

same variety of soybeans grown in different locations (25,26). Also, in closed formula diets containing soybeans, the concentration of soybeans in the diet is not reported and may vary from batch to batch depending upon the supply and demand of dietary ingredients. This could explain why the phytoestrogen content of diets may vary greatly from batch to batch or from the same diet processed by different rodent diet vendors. These findings support the need to use open formula diets prepared from controlled ingredients and the need to monitor these diets for estrogenic substances that affect biological end points. We also agree with Boettger-Tong et al. (1) that rodent diet vendors do not routinely monitor all diets for estrogenic substances including phytoestrogens. Rodent diet vendors producing certified diets for use in comparative estrogenicity and carcinogenicity studies should provide the user with a list of substances assayed, including phytoestrogens, and the results. In our opinion, diets used in comparative estrogenicity or carcinogenicity studies should contain non-detectable levels of estrogenic substances that may alter research results. These diets should be monitored for estrogenic substances and their concentrations reported.

The commentary by Boettger-Tong et al. (1) is important because the authors emphasize the important role of the animal's diet, especially phytoestrogens, when conducting animal bioassays for estrogenicity or studies that are influenced by *in vivo* end points of hormone action. We previously reported that rodent diets significantly differ in estrogenic activity and that a standardized diet with minimal estrogenic activity would be desirable for comparative bioassays for estrogenic substances (5). Results from our second study (20) confirm that a standardized open formula diet should be used for studies that are influenced by exogenous estrogens (4,5). Phytoestrogens were not detected in the AIN-76A and the AIN-93M purified casein diets. Therefore, careful consideration should be given to the use of diets such as these when conducting studies that are influenced by exogenous estrogens. We have also shown that a natural ingredient diet can be formulated to contain less than detectable levels of the phytoestrogens (daidzein and genistein) by omitting soybean and alfalfa meals. The soybean and alfalfa meals were omitted because they may be a source of multiple, yet unidentified, phytoestrogens that may further complicate the interpretation of results from studies which are influenced by exogenous estrogens. We recommend that studies to determine the effects of dietary phytoestrogens on results of toxicologic investigations are

important and timely. National Toxicology Program studies that will help us understand the effect of long-term feeding of a diet with less than detectable levels of daidzein and genistein are presently under way.

In conclusion, we recommend that careful consideration be given to the phytoestrogen content of the diet when conducting studies that are influenced by exogenous estrogens. A standardized open formula diet in which estrogenic substances have been reduced to minimal levels or to less than detectable levels of daidzein and genistein is recommended for use in such studies. In addition, the selected diet should be monitored for estrogenic substances, and their concentrations should be reported.

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#### REFERENCES AND NOTES

- Boettger-Tong H, Murthy L, Chiappetta C, Kirkland JL, Goodwin B, Adlercreutz H, Stancel GM, Mäkelä S. A case of a laboratory animal feed with high estrogenic activity and its impact on *in vivo* responses to exogenously administered estrogens. *Environ Health Perspect* 106:369-373 (1998).
- Thigpen JE, Locklear J, Caviness GF, Stokes WS, Setchell KD. Concentration, source, and role of plant hormones (phytoestrogens) in laboratory animal diets [abstract]. *Contemp Top* 31(4):3 (1992).
- Bickoff EM, Livingston AL, Hendrickson AP, Booth AN. Relative potencies of several estrogen-like compounds found in forages. *Agric Food Chem* 10:410-412 (1962).
- Thigpen JE, Haseman JK, Locklear J, Ahlmark KA, Caviness GF, Williamson RL, Goelz MF, Forsythe D. Comparative estrogenic activity of three new closed formula natural ingredient diets formulated to reduce the concentration of phytoestrogens [abstract]. *Lab Anim Sci* 48(4):415 (1998).
- Thigpen JE, Li LA, Richter CB, Lebetkin EH, Jameson CW. The mouse bioassay for the detection of estrogenic activity in rodent diets: II. Comparative estrogenic activity of purified, certified and standard open and closed formula rodent diets. *Lab Anim Sci* 37(5):602-605 (1987).
- Knapka JJ. Nutrition. In: *The Mouse in Biomedical Research* (Foster HL, Small JD, Fox JG, eds). New York:Academic Press, 1983:51-67.
- Santell RC, Chang YC, Nair MG, Helferich WG. Dietary genistein exerts estrogenic effects upon the uterus, mammary gland, and the hypothalamic/pituitary axis in rats. *Am Soc Nutr Sci* 127:263-269 (1997).
- Rao GN, Ney E, Herbert RA. Influence of diet on mammary cancer in transgenic mice bearing an oncogene expressed in mammary tissue. *Breast Cancer Res Treat* 45:149-158 (1997).
- Adlercreutz H. Phytoestrogens: epidemiology and a possible role in cancer protection. *Environ Health Perspect* 103(suppl 7):103-112 (1995).
- Barrett J. Phytoestrogens: friends or foes? *Environ Health Perspect* 104:478-482 (1996).
- Kurzer MS, Xu X. Dietary phytoestrogens. *Annu Rev Nutr* 17:353-381 (1997).
- Rogers A, Zeisel S, Groopman J. Diet and carcinogenesis. *Carcinogenesis* 14(11):2205-2217 (1993).
- Fitzsimmons JTR, Orson NV, El-Aasser AA. Effects of soybeans and ascorbic acid on experimental carcinogenesis. *Comp Biochem Physiol* 93A:285-290 (1989).
- Barnes S, Grubbs C, Setchell KD, Carlson J.

