# Glyphosate AIR5 Dataset, Renewal Assessment Report (RAR) Proposed Classification and Endpoint Selection

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# Issue: EU Proposed Decrease in the Allowable Daily Intake (ADI) of Glyphosate

**Toxicological Database Has Yielded Differing Interpretations and Conclusions**

| Regulatory Review | Critical Effect for PoD | LOAEL/NOAEL/ADI (mg/kg/day) |
|---|---|---|
| **EU (2021; proposed)** | **Chronic rat salivary "cellular alteration" (Atkinson et al., 1993)** | **100/10/0.1** |
| EU (2015; current) | Rabbit maternal/developmental toxicity | 200/50/0.5 (parotid NOAEL = 100) |
| JMPR (2016) | Salivary parotid (Atkinson et al., 1993) | 300/100/1 |
| EPA (2017) | Rabbit maternal toxicity (diarrhea, few and/or no feces) | 175/100/1 |

# What is the evidence for or against using salivary gland "cellular alteration" as the PoD?

- **Key consideration: The rat salivary gland response is more appropriately interpreted as a non-adverse adaptive response**, as informed by:
  - Organ weight and histological characterization of the repeat-dose dietary salivary gland response
  - Perspectives of other reviewers/reviews of similar response induced by food additive and other substances
  - Consideration of salivary gland biological/physiological function
  - Potential adrenergic mode of action considerations: true systemic effect (other systemic adrenergic responses present?) vs local salivary feedback response (saliva neutralization of glyphosate acid in diet)

# Overview of Key Points on Toxicity Interpretation

Salivary gland response typically regarded as adaptive or adverse?

- Salivary gland weight increases only at 52 weeks with no progression and absence of effect at 104 weeks
- Alterations in basophilic staining only
- Recovery of staining response
- No cytotoxicity, apoptosis, cell proliferation

EU (2021): "A histological finding which is statistically significant *is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes*"

# Conclusions on salivary gland "cellular alteration" (Atkinson et al., 1993)

- Cellular alteration was recorded when:
  - Parotid cells "appeared larger and stained deeply basophilic"
  - Mandibular cells "appeared larger and stained weakly in basophilic manner"
- Authors reported that the histological changes "showed no progress to either proliferative or degenerative changes."

Source: Atkinson et al. 1993, Volume 1, page 54 and Volume 2, page 440.

# INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) 2016

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## Nonproliferative and Proliferative Lesions of the Gastrointestinal Tract, Pancreas and Salivary Glands of the Rat and Mouse

