

## Draft

# Chapter IV. Guidelines for Toxicity Tests

## IV B General Recommendations for Toxicity Studies

### IV B 5. Diets for Toxicity Studies

The effects of diet composition on the responses of experimental animals to xenobiotics have been reviewed.<sup>1-6</sup> Some of the most important effects include:

- ☐ Diet composition may influence experimental results through effects on background rates of toxicology parameters, such as tumor incidence.<sup>7,8</sup>
- ☐ Unrecognized or inadequately controlled nutritional and other dietary variables may alter the outcome and reproducibility of long-term toxicity studies.<sup>8</sup>
- ☐ A number of nutrients and non-nutritive dietary components have been shown to enhance or inhibit carcinogenesis; these include calories or energy, fat, protein, fiber, vitamins C and E, selenium, and lipotropes (methionine, choline, folacin, and vitamin B<sub>12</sub>). Dietary fibers have been shown to reduce, enhance, or have no effect on the toxicity and carcinogenicity of chemicals.<sup>9,10</sup> Detailed reviews of the interactions of nutrients and carcinogens have been reported.<sup>3,11-15</sup>

#### a. Types of Diets

##### i. Natural Ingredient Diets

Natural ingredient diets are the most widely used diets in toxicology research. They are prepared from unrefined plant and animal materials such as wheat, corn, oats, fish meal, soybean meal, or wheat bran and are characterized as open formula or closed formula diets. The percentages of ingredients in open formula diets are known, but the composition of closed formula diets is proprietary information.<sup>16</sup> Natural ingredient diets support growth and reproduction and are economical, commercially available, and satisfactory for studies involving additives that will not affect nutrient balances.

Limitations of natural ingredient diets for toxicity studies include:

- ☐ Variations in types and quantities of nutrients and other dietary components are due to several factors; for example, the composition of fibers may vary with their sources,<sup>17</sup> the mineral content of natural ingredient diets can vary significantly among production batches, and specifications for essential dietary elements are not always met.<sup>18</sup>
- ☐ Diet composition cannot be altered to study the effects of varying a particular nutrient, which makes natural ingredient diets poor choices for research protocols in which nutrition may influence outcome.
- ☐ Nutrient excesses well beyond their requirements, and the presence of other non-nutrients substances in natural ingredient diets support rapid weight gain, pregnancy, and lactation in experimental animals and decrease the effects of many xenobiotics.
- ☐ Finally, common contaminants of natural ingredient diets that can alter the response of laboratory animals to experimental treatment include pesticides and mycotoxins.<sup>13,19</sup>

ii. Purified Diets

The use of purified diets has been recommended to avoid some of the limitations associated with the use of natural ingredient diets.<sup>13,20,21</sup> Purified diets usually contain refined proteins, carbohydrates, and fat. Vitamin and mineral mixtures including highly purified vitamins and inorganic salts also are added to purified diets.<sup>16</sup> AIN-76A, the most commonly used purified diet,<sup>14</sup> was formulated to provide a diet of known composition that was intended to meet the known nutrient requirements of rodents; it supports growth, reproduction (generally, one or two generations), and lactation in a manner similar to natural ingredient diets.<sup>16</sup>

Advantages of using purified diets for toxicity studies include:

- ☐ Ability to reproduce nutrient concentrations from batch to batch, to maintain the nutrient composition of a diet within a narrow range, and to alter the type and composition of dietary components.<sup>20</sup>
- ☐ Use of purified diets usually decreases dietary intake of contaminants such as pesticide residues, heavy metals, enzyme inducers and other agents that may alter the responses of test animals to experimental treatment.<sup>14,20,21</sup>

Disadvantages of using purified diets for toxicity studies include:

- ☐ Difficulty in assessing the impact of purified diets on animal survival and toxicology endpoints because adequate historical data regarding the use of such diets is lacking;
- ☐ Lack of information about the suitability of purified diets for long-term studies, although some researchers have used purified diets successfully for up to 56 weeks;<sup>22,23</sup>
- ☐ Errors that may occur in the preparation of purified diets may be more critical than similar errors in the preparation of natural ingredient diets because, in purified diets, each ingredient may be the sole dietary source of an essential nutrient.<sup>20</sup> In general, practical experiences with purified diets in long-term studies have not been satisfactory.<sup>7,24-27</sup>

**b. Issues to Consider when Selecting and Preparing Diets for Animals in Toxicity Studies**

The following are important issues to consider when selecting diets for animals in toxicity studies:

- ☐ Protein requirements for maintenance and growth of laboratory animals are well characterized,<sup>20</sup> but this is not true for most nutrients. Nutrient needs<sup>28</sup> and metabolism of xenobiotics<sup>29,30</sup> change with age. Hence, the general practice of feeding a single diet throughout the life cycle of experimental animals may be inappropriate--nutritional deficiencies may occur during phases of rapid growth and development in young animals and nutrient excess may occur in older animals.
- ☐ Individual ingredients in purified diets may cause problems in long-term studies. For example, purified diets high in ingredients such as casein and sucrose may stick to the hair of rodents and cause excessive grooming. Purified sugars as the sole source of carbohydrates in diets that are low in dietary fiber may cause diarrhea, resulting in problems of digestion and absorption of other nutrients.
- ☐ For reasons that are incompletely understood, animals may not reproduce well when fed purified diets. The components in natural ingredient diets that are required to support reproduction have not been defined.
- ☐ Toxic chemicals in the diet and induced nutrient deficiencies can lead to decreased food intake by experimental animals and reduced rates of growth and development. When such an effect is expected to occur in a long-term study, pair-feeding can be used to eliminate differences in food intake among

experimental groups; this is the preferred method for ensuring that differences in energy or nutrient intake have not caused the observed experimental results or complicated their interpretation. For example, a moderate restriction of energy intake may increase the life-span, decrease the background cancer rates, and decrease the potency of carcinogens in rodents, thereby potentially modulating the action of a chemical carcinogen. When pair-feeding studies are recommended to eliminate differences in food intake among experimental groups, animals should be single-caged and food consumption should be carefully and accurately determined for each animal in the study.

☐ When the test substance is added to the diet, accurate records of food consumption must be maintained to determine the administered dose and food intake must be equalized across control and experimental groups of animals. When the test substance is a carbohydrate, protein, or fiber that will be added to the diet in large quantities, it must replace a dietary ingredient or the nutrient and energy contents of the diet will be significantly diluted (see **Chapter VII B 1**). The nutrient and energy contents of control diets also must be adjusted to match those of experimental diets. One recommended strategy is to make the control and test diets isocaloric. If food consumption among groups of experimental animals has been equalized, then equal densities of metabolizable energy in the diets will equalize nutrient intake across the groups.<sup>31</sup>

☐ When oil is used as the gavage vehicle for fat-soluble test substances, the necessity of including a vehicle-control group in the study may introduce some problems.<sup>32</sup> If the quantity of oil administered daily by gavage contributes significantly to the total dietary energy of the animals, results for experimental and vehicle-control groups may be significantly different than results for the untreated control group. If a decision is made to administer a test substance by gavage, the volume of oil given as a vehicle should be limited to 0.3 to 0.4 ml/100 g of body weight and the use of a low-fat diet should be considered.

☐ Related issues are discussed in the following chapters: 1) control diets for test animals in **Chapter IV B 1 b-c**; 2) survivorship and recommendations concerning the duration of carcinogenicity bioassays in **Chapter IV C 6 a**; and 3) nutritional concerns for food substitutes (macro-additives) in **Chapter VII B**.

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