Effect of trans-Pacific transport and storage time on macro- and micro-nutrient concentrations of LabDiet® PicoLab Rodent Diet 20, 5053, LabDiet® PicoLab® Mouse Diet 20, 5058, and LabDiet® Monkey Diet, 5037, for 24 months post-manufacture (2015).

LabDiet® 5053, 5058, and 5037 were transported via ocean liner from the US to Taiwan in late September 2013 (arriving early October). Data loggers that record time, temperature and humidity were placed within the container at 3 different locations to monitor conditions during transport. Upon arrival in Taiwan diets were stored at 70°F (21°C) and 50% relative humidity for a total of 24 months post manufacturing. Diets were assayed for macronutrients (data not shown) and micronutrients (Tables 1, 2, and 3) every three months including prior to departing the US (Initial sample) and while in Taiwan (months 3, 6, 9, 12, 18, 21, 24, and 27).

Monitoring of Conditions During Transport

Conditions that can affect the vitamin content of a diet the most are temperature and humidity. Diets shipped to Asia via sea would have higher potential for that exposure than any other location we ship diets.

Figure 1 and 2 illustrate the average temperature and humidity levels, with minimum and maximums, each day the container was in transport. Differences were not detected for logger placement within the container so only the data from the loggers placed in the middle of the container are reported.

Figure 1. Daily temperature in middle of container throughout transportation period.

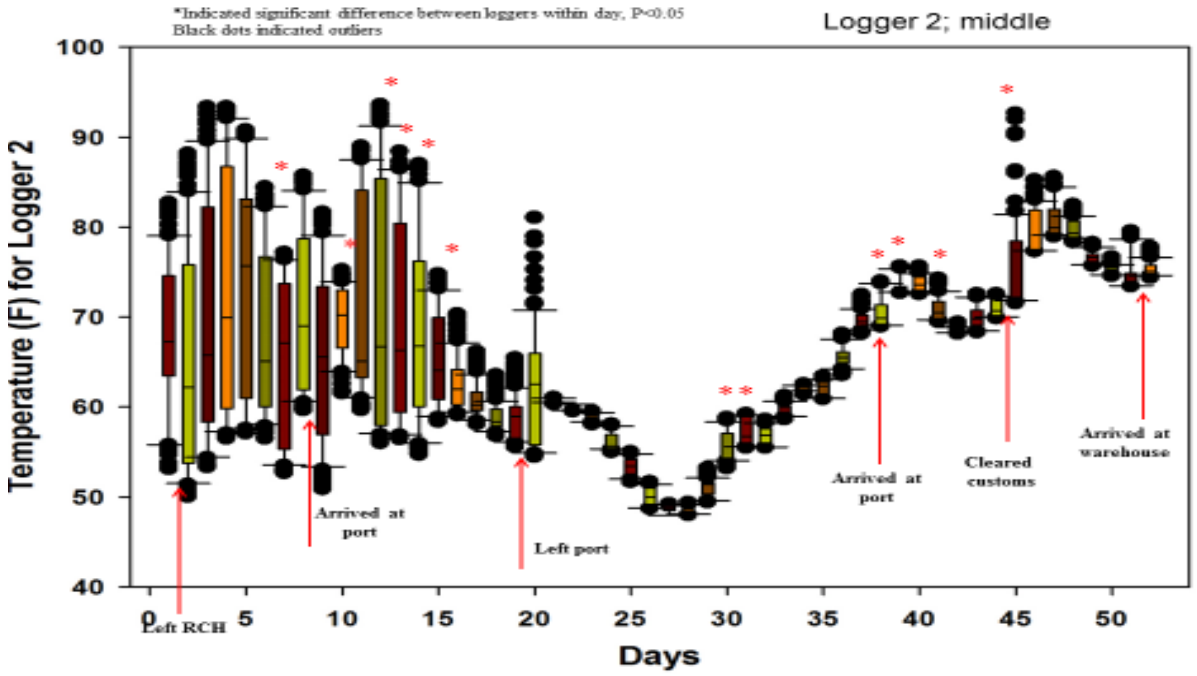
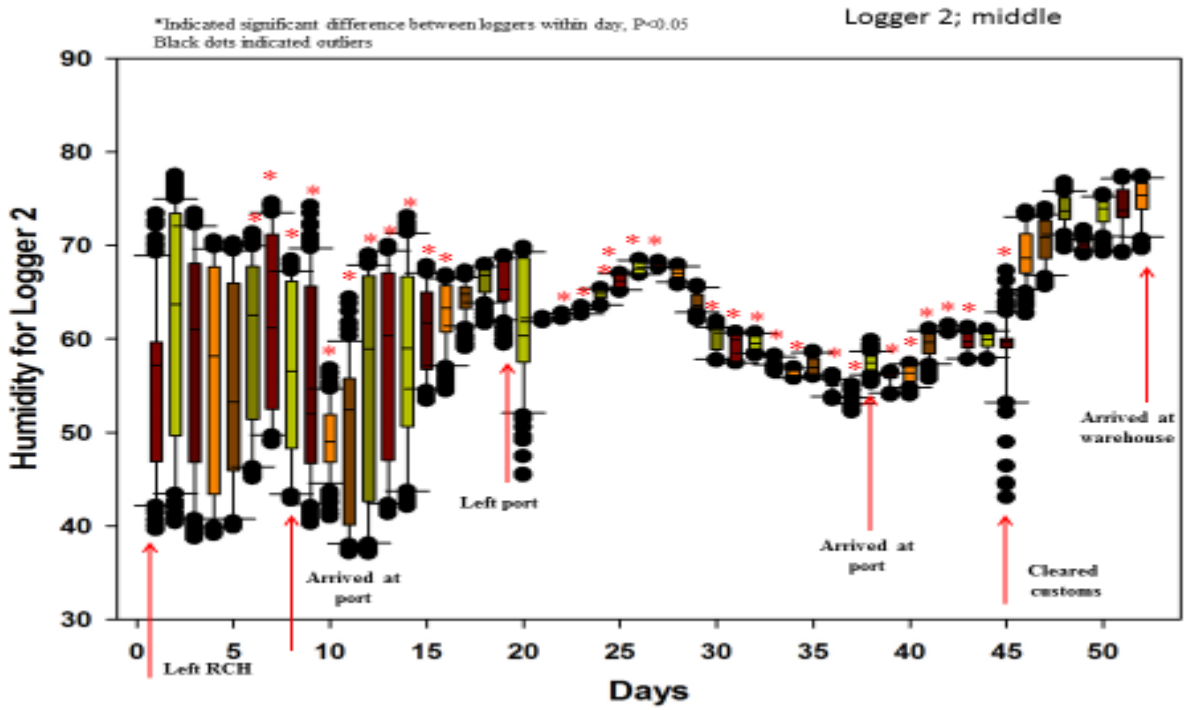


