Appendix N

Endocrine Disruption and Invertebrates

Considerations by the EDSTAC Screening and Testing Work Group (STWG) have predominantly dealt with vertebrate animals for several reasons. The first, and perhaps overriding one, is that the charge given to the work group of focusing on estrogen, androgen, and thyroid hormone actions is not especially relevant to important and well-studied hormones of invertebrates. The purported endocrine disruption effects of public concern are almost exclusively human health or vertebrate wildlife related. The expertise in the work group is, also, predominantly with the vertebrate classes. However, invertebrates represent over 95% of all animals, are ubiquitous, and are tremendously important ecologically and economically. Commercial fisheries of shrimp, crab, and oyster and agriculturally important insect pollination are but a few key examples. Because invertebrates are ubiquitous and are easily adapted for laboratory testing, they can serve as sentinels and surrogates for investigating environmental stress. For these reasons, invertebrates should not be ignored from consideration.

Endocrine disruption has been well studied and well exploited for certain invertebrates, especially the insects. The endocrine systems of insects have been intentionally targeted for insecticidal activity and several insecticides have been developed and used to suppress insect populations by disrupting their normal endocrine functions. Juvenile hormone mimics (e.g., methoprene), antijuvenile hormone analogs (e.g., precocene), chitin synthesis inhibitors (e.g., diflubenzuron), ecdysone analogs (e.g., tebufeno-zide), and molting disruptants (e.g., fenoxycarb) are some examples. These insect growth regulating compounds have also been observed to have adverse effects in related arthropods such as crustaceans, including disrupting normal molting processes, limb regeneration, and reproduction (Christiansen et al., 1977a, b; 1979; Cunningham, 1976; Forward and Costlow, 1978; Landau and Rao, 1980; Nimmo et al., 1980; Touart and Rao, 1987). Other substances like the organotin TBT have caused imposex and intersex conditions in gastropods (Gibbs and Bryan, 1986; Reijnders and Brasseur, 1992) and sewage outfalls have caused intersex conditions in harpacticoid copepods (Moore and Stevenson, 1994), conditions indicative of endocrine disruption.

Although the relevance of estrogen and androgen hormones to invertebrates is unclear, invertebrates may be useful as surrogates for investigating phenomena relevant to these hormones in vertebrates. Estrogens have been reported to play a meaningful role in development and reproduction in echinoderms and molluscs (Takeda, 1979; Brueggemeier et al., 1988; Shirai and Walker, 1988). Daphnids have been used to investigate the effects of xenoestrogens on steroid metabolism (Baldwin et al., 1995; Baldwin et al., 1997) and sex reversal (Shurin and Dodson, 1997). Because of their generally shorter life cycles and relative ease of handling many species in the laboratory, invertebrates could be useful for evaluating endocrine disrupting phenomena. However, additional research is needed before this promise is realized.

There are, therefore, two aspects to considering endocrine disruption for invertebrates, one is relevance to the health of invertebrate organisms themselves and the other is relevance of invertebrates as surrogates for investigating vertebrate-related phenomena. Conventional risk assessment of toxic chemicals such as outdoor use pesticides and high volume industrial chemicals generally include a crustacean reproduction or life cycle test in the data set used in the assessment. Although specific endocrine system endpoints are not considered, the apical nature of these tests may be adequate to detect the adverse consequences of an endocrine disrupting chemical in crustacean arthropods. Additional information is needed to determine what is most useful beyond these conventional tests for the wider invertebrate taxa. As surrogates, more information on the correlation of endocrine phenomena between invertebrates and vertebrates would be helpful. For instance, to what degree does a substance which disrupts ecdysteroid metabolism in crustacea disrupt sex steroid metabolism in vertebrates? Perhaps good correlations may be found, but more comparative information is needed before recommendations of specific invertebrate tests useful for evaluating potential endocrine disrupting activity relevant to vertebrates can be made.

