Committee Report

AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet

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ABSTRACT For sixteen years, the American Institute of Nutrition Rodent Diets, AIN-76 and AIN-76A, have been used extensively around the world. Because of numerous nutritional and technical problems encountered with the diet during this period, it was revised. Two new formulations were derived: AIN-93G for growth, pregnancy and lactation, and AIN-93M for adult maintenance. Some major differences in the new formulation of AIN-93G compared with AIN-76A are as follows: 7 g soybean oil/100 g diet was substituted for 5 g corn oil/100 g diet to increase the amount of linolenic acid; cornstarch was substituted for sucrose; the amount of phosphorus was reduced to help eliminate the problem of kidney calcification in female rats; L-cystine was substituted for DL-methionine as the amino acid supplement for casein, known to be deficient in the sulfur amino acids; manganese concentration was lowered to one-fifth the amount in the old diet; the amounts of vitamin E, vitamin K and vitamin B-12 were increased; and molybdenum, silicon, fluoride, nickel, boron, lithium and vanadium were added to the mineral mix. For the AIN-93M maintenance diet, the amount of fat was lowered to 40 g/kg diet from 70 g/kg diet, and the amount of casein to 140 g/kg from 200 g/kg in the AIN-93G diet. Because of a better balance of essential nutrients, the AIN-93 diets may prove to be a better choice than AIN-76A for long-term as well as short-term studies with laboratory rodents. J. Nutr. 123: 1939–1951, 1993.

INDEXING KEY WORDS:

- *purified diet* • *nutrient requirements*
- *rats* • *mice*

In 1973, an ad hoc committee was formed by the American Institute of Nutrition (AIN) to identify dietary standards for nutritional studies with laboratory rodents. The goal for the committee's work was to establish guidelines that would help scientists with limited experience in experimental nutrition to feel confident with the nutritional aspects of their studies. There was an increasing awareness of a need for nutritionally adequate purified diets that could be used to standardize studies among laboratories. The intent of standardization of test diets for laboratory animals was to reduce the variation inherent in cereal-based or natural ingredient–based diets and to facilitate interpretation of results among experiments and laboratories. The outcome of the committee's deliberations was the now well-known AIN-76 rodent diet. Detailed compositional analysis of this diet and the vitamin and mineral mixes can be found in AIN (1977).

In 1982, a workshop, Nutritional Standards for Laboratory Animal Diets, was sponsored by the International Committee for Laboratory Animal Science at the XII International Congress of Nutrition (Coates 1982a and 1982b). Participants at the workshop expressed concern that poor communication between non-nutritionists and nutritionists caused the former to be "insufficiently aware of the potential influence that a test animal's diet can have on its response to a test compound." Nutritionists, on the other hand, were thought to have inadequately considered the effects of long-term feeding of currently formulated diets. It was the consensus of the workshop participants that a general diet should be formulated that would "enable valid comparisons to be made between results of toxicity or oncogenicity trials in different laboratories."

It is unrealistic, however, to presume that a perfect diet can be formulated, one that can satisfy all circumstances. Moreover, the ingredients that supply the nutrients and the concentrations of individual nutrients themselves are based upon current knowledge in the field and may change with time. This may result in a need to change a standard diet formulation. Shortly after introduction of the first

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formulation of AIN-76, a revision was published (AIN 1980) and designated AIN-76A. Since then, this diet has been used extensively and has served the scientific community well. However, major criticisms of the formulation suggested that further revisions were needed.

At the Federation of American Societies for Experimental Biology (FASEB) meeting in 1988, Forrest Nielsen chaired an ad hoc committee to discuss the issue of whether the AIN-76A diet guidelines needed updating or modification. There were concerns about both nutritional and technical problems with the diet. It was the consensus of the committee to hold a workshop to discuss the shortcomings of the AIN-76A formulation and to decide if changes were warranted.

The workshop was organized by Forrest Nielsen and Philip Reeves, and was held during the 1989 meeting of FASEB. The workshop was composed of 10 discussion groups, each focused on a major nutrient class. Each group was made up of three individuals who were experts in their particular field (see Composition of the AIN-76 Diet Workshop). The groups were asked to review the diet formulation and make recommendations based on new knowledge since the last revision of AIN-76A. A summary of the workshop findings was published in THE JOURNAL OF NUTRITION (Reeves 1989). In that summary, a request was made for further suggestions from the scientific community on how the diet might be improved. Based on suggestions from the workshop participants and on those made in the months following the workshop, new diets were formulated and tested.

It must be emphasized that the new diets were formulated for growth, pregnancy and lactation, and maintenance of rats and mice during normal husbandry. These formulations were based on the following criteria: they can be made from purified ingredients; they conform to or exceed the nutrient requirements set forth by the National Research Council (1978); they can be made with readily available ingredients at reasonable cost; the compositions are consistent and reproducible; and they can be used over a wide range of applications. Any diversions from these criteria such as manipulation of the ingredients for experimental purposes are the responsibility of the individual investigator. See Precautions for a Diet Reformulation.

DIET COMPOSITION

General considerations

Two formulations of the new diet are presented. The AIN-93G diet is recommended to support growth, pregnancy and lactational phases. The AIN-93M diet has a lower protein and fat content and is recommended for adult maintenance. The maintenance diet is based on concerns of investigators in toxicology and oncology research who suggest that a diet with a lower protein and fat content than AIN-76A is more suitable for long-term studies (Coates 1987, Rao 1988). In addition, this follows the general guidelines used in the feeding of livestock, poultry and companion animals where fewer nutrients such as protein and fat are provided to these species when they are held at maintenance.

A list of ingredients for the AIN-93G diet can be found in **Table 1**. A list of recommended mineral elements and the amounts contributed to the diet by the mineral mixes for each diet are found in **Table 2**. The composition of the mineral mix (AIN-93G-MX) that meets the recommendations for AIN-93G is found in **Table 3**. The recommended dietary concentrations of vitamins are listed in **Table 4**, and in **Table 5** is presented the composition of the vitamin mix (AIN-93-VX) that will supply these concentrations.

Table 6 contains the formulation for the AIN-93M diet. The recommended dietary mineral elements and the amounts contributed by the mineral mix are shown in Table 2. **Table 7** is the suggested formula for the mineral mix (AIN-93M-MX) to attain these dietary concentrations. The vitamin mix for the AIN-93M diet is the same as for AIN-93G (Table 5). **Table 8** lists the estimated nutrient composition of the complete diets when composed of the various ingredients and mixes recommended in the previous tables.

