete, notify QC Safety so they may place placards on all newly stacked pallets with a minimum of the following information:
 - A. Feed type.
 - B. Lot number.
 - C. Expiration Date.
 - D. Additional information or comments pertinent to the shipment.
5. QC/Safety will complete a "Feed Inventory Record" for each lot of feed received.

Document History

Rev. #	Change	Reason
12	Reformat	Clarity, new SOP requirements

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP #: 674.01
REPLACES: 674.00
EFFECTIVE DATE: 9-26-12
PAGE 1 OF 3

APPROVED BY:

[Redacted Signature]	9-26-12
QC/Safety	Date:
[Redacted Signature]	09-26-12
Diet Preparation Manager	Date Approved:

**THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
DIET PREPARATION
STANDARD OPERATING PROCEDURE**

TITLE: PREPARATION OF OXYBENZONE / Ethinyl Estradiol (EE₂) DOSED DIETS FOR EXPERIMENT 2186 - 2188.

DIRECTIONS: Upon receipt, the NCTR Regulatory Compliance & Risk Management Office will transfer the Oxybenzone to the Diet Preparation support unit, where all accountability records will be documented and maintained. The Ethinyl estradiol is in custody of the DBT, Chemistry support group.

NOTE: When preparing Oxybenzone and Ethinyl estradiol dosed diets, the following PPE will be worn: Diet Preparation dress attire (scrubs), tyvex suit, fitted dust respirator, surgical gloves, safety shoes, and safety glasses

Oxybenzone Mixing

1. Perform blender clean-up prior to mixing.

Group #	Treatment	Dose Code	Dose Level (PPM)
1	Oxybenzone	IRM	0
2	Oxybenzone	OX2	3000
3	Oxybenzone	OX3	10,000
4	Oxybenzone	OX6	30,000
5	Ethinyl estradiol	EE8	0.05

3. Tare stainless steel feed cans to zero on the scale and weigh out the required amount of 5K96 IR Meal. There is a table on the back of the mixing record to verify that the required amount of feed is added to the mixer. The lot number and feed amount (kg) will be written in the space provided along with initials. Also, the actual amount of feed will be verified by assisting personnel, initialed and dated.

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP #: 674.01
REPLACES: 674.00
EFFECTIVE DATE: 9-26-12
PAGE 2 OF 3

mortar/pestle the feed and Oxybenzone until a whitish colored meal is present in the mortar. Repeat this procedure until all the required amount of Oxybenzone is finely ground into the irradiated meal diet. Secure lid on top of jar and proceed to shake and roll jar for approximately two minutes.

5. With the discharge cover plate in place, add approximately half the 5K96 IR Meal to the Patterson Kelley blender, then add the Oxybenzone premix. Rinse the jar at least two times with 5K96 IR Meal, add the remaining 5K96 IR Meal to the mixer.
6. Secure access cover plates, place screen guard in front of mixer, turn on blender shell and intensifier bar. Blend for required time according to the mixing record with intensifier bar running.
7. After blending feed for required time, turn off blender and intensifier bar. Position blender in upright position and remove cover plates.
8. Remove discharge cover plate. Place an empty feed can under discharge.
9. Process samples and discharge feed into labeled cans with the minimum following information:

Test Article and Dose Level
Batch Number
Lot Number
Expiration Date
Experiment Number
Feed Type and Temperature Storage Requirement
10. Secure can lids with tie downs and place in storage.
11. Complete mixing record.
12. Perform blender clean up and return all records to the office for posting.

Ethinyl Estradiol Diet Mixing

1. Perform blender clean-up prior to mixing diets (see attachment).
2. The DBT Chemistry support unit will weigh the Ethinyl Estradiol and blend it with the 95% ETOH as a carrier to prepare the 0.05 ppm.
3. With the discharge cover plate in place, add the required amount of 5K96, IR Meal to the Patterson Kelley, (PK) blender.
5. Secure access cover plates, place screen guard in front of mixer, turn on blender shell, intensifier bar and vacuum. When vacuum has reached at least 15 Hg, direct inject *the*

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP #: 674.01
REPLACES: 674.00
EFFECTIVE DATE: 9-26-12
PAGE 3 OF 3

0.05 ppm Ethinyl Estradiol/95% ETOH Solution. Rinse with ~500 ml of 95% ETOH. Blend for ~60 minutes with intensifier bar and vacuum running.

