

# **Perchlorate in the Environment: Ecological Considerations**

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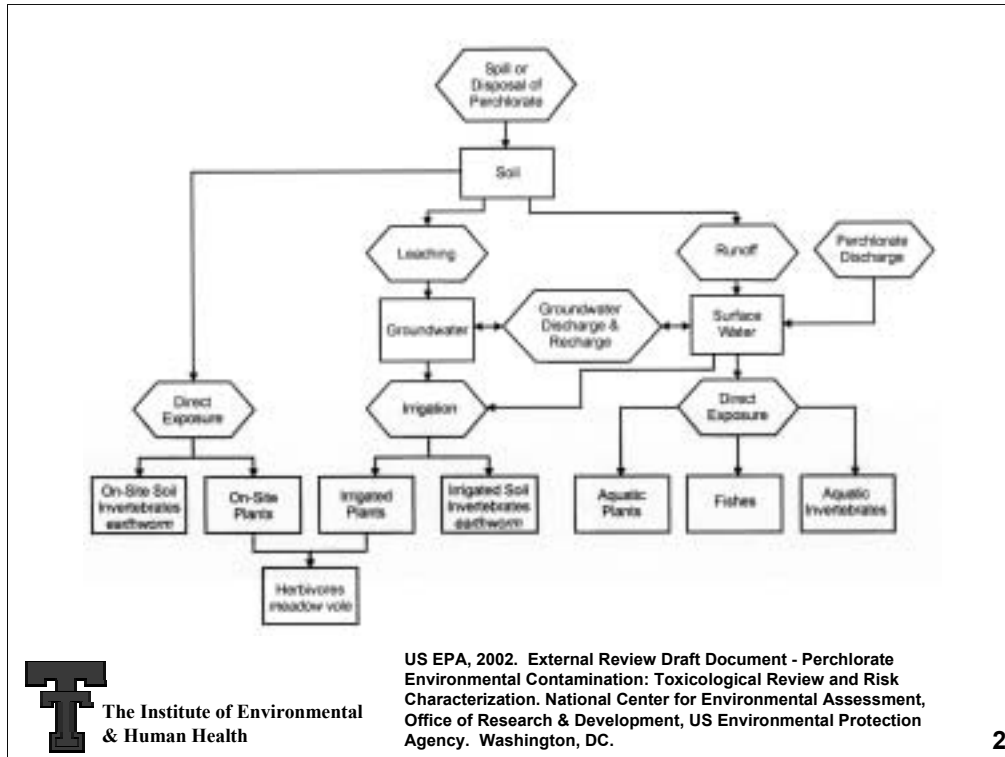


Figure 8-1. A conceptual model of exposure of ecological endpoint receptors to perchlorate. Specific endpoint taxa are identified in *italics*; all other endpoints are defined at the community level. Processes are designated by hexagonal boxes, compartments by rectangular boxes.

# Does Exposure Occur?

- **Parsons, 2001 Biotransport Study**
  - AFIERA and Parsons Engineering sampled 6 sites
  - 965 samples, 18 media types
    - $\text{ClO}_4^-$  detected in both abiotic and biotic matrices at all sites
    - vegetation > animal tissues
    - correlation between surface water and other media types
- **Smith *et al.* 2001. *Ecotoxicology* 10:305-313. Preliminary Assessment of LHAAP**
  - significant exposure among aquatic and terrestrial organisms
    - vegetation 560 - 5,557,000 ppb
    - aquatic insects 810 - 2,040 ppb
    - fish ND – 210 ppb
    - frogs ND – 580 ppb
    - rodents ND – 2,330 ppb



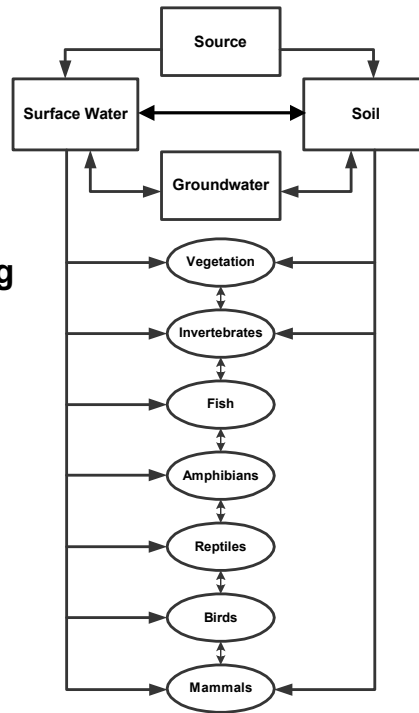
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...Yes

- **Site specific studies indicate numerous receptors are being exposed**

- **direct exposure sources**

- water
- soil
- vegetation
- trophic transfer (?)



