Heavy Metal (HM) concentrations of different commercially available grain-based diets (chows) are variable and increase renal, splenic and hepatic arsenic (As) and cobalt (Co) levels relative to purified diets in weanling, female Sprague-Dawley rats

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BACKGROUND

- Dietary concentrations of heavy metals (HM) can unintentionally affect phenotype, for example gene expression (Kozul, et al.).
- Since chows contain relatively unrefined plant and animal material, HM levels may be higher and more variable compared to purified diets. However, there is little known about the levels and variability in HM levels in chows and purified diets and how dietary HM levels affect tissue HM levels.
- We measured dietary HM levels as well as tissue HM levels in rats fed two chows and three purified diets. Since we noted variation in dietary HM levels in these two chows, we also measured HM levels in a larger number of chows to examine within and between diet variability.

MATERIALS AND METHODS

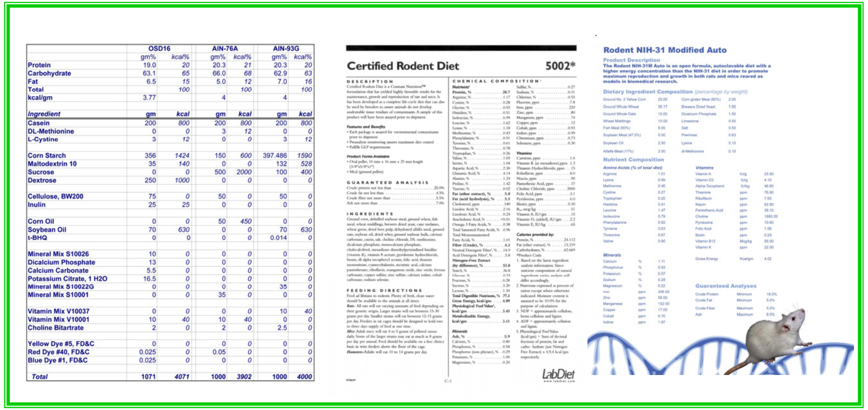
In vivo study:

- Female Sprague-Dawley rats maintained at Taconic Biotechnology, NY (age= 25 d, n=45, singly housed) were fed 1 of 5 diets (n= 9 per group): 2 chow diets (Purina 5002, Zeigler NIH-31M) or 3 purified ingredient diets (Open Standard Diet [OSD], AIN-76A or AIN-93G, Research Diets, Inc.) for 28 days.
- At termination, tissues were frozen and later were analyzed for HM along with the diets by ICP-MS.

Dietary HM Study:

- In a separate study, HM levels were measured in 9 chow diets (5 from Purina, 3 from Harlan and 1 from Zeigler) and 3 purified diets (OSD, AIN-76A, AIN-93G [Research Diets, Inc.]).
- Data are expressed as means ± SE. Statistical significance was determined by one-way ANOVA with Tukey's post-hoc t-test. Different letters indicate significant differences among groups.

Table 1 – Dietary Formulas or Spec Sheets (see attached)