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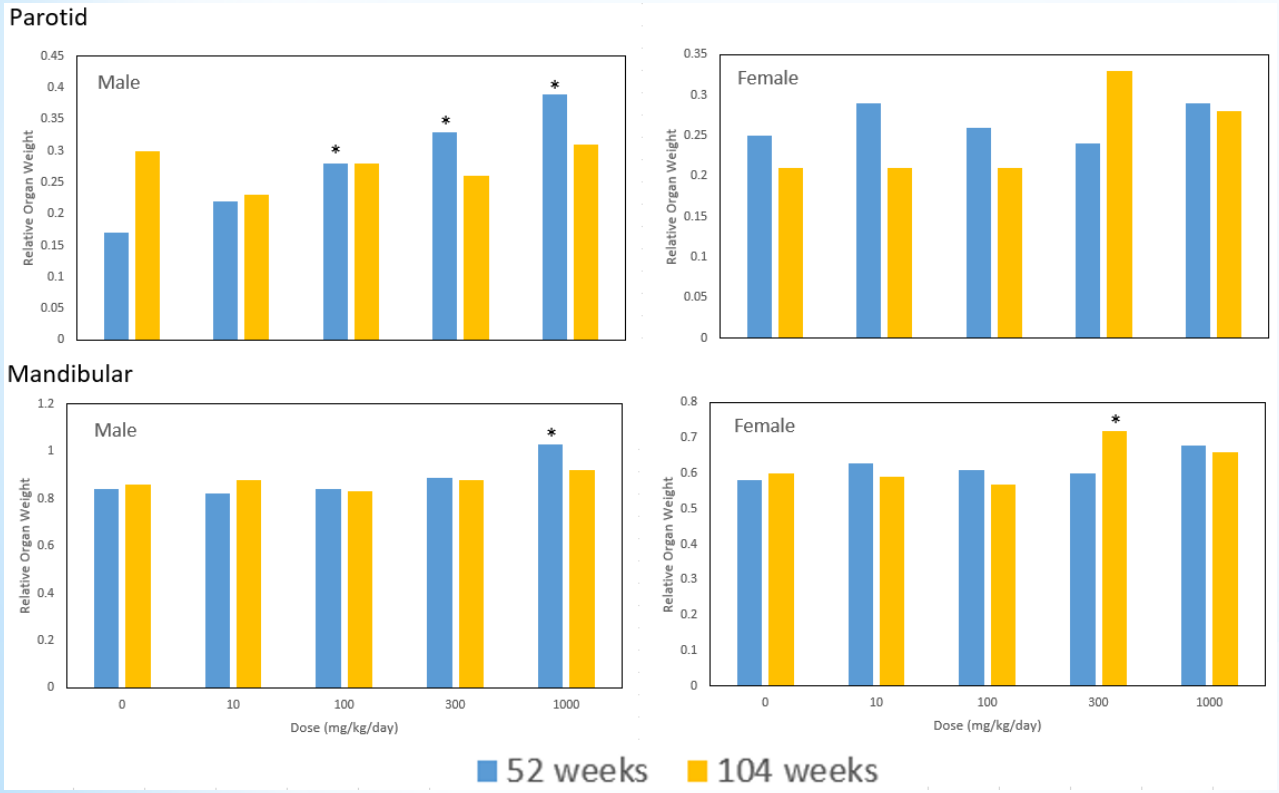
Page 57S: "… several chemicals such as doxylamine (Jackson and Blackwell 1993), triprolidine (Greenman et al. 1995), glyphosate (NTP, 1992a), methyleugenol (NTP, 2000), and diethanolamine (NTP, 1992b) induce basophilic hypertrophic foci in rodents. These foci are considered adaptive hypertrophic lesions rather than precursors of neoplasia."

# Salivary gland *weight* increased in males only at 52 weeks but not 104 weeks (Atkinson et al., 1993)




* – Statistically significant different from controls

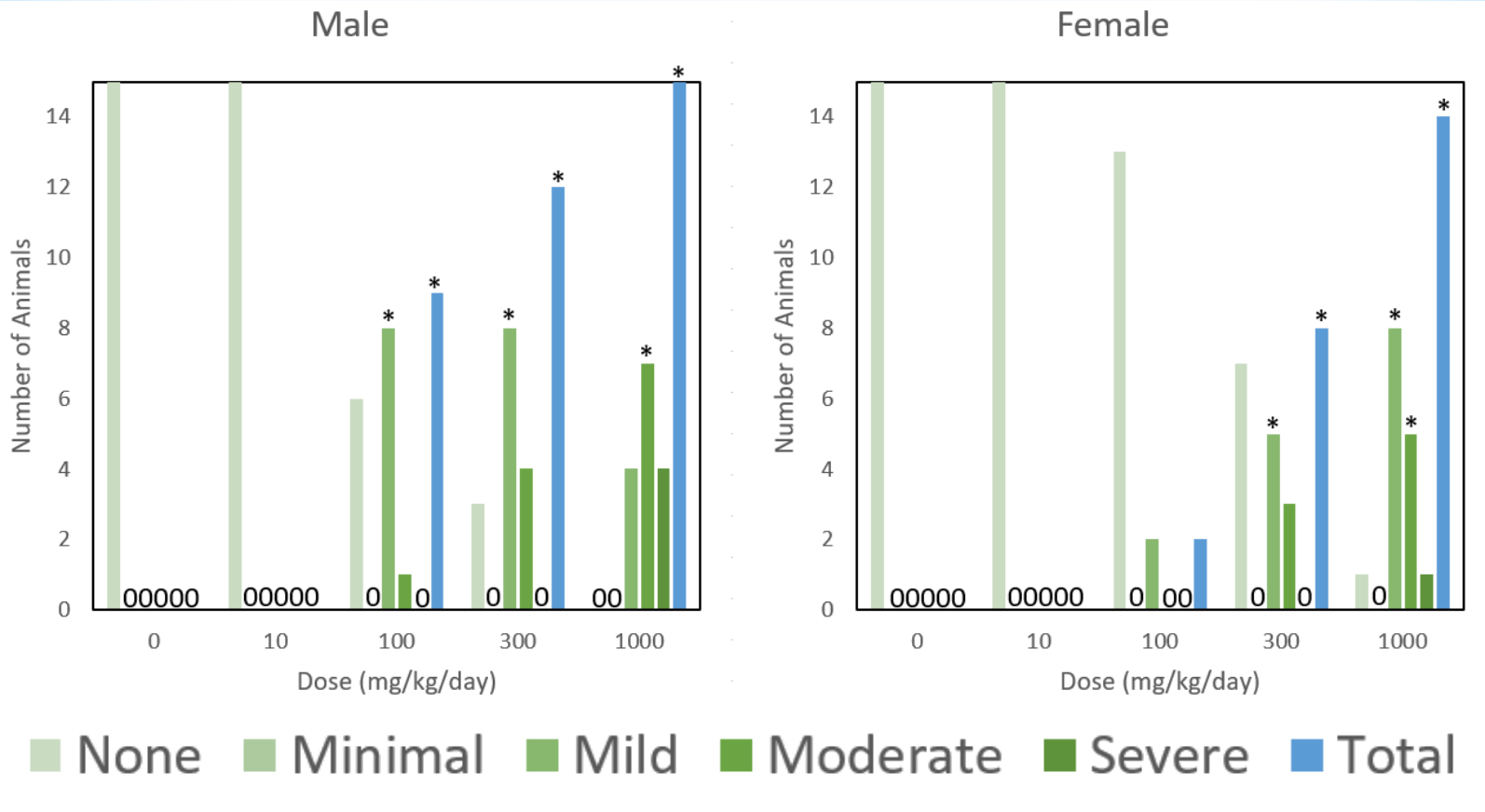
No parotid weight increase at 104 weeks.

