Nonylphenol – Critical Effect

Meeting with Minnesota Department of Health

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Thomas G. Osimitz, PhD, DABT

Alkylphenols and Ethoxylates Research Council (APERC)

Agenda

- Introductions
- Purpose of Meeting
  - Selection of Critical Effects
- Scientific overview
  - Review of histopathology
  - Adverse? Renal toxicity - weight of evidence
- Regulatory overview
  - Comparative assessments
- Recommendations
- Discussion

Preview – Conclusions

- Renal mineralization seen at some dose(s) in all three pivotal studies
  - It was low incidence and low severity
  - No other renal effects accompany the mineralization
- Mineralization is a frequent finding in rat studies
  - Possible mineral imbalance, gut flora, etc.
- Mineralization alone at the low dose in a single study should not be considered a critical effect

Present MDH Assessment

Chronic Non-Cancer Health Based Value ($nHBV_{Chronic}$) = 20 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Chronic Intake Rate, L/kg-d)}}$$

$$= \frac{\text{(0.0049 mg/kg-d) x (0.2)}^{*} \text{ x (1000 µg/mg)}}{\text{(0.045 L/kg-d)}^{**}}$$

= 21.7 rounded to **20 µg/L**

*Relative Source Contribution: MDH 2008, Section IV.E.1.
**Intake Rate: MDH 2008, Section IV.E.1. and US EPA 2019, Exposure Factors Handbook, Tables 3-1, 3-3 and 3-5.

Reference Dose/Concentration:	HED/Total UF = 0.485/100 = 0.0049 mg/kg-d (SD rats)
Source of toxicity value:	Determined by MDH in 2015
Point of Departure (POD):	1.94 mg/kg-d (administered dose $BMDL_{10}$, NTP 1997/Chapin 1999, subchronic exposure)
Dose Adjustment Factor (DAF):	0.25, Body weight scaling, default (US EPA 2011 and MDH 2017)
Human Equivalent Dose (HED):	POD x DAF = 1.94 mg/kg-d x 0.25 = 0.485 mg/kg-d
Total uncertainty factor (UF):	100
Uncertainty factor allocation:	3 for interspecies differences (for toxicodynamics), 10 for intraspecies variability and 3 for subchronic to chronic extrapolation
Critical effect(s):	Renal mineralization in male rats

Present MDH Assessment

Subchronic Non-Cancer Health Based Value ($nHBV_{Subchronic}$) = 40 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Subchronic Intake Rate, L/kg-d)}}$$

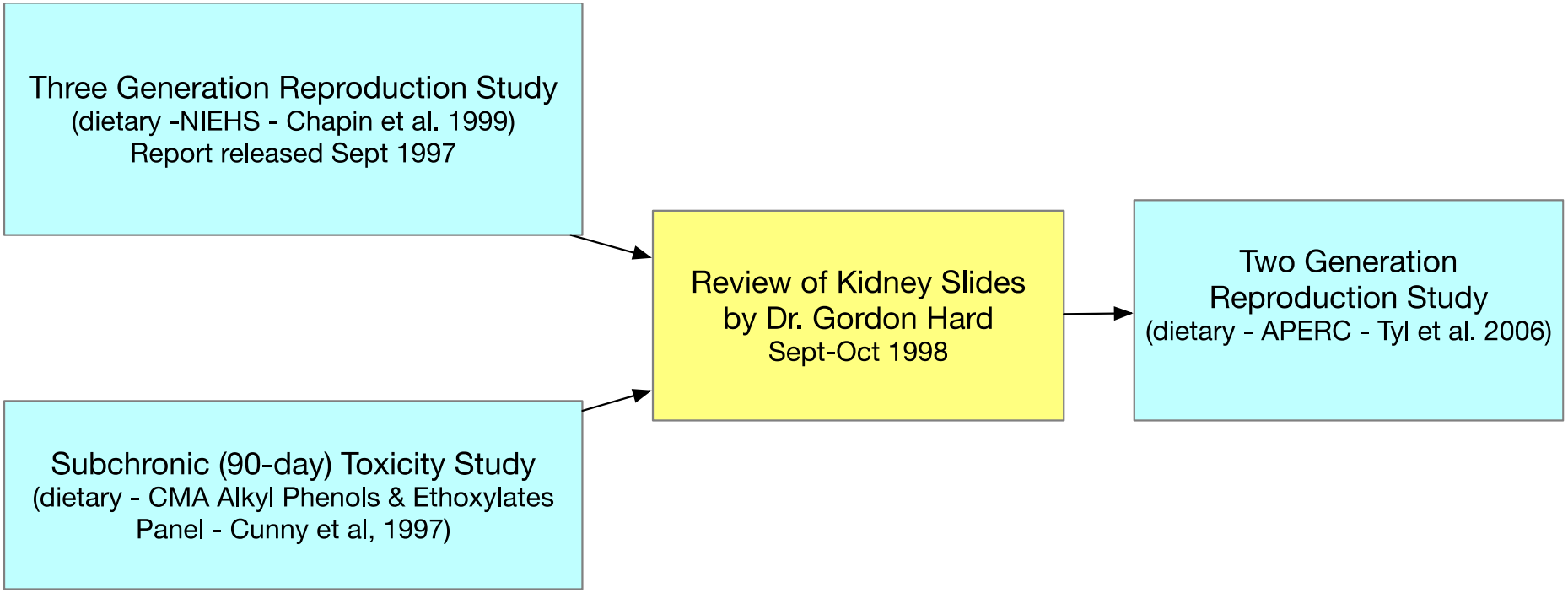
$$= \frac{\text{(0.016 mg/kg-d) x (0.2)}^{*} \text{ x (1000 µg/mg)}}{\text{(0.074 L/kg-d)}^{**}}$$

= 43.2 rounded to **40 µg/L**

*Relative Source Contribution: MDH 2008, Section IV.E.1.
**Intake Rate: MDH 2008, Section IV.E.1. and US EPA 2019, Exposure Factors Handbook, Tables 3-1, 3-3 and 3-5.

