

LabDiet[®]
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International Product Supplies Limited



**Nutrient and Contaminant Levels in
LabDiet[®] Certified Laboratory Animal Feeds**

*Based on data compiled from 1993-2012 by PMI[®] LabDiet[®]
and in conjunction with an independent laboratory.*

Published 2016

*20-Year Technical
Bulletin Update*

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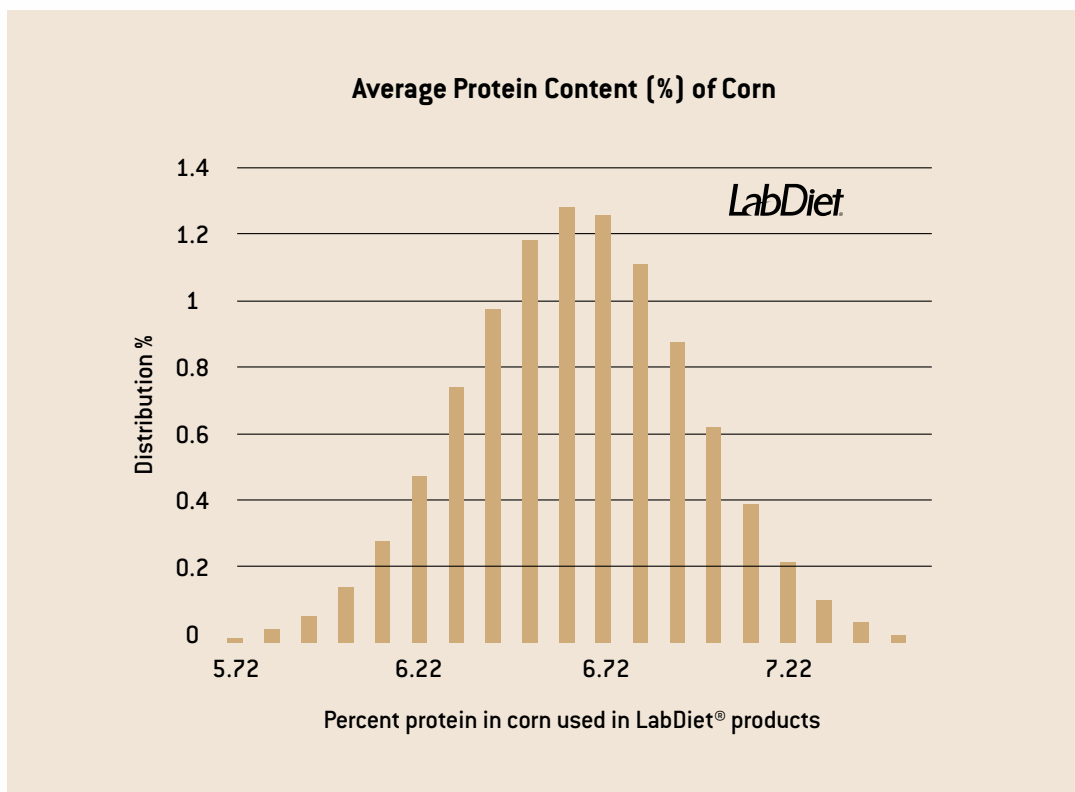
Final Data Report



Quality: Consistency and Control

The integrity of your work is dependent upon controlled experimental protocols. Our responsibility is to provide nutritionally consistent diets through managed formulation, Constant Nutrition®, that enables you to isolate, distinguish, and define breakthroughs. The entire manufacturing process, from ingredient selection to finished product testing, is designed to deliver the most nutritionally consistent diets available. Ingredients are purchased through approved suppliers who understand and adhere to the demands of research diets.

Sophisticated formulation software and near-infrared spectroscopy (NIR) 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 inspected and approved by the quality assurance team prior to release to ensure that quality standards are met. As a result, Certified LabDiet® products are nutritionally consistent and of the utmost quality, enabling you to conduct accurate research by minimizing nutrient variability that can sometimes be associated with fixed formulation diets.



