Case Study: Biological Effects of Nanomaterials

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Aims

- What are the expected target organs and effects on the wildlife?
- Can we use these biological effects to estimate:
  - The extent of the contamination (biological monitoring).
  - Identify exposure route (dietary, aqueous, air)?
  - Type of nanomaterial/causative agent(s)
- Acute effects seem less likely (10 years of production), but is there a new product?
- Focus on cumulative effects.

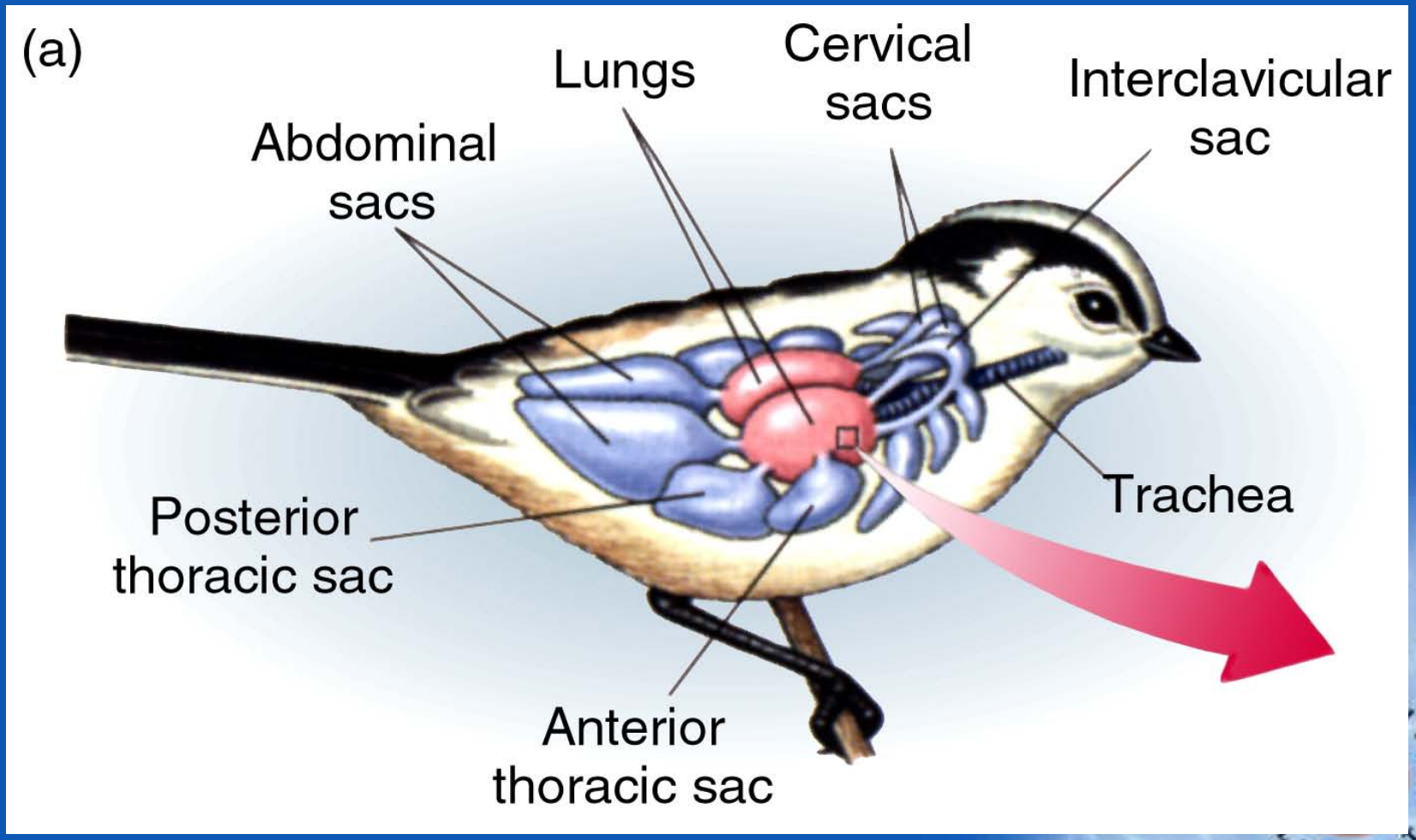
Effects of Chemicals on Respiratory Systems

- Acute effects
  - High respiratory frequency, low respiratory volume (fast & shallow breathing).
  - Mucus production
  - Rapid inflammation and oedema.
  - Loss of respiratory/osmoregulatory functions to cause cardiovascular collapse.
- Chronic effects
  - More subtle pathological change, compensatory hyperplasia, fibrosis.
  - Changes in the numbers/types of epithelial cells
  - Changes in animal locomotion, foraging, bioenergetics

Effects of Chemicals on Gut Function

- Acute effects
  - Empty stomach/food refusal.
  - Vomiting & Diarrhoea
  - Mucus production, inflammation and oedema.
  - Dehydration/acute loss of electrolytes.
- Chronic effects
  - More subtle pathological change in gut mucosa, vacuolation, fusion of villi, fatty change in the liver.
  - Changes in feeding behaviour-food preferences
  - Slower growth, altered body mass indices
  - Changes in immunity

Birds


(a)
Lungs
Cervical sacs
Interclavicular sac
Abdominal sacs
Trachea
Posterior thoracic sac
Anterior thoracic sac

Avian Mortalities

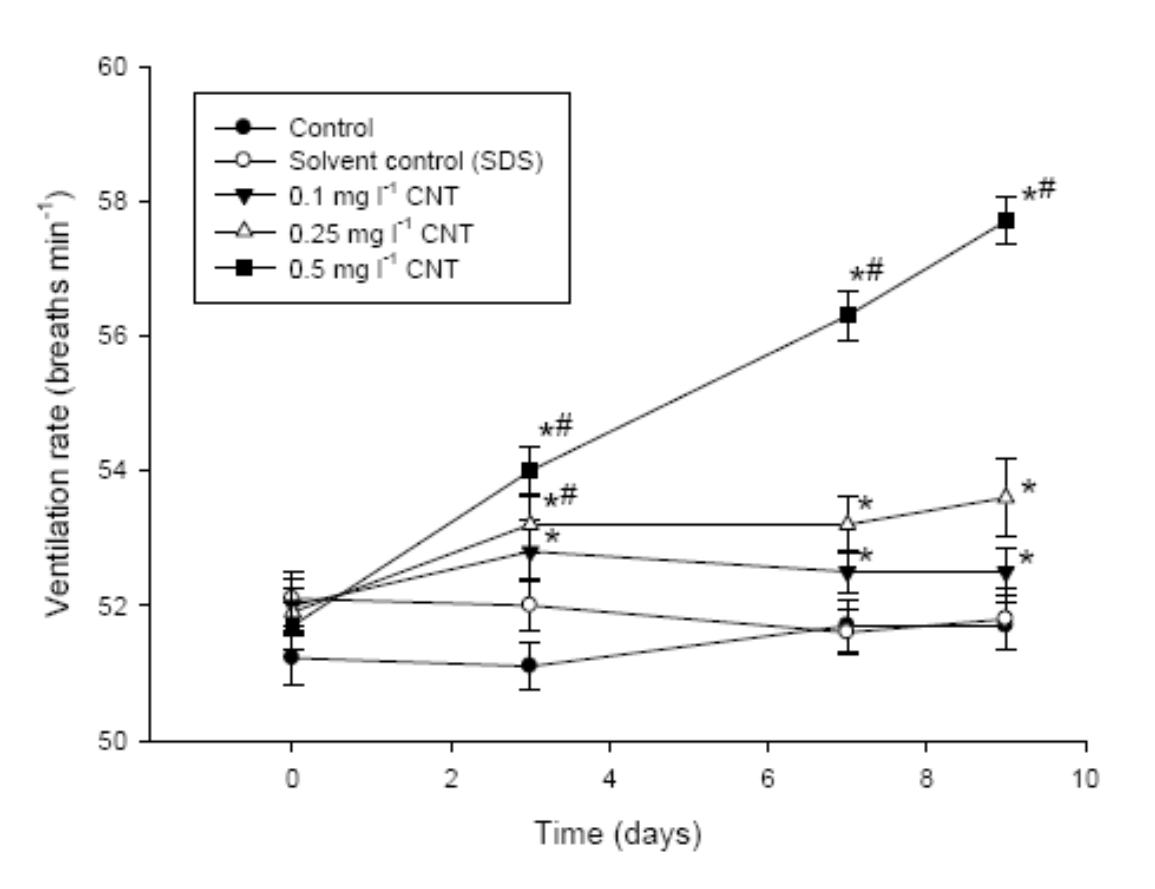
- No toxicology data in the published literature on engineered NPs.
- Assume target organs and effects are similar to other vertebrates (rats, fish).
- Special consideration for birds
  - High respiration rate (exposure via air).
  - NPs in seeds, small insects (food), dust bath (soil exposure)
  - Bioenergetics; high metabolic rates, no fat reserves, short period of starvation = mortality.
  - Predation; loss of habitat (shrubs).
  - Long range transport of metal pollutants (Ek et al. 2004).
  - Birds have natural nanoparticles for navigation (magnetite) in their tissues (e.g., dendrites in beak).

