NTP
National Toxicology Program

Draft NTP Technical Report TR 579 on *N,N*-Dimethyl-*p*-toluidine (DMPT)

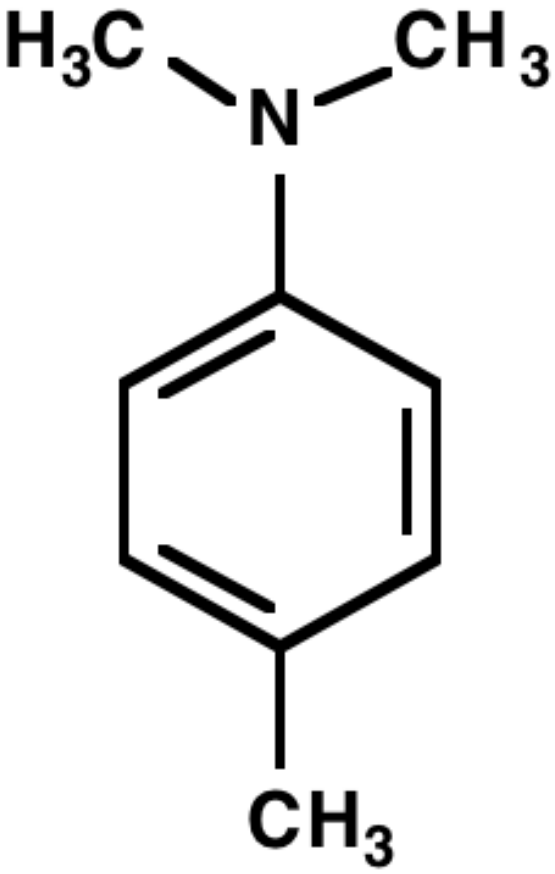
June Dunnick, Ph.D., DABT
National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
February 8-9, 2012

Chemical formula: $C_9H_{13}N$

Molecular weight: 135.21

CAS Number: 99-97-8



N,*N*-Dimethyl-*p*-toluidine (DMPT)

DMPT – in medical devices

- DMPT is a high production chemical with potential for widespread human exposure - used in dental materials and bone cements
  - DMPT accelerator in the redox initiator-accelerator system used to cure methyl methacrylate monomers
  - Polymerization is rarely complete
  - DMPT retained in bone cements and dental materials with potential for exposure to surgical staff, dental prosthetic device manufacturers, and users of DMPT containing medical devices

DMPT – NTP genetic toxicity test results

- Bacterial mutagenicity test results
  - Negative in all strains tested, with and without activation
- Peripheral blood micronucleus test in B6C3F1/N mice
  - 13-week exposure in males and females: negative
  - 4-day exposure in males: negative
- DNA damage (Comet assay) in liver – 4-day exposures
  - Negative in male B6C3F1/N male mice
  - Positive in male Sprague Dawley rats

Overview of DMPT Subchronic and Chronic studies in F344/N rats and B6C3F1/N mice – (oral gavage corn oil)

- **Clinical signs:**
  - Cyanosis and abnormal breathing in subchronic studies
- **Hematologic toxicity:**
  - ↑ Methemoglobin levels
  - Macrocytic, hypochromic, responsive Heinz body anemia
- **Target organ toxicity:**
  - Liver, nasal cavity, hematopoietic system
- **Target organ tumor findings (chronic study):**
  - Liver: male and female rats and mice
  - Nasal cavity: male and female rats
  - Thyroid: male rats
  - Lung & Forestomach: female mice

