A Weight of Evidence Review of the Carcinogenicity of TiO_2

Samuel M. Cohen, M.D., Ph.D.
Havlik-Wall Professor of Oncology
Department of Pathology and Microbiology and Buffett Cancer Center
University of Nebraska Medical center
Omaha, NE 68198-3135, USA
scohen@unmc.edu

PQRI Workshop: TiO_2 Use in Pharmaceuticals-Global Regulatory and Technical Challenges
June 13, 2023
Bethesda, MD

University of Nebraska Medical Center

Disclosure

Research (Blevins et al., 2019) partially funded by TDMA

Outline

- Form of TiO_2
- Route of Administration – Oral vs. Inhalation
- NTP Two Year Bioassay
- Short-Term Studies Regarding Colon
- Conclusions

Forms of TiO_2

Forms: anatase, rutile, brookite

Dietary E171: may be either anatase or rutile form
17-45% nanomaterials, or less

Dietary Unitane 0-220: Anatase form used in NTP bioassay, similar to E171

Inhalation form used in bioassay (Heinrich et al., Inhalation Toxicology, 1995):
80% anatase/20% rutile, 15-40 nm primary particle size

Routes of Administration

Intraperitoneal: not relevant to human exposure

Inhalation: toxicity in rats related to particle effect with inflammation; chronic bioassay in female rats used nanomaterial

Oral:

- Gavage and drinking water: solubility issue; agglomeration
- Dietary: only appropriate route for evaluating dietary exposure; stable

NCI Cancer Bioassay (1979)

- F344 rats, B6C3F1 mice, both sexes
- Treated for 103 weeks followed by 1 week of control diet
- Used Unitane 0-220 TiO_2 – anatase form
  - Similar to E171
- Administered in diet, mixed fresh weekly
- Administered at concentrations of 0, 2.5, and 5.0% (0, 25000, and 50000 ppm)
  - 5.0% is maximum concentration allowed for long term dietary studies of diet
  - 14 day study showed no toxicity up to 10% of diet