Fish Mortalities

Acute aqueous exposure-gill pathology

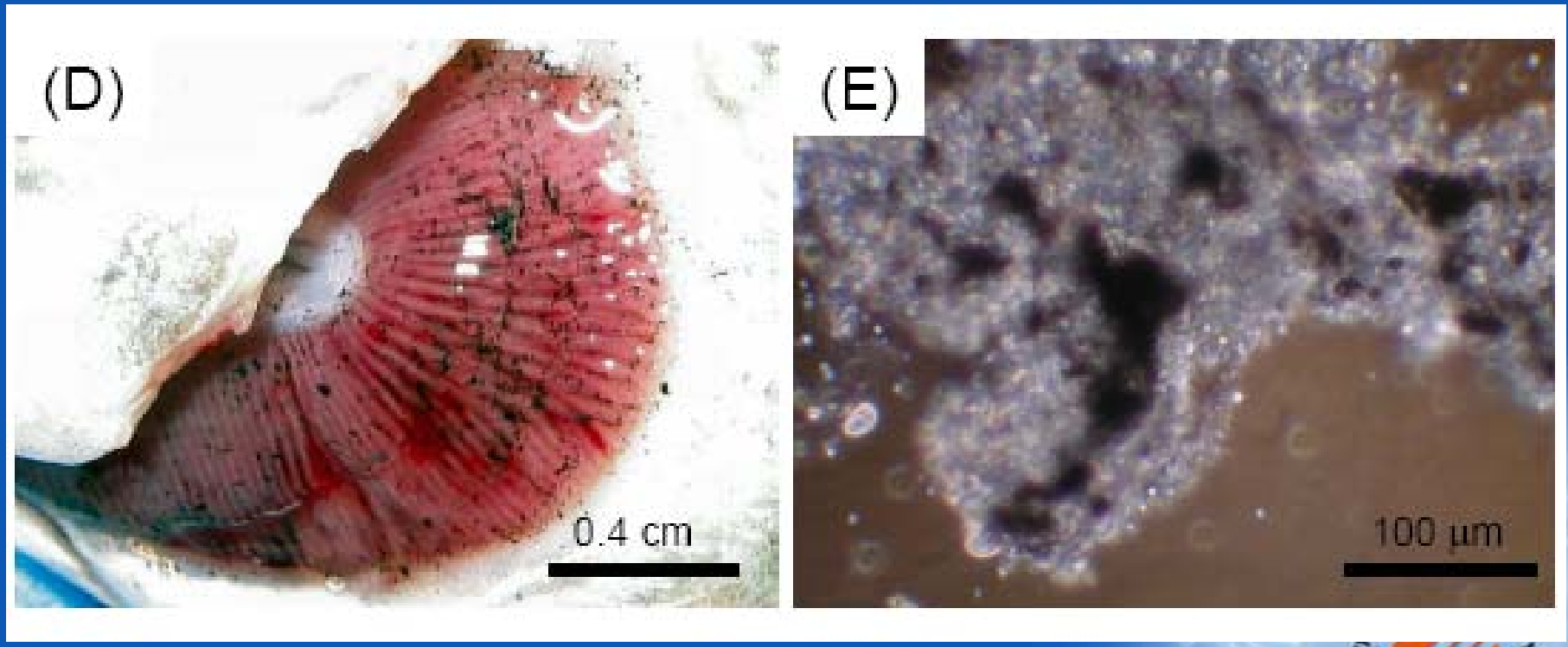
Chronic/diet effects-liver, spleen, brain.

Carbon Nanotubes Are A Respiratory Toxicant To Rainbow Trout



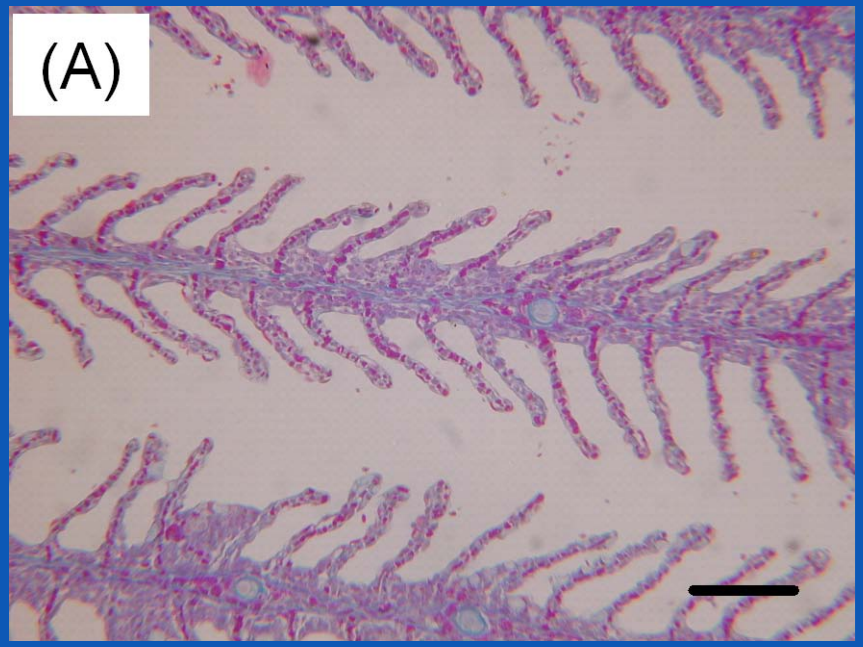
Smith et al. (2007) Aquatic Toxicology, 82, 94-109.