Contents

Introduction	1
Constant Nutrition® You Can Count On	2
Materials and Methods.....	3
Results	
Protein/Amino Acids	4
Fat	6
Minerals – Calcium/Phosphorus.....	7
Vitamins.....	9
Contaminant Analysis	10
Summary.....	12
References	Inside Back Cover

Introduction

Diets used in laboratory animal studies must provide consistent and adequate nutrition.¹ To reduce unwanted variables, feed manufacturers must ensure that all batches of feed meet approved nutritional specifications.² 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 (GLP) Program.

This report is an update to our 2010 published bulletin *"Nutrient and Contaminant Levels in LabDiet® Certified Laboratory Animal Feeds: 15-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 2007 and now 2008 to 2012.



Constant Nutrition® You Can Count On

The PMI® manufacturing process for LabDiet® products ensures consistent levels of nutrients and guarantees minimal concentrations of various contaminants. Constant Nutrition®, a managed formulation technique exclusive to LabDiet®, ensures a consistent level of nutrients by accounting for biological variation of natural feedstuffs versus fixed formulation, 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.

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 diets 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 manage nutrients and reduce unwanted contaminants.

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 2012 demonstrate that the nutritional content and contaminant levels are well managed in Certified LabDiet® products manufactured using Constant Nutrition® 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.

Constant Nutrition®, driven by our managed formulation philosophy, helps to deliver a consistent nutritional product so that researchers are confident in their research data.

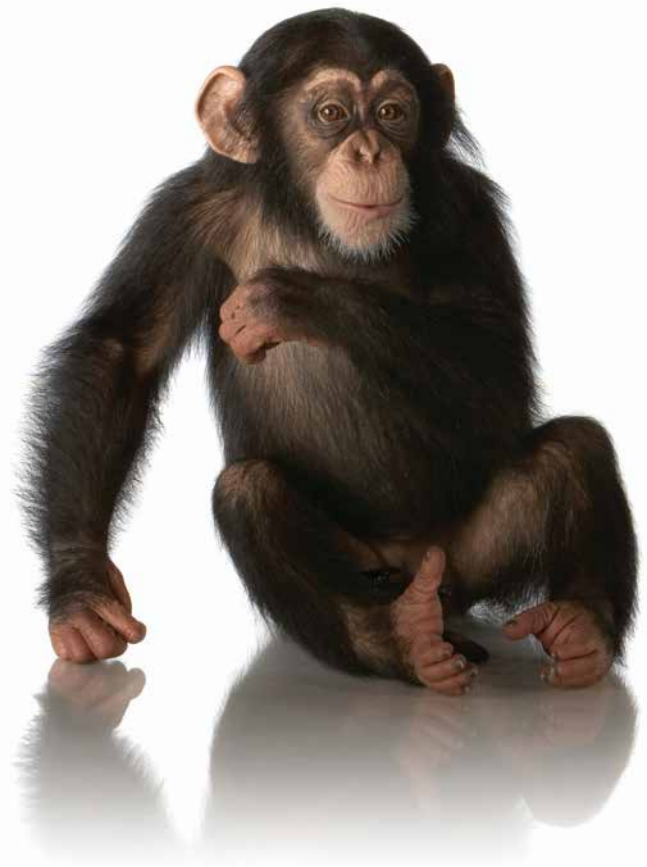
Based on a database maintained by PMI®, the following example shows how widely protein, alone, can vary in a fixed formula:

Fixed-Formula Diet	%	The ingredients could conceivably contain the following protein levels (%):	
		Low	High
Corn	30.0	Corn 6.5	10.0
Wheat	17.0	Wheat 9.0	14.0
Soybean Meal	15.0	Soybean Meal 45.0	50.0
Midds	6.0	Midds 14.5	17.0
Fish	4.0	Fish 57.0	66.0
Oats	4.0	Oats 7.5	12.5
Alfalfa	3.0	Alfalfa 15.5	21.0
Misc	21.0	Misc –	–
	100.0	Calculated protein level in formula above:	
		14.2	17.7

Materials and Methods

In order to measure nutritional consistency of our diets, periodic samples of the following Certified LabDiet® products have been collected for the past 20 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 protein, fat, calcium, phosphorous, lysine, methionine, riboflavin, pantothenate, vitamin B₁₂, lead, arsenic and PCBs. The analytical methods employed were specified by the Association of Analytical Chemists (AOAC) 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 15-year bulletin as well as those collected from 2008 to 2012 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 20 years. Chart 1A 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 1B and 1C. Similar to the first 15 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 20-Year Period