Nutrient composition of AIN-93

Major changes were made in the new diet compared with AIN-76A. The following is a listing of those changes and the rationale for making them. Major changes in dietary ingredients included the form of carbohydrate, the form and amount of fat, and the sulfur amino acid supplement. There were major changes in the mineral mix, including the amount and form of phosphorus, the form of calcium, the amount of manganese, the form and amount of selenium, and the addition of molybdenum. The addition of trace and ultratrace elements with significant, but circumstantial, evidence for essentiality was recommended. Changes in the vitamin mix included the amount of vitamin E, the form and amount of vitamin K, and the amount of vitamin B-12.

Carbohydrate. In the AIN-76 formulation, sucrose is the major carbohydrate. Because of possible adverse effects of using sucrose, recommendations for change were made in the subsequent revision (AIN-76A; AIN 1980). These included substituting glucose for sucrose, replacing half the sucrose with glucose, substituting cornstarch for sucrose, or varying the carbohydrate portion by using a combination of the three. None of these suggested changes was recommended over the others.

TABLE 1

AIN-93G diet formulated for the growth, pregnancy and lactational phases of rodents

Ingredient	g/kg diet
Cornstarch	397.486
Casein (≥85% protein)	200.000
Dextrinized cornstarch (90–94% tetrasaccharides)[1]	132.000
Sucrose	100.000
Soybean oil (no additives)	70.000
Fiber[2]	50.000
Mineral mix (AIN-93G-MX)	35.000
Vitamin mix (AIN-93-VX)	10.000
L-Cystine	3.000
Choline bitartrate (41.1% choline)[3]	2.500
Tert-butylhydroquinone	0.014

[1]Dyetrose (Dyets, Bethlehem, PA) and Lo-Dex 10 (American Maize, Hammond, IN) meet these specifications. An equivalent product may also be used.

[2]Solka-Floc®, 200 FCC (FS&D, St. Louis, MO) or its equivalent is recommended.

[3]Based on the molecular weight of the free base.

TABLE 2

Contribution of mineral elements to the AIN-93G and AIN-93M diets when the recommended mineral mixes AIN-93G-MX and AIN-93M-MX, respectively, are fed at 35 g/kg of the diet

	Diet	
	AIN-93G	AIN-93M
	mg/kg diet	
Essential mineral element		
Calcium	5000.0	5000.0
Phosphorus[1]	1561.0	1992.0
Potassium	3600.0	3600.0
Sulfur	300.0	300.0
Sodium	1019.0	1019.0
Chloride	1571.0	1571.0
Magnesium	507.0	507.0
Iron	35.0	35.0
Zinc	30.0	30.0
Manganese	10.0	10.0
Copper	6.0	6.0
Iodine	0.2	0.2
Molybdenum	0.15	0.15
Selenium	0.15	0.15
Potentially beneficial mineral element		
Silicon	5.0	5.0
Chromium	1.0	1.0
Fluoride	1.0	1.0
Nickel	0.5	0.5
Boron	0.5	0.5
Lithium	0.1	0.1
Vanadium	0.1	0.1

[1]A total of 3000 mg P/kg diet is recommended for each diet. The difference between the contribution of the mix and the recommended dietary amount is made up from the contribution of phosphorus from casein.

Participants in the 1989 workshop suggested that the sucrose content of the new diet be markedly reduced and replaced with starch. It was feared, however, that a diet with a large amount of starch would not pellet properly. Recent tests showed that the inclusion of dextrinized cornstarch (90 to 94% tetrasaccharide: Dyetrose, Dyets, Bethlehem, PA; Lo-Dex 10, American Maize, Hammond, IN)[1] at 130–150 g/kg and sucrose at 100 g/kg in the AIN-93 starch-based diets aided pelleting, reduced heat generation and decreased the time of exposure of the diet to pelleting temperatures. Therefore, the recommended carbohydrate forms and amounts (g/kg diet) for the AIN-93G diet are sucrose, 100; dextrinized cornstarch, 132 and cornstarch, ~400. For AIN-93M, the recommendation is (g/kg diet) sucrose at 100, dextrinized starch at 155, and cornstarch at ~470. These values may vary depending on the addition of premixes (e.g., vitamins) that are made with sucrose or changes in the concentrations of protein and fat.

Fat. The source of fat in AIN-76A is corn oil at 50 g/kg diet. Recent evidence has shown this to be unacceptable because it does not provide sufficient linolenic acid to meet requirements. A change in the fat source is recommended primarily to meet the requirements for an adequate amount and balance of both essential fatty acids, linoleic (*n*-6) and linolenic (*n*-3). Recent studies by Lee et al. (1989) suggest that a (*n*-6):(*n*-3) ratio of 5 and a polyunsaturate:saturate ratio of 2 are the points of greatest influence on tissue lipids and eicosanoid production. Bourre et al. (1989) suggested that the optimal (*n*-6):(*n*-3) ratio is between 1 and 6. Soybean oil is the only single source of dietary fat that comes close to meeting these criteria. The oil contains about 14% saturated fatty acids, 23% monounsaturated fatty acids, 51% linoleic acid and 7% linolenic acid. This gives a (*n*-6):(*n*-3) ratio of 7, and a polyunsaturate:saturate ratio of ~4. Thus, soybean oil is the recommended source of fat in the AIN-93 diets. However, the fatty acid composition of commercial sources must be monitored because of the widespread practice of hydrogenation and the emergence of new cultivars with different fatty acid compositions.

Bourre et al. (1989 and 1990) used the method of dietary titration of 18:2(*n*-6) and 18:3(*n*-3) to determine linoleic and linolenic acid requirements,

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the American Institute of Nutrition and does not imply that other products are not suitable.