Dispersed Nanotubes: Chemistry Changes on Contact With Mucous Ligands

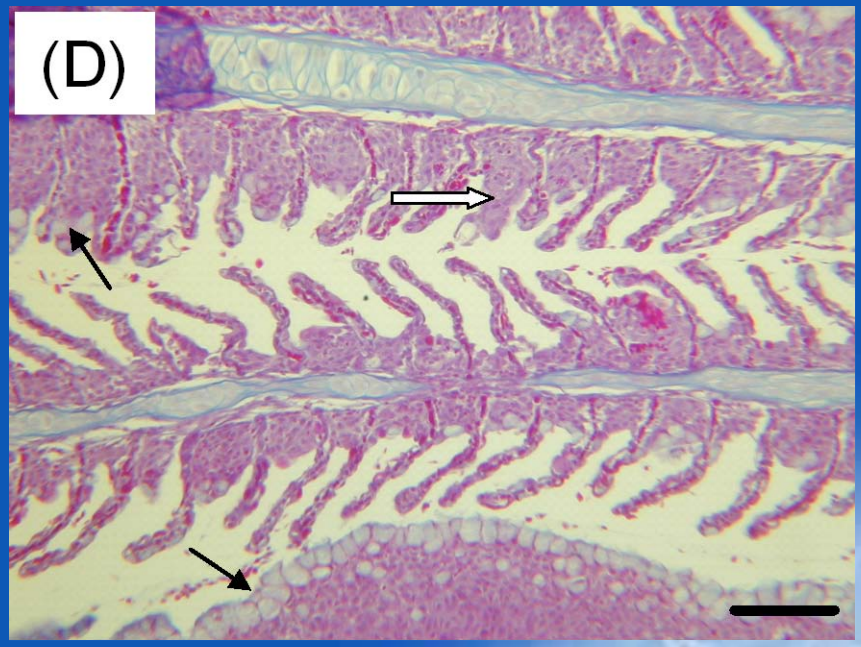


Smith et al. (2007) Aquatic Toxicology, 82, 94-109.

Gill Pathology: Carbon Nanotubes



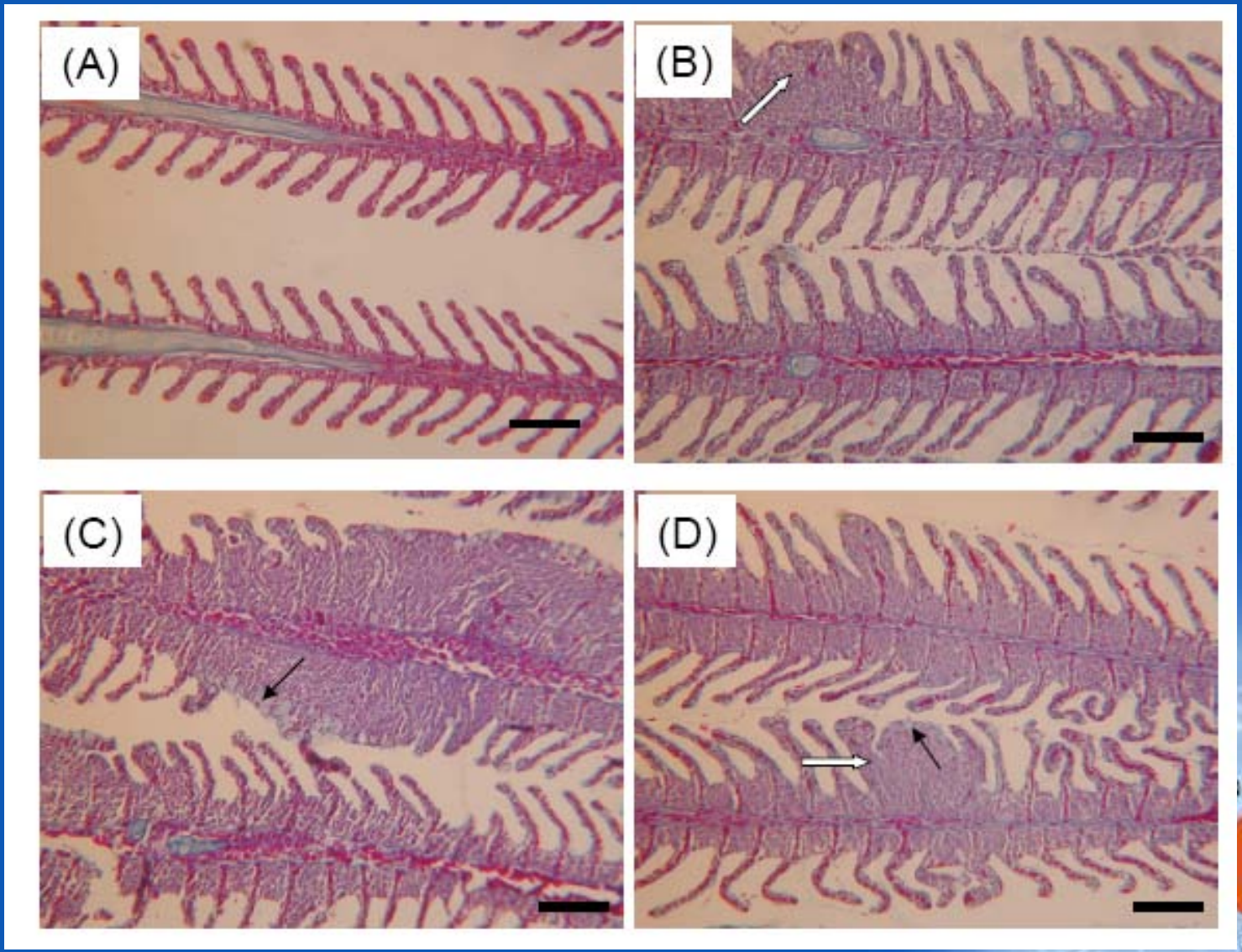
Water only Control



Carbon nanotubes

Smith et al. (2007) Aquatic Toxicology, 82, 94-109.