RESULTS

Figure 1

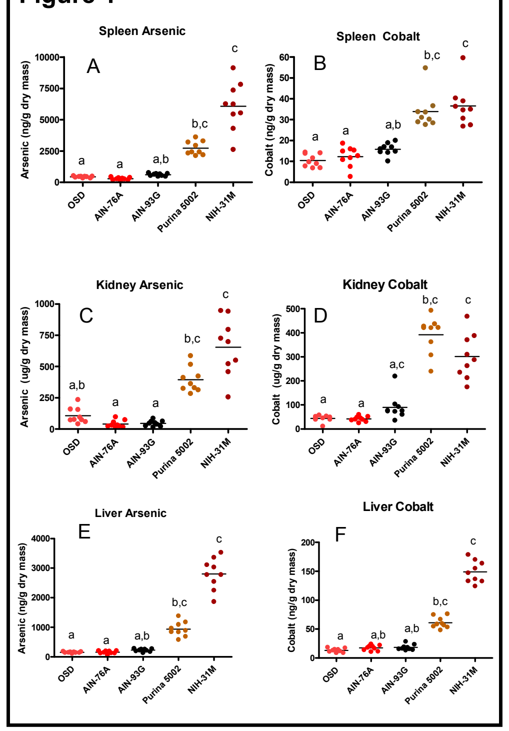


Figure 2

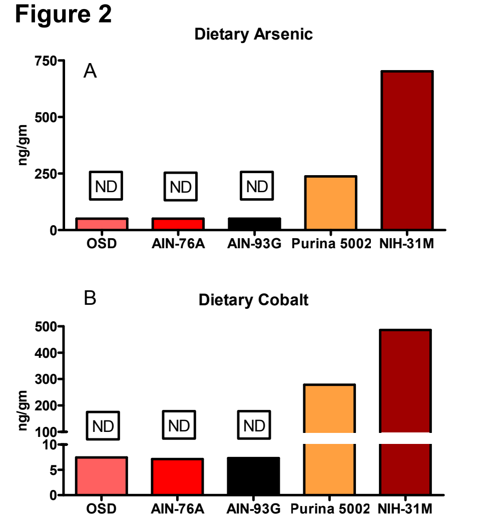


Figure 3 - Chows

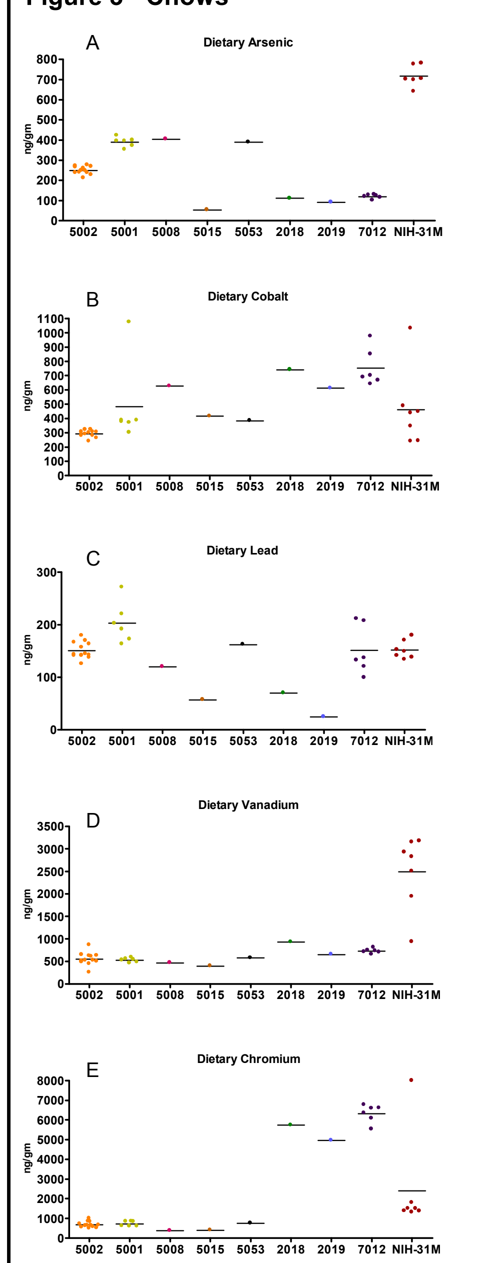


Figure 4 – Purified Diets

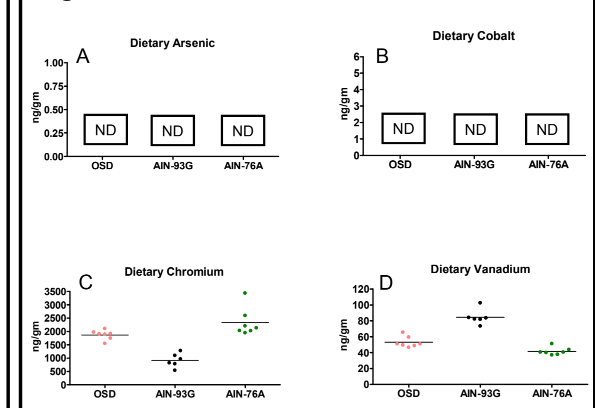
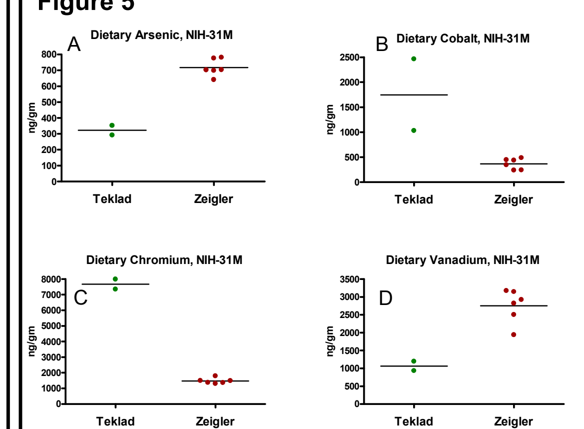


Figure 5



Purified Ingredient Diet

Grain-based Chow

SUMMARY

In vivo study:

- Of the HM measured, the largest tissue differences between diet treatments were found with As and Co. Overall, rats fed purified diets had lower spleen, kidney and liver As and Co levels compared to chow diets (Fig. 1).
- The largest difference in tissue HM levels was found in spleen for As between AIN-76A vs. NIH-31M (278.5 ± 35.3 vs. 6076.3 ± 643.3 ng/gm, respectively; P<0.001; Fig. 1 A).
- As was undetectable in the purified diets (Fig. 2) and tissue As levels were lowest in the purified diets (Fig. 1).
- Dietary As was 3 fold higher in NIH-31M vs. Purina 5002 (702.2 vs. 237.2 ng/gm, respectively, Fig. 2A) and liver As was 3 fold higher in NIH-31M vs. Purina 5002 (2806.1 ± 58.9 vs. 939.7 ± 83.0 ng/gm, P>0.05, Fig 1E).

Dietary HM level study:

