The NIH-07 open-formula nonpurified diet (1) has been the selected diet for National Toxicology Program (NTP) studies since 1980. This diet was formulated about 20 years ago for reproduction, lactation, and growth of rodents in production colonies. This diet, which contains approximately 24% protein, 5% fat, and 3.5% fiber (2), may not be an ideal diet for rats in long-term studies, and some components such as protein may be increasing the severity of chronic diseases. A workshop on diet was held at NIEHS in Research Triangle Park, North Carolina, on 23 June 1993 in conjunction with the NTP Board of Scientific Counselors Technical Report Review Subcommittee meeting. Approximately 40 people from NIEHS, EPA, the Food and Drug Administration, pharmaceutical and chemical industries, and feed manufacturers attended the workshop. The purpose of the workshop was 1) to review the effects of changes in protein, fat, and fiber concentrations of diet on chronic disease (e.g., nephropathy), tumor incidences (e.g., leukemia, mammary and pituitary tumors), and survival of rats, especially Fischer-344 rats, in various 2-year studies conducted by NTP/NIEHS and 2) to discuss modification of diet composition and ingredients. The format of the workshop was 1) presentation of results of NTP/NIEHS studies, related literature information, and proposed concentration of protein, fat, and fiber in the new diet followed by 2) discussion of each diet component by the consultants, members of the NTP Board of Scientific Counselors Subcommittee and other participants.

Protein

The National Research Council (3) recommendation for growth of rats was 12% ideal protein (net protein). Egg protein is considered to be the ideal protein (3), and the biological value of most proteins including casein is ≥90% of the ideal protein for growth and maintenance (3). Accordingly, about 15% crude protein of various sources may be adequate for growth and maintenance of rats (3). A 15% crude protein nonpurified diet (with formulation similar to the NIH-07 diet) was compared with the NIH-07 diet in a 2-year study with F344 rats. Results of this study (4) indicated (1) about 15% protein nonpurified diet is adequate for growth and maintenance of male and female rats, (2) about 30% decrease in protein consumption markedly decreased the severity of nephropathy, and (3) caloric restriction and lowering of body weight may not be necessary to decrease nephropathy. Various studies in the literature with diets containing 15% casein or other purified proteins also showed marked decreases in severity of nephropathy. Based on the results of the above study and literature information (4), there was broad support by the consultants and the other workshop participants for lowering the protein concentration of nonpurified diet for rats to 15%. Twelve percent crude protein in a nonpurified diet was not considered to be adequate for growth and maintenance of rats subjected to stresses of chemical treatment in toxicology and carcinogenesis studies.

The protein content of diet or protein intake influences xenobiotic biotransformations and may decrease or increase the toxic or carcinogenic potential of chemicals. The 15% crude protein diet should not be used for reproduction and diet restriction studies or with a gavage vehicle that has caloric value (e.g., corn oil), as a more than 10% decrease in feed consumption may cause protein deficiency and complicate the interpretation of the toxic and carcinogenic potential of chemicals.

Fat

Fat provides essential fatty acids for growth and maintenance, and about 5% fat in the diet was considered to be necessary (3). Fat has 2.25–2.75 times the caloric value of the carbohydrate or protein. Various studies in the literature indicated that fat, due to its high caloric value, increased the adult body weight and increased the incidences of body-weight–associated tumors such as the anterior pituitary and mammary tumors (3, 6, 8). Other studies concluded that fat alone may contribute to an increased incidence of mammary tumors. When corn oil was given as a gavage vehicle in a number of NTP studies, some changes in body weight and tumor rates were observed in F344 rats (6, 7). These changes included decreases in the incidence or delay in onset of leukemia in male but not in female F344 rats, increased incidences of mammary and anterior pituitary tumors in F344 rats correlated with body weight and not with corn oil gavage, and incidence of pancreatic acinar cell tumors appear to be influenced by a combination of body weight and type of fat in corn oil.

Discussion by the participants of the workshop indicated that the effect of corn oil (fat) in diet may not be the same when given by gavage, and it is difficult to judge the beneficial effect of higher fat content of diet from the available data. Accordingly, the recommendation to increase the fat content of diet was delayed until further review and identification of the beneficial effect of higher fat content (>5%) in diet on decreasing the incidence or delaying the onset of leukemia is completed.