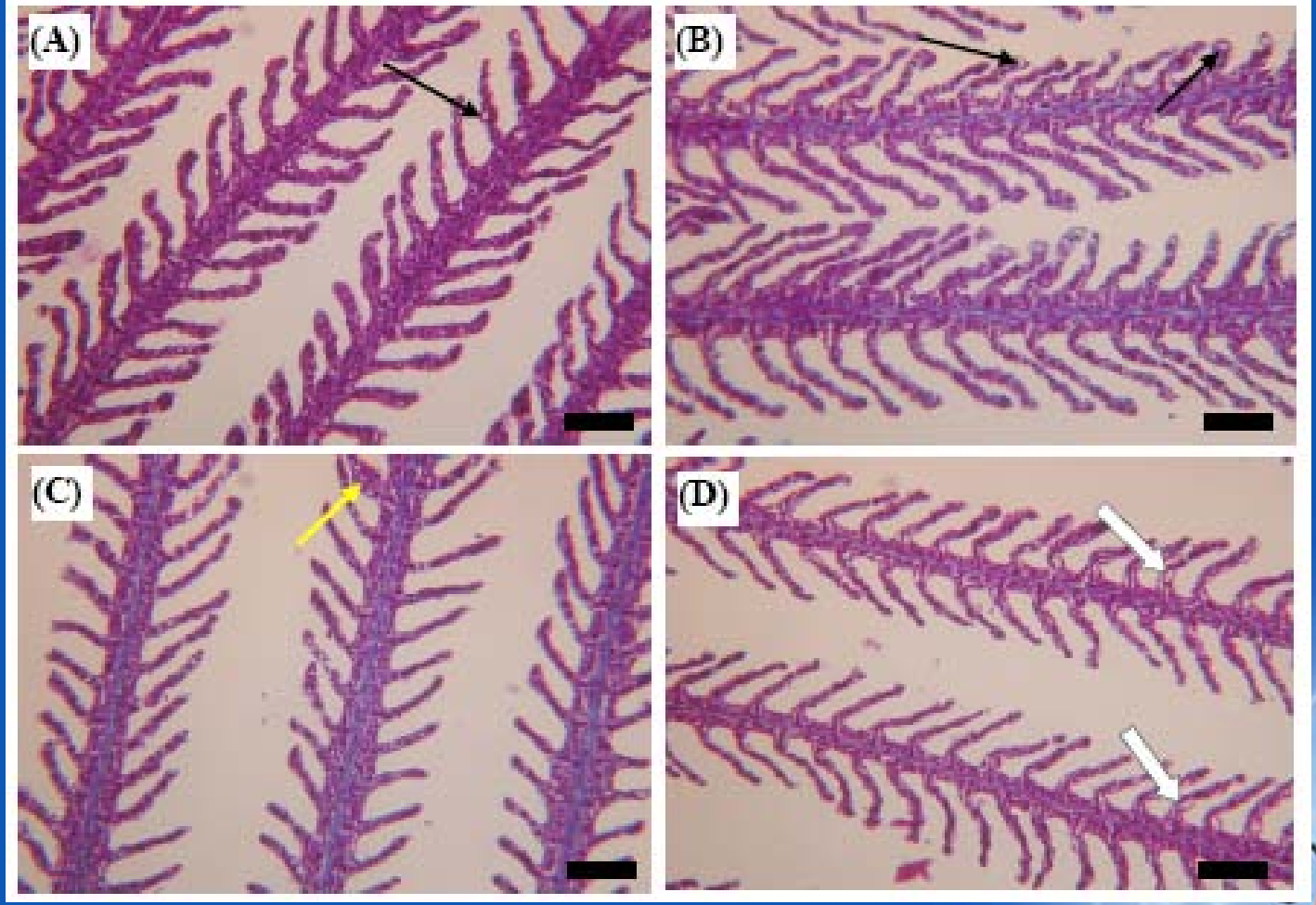
Gill Injury:Waterborne TiO_2 Exposure



Federici et al., 2007. Aquatic Toxicology, 84, 415-430

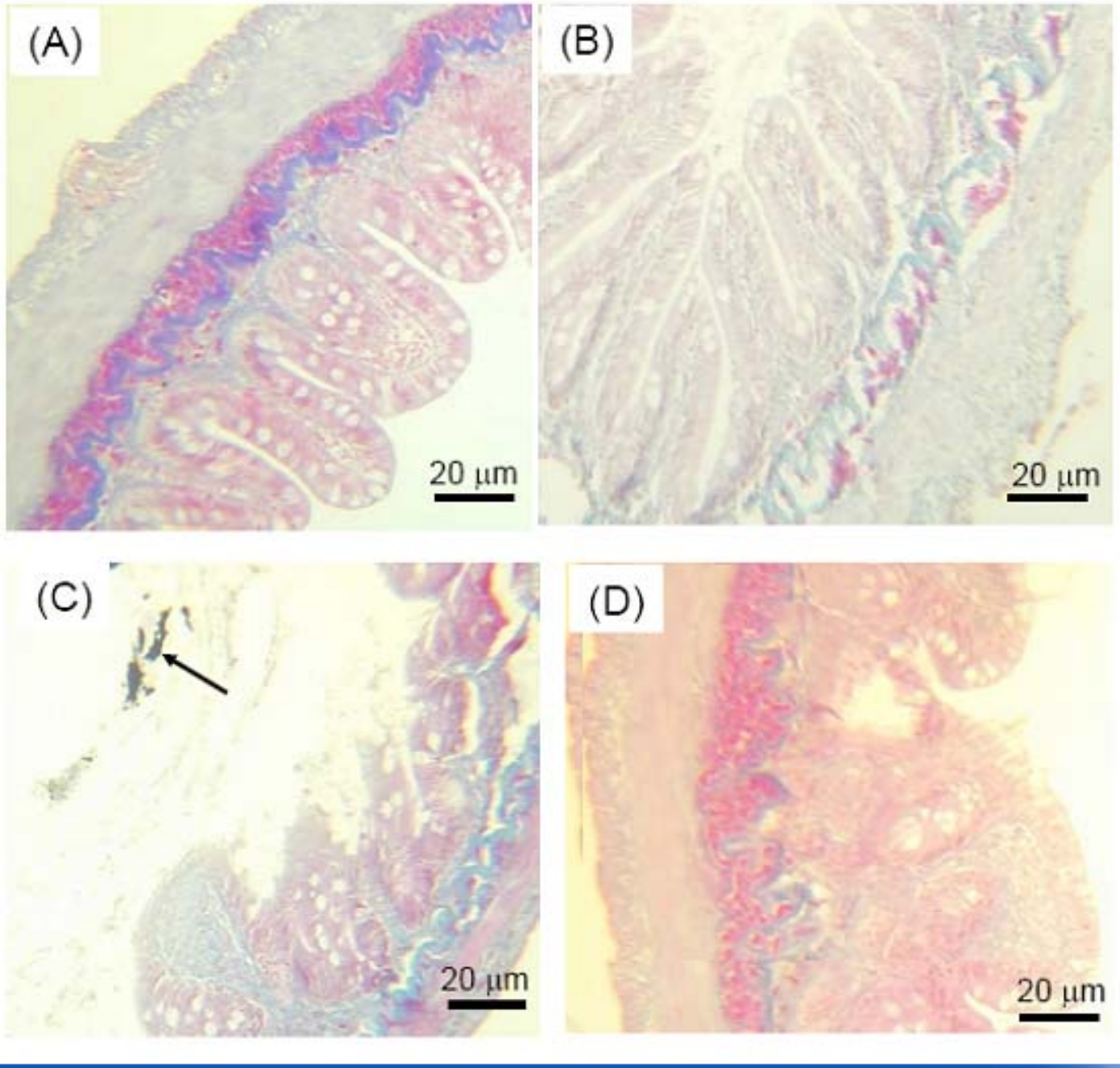
Gill Histology: Dietary Titanium NPs

Ramsden et al.