TABLE 3

Mineral mix (AIN-93G-MX) that supplies the recommended concentrations of elements for the AIN-93G diet

Ingredient	g/kg mix
Essential mineral element	
Calcium carbonate, anhydrous, 40.04% Ca	357.00
Potassium phosphate, monobasic, 22.76% P; 28.73% K[1]	196.00
Potassium citrate, tri-potassium, monohydrate, 36.16% K	70.78
Sodium chloride, 39.34% Na; 60.66% Cl	74.00
Potassium sulfate, 44.87% K; 18.39% S	46.60
Magnesium oxide, 60.32% Mg	24.00
Ferric citrate, 16.5% Fe	6.06
Zinc carbonate, 52.14% Zn	1.65
Manganous carbonate, 47.79% Mn	0.63
Cupric carbonate, 57.47% Cu	0.30
Potassium iodate, 59.3% I	0.01
Sodium selenate, anhydrous, 41.79% Se	0.01025
Ammonium paramolybdate, 4 hydrate, 54.34% Mo	0.00795
Potentially beneficial mineral element	
Sodium meta-silicate, 9 hydrate, 9.88% Si	1.45
Chromium potassium sulfate, 12 hydrate, 10.42% Cr	0.275
Lithium chloride, 16.38% Li	0.0174
Boric acid, 17.5% B	0.0815
Sodium fluoride, 45.24% F	0.0635
Nickel carbonate, 45% Ni	0.0318
Ammonium vanadate, 43.55% V	0.0066
Powdered sucrose	221.026

[1]This amount of potassium phosphate supplies only 1561 mg P/kg diet. The remainder (1440 mg) comes from casein, which contains an average of 0.72% P. The recommended amount of total phosphorus in the diet is 3000 mg/kg.

respectively. They used tissue saturation of 20:4(*n*-6) and 22:6(*n*-3) to make the assessments and concluded that 12 g of linoleic acid and 2 g of α-linolenic acid/kg diet were the minimal requirements for rats. This translates into ~30 g soybean oil/kg in the diet. However, to reach the plateau for maximal concentrations of these fatty acids in many tissues of growing rats, an amount of fat equivalent to 50–60 g soybean oil/kg was required. With a 15% margin of safety, the recommended amount of soybean oil for the AIN-93G diet is 70 g/kg diet. This amount is recommended for both males and females during rapid growth and for adult females during reproduction and lactation. When the animals have completed the rapid growth phase or they are not in the reproductive phase, the amount of soybean oil should be lowered to 40 g/kg diet. The maintenance diet formulation (AIN-93M) that meets this criterion is presented in Table 6.

Often, experimental designs require alterations of the calories derived from fat or the fatty acid composition to mimic human dietary intakes. Adjustments

TABLE 4

Contribution of vitamins to AIN-93G and AIN-93M diets when the recommended vitamin mix AIN-93-VX is fed at 10 g/kg of the diet

Vitamin	U/kg diet
Nicotinic acid, *mg*	30
Pantothenate, *mg*	15
Pyridoxine, *mg*	6
Thiamin, *mg*	5
Riboflavin, *mg*	6
Folic acid, *mg*	2
Vitamin K, μg	750
D-Biotin, μg	200
Vitamin B-12, μg	25
Vitamin A, IU	4000
Vitamin D_3, IU	1000
Vitamin E, IU	75

in fat calories should be made by isocaloric formulation, so that essential nutrients are provided on a caloric basis and not by dilution or substitution (Johnston and Fritsche 1989, Visek and Clinton 1983). There are many common fat sources; thus it is feasible to alter fatty acid composition, but some constraints should be recognized. Fats containing highly saturated fatty acids of 16 carbons or longer chain lengths are poorly digested. High polyunsaturated fatty acid sources require more antioxidants.

TABLE 5

Vitamin mix (AIN-93-VX) that supplies the recommended concentrations of vitamins for AIN-93G and AIN-93M diets

Vitamin	g/kg mix
Nicotinic acid	3.000
Ca Pantothenate	1.600
Pyridoxine-HCl	0.700
Thiamin-HCl	0.600
Riboflavin	0.600
Folic acid	0.200
D-Biotin	0.020
Vitamin B-12 (cyanocobalamin) (0.1% in mannitol)	2.500
Vitamin E (all-*rac*-α-tocopheryl acetate) (500 IU/g)[1]	15.00
Vitamin A (all-*trans*-retinyl palmitate) (500,000 IU/g)[1]	0.800
Vitamin D_3 (cholecalciferol) (400,000 IU/g)	0.250
Vitamin K (phylloquinone)	0.075
Powdered sucrose	974.655

[1]Use of the dry, gelatin-matrix form of these vitamins is recommended.

TABLE 6

AIN-93M diet formulated for maintenance of adult rodents

Ingredient	g/kg diet
Cornstarch	465.692
Casein (≥85% protein)	140.000
Dextrinized cornstarch (90–94% tetrasaccharides)[1]	155.000
Sucrose	100.000
Soybean oil (no additives)	40.000
Fiber[2]	50.000
Mineral mix (AIN-93M-MX)	35.000
Vitamin mix (AIN-93-VX)	10.000
L-Cystine	1.800
Choline bitartrate (41.1% choline)[3]	2.500
Tert-butylhydroquinone	0.008

[1]Dyetrose (Dyets, Bethlehem, PA) and Lo-Dex 10 (American Maize, Hammond, IN) meet these specifications. An equivalent product may also be used.

[2]Solka-Floc®, 200 FCC (FS&D, St. Louis, MO) or its equivalent is recommended.

[3]Based on the molecular weight of the free base.

TABLE 7

Mineral mix (AIN-93M-MX) that supplies the recommended concentrations of elements for AIN-93M diet

Ingredient	g/kg mix
Essential mineral element	
Calcium carbonate, anhydrous, 40.04% Ca	357.00
Potassium phosphate, monobasic, 22.76% P; 28.73% K[1]	250.00
Sodium chloride, 39.34% Na; 60.66% Cl	74.00
Potassium sulfate, 44.87% K; 18.39% S	46.60
Potassium citrate, tri-potassium, monohydrate, 36.16% K	28.00
Magnesium oxide, 60.32% Mg	24.00
Ferric citrate, 16.5% Fe	6.06
Zinc carbonate, 52.14% Zn	1.65
Manganous carbonate, 47.79% Mn	0.63
Cupric carbonate, 57.47% Cu	0.30
Potassium iodate, 59.3% I	0.01
Sodium selenate, anhydrous, 41.79% Se	0.01025
Ammonium paramolybdate, 4 hydrate, 54.34% Mo	0.00795
Potentially beneficial mineral element	
Sodium meta-silicate, 9 hydrate, 9.88% Si	1.45
Chromium potassium sulfate, 12 hydrate, 10.42% Cr	0.275
Boric acid, 17.5% B	0.0815
Sodium fluoride, 45.24% F	0.0635
Nickel carbonate, 45% Ni	0.0318
Lithium chloride, 16.38% Li	0.0174
Ammonium vanadate, 43.55% V	0.0066
Powdered sucrose	209.806

[1]This amount of potassium phosphate supplies only 1992 mg P/kg diet. The remainder (1008 mg) comes from casein which contains an average of 0.72% P. The recommended amount of total phosphorus in the diet is 3000 mg/kg.