Reference Dose/Concentration:	HED/Total UF = 0.485/30 = 0.016 mg/kg-d (SD rats)
Source of toxicity value:	Determined by MDH in 2015
Point of Departure (POD):	1.94 mg/kg-d (administered dose $BMDL_{10}$, NTP 1997/Chapin 1999)
Dose Adjustment Factor (DAF):	0.25, Body weight scaling, default (US EPA 2011 and MDH 2017)
Human Equivalent Dose (HED):	POD x DAF = 1.94 mg/kg-d x 0.25 = 0.485 mg/kg-d
Total uncertainty factor (UF):	30
Uncertainty factor allocation:	3 for interspecies differences (for toxicodynamics), 10 for intraspecies variability
Critical effect(s):	Renal mineralization in male rats
Co-critical effect(s):	None
Additivity endpoint(s):	Renal (kidney) system

Focus - Pivotal Studies



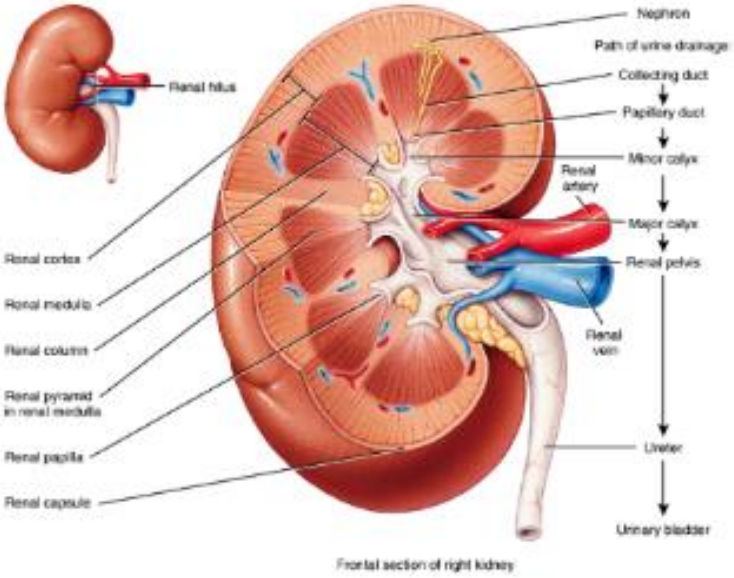
Review of Cunny et al. and Chapin et al.

- Conducted by Gordon Hard, BVSc, PhD, DSc, FRC Pat, FRCVS, FATS (noted renal pathologist)
- Goal: review kidney tissue using same pathologist, criteria, and nomenclature

Renal Mineralization

- Nature of the effect
  - Renal anatomy and pathology
- NP association – study data
- Causes
  - Chemical and non-chemical
- Gauging Adversity
  - LOAEL or LOEL