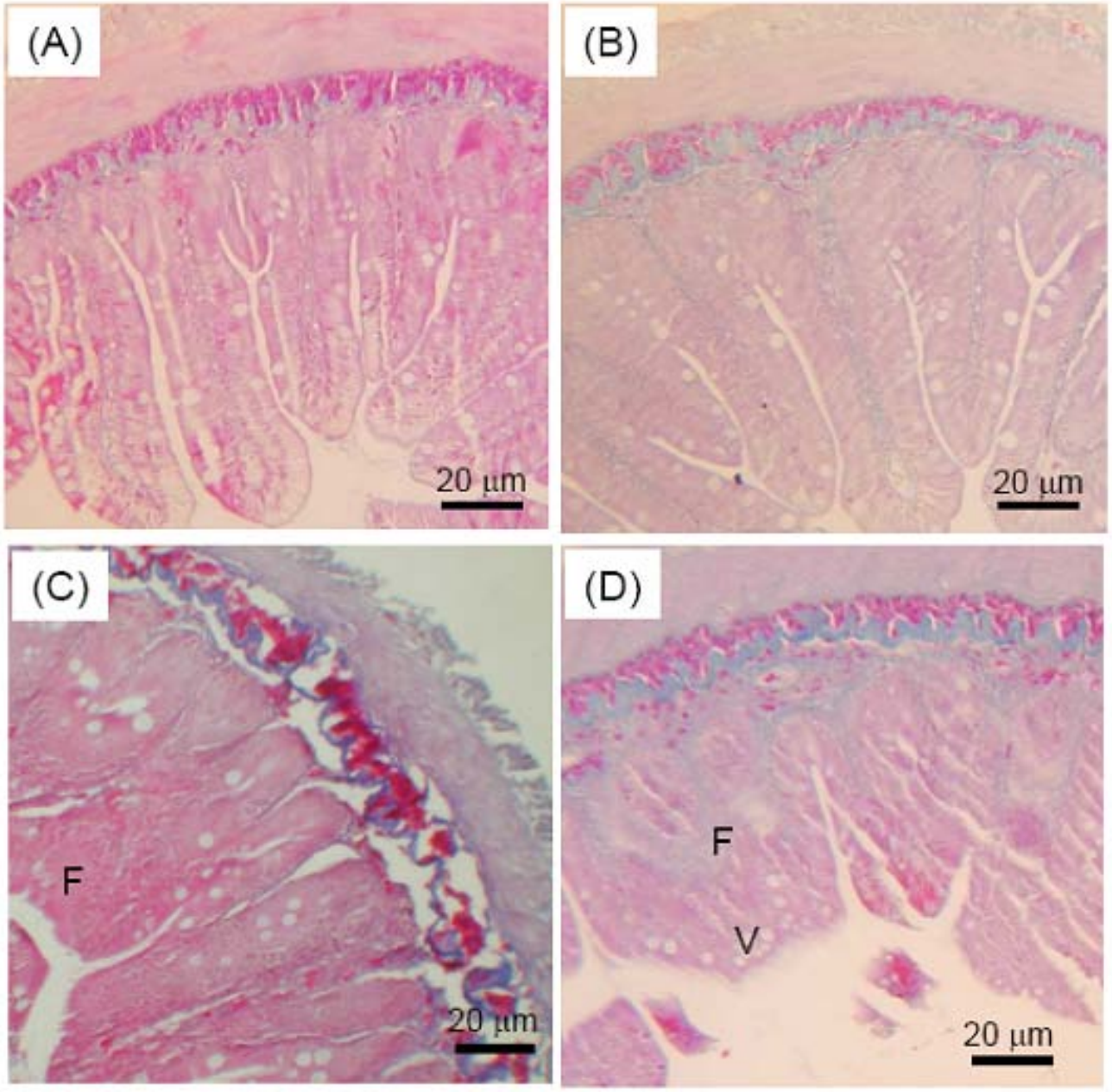
A, control time zero; B, Control after 8 weeks, C, 10 mg/kg TiO_2; D, 100 mg/kg TiO_2 after 8 weeks. Scale bar 80 µm.

Erosion of the Intestine in Trout Exposed to SWCNT



Smith et al. (2007) Aquatic Toxicology, 82, 94-109

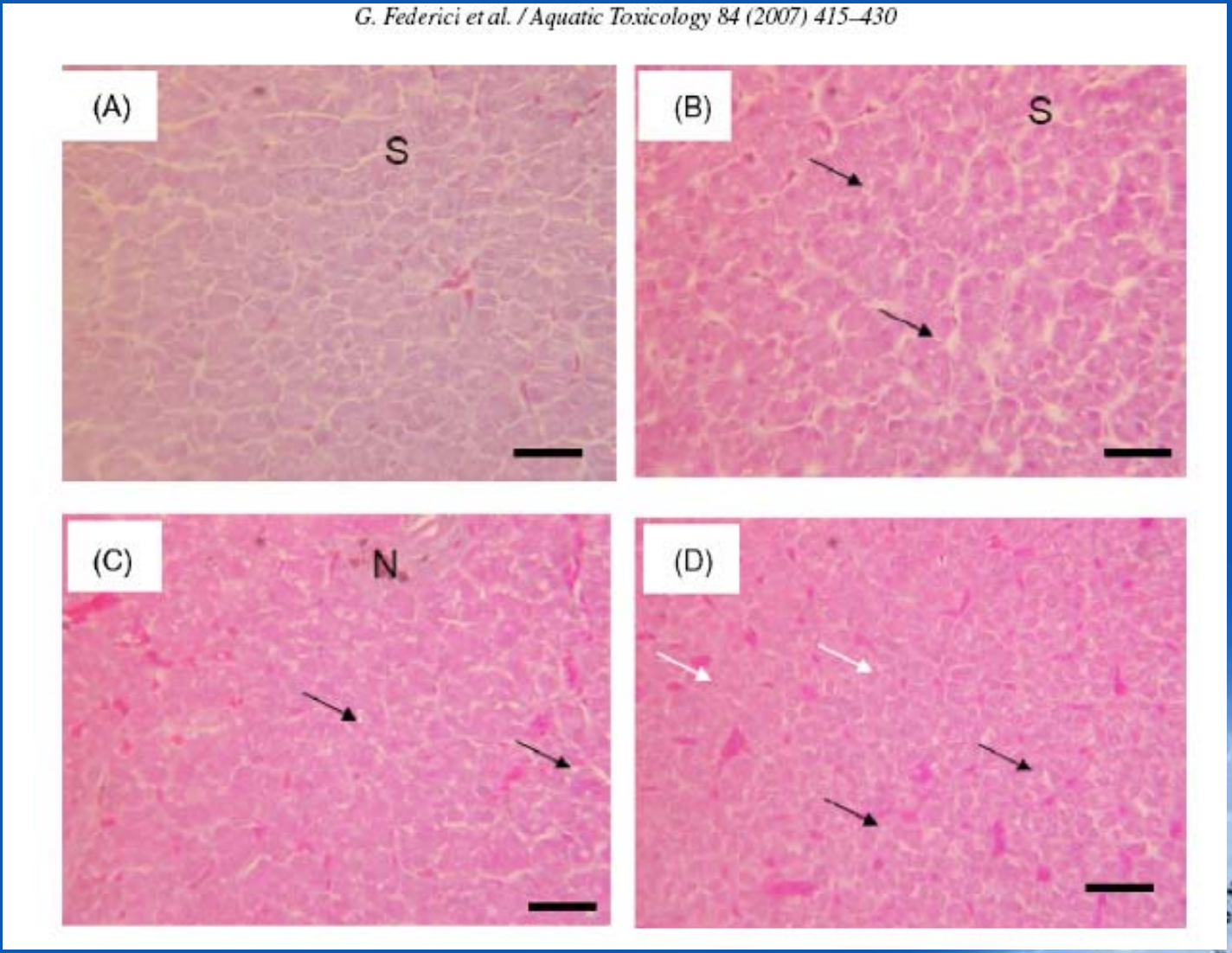
Effect of Drinking TiO_2 NPs on Trout Intestine