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# Perchlorate Effects on Invertebrates

- Acute toxicity (survival and weight loss) of perchlorate to earthworms (*Eisenia foetida*) occurs at concentrations exceeding environmental relevance (> 100 ppm)
- Perchlorate concentrations > 1 ppm reduced cocoon production by earthworms
- In some instances, cocoons produced at high perchlorate concentrations did not hatch upon incubation in clean soil

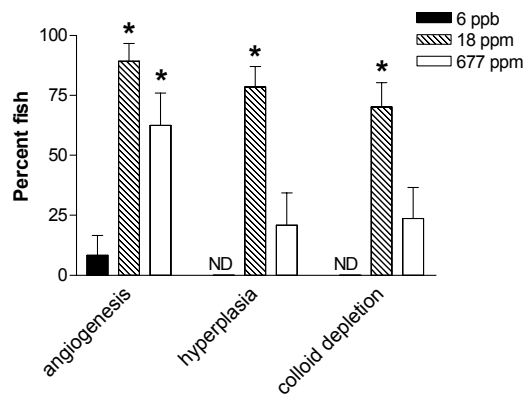


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Unpublished data courtesy of Dr. Todd A. Anderson 5

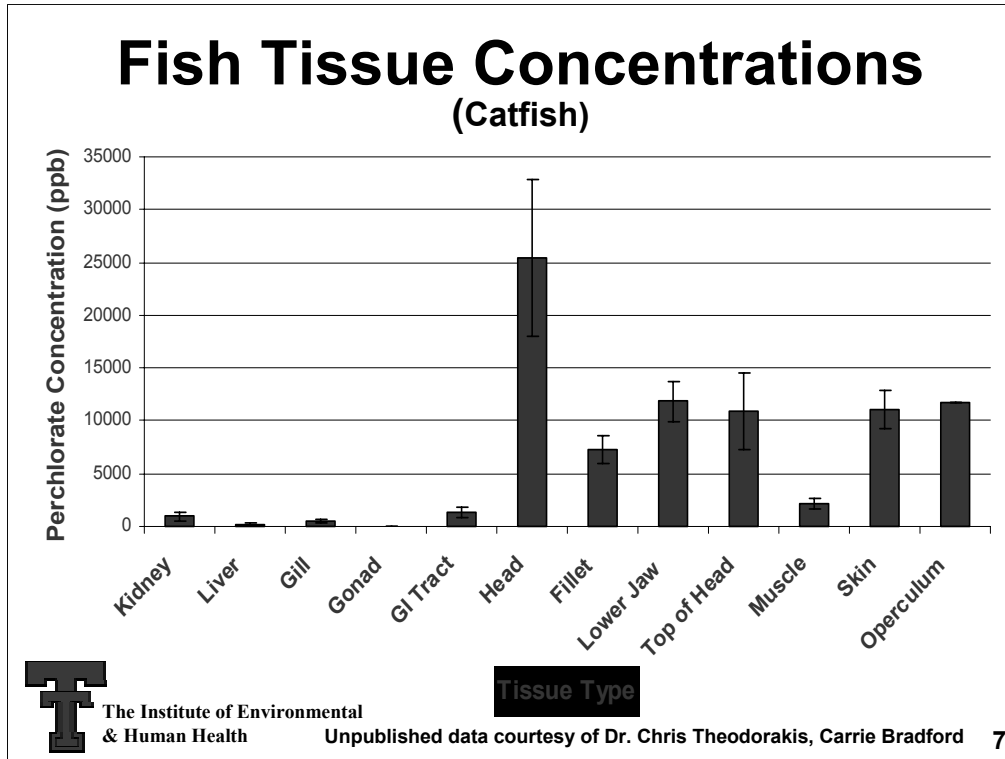
# Fish

- **Not acutely toxic to Zebrafish**
  - zebrafish  $LC_{50}$  869,500 ppb (mean of 2 trials)
  - little or no effect on zebrafish reproduction
    - spawn volume
    - fertilization rates
  - thyroid histological changes apparent