DMPT – Methemoglobin (% of total hemoglobin) at 3 months

Subchronic Studies

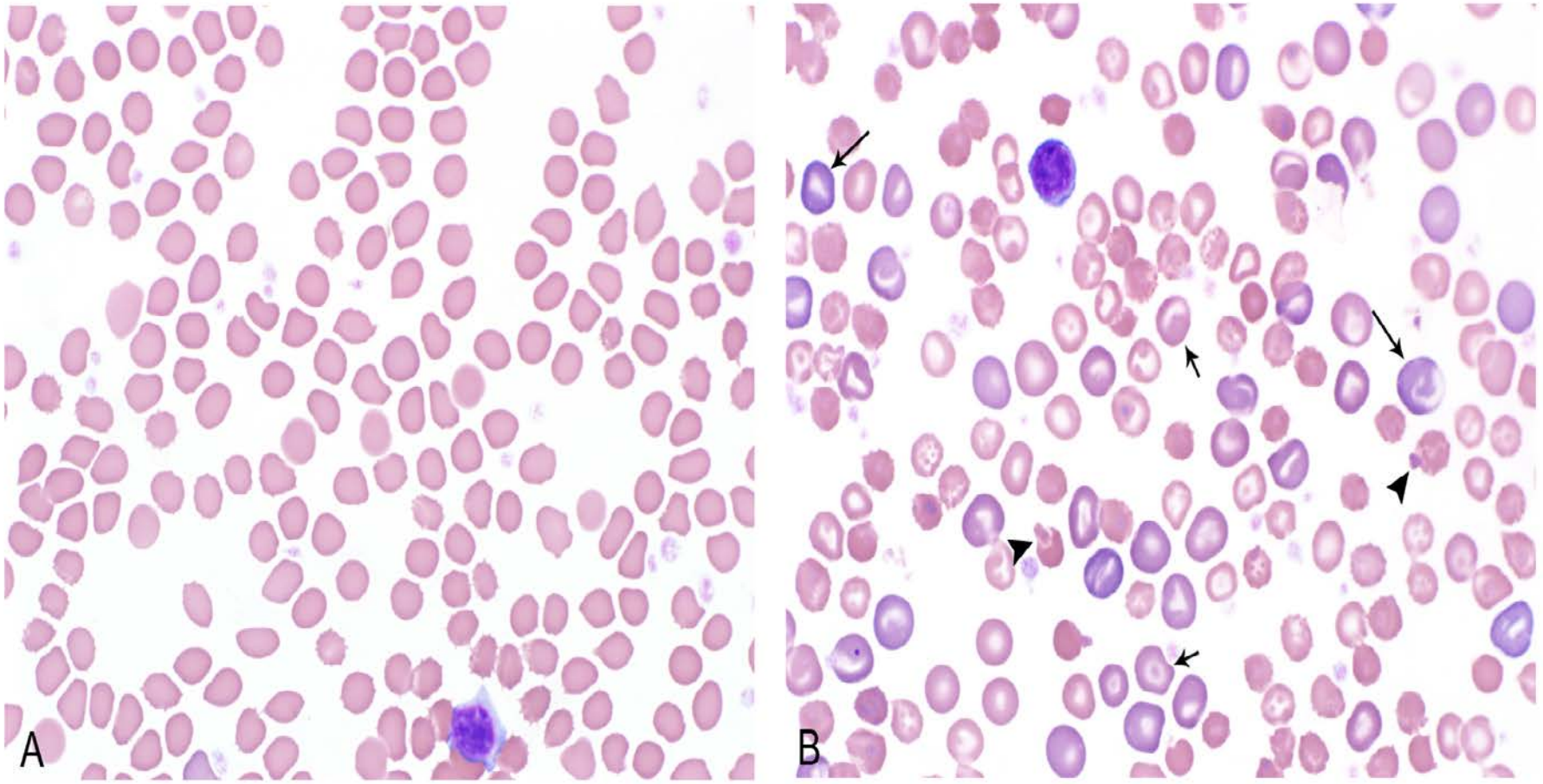
F344/N rats	0	62.5	125	250	500 mg/kg
Males	2.4	10.1**	15.5**	18.2**	17.7**
Females	2.9	11.2**	17.2**	19.7**	16.0**

B6C3F1/N mice	0	15	30	60	125 mg/kg
Males	2.1	2.5	2.8**	3.1**	4.0**
Females	2.1	2.2	2.6*	3.4**	3.9**

Chronic Study

F344/N rats	0	6	20	60 mg/kg
Males	4.7	5.6*	7.9**	17.4**
Females	5.1	5.6	8.4**	17.1**

*p ≤ 0.05; **p ≤ 0.01



Blood smear - Control (A) and High-dose DMPT (B) male F344/N rats - 3 months

Chronic Studies

- F344/N rats – 0, 6, 20, 60 mg/kg
- B6C3F1/N mice – 0, 6, 20, 60 mg/kg

DMPT Chronic Study
Survival and Body Weights in Rats

Dose mg/kg	0	6	20	60
Male rats Terminal sac	37**	37	31	21**
Body weights (% controls)	-	103%	95%	82%
Female rats Terminal sac	33**	42*	33	23
Body weights (% controls)	-	104%	98%	82%