Antioxidant. Dietary sources of fat containing high amounts of polyunsaturated fatty acids are subject to oxidation (Fullerton et al. 1982, Warner et al. 1982). The recommended amount of antioxidant such as butylated hydroxytoluene or ethoxyquin (Santoquin) in AIN-76A using corn oil was 100–200 mg/kg oil (5–10 mg/kg diet). Because of its content of linolenic acid, soybean oil is recommended as the source of fat in AIN-93. Because it contains a higher concentration of 18:3(*n*-3) fatty acids than does corn oil, more antioxidants may be required to prevent lipid oxidation if used under less than ideal conditions, e.g., high environmental temperatures. For normal conditions, however, an antioxidant level of 200 mg/kg oil should suffice.

Because tertiary-butylhydroquinone is very effective in protecting highly unsaturated fish oils (Fritsche and Johnson 1988, Gonzalez et al. 1992, Ke 1977), this antioxidant is recommended for use in AIN-93. Other antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and ethoxyquin also may be used. However, because of its viscosity, ethoxyquin may not disperse adequately during diet preparation.

Protein. Casein at 200 g/kg diet is the sole source of protein in AIN-76A. The recommended amount of high protein (≥85% protein) casein for AIN-93G is also 200 g/kg diet. This provides ~17% protein. Because casein is low in sulfur amino acids, DL-methionine is the recommended supplement to the AIN-76A diet. Compared with other milk proteins, L-cysteine and/or L-cystine and not L-methionine are the amino acids found in small amounts in casein. It was decided, therefore, to recommend L-cystine at 3 g/kg diet instead of methionine as the supplement to the AIN-93 diets. Table 8 shows the estimated amino acid composition of the AIN-93G diet.

For long-term studies using nonpregnant animals, and after completion of the rapid growth phase, the animals should be provided with the AIN-93M diet containing only 140 g casein/kg diet (12% protein) and 1.8 g L-cystine/kg diet. The estimated amino acid composition of this diet is shown in Table 8. It should be noted that changing the amount of casein will require a reformulation of the mineral mix to adjust phosphorus to the recommended amount. Details of the reformulation are provided in Table 7.

Fiber. The amount of fiber in the proposed diets is 50 g/kg, the same concentration as in AIN-76A. Because the composition of available fiber sources can vary, it is recommended that a standardized source be used to assure greater consistency among diets. An example of a source that has been used successfully for many years is supplied under the name Solka-Floc® (200 FCC, FS&D, St. Louis, MO). Some specifications for this product are wood pulp with 90–95%

TABLE 8

Estimated minimal nutrient composition of AIN-93G and AIN-93M rodent diets[1]

Nutrient	Diet	
	AIN-93G	AIN-93M
	U/kg diet	
Total energy,[2] *kcal*	3766.0	3601.0
% as protein	19.3	14.1
% as CHO	64.0	75.9
% as fat	16.7	10.0
Moisture, *g*	66.0	68.0
Total fat, *g*	70.0	40.0
Saturated, *g*	10.8	6.2
Monounsaturated, *g*	16.3	9.3
Polyunsaturated, *g*	40.5	23.1
Linoleic acid, *g*	35.7	10.4
Linolenic acid, *g*	4.8	2.7
Total carbohydrate, *g*	643.7	727.3
Complex carbohydrates, *g*	360.1	421.9
Simple sugars, *g*	236.1	257.9
Cellulose, *g*	47.5	47.5
Total protein, *g*	178.6	125.8
Amino acids (typical analysis)		
Alanine, *g*	4.6	3.3
Arginine, *g*	6.4	4.5
Aspartic acid, *g*	12.2	8.0
Cystine,[3] *g*	3.7	2.4
Glutamic acid, *g*	36.3	25.5
Glycine, *g*	3.2	2.3
Histidine, *g*	4.6	3.3
Isoleucine, *g*	8.5	5.9
Leucine, *g*	15.4	10.9
Lysine, *g*	13.0	9.2
Methionine, *g*	4.6	3.3
Phenylalanine, *g*	8.8	6.2
Proline, *g*	20.5	14.3
Serine, *g*	9.7	6.7
Threonine, *g*	6.7	4.7
Tryptophan, *g*	2.1	1.6
Tyrosine, *g*	9.3	6.6
Valine, *g*	10.0	7.0
Total ash, *g*	41.7	38.9
Minerals		
Calcium, *mg*	5000.0	5000.0
Phosphorus, *mg*	3000.0	3000.0
Magnesium, *mg*	513.0	511.0
Sodium, *mg*	1039.0	1033.0
Potassium, *mg*	3600.0	3600.0
Chloride, *mg*	1631.0	1613.0
Sulfur (inorganic), *mg*	300.0	300.0
Iron, *mg*	45.0	45.0
Zinc, *mg*	38.0	35.0
Manganese, *mg*	10.0	10.0
Copper, *mg*	6.0	6.0
Iodine, *mg*	0.2	0.2
Molybdenum, *mg*	0.15	0.15
Selenium, *mg*	0.18	0.17
Silicon, *mg*	5.0	5.0
Chromium, *mg*	1.0	1.0
Fluoride, *mg*	1.0	1.0
Nickel, *mg*	0.5	0.5
Boron, *mg*	0.5	0.5
Lithium, *mg*	0.1	0.1
Vanadium, *mg*	0.1	0.1
Vitamins		
Nicotinic acid, *mg*	30.0	30.0
Ca pantothenate, *mg*	15.0	15.0
Pyridoxine, *mg*	6.0	6.0
Thiamin, *mg*	5.0	5.0
Riboflavin, *mg*	6.0	6.0
Folic acid, *mg*	2.0	2.0
Biotin, *mg*	0.2	0.2
Vitamin B-12, μg	25.0	25.0
Vitamin K, μg	900.0	860.0
Vitamin E, IU	75.0	75.0
Vitamin A, IU	4000.0	4000.0
Vitamin D, IU	1000.0	1000.0
Other nutrients		
Choline, *mg*	1000.0	1000.0

[1]Values are based on estimates of the nutrient composition of individual ingredients in a nonpelleted formulation.

[2]The estimate of caloric content was based on the standard physiological fuel values for protein, fat, and carbohydrate of 4, 9 and 4, respectively.