Unpublished data courtesy of Dr. Reynaldo Patiño 6

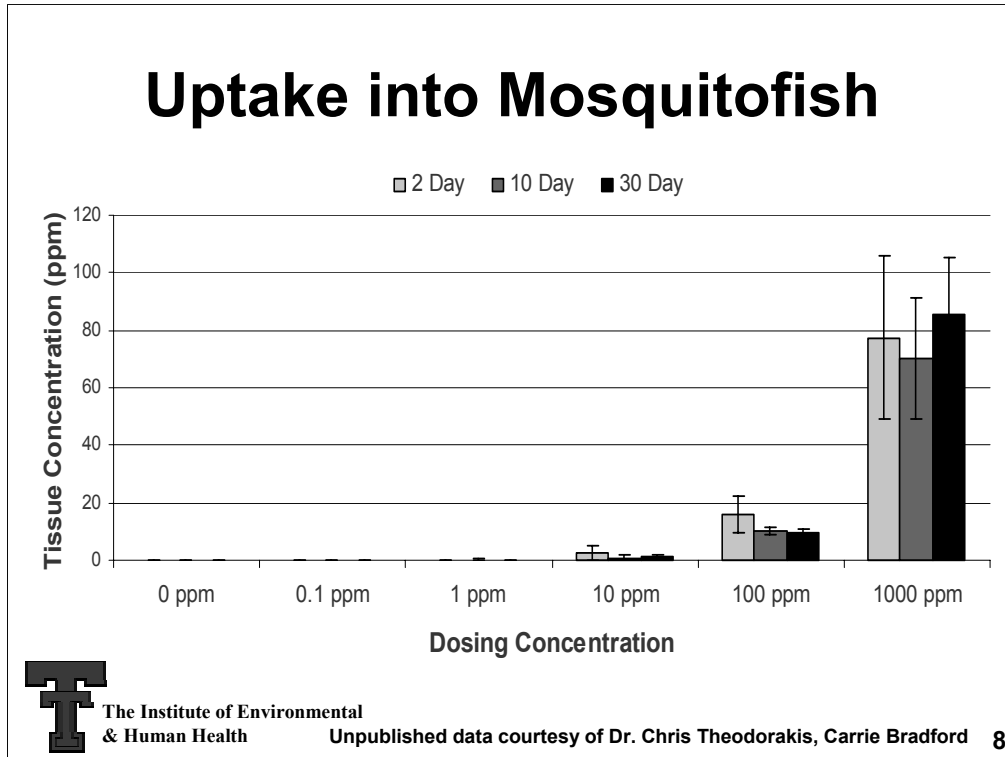
Fig. 5. Effect of ammonium perchlorate at 6 ppb (control), 18 ppm or 677 ppm on the percentage of fish showing signs of thyroidal angiogenesis, hyperplasia and colloid depletion. Bars for each treatment regime represent the mean percentages ( $\pm$  SE) of 6-7 tank replicates (see Fig. 4). Planned comparisons were made between the control and the 18-ppm group, and the control and 677-ppm group using Kruskal-Wallis and Median tests (critical  $\alpha$  adjusted to 0.025). Statistically significant differences from the control group are shown with an asterisk. See text for details of statistical analyses and results.



Concentrations of perchlorate in channel catfish tissues from fish exposed to perchlorate in water.

catfish that weigh approximately 120-150 g, were used for these experiments.

Catfish were placed in aquaria containing 100 ppm sodium perchlorate, with one fish per aquaria and twenty replicate aquaria.