Figure 2. Daily relative humidity in middle of container during transportation period.



The recommended storage conditions for diets are 21°C (70°F) and 50% or less relative humidity; however, it is clear from Figures 1 and 2 that during transport these conditions are exceeded.

Review of Assayed Diets

Significant losses (>50%) were not observed for vitamin E, thiamin, calcium, phosphorous or selenium for all three diets across the 24 month period. This is expected as all are considered to be relatively stable micronutrients at the temperature and humidity levels observed during transport or storage.

Vitamin A concentrations decreased over the 24-month period ranging from 46.8% (5053) to 69.5% (5058) loss. For diet 5058, the average loss of vitamin A per month was 2.9% which is much lower than the estimated 8% per month estimated in Tobin et al. (2007). When the 24-month value is compared to animal requirements (NRC, 1995) rodent diets 5053 and 5058 have 2.3 times and 2.5 times more vitamin A remaining than is required by mice, respectively. Ending vitamin A levels in LabDiet® 5037 were lower than the listed nutrient requirement for non-human primates suggesting that unless vitamin A becomes more stable with changes in technology, storing and utilizing this particular diet for longer than 9-12 months from the date of manufacture may not be suggested. We know there is considerable variation with assaying vitamin A and the NRC requirement is based on limited data, but would still caution on extending the shelf life beyond 9 months for 5037 based on this analyzed data.

Across all diets one of the greatest micronutrient losses seen was in niacin which averaged a 54.2% loss. While this loss may be accurate a few things must be considered when interpreting these values. First, the Initial (1°) sample was analyzed at a different laboratory than the other

samples. Variation in assay techniques may contribute to differences seen from the initial value to the others. We knew such a variation could impact the data; however, we did not have the ability to use the same lab for samples that were assayed in the U.S. prior to shipment and those assayed in China following transport. Additionally, the majority of loss observed was between the initial sample and the 3 month time point with very little loss occurring from 3 months to 24 months (%, 4.5%, and 9.2% for 5053, 5058, and 5037, respectively). Published data has also shown niacin to be a relatively stable vitamin with an estimated loss after 6 months storage of 13.6 to 20% (Tobin, 2007; Coelho, 2002). Regardless of the amount lost all niacin levels remained above the requirements for both rodents and primates (NRC, 1995; NRC, 2003).

Table 1. LabDiet® PicoLab® Rodent Diet 20, 5053.

		Time, months[1]									
Nutrient	**Min Nutr. Requirements[2]**	**Initial[3,4]** (1°)	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**% Loss**
Vitamin A, IU/g	2.4	10.1	13.3	9.0	11.8	8.8	7.9	8.5	6.2	5.4	46.8
Vitamin E, IU/kg	27.0	93.0	142.9	149.8	145.4	135.5	126.8	103.8	100.4	101.5	0
Thiamin, mg/kg	5.0	12.6	12.7	14.5	15.5	13.2	16.7	13.7	13.0	14.1	0
Niacin, mg/kg	15.0	111.0	58.3	50.3	36.9	54.1	46.6	47.9	45.6	65.9	40.6
Calcium, %	0.5	0.9	0.8	1.0	0.9	0.9	0.8	0.9	0.9	0.9	4.5
Phosphorous, %	0.3	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.7	0.6	10.8
Selenium, mg/kg	0.2	0.4	0.5	0.9	0.8	0.6	0.7	0.8	0.9	0.6	0

[1] Samples were assayed by Eurofins (Jiangsu Province, P.R.China) upon arrival to Taiwan
[2] Nutrient requirements of mice according to Nutrient Requirements of Laboratory Animals (NRC), 1995.
[3] Initial values are assayed values taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.
[4] Initial samples were assayed by Covance Laboratories (Madison, WI) prior to diets being transported to Taiwan

Table 2. LabDiet® PicoLab® Mouse Diet 20, 5058.