Control male parotid weight increases between 52 and 104 weeks

Mandibular weight increased at 104 weeks only in females at 300 mg/kg/day.

Source: Table B.6.5.7-6 from RAR Volume 3 – B.6.5 (AS), page 83

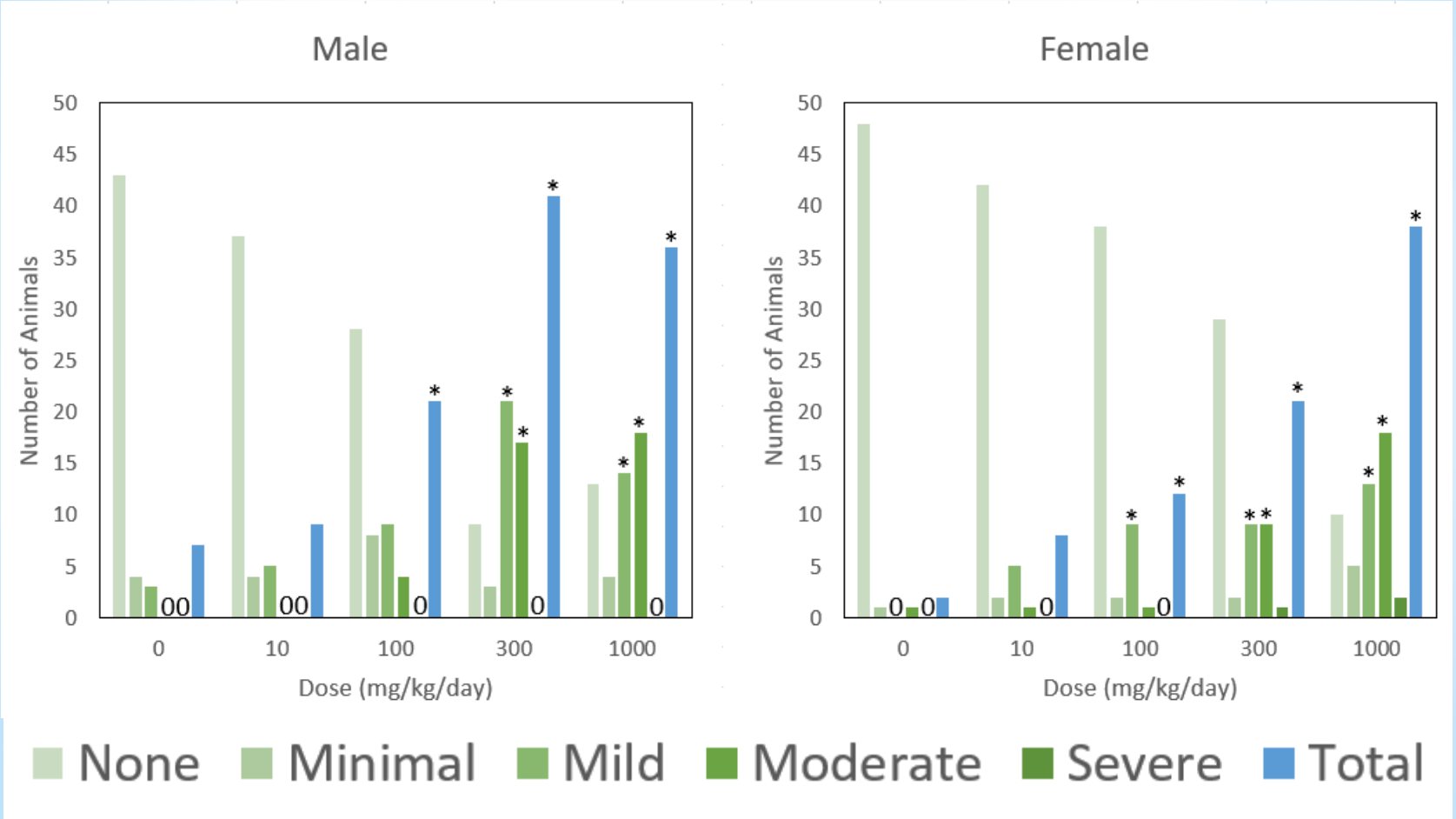
# Parotid *cellular alteration histopathology* intensity increased with dose at 52 weeks (Atkinson et al., 1993)




