

**Supporting Data for Inotiv Teklad Standard Diets nine (9) and twelve (12) month Shelf Life Assurance**

This document will share data used to establish a nine or twelve month use period (shelf life or expiration) for Teklad standard natural ingredient diets.

An accompanying appendix contains tables and figures mentioned in this text, beginning on page 9.

**Shelf life labeling for Teklad Standard Diets sold in North America PRIOR to June 1, 2026  
US & Canada:**

- Diet packaging (label or bag closure tape) includes date of manufacture only, with following exceptions:
  - Irradiated cases (vacuum packaged) - 9 month expiration date
  - Some customer-specific diets include 9 month expiration date
- Use period of 6 months (per *Guide for the Care and Use of Laboratory Animals, 8<sup>th</sup> edition, 2011*) or other institutional protocol

**Mexico:**

- Diet packaging (label or sew strip) includes date of manufacture and 6 month expiration

**Shelf life labeling for Teklad Standard Diets sold in North America AFTER June 1, 2026**

Based on the information contained in this document, our shelf life assurances for North American destinations will be as follows as long as diet is appropriately stored.

<b>Product Type</b>	<b>Shelf Life Assurance</b>
<b>Conventional – pelleted and extruded</b> - All diets, including Non-human Primate Diet and Guinea Pig diet (using stabilized Vitamin C)	<b>9 months</b>
<b>Irradiated – pelleted and extruded</b> - Most diets	<b>12 months</b>
<b>Exceptions</b> - 2914 Irradiated Global Rodent Maintenance Diet - 2940 & 2941 Irradiated Global Guinea Pig Diets	<b>9 months</b> <b>9 months</b>

### Shelf life labeling for Teklad Standard Diets sold to destinations other than North America PRIOR to April 1, 2026

- UK, EU, India - Diet packaging (label or bag closure tape) includes date of manufacture and 9 month expiration
- Korea, Australia/New Zealand, Hong Kong, Israel, Japan, Singapore, Indonesia
  - Diet packaging (label or sew strip) includes date of manufacture only (except irradiated cases with 9 month expiration)
  - Use period either follows primary North American guidance (6 months) or is left to user preference or country guidance

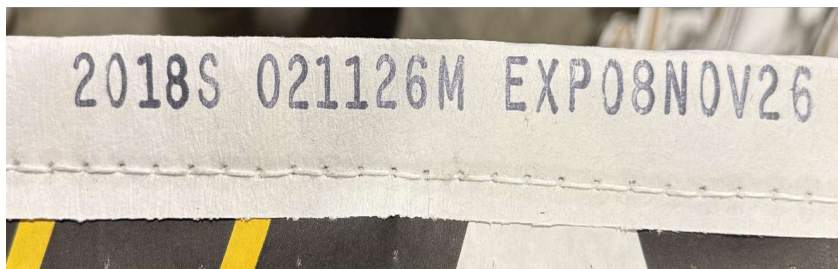
### Shelf life labeling for Teklad Standard Diets sold to International destinations (outside of North America) AFTER to April 1, 2026

Based on the information contained in this document, our shelf life assurances for non-US and Canada destinations will be as follows as long as diet is appropriately stored.

Product Type	Shelf Life Assurance
<b>Conventional – pelleted and extruded</b> - Most diets, including Non-human Primate Diets (using stabilized Vitamin C)	<b>12 months</b>
<b>Irradiated – pelleted and extruded</b> - Most diets	<b>12 months</b>
<b>Exceptions</b> - 2014 Global Rodent Maintenance Diet - 2914 Irradiated Global Rodent Maintenance Diet - 2040 & 2041 Global Guinea Pig Diets - 2940 & 2941 Irradiated Global Guinea Pig Diets	<b>9 months</b> <b>9 months</b> <b>9 months</b> <b>9 months</b>

NOTE: In some circumstances, orders to customers outside of North America will be fulfilled from domestic stock rather than new production. In those cases, the North America expirations will be on product packaging.

Example of dates on bag closure tape – Product code followed by lot number (manufacture date) and expiration date.



- Date of manufacture – format is MMDDYY with M suffix (for non-certified diet) and MA suffix for certified diet
- Expiration date – format is DDMMYY (month is 3 letter abbreviation)
- 9 months is calculated as date of manufacture + 270 days
- 12 months is the next calendar year, with same month and date

Shelf life is evaluated by monitoring changes in quality relative to some standard. The primary shelf life considerations for laboratory animal diets are **vitamin stability** and **microbiological quality**.

Shelf life determination is an exercise in risk assessment. This assessment was done at a theoretical level and via analysis of aged diet samples stored in conditions that mimic customer's storage practices.

### Description of Data

**Tables 1 and 2** (appendix) describe the data sources used to establish the new shelf life assurance period for Teklad standard diets. Primary data sources (Table 1) are those where data was acquired in an intentional way. Later time points were compared to measurements taken post-production or a frozen sample of the same lot.

These studies tested diets across the product line (rodent, guinea pig, rabbit, canine, NHP, and poultry diets) and covered pelleted and extruded manufacturing methods and included irradiated diets. Vitamins (A, E, C, thiamin, riboflavin, pyridoxine) were measured in the primary studies. Measurement of these vitamins, as well as a few others, were also part of the historical dataset. Water activity, a proxy for risk of microbiological growth and other reactions that could reduce product quality, was measured in some studies from the primary dataset.

To address the potential impact of ocean transit on vitamin retention, the primary dataset included analysis of US manufactured diets that were shipped to Singapore via ocean vessel, stored in Singapore, and returned to the US at various timepoints. Duplicate samples were retained in the US for comparison. There was no obvious difference in vitamin retention over time between the Singapore and US samples, and the data was combined in the final evaluation.

### Formulation – Vitamin Excess

Vitamin levels in standard natural ingredient diets are generous relative to estimated requirements. Formulated vitamin content exceeds estimated requirements by a range of 1.5 – 10X, and sometimes more, across multiple laboratory animal species. This is shown in **Table 3** (appendix).

The magnitude of this relative formulated excess is important, because even with some expected loss of vitamins over time, this provides assurance of suitability throughout the use period.

Information regarding the vitamin requirements of all lab animal species is inconsistent. The current state of our knowledge as to laboratory animal species minimum needs can be summarized as follows:

- Well-defined for livestock (poultry, swine)
- Reasonably well-defined for rodents, canines, felines
- More limited for primates and guinea pigs
- Very limited for rabbit and ferret

In the absence of definitive requirements, we are left with levels that have been determined to be adequate based on normal growth and other physiological responses. That can make it difficult to make definitive statements about vitamin adequacy, however, for the most part this uncertainty has led to erring on the side of caution and generous supplementation.

**Table 3** (appendix) shows that with the exception of vitamin A in guinea pig diets, vitamin levels are in excess of estimated requirements. The vitamin A estimate does not include the contribution of beta-carotene coming from alfalfa meal. When this is included, the formulated fold excess is approximately 1.6. The 2040 and 2041 Teklad Guinea Pig diets have been used for many years around the world and out to 9 months routinely; additionally, the 2040 diet is used internally for breeding purposes.