*p ≤ 0.05; **p ≤ 0.01; N = 50

DMPT Chronic Study
Survival and Body Weights in Mice

Dose mg/kg	0	6	20	60
Male mice Terminal sac	34	36	31	36
Body weights (% controls)	-	97%	91%	84%
Female mice Terminal sac	43*	40	39	32*
Body weights (% controls)	-	97%	102%	72%

*p ≤ 0.05; N = 50

DMPT Chronic Study - Selected Nonneoplastic Lesions Liver

Dose mg/kg	0	6	30	60
Liver – Hepatocellular Hypertrophy Male rats	0	0	6*	31**
Female rats	0	0	6*	22**
Male mice	1	9**	11**	16**
Female mice	0	11**	10**	17**

*p ≤ 0.05; **p ≤ 0.01; N = 49-50

DMPT Chronic Study
Neoplastic Lesions – Liver, Male Rats

Dose mg/kg	0	6	20	60
Hepatocellular Adenoma	0	0	1 (2%)	1 (2%)
Hepatocellular Carcinoma[a]	0**	0	1 (2%)	6** (12%)
Hepatocellular Adenoma or Carcinoma[b]	0**	0	2 (4%)	6** (12%)

**p ≤ 0.01; N = 50

Historical controls, gavage corn oil
[a]0/299
[b]3/299 (1.0% ± 1.1%), range 0%-2%

Historical controls, all routes
[a]5/1249 (0.4% ± 1.0%), range 0%-4%
[b]23/1249 (1.8% ± 1.9%), range 0%-6%

DMPT Chronic Study
Neoplastic Lesions – Liver, Female Rats

Dose mg/kg	0	6	20	60
Hepatocellular Adenoma[a]	0*	1 (2%)	1 (2%)	3 (6%)
Hepatocellular Carcinoma (includes multiple)[b]	0**	0	0	4* (8%)
Hepatocellular Adenoma or Carcinoma[c]	0**	1 (2%)	1 (2%)	7** (14%)

*p ≤ 0.05; **p ≤ 0.01; N = 49-50

Historical controls, gavage corn oil
[a]1/300 (0.3% ± 0.8%), range 0% -2%
[b]0/300
[c]1/300 (0.3% ± 0.8%), range 0% -2%

Historical controls. all routes
[a]11/1200 (0.9% ± 1.6%), range 0%-4%
[b]1/1200 (0.1% ± 0.4%), range 0%-2%
[c]12/1200 (1.0% ± 1.6%), range 0%-4%

DMPT Chronic Study - Neoplastic Lesions – Liver, Male Mice

Dose mg/kg	0	6	20	60
Hepatocellular Adenoma, Multiple	17 (34%)	19 (38%)	27* (54%)	26* (52%)
Hepatocellular Carcinoma (includes multiple)[a]	22** (44%)	25 (50%)	30 (60%)	36** (72%)
Hepatocellular Adenoma or Carcinoma[b]	38** (76%)	44 (88%)	47** (94%)	48** (96%)
Hepatoblastoma (includes multiple)[c]	1 (2%)	5 (10%)	10** (20%)	8* (16%)
Hepatocellular Adenoma or Carcinoma or Hepatoblastoma[d]	38** (76%)	45 (90%)	48** (96%)	48** (96%)

*p ≤ 0.05; **p ≤ 0.01; N = 50

Historical controls, gavage corn oil

[a]116/350 (33.1% ± 10.5%), range 16%-44%
[b]239/350 (68.3% ± 8.9%), range 56%-78%
[c]14/350 (4.0% ± 2.8%), range 0%- 8%
[d]242/350 (69.1% ± 8.0%), range 58%-78%

Historical controls, all routes

[a]339/1149(34.7% ± 10.8%), range 16%-56%
[b]844/1149 (73.5% ± 11.3%), range 52%-90%
[c]61/1149 (5.3% ± 7.1%), range 0%-34%
[d]852/1149 (74.2% ± 11.5), range 52%-92%