* – Statistically significant difference from controls
0 – Zero animal

Source: Atkinson et al., 1993. Vol 1, Table 52

# Parotid *histopathology* intensity in males partially recovers at 104 weeks (Atkinson et al., 1993)




* – Statistically significant difference from controls
0 – Zero animal

Source: Atkinson et al., 1993. Vol 1, Table 51

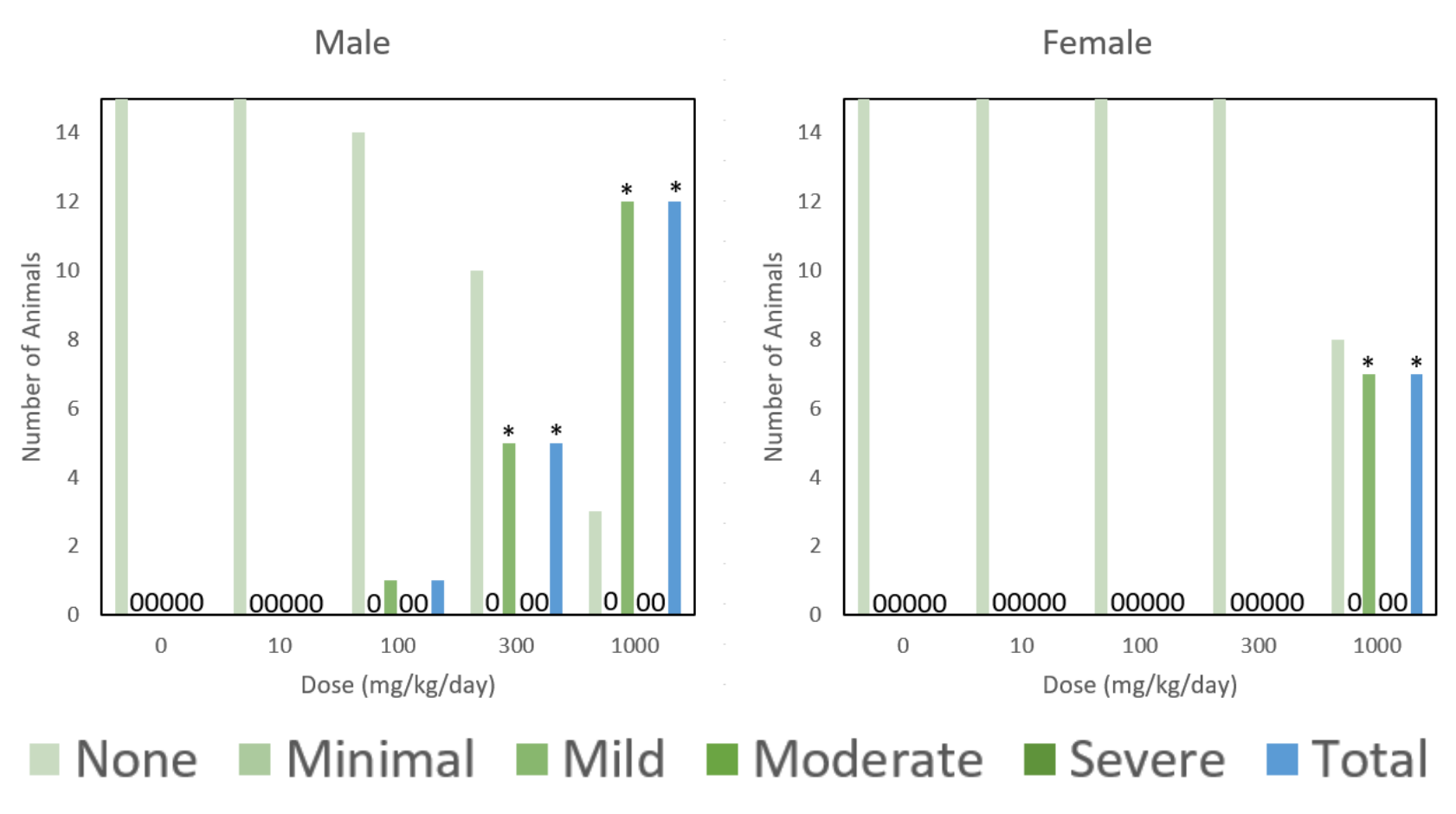
# Weighted mean 104 week male parotid intensity is statistically significant but "minimal" at 100 mg/kg/day