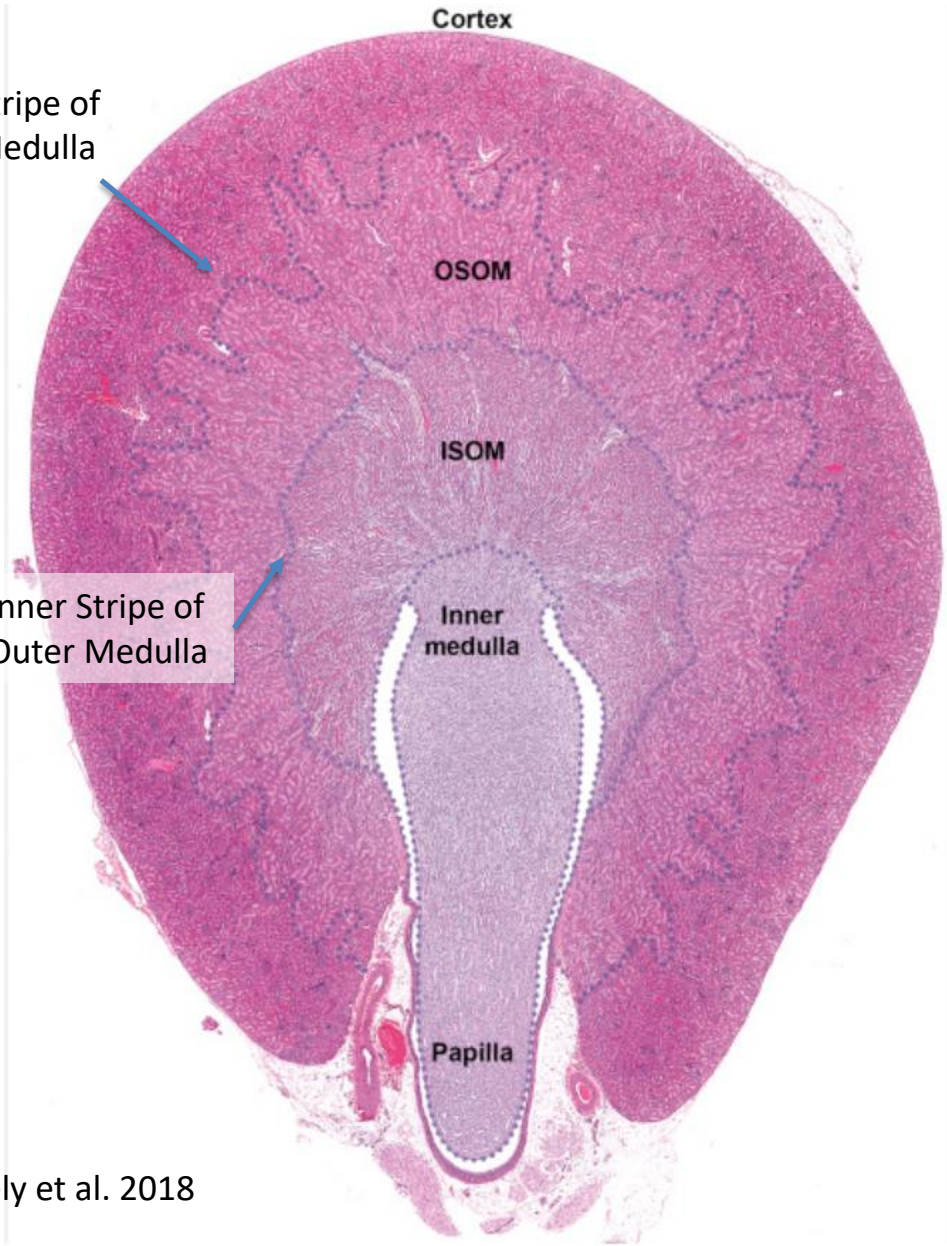
Renal Orientation



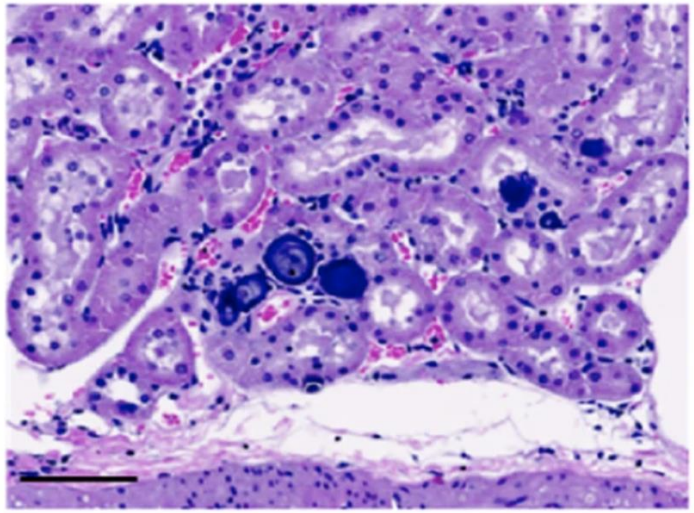
Maurya et al. 2018

Outer Stripe of Outer Medulla

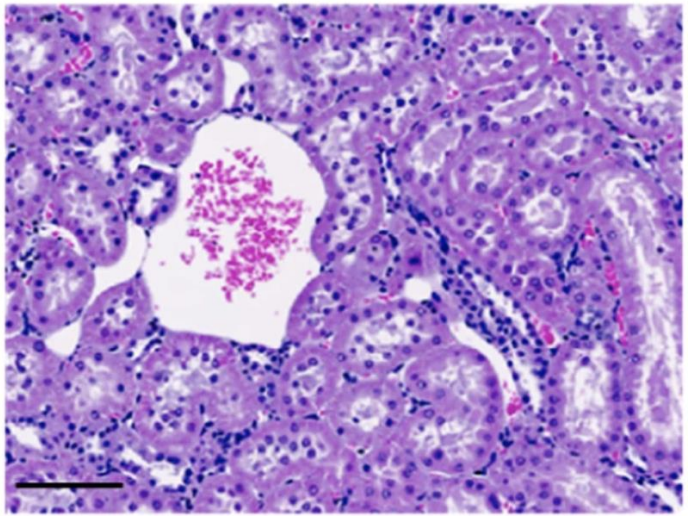
Inner Stripe of Outer Medulla



Seely et al. 2018



Mineralization (tubular)