DMPT Chronic Study
Neoplastic Lesions – Liver, Female Mice

Dose mg/kg	0	6	20	60
Hepatocellular Adenoma (includes multiple)[a]	17** (34%)	19 (38%)	37** (74%)	44** (88%)
Hepatocellular Carcinoma (includes multiple)[b]	6** (12%)	13* (26%)	18** (36%)	31** (62%)
Hepatocellular Adenoma or Carcinoma[c]	20** (40%)	25 (50%)	42** (84%)	45** (90%)
Hepatoblastoma[d]	0**	1 (2%)	0	4* (8%)
Hepatocellular Adenoma or Carcinoma or Hepatoblastoma[e]	20** (40%)	26 (52%)	42** (84%)	45** (90%)

*p ≤ 0.05; **p ≤ 0.01 N = 50

Historical controls, gavage corn oil

[a]75/347 (21.6% ± 10.8%), range 6%-34%
[b]29/347 (8.3% ± 5.5%), range 2%-18%
[c]91/347 (26.2% ± 12.7%), range 8%- 40%
[d]1/347 (0.3% ± 0.8%), range 0%-2%
[e]91/347 (26.2% ± 12.7%), range 8%-40%

Historical controls, all routes

[a]380/1195(31.8% ± 21.4%), range 2%-78%
[b]144/1195 (12.1% ± 10.8%), range 0%-46%
[c]444/1195 (37.2% ± 22.9%), range 6%-82%
[d]4/1195 (0.3% ± 0.8), range 0%-2%
[e]444/1195 (37.2% ± 22.9), range 6%-82%

DMPT Chronic Study – Selected Nonneoplastic Lesions Nasal Cavity

Dose mg/kg	0	6	30	60
Nasal Cavity **Male rats** Transitional Epithelium - Hyperplasia	1	1	11**	46**
Female rats Transitional Epithelium - Hyperplasia	0	1	6*	33*
Male mice Olfactory Epithelium – Metaplasia, respiratory	10	10	5	49**
Female mice Olfactory Epithelium – Metaplasia, respiratory	1	6*	14**	46**

*p ≤ 0.05; **p ≤ 0.01; N = 49-50

DMPT Chronic Study
Neoplastic Lesions – Nose, Male Rats

Dose mg/kg	0	6	20	60
Transitional Epithelium - Adenoma[a]	0**	3 (6%)	2 (4%)	11** (22%)
Transitional Epithelium - Carcinoma	0*	0	0	2 (4%)
Transitional Epithelium - Adenoma or Carcinoma	0**	3 (6%)	2 (4%)	13** (27%)
Glands, Olfactory Epithelium - Adenoma	0	0	0	1 (2%)

*p ≤ 0.05; **p ≤ 0.01; N = 49-50

Historical controls, gavage corn oil
[a]0/299

Historical controls, all routes
[a]0/1248

DMPT Chronic Study
Neoplastic Lesions – Nose, Female Rats

Dose mg/kg	0	6	20	60
Transitional Epithelium - Adenoma[a]	0	1 (2%)	0	2 (4%)

N = 49-50

Historical controls, gavage corn oil
[a]0/299

Historical controls, all routes
[a]1/1196 (0.1% ± 0.4%), range 0% - 2%

DMPT Chronic Study
Neoplastic Lesions – Thyroid, Male Rats

Dose mg/kg	0	6	20	60
Follicular Cell - Adenoma[a]	1 (2%)	0	1 (2%)	3 (6%)
Follicular Cell - Carcinoma[b]	0	2 (4%)	1 (2%)	2 (4%)
Follicular Cell - Adenoma or Carcinoma[c]	1 (2%)	2 (4%)	2 (4%)	4 (8%)

N = 49-50

Historical controls, gavage corn oil
[a]6/299 (2.0% ± 1.3%), range 0%-4%
[b]3/299 (1.0% ± 1.7%), range 0%-4%
[c]9/299 (3.0% ± 2.1%), range 0%-6%

Historical controls, all routes
[a]13/1239 (1.0% ± 1.7%), range 0%-6%
[b]10/1239 (0.8% ± 1.5%), range 0%-4%
[c]23/1239 (1.9% ± 2.2%), range 0%-6%

DMPT Chronic Study – Selected Nonneoplastic lesions Lung, Female Mice

Dose mg/kg	0	6	20	60
Female mice - lung				
Alveolar Epithelium - Hyperplasia	2	3	8*	2
Alveolus – Infiltration Cellular, Histiocyte	1	0	0	7*
Bronchiole, Epithelium - Regeneration	0	0	0	5*
Bronchus, Epithelium - Regeneration	0	0	0	5*
Bronchus, Necrosis	0	0	0	5*