### Methodology

For every diet in the product line, the fold vitamin excess relative to estimated requirements at 6, 9 and 12 months post-manufacturing was calculated. This approach allows for estimates for vitamins not measured, and for a comparison between theoretical and measured vitamin retention.

This involved the following steps:

1. Calculation of vitamin retention (as a fraction) using the equation:

$$\text{Vitamin retention} = \text{premix retention} \times \text{manufacturing retention} \times \text{finished product retention in storage}$$

Tables published by M. Coelho (of vitamin manufacturer BASF) were used. Expected losses due to irradiation were also accounted for.

2. Multiply the formulated vitamin levels by their respective fraction to obtain the calculated vitamin levels at 6, 9 and 12 months.
3. Divide the calculated vitamin levels by the estimated species requirement to obtain the fold vitamin excess at these time points.

Ranges are shown in **Tables 4a and 4b** (appendix).

### Comparison between calculated vitamin retention and measured vitamin retention

**Table 5** shows the calculated and measured **vitamin retention (% of formulated or post-production)** for five measured vitamins from the primary dataset.

	Vitamin A	Vitamin E	Thiamin	Vit. B6	Riboflavin
Calc. retention - 6 mo.	49 - 67%	70 - 81%	51 - 68%	63 - 78%	74 - 84%
Calc. retention - 9 mo.	39 - 53%	62 - 72%	43 - 58%	56 - 70%	69 - 78%
Calc. retention - 12 mo.	27 - 37%	54 - 62%	33 - 44%	48 - 60%	64 - 73%
Measured retention - 6 mo.	88% (79% – 97%; n=6)	71% (66% - 76%; n=6)	98% (72 - 125%; n=14)	105% (92 - 130%; n=8)	88% (64 - 125%; n=6)
Measured retention - 9 mo.	80% (56% – 100%; n=8)	85% (57% - 119%; n=16)	91% (66 - 117%; n=16)	106 % (92 - 130%; n=8)	83 % (55 - 125%; n=9)
Measured retention - 12 mo.	58% (31% – 85%; n=8)	91% (65% - 102%; n=7)	89% (68 - 120%; n=16)	102 % (81 - 122%; n=8)	80 % (50 - 106%; n=9)

- The ranges for calculated encompass impact of pelleting, extrusion, and irradiation
- Actual (measured) ranges are wider which reflect variation seen in vitamin analysis
- Measured retention is always compared to the post-production measurement
- Calculated retention is always compared to theoretical (formulated) value

**Table 6** compares the measured and calculated values of **fold vitamin excess** for specific but representative diets.

Diet Code	Species & Attribute	Fold EX at 12 months Measured   Calculated			
		Vit. A	Vit. E	Thiamin	Vit. B6
2918	Rodent Irradiated	2.6   2.2	3.0   2.1	3.5   1.5	2.1   1.5
2019S	Rodent Extruded Autoclavable	4.7   3.9	3.4   2.7	32.0   12.0	4.0   2.5
2016	Rodent Conventional	3.5   2.4	2.2   2.3	4.5   1.9	2.5   1.8
2014	Rodent Maintenance Conventional	1.0   1.0	2.3   2.5	2.9   1.3	6.6   6.0
2027	Canine	1.0   0.6	1.2   1.3	13.5   6.6	5.4   3.6
2050	Primate	1.7   0.7	0.5   0.6	8.3   2.5	3.6   2.4
3961	Poultry Irradiated*	3.5   2.7	3.7   2.2	17.9   4.9	2.7   1.6
2031	Rabbit Conventional	1.0   0.9	2.3   2.7	12.8   3.7	7.5   5.4

- \*Riboflavin measured, not Vitamin E
- For thiamin, on average the measured fold excess values were ~60% higher than the calculated values
- For vitamin B6, on average the measured fold excess values were ~30% higher than the calculated values
- For vitamin A, on average the measured fold excess values were ~20% higher than the calculated values
- For vitamin E, on average the measured fold excess values were ~equivalent to calculated values

Plots of vitamin retention from post-production (PP) out to 18 months are shown in **Figures 1 through 6** (appendix) for vitamin A, E, thiamin, B<sub>6</sub>, Riboflavin, and vitamin C. The data is from the primary dataset and is an extension of the measured data presented in table 5. Vitamin levels are expressed as % retention relative to the measurement done post-production or the frozen retained sample.

The table below is summary of the slopes (the monthly degradation rate), R-squared (fit) and intercept (PP value set at 100%)

	Vit. A	Vit. E	Thiamin	Vit. B6	Riboflavin	Vit. C
slope	-3.70	-0.36	-1.21	-1.62	-1.20	-0.16
R <sup>2</sup>	0.93	0.06	0.94	0.54	0.44	0.03
Y Int	103.0	90.6	102.1	110.6	93.2	96.1

Vitamin A degraded at the fastest rate in this set of vitamins (3.7% per month). Vitamin A levels were the limiting factor in assuring suitability for use out to 9 or 12 months.

Comparison of post-production values to formulated levels revealed that ~20% loss of vitamins A, E, and B<sub>6</sub> occurs with manufacturing (which in this dataset is not separated from effects of irradiation). For stabilized vitamin C, that loss is generally less than 15%, and for riboflavin less than 10%. There was a wide range of post-production retention for thiamin. This is likely due to an underestimation of background levels in certain diets. When those high retentions are removed, loss with processing is ~15%.

Storage conditions for the samples from this dataset were as follows:

- Temperature: 65°F – 75°F/18 – 24°C
  - as high as 80°F/27°C in a small number of samples stored in Israel
- Relative humidity (RH): 35% - 65%

For vitamins other than vitamin A, loss during storage was under 2% per month. The estimates of monthly losses in storage by Coelho range from 2-4%, apart from vitamins A and K which are higher.

#### Summary of vitamin retention findings

- The calculated fold vitamin excess is over 1 for most vitamins at the 12 month point.
- The measured fold vitamin excess is over 1 in most cases at the 12 months point.
- The significance of deviations and other considerations are noted in **Table 7** (appendix).

#### Impact of storage conditions –humidity & water activity

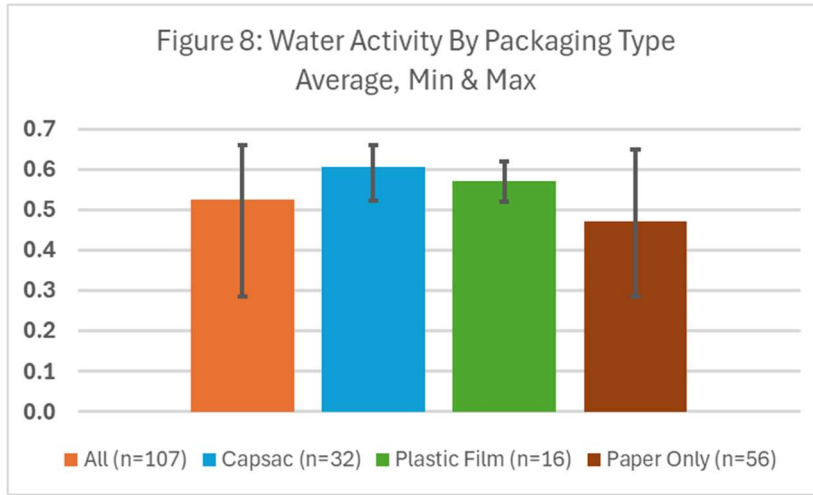
The logic of choosing 65% RH as an upper limit is that this will minimize the likelihood of mold growth with extended storage. This is relevant primarily to diet that is not irradiated. Irradiation destroys mold spores, and irradiated diet packaging (referred to as a capsac) is impermeable so there will not be exchange with room air. Therefore, fluctuations in humidity in the storage area will not impact the quality of these diets.