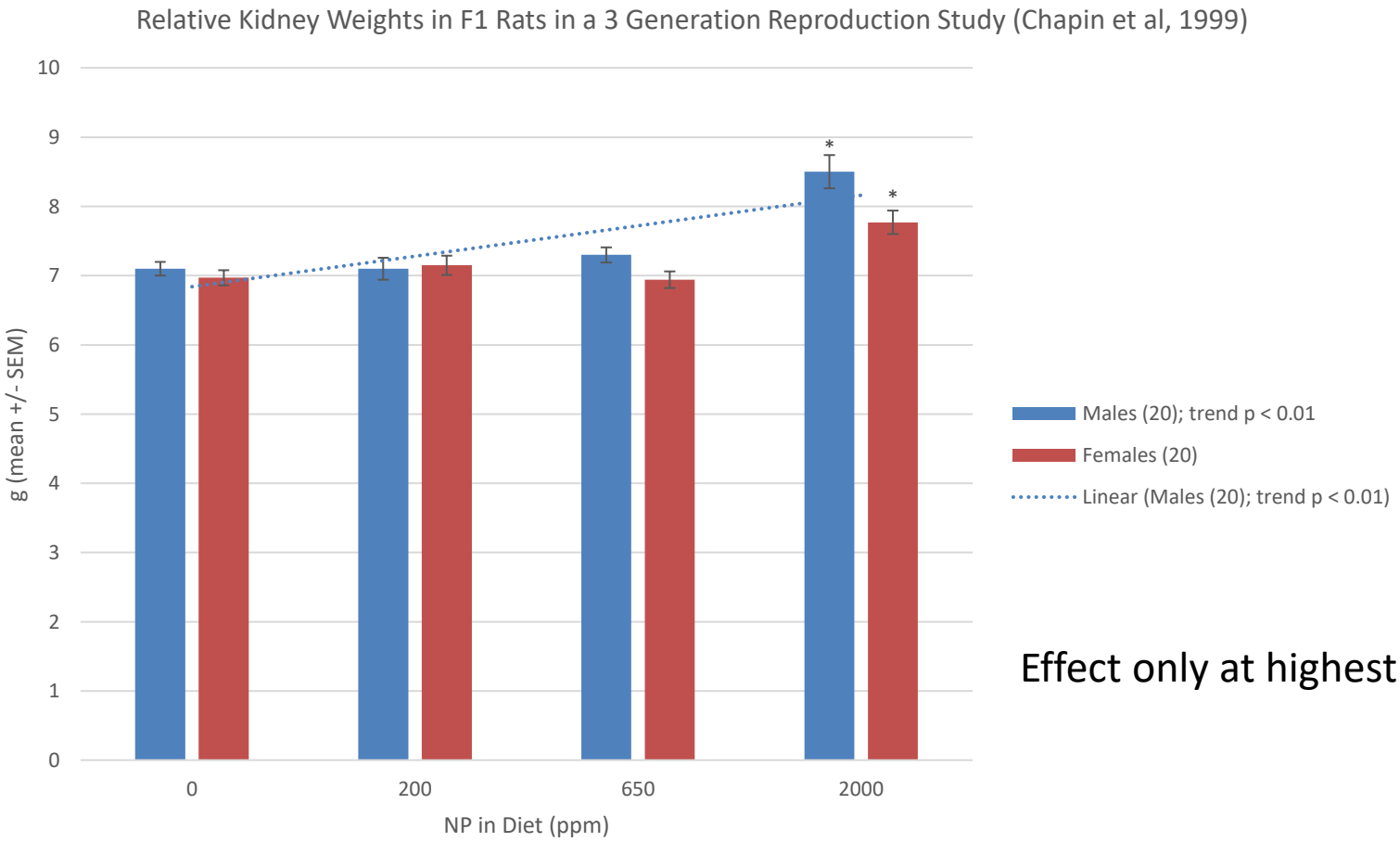


Normal tubular histology

Byun et al. (2022)

Chapin et al. – Closer Look

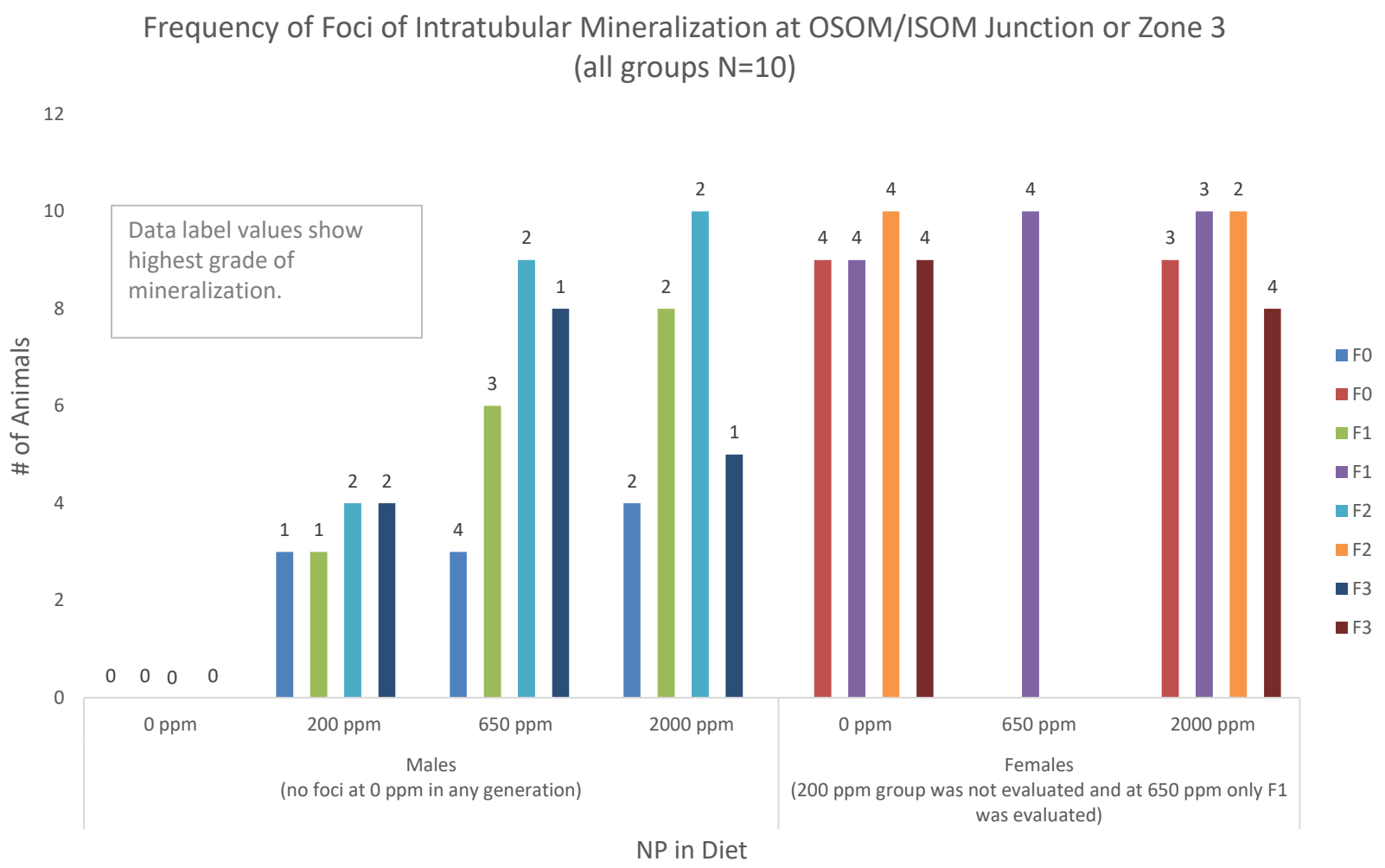
(From Hard, 1998)



Effect only at highest dose

Chapin et al. – Closer Look

(From Hard, 1998)



Mineralization at low grade and low incidence in males at 200 ppm

Chapin et al. – Closer Look

(From Hard, 1998)

Other changes which showed an association with treatment in high-dose (and sometimes mid-dose) rats of the F1, F2 and F3 generations (but not in the 90-day study or F0 generation) were cystic tubules and/or fibrotic areas, scattered or sporadic dilated tubules, granular or cellular casts, and foci of mononuclear cell inflammation, all at the OSOM/ISOM junction or in zone 3. In addition, some treated animals in the F2 and F3 generations had tubular involvement suggestive of pyelonephritis. The PWG identified hydronephrosis above control levels in the males of the F1, F2 and F3 generations, which was generally confirmed during this review. However, the PWG did not consider the observed hydronephrosis to have a definitive treatment relationship. Nevertheless, this condition can reflect chronic inflammation or obstruction in the lower urinary tract (Hard et al., 1998), and possibly may be a further indication of some intercurrent process superimposed on the treatment.

