

**Nutrient and Contaminant Levels in  
LabDiet<sup>®</sup> Certified Laboratory Animal Feeds**

*Based on data compiled from 1993-2007 by Purina<sup>®</sup> LabDiet<sup>®</sup>  
and in conjunction with an independent laboratory.*

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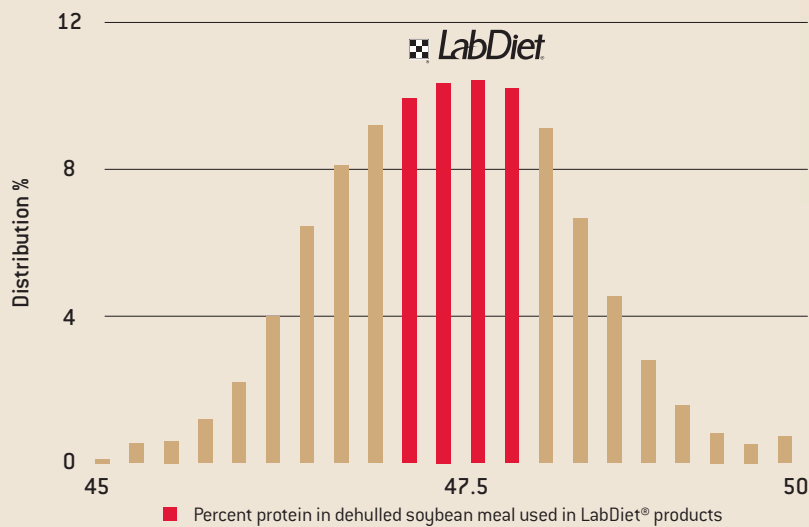
## Quality: Consistency and Control

The integrity of your work is dependent upon controlled experimental protocols. Our responsibility is to provide nutrition, through our Constant Nutrition® program, that enables you to isolate, distinguish, and define breakthroughs. The entire manufacturing process, from ingredient selection to date-coding of packaged products, is designed to deliver the most consistent nutritional diets available. Ingredients are purchased through approved suppliers who understand and adhere to the demands of research diets.

Computerized formulation and near-infrared spectroscopy allows PMI Nutrition International to maintain the most stringent feed ingredient standards in the industry. Ingredients are analyzed daily for nutritional content as well as non-nutritional contaminants. LabDiet® products are then monitored before approval and release to ensure that quality standards are met. As a result, Certified LabDiet® products provide a managed formulation program, Constant Nutrition® program, that enables you to conduct accurate research by minimizing nutrient variability that can sometimes be associated with fixed formulation diets.



Average Protein Content (%) of Dehulled Soybean Meal



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## Introduction

Animal diets used in laboratory studies must provide consistent and proper nutrition.<sup>1</sup> To assist in experimental control, feed manufacturers must ensure that all batches of feed meet approved nutritional profiles.<sup>2</sup> Certified diets for laboratory animals are commonly used in toxicological studies where control of contaminants, such as pesticides and heavy metals, is imperative. Certified LabDiet® products manufactured by PMI are analyzed prior to sale and guaranteed not to exceed established maximum concentrations of key contaminants. PMI controls variables that could adversely affect study animals and possibly lead to inaccurate conclusions. Certified LabDiet® products meet the recommendations of the FDA's Good Laboratory Practices Program.

This report is an update to our 2004 published bulletin "*Nutrient and Contaminant Levels in LabDiet® Certified Laboratory Animal Feeds: 10-Year Technical Bulletin*" which reports the results of our ongoing efforts to provide diets that are consistent from batch to batch as well as year to year. The data reported in this bulletin includes that from 1993 to 2002 and now 2003 to 2007.



## Constant Nutrition® You Can Count On

PMI's manufacturing process for LabDiet® products ensures consistent levels of nutrients and guarantees minimal concentrations of various contaminants. The Constant Nutrition® program, a managed formulation program exclusive to LabDiet®, ensures a consistent level of nutrients by accounting for biological variation of natural feedstuffs versus fixed formulations, which does not account for that inherent variation resulting in variable nutrients from batch to batch. Therefore, managed formulation requires ingredients be assayed daily and formulations refined to ensure constant nutrient delivery. Alternatively fixed formulation does not consider nutrient variation, thus assays may not be conducted to account for nutrient variability.

