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Society of Toxicologic Pathology Position Paper: Diet as a Variable in Rodent Toxicology and Carcinogenicity Studies

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Diet is an important variable in rodent toxicology and carcinogenicity studies. Several diet models are now available for selection in rodent toxicology studies. Abundant data have clearly established a significant correlation between average food (calorie) consumption, nutrient composition of the diet, adult body weight, the onset and incidences of specified pathological endpoints, and survival (2, 3). Ad libitum feeding of common diets to rodents on long-term studies results in overeating, excessive caloric intake, higher body weights, decreased activity patterns, and earlier onset and higher incidences of some spontaneous degenerative diseases (nephropathy and cardiomyopathy), neoplasia (eg, pituitary and mammary tumors), and reduced survival. Reduced survival of the control animals in 2-year studies, often significantly less than 50%, has heightened the industry's awareness of these diet-related issues (2).

The overeating problems in long-term rodent studies have been addressed by two different approaches: dietary modification (change the nutrient contents of the diet) and dietary restriction (control the amount of food the animals can eat). Some of the early studies using dietary modification were inconclusive, but more recently scientists at the National Toxicology Program and others have shown that control of the formulation of an ad libitum, nonpurified diet source for laboratory animals (diet modification) has a significant effect on survival and the incidence and severity of several dietary and possibly age-associated pathology. More specifically, a diet (NTP-2000) with lower protein and higher fat and fiber (~15% protein, 7–8.5% fat, and 9–14% crude fiber), when fed ad libitum to F344 rats, enhanced survival and reduced the incidence and/or severity of several common age-related conditions, including nephropathy when compared with rats fed NIH-07 (7, 8). Data is currently being accumulated on the background incidences of tumors in rodents fed the NPT-2000 diet. NIH-07, the traditional NTP diet, contains approximately 23% protein, 5% fat, and 3.4% crude fiber. The NTP has adopted NTP-2000 as their standard ad libitum rodent diet.

Studies using dietary restriction (caloric restriction) showed clear effects on rodent survival and the occurrence of spontaneous neoplastic and nonneoplastic lesions (2). Since the 1930s, it has been shown that dietary restriction can profoundly increase the longevity of rodents. Moderate caloric restriction of a balanced diet (70–80% of the maximum unrestricted ad libitum food intake) has been shown to significantly enhance survival in long-term rodent studies, reduce the onset and severity of spontaneous nonneoplastic lesions such as nephropathy (4) and cardiomyopathy (6), and delay the onset of age- or diet-related tumors such as mammary and pituitary gland tumors (5). These beneficial effects have been apparent in experiments with multiple rodent strains on multiple diets, including the traditional high-protein diets (2). Thus, the use of moderate dietary restriction has been recommended as an operationally simple procedure that significantly improves survival, controls adult body weight, reduces age-related renal, endocrine, and cardiac diseases, increases xenobiotic exposure time, and increases the statistical sensitivity of expensive, chronic bioassays to detect treatment effects in chronic rodent studies. It should be noted that other laboratory animals used for toxicology studies (primates and canines) have always been subjected to calorically controlled diets.

Some additional factors for consideration include altered sensitivity to carcinogenesis and dose selection in toxicity studies. Feeding different diets or changing the feeding technique will not only modify longevity, spontaneous disease and tumor profiles, but may also change the responses to test substances. Animals on moderate dietary restriction may exhibit altered sensitivity and tissue vulnerability for established xenobiotics (1, 2). The dietary restricted

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animals are "healthier," more robust animals and demonstrate different tolerance to carcinogens and to shorter-term xenobiotic toxicity. The possibility exists that the carcinogenic potential of a weak carcinogen may be decreased (by increased tolerance) or increased (by longer xenobiotic exposure) in dietary controlled animals. Therefore, the selection of low-dose, mid-dose, and maximum-tolerated dose levels must be derived from studies of similarly fed animals, regardless of the chosen diet or feeding technique.

The goal in toxicity studies is to control as many variables as possible while assessing toxicologic or carcinogenic endpoints of xenobiotic exposure. As the providers of pathologic endpoints for toxicology studies, the members of the Society of Toxicologic Pathology (STP) understand the importance of controlling study variables and that diet is one of these important factors. Dietary caloric restriction or variably formulated diets (diet modification) represent important and different nutritional models available for incorporation into study design, and might appropriately be called *dietary optimization*. Whenever scientifically appropriate, the STP recommends that dietary optimization be employed. The STP anticipates that the selection of dietary restriction regimens and/or diet modification will increase as scientists and regulators discuss and better understand the issues. The selection of a nutritional model should include a clear understanding of study endpoints. The important ramifications of nutritional model changes on such factors as dose selection, sensitivity of toxicology and pathology endpoints, and historical control data should be clearly understood and anticipated.

References

1. Abdo KM, Kari FW (1997). The sensitivity of the NTP bioassay for carcinogen hazard evaluation can be modulated by dietary restriction. *Exp Toxic Pathol* 48: 129–137.
2. Hart RW, Newmann DA, Robertson RT (eds.) (1995). *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*. ILSI Press, Washington, DC.
3. Keenan KP, Ballam GC, Soper KA, Laroque P, Coleman JB, Dixit R (1999). Diet, caloric restriction, and the rodent bioassay. *Toxicol Sci* 52 (Suppl): 24–34.
4. Keenan KP, Coleman JB, McCoy CL, Hoe C-M, Soper KA, Laroque P (2000). Chronic nephropathy in ad libitum overfed Sprague-Dawley rats and its early attenuation by increasing degrees of dietary (caloric) restriction to control growth. *Toxicol Pathol* 28: 788–798.
5. Keenan KP, Laroque P, Ballam GC, Soper KA, Dixit R, Mattson BA, Adams SP, Coleman JB (1996). The effects of diet, *ad libitum* overfeeding, and moderate dietary restriction on rodent bioassay: The uncontrolled variable in safety assessment. *Toxicol Pathol* 24: 757–768.
6. Kemi M, Keenan KP, McCoy C, Hoe C-M, Soper KA, Ballam GC, vanZwieten MJ (2000). The relative protective effects of moderate dietary restriction versus dietary modification on spontaneous cardiomyopathy in male Sprague-Dawley rats. *Toxicol Pathol* 28: 285–296.
7. Rao GN (1997). New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J Nutr* 127: 842S–846S.
8. Rao GN, Morris RW, Seely JC (2001). Beneficial effects at NTP-2000 diet on growth, survival and kidney and heart diseases of Fischer 344 rats in chronic studies. *Toxicol sci* (in press).