Glyphosate ADI Endpoint
(Salivary Gland Changes),
Adverse or Adaptive?

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Disclosure

- The author works for Exponent, a scientific and engineering consulting firm, and for Georgetown University School of Medicine, an institution of medical education

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Take Home Message

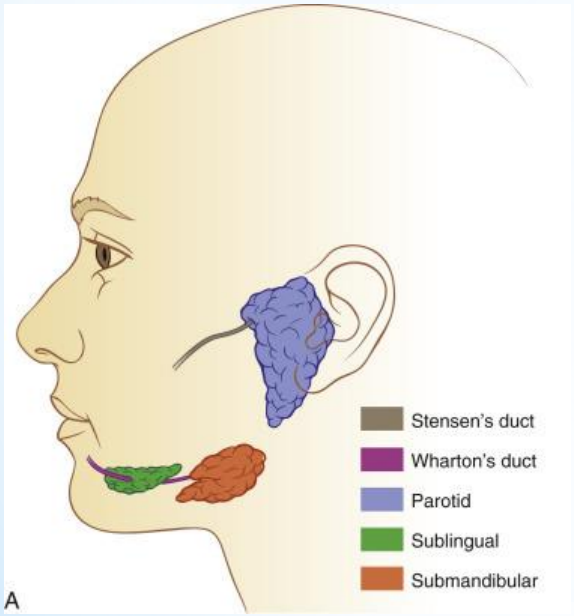
The findings of salivary gland acinar cell changes subsequent to repeated dietary exposure of glyphosate are adaptive and should not be considered adverse effects

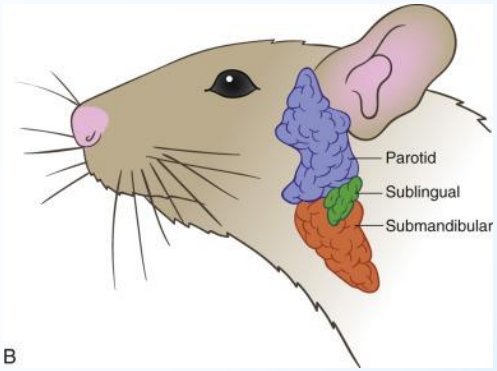
Findings at Issue

- Altered morphology in (parotid) salivary acinar cells after dietary exposure to glyphosate technical
  - Increase in acinar cell size
  - Change in staining intensity
  - No indications of necrosis/cellular debris or proliferation
- Interpreted to be adverse findings and used to set new proposed ADI

