

Notes for Appendix 6 - Multi-chemical study - RTI - summary table

Thyroid

A limited number of histopathologic changes were observed in both control and treated animals. For the most part, these changes were typical of the spontaneous type of microscopic pathology that can be observed at this age and in this strain of rat. The following chemicals were not associated with any treatment-related histopathologic changes: Component 1 = Atrazine (75 and 150 mg/kg), Fenarimol (50 mg/kg), and Methoxychlor (25 and 50 mg/kg); Component 2 = Bisphenol A (400 mg/kg).

TREATMENT-RELATED FINDINGS BY CHEMICAL

Fenarimol:

Exposure to Fenarimol (250 mg/kg) was associated with follicular cell hypertrophy of the thyroid gland. This lesion was characterized by a subtle increase in cell size, particularly in the height of the follicular lining cells. In addition, the lumen of affected thyroid follicles was slightly reduced in size and the amount and staining intensity of the thyroid colloid was less. The incidence and severity of follicular cell hypertrophy are presented in Table 3 of Histopathology Report. As can be seen in the Table 3, 5 out of 15 animals had minimal follicular cell hypertrophy. The toxicological or biological significance of this lesion is not clear since only 1/3 of the animals had hypertrophy and there was no apparent increased thyroid weight changes noted. However in this dose group, the level of thyroxine hormone (4) was decreased and thyroid stimulating hormone (TSH) was increased.

Propylthiouracil:

Administration of both 2 and 25 mg/kg Propylthiouracil was associated with the presence and dose-related increased severity of thyroid follicular cell hypertrophy/hyperplasia which was clearly related to increased thyroid weights and levels of TSH and decreased levels of T4.

Follicular cell hypertrophy/hyperplasia was characterized by a spectrum of histologic changes including the increased size and apparent number of follicular cells, the reduction in follicular lumen size, the presence of pale staining colloid and/or the reduction or absence of colloid within some thyroid follicles. The severity of hypertrophy/hyperplasia was subjectively based on a number of criteria: minimal = multifocal follicles affected, size and number of follicular cells slightly enlarged and increased; mild = diffuse change with further increased cell numbers of follicular cell mitoses; marked = increased mitotic rate, some degenerative cells (deeply eosinophilic cytoplasm and pyknotic nuclei) within the follicular epithelium, and obvious thyroid gland size and shape enlargement. No alteration of the thyroid vasculature was noted. The incidence and severity of thyroid hypertrophy/hyperplasia is presented in Table 5 of report.

Results of the microscopic examination of the thyroid gland are compatible with previous reports on the direct action of Propylthiouracil on the thyroid gland (Thomas and Williams, 1999).

Ovary

A limited number of histopathologic changes were observed in both control and treated animals. For the most part, these changes were typical of the spontaneous type of microscopic pathology that can be observed at this age and in this strain of rat. During the microscopic examination of ovaries, attempts to quantify the number of corpora lutea were not performed because of the variation that one may observe in any one cross-section of ovary. However, each ovary was examined for the presence of primary, growing and pre-ovulatory follicles.

The following chemicals were not associated with any treatment-related histopathologic changes: Component 1 = Atrazine (75 and 150 mg/kg), Fenarimol (50 mg/kg), and Methoxychlor (25 and 50 mg/kg); Component 2 = Bisphenol A (400 mg/kg).