[3]Includes L-cystine addition to diet.

cellulose, 5–10% hemicellulose and 5% moisture. The average fiber length is 35 μm and ≥75% passes through a 200-mesh sieve. It is recommended that a fiber source such as Solka-Floc® or its equivalent be used in the AIN-93 diets. It should be noted that the less expensive, nonpurified sources of fiber often contain higher concentrations of essential mineral elements such as iron and manganese and heavy metals such as lead than the more expensive purified sources. If an experimental design requires low dietary content of a particular mineral element, alternative sources of fiber should be considered.

Mineral mix. The major changes in the AIN-93 mineral mix compared with AIN-76A include lowering of the phosphorus and manganese contents, changing the form and amount of selenium and adding trace and ultratrace elements molybdenum, boron, fluoride, lithium, nickel, silicon and vanadium.

Phosphorus. A major problem with the AIN-76A diet is its propensity to produce calcium deposits in the kidneys of female rats (Hoek et al. 1988, Mars et al. 1988, Nguyen 1982). Many studies have suggested that a low molar ratio of calcium to phosphorus is probably the cause (Adams et al. 1989, Shah and Belonje 1991, Shah et al. 1986). The molar ratio of Ca:P in AIN-76A is ~0.75, considering the contribution of phosphorus from casein. To solve the problem of kidney calcification, calcium and phosphorus forms and the amount of phosphorus in the AIN-93 diets were changed from those in AIN-76A. $CaHPO_4$ was

replaced with $CaCO_3$ to supply calcium, and KH_2PO_4 was used to supply phosphorus. The amount of calcium and phosphorus salts listed for the AIN-93G-MX mineral mix (Table 3) will provide 5 g Ca/kg diet and 1.56 g P/kg. Casein contains an average of 7.2 g P/kg. With casein at 200 g/kg diet, the phosphorus concentration is increased by 1.44 g/kg diet, which results in a total phosphorus concentration in the AIN-93G diet of ~3 g/kg. The molar ratio then is 1.3, or 70% higher than in AIN-76A. The formulation of the mineral mix for AIN-93M adjusts the amount of phosphorus to compensate for the reduced amount of phosphorus coming from casein. However, the amounts of calcium and phosphorus remain the same as in AIN-93G. The recommended amounts of individual elements to be added to AIN-93G and AIN-93M from the respective mineral mixes can be found in Table 2.

Experiments showed that female rats fed the AIN-93G diet for 12 wk exhibited no increase in kidney calcium when compared with those fed a cereal-based diet (Laboratory Rodent Diet 5001, Ca:P molar ratio, 1.3; PMI feeds, Richmond, IN) (PMI Feeds 1992, Reeves et al. 1993). On the other hand, rats fed the AIN-76A diet (Ca:P molar ratio, 0.75) had 23 times more calcium in their kidneys than rats fed the cereal-based diet. Calcium concentration in the kidneys of male rats was not affected by either diet. In another experiment, both weanling female rats and mice were fed the AIN-93G diet for 16 wk. There were no changes in kidney calcium compared with similar animals fed a cereal-based diet (Certified Rodent Diet 5002; Ca:P molar ratio, 0.86; PMI Feeds) (Reeves et al. 1993).

In recent long-term studies through three successive generations of rats, Ritskes-Hoitinga et al. (1993) described the influence of dietary phosphorus concentrations (2 g vs. 4 g phosphorus/kg diet; 5 g calcium/kg diet) on kidney calcification, reproduction and bone mineralization. They concluded that 2 g phosphorus/kg diet prevented excessive kidney calcification in female rats, sustained reproduction, but delayed bone mineralization of first and second generation offspring after 4–12 wk. Although long-term, successive generation studies have not been done with the AIN-93 diets, the dietary concentration of phosphorus at 3 g/kg is not expected to cause problems with regard to bone mineralization.

There may be factors other than the Ca:P molar ratio involved in the calcification of the kidney in female rats. As noted previously, female rats did not have abnormal calcium concentrations in their kidneys when fed a commercially prepared cereal-based diet with a Ca:P molar ratio of <1. Cereal-based diets typically contain threefold more potassium and magnesium than purified diets. High dietary magnesium has been shown to prevent kidney calcification in rats fed diets with low molar ratios of calcium to phosphorus (Shah et al. 1980).

Manganese. The manganese supplement in the mineral mix of AIN-76A provides ~50 mg Mn/kg diet. Recent reports (Lee and Johnson 1988) showed that less than one-tenth this amount supported good growth in rats with no signs of deficiency. The current recommendation for manganese, with a margin of safety, is 10 mg/kg diet.

Selenium. Selenium is supplied in the AIN-76A diet at 0.1 mg/kg as the selenite form. Recent studies found the minimal requirement for selenium in growing rats by using the maximization of liver glutathione peroxidase (GSHPx) activity and GSHPx mRNA concentrations as criteria (Evenson et al. 1992, Sunde et al. 1992). The minimal dietary concentration of selenium that satisfied both criteria was 0.1 mg/kg diet. Eckhert et al. (1993) showed that 0.2 mg Se/kg diet provided greater protection from capillary degeneration in the retina of male rats than 0.1 mg/kg.

Based on these studies, 0.15 mg Se/kg diet is recommended for AIN-93. Analyses of the selenium content of major dietary ingredients such as casein and L-cystine showed that these components could contribute enough selenium to bring the total in this diet to ~0.18 mg/kg. Selenate is the recommended form of added dietary selenium because it is less likely than selenite to cause oxidation of other dietary components (NRC 1983). Also, it has been shown that selenium from selenate is absorbed more efficiently than selenium from selenite (Vanderland et al. 1992).

Pregnancy and lactation seem to elevate the selenium requirement. Smith et al. (1986 and 1987) used liver GSHPx activity to find the selenium requirements for pregnant and lactating rats. Their findings suggest that as much as 0.5 mg/kg is required to maximize GSHPx activity in dams and pups. Therefore, if the diet is to be used during pregnancy and lactation, a higher concentration of selenium might be considered. Caution should be exercised, however, because selenium in higher concentrations can be toxic. It should be noted also that commercially available cereal-based diets are reported to contain ~0.2 mg Se/kg and support reproduction and lactation adequately.