Manufactured for a wide variety of animals, key nutrients of LabDiet® products such as protein, fat, crude fiber, calcium, phosphorus, and vitamin C (in monkey and guinea pig rations only) are maintained within established ranges. Concentrations of 30 contaminants are maintained below established levels. Certified LabDiet® products are a key component of animal research studies. As a result, it is essential we control unwanted nutrient- and contaminant-induced variables.

Large discoveries are often built upon data generated over years of research. LabDiet® products have played an important role in building solid baseline data for many research areas.



Combined data from 1993 to 2007 demonstrate that the nutritional content and contaminant levels are well controlled in Certified LabDiet® products manufactured using the Constant Nutrition® program by PMI. Fixed formulation is most appropriately reserved for the equine and pet food industries. It can result in unknown, and sometimes radically variable, nutrient levels as demonstrated in the adjacent table.

PMI's Constant Nutrition® program, driven by our managed formulation philosophy, helps to deliver a consistent nutritional package so that researchers can succeed with reaching their goals.

*Based on a database maintained by PMI®, the following example shows how widely protein, alone, can vary in a fixed formula:*

Fixed-Formula Diet		The ingredients could conceivably contain the following protein levels (%):		
	%		Low	High
<b>Corn</b>	30.0	<b>Corn</b>	6.5	10.0
<b>Wheat</b>	17.0	<b>Wheat</b>	9.0	14.0
<b>Soybean Meal</b>	15.0	<b>Soybean Meal</b>	45.0	50.0
<b>Midds</b>	6.0	<b>Midds</b>	14.5	17.0
<b>Fish</b>	4.0	<b>Fish</b>	60.0	60.0
<b>Oats</b>	4.0	<b>Oats</b>	12.0	12.0
<b>Alfalfa</b>	3.0	<b>Alfalfa</b>	17.0	17.0
<b>Misc</b>	21.0	<b>Misc</b>	–	–
	<b>100.0</b>	<b>Calculated protein level in formula above:</b>	<b>14.5</b>	<b>17.3</b>

## Materials and Methods

In order to measure nutritional consistency and derivation of limits of our diets, periodic samples of the following Certified LabDiet® products have been taken over the past 15 years: Certified Rodent Diet 5002, Certified Canine Diet 5007, Certified Guinea Pig Diet 5026, Certified Primate Diet 5048, and Certified Rabbit Diet 5322.

Representative feed samples were sent to an independent laboratory for analysis.\* Samples were assayed for proximate constants, essential amino acids, vitamins, and macro and trace minerals. The analytical procedures employed were specified by the Association of Analytical Chemists or were procedures that provide equivalent results. The average analyzed values for nutrients were compared with calculated formulation values. The standard deviation of the analysis for each nutrient was tabulated.



\*Nestle-Purina Laboratories, 801 Chouteau Avenue, St. Louis, Missouri 63102.

# Results

## Protein/Amino Acids

The calculated and analyzed crude protein concentrations of Certified Diets from our 10-year bulletin as well as those collected in the past 5 years are provided in Table 1A. Analyzed concentrations of crude protein tend to be slightly greater than that of the calculated values. The consistency of crude protein in certified diets is evident based on the data collected for the past 15 years. Chart 1B indicates the consistency of protein in 5002 during the same period. Ingredients are analyzed daily for crude protein using NIR to ensure our formulations and finished products never fall below the level guaranteed by the tag.

Proteins are composed of amino acids. Laboratory animals have specific dietary requirements for numerous amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. All of these must be in sufficient concentrations in the diet to ensure proper animal performance (growth, gestation and lactation). Because lysine and methionine are rate limiting amino acids, their concentrations over time are exhibited in Tables 1C and 1D. Similar to the first 10 years, data collected in the past 5 years illustrates a very consistent product from batch to batch.