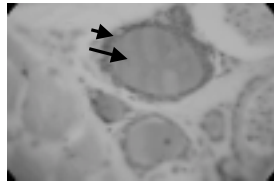


No difference in uptake depending upon time period

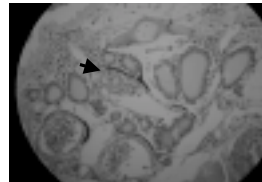
It does not appear that perchlorate bioaccumulates in fish tissues (Table 2). Two-way ANOVA indicated that there was no difference in perchlorate uptake depending upon the time period, but there was a difference depending upon the concentration of the dose.



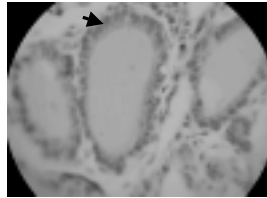
## Mosquitofish Thyroid Histology



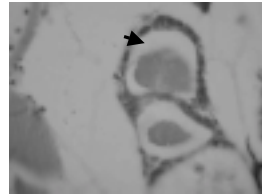
Normal Follicle



Hyperplastic  
Follicle



Enlarged Follicular  
Epithelium  
(Hypertrophy)



Depleted Colloid



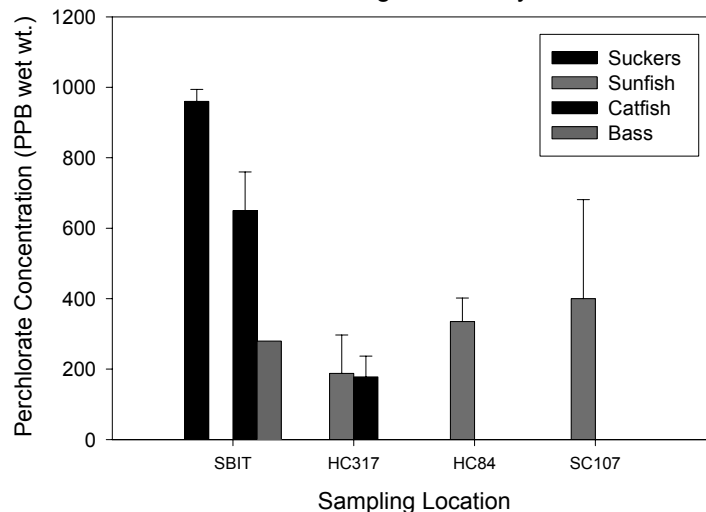
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Photos courtesy of Dr. Chris Theodorakis, Dr. Jaques Rinchard 9

Perchlorate induces increase in epithelial cell height, and hyperplasia seen in most fish exposed to perchlorate for 30 days

## Site/Species Specific Exposure

Perchlorate in Fish Heads Collected  
Near McGregor, TX May 2001



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Catfish:  $[\text{ClO}_4^-]_{\text{head}} / [\text{ClO}_4^-]_{\text{fillet}} \approx 3.5$

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ND = not detected by the analytical procedure [detection limit in dry tissue = 100 ppb]

Preliminary data on perchlorate concentrations in tissues from aquatic organisms within the Lake Waco and Lake Belton watersheds. Perchlorate concentrations are in ng/g (ppb) expressed based on tissue dry weight. Data represent the results of analyses of individual tissues (heads) with the number of analyses indicated parenthetically.

Respective wet weight concentrations are presented in brackets. Samples were collected in May, 2001 from areas previously identified through water quality analyses as having received perchlorate.

### Subsequent sampling

Fillets (muscle) from edible-size fish were collected within the Lake Waco and Lake Belton Watersheds during August/September, 2001 from locations mutually agreed upon by BRA and the Investigators. Only four (4) fillets tested positive for quantifiable perchlorate residues (Table 3). Two additional fish had trace quantities of perchlorate (perchlorate levels detectable, but below the limit of quantitation). It does not appear that perchlorate accumulates in muscle tissue to the

extent that it appears to accumulate in other tissues. Perchlorate contamination in fish fillets from this collection was not as widespread as indicated by our previous analysis of fish heads from smaller fish collected in May, 2001, a period of active surface water flow. It is possible that more of the fillets contained perchlorate, but the tissue concentrations were below of limit of detection (170 ppb in wet tissue).