15- and 20-Year Average Protein Levels (%) in Certified LabDiet®						
DIET	Calculated Value %	# Samples 15 Years	15-Year Average	# Samples 20 Years	20-Year Average	2008-2012 4th 5-year Average
Certified Rodent Diet 5002	20.70	48	21.23+/-0.40	62	21.19+/-0.42	21.01+/-0.47
Certified Canine Diet 5007	25.50	47	26.70+/-0.57	62	26.60+/-0.60	26.16+/-0.51
Certified Guinea Pig Diet 5026	19.90	47	20.07+/-0.51	61	20.00+/-0.60	19.64+/-0.82
Certified Primate Diet 5048	25.80	47	26.81+/-0.61	60	26.73+/-0.63	26.49+/-0.63
Certified Rabbit Diet 5322	17.10	47	17.36+/-0.64	61	17.26+/-0.68	16.87+/-0.72

+/- Standard Deviation

Chart 1A.
Protein Levels in Certified Rodent Diet 5002 Over 20-Year Period

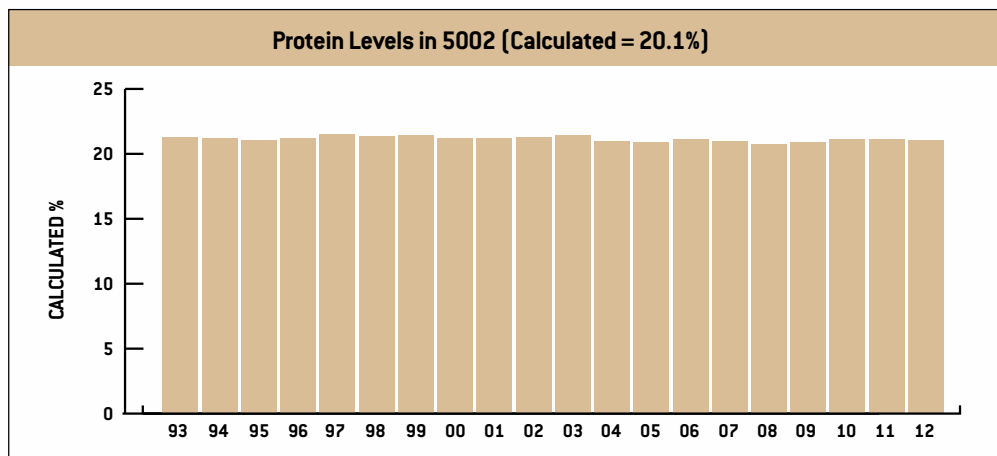


Table 1B.
Lysine Levels in
Certified LabDiet®
Products Over
20-Year Period

Lysine % in Certified LabDiet®						
DIET	Calculated Value %	# Samples 15 Years	15-Year Average	# Samples 20 Years	20-Year Average	2008-2012 4th 5-year Average
Certified Rodent Diet 5002	1.19	47	1.16+/-0.05	62	1.15+/-0.05	1.14+/-0.05
Certified Canine Diet 5007	1.10	47	1.25+/-0.06	61	1.25+/-0.06	1.25+/-0.05
Certified Guinea Pig Diet 5026	1.10	47	1.04+/-0.11	61	1.04+/-0.10	1.04+/-0.09
Certified Primate Diet 5048	1.26	47	1.22+/-0.05	60	1.21+/-0.05	1.21+/-0.06
Certified Rabbit Diet 5322	0.92	47	0.87+/-0.05	61	0.86+/-0.05	0.83+/-0.05