Molybdenum and chromium. Chromium but not molybdenum is added to the AIN-76A diet. Molybdenum is an essential component of the enzymes xanthine oxidase/dehydrogenase, aldehyde oxidase and sulfite oxidase. Wang et al. (1992) used the maximization of several criteria including enzyme activity and tissue concentration of molybdenum to assess the molybdenum requirement of female rats. They found that 0.1 mg Mo/kg diet was required to maximize the activity of the oxidase enzymes but 0.2 mg/kg was required to maximize liver and brain concentrations of molybdenum. However, to maximize tissue concentrations of a trace element may not be a

valid criterion for requirement. Based on these results, the molybdenum supplement for the AIN-93 diet was set at 0.15 mg/kg.

Because recent investigations (Flatt et al. 1989, Holdsworth and Neville 1990) Could not corroborate earlier suggestions that chromium functioned in glucose metabolism, a biochemical function for chromium has not been firmly established. Therefore, chromium is discussed with the other ultratrace elements whose status is similar.

Ultratrace elements. The discussion group at the 1989 Workshop recommended adding several ultratrace elements and silicon to the new diet. The list included chromium, fluoride, boron, vanadium, arsenic, nickel, lithium and tin. Although biochemical functions have not been described, and essentiality has not been firmly established for any of these elements, feeding diets with very low quantities of some of them may result in negative effects on growth, reproductive performance and on various physiological characteristics in a variety of animals (Hunt et al. 1993, Mertz 1986 and 1987, Pickett and O'Dell 1992). The final suggestions for the addition of ultratrace elements to the diet and their amounts can be found in Tables 2, 3 and 7 under the heading "Potentially Beneficial Mineral Elements."

Many of the ultratrace elements are found in plentiful quantities in the natural ingredients that make up cereal-based diets, but their concentrations in purified diets are often very low, and in chemically defined diets, they may be completely absent. Purified diets without added ultratrace elements support growth and reproduction, but investigators have noted that animals exposed to stress, toxins, carcinogens or diet imbalances display more negative effects when fed purified diets than when fed cereal-based diets (Bounous 1987, Boyd 1972 and 1983, Evers 1982, Gans 1982, Hafez and Kratzer 1976, Longnecker 1981). This suggests that detrimental effects may occur with the omission of some substances found in the more natural, cereal-based diets; some of these substances may be the ultratrace elements. It should be pointed out that many naturally occurring organic compounds in cereal-based diets can cause induction of the cytochromes P-450 drug/toxin metabolizing system and mixed function oxidases (Parke 1978, Wattenberg 1975). This might play a significant role in lessening the effects of drugs and toxins in animals fed cereal-based diets. Recent studies showed that the deethylation of 7-ethoxyresorufin, catalyzed by a form of cytochrome P-450, was not detectable in the intestine of rats fed a purified diet similar to AIN-76A, but there was a substantial amount of activity in the intestines of similar rats fed a commercially prepared cereal-based diet (W. T. Johnson, unpublished data). However, stimulation of the P-450 detoxication system is not likely to explain the enhanced detrimental effects of diet imbalances.

Many of the ultratrace elements are natural components of the ingredients in the proposed diet (**Table 9**). Although the ingredients may provide adequate amounts of ultratrace elements, their concentrations can be highly variable from lot to lot and may be lower than those suggested by some investigators to be optimal for rats. The addition of small amounts of ultratrace elements should alleviate the concern that amounts present are too low, or variation from diet to diet would result in variable responses to experimental manipulation. However, the additions are low enough that the total amounts in the diet, including endogenous concentrations, are considered nontoxic. Cereal-based diets contain much higher concentrations of ultratrace elements than those found in the AIN-93 diets, but their availability from the former may be much less.

Because the essentiality of the ultratrace elements has not been firmly established by defined biochemical functions, some investigators may choose not to add one or more of them to the diet. Also, the constraints of an experimental design may nullify the addition of one or more of the ultratrace elements to the diet. In these cases, an equal substitution of powdered sucrose should be made for the amount of ultratrace elements left out of the mineral mix. The mix should then be incorporated into the diet as before, at 35 g/kg diet. Any changes made in the mineral mix should be thoroughly described, and the mix and the diet into which it is incorporated, should be designated as "modified."

It will always be in the best interest of the investigator who works with living systems to factor in and, whenever possible, minimize fluctuations in the concentrations of any dietary component. Ultratrace elements are of particular concern because so little goes so far. At present, the best approach to accomplishing this task is to monitor the nutrient concentrations by analysis, and take steps to reduce the concentrations if they are considered excessive, or increase them if they are suboptimal. Table 9 shows a comparison among concentrations of some dietary mineral elements including ultratrace elements in a cereal-based diet and two purified diets, one with and one without added ultratrace elements.

Vitamin mix. Three changes were made in the vitamin mix from that in AIN-76A: the form and amount of vitamin K, the amount of vitamin E and the amount of vitamin B-12. The contribution that the vitamin mix makes to the diet for each vitamin is shown in Table 4.

Vitamin K. Although no changes for vitamin K were suggested at the 1989 workshop, several concerns were voiced about the problem of vitamin K deficiency when using the AIN-76A diet that contains 500 μg menadione/kg. Kindberg and Suttie (1989) measured plasma prothrombin concentration and liver peptide and protein carboxylase activities, and

TABLE 9

Representative analyses of macro, trace and ultratrace elements in commercially prepared Laboratory Rodent Diet-5001 (LRD-5001), AIN-76A and AIN-93G with and without ultratrace elements (UTE)[1]

Element	Diets[2]			
	LRD-5001	AIN-76A	AIN-93G[3]	AIN-93G w/o UTE
	mg/kg diet			
Calcium	9200.0 ± 80	4800.0 ± 130	4950.0 ± 40	5062.0 ± 87
Phosphorus	6500.0 ± 85	5100.0 ± 146	3020.0 ± 100	3100.0 ± 90
Magnesium	1860.0 ± 19	510.0 ± 7	520.0 ± 10	470.0 ± 9
Sodium	3032.0 ± 113	927.0 ± 31	1130.0 ± 54	1004.0 ± 85
Potassium	10212.0 ± 330	3252.0 ± 103	3273.0 ± 107	3697.0 ± 161
Zinc	66.0 ± 0.1	42.0 ± 3	39.0 ± 1.0	44.0 ± 1.0
Copper	14.0 ± 0.1	6.5 ± 0.5	6.0 ± 0.7	6.5 ± 0.1
Iron	270.0 ± 0.7	49.0 ± 4	45.0 ± 1.5	42.9 ± 2.0
Manganese	65.0 ± 2	53.0 ± 3	12.3 ± 0.4	10.3 ± 0.2
Molybdenum	4.78 ± 0.2	0.37 ± 0.01	1.01 ± 0.06	0.62 ± 0.14
Silicon[4]	731.0 ± 61	4.0 ± 6.1	8.3 ± 1.8	2.8 ± 0.9
Nickel	2.3 ± 0.1	0.24 ± 0.03	1.2 ± 0.07	0.04 ± 0.02
Chromium	1.2 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	Not done
Vanadium	1.1 ± 0.04	0.07 ± 0.01	0.35 ± 0.01	0.06 ± 0.01
Boron	14.0 ± 1	0.18 ± 0.04	0.5 ± 0.08	0.14 ± 0.06
Arsenic	0.23 ± 0.05	0.005 ± 0.002	0.20 ± 0.05	Not done