Liver Histology

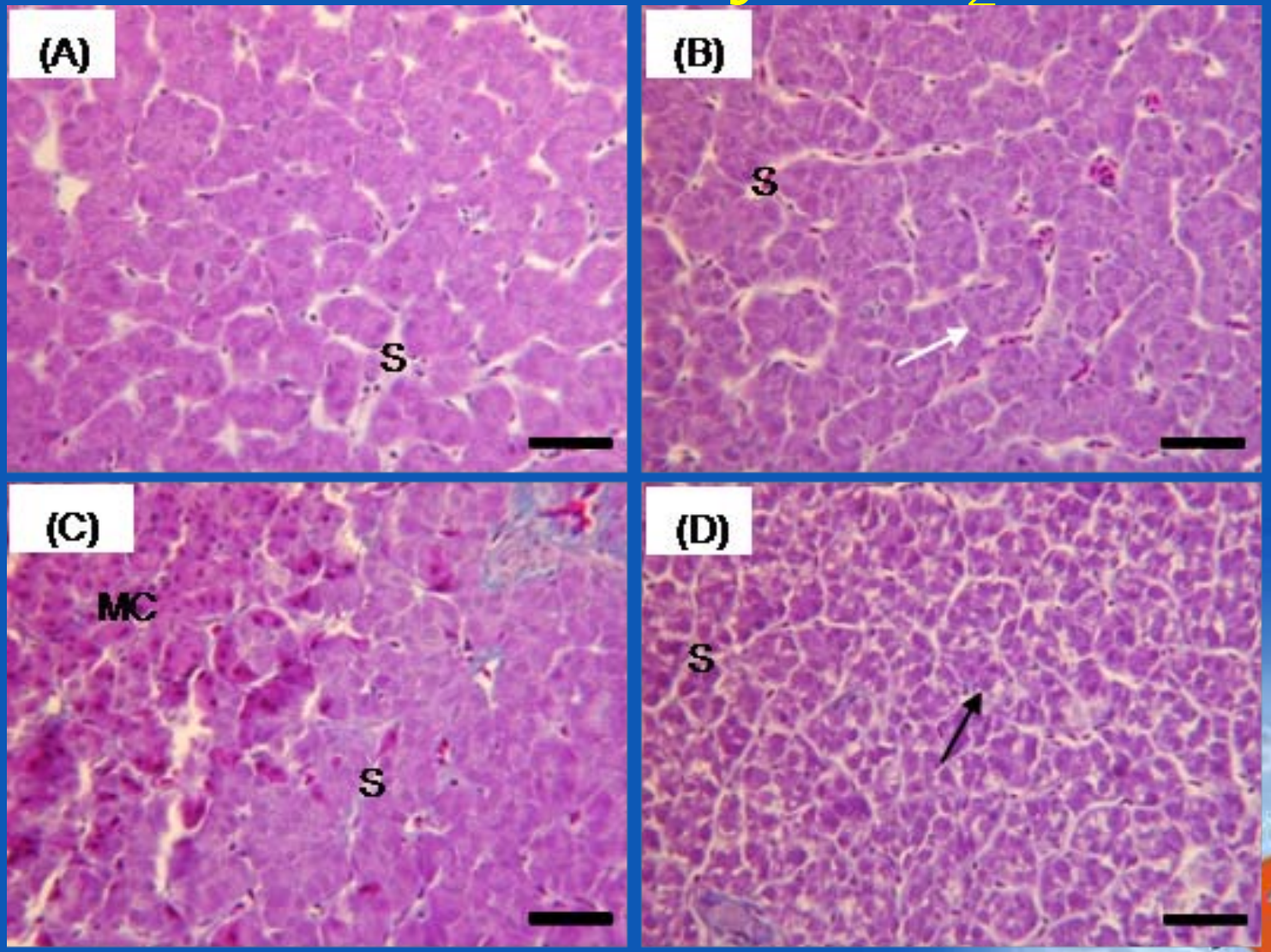
- Dietary metals (e.g., Cu, Fe, Zn):
  - Changes in % sinusoid space
  - Glycogen deposition/loss of glycogen mobilisation.
  - Fatty change and mild lipidosis
- Waterborne TiO_2 NPs:
  - Loss of sinusoid space
  - Foci of lipidosis
  - Changes in nuclear morphology (early stage necrosis/apoptosis)
- Dietary TiO_2 NPs in liver:
  - Condensed chromatin in nucleus/no micronuclei
  - Eosinic red cells/damaged red cells in the sinusoids.
  - Foci of morphological change = hepatitis in 10 mg/kg TiO_2 treatment.
  - fatty change and foci of lipidosis at the highest TiO_2 inclusion.

Liver Histology: Waterborne TiO_2 NPs



Liver morphology in trout after 14 days of exposure to (A) 0, (B) 0.1 (C) 0.5, and (D) 1.0 mg l^{-1} TiO_2 NPs. Sinusoid space (*S*), foci of liposis *(black arrow)*. Some cells showed nuclear fragments (white arrows), necrotic cells *(N)*. Scale bar = 50μm, sections 8μm thickness, Mallory's trichrome.

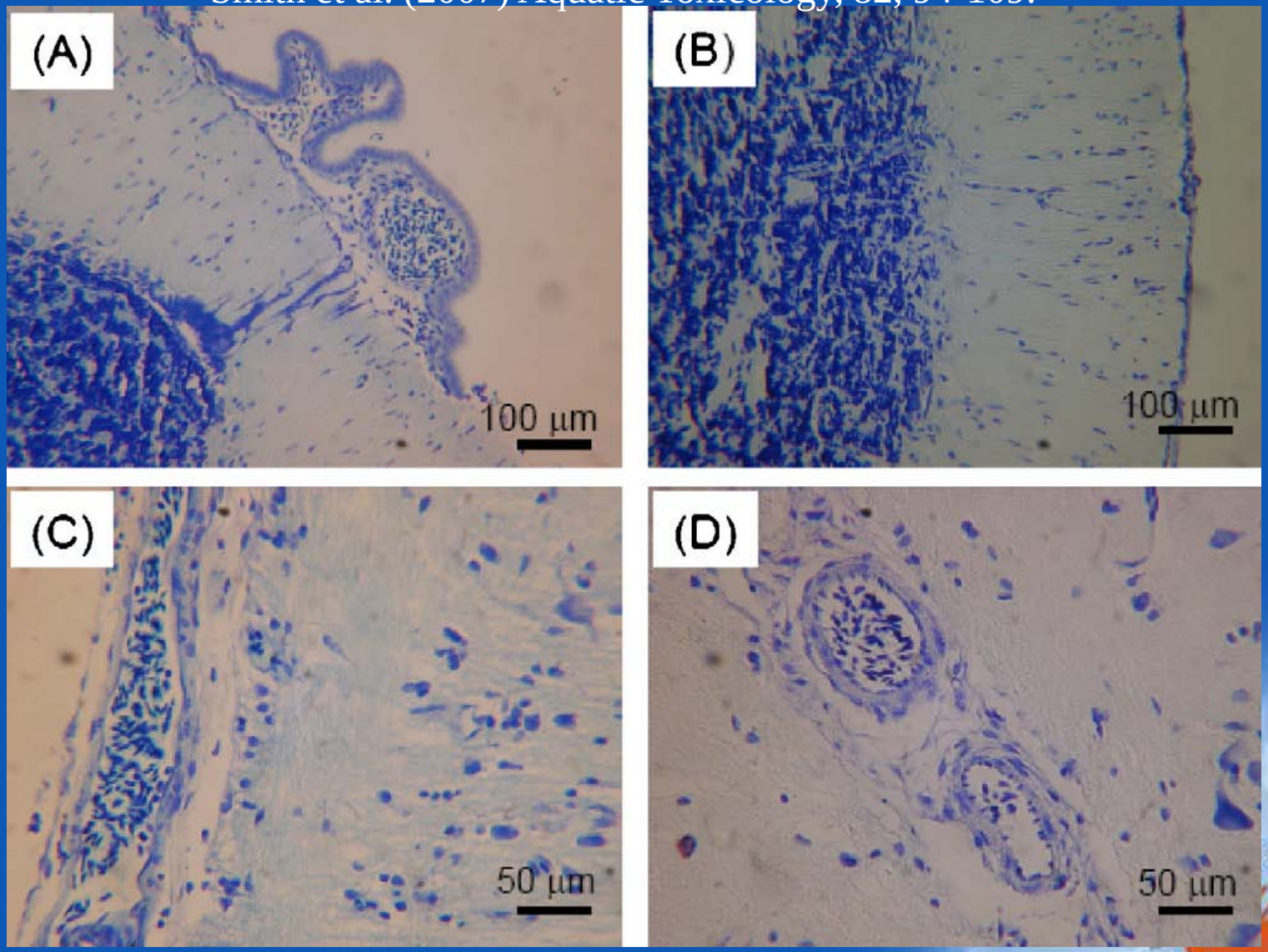
Liver: Dietary TiO_2



Liver morphology in rainbow trout fed 0, 10 or 100 mg kg^{-1} TiO_2 NPs for 8 weeks (A) 0 (control), (B) and (C) 10, and (D) 100 mg kg^{-1} TiO_2 NPs.