Catfish head/fillet ratio calculated from dosing study @ 100 PPM for 5 days.

# Amphibians

- **Perchlorate not acutely lethal**
  - 5-day  $LC_{50}$   $510 \pm 36$  ppm
  - 70-day  $LC_{50}$   $223 \pm 13$  ppm
- **Perchlorate  $\geq 147$  ppb prevented forelimb emergence and tail resorption over 70-d exposure**



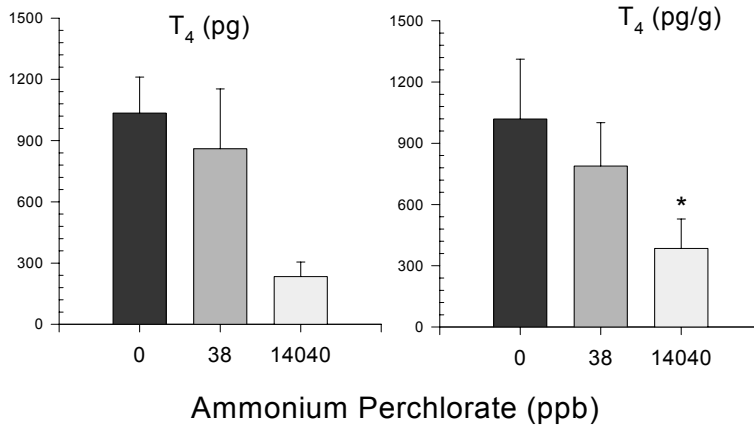
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Goleman, WL, Urquidi LJ, Anderson, TA, Kendall, RJ, Smith, EE, Carr, JA. 2002. Environ Toxicol Chem. 21: 424-430.

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# Whole Body T<sub>4</sub> Levels

70-d AP exposure-T<sub>4</sub>

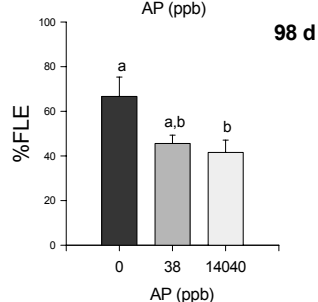
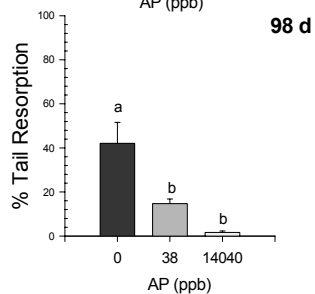
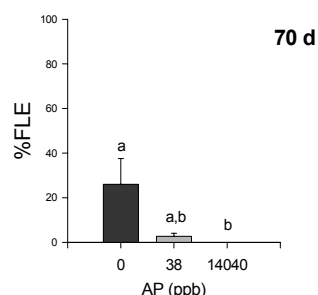
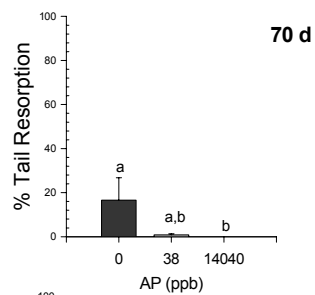


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Goleman, W, Carr, JA, Anderson, TA. 2002.  
Environ Toxicol Chem. 21:590-597.