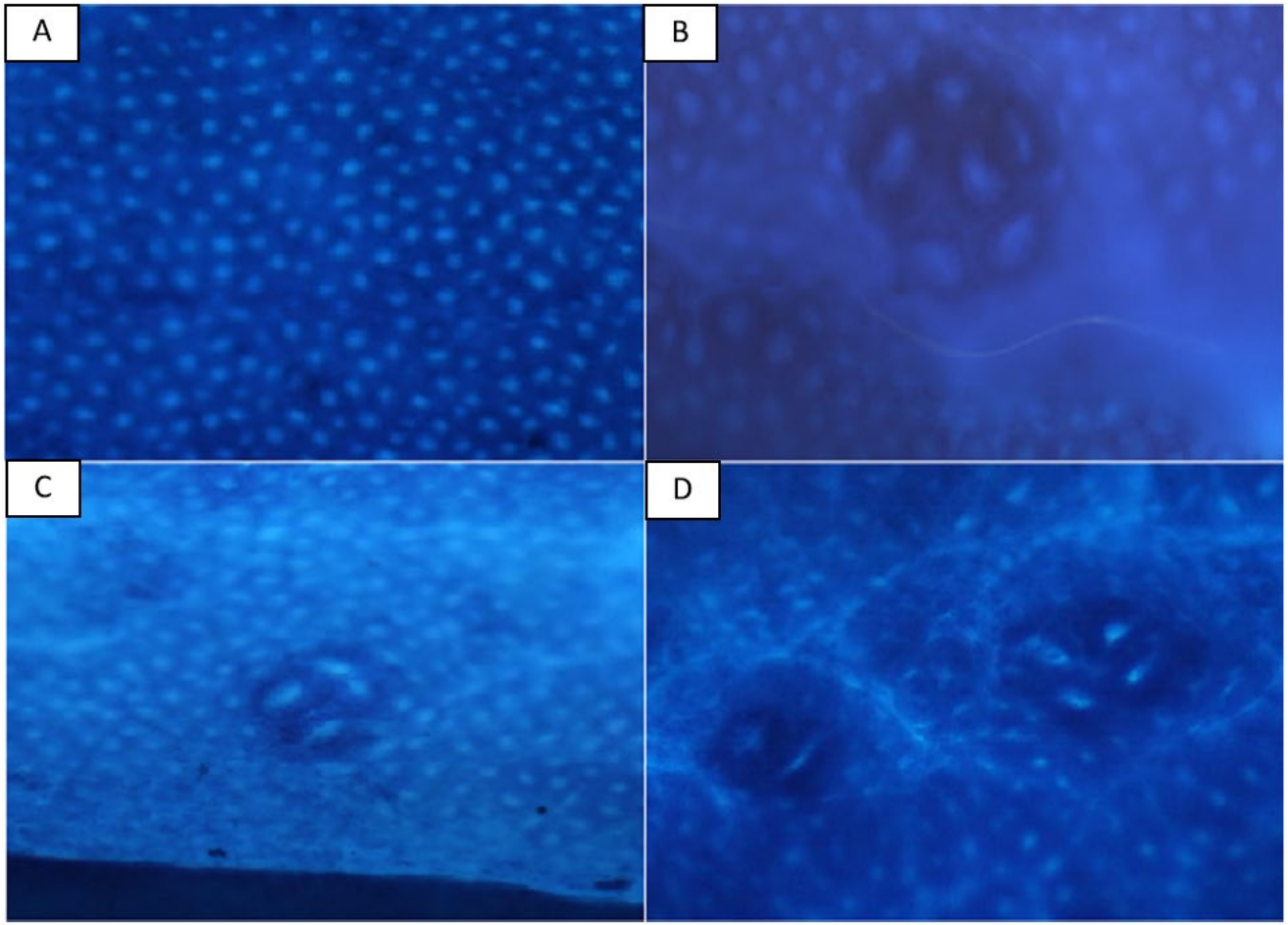
NTP Cancer Bioassay – Results

- No effect on survival
- No effect on weight gain
- No increased incidences of any tumors above historical controls
- No increased evidence of non-neoplasic effects above historical controls
- Specifically, no increased incidences of preneoplastic changes such as hyperplasia

Aberrant Crypt Foci

- Used as a precursor for colon cancer in rodents
- Evidence is contradictory
- No good historical control database
- Large foci not well defined
- Dysplastic foci not well defined

Aberrant Crypt Foci



Shwter et al., J. Ethnopharmacology 193: 195-206, 2016

Bettini et al.: Scientific Reports, 2017

- Claimed increased ACF with E171 and immune effects
- Dose: 0.2 or 10 mg/kg., drinking water, 100 days
- Issues:
  - Administered in drinking water (insoluble)
  - Administered after DMH (potent genotoxic colon carcinogen)
  - No groups without DMH
  - No historical range for ACF with DMH
    - Highly variable
  - No standardization of tissue sample selection
  - Results not evaluated blinded

Urrutia – Ortega et al.: Food and Chemical Toxicology, 2016

- Colitis associated colon cancer model in mice: wide variation in tumor size and number
- Colitis by 2% dextran sulfate sodium in water, plus azoxymethane (AOM) i.p.
- E171 administered by gavage in water, 5 mg/kg.
- 11 weeks on study
- Tumors assessed by gross measurement and number
- Assessment of results not blinded
- 6 mice per group
- Goblet cell assessment: sample not standardized nor controlled for tangential sectioning

Urrutia – Ortega et al.: Results

- No tumors with control or E171 alone
- 7 tumors with colitis treatment and 21 tumors by colitis + E171
- Decreased goblet cells with E171 treatment

Blevins et al.: Food & Chemical Toxicology, 2019

Attempt to replicate Bettini et al.