Brain Injury: Waterborne CNT

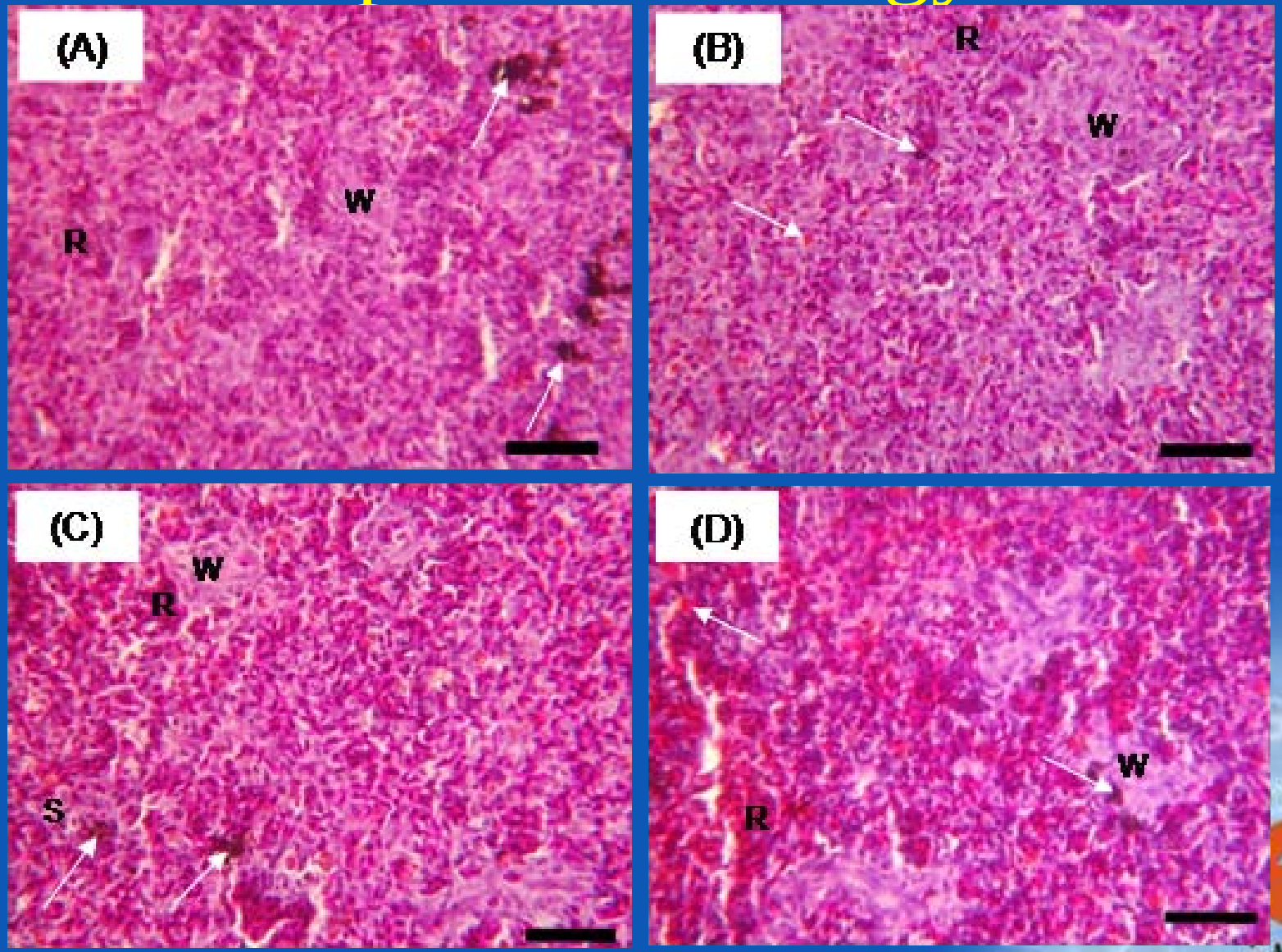
Smith et al. (2007) Aquatic Toxicology, 82, 94-109.



Blood vessel abnormality on the ventral surface of the cerebellum in a fish exposed to 0.5mg l^{-1}SWCNT (panel A) compared to solvent control (panel B). Blood vessels in the ventral region of the brain were normal in same fish from each treatment (panels C&D). 8µm thickness, toluidine blue.

Fig. 8.

