Potential for Endocrine Modulation

In Vitro Assays

A number of *in vitro* assays have been developed to assess potential endocrine modulating effects of a chemical. The primary use of these *in vitro* assays in hazard identification is to screen large numbers of chemicals to determine which ones should be further studied in more definitive *in vivo* testing. As with any screening strategy, these assays are generally designed such that any errors are likely to be false positives rather than false negatives. When a positive result is reported in these assays, *in vivo* work is indicated to confirm, characterize, and quantify the true nature of the endocrine-modulating properties of the chemical. The recent concern over endocrine modulation and the availability of inexpensive screens is leading to the testing of chemicals in these *in vitro* assays regardless of the size and reliability of the more definitive *in vivo* database.

Petit *et al.* (1997) tested glyphosate and 48 other chemicals in two complementary assays: one measuring activation of the estrogen receptor from rainbow trout in a yeast system, and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had no estrogenic activity in either assay.

In Vivo Studies

The repeat-dose *in vivo* toxicology studies required by the U.S. EPA and other key worldwide regulatory agencies detect modulation of endocrine system activity (Carney *et al.*, 1997; Stevens *et al.*, 1997, 1998). These studies are more apical and predictive than *in vitro* screening assays as they assess a variety of endocrine-sensitive endpoints in animals that are capable of metabolic activation and/or detoxification. These studies also use extended exposure periods encompassing various stages of endocrine development. Endocrine active substances affecting a single or multiple endocrine target sites invariably initiate direct or compensatory biochemical, cellular, and/or histopathological processes which will be detected in standard toxicology studies required for pesticide registration in Canada, Europe, Japan, and the United States. A comprehensive histopathological assessment of endocrine tissues combined with gross organ pathology and organ weight data should detect all adverse endocrinopathies.

The standard toxicology studies that provide valuable information on potential endocrine-modulating effects include subchronic, chronic, developmental, and reproduction studies. The multi-generation rat reproduction study is the most definitive study for evaluating the potential of substances to produce endocrine-modulating effects in humans and other mammals (U.S. EPA, 1998b). This study evaluates effects on gonadal development/ function, estrous cycles, mating behavior, fertilization, implantation, *in utero* development, parturition, lactation, and the offsprings' ability to survive, develop, and successfully reproduce. A comprehensive histopathological assessment of all major organ systems also is a prominent feature of these studies. Developmental toxicity studies evaluate effects on many of these same processes, while

subchronic and chronic studies incorporate numerous direct and indirect evaluations of endocrine and reproductive tissues such as target organ weights and a comprehensive assessment of endocrine organ pathology.

There were no findings in the subchronic, chronic, developmental, or reproductive toxicity studies indicating that glyphosate or AMPA produced any endocrine-modulating effects (see Tables 3 and 4). Histopathological observations of endocrine and reproductive tissues from animals in a chronic and a 2-generation toxicity study are presented in Tables 3 and 4 to illustrate the magnitude and comprehensive nature of these assessments for those not familiar with standard long-term toxicology studies. The data clearly indicate that glyphosate exposure had no adverse histological consequence on any reproductive or endocrine tissue from either male or female rats even at exaggerated dosage levels. Negative results also were obtained in a dominant lethal study conducted at very high doses. While this latter test is typically used to assess genetic toxicity, substances that affect male reproductive function through endocrine modulating mechanisms can also produce effects in this type of study. To summarize, no effects were observed in two independent, multi-generation reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and AMPA are not reproductive toxicants and do not perturb the endocrine system. The U.S. EPA (1998a) reviewed these studies and also concluded that there were no effects that suggest that glyphosate produces endocrine-modulating effects.

The results of subchronic and developmental toxicity tests on POEA also showed no evidence of endocrine modulation. In addition, the metabolism of POEA would be expected to produce short chain carboxylic acids and similar derivatives, which are not considered to be endocrine modulators. The lack of any indications of hormonal activity in subchronic toxicity studies with Roundup® herbicide supports the conclusion that POEA does not possess endocrine modulating activity.

Summary

The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies. The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies. Results from standard studies with AMPA, Roundup® herbicide and the POEA surfactant also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup® herbicide does not result in adverse effects on endocrine systems in humans and other mammals.