Soybeans inhibit mammary tumors in models of breast cancer. *Clin Biol Res* 347:239-253 (1990).

- Barnes S. Effects of genistein *in vitro* and *in vivo* models of cancer. *J Nutr* 125:777S-783S (1995).
- Pollard M, Luckert PH. Influence of isoflavones in soy protein isolates on development of induced prostate-related cancers in L-W rats. *Nutr Cancer* 28(1):41-45 (1997).
- Lamartiniere CA, Moore J, Holland M, Barnes S. Neonatal genistein chemoprevents mammary cancer. *Proc Soc Exp Biol Med* 208:120-123 (1995).
- Tennant RW, French JE, Spalding JW. Identifying chemical carcinogens and assessing potential risk in short-term bioassays using transgenic mouse models. *Environ Health Perspect* 103:942-950 (1995).
- Barrett JC. Prevention of environmentally related diseases [editorial]. *Environ Health Perspect* 102:812-813 (1994).
- Thigpen JE, et al. Unpublished data.
- Reeves PG, Nielson FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123(11):1939-1951 (1993).
- Ad Hoc Committee on Standards. Second report of the Ad Hoc Committee on Standards for Nutritional Studies. *J Nutr* 110:1726 (1980).
- Rao GN. New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam Appl Toxicol* 32:102-108 (1996).
- Rao GN. New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J Nutr* 127:842S-846S (1997).
- Eldridge AC. Determination of isoflavones in soybean flours, protein concentrates, and isolates. *J Agric Food Chem* 30(2):353-355 (1982).
- Eldridge AC, Kwolok WF. Soybean isoflavones: effect of environment and variety on composition. *J Agric Food Chem* 31(2):394-396 (1983).

## The TEF Approach for Hexachlorobenzene

Van Birgelen (1) argues that hexachlorobenzene (HCB) has dioxinlike activity to the extent that its toxicity may be evaluated using a so-called toxic equivalency factor (TEF), which treats the toxic potency of HCB as a fraction of the potency of 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD). She suggests a TEF of 0.0001 based on comparisons of *in vitro* effects including binding affinity for the aryl hydrocarbon (Ah) receptor and effective concentrations for ethoxyresorufin *O*-deethylase induction and uroporphyrin accumulation in chicken hepatocytes. Van Birgelen (1) reported that these measures were used because "no *in vivo* studies designed for estimating a TEF value are available." This may come as something of a surprise to researchers at the EPA, who derived a potency factor (PF) for the carcinogenic activity of HCB based on studies of hepatocellular carcinoma in female Sprague Dawley rats (2) using a protocol similar to the one that supports earlier estimates of the carcinogenic activity of TCDD (3). The EPA reports that the upper 95th percent confidence bound on the slope fitting the HCB data to a linearized multistage model of carcinogenesis is 1.6 per milligram of HCB per kilogram body weight per day (mg/kg/day) (4).