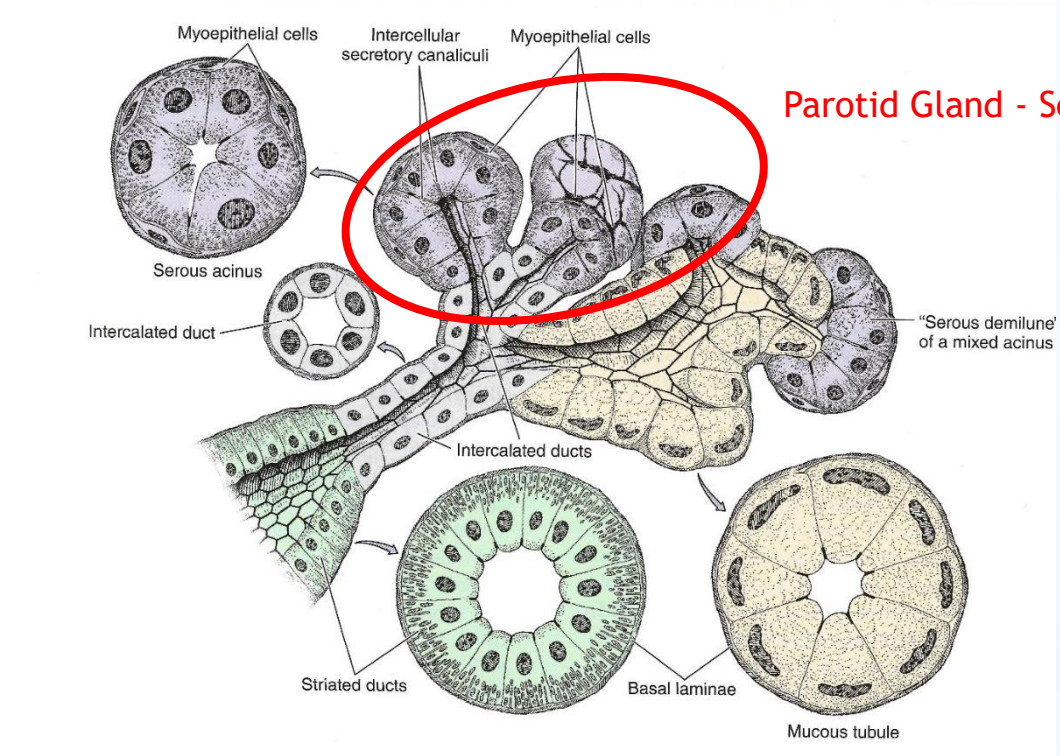
Parotid Gland

Salivary Gland Locations in Humans and Rats





Salivary Gland Characteristics



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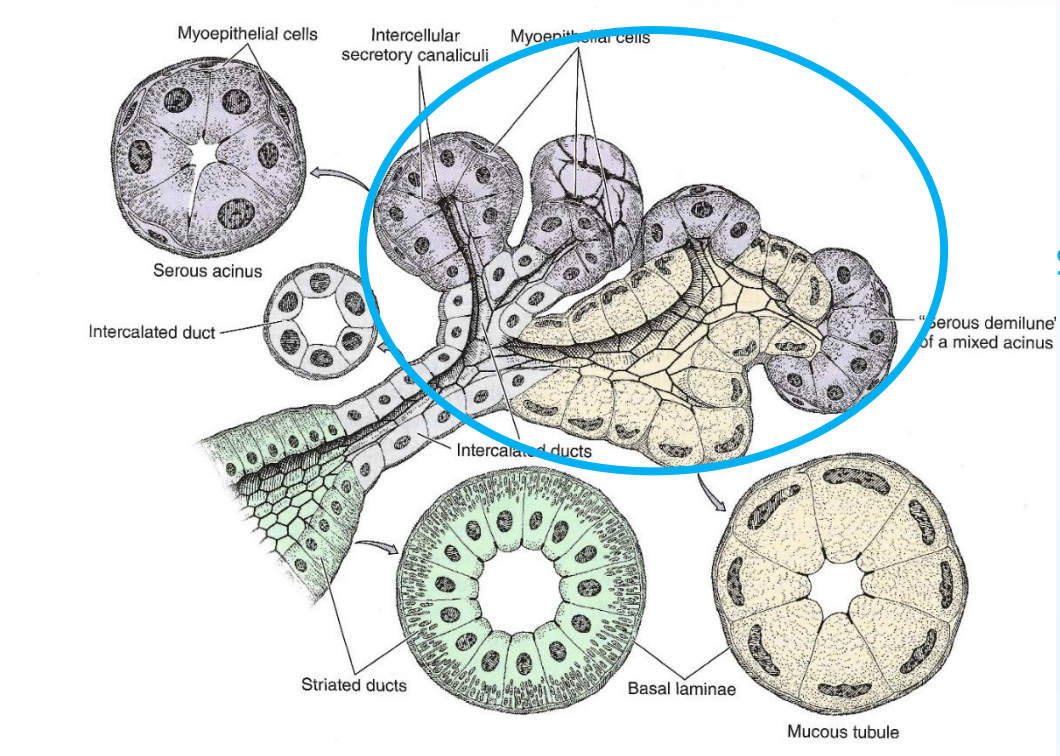
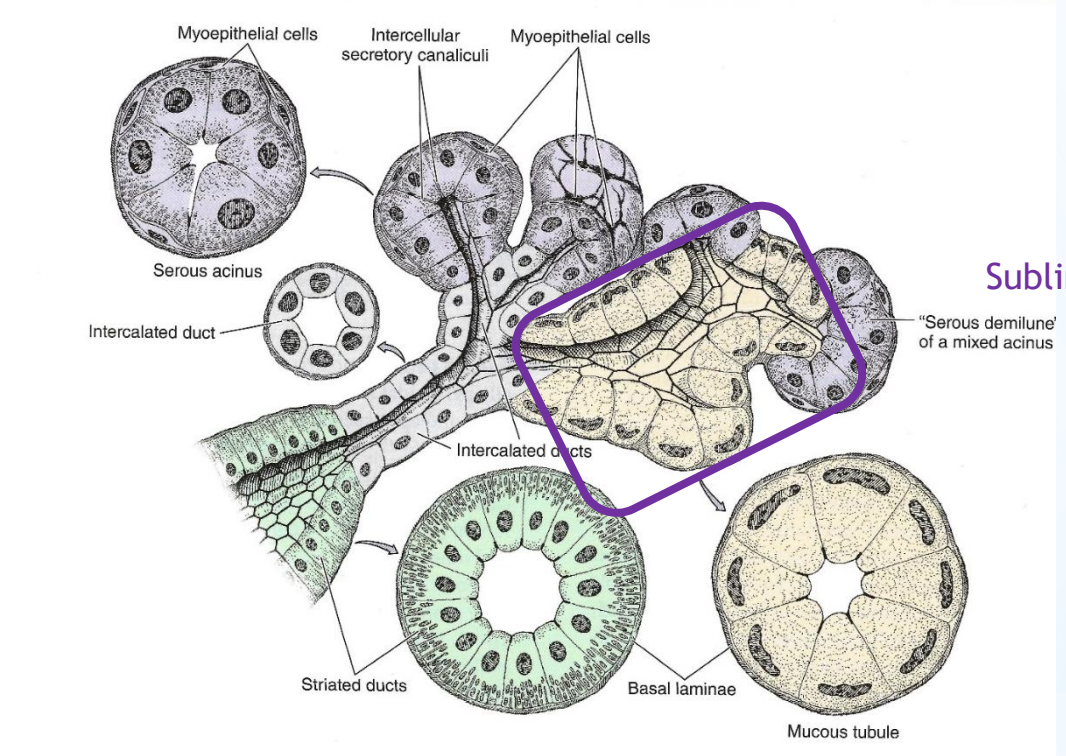


Submandibular Gland – Mixed
(Serous & Mucous)

