

PART IV  
CONCLUSIONS

## CONCLUSION

We hypothesized that TCDD and DES may act via the same mechanisms(s) to effect alterations in the fetal mouse thymus and liver as they have comparable effects on thymocyte progenitor cells, fetal thymic atrophy, thymic cellularity, and thymocyte subpopulations. We further hypothesized that these two compounds may target the same genes involved in cell cycle progression and apoptosis. To test these hypotheses, late gestation exposure to TCDD at 5 and 10  $\mu\text{g}/\text{kg}$  or DES at 48  $\mu\text{g}/\text{kg}$  resulted in a reduction in fetal thymic weights, a reduction in the overall number of thymocytes present per organ, and alterations in percentages of thymocytes in all phenotypes present characterized by CD4 and CD8 cell surface antigens. The percentages of CD4CD8 double negative cells were increased while the percentages of CD4CD8 double positive cells were reduced after either TCDD or DES exposure. Co-identification of TCDD- or DES-treated cells with 7-AAD demonstrated an overall decrease in thymocyte viability with concomitant increase in early apoptosis. However, the subpopulations affected by this change were unique to each treatment. These results suggest that TCDD and DES may have different targets in the fetal phenotypic subpopulations, although thymocyte apoptosis appears to be contributing mechanism in thymic atrophy for both chemicals. Histologically, increased numbers of apoptotic bodies were identified, confirming the results seen in flow cytometry, as well as decreased numbers of mitotic figures, suggesting a decline in the proliferative activity of the cells after either TCDD or DES exposure. Disruption of thymic cortico-medullary architecture was also noted after exposure to these chemicals. The persistence of this change after birth, its implication in

thymocyte development, as well as its potential role in long term thymocyte functionality require further investigation.

The histologic evaluation of the fetal liver after late gestation exposure to TCDD or DES further demonstrated the responsiveness of both the developing hepatocytic and hematopoietic cell populations to both of these compounds. The histologic alterations noted were similar and included hepatocytic cytomegaly, increased cytoplasmic basophilia, and increased variability in the hepatocytic nucleoli. The hematopoietic compartment was also affected, and an overall diminution in hematopoiesis was noted, although thrombopoiesis appeared unaffected by either chemical. Interestingly, study results evaluating p53, Bcl-2, PCK $\alpha$ , and c-jun mRNA expression suggested different molecular targets for each chemical. TCDD's proliferative effects on the hepatocytes appeared to predominate among gene expression changes, with increased c-jun and decreased p53 expression. The modest decrease in PCK $\alpha$  was the only shared effect of TCDD and DES for the four genes selected, suggesting that both chemicals may cause a nonspecific alteration in the phosphorylation of substrates requisite to a variety of cellular functions. DES had no effect on the expression of p53, however it downregulated the expression of Bcl-2 and c-jun as well as PCK $\alpha$ . These results after DES treatment may reflect primarily the alterations noted histologically of the hematopoietic population. These data have described some of the similarities and subtle differences of the immunotoxic effects of TCDD and DES in the fetal mouse thymus and liver. Further studies identifying, enumerating, and separating the different hematopoietic subpopulations in the fetal liver from the hepatocellular component would be initial steps to further elucidate the impact these two chemicals have on the developing immune

system. Furthermore, assays to target the effects on fetal hepatocytes, which may make an indirect contribution to hematopoiesis, need to be employed to discover their potential role in the developmental immunotoxicity of TCDD and DES