No invertebrate assays, therefore, have been evaluated for use in T1S for detecting estrogen, androgen, or thyroid hormone disruption. Invertebrate tests have been proposed for T2T. It is recommended that a workshop of invertebrate endocrinologists and toxicologists be convened to address first, the suitability of invertebrate assays for estrogen and androgen (not thyroid) for use in a screening battery, and second, future improvements to the broader consideration of endocrine disruption in the environment and the utility of invertebrates as surrogate test organisms.

References:

Baldwin, W.S., D.L. Milam, and G.A. LeBlanc, "Physiological and biochemical perturbations in *Daphnia magna* following exposure to the model environmental estrogen diethylstilbestrol," *Environ.Toxicol.Chem.*, 14, 1995, pp. 945-952.

Baldwin, W.S., S.E. Graham, D. Shea, and G.A. LeBlanc, "Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol," *Environ.Toxicol.Chem.*, 16, 1997, pp. 1905-1911.

Brueggemeier, R.W., G.D. Yocum, and D.L. Denlinger, "Estranes, androstanes, and pregnanes in insects and other invertebrates," *Physiological Insect Ecology*, 1988, pp. 885-898.

Christiansen, M.E., J.D. Costlow, Jr., and R.J. Monroe, "Effects of the juvenile hormone mimic ZR-515 (Altosid) on larval development of the mud-crab, *Rhithropanopeus harrisii* in various salinities and cyclic temperatures," *Marine Biol.*, 39, 1977, pp. 269-279.

Christiansen, M.E., J.D. Costlow, Jr., and R.J. Monroe, "Effects of the juvenile hormone mimic ZR-512 (Altozar) on larval development of the mud-crab *Rhithropanopeus harrisii* at various cyclic temperatures," *Marine Biol.*, 39, 1977, pp. 281-288.

Christiansen, M.E., J.D. Costlow, Jr., and R.J. Monroe, "Effects of the insect growth regulator Dimilin (TH-6040) on the larval development of two estuarine crabs," *Marine Biol.*, 50, 1979, pp. 29-36.

Cunningham, P.A., "Effects of Dimilin (TH-6040) on reproduction in the brine shrimp *Artemia salina,"* *Environm. Entomol.*, 5, 1976, pp. 701-706.

Forward, R.B., Jr. and J.D. Costlow, Jr.). "Sublethal effects of insect growth regulators upon crab larval behavior," *Water, Air, Soil Pollution*, 9, 1978, pp. 227-238.

Gibbs, P.E., and G.W. Bryan, "Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints," *J. Mar. Biol. Assoc.*, UK 66, 1986, pp. 767-777.

Landau, M. and K.R. Rao, "Toxic and sublethal effects of precocene II on the early developmental stages of the brine shrimp *Artemia salina* (L.) and the barnacle Balanus eburneus Gould," *Crustaceana,* 39, 1980, pp. 218-221.

Moore, C. G. and J.M. Stevenson, "Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland,". *J. Nat. History*, 28, 1994, pp. 1213-1230.

Nimmo, D.R., T.L. Hamaker, J.C. Moore, and R.A. Wood, "Acute and chronic effects of Dimilin on survival and reproduction of Mysidopsis bahia," *Aquatic Toxicology*, ASTM, 1980, pp. 366-376.

Reijnders, P. J. H. and S.M.J.M. Brasseur, "Xenobiotic induced hormonal and associated disorders in marine organisms and related effects in humans; an overview," *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*, 1992, pp. 159-174.

Shirai, H. and C.W. Walker, "Chemical control of asexual and sexual reproduction in echinoderms," *Endocrinology of Selected Invertebrate Types*, 1988, pp. 453-476.

Shurin, J.B. and S.I. Dodson, "Sublethal toxic effects of cyanobacteria on nonylphenol on environmental sex determination and development in *Daphnia,"* *Environ. Toxicol. Chem.,* 16, 1997, pp. 1269-1276.

Touart, L. W. and K.R. Rao, "The influence of diflubenzuron on survival, molting and limb regeneration in the grass shrimp, *Palaemonetes pugio*," *Pollution Physiology of Estuarine Organisms*, 1987, pp. 333-349.