Water activity ( $A_w$ ; also called active water) is a measurement of the availability of water for biological reactions. It is expressed as the ratio of the vapor pressure in a food to the vapor pressure of pure water. For laboratory animal diet, the preferred range for water activity is 0.4 – 0.7, as this will limit the risk of microbiological growth and other reactions. If diet in its packaging is able to interact with room air, then changes in ambient humidity would lead to changes in water activity.

Packaging for conventional diet (non-irradiated) is in 2 or 3 ply paper packaging, and for some diets (particularly those that are fat-coated) with a plastic film liner that adheres to the paper. Conventional diet in the 2 or 3 ply paper packaging has some permeability, and we have measured changes in  $A_w$  in samples taken from bags under variable RH.

**Figure 7** (appendix) shows that changes in RH in the storage area did not impact irradiated diet or diet packaged in bags with the plastic film liner. Changes in water activity for diets in paper packaging correlated with seasonal changes in RH in the storage area (range of 35% to 65%). The lowest  $A_w$  values were measured in winter and highest in summer.

**Figure 8** is compilation of water activity values from both datasets. Measurements were made at timepoints ranging from post-manufacturing through 18 months. The bars show the upper and lower limit of  $A_w$  values.



The majority of the variability in  $A_w$  is in diet packaged in paper only. All values are less than 0.7 upper limit, therefore there is little risk for microbiological growth in non-irradiated diet when RH in storage area stays below 65%.

The 2031 diet had microbiology measured at the same time as  $A_w$  to check for changes in microbiological load. 2031, by virtue of high alfalfa meal inclusion has the highest bioburden which is why this diet was monitored. There were no meaningful changes in basic microbiology.

Season	Diet Age	$A_w$	APC, CFU/g	Mold, CFU/g	Yeast, CFU/g
Winter	PP	0.36	4100	<10	<10
Fall	9 mon	0.53	710	<10	<10
Winter	12 mon	0.29	4700	<10	<10
Summer	18 mon	0.61	3500	<10	<10

#### Overall summary of findings & conclusion

- The calculated fold vitamin excess is over 1 for most vitamins at the 9 and 12 month points.
- The measured fold vitamin excess is over 1 in most cases at the 9 and 12 month points.
- When diet is stored in a range of cool and dry conditions, vitamin retention is adequate to meet various species needs out to 9 or 12 months.
- Irradiated product packaging protects against fluctuations in humidity in storage areas.

#### Comment on the different recommended use periods for North America and International

The reasoning for 12 months for irradiated diets (vs 9 months for conventional diets) is that packaging offers protection against degradative processes. However, the data supports use of most Teklad standard diets (conventional and irradiated) to 12 months. Twelve months is particularly beneficial to customers in international markets due to transit time and costs, and there is already some regular use to 12 months. In the North American market, the other standard diet manufacturer utilizes a 9 month expiration for conventional diets and 12 months for irradiated diet. By setting our assurances similarly, this will help our customers to standardize their use practices across vendors.

**For North America:**

**Inotiv assures shelf life of Teklad Natural Ingredient diets to be 9 months (conventional diets) or 12 months (irradiated diets) from the date of manufacture (exceptions noted on first page of this document), under the following storage conditions.**

**For International:**

**With the exceptions noted on the second page of this document, Inotiv assures shelf life of Teklad Natural Ingredient diets to be 12 months from the date of manufacture, under the following storage conditions.**

- Temperature at or below 70°F/22°C (minor excursions acceptable)\*
- Relative humidity (RH) at or below 65%
- Clean and free of pests, in original packaging or in a container that prevents continuous exposure to light and minimal exposure to air

**Compilation prepared by:****Barbara Mickelson, PhD****Nutritionist, Technical Services Manager****Inotiv Teklad Diets****Primary resources**

Coelho, M. (2002). Vitamin stability in premixes and feeds: A practical approach in ruminant diets. In Proceedings 13th Annual Florida Ruminant Nutrition Symposium.

National Research Council. (1995). Nutrient requirements of laboratory animals. National Academies Press.

National Research Council. (2003). Nutrient requirements of Nonhuman Primates. National Academies Press.

National Research Council. (2006). Nutrient requirements of dogs and cats. National Academies Press.

National Research Council. (1998 & 2012). Nutrient requirements of Swine. National Academies Press.

National Research Council. (1994). Nutrient requirements of Poultry. National Academies Press.

Leeson, S. and Summers, J.D. (2005) Commercial Poultry Nutrition. 3rd Edition, University Books.

Blas, C., & Wiseman, J. (Eds.). (2010). Nutrition of the Rabbit. CABI.

Tobin, G, KA Stevens and RJ Russell. 2007. Nutrition (Chapter 10). In: The Mouse in Biomedical Research. 2<sup>nd</sup> edition. Elsevier, New York, NY.

*\* It is not possible to definitively quantify what constitutes a minor excursion in temperature or relative humidity. More risk is likely when deviations are of greater magnitude and duration. With respect to risk for vitamin degradation due to temperature increases, temperatures would need to rise beyond the typical 5 -10 ° F (up to 5 °C) range expected in facilities that have adequate temperature and humidity control. Small changes that could occur in several months can be obscured by measurement error. Packaging can minimize changes due to fluctuations of RH. It would be best practice for RH to not exceed 70% on a regular basis so that conventional diet in the 2-3 ply paper packaging does not experience appreciable increases in water activity.*

**Date: May 2026**



## Appendix to: Supporting Data for Inotiv Teklad Standard Diets nine (9) and twelve (12) month Shelf Life Assurance - Tables and figures

**Table 1: Primary\* data sources**

Source	Time Period	Scope	Tested (product & assay)
Singapore Transit & US Stored	2010 - 2011	Diets shipped on container to Singapore; Pre-packaged samples shipped back by air at 3, 6, 9, 12 mo. Identical set of samples kept in US at RT and frozen storage	2018, 2918, 2041 Vitamin A, E, Thiamin, Riboflavin, Vitamin C, water activity
Israel	2011 (9 mo), 2012 (12 mo)	Samples returned from Israel (storage temp up to 80°F/27°C). Used US retained sample (frozen) for post-production comparison	2918, 2018S, 7078S Vitamin A, E, Thiamin, Riboflavin
Current - US	2024 - 2025	8 diets to cover manufacturing method, processing, additional species and other attributes. Testing at 6, 9, 12, 15, 18 months	2918, 2019S, 2016, 2014, 2027, 2031, 2050, 3961 Vitamin A, E, Thiamin, B6, Vitamin C, water activity, anisidine value