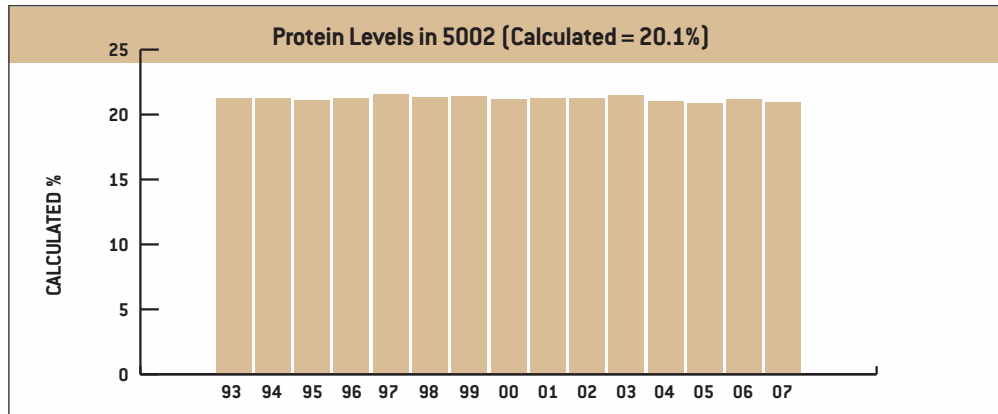


**Table 1A.**  
Protein Levels in Certified LabDiet Products Over 15-year Period

10- and 15-Year Average Protein Levels (%) in Certified LabDiets®						
DIET	Calculated Value %	# Samples 10 Years	10-Year Average	# Samples 15 Years	15-Year Average	2003-2007 3rd 5-year Average
Certified Rodent Diet 5002	20.10	30	21.29+/-0.41	47	21.23+/-0.40	21.09+/-0.35
Certified Canine Diet 5007	25.50	30	26.82+/-0.57	47	26.70+/-0.57	26.39+/-0.45
Certified Guinea Pig Diet 5026	18.50	29	20.13+/-0.47	47	20.07+/-0.51	19.80+/-0.59
Certified Primate Diet 5048	25.00	29	26.96+/-0.61	47	26.81+/-0.61	26.55+/-0.51
Certified Rabbit Diet 5322	16.20	29	17.48+/-0.68	47	17.36+/-0.64	17.08+/-0.41

+/- Standard Deviation

**Chart 1B.**  
Protein Levels in Certified Rodent Diet 5002 Over 15-Year Period



**Table 1C.**  
**Lysine Levels in**  
**Certified LabDiet**  
**Products Over a**  
**15-year Period**

Lysine % in Certified LabDiets®						
DIET	Calculated Value %	# Samples 10 Years	10-Year Average	# Samples 15 Years	15-Year Average	2003-2007 3rd 5-year Average
Certified Rodent Diet 5002	1.20	30	1.15+/-0.06	47	1.16+/-0.05	1.16+/-0.04
Certified Canine Diet 5007	1.10	30	1.24+/-0.07	47	1.25+/-0.06	1.26+/-0.06
Certified Guinea Pig Diet 5026	1.00	29	1.03+/-0.13	47	1.04+/-0.11	1.04+/-0.06
Certified Primate Diet 5048	1.20	29	1.21+/-0.56	47	1.22+/-0.05	1.22+/-0.04
Certified Rabbit Diet 5322	0.80	29	0.88+/-0.06	47	0.87+/-0.05	0.86+/-0.04

+/- Standard Deviation

**Table 1D.**  
**Methionine Levels in**  
**Certified LabDiet**  
**Products Over a**  
**15-year Period**

Methionine % in Certified LabDiets®						
DIET	Calculated Value %	# Samples 10 Years	10-Year Average	# Samples 15 Years	15-Year Average	2003-2007 3rd 5-year Average
Certified Rodent Diet 5002	0.40	30	0.41+/-0.02	47	0.42+/-0.02	0.43+/-0.02
Certified Canine Diet 5007	0.40	30	0.41+/-0.03	47	0.42+/-0.03	0.43+/-0.03
Certified Guinea Pig Diet 5026	0.40	29	0.39+/-0.07	47	0.38+/-0.05	0.38+/-0.02
Certified Primate Diet 5048	0.50	29	0.47+/-0.02	47	0.47+/-0.03	0.49+/-0.03
Certified Rabbit Diet 5322	0.40	29	0.34+/-0.02	47	0.34+/-0.02	0.35+/-0.03