| Dose (mg/kg/day) | Weighted mean intensity |
|---|---|
| 0 | 0.2 |
| 10 | 0.3 |
| 100 | 0.8* (< minimal) |
| 300 | 1.9* |
| 1000 | 1.8* |

0 = 0; ± = minimal = 1; + = mild = 2; ++ = moderate = 3; +++ = severe = 4
* = statistically significant

EU (2021): "A histological finding which is statistically significant *is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes*

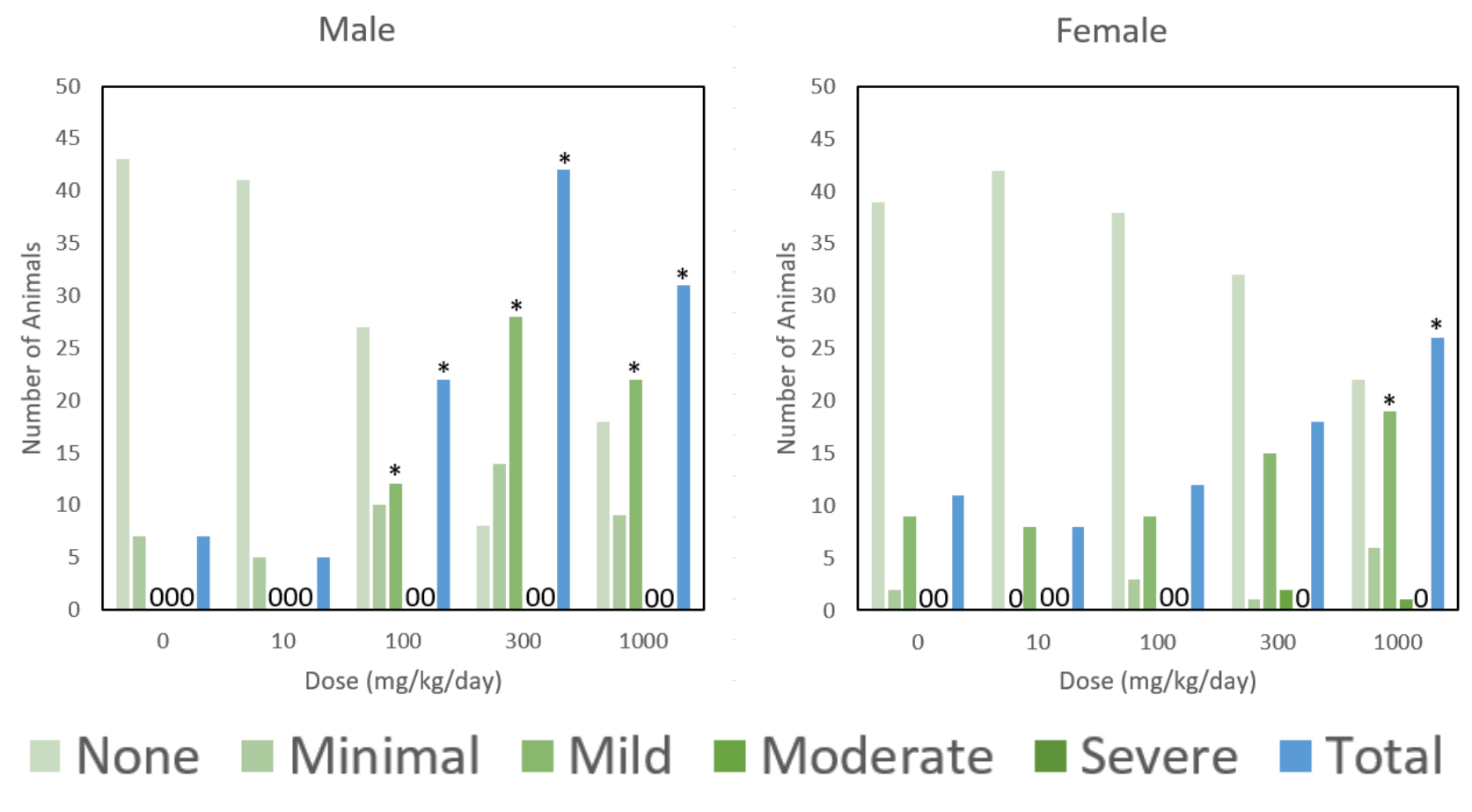
# Mandibular *histopathology* intensity increased < parotid at 52 weeks (Atkinson et al., 1993)