*p ≤ 0.05; N = 50

DMPT Chronic Study
Neoplastic Lesions – Lung, Female Mice

Dose mg/kg	0	6	20	60
Alveolar/bronchiolar Adenoma[a] (includes multiple)	2** (4%)	4 (8%)	8* (16%)	12** (24%)
Alveolar/bronchiolar Carcinoma[b]	0	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar Adenoma or Carcinoma[c]	2** (4%)	5 (10%)	9* (18%)	13** (26%)

*p ≤ 0.05; **p ≤ 0.01; N = 50

Historical controls, gavage corn oil
[a]16/346 (4.6% ± 3.1%), range 0%-8%
[b]7/346 (2.0% ± 2.0%), range 0%-4%
[c]23/346 (6.7% ± 3.2%), range 2%-12%

Historical controls, all routes
[a]60/1196(5.0% ± 3.6%), range 0%-12%
[b]44/1196 (3.7% ± 3.3%), range 0%-14%
[c]100/1196 (8.4% ± 4.3%, range 2%-22%

DMPT Chronic Study
Neoplastic Lesions – Forestomach, Female Mice

Dose mg/kg	0	6	20	60
Epithelium, Hyperplasia	3 (6%)	5 (10%)	12** (24%)	17** (34%)
Squamous Cell Papilloma (includes multiple)[a]	1* (2%)	5 (10%)	6* (12%)	7* (14%)
Squamous Cell Carcinoma[b]	0	1 (2%)	0	0
Squamous Cell Papilloma or Carcinoma[c]	1 (2%)	6 (12%)	6* (12%)	7* (14%)

*p ≤ 0.05; **p ≤ 0.01; N = 50

Historical controls, gavage corn oil
[a]12/348 (3.5% ± 1.5%), range 2%-6%
[b]0/348
[c]12/348 (3.5% ± 1.5%), range 2%-6%

Historical controls, all routes
[a]22/1198(1.8% ± 1.7%), range 0%-6%
[b]1/1198 (0.1% ± 0.4%), range 0%-2%
[c]23/1198 (1.9% ± 1.6%, range 0%-6%

DMPT Chronic Study – Selected Nonneoplastic lesions Hematopoietic system – Spleen, Bone marrow, Lymph Node

Dose mg/kg	0	6	30	60
Hematopoietic system				
Male rats				
Spleen Capsule - Hypertrophy, mesothelium	0	1	3	39**
Bone Marrow - Hyperplasia	17	13	28**	50**
Lymph Node - Infiltration Cellular, Histiocyte (mesenteric)	21	23	30*	34**
Female rats				
Spleen Capsule - Hypertrophy, Mesothelium	1	14**	10**	16**
Bone Marrow - Hyperplasia	18	13	18	49**
Female mice				
Bone Marrow - Hyperplasia	5	14*	15**	14**
Lymph Node - Atrophy (mesenteric)	1	5	5	12*