+/- Standard Deviation



## Fat

Fat was analyzed using 2 different procedures: ether extract and acid hydrolysis. Ether extraction of fat measures neutral fat and is an appropriate assay for most feeds. However, fat in extruded animal foods, such as canine and primate diets, must be hydrolyzed with dilute acid (acid hydrolysis) to free the fat molecules, which is then extracted and measured. Acid hydrolysis of fat also removes bound fat from ingredients, along with other forms of lipids (i.e. phospholipids). Consequently, fat measured by acid hydrolysis is generally 1 or 2 percentage units greater than fat measured by ether extract.

Calculated and analyzed crude fat levels for LabDiet® 5002 and the extruded primate diet, LabDiet® 5048, are provided in Table 2. Over time, crude fat concentrations have remained relatively constant and tend to be similar to calculated values. Consistent dietary fat is important to maintain steady energy levels and feed consumption.

You may note that calculated fat (ether extract) values do not account for fat bound by the extrusion process. Thus, the analyzed values, by ether extract, for extruded products (Canine, Feline, and Primate) will be less than the calculated values. Although one extraction process works better for various diet forms (pelleted vs. extruded), we continue to use both extraction methods when analyzing for crude fat regardless of form.

**Table 2.**  
**Fat Levels in Certified Rodent Diet 5002 and Certified Primate Diet 5048 Products Over 15-Year Period**

	Certified Rodent Diet 5002		Certified Primate Diet 5048	
	Fat E.E., % <sup>1</sup>	Fat A.H., % <sup>2</sup>	Fat E.E., % <sup>1</sup>	Fat A.H., % <sup>2</sup>
<b>10-Year Average</b>	4.61+/-0.45	5.78+/-0.25	3.45+/-0.71	6.35+/-0.62
<b>2003</b>	4.67+/-0.35	5.71+/-0.25	3.69+/-0.20	6.52+/-0.44
<b>2004</b>	4.74+/-0.10	5.61+/-0.29	3.06+/-0.10	6.19+/-0.48
<b>2005</b>	4.46+/-0.10	5.60+/-0.26	4.04+/-0.10	6.23+/-0.80
<b>2006</b>	4.75+/-0.05	5.54+/-0.23	2.86+/-0.21	6.01+/-0.74
<b>2007</b>	5.08+/-0.34	5.85+/-0.27	3.62+/-0.28	5.97+/-0.28
<b>15-Year Average</b>	4.65+/-0.40	5.74+/-0.27	3.60+/-1.13	6.29+/-0.63
<b>Calculated Value, %</b>	<b>4.50</b>	<b>5.10</b>	<b>4.70</b>	<b>5.60</b>
<sup>1</sup> Ether Extract <sup>2</sup> Acid Hydrolysis    +/- Standard Deviation				