* – Statistically significant difference from controls
0 – Zero animal

Source: Atkinson et al., 1993. Vol 1, Table 52

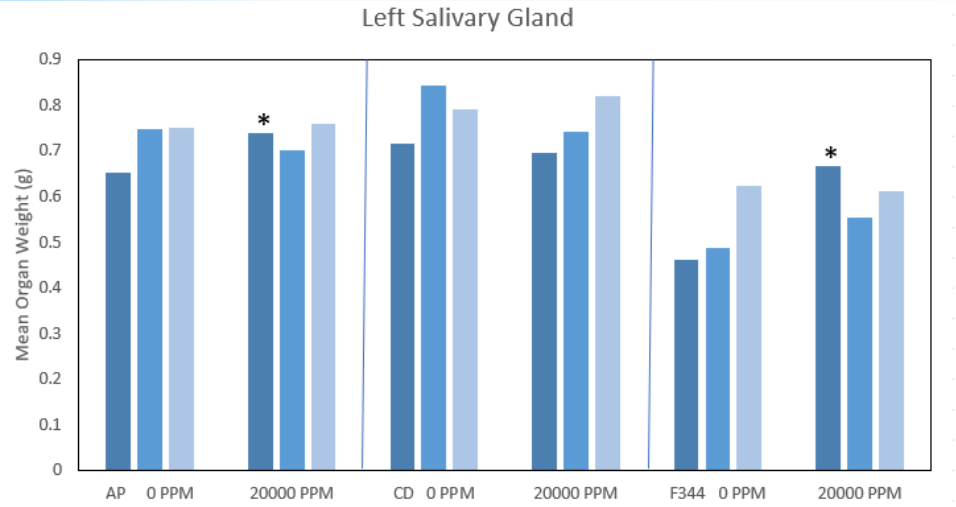
# Mandibular *histopathology* intensity in males increased slightly at 104 weeks (Atkinson et al., 1993)