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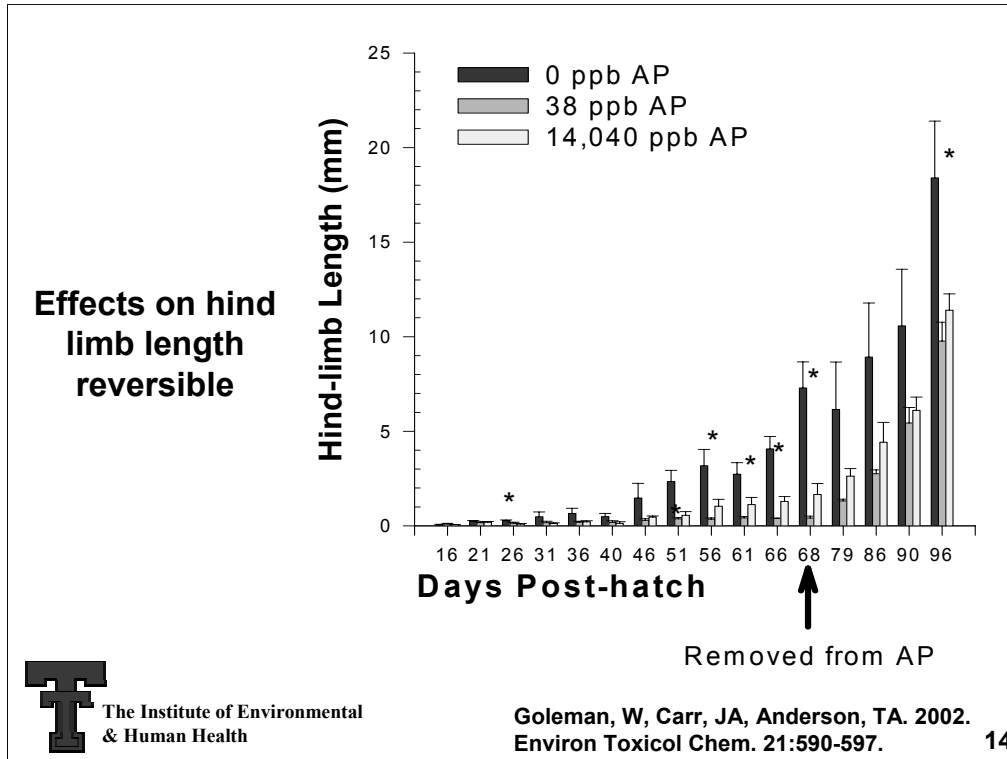
# Tail Resorption      Forelimb Emergence



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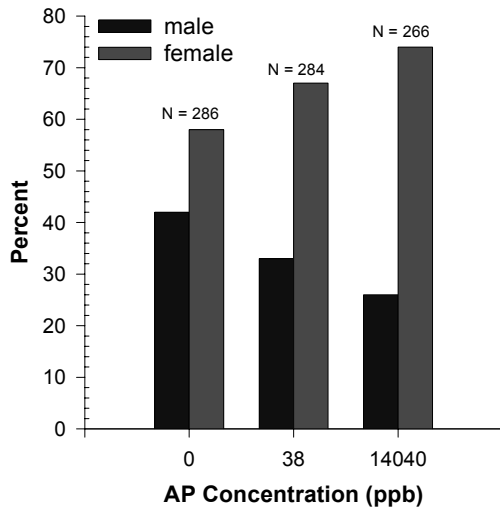
Goleman, W, Carr, JA, Anderson, TA. 2002.  
Environ Toxicol Chem. 21:590-597.

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- Hindlimb measurements were recorded every 5 days.