+/- Standard Deviation

Table 1C.
Methionine Levels in
Certified LabDiet®
Products Over
20-Year Period

Methionine % in Certified LabDiet®						
DIET	Calculated Value %	# Samples 15 Years	15-Year Average	# Samples 20 Years	20-Year Average	2008-2012 4th 5-year Average
Certified Rodent Diet 5002	0.43	47	0.42+/-0.02	62	0.42+/-0.02	0.44+/-0.02
Certified Canine Diet 5007	0.44	47	0.42+/-0.03	62	0.42+/-0.03	0.42+/-0.01
Certified Guinea Pig Diet 5026	0.40	47	0.38+/-0.05	61	0.38+/-0.05	0.37+/-0.05
Certified Primate Diet 5048	0.48	47	0.47+/-0.03	61	0.48+/-0.03	0.49+/-0.02
Certified Rabbit Diet 5322	0.35	47	0.34+/-0.02	61	0.34+/-0.02	0.34+/-0.02

+/- 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 values (in red) for ether extraction 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 value. 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 20-Year Period

	Certified Rodent Diet 5002		Certified Primate Diet 5048	
	Fat E.E., % ¹	Fat A.H., % ²	Fat E.E., % ¹	Fat A.H., % ²
15-Year Average	4.65+/-0.40	5.74+/-0.27	3.60+/-1.13	6.29+/-0.63
2008	5.28+/-0.09	6.02+/-0.26	3.19+/-0.39	5.79+/-0.51
2009	5.13+/-0.06	5.84+/-0.22	2.98+/-0.37	5.51+/-0.53
2010	5.06+/-0.12	5.76+/-0.28	3.48+/-0.36	5.84+/-0.51
2011	5.19+/-0.43	5.96+/-0.28	3.14+/-0.09	6.03+/-0.63
2012	4.83+/-0.86	6.06+/-0.39	3.29+/-0.98	5.91+/-0.60
20-Year Average	4.78+/-0.52	5.78+/-0.28	3.37+/-1.20	6.15+/-0.65
Calculated Value, %	5.00	6.30	5.00	6.60
<small>¹Ether Extract ²Acid Hydrolysis +/- Standard Deviation</small>				



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, 5026, 5048 and 5322. Analyzed values for calcium and phosphorus from 2008 to 2012 are consistent with values tabulated and published in the *15-Year Technical Bulletin* further demonstrating nutrient consistency due to Constant Nutrition® formulation.