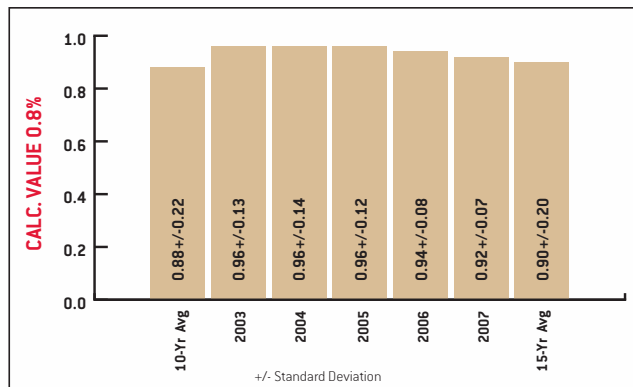


## Minerals – Calcium/Phosphorus

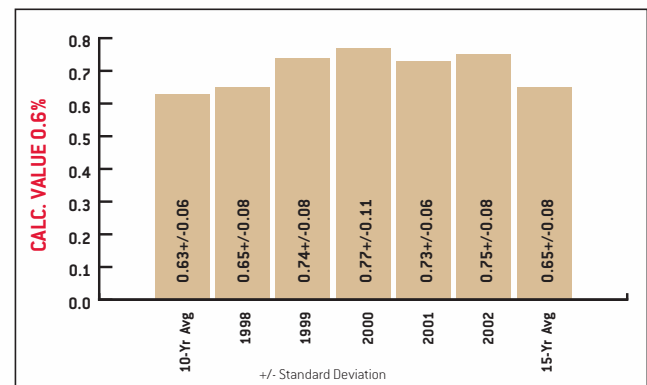
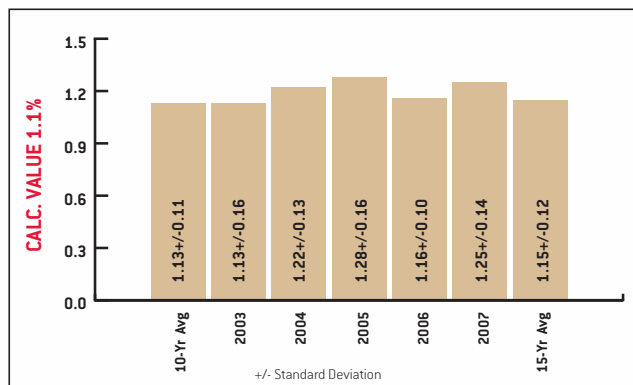
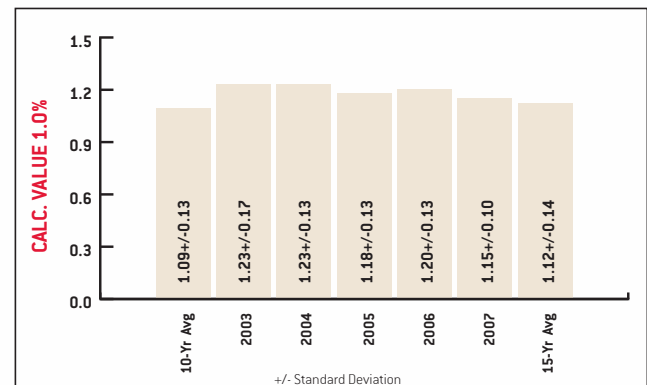
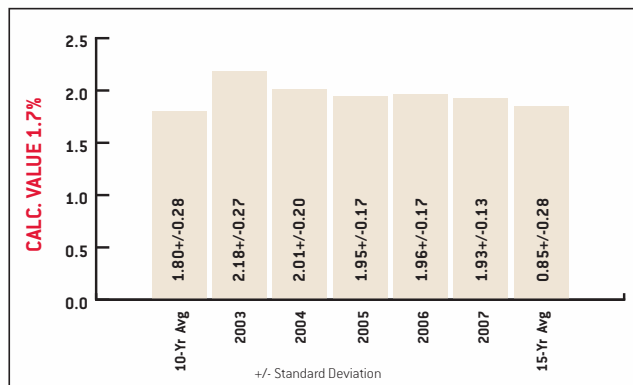
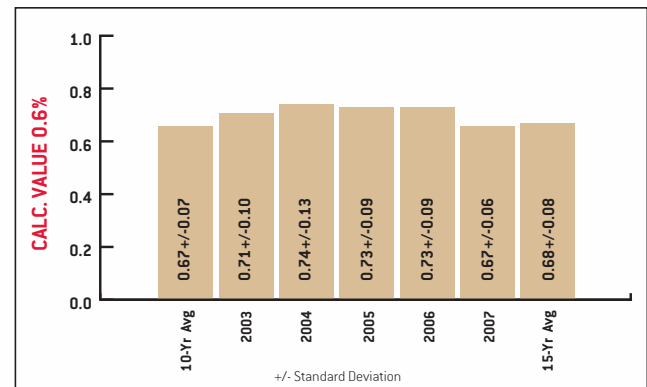
Calcium and phosphorus are the primary macro minerals in mammals and are necessary for maintenance of bone, teeth, nerve function, energy, metabolism and milk production. Minerals are also cofactors for various enzymes. Calcium and phosphorus must be delivered to laboratory animals at proper dietary concentrations with a calcium:phosphorus ratio maintained at 1:1 or greater.