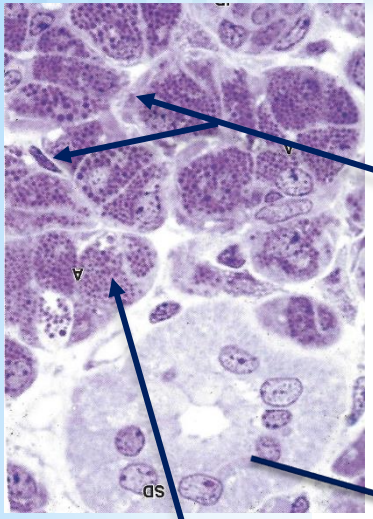
Salivary Gland Characteristics



Sublingual Gland - Mucous

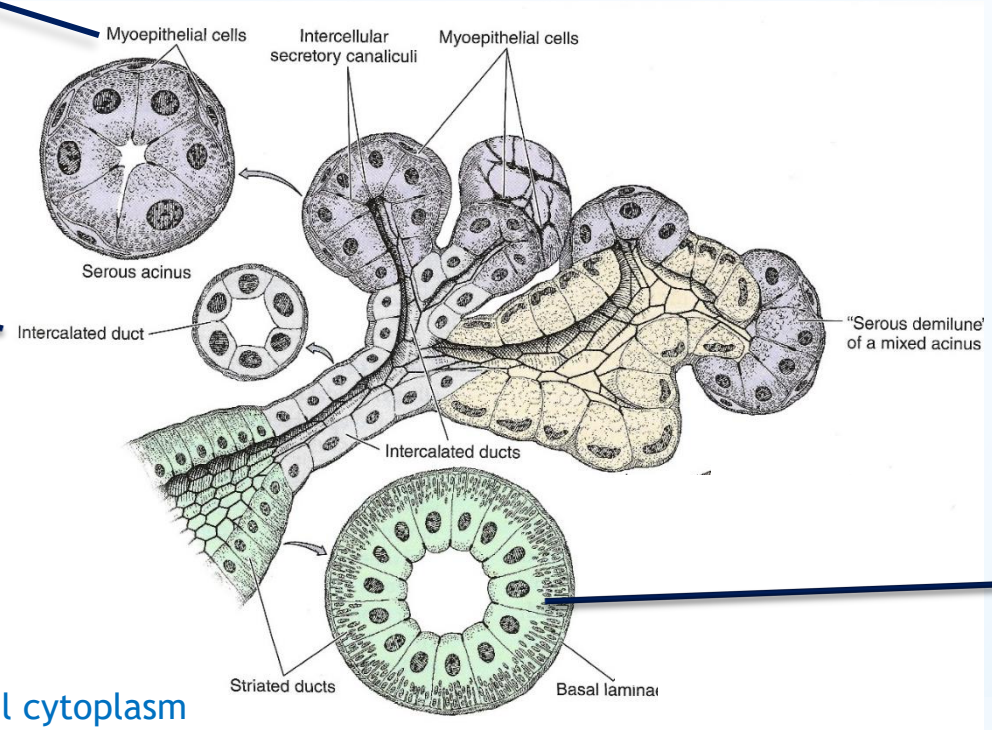
Serous secreting acinar cells of are the target of concern
Parotid gland is totally serous and most affected gland

Parotid Gland Anatomical Details

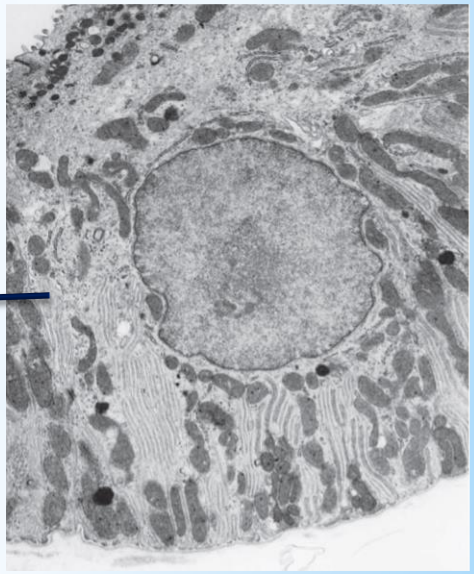


Mescher, 2016

Zymogen granules (amylase)