Chart 3A.
Calcium Levels (%) in Certified LabDiet® Products Over 20-Year Period

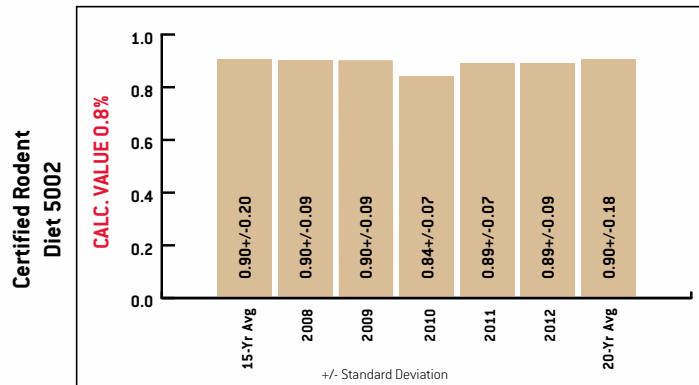
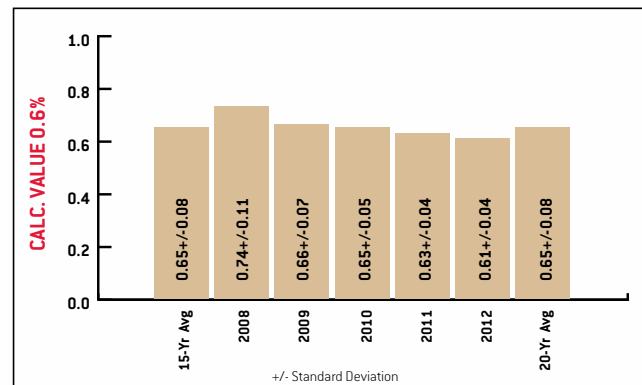
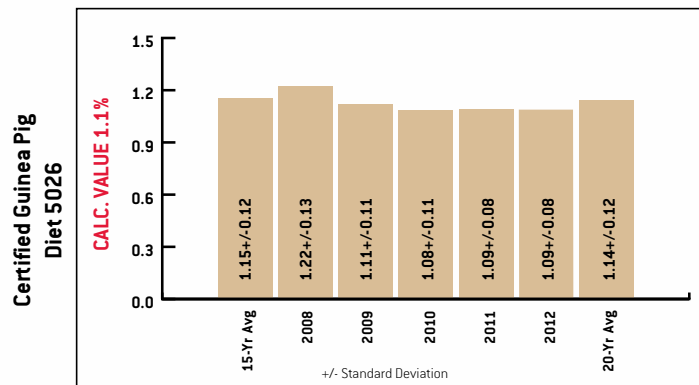
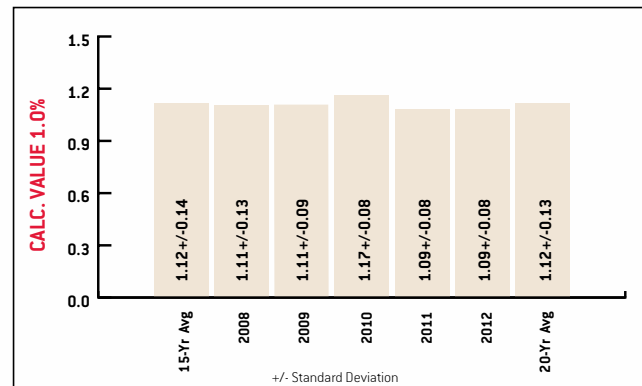
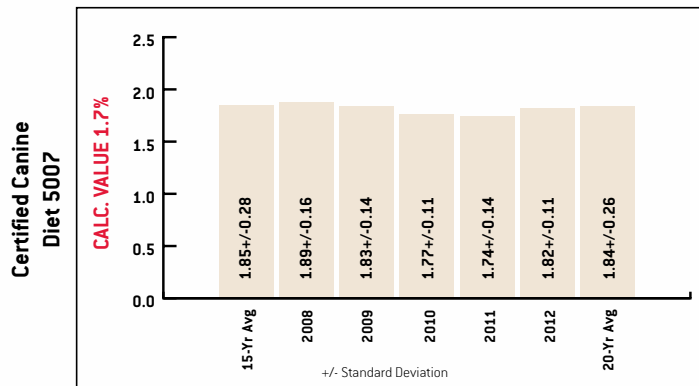
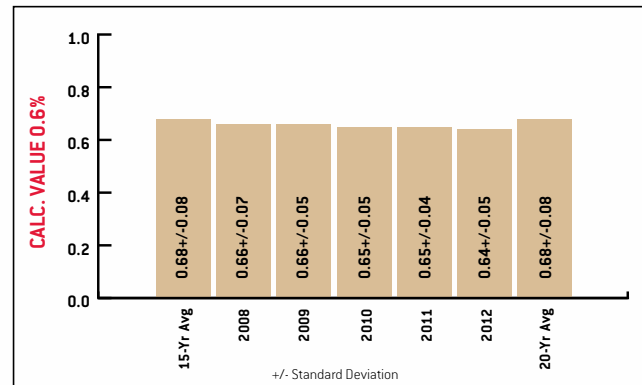


Chart 3B.
Phosphorus Levels (%) in Certified LabDiet® Products Over 20-Year Period



Minerals – Calcium/Phosphorus (cont.)