		Time, months[1]									
Nutrient	**Min Nutr. Requirements[2]**	**Initial[3,4]** (1°)	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**% Loss**
Vitamin A, IU/g	2.4	19.1	8.5	11.0	10.7	8.0	9.0	8.9	5.2	5.8	69.5
Vitamin E, IU/kg	27.0	69.3	79.3	80.7	87.9	76.9	66.0	57.9	47.5	54.0	22.0
Thiamin, mg/kg	5.0	10.3	12.2	12.7	13.2	10.5	11.3	11.6	12.0	12.0	0
Niacin, mg/kg	15.0	103.0	46.0	53.6	34.6	55.4	44.3	44.8	38.7	41.4	59.8
Calcium, %	0.5	0.9	0.9	0.8	0.9	0.8	0.7	0.8	0.8	0.8	8.4
Phosphorous, %	0.3	0.6	0.7	0.6	0.7	0.6	0.5	0.6	0.6	0.5	13.0
Selenium, mg/kg	0.2	0.3	0.5	0.7	0.9	0.6	0.7	0.8	0.7	0.5	0

[1]Samples were assayed by Eurofins (Jiangsu Province, P.R.China) upon arrival of diets to Taiwan
[2] Nutrient requirements of mice according to Nutrient Requirements of Laboratory Animals (NRC), 1995
[3] Initial values are assayed values taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.[4] Initial samples were assayed by Covance Laboratories (Madison, WI) prior to diets being transported to Taiwan

Table 3. LabDiet® Monkey Diet Jumbo, 5037.

Nutrient	Min Nutr. Requirements[2]	Time, months[1] Initial[3, 4] (1°)	3	6	9	12	15	18	21	24	% Loss
Vitamin A, IU/g	8.0	14.1	12.8	11.5	12.3	8.2	9.0	9.5	7.2	6.6	53.4
Vitamin E, IU/kg		96.2	134.9	133.0	144.7	129.2	130.3	115.8	50.8	101.3	0
Thiamin, mg/kg	3.0	9.6	8.5	11.9	11.6	11.6	13.9	11.3	12.1	11.4	0
Niacin, mg/kg	25.0	155.0	72.6	NA	63.0	77.9	71.8	66.5	68.0	58.4	62.3
Calcium, %	0.8	1.0	1.0	1.0	1.1	0.9	0.8	1.0	0.9	1.0	1.96
Phosphorous, %	0.6	0.7	0.6	0.6	0.7	0.6	0.6	0.6	0.7	0.6	9.09
Selenium, mg/kg	0.3	0.3	0.5	0.7	0.7	0.5	0.6	0.8	0.8	0.4	0

[1] Samples were assayed by Eurofins (Jiangsu Province, P.R.China) upon arrival of diets to Taiwan
[2] Nutrient requirements of non-human primates according to Nutrient Requirements of Nonhuman Primates (NRC), 2003
[3] Initial values are assayed values taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.
[4] Initial samples were assayed by Covance Laboratories (Madison, WI) prior to diets being transported to Taiwan

In summary, we did not find significant decreases in nutrients from the initial and the 3 month samples assayed even when the diets were exposed to conditions one would not typically experience in a facility or even during transport within North America or to Europe. The loss, for only some vitamins, was gradual over time suggesting that time also plays a factor in that loss and not just the level of humidity or the temperature. In more extreme conditions, like with autoclaving, temperature appears to play more of a role in nutrient loss than time.

As illustrated by Toben et al. (2007) and other studies discussed in this document marginal loss in micronutrients can be expected over a period of time when stored under proper conditions, so it can be expected that some loss would occur during the current 24 month test period. These results indicate LabDiet® products are formulated to meet or safely exceed animal nutrient requirements for as much as 9 months, and potentially longer for some diets.

Coelho, M. 2002. Vitamin Stability in Premixes and Feeds. A practical approach in ruminant diets. Proc. 13th. Annual Florida Ruminant Nutrition Symp. 127-145.

NRC. 2003. The Nutrient Requirements of Nonhuman Primates. 2nd rev. ed. Natl Acad Press, Washington DC.

NRC. 1995. The Nutrient Requirements of Laboratory Animals. 4th rev. ed. Natl Acad Press, Washington, DC.

Tobin G, KA Stevens and RJ Russell. 2007. Nutrition In: The mouse in biomedical research. 2ed. Elsevier, New York, NY. 3:343-344.