\*Data that was acquired in an intentional way and where post-production (or proxy) testing was available for comparison

**Table 2: Supporting (Historical) Data Sources**

Source	Time Period	Scope	Tested (product & assay)
Europe (Teklad formulas produced in UK)	1990's-2000	Testing of older diet n=8 (6 months) and n=6 (9 months)	Rodent, Guinea Pig Vitamin A, E, Thiamin, B6, Folic acid, Vitamin C
Israel	2017 (12-15 months)	Samples returned from Israel (higher temp storage).	2918, 2018S, 7078S Vitamin A, E, Thiamin, B6, Riboflavin, Vitamin C
US	2024	Testing of aged diet – 12, 18 and 24 months	2914, 2055 Vitamin A, E, Thiamin, B6, Vitamin C, water activity
US made shipped to EU	2007 - 2021	Post-production (sometimes post-irradiation) testing of various diets	Rodent, Rabbit, Guinea Pig, Primate, Canine (n>600) Vitamin A, Vitamin E, Vitamin C
Other	2009, 2014	Testing of Primate diet, time points ranging from 3 to 16 months	Primate Diet Vitamin D
Other	Various	Post-autoclave testing	Autoclavable Rodent Thiamin, Vitamin A

**Table 3. Demonstration of fold vitamin excess (Teklad formulated standard diets) compared to estimated species requirements or adequate intake**

	Rodent	Rabbit	Guinea Pig	NHP	Canine	Feline	Poultry	Swine
Vitamin A	6.5	2.5	0.7	2.4	2.0	2.0	8.2	7.5
Vitamin D	1.5	1.9	1.5	3.2	4.2	4.4	6.8	6.8
Vitamin E	3.7	4.3	3.3	1.1	2.3	2.9	4.5	6.9
Vitamin K	50.0	50.0	10.0	26.0	1.3	9.0	24.0	100.0
Thiamin	4.3	8.5	9.0	6.0	16.2	2.4	14.0	17.0
Riboflavin	5.0	3.2	6.0	3.0	2.1	2.5	3.5	4.3
Niacin	4.7	1.4	7.2	3.7	5.1	1.8	2.3	1.9
Pyridoxine	3.0	9.5	6.0	4.3	6.4	4.5	3.1	6.3
Pantothenic acid	3.3	2.3	2.0	2.3	1.6	3.3	2.8	3.2
Vitamin B12	3.2	8.0	NA	1.3	4.6	2.5	3.5	5.3
Biotin	2.0	2.3	2.2	1.7	NA	1.9	1.7	4.0
Folate	4.0	1.0	1.3	4.5	8.9	1.3	6.6	13.3
Vitamin C			5.3	4.6				

NA – no established requirement

Guinea Pig - When including estimate for vitamin A (as beta-carotene from alfalfa meal) for GP diets, the vitamin excess relative to estimated requirements is ~1.6

**Table 4a. Demonstration of fold vitamin excess at 6,9,12 months compared to estimated species requirements or adequate intake. The table reflects all Teklad diets currently sold to international destinations.**

	Species							
	Calculated fold excess at:	Rodent	Auto-Rodent	Rodent-Maint.	Rabbit	Guinea Pig	NHP	Swine
Vit. A	6 mo	3.2 - 4.4	7.1 - 8.7	1.6 - 1.7	1.5 - 2.1	0.2 - 0.5	1.3	5.0
	9 mo	2.5 - 3.5	5.6 - 6.9	1.4 - 1.4	1.2 - 1.7	0.2 - 0.4	1.0	4.0
	12 mo	1.8 - 2.4	3.9 - 4.9	0.9 - 1	0.8 - 1.2	0.1 - 0.3	0.7	2.8
Vit. D	6 mo	0.9 - 1	1.3 - 1.4	0.4 - 0.4	1.2 - 1.3	0.6 - 1	2.1	4.7
	9 mo	0.7 - 0.8	1.1 - 1.1	0.3 - 0.3	0.9 - 1	0.4 - 0.8	1.7	3.8
	12 mo	0.5 - 0.6	0.8 - 0.8	0.2 - 0.2	0.7 - 0.8	0.3 - 0.6	1.3	2.8
Vit. E	6 mo	2.6 - 3	3.5 - 3.6	2.9 - 3.2	3.2 - 3.5	2.2 - 2.6	0.8	5.6
	9 mo	2.3 - 2.6	3.1 - 3.2	2.6 - 2.9	2.8 - 3.1	1.9 - 2.3	0.7	4.9
	12 mo	2 - 2.3	2.7 - 2.8	2.2 - 2.5	2.4 - 2.7	1.7 - 2	0.6	4.3
Vit. K	6 mo	5.8 - 15.1	19.4 - 30.2	3.6 - 6	9.1 - 15.1	1.1 - 3	5.0	30.2
	9 mo	3 - 7.9	10.1 - 15.8	1.9 - 3.2	4.7 - 8.3	0.6 - 1.6	2.6	15.8
	12 mo	0.7 - 1.7	2.2 - 3.4	0.4 - 0.7	1 - 3	0.1 - 0.3	0.6	3.4
Thiamin	6 mo	2.1 - 2.9	18.5 - 19.8	1.6 - 2	4.6 - 18.5	3.8 - 6.1	3.2 - 3.8	11.5
	9 mo	1.8 - 2.5	15.8 - 17	1.4 - 1.7	3.9 - 16.2	3.3 - 5.2	2.7 - 3.2	9.9
	12 mo	1.4 - 1.9	12 - 12.9	1.1 - 1.3	3 - 12.8	2.5 - 4	2.1 - 2.5	7.5
Riboflavin	6 mo	3.7 - 4.2	7.4 - 7.5	1.5 - 1.6	2.3 - 2.6	3.2 - 4.9	2.3 - 2.5	3.5
	9 mo	3.4 - 3.9	7 - 7	1.4 - 1.5	2.2 - 2.5	3 - 4.6	2.1 - 2.3	3.3
	12 mo	3.2 - 3.6	6.4 - 6.5	1.3 - 1.4	2 - 2.3	2.8 - 4.3	2 - 2.2	3.0
Niacin	6 mo	3 - 3.7	5.4 - 6.1	2.6 - 2.9	0.8 - 1.1	3.9 - 5.8	2.2 - 2.6	1.5
	9 mo	2.9 - 3.6	5.2 - 5.9	2.5 - 2.8	0.8 - 1	3.7 - 5.5	2.1 - 2.5	1.5
	12 mo	2.8 - 3.4	5 - 5.7	2.4 - 2.7	0.7 - 1	3.6 - 5.3	2 - 2.4	1.4
Vit. B6	6 mo	1.9 - 2.3	3.2 - 3.4	6.7 - 7.8	6 - 7	2.9 - 4.7	3 - 3.1	5.0
	9 mo	1.7 - 2.1	2.9 - 3	6 - 7	5.4 - 6.3	2.6 - 4.2	2.7 - 2.8	4.4
	12 mo	1.5 - 1.8	2.5 - 2.6	5.1 - 6	4.6 - 5.4	2.2 - 3.6	2.3 - 2.4	3.8
Pant. acid	6 mo	2.4 - 2.8	11.2 - 11.8	1.3 - 1.4	1.5 - 3.3	1.1 - 1.7	1.6 - 1.9	2.7
	9 mo	2.2 - 2.6	10.5 - 11.1	1.2 - 1.3	1.4 - 3.1	1.1 - 1.6	1.5 - 1.7	2.5
	12 mo	2.1 - 2.4	2.9 - 10.3	1.1 - 1.3	1.3 - 2.9	1 - 1.5	1.4 - 1.6	2.4
Vit. B12	6 mo	2.2 - 2.7	2 - 5.1	0.9 - 1	5.7 - 7.5	NA	1.1 - 1.3	4.5
	9 mo	2 - 2.4	1.8 - 4.5	0.8 - 0.9	5.1 - 7.5	NA	1 - 1.2	4.0
	12 mo	1.7 - 2.1	3.7 - 3.9	0.7 - 0.8	4.4 - 5.9	NA	0.8 - 1	3.5
Biotin	6 mo	1.1 - 1.6	3 - 3.5	0.9 - 1	1.5 - 2.3	1.3 - 1.7	0.7 - 1.1	3.1
	9 mo	1.1 - 1.5	2.9 - 3.4	0.8 - 1	1.4 - 2.2	1.2 - 1.6	0.7 - 1.1	3.0
	12 mo	1.1 - 1.5	2.8 - 3.3	0.8 - 0.9	1.4 - 2.1	1.2 - 1.6	0.7 - 1	2.9
Folate	6 mo	2.1 - 2.9	5.5 - 6.5	1.2 - 1.4	0.6 - 0.8	0.6 - 1	2.8	9.6
	9 mo	1.8 - 2.5	4.9 - 5.7	1.1 - 1.3	0.5 - 0.7	0.5 - 0.8	2.4	8.4
	12 mo	1.5 - 2.1	4 - 4.7	0.9 - 1	0.4 - 0.6	0.4 - 0.7	2.0	7.0