- HM levels varied widely in chows: (Fig. 3): As (52.6 to 776 ng/gm), Co (240 to 1075 ng/gm), Lead (Pb) (24.3 to 271 ng/gm), Vanadium (V) (394.9 to 3175 ng/gm) and Cr (369 to 6773 ng/gm).
- In purified diets, HM levels were lower compared to chows (Fig. 4): As (Undetectable to 5.9 ng/gm), Co (3.0 to 5.6 ng/gm), (V) (37.6 to 84.0 ng/gm) and Cr (536 to 2594 ng/gm) [Cr is added to purified diets purposely]. Pb was largely undetected in purified diets and was not graphed.
- HM levels in batches of NIH-31M chow varied depending on the manufacturer (Fig. 5), the greatest difference being Cr: 1472 ± 71.1 (Zeigler) vs. 7670 ± 318 ng/gm. (Teklad)(Fig. 5C). Even lots from the same manufacturer showed variability for Co (1031 vs. 2464 ng/gm; Teklad) (Fig. 5B) and V: (1937 vs. 3175 ng/gm, Zeigler) (Fig. 5D).

CONCLUSIONS

- Compared to purified diets, chow HM levels are not only more variable but are also up to ~190 fold higher.
- Even within the same chow (NIH-31M) there was both within and between manufacturer variation (up to 5 fold).
- Since HM tissue levels directly reflect dietary concentrations, purified diets can be used to limit tissue accumulation, and likely reduce changes in the toxicological phenotype caused by these metals.