Observed mineral concentrations are provided in Charts 3A and 3B for diets 5002, 5007 and 5026. Analyzed values for calcium and phosphorus for the past 5 years are consistent with values tabulated and published in the *10-Year Technical Bulletin* further demonstrating nutrient consistency due to Constant Nutrition® formulation.

**Chart 3A.**  
**Calcium Levels (%) in Certified LabDiet® Products Over 15-Year Period**



**Chart 3B.**  
**Phosphorus Levels (%) in Certified LabDiet® Products Over 15-Year Period**



## Minerals – Calcium/Phosphorus (cont.)

Chart 3A.  
Calcium Levels (%) in Certified LabDiet® Products Over 15-Year Period

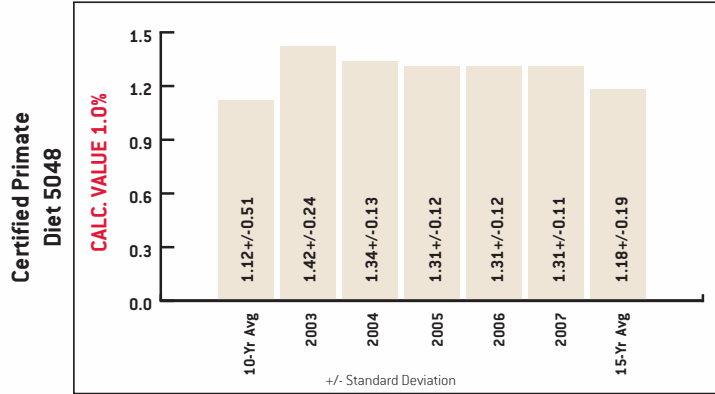
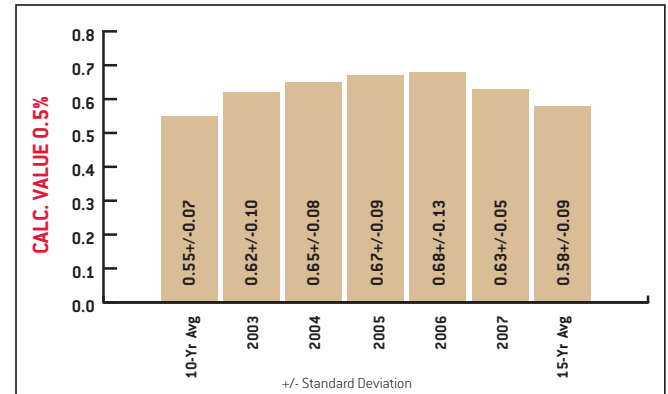
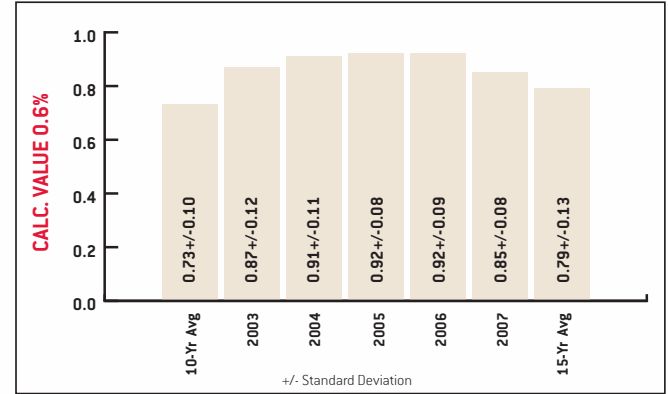


Chart 3B.  
Phosphorus Levels (%) in Certified LabDiet® Products Over 15-Year Period



## Vitamins

Riboflavin, pantothenic acid, and vitamin B<sub>12</sub> analyzed in Certified LabDiet® products are provided in Tables 4A, 4B and 4C, respectively. Values tabulated during the first 10 years of our collection period are consistently similar to those collected within the past 5 years. Some variation has been observed when comparing calculated values to analyzed values. Analytical and sampling variation may explain some of the discrepancies seen between these values.