Fiber

There were no requirements or recommendations (3) on fiber content of diets for rats and mice. The fiber content of NIH-07 diet was about 3.5% (2). A nonpurified diet similar to NIH-07 diet with approximately 7% fat and approximately 10% fiber showed the following effects (Rao GN, et al., unpublished data), when fed to F344 rats for 2 years 1) higher caloric density of diet (due to higher fat content) when lowered with crude fiber, decreased the adult body weight, 2) increased fat content of diet appeared to decrease the incidence of leukemia in male but not in female F344 rats, 3) increased fiber content of diet appeared to slow the growth of mammary tumors in females, 4) increased fiber content may have decreased the incidence of adrenal pheochromocytomas in males, and 5) increased fiber content of diet may have increased the incidence of mesothelioma and associated mortality in males. An ongoing study with diets containing 8% fat (mostly from corn oil or safflower oil) and 14% fiber significantly lowered the body weight gain of both male and female F344 rats. Final results of this study will be available in 1994.

There were no studies on the influence of fiber on mammary tumor development in animal models. Human studies (8) indicated that fiber may protect against breast cancer by influencing estrogen metabolism. The beneficial effect appears to be due to binding of estrogens (deconjugated in the gut by intestinal microflora enzymatic activity) to dietary fiber, thus decreasing enterohepatic recirculation and blood levels while increasing the excretion.

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of estrogens through feces.

Fiber, due to its low caloric value, appears to lower body weight gain. However, discussion by the participants of the workshop indicated that fiber may decrease the intestinal transit time and decrease chemical absorption, change gut flora and associated metabolism of chemicals, and bind and decrease the availability of some chemicals. A decision to increase the fiber content of diet was delayed until further review of beneficial effects of fiber on delaying the development of mammary tumors is completed.

Other proposed changes in future rodent diets included 1) decrease the vitamin D content from 5000 to 1000 IU/kg of diet, as 1000 IU is adequate for growth and maintenance (3), 2) increase the vitamin B12 content from 20 to 60 ppb, as 50 ppb may be necessary for growth (3), eliminate dried skim milk from the formulation, as it is no longer available as a commodity for feed formulation and substitute dried whey if necessary, and 4) if the fiber content of the diet is increased, beet pulp may not be an appropriate ingredient because it has high water-holding capacity and may increase the bulk of intestinal contents. The participants, including the consultants, did not express any objections to the above changes. Protein, fat, fiber, and various ingredient concentrations of the proposed new diet along with NIH-07 diet are given in Table 1. The appropriateness of the proposed new diet for mice in long-term studies was not discussed.

The adult body weights of most strains/stocks of rats commonly used for toxicity and carcinogenicity studies increased during the last 10–20 years. Higher adult body weight increased the incidences of tumors in endocrine tissues, especially the anterior pituitary and mammary tumors and decreased the life span (5). There have been no major changes in diets for rats and mice for over 10 years. The major reason for gradual increase in adult body weight appears to be intentional or inadvertent selection of breeding stock for early reproduction and faster growth to satisfy user preference for larger rodents at low cost. Recommendations (9) for permanent resolution of the problem include selecting slower-growing breeders in the production colonies to lower the body weight of the progeny and modifying diets so they are adequate for growth and maintenance but do not contain excess growth-enhancing nutrients. NTP is pursuing these procedures to lower the adult body weight of rats in chemical toxicity and carcinogenicity studies.

### Table 1. Proposed composition of the new open-formula nonpurified diet

| Nutrients or ingredients | NIH-07 | New diet |
|--------------------------|--------|----------|
| Crude protein            | 23     | 15       |
| Crude fat                | 5      | 10       |
| Crude fiber              | 3.5    | 10       |
| Ground hard-winter wheat | 23.0   | 20.4     |
| Ground #2 yellow shelled corn | 24.5 | 20.4     |
| Wheat middlings          | 10.0   | 15.0     |
| Soybean meal (48% protein) | 12.0 | 5.0      |
| Fish meal (60% protein)  | 10.0   | 4.0      |
| Dried whey               | 0.0    | 2.0      |
| Dried milk               | 5.0    | 0.0      |
| Alfalfa meal (17% protein) | 4.0  | 6.0      |
| Corn gluten meal (60% protein) | 3.0 | 1.0      |
| Dried brewer’s yeast     | 2.0    | 1.0      |
| Purified cellulose       | 0.0    | 5.0      |
| Oat hulls                | 0.0    | 10.0     |
| Dry molasses             | 1.5    | 0.0      |
| Corn oil                | 0.0    | 3.6      |
| Safflower oil            | 0.0    | 3.6      |
| Fish oil                | 0.0    | 0.5      |
| Soy oil                 | 2.5    | 0.0      |
| Salt                    | 0.5    | 0.3      |
| Dicalcium phosphate      | 1.25   | 0.4      |
| Calcium carbonate        | 0.0    | 0.6      |
| Ground limestone         | 0.5    | 0.0      |
| Methionine              | 0.0    | 0.2      |
| Vitamin and mineral premixes | 0.25 | 1.05     |

*Vitamin and mineral premix composition is given by the American Institute of Nutrition (1). Vitamin and mineral premix composition is given by Rao et al. (4).*

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