Chart 3A.
Calcium Levels (%) in Certified LabDiet® Products Over 20-Year Period

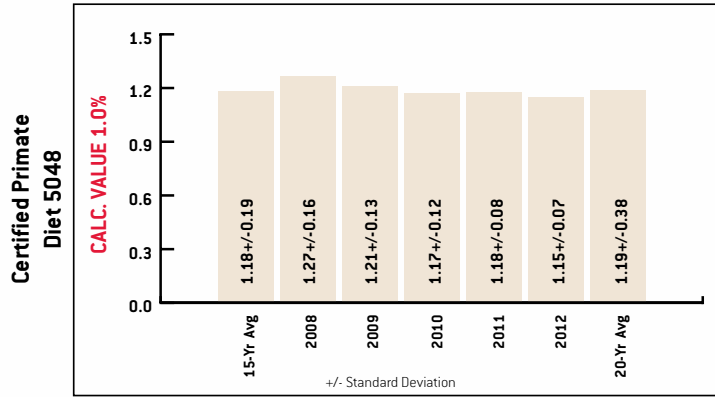
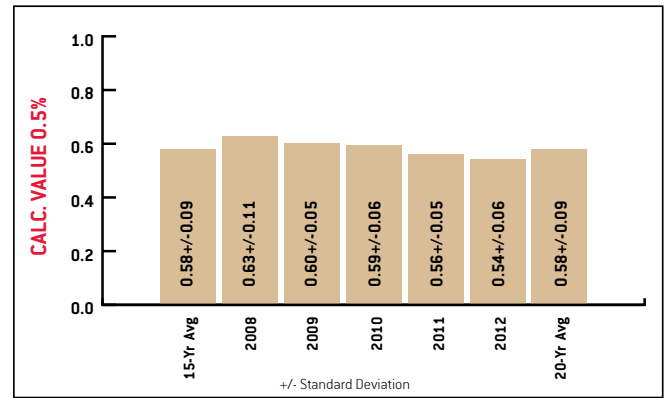
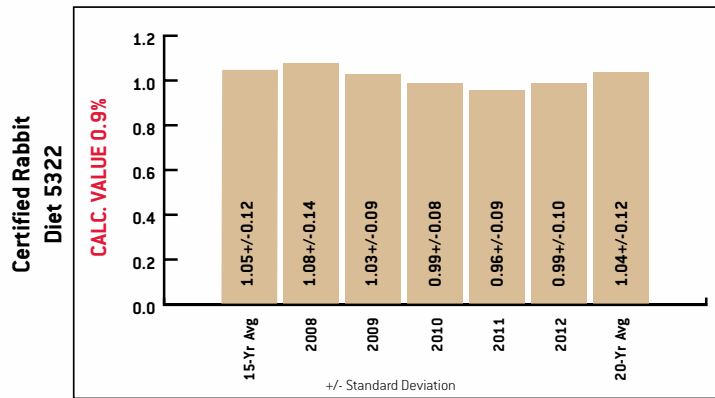
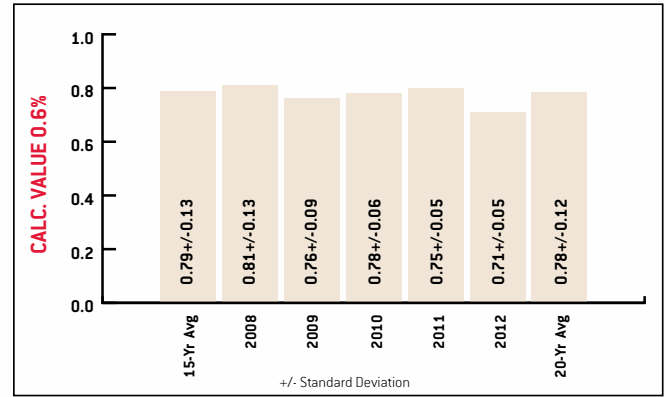


Chart 3B.
Phosphorus Levels (%) in Certified LabDiet® Products Over 20-Year Period



Vitamins

Riboflavin, pantothenic acid, and vitamin B₁₂ analyzed in Certified LabDiet® products are provided in Tables 4A, 4B and 4C, respectively. Values tabulated during the first 15 years of our collection period are consistently similar to those collected from 2008 to 2012. 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. We also know that some loss can occur during manufacturing. This loss is taken into account when formulating the diet so that no deficiencies occur.

Vitamin Levels in Certified LabDiet® Products Over 20-Year Period

Table 4A.
Riboflavin Levels in Certified LabDiet® Products Over 20-Year Period

Riboflavin Levels (PPM)			
DIET	Calculated Value	15-Year Average	20-Year Average
5002	8.10	7.81+/-1.30	7.75+/-1.22
5007	4.50	4.70+/-0.89	4.83+/-1.01
5026	6.10	6.76+/-1.47	6.74+/-1.50
5048	8.70	8.55+/-1.62	8.70+/-1.70
5322	5.50	6.49+/-1.53	6.45+/-1.55
+/- Standard Deviation			