### Vitamin Levels in Certified LabDiet Products Over 15-Year Period

**Table 4A.**  
Riboflavin

Riboflavin Levels (PPM)			
DIET	Calculated Value	10-Year Average	15-Year Average
5002	8.00	7.80+/-1.49	7.81+/-1.30
5007	4.50	4.61+/-0.84	4.70+/-0.89
5026	6.00	6.63+/-1.53	6.76+/-1.47
5048	8.50	8.44+/-1.79	8.55+/-1.62
5322	5.00	6.44+/-1.81	6.49+/-1.53
+/- Standard Deviation			

**Table 4B.**  
Pantothenic Acid

Pantothenic Acid Levels (PPM)			
DIET	Calculated Value	10-Year Average	15-Year Average
5002	17.00	17.19+/-1.97	17.08+/-1.30
5007	20.00	20.73+/-2.30	20.81+/-3.70
5026	19.00	21.50+/-1.82	21.61+/-3.27
5048	50.00	56.34+/-4.67	55.95+/-6.69
5322	19.00	21.52+/-1.96	22.10+/-2.79
+/- Standard Deviation			

**Table 4C.**  
Vitamin B<sub>12</sub>

Vitamin B <sub>12</sub> Levels (MCG/KG)			
DIET	Calculated Value	10-Year Average	15-Year Average
5002	19.80	26.70+/-8.68	27.34+/-8.32
5007	26.90	37.57+/-11.95	37.85+/-11.82
5026	13.20	19.48+/-5.47	20.70+/-6.85
5048	46.00	48.24+/-8.09	49.57+/-9.29
5322	6.60	14.83+/-5.13	15.37+/-5.26
+/- Standard Deviation			

## Contaminant Analysis

Heavy metal and/or chemical contamination of lab animal diets used for GLP studies is unacceptable. Thus, these substances must be closely monitored. Composite samples from each manufacturing lot of Certified LabDiet® product are analyzed for 30 contaminants, including heavy metals, aflatoxin, chlorinated hydrocarbons and organophosphates. The certification profile has a maximum allowable concentration for each contaminant measured. Analyzed concentrations of all 30 contaminants were consistently below maximum allowable levels. Concentrations of arsenic, malathion, lead, and PCBs are exhibited in Tables 5A, 5B, 5C and 5D, respectively. For all contaminant assays, the lower limit of detection

is considerably lower than our guaranteed maximum limits. For many of the contaminants analyzed concentrations fall well below that detection limit. Because of our tight controls, data collected during the 15-year period is consistent and reliable.

In the periodic samples submitted to an independent laboratory for this study, there was no derivation of limits of aflatoxin detected. This indicates that our Certified LabDiet® program for controlling mycotoxin contamination has achieved its intended goal.

### Contaminant Analysis of Certified Labdiet® Products Over 15-Year Period

**Table 5A.**  
Arsenic Concentrations  
in Certified LabDiet®  
Products Over Test  
Period of 1993-2007

Arsenic, ppm					
YEAR	5002	5007	5026	5048	5322
<b>10-Year Average</b>	0.24+/-0.14	0.27+/-0.16	0.23+/-0.15	0.23+/-0.14	0.21+/-0.15
<b>2003</b>	0.10+/-0.12	<0.02	0.04+/-0.12	0.07+/-0.18	<0.02
<b>2004</b>	0.03+/-0.09	0.04+/-0.13	0.17+/-0.11	0.02+/-0.07	0.15+/-0.14
<b>2005</b>	0.03+/-0.14	<0.02	0.25+/-0.03	<0.02	0.23+/-0.17
<b>2006</b>	<0.02	<0.02	0.19+/-0.14	0.03+/-0.07	0.33+/-0.11
<b>2007</b>	<0.02	<0.02	0.29+/-0.16	<0.02	0.47+/-0.22
<b>15-Year Average</b>	0.21+/-0.15	0.24+/-0.17	0.22+/-0.15	0.19+/-0.16	0.19+/-0.15
<b>Max. Conc. Levels</b>	1.00 PPM	1.00 PPM	1.00 PPM	1.00 PPM	1.00 PPM
<b>Limits of Detection</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>