- Administered in diet instead of drinking water
- More doses of E171 (0, 40, 400, 5000 ppm, approximately 1, 3, 20, 250 µg/kg (measured)
- Groups with and without pretreatment with DMH
- Standardized tissue sampling
- All evaluations blinded
- Entire colon not examined for ACF so that immune evaluation could also be performed

Blevins et al.: Results

Group	Treatment	Total cm2 Evaluated	Mean No. of ACF/cm2,[a]	Mean No. of ABC/cm2,[a]
1	0 ppm E 171	25.4	0.8 ± 0.5	1.9 ± 1.1
2	40 ppm E 171	19.7	0.1 ± 0.1	0.2 ± 0.2
3	400 ppm E 171	27.6	0.9 ± 0.4	2.1 ± 1.1
4	5000 ppm E 171	23.9	0.9 ± 0.5	2.7 ± 1.6
5	180 mg/kg DMH · 2HCl	24.2	5.4 ± 1.2[b]	17.1 ± 4.1[b]
6	180 mg/kg DMH · 2HCl + 40 ppm E 171	27.3	5.3 ± 1.3[c]	14.8 ± 3.9[c]
7	180 mg/kg DMH · 2HCl + 400 ppm E 171	30.4	7.2 ± 1.3[d]	19.7 ± 3.6[d]
8	180 mg/kg DMH · 2HCl + 5000 ppm E 171	26.9	10.1 ± 2.6[e]	28.4 ± 6.6[e]