REFERENCES

Kozul, C, et al. Chemico-Biological Interactions, 173: 129-140, 2008

Certified Rodent Diet 5002*

DESCRIPTION

Certified Rodent Diet is a Constant Nutrition® formulation that has yielded highly favorable results for the maintenance, growth and reproduction of rats and mice. This diet is formulated using the unique and innovative concept of Constant Nutrition®, paired with the selection of highest quality ingredients to assure minimal inherent biological variation in long-term studies. It has been developed as a complete life-cycle diet. A sample of this product will have been assayed and approved prior to shipment to assure GLP compliance.

Features and Benefits

- Constant Nutrition® formula helps minimize nutritional variables
- High quality animal protein added to create a superior balance of amino acids for optimum performance
- Each package is assayed for environmental contaminants prior to shipment
- Preanalysis monitoring, Constant Nutrition® formulation, along with selection of highest quality ingredients, assures maximum diet control
- Fulfills GLP requirements

Product Forms Available

- Oval pellet, 10 mm x 16 mm x 25 mm length (3/8"x5/8"x1")
- Meal (ground pellets)

GUARANTEED ANALYSIS

Crude protein not less than	20.0%
Crude fat not less than	4.5%
Crude fiber not more than	5.5%
Ash not more than	7.0%

INGREDIENTS

Ground corn, dehulled soybean meal, ground wheat, fish meal, wheat middlings, brewers dried yeast, cane molasses, wheat germ, dried beet pulp, dehydrated alfalfa meal, ground oats, soybean oil, dried whey, ground soybean hulls, calcium carbonate, casein, salt, choline chloride, DL-methionine, dicalcium phosphate, monocalcium phosphate, cholecalciferol, menadione dimethylpyrimidinol bisulfite (vitamin K), vitamin A acetate, pyridoxine hydrochloride, biotin, dl-alpha tocopheryl acetate, folic acid, thiamin mononitrate, vitamin B_{12} supplement, nicotinic acid, calcium pantothenate, riboflavin, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate, sodium selenite.

FEEDING DIRECTIONS

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

Rats- All rats will eat varying amounts of feed depending on their genetic origin. Larger strains will eat up to 30 grams per day. Smaller strains will eat up to 15 grams per day. Feeders in rat cages should be designed to hold two to three days supply of feed at one time.

Mice-Adult mice will eat up to 5 grams of pelleted ration daily. Some of the larger strains may eat as much as 8 grams per day per animal. Feed should be available on a free choice basis in wire feeders above the floor of the cage.

Hamsters-Adults will eat up to 14 grams per day.

CHEMICAL COMPOSITION[1]

Nutrients[2]

Protein, %	**20.7**
Arginine, %	1.17
Cystine, %	0.28
Glycine, %	0.92
Histidine, %	0.51
Isoleucine, %	0.99
Leucine, %	1.62
Lysine, %	1.18
Methionine, %	0.43
Phenylalanine, %	0.91
Tyrosine, %	0.61
Threonine, %	0.78
Tryptophan, %	0.26
Valine, %	1.03
Serine, %	1.04
Aspartic Acid, %	2.30
Glutamic Acid, %	4.14
Alanine, %	1.24
Proline, %	1.42
Taurine, %	0.02
Fat (ether extract), %	**5.0**
Fat (acid hydrolysis), %	**5.5**
Cholesterol, ppm	140
Linoleic Acid, %	2.16
Linolenic Acid, %	0.24
Arachidonic Acid, %	<0.01
Omega-3 Fatty Acids, %	0.38
Total Saturated Fatty Acids, %	0.96
Total Monounsaturated Fatty Acids, %	1.01
Fiber (Crude), %	**4.3**
Neutral Detergent Fiber[3], %	14.9
Acid Detergent Fiber[4], %	5.8
Nitrogen-Free Extract (by difference), %	**53.8**
Starch, %	36.8
Glucose, %	0.23
Fructose, %	0.28
Sucrose, %	3.20
Lactose, %	1.34
Total Digestible Nutrients, %	**77.3**
Gross Energy, kcal/gm	**4.09**
Physiological Fuel Value[5], kcal/gm	**3.43**
Metabolizable Energy, kcal/gm	**3.11**