**Guinea Pig:** When including estimate for vitamin A (as beta-carotene from alfalfa meal) for GP diets, the fold excess range at 6 months is 0.9 – 1.1, at 9 months is 0.7 – 0.9 and at 12 months is 0.5 – 0.6.

**Conclusion:** The calculated fold vitamin excess compared to estimated requirements is over 1 for most vitamins at the 12 month point.

**Table 4b. Demonstration of fold vitamin excess at 6,9,12 months compared to estimated species requirements or adequate intake. The table reflects all diets within Teklad standard diet line (global and traditional).**

	Calculated fold excess at:	Species									
		Rodent	Auto-Rodent	Rodent-Maint.	Rabbit	Guinea Pig	NHP	Canine	Feline	Poultry	Swine
Vit. A	6 mo	1.6-4.6	7.1-10.8	1.6-1.7	1.5-2.1	0.2-0.5	1.2-1.3	1.1	1.1	4.9	1.7-5
	9 mo	1.3-3.6	5.6-8.5	1.4-1.4	1.2-1.7	0.2-0.4	0.9-1	0.9	0.8	3.9	1.4-4
	12 mo	0.9-2.6	3.9-6	0.9-1	0.8-1.2	0.1-0.3	0.7-0.7	0.6	0.6	2.7	1-2.8
Vit. D	6 mo	0.9-3.5	1.3-2.9	0.4-0.4	1.2-1.3	0.6-1	1.9-2.1	2.8	2.9	4.2	3.8-4.7
	9 mo	0.7-2.8	1.1-2.3	0.3-0.3	0.9-1	0.4-0.8	1.5-1.7	2.2	2.4	3.4	3.1-3.8
	12 mo	0.5-2.1	0.8-1.7	0.2-0.2	0.7-0.8	0.3-0.6	1.2-1.3	1.7	1.8	2.5	2.3-2.8
Vit. E	6 mo	1.2-4	1.2-4.2	2.9-3.2	3.2-3.5	2.2-2.6	0.7-0.8	1.7	2.2	3.3	5-6.3
	9 mo	1.1-3.6	1.1-3.7	2.6-2.9	2.8-3.1	1.9-2.3	0.6-0.7	1.5	2.0	2.9	4.4-5.6
	12 mo	0.9-3.1	0.9-3.2	2.2-2.5	2.4-2.7	1.7-2	0.6-0.6	1.3	1.7	2.5	3.9-4.9
Vit. K	6 mo	0.7-15.1	6.6-30.2	3.6-6	9.1-15.1	1.1-3	3-5	0.2	1.6	4.3	5.8-30.2
	9 mo	0.4-7.9	3.5-15.8	1.9-3.2	4.7-8.3	0.6-1.6	1.6-2.6	0.1	0.9	2.3	3-15.8
	12 mo	0.1-1.7	0.8-3.4	0.4-0.7	1-3	0.1-0.3	0.3-0.6	0.3	0.2	0.5	0.7-3.4
Thiamin	6 mo	2.1-5.4	12.9-20	1.6-2	4.6-18.5	3.8-6.1	3-3.8	10.2	1.5	7.6	9.2-21.7
	9 mo	1.8-4.6	11-17.1	1.4-1.7	3.9-16.2	3.3-5.2	2.6-3.2	8.8	1.3	6.5	7.9-18.6
	12 mo	1.4-3.5	8.4-13	1.1-1.3	3-12.8	2.5-4	2-2.5	6.6	1.0	4.9	6-14.1
Riboflavin	6 mo	1.5-4.2	1.9-7.5	1.5-1.6	2.3-2.6	3.2-4.9	2.3-2.5	1.8	2.1	2.6	1.8-3.5
	9 mo	1.4-3.9	1.8-7	1.4-1.5	2.2-2.5	3-4.6	2.1-2.3	1.7	2.0	2.4	1.7-3.3
	12 mo	1.3-3.6	1.7-6.5	1.3-1.4	2-2.3	2.8-4.3	2-2.2	1.6	1.8	2.2	1.5-3
Niacin	6 mo	3-4.2	4.6-7	2.6-2.9	0.8-1.1	3.9-5.8	2.2-2.6	3.4	1.2	1.6	1.2-1.5
	9 mo	2.9-4	4.4-6.7	2.5-2.8	0.8-1	3.7-5.5	2.1-2.5	3.3	1.2	1.6	1.2-1.5
	12 mo	2.8-3.8	4.3-6.5	2.4-2.7	0.7-1	3.6-5.3	2-2.4	3.2	1.1	1.5	1.1-1.4
Vit. B6	6 mo	1.2-2.3	1.2-3.4	6.7-7.8	6-7	2.9-4.7	2.7-3.1	4.7	3.3	2.1	3-5
	9 mo	1.1-2.1	1.1-3	6-7	5.4-6.3	2.6-4.2	2.4-2.8	4.2	3.0	1.8	2.7-4.4
	12 mo	0.9-1.8	0.9-2.6	5.1-6	4.6-5.4	2.2-3.6	2.1-2.4	3.6	2.6	1.6	2.3-3.8
Pant. acid	6 mo	1.6-2.8	3.3-11.8	1.3-1.4	1.5-3.3	1.1-1.7	1.6-1.9	1.3	2.5	2.1	2.4-2.7
	9 mo	1.5-2.6	3.1-11.1	1.2-1.3	1.4-3.1	1.1-1.6	1.5-1.7	1.2	2.4	2.0	2.2-2.5
	12 mo	1.4-2.4	2.9-10.3	1.1-1.3	1.3-2.9	1-1.5	1.4-1.6	1.1	2.2	1.9	2.1-2.4
Vit. B12	6 mo	1.4-2.7	2-5.1	0.9-1	5.7-7.5	NA	0.9-1.3	2.2	3.2	2.5	3.6-4.5
	9 mo	1.3-2.4	1.8-4.5	0.8-0.9	5.1-7.5	NA	0.8-1.2	2.0	2.9	2.3	3.3-4
	12 mo	1.1-2.1	1.6-3.9	0.7-0.8	4.4-5.9	NA	0.7-1	1.1	2.5	1.9	2.8-3.5
Biotin	6 mo	1.1-1.6	1.2-3.5	0.9-1	1.5-2.3	1.3-1.7	0.7-1.1	NA	1.1	1.1	2.7-12.2
	9 mo	1.1-1.5	1.1-3.4	0.8-1	1.4-2.2	1.2-1.6	0.7-1.1	NA	1.1	1.1	2.6-11.8
	12 mo	1-1.5	1.1-3.3	0.8-0.9	1.4-2.1	1.2-1.6	0.7-1	NA	1.1	1.1	2.5-11.4
Folate	6 mo	1.8-2.9	1.4-6.5	1.2-1.4	0.6-0.8	0.6-1	2.4-2.8	5.5	0.4	4.0	6.5-9.6
	9 mo	1.6-2.5	1.3-5.7	1.1-1.3	0.5-0.7	0.5-0.8	2.1-2.4	4.8	0.3	3.5	5.7-8.4
	12 mo	1.3-2.1	1-4.7	0.9-1	0.4-0.6	0.4-0.7	1.7-2	4.0	0.3	2.9	4.7-7