Mescher, 2016

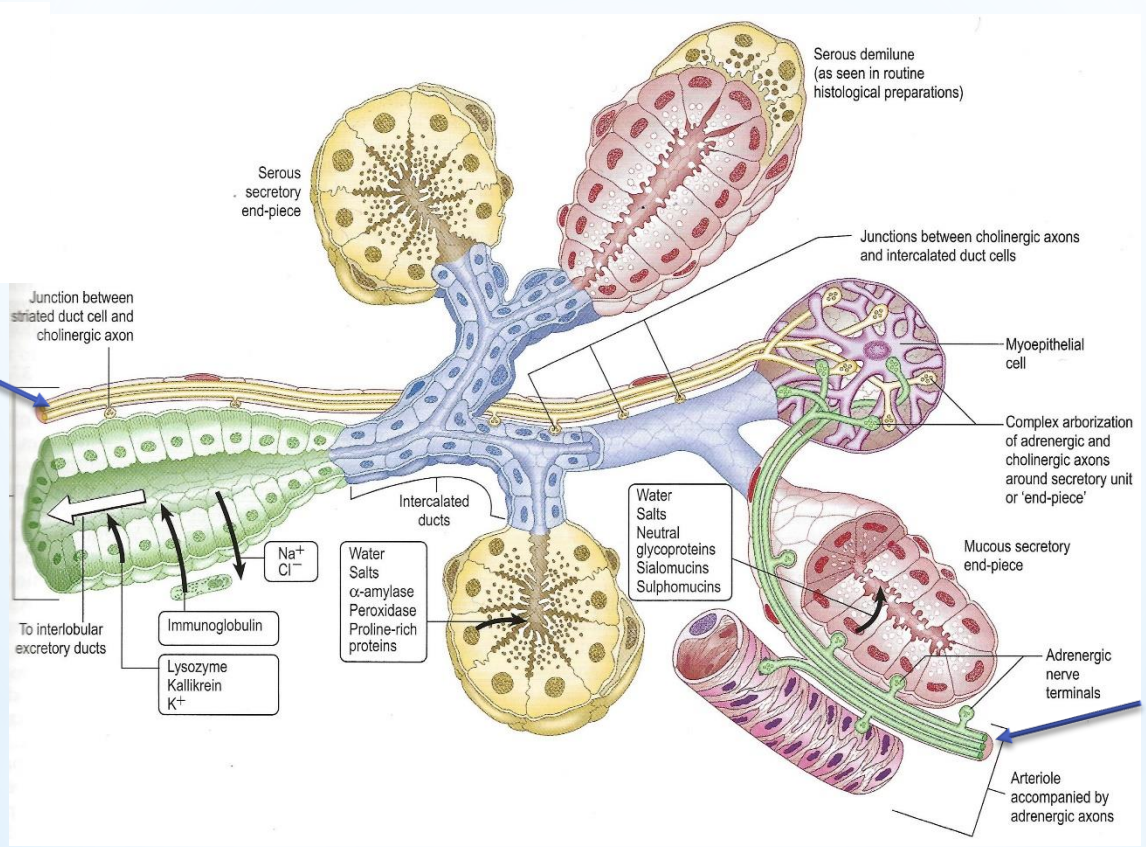


Amano et al, 2012

- Zymogen granules fill cytoplasm
- Myoepithelial cells controlled by cholinergic nerves
- Striated ducts have many mitochondria for transport of bicarbonate

Innervation of Salivary Gland Acini and Ducts

Parasympathetics via Cranial Nerves to Ducts & Acini



Sympathetics via Arteriole to Blood Vessels and Acini

Innervated by Both Cholinergic and Adrenergic Nerves

- Saliva production is ***stimulated*** by both parasympathetic (cholinergic) and sympathetic (adrenergic) nerves
  - Parasympathetic: related to eating tasty/savory foods
  - Sympathetic: related to spicy, hot foods and sour/astringent stimuli
- Saliva production is ***reduced*** by sympathetic nerves causing vasoconstriction of blood vessels

Saliva

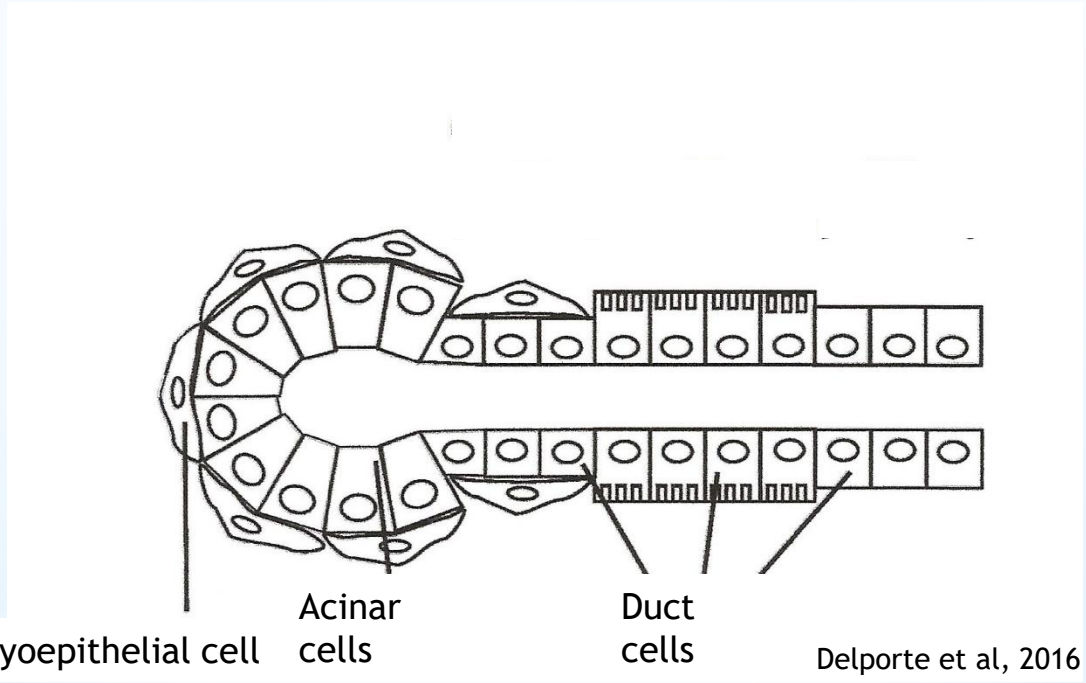
Why is saliva secreted?

- Oral health – resting
  - Low rate of production
  - Protection of teeth
  - Parotid saliva contains bicarbonate (neutralizes acid pH)
- Digestive – stimulated
  - Increased flow
  - Release of amylase to begin digestion of starches
  - (Mucus – released from other salivary glands)
- Response to stimuli
  - Gustatory – from tastebuds
  - Mechanoreceptors – chewing
  - Nociceptive – Pain; sour (acidity); astringency

How is Parotid Saliva Formed?