There is no PF for TCDD currently available in the Integrated Risk Information Service database (3). However, the PF originally derived from the aforementioned study was 156,000 mg/kg/day and may be modified downward to approximately 100,000 mg/kg/day, based on reinterpretation of histopathologic data. Applying van Birgelen's TEF to these values would suggest a PF for the carcinogenic action of HCB (under the linearized multistage assumptions) to be approximately 10–16 mg/kg/day, or about an order of magnitude more potent than is suggested by the *in vivo* data. It is possible that the difference lies in pharmacokinetic or pharmacodynamic factors that exist in the whole animal, which cannot be captured in cellular assays.

As van Birgelen points out (1), the World Health Organization indicates a preference for long-term *in vivo* studies over *in vitro* measures when setting a TEF (5). The HCB example illustrates the extreme caution that should be exercised when applying a TEF based solely on *in vitro* information, particularly when major public health claims are being made.

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#### REFERENCES AND NOTES

1. Van Birgelen APJM. Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. *Environ Health Perspect* 106:683–688 (1998).
2. Ertuk E, Lambrecht RW, Peters HA, Cripps DJ, Gocmen A, Morris CR, Bryan GT. Oncogenicity of hexachlorobenzene. In: *Hexachlorobenzene: Proceedings of an International Symposium* (Morris CR, Cabral JRP, eds). IARC Scientific Publication No 77. Lyon:International Agency for Research on Cancer, 1986;417–423.
3. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnar SD, et al. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol Appl Pharmacol* 46:279–303 (1978).

4. Integrated Risk Information Service. Available: <http://www.epa.gov/ngispgm3/iris/subst/irisbak/0374.htm> [last revised 1 November 1996].
5. Ahlborg UG, Becking GC, Birnbaum LS, Brower A, Derks HJGM, Feeley M, Golour G, Hanberg A, Larsen JC, Liem AKD, et al. Toxic equivalency factors for dioxinlike PCBs. *Chemosphere* 28:1049–1067 (1994).

#### Response: Hexachlorobenzene

I appreciate Schwab's comments regarding caution in the use of a toxic equivalency factor (TEF) for hexachlorobenzene (HCB) based on results of *in vitro* studies. The dioxinlike effects of HCB include cytochrome P4501A induction and binding to the aryl hydrocarbon (Ah) receptor. In addition, HCB has been shown to bioaccumulate. These three factors are a prerequisite to include a compound in the TEF concept, which compares the potency of a dioxinlike compound to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TEFs are consensus values based on available data on relative potency values for specific compounds (1). TEF values are used to estimate the total dioxin activity in environmental and human samples by multiplying the TEF value by the concentration of each compound, leading to a certain amount of toxic equivalents (TEQs) for each compound. The summation of all TEQs in a certain mixture expresses the total dioxin activity of this mixture. Based on the binding affinity of HCB to the Ah receptor, *in vitro* cytochrome P4501A induction, and porphyrin accumulation, a relative potency of 0.0001 for HCB was estimated (2). Using this relative potency value suggested that HCB could lead to a considerable contribution to the dioxin activity of human milk in some countries. I did not estimate the slope factor for HCB that is used in carcinogenicity assessment. The slope factor is the result of the application of a low-dose extrapolation procedure

and is presented as the risk per milligram per kilogram of body weight per day (mg/kg/day) (3).

Schwab's comments included the comparison of these slope factors (although potency factors are mentioned) for HCB (1.6 per mg/kg/day) and TCDD (100,000 or 156,000 per mg/kg/day). The slope factors for TCDD are not available in the Integrated Risk Information Service database (3), as Schwab mentioned. He points out correctly that the ratio between these two slope factors is different from the suggested relative potency value for HCB. He assumes by using this approach that TEFs would predict the carcinogenic potential of dioxinlike compounds. However, no studies have been performed to verify this approach. Studies are currently under way to determine whether relative potency values based on biochemical effects are predictive for carcinogenesis in female Sprague Dawley rats for various dioxinlike compounds (4).

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#### REFERENCES AND NOTES

1. Van den Berg M, Birnbaum L, Bosveld BTC, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792 (1998).
2. van Birgelen APJM. Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. *Environ Health Perspect* 106:683–688 (1998).
3. Integrated Risk Information System. Available: <http://www.epa.gov/ngispgm3/iris/subst/irisbak/0374.htm> [last revised 1 November 1996].
4. van Birgelen APJM, DeVito MJ, Orzech D, Walker N, Birnbaum LS, Bucher J, Lucier G. Design of 2-year bioassays with dioxin-like compounds in female Sprague Dawley rats. *Organohalogen Compounds* 34:154–159 (1997).

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