**Guinea Pig:** When including estimate for vitamin A (as beta-carotene from alfalfa meal) for GP diets, the fold excess range at 6 months is 0.9 – 1.1, at 9 months is 0.7 – 0.9 and at 12 months is 0.5 – 0.6.

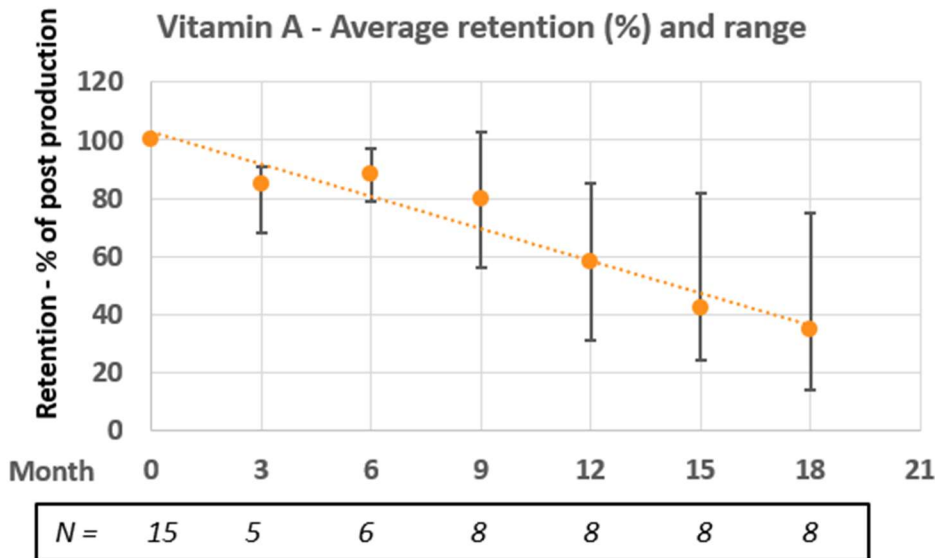
**Conclusion:** The calculated fold vitamin excess compared to estimated requirements is over 1 for most vitamins at the 12 month point.

**Table 7. Significance of fold vitamin excess values near or less than 1, by vitamin and type of diet/species**

Vitamin	Diet Type	Comment
Vit. A	Rodent Maintenance Guinea Pig & Rabbit  NHP General	<ul style="list-style-type: none"> <li>- Vitamin A is intentionally lower in 2014/2914 to reduce risk of excessive vitamin A accumulation in the liver with long-term feeding</li> <li>- Doesn't consider contribution of alfalfa meal, and current feeding practices suggest little risk</li> <li>- Value used for estimated requirement is not the biological minimum</li> <li>- Autoclavable diets – 4X remaining at 12 months covers expected loss with autoclaving</li> </ul>
Vit. D	Multiple species	<ul style="list-style-type: none"> <li>- The estimated requirements across species are not biological minimums</li> <li>- Measurements of aged diet show good stability. 18 samples of primate diet batches (unique lots) were measured from 3 to 16 months post-production. Measured levels ranged from 70 to 140% of theoretical.</li> <li>- NHP's raised in outdoor enclosures can produce vitamin D.</li> </ul>
Vit. E	NHP Rodent	<ul style="list-style-type: none"> <li>- Value used for estimated requirement is not biological minimum</li> <li>- Low end for rodent diets are NIH open formula diets</li> </ul>
Vit. K	Multiple species	<ul style="list-style-type: none"> <li>- Supplementation levels high due to expected degradation</li> <li>- For some species a requirement is not established</li> <li>- Contribution from microbiota, particularly through coprophagy and cecotrophy (rabbits)</li> <li>- Low end for rodent diets are NIH open formula diets</li> </ul>
Thiamin	Multiple species	<ul style="list-style-type: none"> <li>- No concerns theoretical or measured; more stable than literature states</li> <li>- Autoclavable diets – 12X remaining at 12 months covers expected loss with autoclaving</li> </ul>
Riboflavin	Multiple species	<ul style="list-style-type: none"> <li>- No concerns theoretical or measured</li> </ul>
Niacin	Rabbit	<ul style="list-style-type: none"> <li>- Value used for estimated requirement is not biological minimum</li> <li>- Cecotrophy supplies B vitamins</li> </ul>
Vit. B6	Rodent	<ul style="list-style-type: none"> <li>- Low 12 month value from NIH open formula diets</li> </ul>
Pant. acid	Multiple species	<ul style="list-style-type: none"> <li>- No concerns theoretical</li> </ul>
Vit. B12	Rodent Maint.  NHP	<ul style="list-style-type: none"> <li>- Contribution from microbiota through coprophagy; difficult to create deficiency</li> <li>- Value used for estimated requirement is not biological minimum; consider contribution of microbiota.</li> </ul>
Biotin	Rodent Maint. NHP	<ul style="list-style-type: none"> <li>- Contribution of microbiota through coprophagy</li> <li>- Value used for estimated requirement is not biological minimum; consider contribution of microbiota</li> </ul>
Folate	Guinea Pig & Rabbit	<ul style="list-style-type: none"> <li>- Background levels likely underestimated</li> <li>- Contribution from microbiota through coprophagy and cecotrophy</li> </ul>