[1]Values are means ± SD of 4 to 10 replicates of diet samples picked at random from individual containers as supplied by the vendor. Samples for the determination of macro and trace elements were dry-ashed in a muffle furnace at 450°C and the ash dissolved with 15% aqua regia. Samples for the determination of the ultratrace elements were treated with concentrated nitric acid saturated with magnesium nitrate, and the mixture slowly heated to dryness before ashing. The analysis of boron was done according to the procedures of Hunt and Shuler (1989).

[2]Laboratory Rodent Diet-5001 was purchased from PMI Feeds, Richmond, IN; AIN-76A was purchased from Teklad Premier, Madison, WI; AIN-93G with UTE was supplied gratis by Dyets, Bethlehem, PA; AIN-93G without UTE was made in house.

[3]Ultratrace elements were added to the diet in the following amounts (mg/kg): Mo, 0.2; Ni, 1.0; Cr, 1.0; V, 0.2; B, 0.5; As, 0.2; F, 1.0; Sn, 0.5. This diet was analyzed before the decision was made to exclude As from the diet and to lower the amount of Ni to 0.5 mg/kg.

[4]Si added to the diet as the mineral salt is difficult to recover unless a special procedure is used during the ashing process (Lichte et al. 1980). Otherwise, only endogenous Si is recovered.

showed that 500 μg phylloquinone (vitamin K)/kg diet did not maintain these criteria at normal levels as higher concentrations did. Another concern was the possibility that the amount of menadione required to overcome this problem might be toxic to the animal. It was decided, therefore, to use vitamin K instead of menadione as the form of vitamin K in the new diet. The amount to use was arbitrarily set at 75 mg vitamin K/kg vitamin mix. This results in 750 μg/kg diet. Casein contains an average of ~800 μg vitamin K/kg. At 200 g/kg diet, this will add an additional 160 μg for a total of ~900 μg vitamin K/kg.

Vitamin B-12. Vitamin B-12 (cyanocobalamin) concentration in AIN-76A is 10 μg/kg diet. Based on the urinary excretion of the methylmalonic acid, a method of assessing vitamin B-12 status of young rats, Thenen (1989) found that 10 μg/kg diet was not sufficient to meet their requirements. Therefore, the vitamin B-12 concentration of the AIN-93 diets was increased to 25 μg/kg diet.

Vitamin E. The recommendation for vitamin E in AIN-76A was 50 IU all-*rac*-α- tocopheryl acetate/kg diet. The recommendation of the fat-soluble vitamins group at the workshop was to increase the amount of vitamin E if the total amount of fat was increased. This was based on the concept that an increase in the amount of polyunsaturated fatty acids increases the need for vitamin E (Harris et al. 1963). The AIN-93G diet contains 70 g soybean oil/kg diet instead of 50 g corn oil/kg diet. This not only increases the amount of polyunsaturated fatty acids but the amount of linolenic acid as well. Thus, the amount of vitamin E was increased to 75 IU/kg. The ester form in a dry, gelatin-matrix is recommended because of its stability and reliability.

Because vitamin E might be beneficial in alleviating some age-associated biological and pathological changes caused by increased peroxidation and eicosanoid production (Meydani et al. 1986 and 1992), it was decided to retain 75 IU/kg in the maintenance diet (AIN-93M) even though the amount of oil was reduced to 40 g/kg. Blumberg (1987) suggested that the requirement for vitamin E for the maintenance of cellular function and integrity may increase during the aging process. Vitamin E also has been found to enhance the immune response in aged mice (Meydani

et al. 1987) and elderly human subjects (Meydani et al. 1990).

Vitamin A. The amount of vitamin A has not been changed from the previous diet. However, it is recommended that the dry, gelatin-matrix form of retinyl palmitate be used as the form of vitamin A. This form is stable in products with high moisture and/or high mineral contents.

Choline. The third edition of the National Research Council Handbook No. 10, Nutrient Requirements of Laboratory Animals (NRC 1978) recommended 1 g choline/kg diet for rats. The AIN-76A diet contains 2 g choline bitartrate/kg, which gives only ~0.8 g choline/kg diet when expressed as the free base. The amount of choline bitartrate for AIN-93 was increased to 2.5 g to give 1 g choline/kg diet.

PRECAUTIONS FOR DIET REFORMULATION

It is assumed that the diets presented in this report will be used from time to time for nutritional studies that require alterations in one or more of the major ingredients such as the fat or the protein source. In these cases, it is imperative that the investigator be aware of the composition of the component(s) being substituted and make necessary adjustments to ensure adequate amounts of nutrients.

For example, because of their low zinc content egg white solids are usually substituted for casein for zinc deficiency studies. Egg white solids contain large amounts of sodium, potassium, and chloride and low phosphorus. Consequently, the mineral mix must be reformulated to meet the recommended amounts of these nutrients given in this report, i.e., no NaCl, potassium and higher phosphorus in the mineral mix. In addition, egg white solids contain adequate amounts of all amino acids, including the sulfur amino acids, and none should be added to the diet. If isolated soybean protein (85–87% protein) is used, the mineral mix should be reformulated to lower the sodium content and to supply adequate phosphorus. Much of the phosphorus in soybean protein is in the form of phytate with limited bioavailability. A combination of L-methionine and L-cystine could be used to supplement sulfur amino acids.

Another example was given earlier and bears emphasis. When experimental designs require alterations in calories derived from fat or alterations in fatty acid composition, adjustments in fat calories should be made by isocaloric formulation, so that essential nutrients are provided on a caloric basis and not by dilution or substitution. Because there are many common fat sources, it is feasible to alter fatty acid composition, but it should be recognized that fats containing highly saturated fatty acids with chain lengths longer than 16 are poorly digested. They also may require higher concentrations of antioxidants.