Blevins et al.: Results

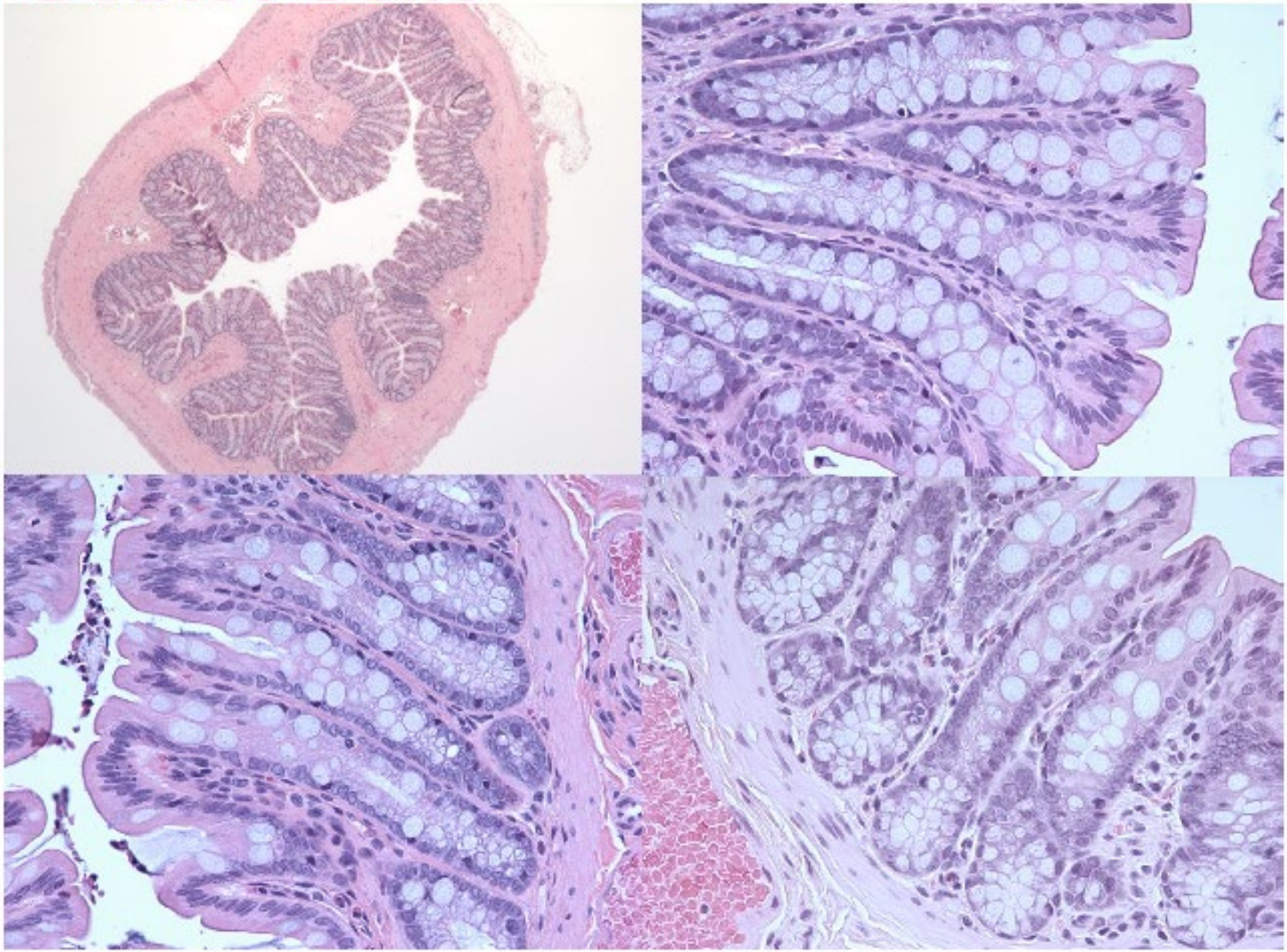
Colon Tumors

- DMH + 0 ppm: 1 rat with 2 invasive adenocarcinomas
- DMH + 40 ppm: 1 rat with 1 adenoma
- DMH + 400 ppm: 1 rat with 1 adenoma

Blevins et al.: Goblet cells

Group	Treatment	Mean Length of Gland (units)[a,b]	Mean No. of Goblet Cells/Unit[b]
1	0 ppm E 171	4.6 ± 0.1	6.5 ± 0.5
2	40 ppm E 171	5.2 ± 0.2	5.9 ± 0.2
3	400 ppm E 171	4.9 ± 0.3	6.4 ± 0.3
4	5000 ppm E 171	5.3 ± 0.2	6.2 ± 0.3
5	180 mg/kg DMH · 2HCl	5.5 ± 0.3[c]	6.2 ± 0.4
6	180 mg/kg DMH · 2HCl + 40 ppm E 171	5.0 ± 0.2	5.9 ± 0.4
7	180 mg/kg DMH · 2HCl + 400 ppm E 171	5.3 ± 0.3	6.0 ± 0.4
8	180 mg/kg DMH · 2HCl + 5000 ppm E 171	5.5 ± 0.2	6.2 ± 0.2

Goblet Cells



Bischoff et al.: Nanomaterials, 2022

- Transgenic mouse intestinal cancer model
- NCI recommends not using this model for carcinogenesis evaluation
- Marked variability in tumor incidences, benign tumors
- E171 administered in drinking water or by gavage (in water)
- Transcriptomic analysis faulty
- **No statistically significant findings**
- Critique in Letter to the Editor by Kaminski and Cohen, 2023

EOGRT Reproductive Study

- Extend one generation reproductive study
- Performed under GLP; 10 males and 10 females/group
- Dietary E171 at 0, 100, 300, and 100 mg/kg for 122 days
- ACF evaluated in FO rats
- No definite ACF; 7 rats with minimally increased variability in crypt sizes; in all groups

Akagi et al., Particle and Fibre Toxicology, in press

Oral Toxicological Study of Titanium Dioxide Nanoparticles with a Crystallite Diameter of 6 nm in Rats

- 28 and 90 day dietary studies in rats
  - 28 day: 0, 10, 100, 1000 mg/kg bw/day
  - 90 day: 0, 100, 300, 1000 mg/kg bw/day
- No treatment related adverse effects on survival, body weight, hematology, urinalysis, serum chemistries, organ weights

Akagi et al., Particle and Fibre Toxicology, in press (continued)

- Particles observed in GI lumen and in nasal cavity epithelium and stroma
- Particles in Peyer's patches, cervical and mediastinal lymph nodes, and bronchus-associated lymphoid tissue
- No inflammation around particles
- Colon: no increase in proliferation and no increase of β-catinin expression nor nuclear/cytoplasmic translocation
- Negative *in vivo* genotoxicity: MN or γ-H2AX in hepatocytes

Human Carcinogenesis

- DNA reactivity (genotoxicity)
- Immunosuppression
- Estrogenic activity
- Cytotoxicity and regenerative proliferation
- E171 has none of these properties

Conclusions

- NTP two-year bioassay negative for increased neoplastic or preneoplastic lesions in rats and mice at doses up to 5% of diet.
- No credible evidence for an effect on altered crypt foci in short-term assays
- E171 is not carcinogenic when administered orally