# Alterations in Sex Ratios



AP (PPB)	$\chi^2$	$\rho$
0	3.41	= 0.06
38	16.7	< 0.0001
14040	31.7	< 0.0001

**Thyroid hormones required for upregulation of androgen receptors**

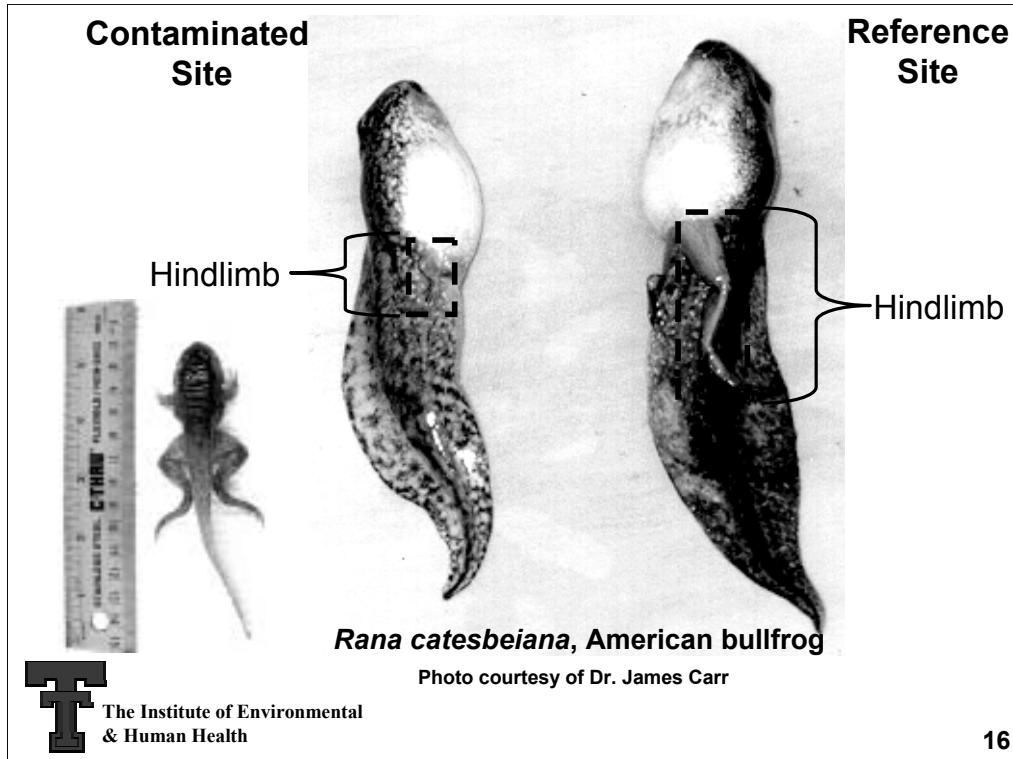
(Robertson and Kelley, 1996, Dev. Biol. 176: 108-123)



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Goleman, W, Carr, JA, Anderson, TA. 2002. Environ Toxicol Chem. 21:590-597.

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Bullfrog tadpoles from AP-exposed site had

- shorter hindlimbs

- a shorter hindlimb/snout-vent length ratio

- and had not yet progressed to late prometamorphosis/metamorphic climax

Smaller thyroid gland, probably related to developmental stage, as the thyroid increases in size during late prometamorphosis and metamorphic climax in some species.

These tadpoles over-winter, and exposure history throughout entire larval period is unknown.



# Thyroid Histopathology in Wild Chorus Frogs

Site	[ClO <sub>4</sub> <sup>-</sup> ] (ppb)	Gosner stage	Colloid Depletion	Hypertrophy
Ref.	ND	33-34	0.02 ± 0.02 (n=8)	0.00 ± 0.00 (n=8)
Cont.	9,802	33-34	0.71 ± 0.20* (n=15)	0.79 ± 0.20* (n=15)



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Carr, JA, Urquidi, LJ, Goleman, WL, Hu, F, Smith, PN, and Theodorakis, CW. 200X. Ammonium perchlorate disruption of thyroid function in natural amphibian populations: Assessment and potential impact. "Multiple Stressor Effects in Relation to Declining Amphibian Populations, ASTM STP 1443, G. Linder, Ed., ASTM International, West Conshohocken, PA (in review).

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*Pseudacris triseriata*

<sup>1</sup>After Gosner (1960).

<sup>2</sup>Mean ± standard error of scores made from all follicles present in three sections from the rostral, middle, and caudal regions of the thyroid gland.

<sup>3</sup>Statistically different from reference site (site C) based on Student's *t*-test, *P* < 0.05.

ND, not detectable.

# Mammals

- Extensive testing with laboratory rodent models
- Thuett et al., *J Toxicol Environ Health* (in press) examined effects on weanling deer mice
  - no change in  $T_3$ , increase in  $T_4$ , histological changes
  - very low tissue concentrations in rodents dosed via water
- Raccoons inhabiting LHAAP
  - elevated perchlorate concentrations in food items
  - no detectable perchlorate in plasma (n=59)
  - no correlations/alterations in  $T_3 / T_4$  and TSH
- Why do we see elevated residues in wild rodent tissues?



# Ecotoxicological Data Gaps

- Trophic transfer studies / food crops
- Avian toxicity studies
  - transfer of perchlorate to eggs
- Amphibian population studies
- Effects on endothermic organisms
- Effects on reptiles



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  - US EPA
  - TNRCC
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