DIET PREPARATION

There are perhaps as many different ways to prepare a purified diet as there are individuals who attempt it (Baker 1987). Because most nutrition researchers today do not have the means to prepare diets, this task is often left to commercial suppliers. When diet preparation is necessary, however, some basic techniques are essential. The goal is to prepare a diet that is homogeneous, especially with regard to those constituents in extremely low concentrations, and has a minimal loss of labile nutrients. From time to time, conflicts between the nutritional and technical aspects of diet preparation will arise. A particular form of a nutrient that provides a technical advantage to mixing may not always be the most biologically available form. For example, from a technical standpoint, finely powdered silicon dioxide makes an excellent mix component, but silicon in this compound is almost totally unavailable to the animal. On the other hand, sodium metasilicate, as a form of nutritionally available silicon, can be difficult to blend because of its hydrophilicity.

Ingredients and sources

The dietary ingredients should be of the highest quality and purchased from reputable suppliers. If the diet is to be used for long periods, large batches of each ingredient should be purchased at once to avoid lot-to-lot variation in composition. A lot analysis of each ingredient should be requested from the supplier. If none is available, an in-house analysis should be done. The purity of vitamins and minerals can be variable, and each batch should be analyzed if it is important to have precise amounts of a particular nutrient. Several mineral forms are hygroscopic, and, if purchased in the anhydrous form, they should be stored in a desiccator or thoroughly dried before use. Because some vitamins are subject to deterioration in light, they should be stored in the dark and the supply replenished periodically. Fat sources, especially those highly unsaturated, are subject to oxidation and should be stored in a refrigerator and checked periodically for peroxidation.

Pre-mix preparation

Because of the extremely small quantities of some dietary vitamins and minerals, it is very important to prepare these in premixed batches. For example, most of the vitamins are present in the diet in very low concentrations. A vitamin premix is prepared by thoroughly mixing the required quantities of each

vitamin into powdered sucrose before adding them to the total diet. For the AIN-93 diet, it is recommended that the vitamin mix be prepared so that a 10 g addition/kg total diet will provide the required vitamin concentrations. It is important that the vitamin premixes be stored in airtight packages, e.g., zip-lock plastic bags with excess air expressed. If used over a short period (<1 mo), they can be stored at 4°C in the dark. If long storage is anticipated, they should be frozen (–20 to –80°C). It is not recommended that vitamin mixes be stored longer than 6 mo under the best of conditions. Also, one should be aware that the slight vibration of the refrigerator or freezer motor will cause particle separation in mixes that are stored for long periods. If this occurs, the premix should be mixed again before use.

Because most forms of choline are hygroscopic, this compound should not be added to the vitamin premix; it will cause the mix to clump. Although choline bitartrate is less hygroscopic than other forms, it should be mixed briefly with part of the sucrose or starch component before being incorporated into the total diet.

The components of the mineral mix should be reduced to a powder before they are combined. Many mineral forms are crystalline and should be individually ground to a powder in a mortar and pestle or in a ball-mill. An alternative method is to put all the components of the mix into a large ball-mill, which will reduce all ingredients to a powder. This procedure will ensure a homogeneous mixture of even the smallest components. As pointed out before, several mineral forms are hygroscopic, and in high humidity, the mineral mix may clump and take on the consistency of concrete. Under these conditions, the mineral mix should be prepared with the least hygroscopic but biologically available forms of the mineral. Storing the mix in a desiccator at room temperature also reduces the risk of clumping.

Mixing the diet

To limit the loss of vitamins, the diet should be mixed under reduced light conditions. One method of mixing the diet is to place all ingredients except the oil into the mixing bowl, being careful to keep the vitamins and minerals separated. The bowl with cover is attached to the mixer and the components mixed for 5 min at the slowest speed to reduce dust. Then the oil component is slowly added and the diet mixed for about 15 min at slow speed. Over-mixing will result in heat generation and possible loss of vitamins and oxidation of fatty acids. The preceding description is only an example. Time to blend depends upon volume and density of the diet and on the type of mixer used. The technical service department of the equipment source should be able to give advice on the best performance.

Pelleting the diet

Proper pelleting of a diet such as AIN-93 is a difficult task and depends very much on the type and amount of ingredients in the diet, and on the pelleting equipment. Improper use of the equipment can result in heat generation and loss of nutrients. This task probably should be left to the experts in your laboratory or to the commercial suppliers.

DIET STORAGE

Diets should be stored at 4°C in plastic containers with tight-fitting lids. They should be refrigerated for no longer than 3 mo at a time (Fullerton et al. 1982), and frozen if stored for longer periods. As a rule of thumb, diets should not be stored for more than 6 mo under the best of conditions. Even then, deterioration should be monitored periodically. This is especially important if constraints of the experimental protocol prohibit the use of antioxidants. Fritsche and Johnston (1988) suggested guidelines to limit autoxidation of highly unsaturated oils in purified diets during storage and feeding. See also the publication by Warner et al. (1982).

DIET NOMENCLATURE

The report of the AIN ad hoc committee on standards for nutritional studies recommended a list of terms and definitions for the characterization of diets for laboratory animals (AIN 1977). They are: diets based on formulations composed predominantly of unrefined plant and animal materials should be designated as cereal-based, unrefined or nonpurified diets; diets composed primarily of refined ingredients such as commercially available proteins, carbohydrates and fats, with mineral and vitamin mixtures added should be called purified diets; and those diets made up of chemically pure sources of nitrogen, carbohydrates, fats, vitamins, minerals and other ingredients should be called chemically defined diets. These terminologies are not completely accurate, but it is believed that their continued use will promote standardization in reporting diet composition in the literature and help eliminate many inaccuracies.

GUIDELINES FOR DESCRIBING EXPERIMENTAL DIETS

Investigators are encouraged to give a complete description of their experimental diets when they are presented in publications. This will help develop some degree of uniformity among laboratories. Under

no circumstances, however, should a purified diet be labeled AIN-93G or AIN-93M unless the specific published formula has been used. With any change in these published formulae, the diet should be labeled as "modified" and a complete description of the modification given. The Experimental Animal Nutrition Committee of AIN published guidelines for describing diets for experimental animals (Beitz 1984). These guidelines are still applicable today and their use is recommended.

DIET TESTING

During the development of the AIN-93 diets, feeding trials were conducted to determine the effects of each new formulation on weight gain, calcification of kidneys and bone mineralization in rats and mice. The article by Reeves et al. (1993) describes some of the results of those trials.

WORKSHOP PARTICIPANTS

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