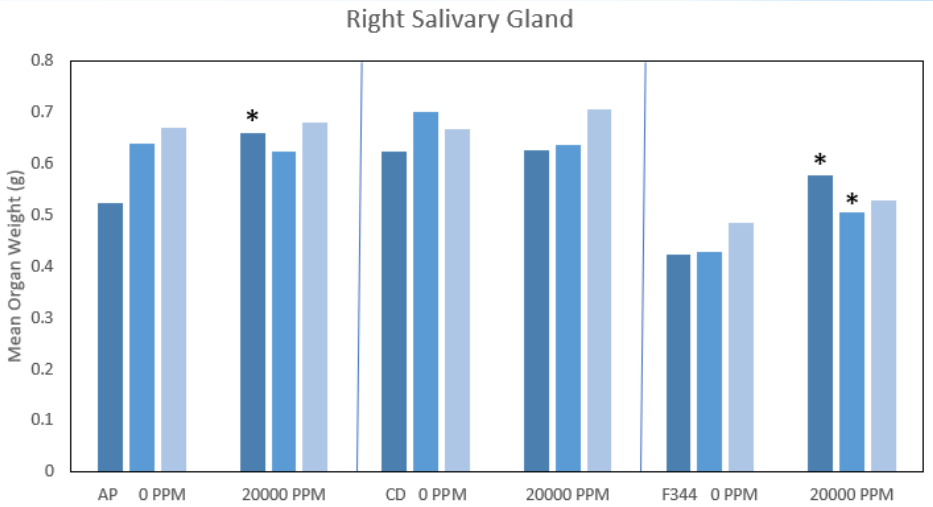



* – Statistically significant difference from controls
0 – Zero animal

Source: Atkinson et al., 1993. Vol 1, Table 51

# Salivary gland *weight* recovers 4 and 13 weeks after 4 week glyphosate dietary exposure in AP, CD and F344 rats (Allen, 1996)