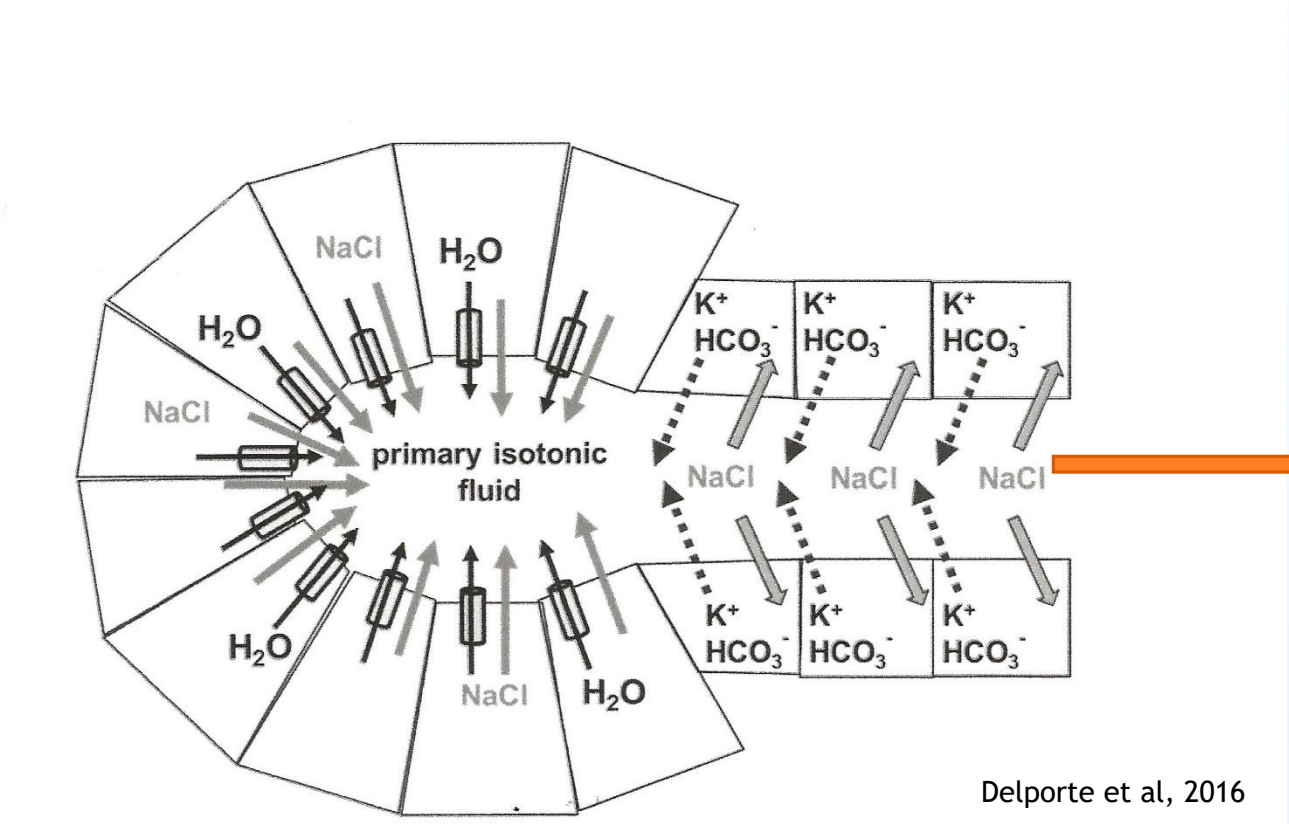
- Immature saliva
  - Formed in acini
  - Transudate of blood plasma
  - Isotonic
- Complete saliva
  - Modified in ducts
  - Enriched in bicarbonate
  - Hypotonic

Identification of Saliva Modification Regions



Delporte et al, 2016

Maturation of Saliva



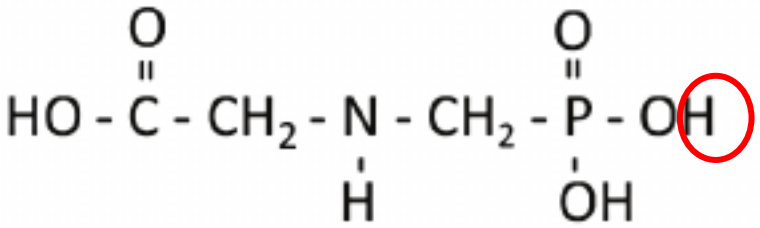
Duct epithelial cells are impervious to water

What initiates saliva secretion?

- Central mechanisms (Higher CNS centers)
  - Memory
  - Smell
  - Sight
- Gustatory mechanisms
  - Taste
  - Texture in oral cavity
- Local mechanisms
  - Mechanoreceptors – Project to brainstem
    - Chewing
  - Nociceptors – Brainstem & Local effects at myoepithelial cells
    - Pain
    - Sour (acidity)
    - Astringency