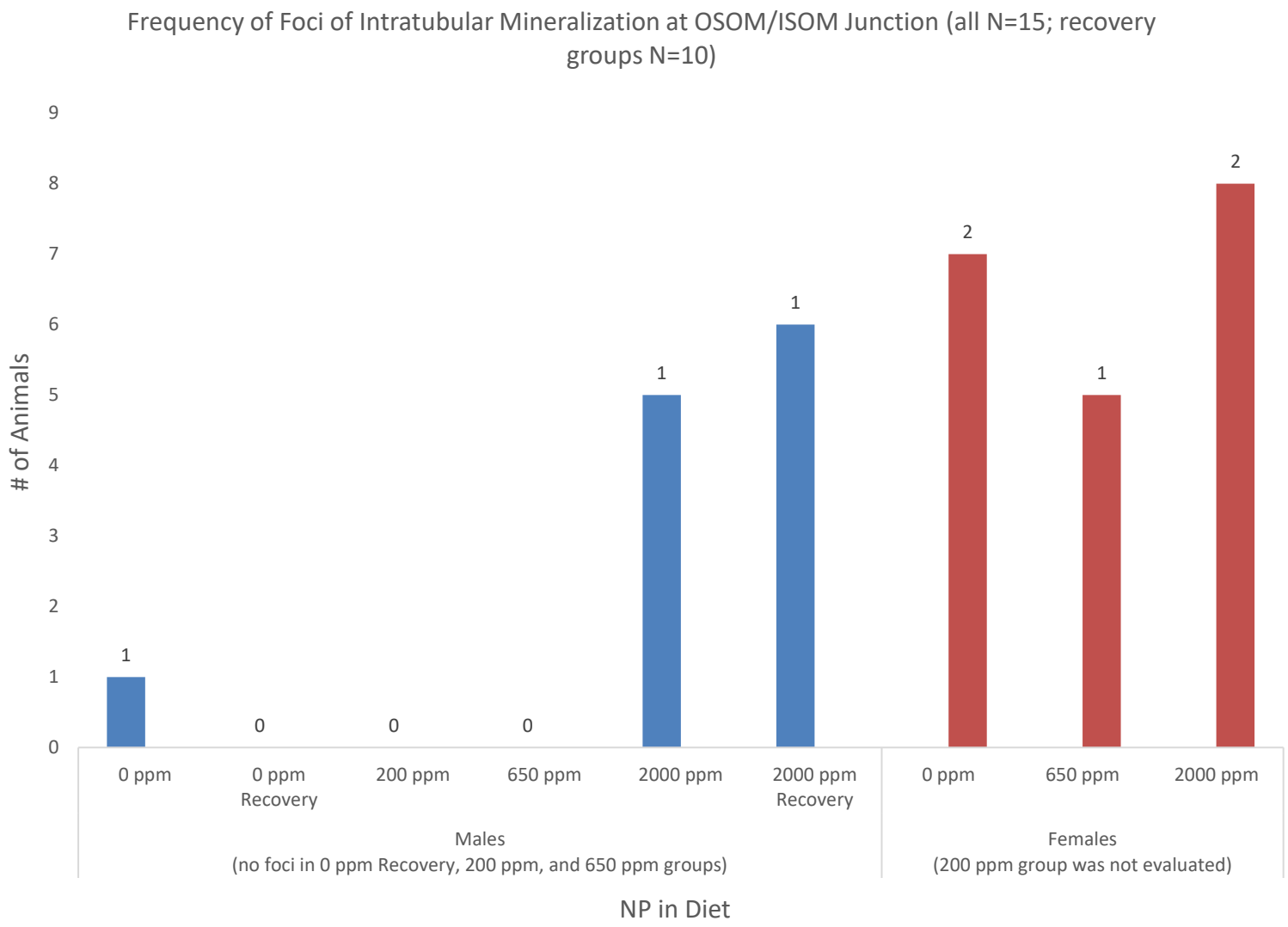
Cunny et al. – Closer Look

(from Hard, 1998)

- "The only treatment-related pathological effect observed was an increase in the frequency of deposits of intratubular mineralization in the P3 (straight) of the proximal tubule at the OSOM/ISOM junction in the high does males. In this group, 11 of 25 rats had such mineral deposits compared to none in the lower dose groups and 1 of 25 control rats. A similar treatment related effect not observed in female rats because foci of intratubular mineralization in all groups, controls were comparable."

Cunny et al. – Closer Look

(from Hard, 1998)



Mineralization in all female dose groups, but only high dose in males

Cunny et al. – Closer Look

(from Hard, 1998)