6. After blending feed for ~60 minutes turn off blender, intensifier bar, and release vacuum pressure. Position blender in upright position and remove cover plates.
7. Remove discharge cover plate. Place an empty feed can under discharge.
8. Process samples and discharge feed into labeled cans with the minimum following information:

Test Article and Dose Level
Batch Number
Lot Number
Expiration Date
Experiment Number
Feed Type and Temperature Storage Requirement
9. Secure can lids with tie downs and place in storage.
10. Complete mixing record.
11. Perform blender clean up and return all records to the office for posting.

Document History

Rev. #	Change	Reason
.00	.01	Correct dose codes as per QC audit.

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP # 1006.09
REPLACES SOP# 1006.08
EFFECTIVE DATE: 2-24-12
PAGE 1 OF 2

APPROVED BY: *[Signature]*

<i>[Redacted]</i>	2-24-12
QC/Safety	Date:
<i>[Redacted]</i>	02/23/12
Diet Preparation, Manager	Date Approved:

THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
DIET PREPARATION
STANDARD OPERATING PROCEDURE

TITLE: QUALITY CONTROL ROUTINE CHEMICAL AND MICROBIOLOGICAL
FEED SAMPLING

DIRECTIONS:

Commercial Manufactured Feed Received

Diet Preparation receives a variety of feeds; NIH-41 Irradiated Pellets, Jumbo Monkey Chow, Primate Banana Pellets, and other certain specialty diets, usually ordered by Study Directors.

NOTE: Take a microbiological samples first if the same packaging container is going to be sampled for both chemistry and microbiology.

NCTR Standard Irradiated Diet

Chemical Sampling

Frequency

1. Sample each lot and insure sample is analyzed for Organophosphates and chlorinated insecticides. (Routine Assay).

Routine Sampling Method of Each Lot Number

1. Randomly select bags to be sampled. Number of bags sampled is equal to the square root of the total number of bags received per lot.
2. Cut open the center of each bag. Remove a sample amount approximately 100 grams and place in a labeled sample container.
3. Sample label should indicate feed type, lot number, sample number, and sample date.

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP # 1006.09
REPLACES SOP# 1006.08
EFFECTIVE DATE: 2-24-12
PAGE 2 OF 2

4. Tape bags shut and complete SCR. Refer to SOP #1312

Microbiological Sampling

Frequency

1. Sample as necessary or required.

Routine Sampling Method of Each Lot Number

1. Randomly select a bag or box to be sampled.
2. Lightly spray with 5% bleach solution, cut open the bag or box with a 6 inch incision towards top of the bag. Sample feed and place in a labeled sample specimen cup.
3. Label sample cup indicating feed type, lot number, sample number, and sample date.
4. Tape bag and box shut and complete SCR. Refer to SOP #1312.

Document History

Rev. #	Change	Reason
09	Reformat, revise	Clarity, new SOP requirements.

Diet Preparation Services Support
NCTR Experiment 2186, 2188

SOP # 1312.02
REPLACES SOP # 1312.01
EFFECTIVE DATE: 2-24-12
PAGE 1 OF 1

APPROVED BY:

	2-24-12
QC/Safety	Date:
	02/23/17
Diet Preparation, Manager	Date Approved:

**NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
DIET PREPARATION
STANDARD OPERATING PROCEDURE**

TITLE: SAMPLE COLLECTION RECORD (SCR)

DIRECTIONS:

1. Record required information on a SCR record
 - A. Initials of person completing the SCR sheet.
 - B. Sample submission date.
 - C. Sample collection date.
 - D. Sample collection location.
 - E. Sample type (feed, surface swabs, air, water, etc.).
 - F. Sample sub-type (autoclaved, irradiated, shipment lot, processed, other).
 - G. Check "Chemistry" or "Microbiology" on SCR sheet.
 - H. Fill in appropriate information in the lower sections of the SCR.
2. After completion of the SCR record information, attach identical numerical label to sample.
3. Record the SCR number on appropriate Diet Preparation record.
4. After recording the SCR number, transport sample(s) and SCR record(s) to the appropriate NCTR department.

Document History

Rev. #	Change	Reason
02	Reformat, revise	Clarity, new SOP requirements.