+/- Standard Deviation

**Table 5B.**  
Malathion Concentrations  
in Certified LabDiet®  
Products Over Test  
Period of 1993-2007

Malathion, ppm					
YEAR	5002	5007	5026	5048	5322
<b>10-Year Average</b>	0.05+/-0.06	0.04+/-0.06	0.04+/-0.05	0.04+/-0.06	0.05+/-0.06
<b>2003</b>	<0.02	<0.02	0.02+/-0.02	0.02+/-0.03	0.05+/-0.05
<b>2004</b>	<0.02	<0.02	<0.02	<0.02	<0.02
<b>2005</b>	<0.02	<0.02	<0.02	<0.02	<0.02
<b>2006</b>	0.02+/-0.02	<0.02	0.02+/-0.02	<0.02	0.02+/-0.02
<b>2007</b>	0.03+/-0.05	<0.02	0.05+/-0.06	<0.02	0.07+/-0.07
<b>15-Year Average</b>	0.04+/-0.05	0.03+/-0.05	0.04+/-0.05	0.03+/-0.05	0.04+/-0.06
<b>Max. Conc. Levels</b>	0.5 PPM	0.5 PPM	0.5 PPM	0.5 PPM	0.5 PPM
<b>Limits of Detection</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>

+/- Standard Deviation

**Contaminant Analysis of Certified Labdiet® Products Over 15-Year Period**

**Table 5C.**  
**Lead Concentrations**  
**in Certified LabDiet®**  
**Products Over Test**  
**Period of 1993-2007**

<b>Lead, ppm</b>					
<b>YEAR</b>	<b>5002</b>	<b>5007</b>	<b>5026</b>	<b>5048</b>	<b>5322</b>
<b>10-Year Average</b>	0.22+/-0.05	0.83+/-0.09	0.27+/-0.08	0.18+/-0.05	0.30+/-0.09
<b>2003</b>	0.13+/-0.05	0.36+/-0.29	0.18+/-0.07	0.11+/-0.05	0.19+/-0.07
<b>2004</b>	0.18+/-0.03	0.28+/-0.04	0.26+/-0.04	0.17+/-0.02	0.27+/-0.04
<b>2005</b>	0.20+/-0.03	0.31+/-0.05	0.25+/-0.05	0.19+/-0.03	0.29+/-0.05
<b>2006</b>	0.19+/-0.03	0.29+/-0.04	0.28+/-0.03	0.18+/-0.02	0.28+/-0.04
<b>2007</b>	0.20+/-0.03	0.31+/-0.04	0.32+/-0.05	0.21+/-0.03	0.31+/-0.04
<b>15-Year Average</b>	0.21+/-0.05	0.68+/-0.25	0.73+/-0.08	0.18+/-0.05	0.29+/-0.08
<b>Max. Conc. Levels</b>	1.5 PPM	1.5 PPM	1.5 PPM	1.5 PPM	1.5 PPM
<b>Limits of Detection</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>

+/- Standard Deviation

**Table 5D.**  
**PCBs Concentrations**  
**in Certified LabDiet®**  
**Products Over Test**  
**Period of 1993-2007**

<b>PCBs, ppm</b>					
<b>YEAR</b>	<b>5002</b>	<b>5007</b>	<b>5026</b>	<b>5048</b>	<b>5322</b>
<b>10-Year Average</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>2003</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>2004</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>2005</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>2006</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>2007</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>15-Year Average</b>	<0.15	<0.15	<0.15	<0.15	<0.15
<b>Max. Conc. Levels</b>	0.15 PPM	0.15 PPM	0.15 PPM	0.15 PPM	0.15 PPM
<b>Limits of Detection</b>	<b>0.15</b>	<b>0.15</b>	<b>0.15</b>	<b>0.15</b>	<b>0.15</b>

+/- Standard Deviation



## Summary

The cumulative results of what is now a 15-year collection period demonstrates that nutrient and contaminant concentrations are consistent with our Certified LabDiet® products. LabDiet® has placed strict constraints on our certified products through the use of our managed formulation program of Constant Nutrition®, approved suppliers, daily nutrient analyses, and defined manufacturing protocols. In the end, this translates to greater control and more consistency with your research experiments.



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