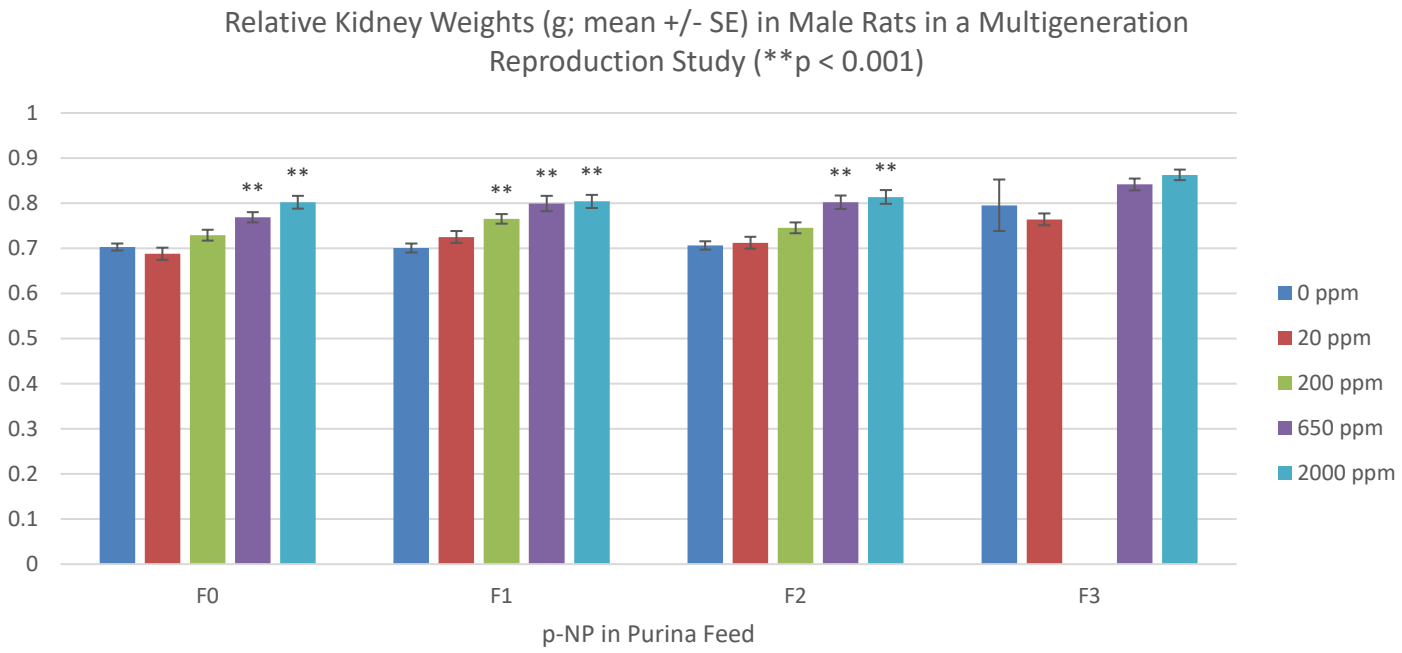
- Mineralization may represent calcium phosphate formation - frequently associated with a decrease in the dietary calcium/phosphorus ratio below 1.0. The rat is considered less able than other species to cope with disturbance in calcium homeostasis, with female rats more prone to renal tubular mineralization than male rats, “as estrogen levels may play a role in the process” (Hard, 1998; p. 8).

Tyl et al. – Another Look

- This study evaluated the potential for dietary para-nonylphenol (NP; CAS No. 84852-15-3) to affect parental fertility and growth and development of three offspring generations in CD (SpragueDawley [SD]) rats, including sperm counts across generations to determine the validity of equivocal reductions observed in the F2 generation by R. E. Chapin et al. (1999, Toxicol. Sci. 52, 80–91). Male rat kidney toxicity was also examined based on inconsistent observations in NP-exposed rats at 2000 ppm but not at 200 or 650 ppm in Purina 5002 (H. C. Cunny et al., 1997, Regul. Toxicol. Pharmacol. 26, 172–178) and at all of these NP concentrations in NIH-07 diet

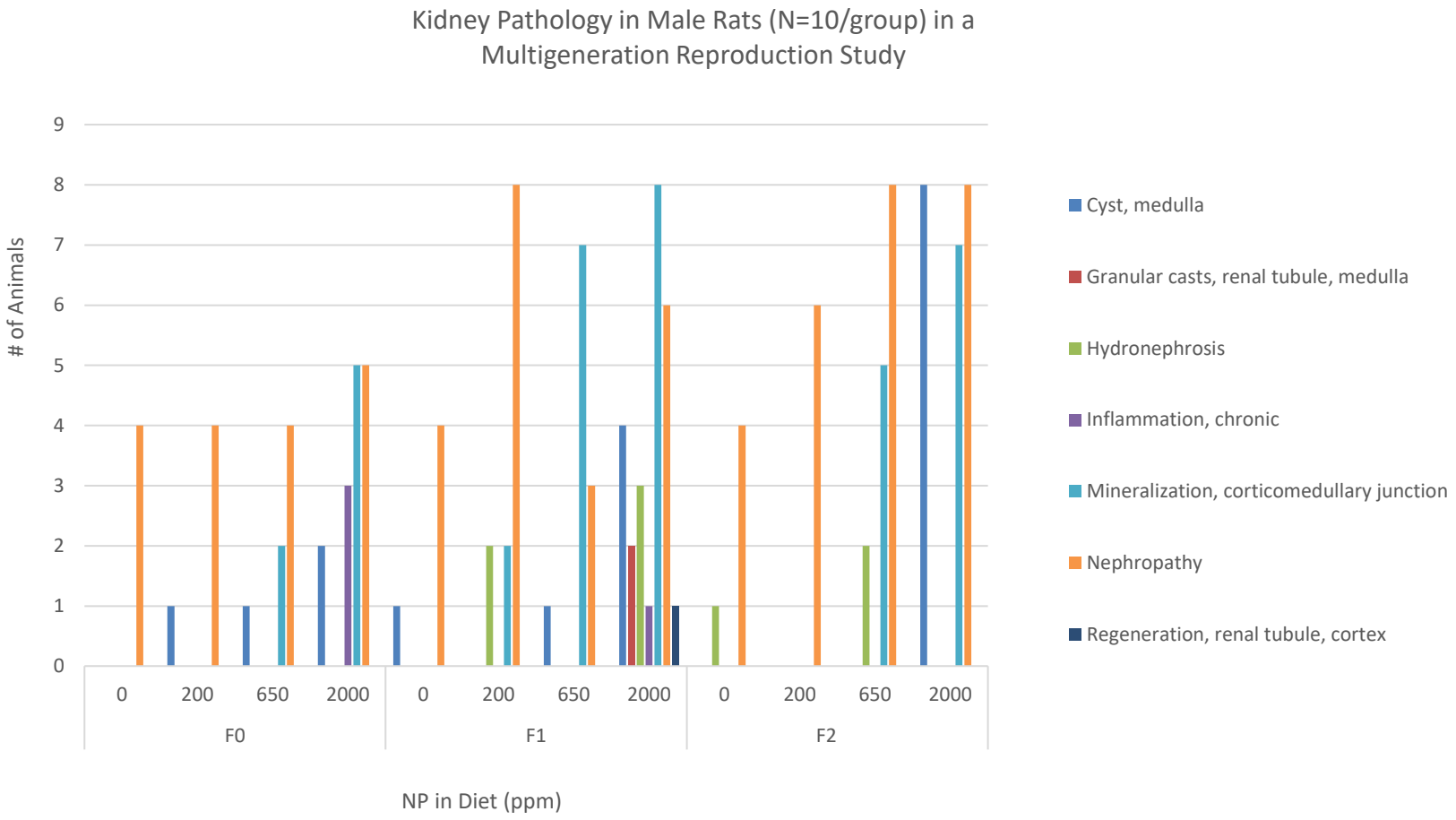
- Kidney toxicity (histopathology) occurred at 650 and 2000 ppm with no clear difference for the two diets.