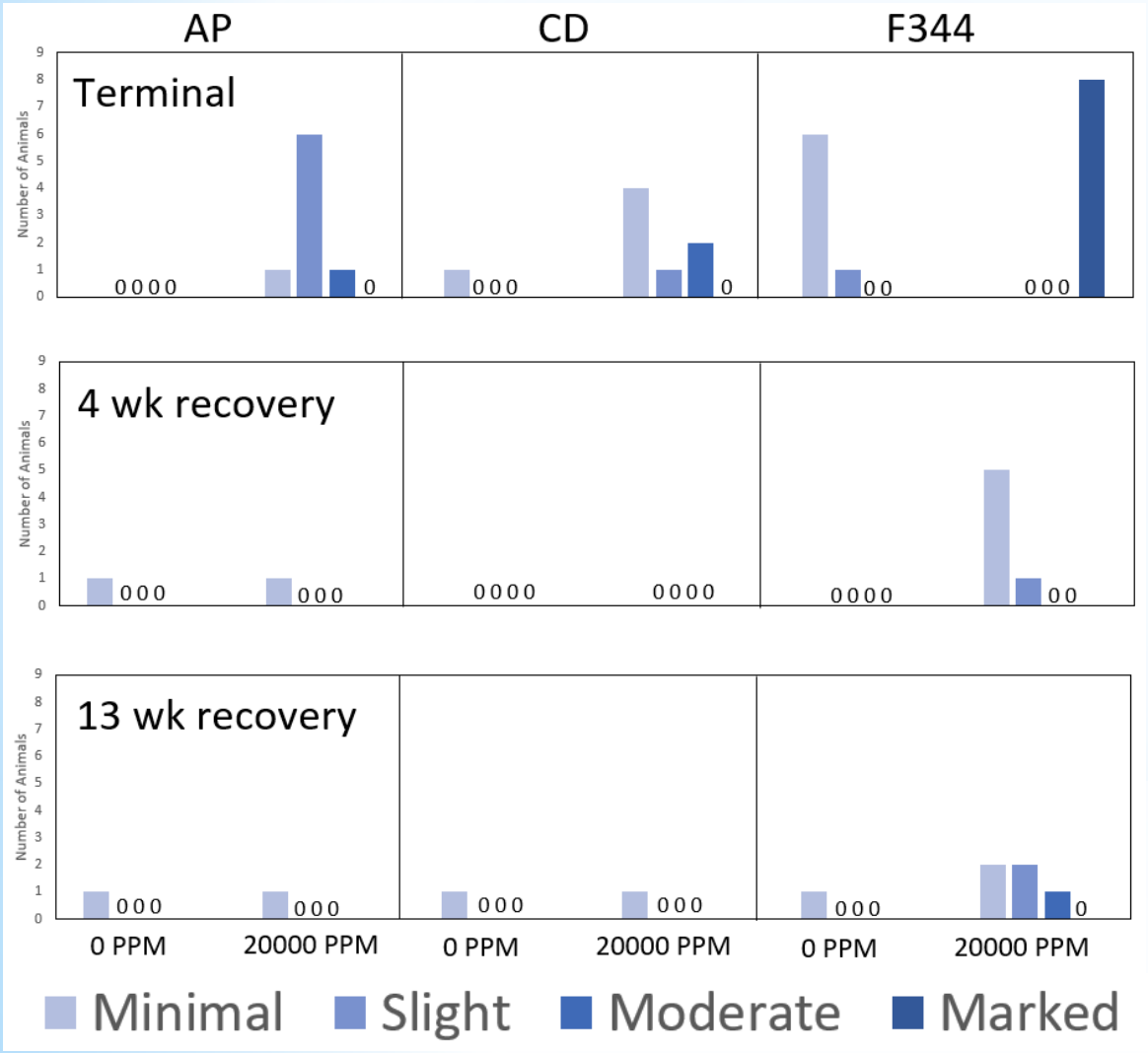




Terminal 4 wk recovery 13 wk recovery

* – Statistically significant difference from controls

Source: Table B.6.8.2.3-3 from RAR Volume 3 – B.6.7 – B6.10 (AS), page 336

# Salivary gland *histopathology* recovers by 4 weeks after 4 weeks glyphosate dietary exposure in AP, CD and F344 rats (Allen, 1996)




0 – Zero animal

Source: Table B.6.8.2.3-4 from RAR Volume 3 – B.6.7 – B6.10 (AS), page 337

# Other compounds induce similar parotid effects after high-dose diet administration

- Inoue et al. (2014): Rat 4 wk *diet* grape skin extract: basophilic hypertrophy
  - *Gavage: No response*
  - "adaptive non-adverse response that is reversible"
- Fujiwara et al. (2013): Rat 13 wk *diet* apple polyphenol extract: hypertrophy only
  - "transient physiological adaptive response to an oral stimulus by consecutive lowering of pH"
- Lina et al. (2013): Rat 13 wk *diet* apple polyphenol extract: basophilic hypertrophy
  - "not considered adverse…reflected local reversible and adaptive…to direct contact"

# Key mode of action considerations

- Not genotoxic
- No evidence of cytotoxicity, apoptosis and cell proliferation
- Histopathology shows only cellular alteration: basophilic hypertrophy
- Organ weight and histopathology are reversible
- NTP 1992 limited mode of action study implied potential systemically-mediated adrenergic effects – is this a true systemic response or an integral mode of action key event of local physiological feedback loop response of parotid salivary gland to a high acid content diet?

# NTP Mode of Action Study: 50,000 ppm glyphosate similar to isoproterenol (Beta-adrenergic agonist)?

**TABLE 11 Incidence and Severity of Cytoplasmic Alteration of the Salivary Glands of F344/N Rats in the 14-Day Mechanism Study of Glyphosate**

| Group (Feed/Pump) | Parotid | Submandibular | Sublingual |
|---|---|---|---|
| 1 (control diet/vehicle) | 1/4 (1.0) * | 0/4 | 0/3 |
| 2 (glyphosate/vehicle) | 4/4 (4.0) | 4/4 | 0/4 |
| 3 (glyphosate/propranolol) | 3/4 (1.5) | 4/4 | 0/2 |
| 4 (control diet / isoproterenol) | 4/4 (2.7) | 0/4 | 0/1 |
| 5 (control diet/isoproterenol + propranolol) | 4/4 (2.0) | 0/4 | 0/4 |

* Average severity grades for parotid gland lesions in affected animals, based on the following scale:
1=Focal change; 2=Multifocal, confluent change; 3=Diffuse change;
4=Diffuse change with intense basophilia and marked acinar swelling.

Table from NTP 1992, page 29.

# NTP study: Local or systemic mode of action inference?

- Attenuation of glyphosate and isoproterenol salivary gland effects by propranolol potentially implies glyphosate *systemic* adrenergic response
  - Incorrect inference?
- Glyphosate safety pharmacology study
  - *No effects* on increased heart rate similar to isoproterenol

# Mode of action hypothesis

- Parotid salivary gland response is a local non-systemic oral cavity chemo- and/or mechano-receptor mediated *adaptive non-adverse* physiological response to repeated ingestion of highly acidic glyphosate acid diet
- John DeSesso presentation will offer supporting perspectives