Glyphosate

Molecular Structures of Glyphosate and Its Salt



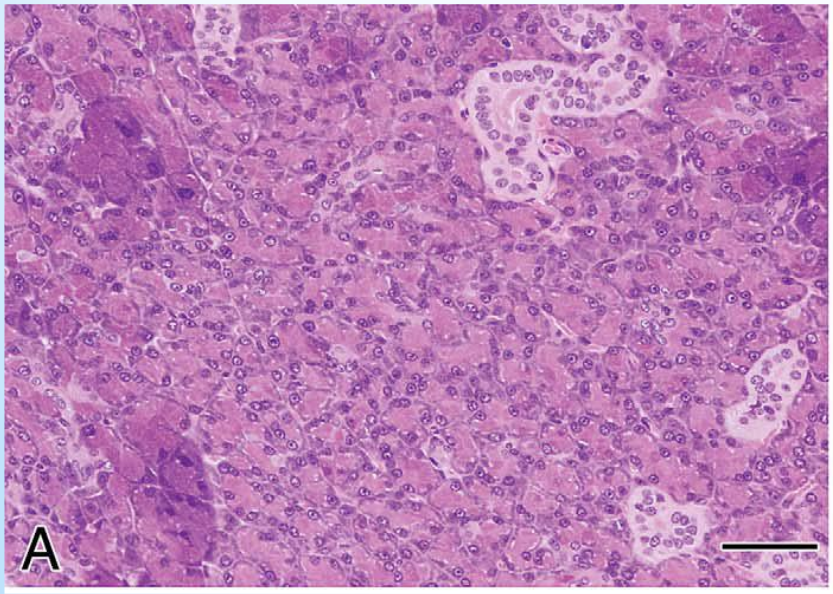
Glyphosate: N-(phosphonomethyl) glycine

A phosphonic acid

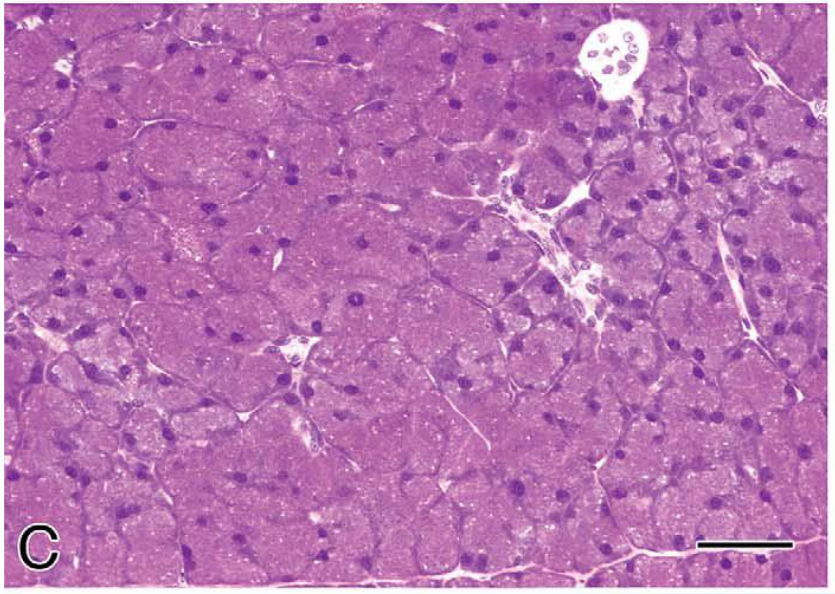
- Safety tests performed on Glyphosate Technical (acid)
- Products formulated with Glyphosate salts

Findings in Parotid Glands after Dietary Glyphosate Exposure

- Non-degenerative parotid acinar cell changes in 2-year assay at doses of 300 and 1,000 mg/kg/day (Atkinson et al, 1993)
  - Seven other 2-year studies reported no salivary effects
    - Reported on "salivary glands" (not specific)
    - Glands not weighed



Control



Enlarged acinar cells (GSE)

Inoue et al, 2014

Parotid Acinar Cell Changes Are Reversible (Allen, 1996)

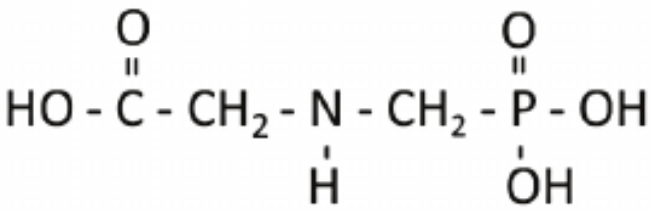
- Three strains of male rats (N=24/strain) received dietary glyphosate (20,000 ppm) for 28 days
  - Sprague Dawley CD
  - Sprague Dawley derived AP
  - Fischer 344
- Termination schedule
  - 8 rats/strain after 28 days of dosing
  - 8 rats/strain after 4 weeks of recovery
  - 8 rats/strain after 13 weeks of recovery
- Parotid acinar cell changes decreased/disappeared with recovery time
  - Complete recovery at 4 weeks for Sprague Dawley strains
  - Fischer 344 minor changes at 13 weeks; possible random variation

Are parotid cell effects due to acidity or the glyphosate moiety?

Citric Acid

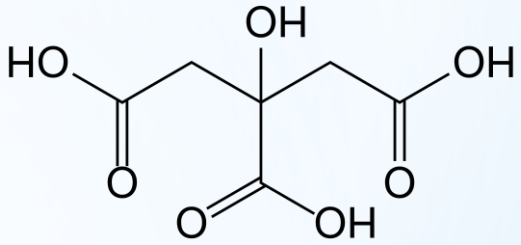
Citric Acid as Surrogate for Glyphosate Acid