Table 4B.
Pantothenic Acid Levels in Certified LabDiet® Products Over 20-Year Period

Pantothenic Acid Levels (PPM)			
DIET	Calculated Value	15-Year Average	20-Year Average
5002	17.00	17.08+/-1.30	17.28+/-2.44
5007	20.00	20.81+/-3.70	21.39+/-3.95
5026	21.00	21.61+/-3.27	22.38+/-3.51
5048	60.00	55.95+/-6.69	57.18+/-7.53
5322	19.00	22.10+/-2.79	22.1+/-2.73
+/- Standard Deviation			

Table 4C.
Vitamin B₁₂ Levels in Certified LabDiet® Products Over 20-Year Period

Vitamin B₁₂ Levels (MCG/KG)			
DIET	Calculated Value	15-Year Average	20-Year Average
5002	51.00	27.34+/-8.32	33.06+/-13.41
5007	27.00	37.85+/-11.82	39.00+/-11.42
5026	14.00	20.70+/-6.85	20.90+/-6.33
5048	49.00	49.57+/-9.29	50.49+/-10.52
5322	7.00	15.37+/-5.26	15.67+/-5.36
+/- 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, a greater percentage fell below the limit of detection than were actually quantified. For example, looking at arsenic, only the averages of those samples that assayed above the limit of detection (LOD) are reported in Table 5A.

Contaminant Analysis of Certified Labdiet® Products Over 20-Year Period

Table 5A.
Arsenic Concentrations in Certified LabDiet® Products Over 20-Year Period

Arsenic, ppm ^{1,2}					
YEAR	5002	5007	5026	5048	5322
15-Year Average	0.29+/-0.07	0.36+/-0.11	0.30+/-0.06	0.30+/-0.08	0.28+/-0.06
2008	0.21+/-0.01	<0.20	0.36+/-0.13	0.21+/-0.05	0.36+/-0.15
2009	0.21	<0.20	0.30+/-0.08	0.21	0.26+/-0.03
2010	0.22+/-0.01	<0.20	0.25+/-0.03	0.21	0.24+/-0.02
2011	<0.20	<0.20	0.27+/-0.07	0.21	0.31+/-0.13
2012	0.24+/-0.02	<0.20	0.29+/-0.07	0.21+/-0.01	0.30+/-0.09
20-Year Average	0.27+/-0.06	0.36+/-0.11	0.30+/-0.07	0.28+/-0.08	0.29+/-0.10
Max. Allowable Conc.	1.00 PPM	1.00 PPM	1.00 PPM	1.00 PPM	1.00 PPM
Limits of Detection	0.20	0.20	0.20	0.20	0.20

+/- Standard Deviation

¹ The average percentage of samples above the limit of detection for arsenic for 5002, 5007, 5026, 5048 and 5322, respectively:
 1993-1997: 93%, 95%, 93%, 89%, 91%
 1998-2002: 56%, 60%, 52%, 61%, 51%
 2003-2007: 15%, 2%, 66%, 9%, 63%
 2008-2012: 4%, 0%, 64%, 7%, 62%

² Values only represent concentrations measured above the limit of detection. See text regarding the percentage of samples assayed below the limit of detection.

Table 5B.
Malathion Concentrations in Certified LabDiet® Products Over 20-Year Period

Malathion, ppm ¹					
YEAR	5002	5007	5026	5048	5322
15-Year Average	0.06+/-0.04	0.06+/-0.04	0.06+/-0.04	0.06+/-0.06	0.06+/-0.04
2008	0.05+/-0.05	0.04+/-0.02	0.05+/-0.02	0.06+/-0.04	0.05+/-0.03
2009	0.04+/-0.04	0.04+/-0.02	0.09+/-0.07	0.06+/-0.04	0.06+/-0.03
2010	0.05+/-0.05	0.04+/-0.02	0.04+/-0.01	0.03+/-0.01	0.04+/-0.01
2011	0.06+/-0.03	0.05+/-0.02	0.03+/-0.01	0.05+/-0.03	0.05+/-0.03
2012	0.04+/-0.06	0.07+/-0.07	0.03+/-0.01	0.03+/-0.01	0.04+/-0.02
20-Year Average	0.06+/-0.04	0.06+/-0.04	0.05+/-0.04	0.06+/-0.05	0.06+/-0.04
Max. Allowable Conc.	0.5 PPM	0.5 PPM	0.5 PPM	0.5 PPM	0.5 PPM
Limits of Detection	0.02	0.02	0.02	0.02	0.02