TREATMENT-RELATED FINDINGS BY CHEMICAL

Bisphenol A:

Exposure to Bisphenol A (600 mg/kg) was associated with the presence of ovarian hypoplasia in 3 out of 14 animals examined. Ovarian hypoplasia was characterized by the complete absence of corpora lutea (CL's) and an apparent reduction or absence of the large pre-ovulatory follicles (Graffian Follicles). Mild hypoplasia seemingly had fewer large follicles and with moderate hypoplasia no large pre-ovulatory follicles were observed. This appearance suggested that some inhibition or delay of follicle development and/or ovulation had occurred. Hypoplasia was used in this context since evidence of complete ovarian maturity and subsequent atrophy was not observed (Davis et al, 1999). According to the organ weight data, animals exposed to 400 and 600 mg/kg of Bisphenol A had lower ovary weights but also had significantly lower body weights. It has been suggested that significantly reduced body weights, associated with chemical administration, may result in "stress" to the animal decreasing the frequency and amplitude of luteinizing hormone (LH) thus altering the reproductive cycle (Yuan et al, 2002). Although the small incidence of ovarian hypoplasia might have been associated with decreased body weights, it is not clear if other mechanisms might be involved as well.

Ketoconazole:

Exposure to 50 and 100 mg/kg of Ketoconazole was associated with cytoplasmic vacuolization of ovarian corpora luteal cells and ovarian hypoplasia in the 100 mg/kg dosed animals. Cytoplasmic vacuolization was characterized by the presence of clear, variably sized vacuoles within the cytoplasm of corpora luteal cells. Occasionally, small cytoplasmic vacuoles may be noted in some degenerating cells within normal corpora lutea, but the vacuoles in Ketoconazole-exposed animals tended to be larger and more dispersed within the corpora lutea. The severity of cytoplasmic vacuolization was graded according to a subjective evaluation based on the average number and size of the

vacuolated cells in a corpora lutea (minimal = 6-25%; mild = 26-50%; moderate = 51-75%; and marked 76-100%). The pathogenesis of the vacuolization could not be determined but was thought to involve the alteration of steroid metabolism at the cellular level.

As previously mentioned, 5 out of 15 high-dosed animals also had mild ovarian hypoplasia which meant there were no corpora lutea present and subsequently, no cytoplasmic vacuolization to evaluate and grade. The incidence and severity of corpora luteal cytoplasmic vacuolization and ovarian hypoplasia is presented in Table 4 of report. According to the organ weight data, some evidence of decreased ovarian weight was apparent in the 100 mg/kg dosed animals. This may be related to the ovarian hypoplasia seen at this dose level. However, the apparent cytoplasmic vacuolization apparently did not result in any ovarian weight change.

Uterus

A limited number of histopathologic changes were observed in both control and treated animals. For the most part, these changes were typical of the spontaneous type of microscopic pathology that can be observed at this age and in this strain of rat. The following chemicals were not associated with any treatment-related histopathologic changes: Component 1 = Atrazine (75 and 150 mg/kg), Fenarimol (50 mg/kg), and Methoxychlor (25 and 50 mg/kg); Component 2 = Bisphenol A (400 mg/kg).

TREATMENT-RELATED FINDINGS BY CHEMICAL

Methoxychlor:

Administration of Methoxychlor was associated with the presence of epithelial hyperplasia of the uterine epithelium.

In control animals, the uterine surface epithelial lining was characterized by having a single layer of columnar cells with a cytoplasmic to nuclear ratio of around 1.5. The height and presence of vacuolar to necrotic changes and the presence of inflammatory cells depended upon the stage of the estrous (reproductive) cycle. In addition, mitotic figures were common in the estrous portion of the cycle (Yuan and Foley, 2002).

In the 50 mg/kg/day dosed group, some animals had changes of the uterine surface epithelium that were diagnosed as epithelial hyperplasia. In these cases, the lining epithelium was generally diffusely affected. This change was characterized by taller columnar cell height and nuclear crowding indicative of more cells. In addition, these cells tended to have more cytoplasm (hypertrophy) which appeared lightly basophilic. Mitotic figures were occasionally noted, however, vacuolar to necrotic changes normally associated with the estrous cycle were not observed.

All cases of epithelial hyperplasia were diagnosed as minimal which was a change barely detectable. The appearance of the lesion suggested a hormonal imbalance as a possible mechanism.

The incidence and severity of uterine epithelial hyperplasia is presented in Table 6 of report.