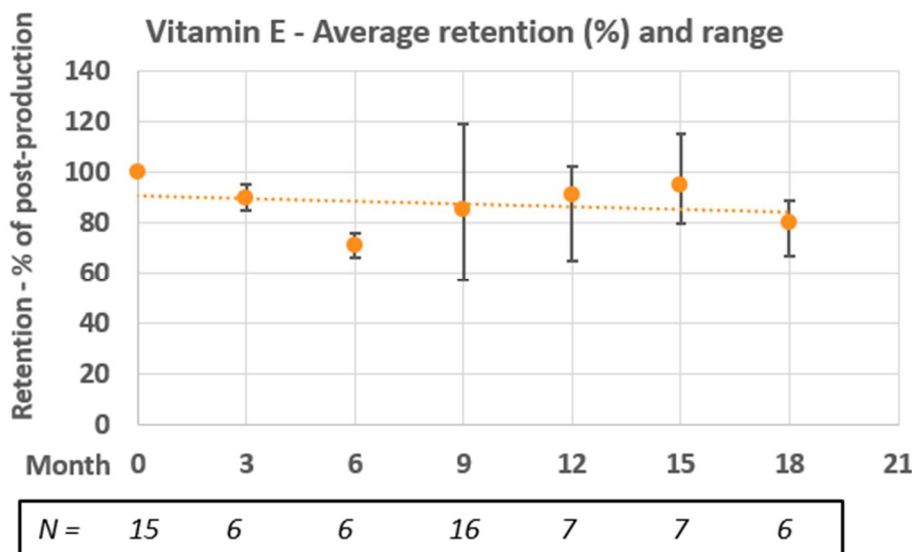
**For figures 1 through 6:** Measured vitamin levels are expressed as percent retention relative to post-production (PP) or frozen retain sample. The bars represent the range of the data (lower and upper). Sample number is shown for each time point.

Figure 1: Vitamin A – Average retention (%) and range



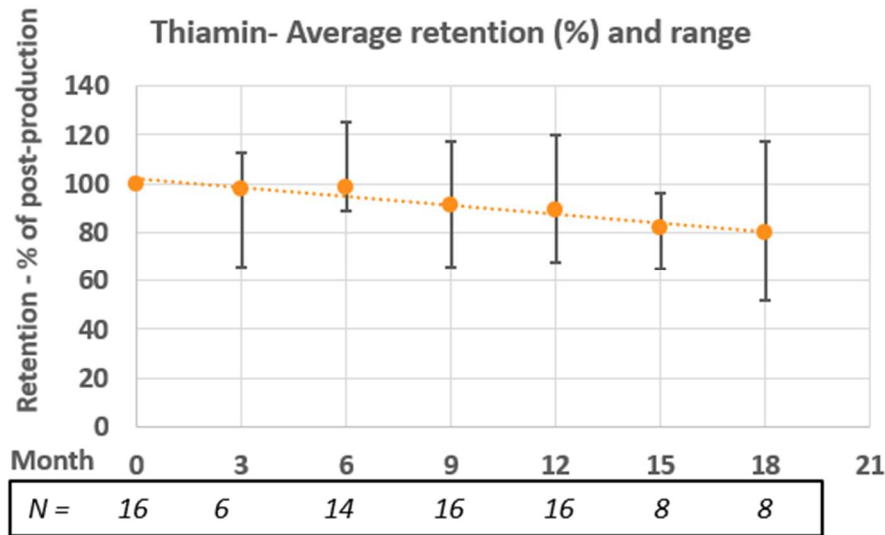
- Diet types measured: rodent, rodent irradiated, rabbit, GP, canine, NHP, irradiated poultry
- Post-production value is 80% of theoretical (on average)
- Slope show 3.7% loss per month with good fit ( $R^2=93\%$ ); rate of loss may be greater after 9 months vs before 9 months
- % retention broadens with time

Figure 2: Vitamin E – Average retention (%) and range



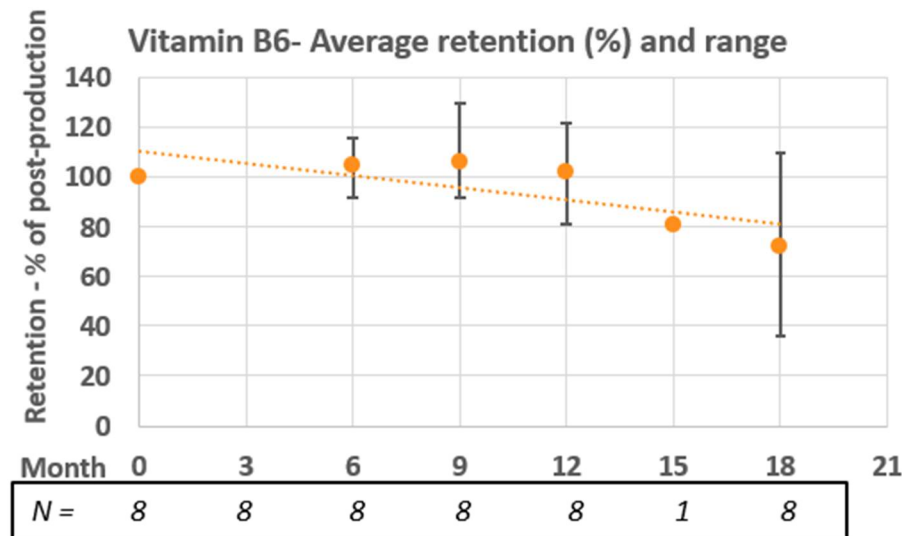
- Diet types measured: rodent, rodent irradiated, rabbit, GP, canine, NHP
- Post-production value is 82% of theoretical (on average)
- Slope shows 0.36% loss per month with poor fit ( $R^2=6\%$ )
- Vitamin E is stable during storage