+/- Standard Deviation

¹ The average percentage of samples above the limit of detection for malathion for 5002, 5007, 5026, 5048 and 5322, respectively:
 1993-1997: 80%, 71%, 64%, 65%, 73%
 1998-2002: 65%, 41%, 62%, 41%, 67%
 2003-2007: 30%, 16%, 44%, 16%, 40%
 2008-2012: 28%, 21%, 37%, 16%, 35%

For LabDiet® 5002, 5007, and 5048 a maximum of only 7% of the samples assayed above the LOD. For LabDiet® 5026 and 5322, higher alfalfa containing diets, the maximum percentage of samples that assayed above the LOD was 64%. Because of our tight controls, data collected during the 20-year period is consistent and reliable.

Malathion levels have also decreased in diets over the 20 year period. From 2008 to 2012, a maximum of 37% of the samples assayed above the LOD while 63% of the samples were below the LOD. The percentage of samples that assayed above the LOD was even lower for 5002, 5007 and 5048 (max of 28% > LOD).

In the 20 year period, the percentage of samples assaying above the LOD for both arsenic and malathion have decreased in all diets demonstrating that even raw material suppliers understand the importance of reducing contaminants in ingredients and subsequently animal feeds.

In the periodic samples submitted to an independent laboratory for this study, there was no 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 20-Year Period

Table 5C.
Lead Concentrations in Certified LabDiet® Products Over 20-Year Period

Lead, ppm					
YEAR	5002	5007	5026	5048	5322
15-Year Average	0.21+/-0.05	0.68+/-0.25	0.27+/-0.01	0.18+/-0.05	0.29+/-0.08
2008	0.21+/-0.03	0.31+/-0.03	0.33+/-0.05	0.20+/-0.03	0.32+/-0.03
2009	0.21+/-0.02	0.33+/-0.03	0.31+/-0.03	0.22+/-0.02	0.33+/-0.03
2010	0.21+/-0.04	0.31+/-0.05	0.30+/-0.07	0.22+/-0.02	0.30+/-0.07
2011	0.19+/-0.04	0.27+/-0.06	0.29+/-0.05	0.23+/-0.04	0.29+/-0.05
2012	0.19+/-0.03	0.33+/-0.04	0.35+/-0.04	0.25+/-0.02	0.34+/-0.04
20-Year Average	0.21+/-0.05	0.61+/-0.27	0.28+/-0.07	0.19+/-0.05	0.29+/-0.08
Max. Conc. Levels	1.5 PPM	1.5 PPM	1.5 PPM	1.5 PPM	1.5 PPM
Limits of Detection	0.05	0.05	0.05	0.05	0.05

+/- Standard Deviation

Table 5D.
PCBs Concentrations in Certified LabDiet® Products Over 20-Year Period

PCBs, ppm					
YEAR	5002	5007	5026	5048	5322
15-Year Average	<0.15	<0.15	<0.15	<0.15	<0.15
2008	<0.15	<0.15	<0.15	<0.15	<0.15
2009	<0.15	<0.15	<0.15	<0.15	<0.15
2010	<0.15	<0.15	<0.15	<0.15	<0.15
2011	<0.15	<0.15	<0.15	<0.15	<0.15
2012	<0.15	<0.15	<0.15	<0.15	<0.15
20-Year Average	<0.15	<0.15	<0.15	<0.15	<0.15
Max. Conc. Levels	0.15 PPM	0.15 PPM	0.15 PPM	0.15 PPM	0.15 PPM
Limits of Detection	0.15	0.15	0.15	0.15	0.15

+/- Standard Deviation

Summary

The cumulative results of what is now a 20-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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Committed to providing premium products to research professionals around the world, LabDiet® conducts innovative laboratory animal research to provide Constant Nutrition® that will make your research a success.



LabDiet®
Your work is worth it.®

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