Minerals

Ash, %	**5.9**
Calcium, %	0.80
Phosphorus, %	0.58
Phosphorus (non-phytate), %	0.29
Potassium, %	1.00
Magnesium, %	0.20
Sulfur, %	0.27
Sodium, %	0.31
Chlorine, %	0.55
Fluorine, ppm	7.8
Iron, ppm	220
Zinc, ppm	80
Manganese, ppm	74
Copper, ppm	12
Cobalt, ppm	0.93
Iodine, ppm	0.99
Chromium, ppm	0.73
Selenium, ppm	0.30

Vitamins

Carotene, ppm	1.6
Vitamin K (as menadione), ppm	1.3
Thiamin Hydrochloride, ppm	15
Riboflavin, ppm	8.0
Niacin, ppm	90
Pantothenic Acid, ppm	17
Choline Chloride, ppm	2000
Folic Acid, ppm	3.1
Pyridoxine, ppm	6.0
Biotin, ppm	0.30
B_{12}, mcg/kg	51
Vitamin A, IU/gm	15
Vitamin D_3 (added), IU/gm	2.2
Vitamin E, IU/kg	65

Calories provided by:

Protein, %	24.112
Fat (ether extract), %	13.219
Carbohydrates, %	62.669

*Product Code

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.
2. Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.
3. NDF = approximately cellulose, hemi-cellulose and lignin.
4. ADF = approximately cellulose and lignin.
5. Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbo- hydrate (use Nitrogen Free Extract) x 4,9,4 kcal/gm respectively.

LabDiet
www.labdiet.com

Rodent NIH-31 Modified Auto

Product Description

The Rodent NIH-31M Auto is an open formula, autoclavable diet with a higher energy concentration than the NIH-31 diet in order to promote maximum reproduction and growth in both rats and mice reared as models in biomedical research.

Dietary Ingredient Composition *(percentage by weight)*

Ground No. 2 Yellow Corn	20.00	Corn gluten Meal (60%)	2.00
Ground Whole Wheat	35.17	Brewers Dried Yeast	1.00
Ground Whole Oats	10.00	Dicalcium Phosphate	1.50
Wheat Middlings	10.00	Limestone	0.50
Fish Meal (60%)	9.00	Salt	0.50
Soybean Meal (47.5%)	5.00	Premixes	0.63
Soybean Oil	2.50	Lysine	0.10
Alfalfa Meal (17%)	2.00	dl-Methionine	0.10

Nutrient Composition

Amino Acids (% of total diet)

Arginine	1.01
Lysine	0.99
Methionine	0.46
Cystine	0.27
Tryptophan	0.20
Histidine	0.41
Leucine	1.47
Isoleucine	0.79
Phenylalanine	0.82
Tyrosine	0.63
Threonine	0.67
Valine	0.90

Minerals

Calcium	%	1.11
Phosphorus	%	0.93
Potassium	%	0.57
Sodium	%	0.28
Magnesium	%	0.22
Iron	ppm	206.00
Zinc	ppm	58.00
Manganese	ppm	152.00
Copper	ppm	17.00
Cobalt	ppm	0.70
Iodine	ppm	1.97

Vitamins

Vitamin A	IU/g	25.90
Vitamin D3	IU/g	4.10
Alpha-Tocopherol	IU/kg	46.80
Thiamine	ppm	76.90
Riboflavin	ppm	7.60
Niacin	ppm	82.80
Pantothenic Acid	ppm	38.10
Choline	ppm	1880.00
Pyridoxine	ppm	10.60
Folic Acid	ppm	1.56
Biotin	ppm	0.29
Vitamin B12	Mcg/kg	56.00
Vitamin K	ppm	22.00
Gross Energy	Kcal/gm	4.02

Guaranteed Analyses

Crude Protein	Minimum	18.0%
Crude Fat	Minimum	5.0%
Crude Fiber	Maximum	5.0%
Ash	Maximum	8.0%