Presentations

Back to the Listing

2,4-Dichlorophenoxyacetic Acid (2,4-D): Evaluation of Reproductive/Endocrine Endpoints in a Dietary Extended One-generation Study in Crl:CD(SD)Rats.

B.H. Neal[2], J.S. Bus[2], C.L. Zablotny[2], B.L. Yano[2], J.K. Passage[2], and M.S. Marty[2].
[1]Exponent, Alexandria, VA; [2]The Dow Chemical Company, Midland, MI.

ABSTRACT

An advantage of the extended one-generation study is that it examines multiple life stages, and offers flexibility to verify age-related sensitivity and/or reproducibility of results. Dietary doses to CD rats were 0, 100, 300, 600 (female) or 800 (male) ppm 2,4-D. P1 males exposed for ~ 11 weeks showed decreased seminal vesicle and prostate weights (≥300 ppm), but there were no associated effects on reproductive function, sperm parameters or pathology. Control relative organ weights exceeded historical control. Decreased testes weights were seen in PND 22 F1 weanlings; 800 ppm F1 males had slightly delayed preputial separation. In both cases, affected offspring had decreased body weights; PND 22 pups showed no associated pathology. Evaluation of groups of F1 males at PND 70 and PND 139 showed no effects on male reproductive organ weights, pathology, and/or sperm parameters. The F1 males, exposed *in utero*, via lactation and in the diet, did not reproduce P1 organ weight effects, despite longer exposures at higher doses (mg/kg/day). Thyroid hormones (TH), weights and/or pathology were assessed in P1 adults, GD 17 dams, F1 PND 4 and 22 pups, and F1 PND 70 and 139 adults. High-dose GD 17 dams had non-significant TH changes and altered pathology (3 of 12), indicating an adaptive change during the demanding period of gestation. In contrast, TH changes in high- and mid-dose PND 22 pups had no corresponding thyroid weight changes or pathology and were deemed not biologically significant. There were no other effects on endocrine-sensitive endpoints, including anogenital distance or nipple retention, vaginal opening, estrous cyclicity, reproductive indices or litter parameters and mating of a second generation was not triggered. Thus, this integrated study design supports the conclusion that 2,4-D did not induce reproductive toxicity; endocrine toxicity, seen as adaptive thyroid changes on GD 17, only occurred at a nonlinear toxicokinetic dose. Study sponsored by Industry Task Force II on 2,4-D Research Data.

INTRODUCTION

The extended one-generation study presents a new experimental design to examine general toxicity, developmental neurotoxicity, developmental immunotoxicity, endocrine modulation, and reproductive toxicity in a single study. The study design is based on the work of Cooper *et al.* (*Crit. Rev. Toxicol.* 36:69-98; 2006 – see poster 419 for study design). One advantage of this study design is the opportunity to examine patterns of effects across the parental generation and multiple cohorts of offspring, which facilitates a weight of evidence approach to data interpretation.

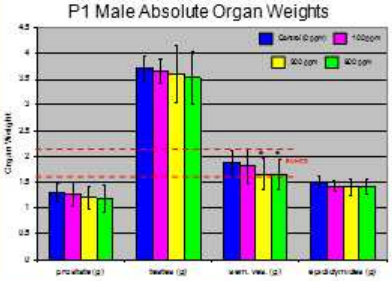
RESULTS

Potential Reproductive Toxicity

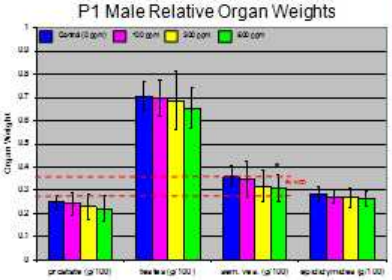
P1 Male Reproductive and Accessory Sex Gland (ASG) Weights (ANOVA; α = 0.05):

- Seminal vesicle weights significantly decreased at 800 ppm (abs. and rel.) and 300 (abs. only)
- Non-significant decrease in rel. prostate (11%) at 800 ppm

P1 Male Absolute Organ Weights



P1 Male Relative Organ Weights



Interpreting P1 Male Organ Weight Changes:

- Control values for relative weights were above HCD; treated groups were within HCD
- No corresponding effects on P1 sperm parameters
- No corresponding histopathological findings in P1 tissues
- P1 male organ weight differences not replicated in F1 adult males (Sets 1a, 3)

Conclusion: No exposure-related effects on P1 Male Reproductive or ASG weights

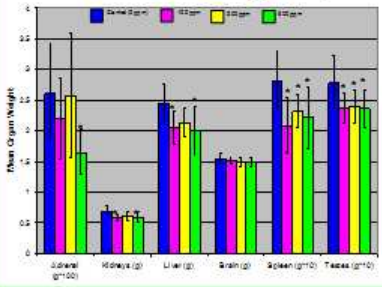
No exposure-related effects on the following reproductive endpoints:

- P1 male or female reproductive indices
- Gestation Length
- Pup Sex Ratio
- Litter size
- Litter survival
- Estrous cyclicity in P1 or Set 3 females
- Time to mating
- Corpora lutea (GD 17 satellite dams)
- Pre-implantation loss (GD 17 satellite dams)
- Post-implantation loss (GD 17 satellite dams)
- Quantitative ovarian follicle counts
- Reproductive organ weights: P1, Sets 1a, 3
- Reproductive organ histopathology: P1, Sets 1a, 3
- Sperm parameters (motility, counts, morphology) in P1 or Set 3 males
- Nursing behavior and presence/absence of milk bands