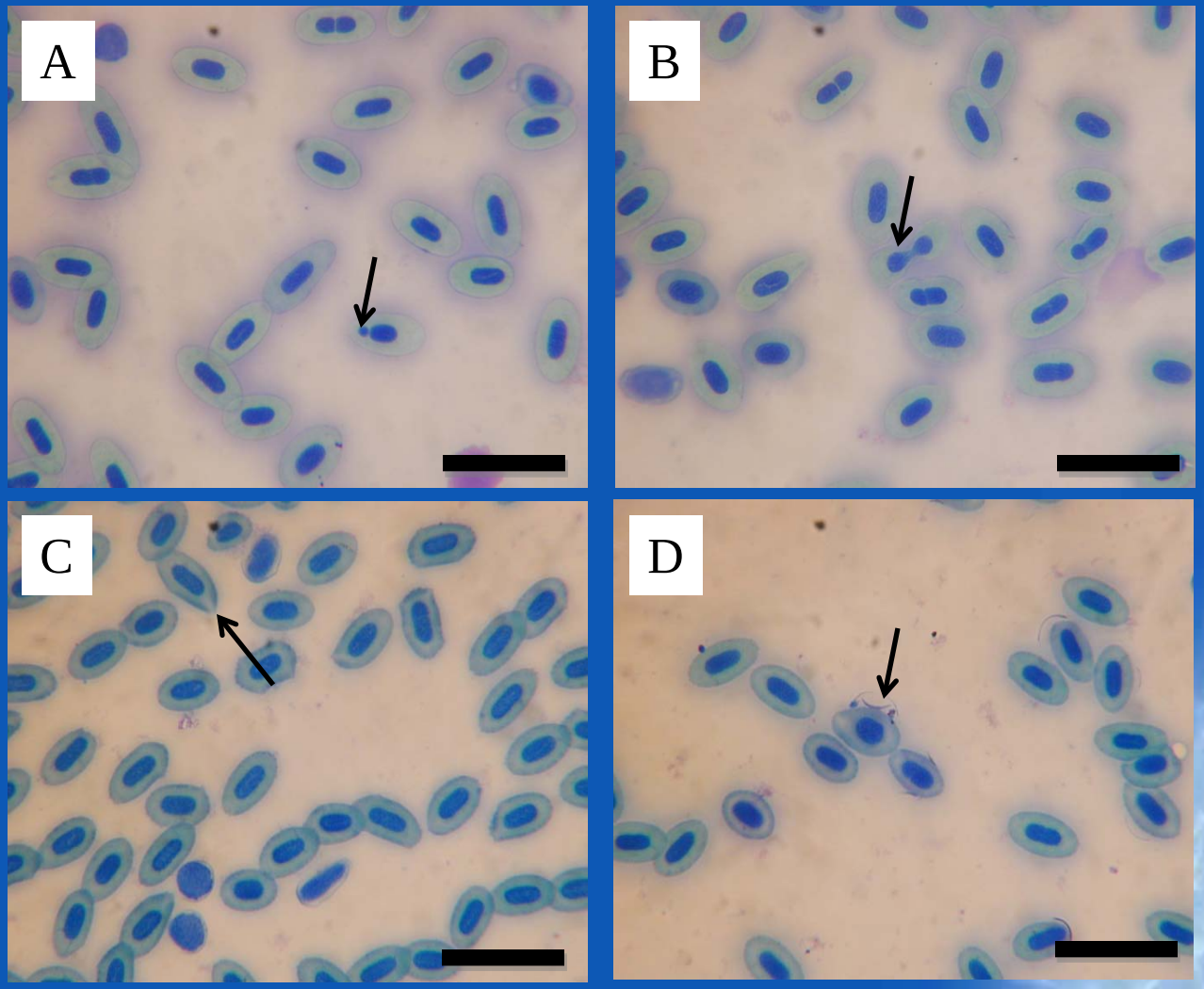
Spleen Pathology



Spleen morphology in rainbow trout fed 0, 10 or 100 mg kg^{-1} TiO_2 NPs for 8 weeks (A) 0 (control), (B) 10, and (C) 100 mg kg^{-1} TiO_2 NPs; and (D) in the 100 mg kg^{-1} TiO2 NP treatment after a further 2 weeks on the control diet (post-exposure phase).

Blood Cell Morphology

Voskou et al (2009)



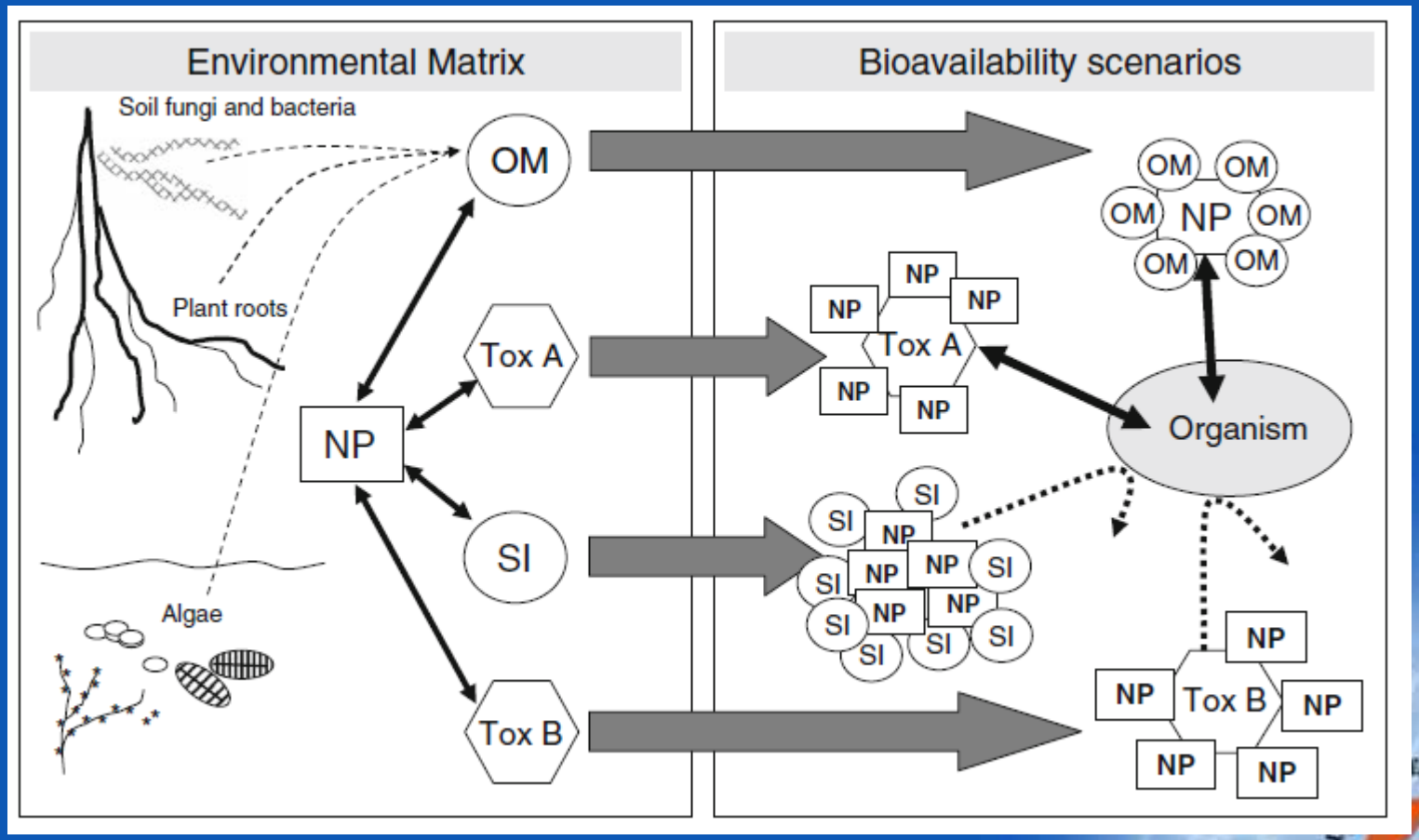
Peripheral blood smears of rainbow trout (Giemsa stain), bar 30μm.

Amphibian Deformity

- No published data on engineered NP-induced deformities in amphibians.
- Causes of deformity
  - Endocrine toxicity during development
  - Direct toxicity to developing tissues; abnormal tissue repair.
  - Threshold effects with other chemicals (atrazine, nitrates ,etc)
- Why no effects on fish?
  - There are effects; not looking in the right place/right time
  - Wait for it to appear; rapid life cycle in amphibians compared to some fish
  - Sample size-big data set to be sure the incidence is not natural variation.
- Differences in exposure less likely
  - Both fish & amphibians in the water
  - Similar invertebrate diets (caveat-terrestrial invertebrates)

Terrestrial Plants

Navarro et al. (2008) Ecotoxicology, 17:372–386



Which NP is toxic?

- Target organs and pathologies are similar for different types of NPs.
- Even similar to other chemicals
- Novel/unique effects-brain injury from CNT
- Measurement of metal content of tissue for metal NPs.
- Tissue detection of carbon-based NPs?
- Not an NP: “delivery vehicle” effect for other contaminants (Baun et al.).

Conclusions

- Histopathology identifies the gill/lung, liver, spleen, brain and intestine as target organs for nanomaterials in animals.
- Not easy to differentiate effects of the different NPs.
- NP effects similar to metals/other organic chemicals.
- Measuring accumulated dose is problematic.
- Biomonitoring on the basis of biological effect, but cannot identify the specific contaminant with this approach alone.
- Big knowledge gaps on birds, amphibians, small mammals and plants.

Any Questions?