Glyphosate Acid



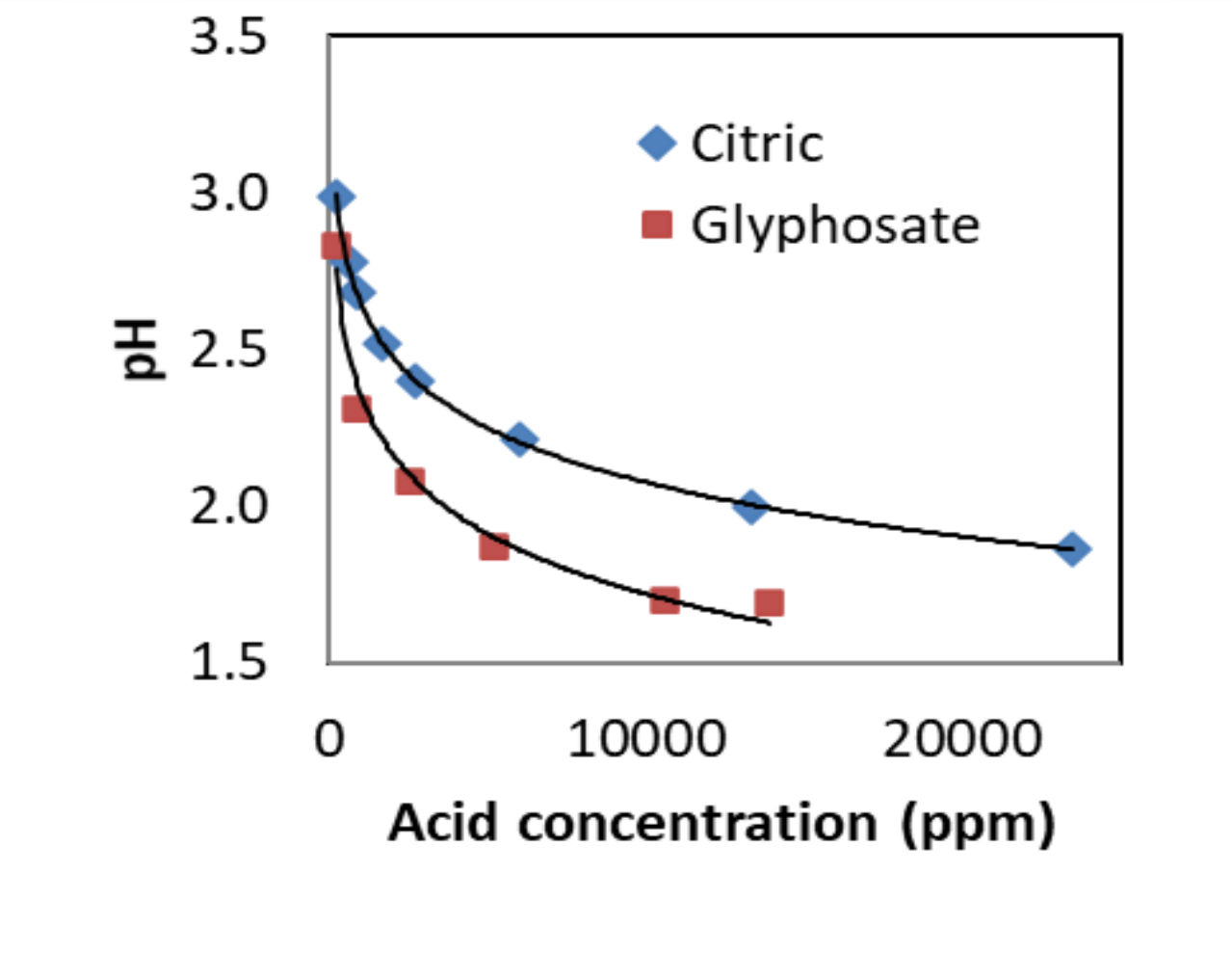
Molecular weight 169.07 Daltons

Citric Acid

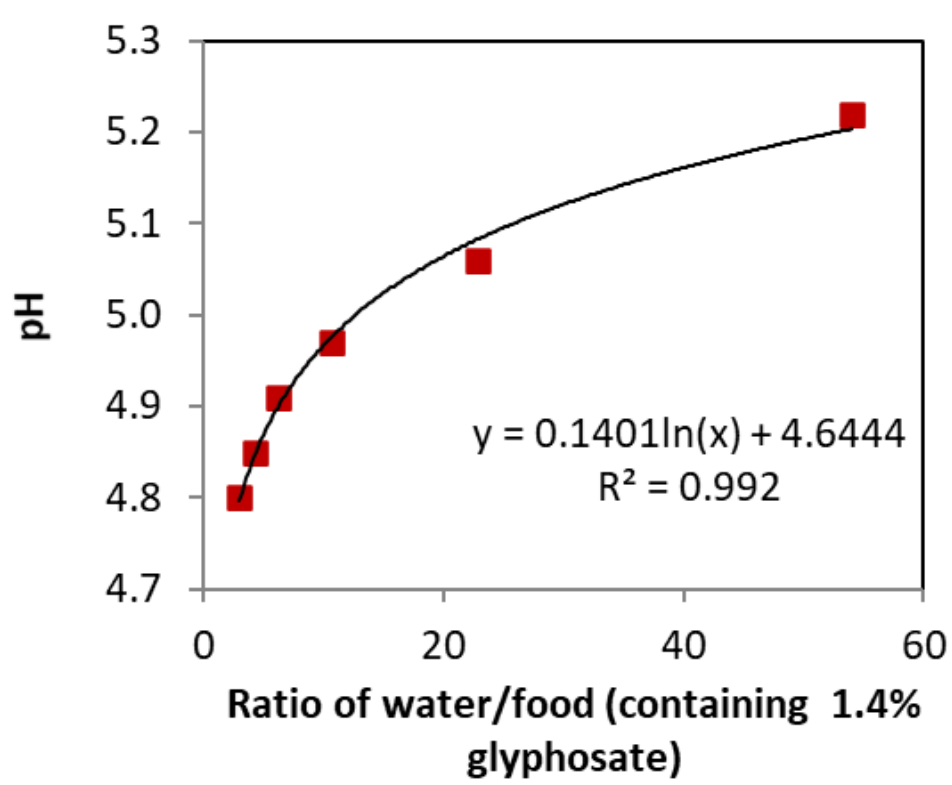


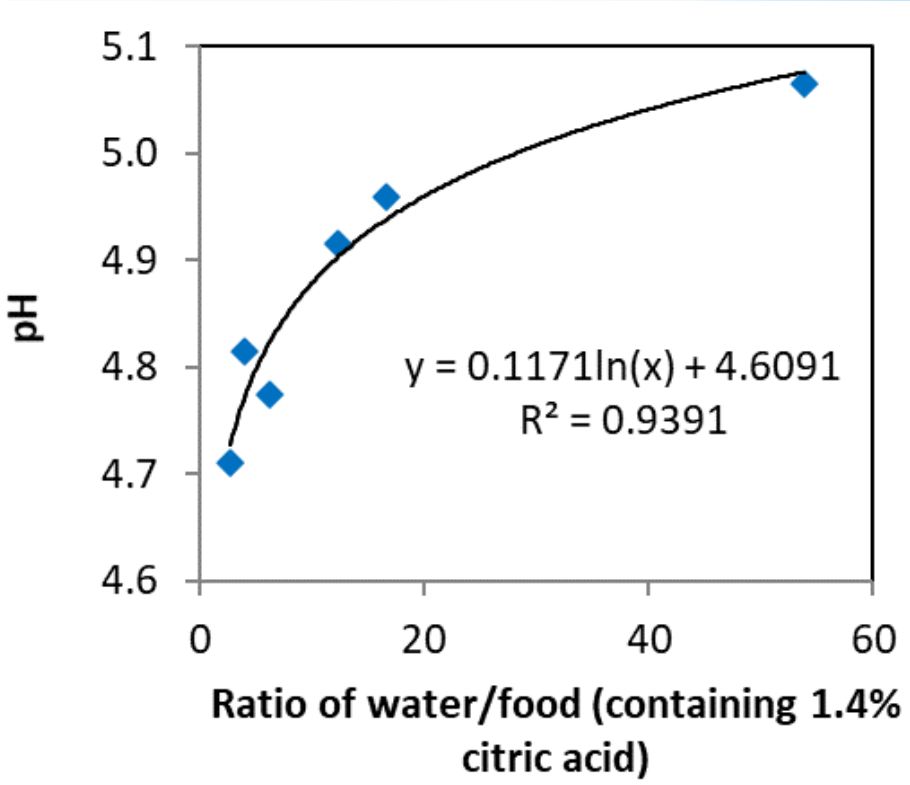
Molecular weight 192.1 Daltons

Glyphosate and Citric Acid:
Similar Concentrations in Water vs. pH Curves



Glyphosate and Citric Acid:
Feed-Water Slurry (14,000 ppm) vs. pH Curves Are Similar





Incidence and Severity of Parotid Acinar Cell Changes after Citric Acid

Exposure	Diet Control	Diet Low pH	Diet High pH*
Parotid Gland	10	10	10
Cellular Changes (Overall)	7	10	9
Incidence (%)	70	100	90
Severity Minimal	5	0	4
Severity Mild	2	6	5
Severity Moderate	0	4	0
Average Severity Score	0.9	**2.4**	1.4

*Trisodium citrate incorporated into high pH diet

Saltmiras et al, 2011

- Acinar cell changes seen only with low pH dietary exposure
- Exposure in rats would be virtually continuous
- No acinar cell changes when exposed via oral gavage
- Changes are due to local (rather than systemic) effects

Other Organic Acids

Selected Other Organic Acids that Cause Adaptive Changes in Parotid Gland

- Grape seed extract (GSE)
  - Inoue K, Morikawa T, Matsuo S, et al. 2014[a]
- L-Aspartic acid
  - Tada Y, Yano H, Takahashi K et al, 2008[b]
- Polyphenols
  - Gho F, Pena-Neira A, Lopez-Solis, 2007
  - Lina B, Reus A, Hasselwander et al. 2012
  - Fujiwara K, Nakashima S, Sami M et al, 2013
- Tannins
  - Mehansho H, Clements S, Sheares B, et al 1985
  - Jansman A, Frohlich A, Marquardt R, 1994
  - Lamy E, Baptista E, Coelho e Silva, 2010

[a]National Institute of Health Sciences, Tokyo
[b]Metropolitan Institute of Public Health, Tokyo

Conclusions

Conclusions

- Glyphosate technical is an organic acid
- Parotid salivary gland acinar changes occur only after dietary exposure to glyphosate technical
- Parotid salivary gland changes are reversible upon withdrawal of exposure
- Similar, reversible changes in parotid gland acinar cells occur after dietary exposure to other organic acids
- Parotid acinar cell changes after dietary glyphosate exposure are non-adverse, adaptive reactions that should not be used as a basis for classification

Similar Opinions by Expert Groups and Scientific Authorities

- INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice)Project
  - International initiative of Societies of Toxicologic Pathology
    - Europe
    - Great Britain
    - Japan
    - North America
- Investigations of food additives
  - National Institute of Health Sciences, Japan
  - Tokyo Metropolitan Institute of Public Health

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