Recent studies have indicated that a number of ubiquitous chemicals found in the environment are linked to a myriad of health concerns, including reproductive abnormalities and increases in infertility, cancer, and obesity via their actions as exogenous hormones. Because of the importance of evaluating the effects of these chemicals in living model organisms, maintaining the sensitivity of various bioassays as indicators of hormone action is critical. One source of variability is the isoflavones (a type of hormonally active phytoestrogen) in soy-based rodent diets. If the background levels of these diets are too high, they can interfere with the body’s response to an endocrine disrupting chemical. Our studies showed that variability within the phytoestrogen levels in different brands and lots of diets can cause changes in sensitive bioassays such as the fetal estradiol levels, uterotrophic response to estrogen, the timing of the onset of puberty, and the amount of gonadal fat as adults. In addition, maintaining the animals on various brands with varying background levels of phytoestrogens yielded different endpoints when the animals were exposed to a prenatal dose of the known endocrine disrupting chemical diethylstilbestrol. We conclude that sensitive endpoints used to determine the effects of environmental endocrine disrupting chemicals are affected by varying background levels of phytoestrogens.