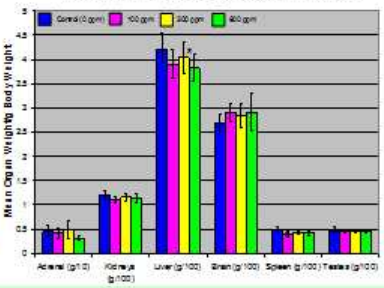
Potential Anti-androgenicity

Decreased testes weight in F1 weanlings (ANOVA; α = 0.05)

PND 22 Male Absolute Organ Weights



PND 22 Male Relative Organ Weights



Interpreting F1 Male Decreased Organ Weights:

- Affected weanling organ weights correlated with body weight effects (see Table below)
- Pups do not conserve testis weights as do adults (Carney *et al.*, *Toxicol. Sci.* **82:**237, 2004)
- No organ weight changes in F1 adult males (Sets 1a and 3)
- No corresponding histopathological findings in PND 22, Sets 1a or Set 3 (except adult kidney)

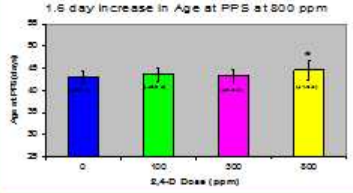
Correlation between Organ Wts and Terminal Body Wts in Weanlings

Tissue	Spearman Correlation Coefficient (r)
Adrenals	0.33
Kidneys	0.86
Liver	0.86
Brain	0.44
Spleen	0.77
Testes	0.79

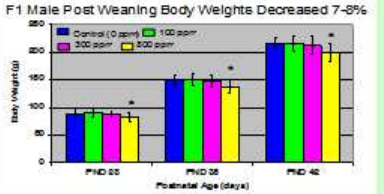
Potential Anti-androgenicity

Delayed belanopreputial separation in F1 offspring related to decreased body wt.

1.6 day increase in Age at PPS at 500 ppm



F1 Male Post Weaning Body Weights Decreased 7-8%



No exposure-related effects on the following (anti)androgen endpoints:
- F1 anogenital distance
- F1 Nipple/areolae retention
- No changes in sperm parameters in either generation

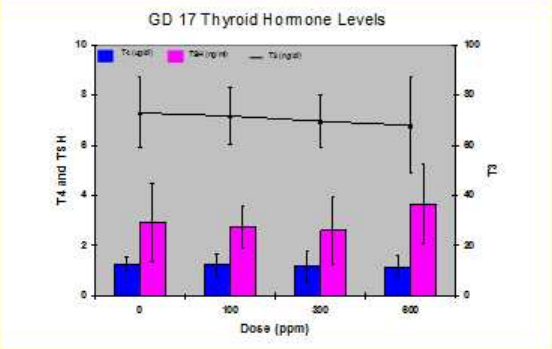
Conclusion: Overall, data do not support androgenicity/anti-androgenicity by 2,4-D

Potential (Anti)Estrogenicity
- No effects on vaginal opening
- No effects on estrous cyclicity
- No effects on uterine weights
- No effects uterine or ovarian histopathology
- No effects on quantitative ovarian follicle counts

Conclusion: Overall, data do not support estrogenicity/anti-estrogenicity by 2,4-D

Potential (Anti)Thyroid effects

Thyroid histopathology in P1 Satellite GD 17 Females
- Thyroid hormone analyses (GD 17 dams): no significant effects, but potential exposure related pattern at 600 ppm
- Increased TSH, slightly decreased T3 and T4 (ANOVA; $\alpha = 0.05$)



- No effect on thyroid weights (ANOVA; $\alpha = 0.05$)
- Histopathology: Very slight decreased follicle size/colloid depletion at 600 ppm in 3/12 females
- No evidence of degenerative changes, hypertrophy or hyperplasia
- No replication of histopathology in P1 LD 22 females

F1 PND 22 Weanling Thyroid Assessment
- Incomplete pattern in thyroid hormone changes
  - Significant decrease in T3 at 300 ppm in males only; no dose response
  - Significant decrease in T4 at 600 ppm in males only
  - No corresponding changes in TSH
  - No significant changes in T3, T4 or TSH in females
- No exposure-related effect on thyroid weights, histopathology, gross brain measurements or brain histopathology

Thyroid assessments in P1 adults, F1 PND 4 Cull Pups, Set 1a and Set 3 Adults
- No exposure-related effects on thyroid parameters
- No exposure-related effects on motor activity, auditory startle, gross brain measurements, morphometrics, myelin deposition or brain histopathology (F1 Set 1B Adults)

Conclusion: Exposure-related thyroid effects in dams on GD 17 only; Considered an adaptive non-adverse response during the demanding period of gestation

CONCLUSION

2,4-D did not adversely affect reproduction, did not alter androgen- or estrogen-sensitive endpoints, and altered thyroid endpoints only in females at an exposure level (600 ppm) that clearly resulted in nonlinear toxicokinetics.

Copyright 2012 Industry Task Force II on 2,4-D Research Data