Tyl et al. – Another Look



Effects if any only at highest 2 or 3 doses

Tyl et al. – Another Look



No mineralization at low dose (200 ppm) in F0, F2, and only 2/10 males in F1

Perspectives on Mineralization

Perspectives on Mineralization

“Renal mineralization is usually seen in female rats fed a semisynthetic diet but is also seen with regular laboratory feed (Figure 11.38). Imbalances of calcium, phosphorus (excessive phosphorus in the diet), chloride, magnesium, protein, and lipid have been incriminated or been shown to cause renal mineralization. The severity of mineralization is both sex and strain dependent ovariectomy prevents renal mineralization, whereas gonadectomized males and females receiving estradiol benzoate develop renal mineralization quickly. Mineralization may be observed with other forms of renal disease including hyaline droplet nephropathy, dystrophic calcification, and end-stage CPN disease.”

Seely et al. 2018

Perspectives on Mineralization

"Mineralization is commonly observed in the area of the outer stripe and inner stripe of the outer medulla."

"Comment: Mineralization is more commonly associated with spontaneous and minute background findings of basophilic deposits in the renal cortex, medulla, or papilla of rats and mice. In general, these deposits have no pathologic significance. However, mineralization may also be seen as a consequence to degeneration and necrosis*. Mineralization may be induced by chemicals, hormones, or diet."

"Recommendation: Mineralization should be diagnosed and graded. If small deposits of focal mineralization are recognized as a spontaneous background finding, they need not be diagnosed and the pathologist should use his or her judgment in deciding whether or not they are prominent enough to warrant diagnosis. When diagnosed, the pattern of the mineralization (e.g., linear papillary mineralization, focal medullary mineralization) should be described in the pathology narrative."

*No evidence for this in NP studies

NTP Non-neoplastic lesion atlas

Perspectives on Renal Effects (including mineralization) in Rats

"Comparing previous studies with this one (where the dose and route of exposure of NP are the same, but the diet is not),the striking difference in the severity of Polycystic Kidney Disease (PKD) observed leads to the conclusion that the renal toxicity of NP is highly dependent on the diet on which the animals are maintained. Furthermore, there appear to be some protective effects associated with soy-meal supplementation, although the dietary factors responsible are unknown.

Because of the reported weak estrogenic activity of NP, it is possible that the minimal mineralization observed in the 3 male groups exposed to the highest doses was an "estrogenic" effect of NP on kidney tubules. This seems more plausible than the possibility that it was a sequela of tubular epithelial necrosis associated with the toxicity of the NP-dietary interaction (e.g., PKD), because severe PKD occurred in 100% of the 2000-ppm group, but mineralization was observed in only 40% of the same group. Furthermore, mineralization was present the 500-ppm group that, like the control and the 3 other lower-dose groups, did not have PKD."

Laterdresse et al. 2001

Regulatory Perspectives

- Danish Environmental Protection Agency (Nielsen, et al. 2000)
- Environment Canada (2001,2002)
- US Forest Service (2003)
- USEPA (2009)

Denmark (Nielsen)

- Nielsen, et al. (2000) conclude with regards to Chapin et al. (1999):

  "Consequently, the conclusion has been drawn from this study that there is a **LOEL** (emphasis added) for repeated exposure of 15 mg/kg/day, based on histopathological changes in the kidneys. Since renal tubular degeneration and/or dilatation are common findings in untreated rats, and as they were not accompanied by other related signs or symptoms in the affected rats, they are not considered signs of severe toxicity by the rapporteur."

USEPA

- Hazard Characterization Document – September 2009

  "Toxicity was manifested as reductions in terminal body weights at 650 ppm in F2 males (8%) and F1 females (7%) and on post-natal days 55-58 in F3 females (10%) and at 2000 ppm in F1 female (9%), F2 (7%), and post-natal day 55-58 F3 (7%) males and F0 (9%), F1 (12%), F2 (10%), and post-natal day 55-58 F3 (11%) females. Increased relative kidney weights were observed at 650 ppm and/or 2000 ppm in adult males from the F0, F1, and F2 generations and in the F1 2000 ppm adult females. A treatment-related increase in the incidence of renal tubular degeneration/dilatation was seen in the 200, 650, and 2000 ppm males from all generations and in the 2000 ppm females from the F1, F2, and F3 generations and in the 200 and 650 ppm females in the F3 generation."