**Figure 3: Thiamin – Average retention (%) and range**



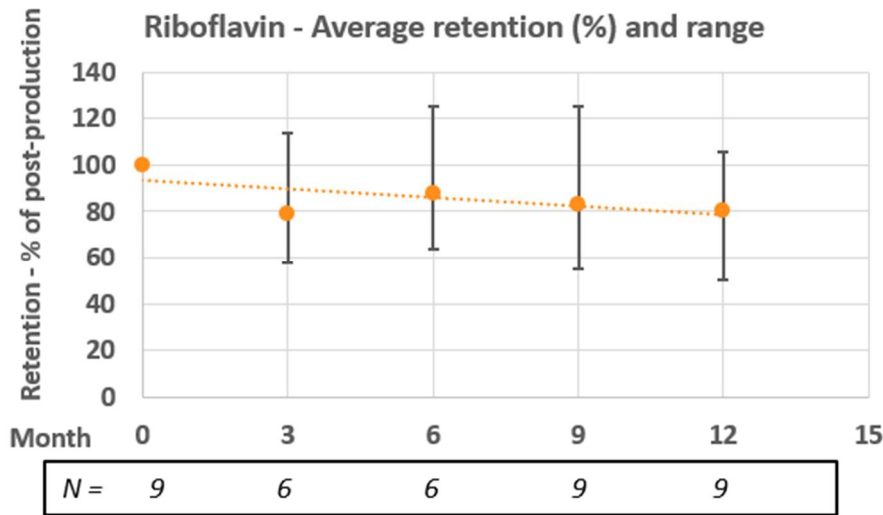
- Diet types measured: rodent, rodent irradiated, rabbit, GP, canine, irradiated poultry
- Post-production value is 98% of theoretical (on average) but with big range; when diets where background thiamin is presumed to be underestimated are removed, the post-production retention is ~85%
- Slope shows 1.2 % loss per month with good fit ( $R^2=94\%$ )
- Thiamin loss during storage is very gradual

**Figure 4: Pyridoxine (vitamin B<sub>6</sub>)– Average retention (%) and range**



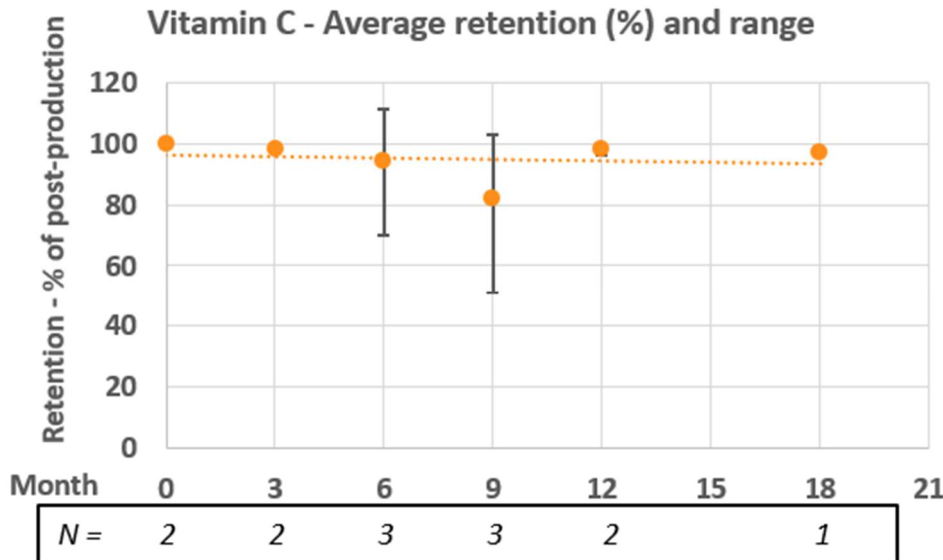
- Diet types measured: rodent, rodent irradiated, rabbit, GP, canine, irradiated poultry
- Post-production value is 81% of theoretical (on average)
- Slope shows 1.6 % loss per month with moderate fit ( $R^2=54\%$ )
- Vitamin B<sub>6</sub> is stable during storage; loss is not really apparent until after 12 months

**Figure 5: Riboflavin - Average retention (%) and range**



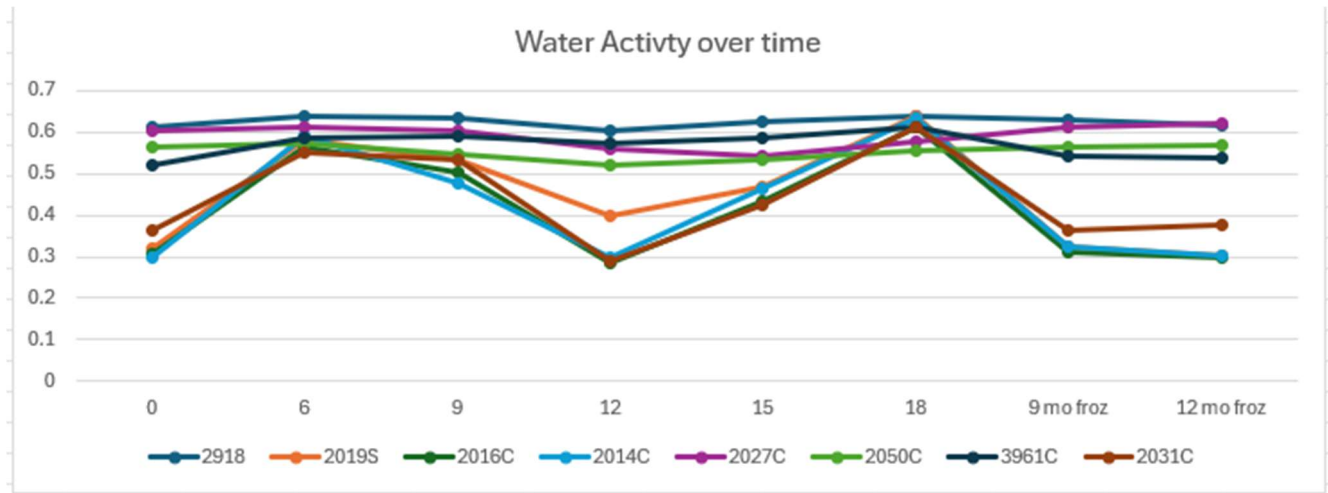
- Diet types measured: rodent, rodent irradiated, rabbit, GP, irradiated poultry
- Post-production value is 93% of theoretical (on average)
- Slope shows 1.2% loss per month with moderate fit ( $R^2=44\%$ )
- Vitamin B<sub>6</sub> loss during storage is very gradual

**Figure 6: Vitamin C- Average retention (%) and range**



- Diet types measured: NHP, GP
- Post-production value is 91% of theoretical (on average)
- For much larger dataset (n=478), PP on average is 86% of formulated
- Slope shows 0.16% loss per month with poor fit ( $R^2=3\%$ )
- Limited number of samples in this dataset, but other data confirms that stabilized vitamin C shows little loss over time
- GP and NHP diets formulated to contain ~4-5X requirement

**Figure 7: Water activity over time in storage**



Water activity of irradiated diet (capsac packaging – 2918 and 3961C) and diets where there is a plastic film liner (2050C and 2027C) stays constant over time, while non-irradiated diet in paper packaging (2019S, 2016C, 2014C, 2031C) does fluctuate. At each time point, a new bag was opened for sampling. The lowest  $A_w$  values were measured in winter and highest in summer.