- Mineralization not included in critical effect determination

Environment Canada (2001)

- “The renal lesions identified in the [Chapin et al., 1999] multigeneration study were described as being of minimal to mild severity, even at the higher dose levels, and were interpreted by the authors as a slight acceleration of the tubular nephropathy normally seen in this strain of rats Chapin. There was also no effect on serum urea nitrogen or creatinine at this dose in the subchronic study (Cunny et al., 1997), suggesting that renal function was not affected (though urinalysis was not conducted in any study, and plasma urea concentration is not a sensitive marker of nephropathy). Based on these considerations, it seems likely that the LOEL of 12 mg/kg-bw per day is close to a No-Observed-Adverse-Effect-Level (NOAEL) for effects on the kidney…”

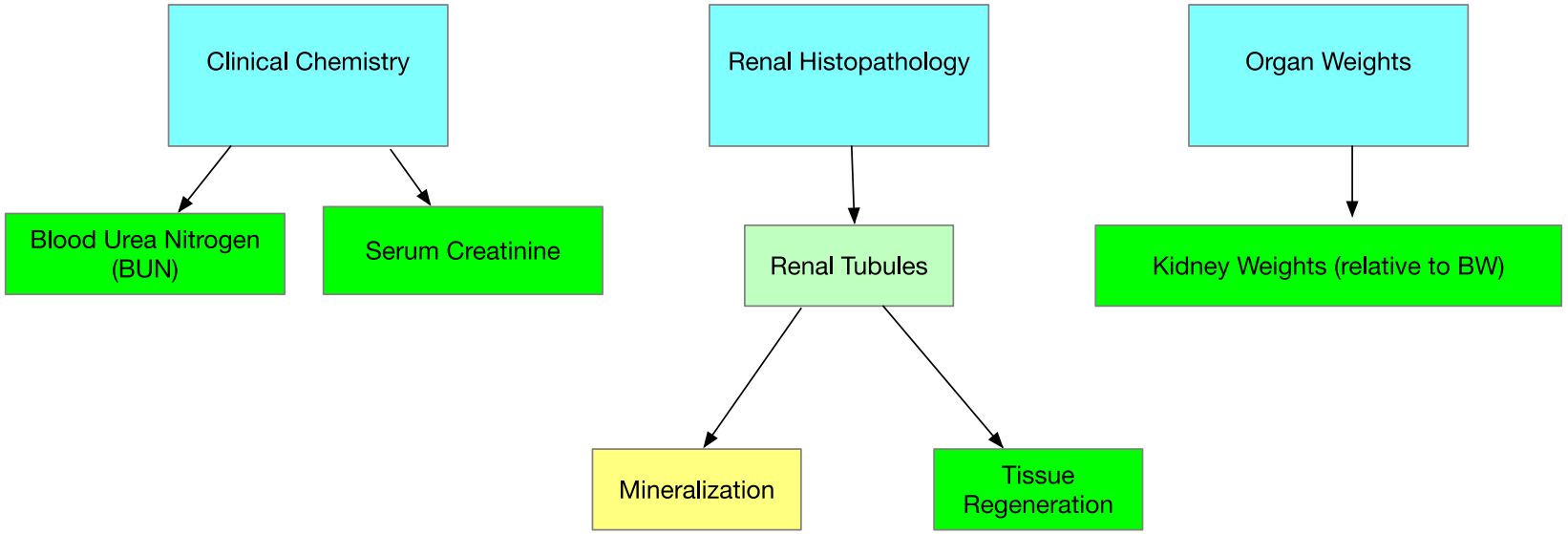
Environment Canada (2002) Canadian Environmental Quality Guidelines for PA & Ethoxylates

- In a multigenerational study, Chapin et al. (1999) examined the effects of nonylphenol administered through dosed food on Sprague Dawley rats (Rattus norvegicus). At a diet concentration of 650 mg·kg -1 (i.e., a dose of 30-108 mg·kg -1 body weight) vaginal opening at an earlier age was observed in the F1 generation. Significant effects observed at a diet concentration of 2000 mg·kg -1 (i.e., a dose of 100-360 mg·kg -1 body weight) included increased relative kidney weights and decreased adult ovary weights in the F1 generation, and increased estrous cycle length in both the F1 and F2 generations.
- Mineralization not included in critical effect determination

US Forest Service

- “The decision by Environment Canada (2001) to utilize the 12 mg/kg/day figure as a NOAEL is further reinforced by the results of Nagao et al 2001 and a recent study by Latendresse et al 2001, in which kidney effects (polycystic kidney disease) were seen in Sprague Dawley rats fed NP at doses at or above 1,000 ppm in soy- free feed. Latendresse et al determined a NOAEL for this kidney effect at 500 ppm, which is similar to what was determined in Cunny et al 1997 (a NOEL of 650 ppm based on kidney effects). An interesting side note to Latendresse et al 2001 is that it appeared that the soy- free diet exacerbated the kidney effects, and the authors surmise that soy in the diet could act to ameliorate these effects.”

Elements of Weight of Evidence Assessment



Overall Weight of Evidence Mineralization

- Renal mineralization seen at some dose(s) in all three pivotal studies
  - It was low incidence and low severity
  - No other renal effects accompany the mineralization
- Mineralization is a frequent finding in rat studies – (mineral imbalance, gut flora, etc.)
- Finding alone (without other indications of renal toxicity should not be considered a critical effect)

Recommendations

- The critical effects in the multi-generation reproduction studies
  - Acceleration of vaginal opening in females (Chapin et al. 1999)
  - Toxicologically significant changes in the kidney from males (Chapin et al. 1999; Nagao et al. 2001; NCTR 2009; Tyl et al. 2006), both of which occurred at doses of >200 ppm.
- Note: no vaginal effects were observed in a five-generation study at doses up to and including 750 ppm (the highest dose tested), whereas kidney effects were seen only at 750 ppm (NCTR 2009).
- Point of Departure = 200 ppm in the diet, equating to approximately 13 mg/kg bodyweight/day