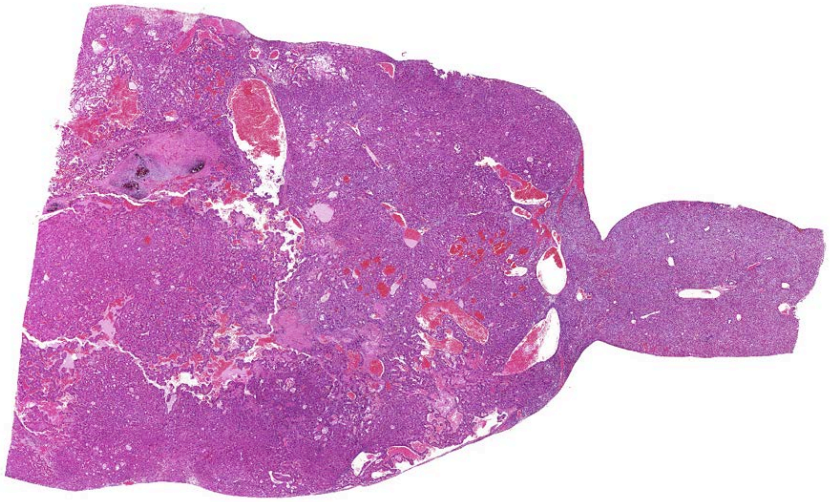
*p ≤ 0.05; **p ≤ 0.01; N = 49-50

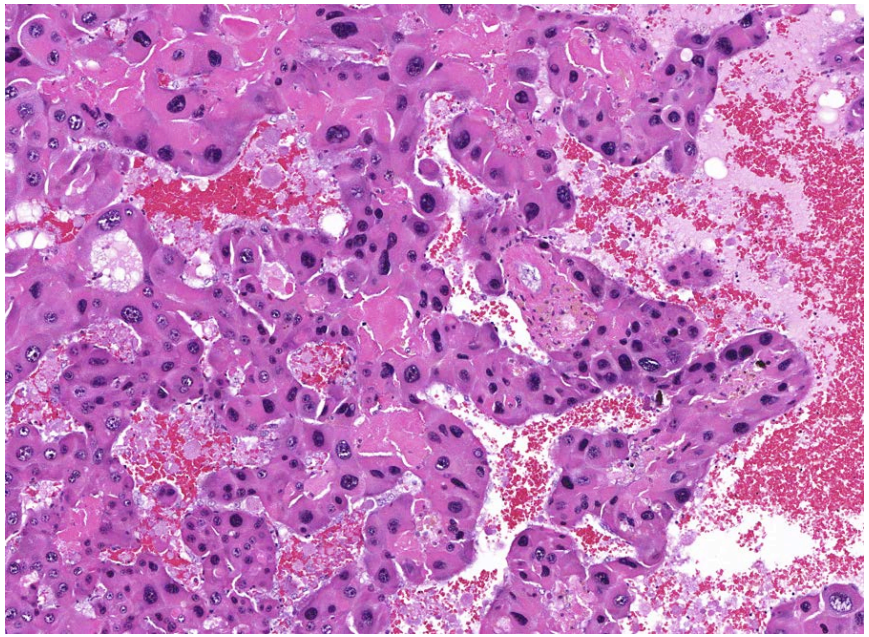
DMPT Chronic Study – Selected Nonneoplastic lesions Kidney

Dose mg/kg	0	6	20	60
Male rats Nephropathy	49 [1.4]	49 [2.0]	48 [2.5]	49 [2.7]
Pigmentation	24 [1.2]	46** [1.0]	37** [1.2]	44** [1.6]
Female Rats Nephropathy	28 [1.1]	38* [1.2]	38* [1.2]	41** [1.8]
Pigmentation	41 [1.0]	45 [1.0]	43 [1.0]	49** [1.4]

*p ≤ 0.05; **p ≤ 0.01; N = 49-50

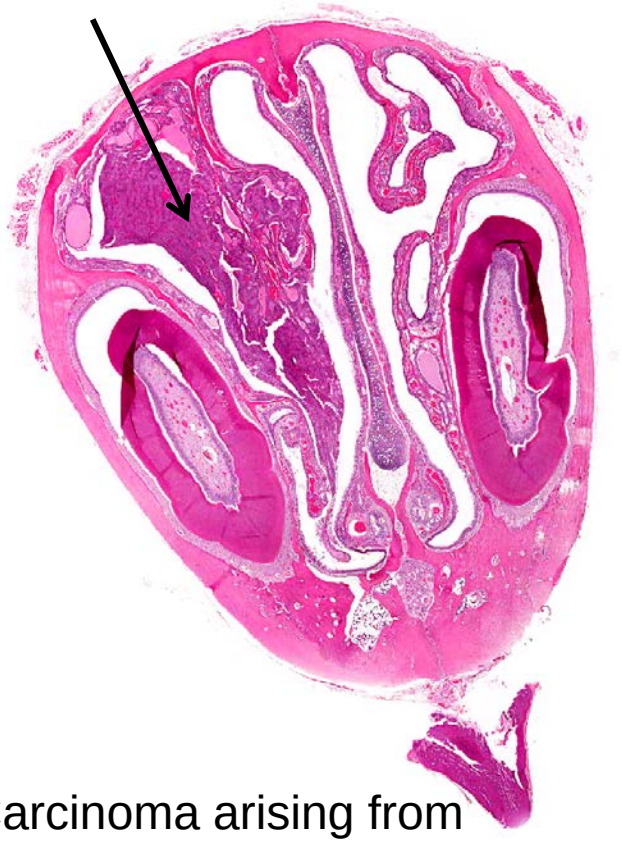
DMPT Chronic Study – Liver - Hepatocellular Carcinoma from a High Dose Male Rat



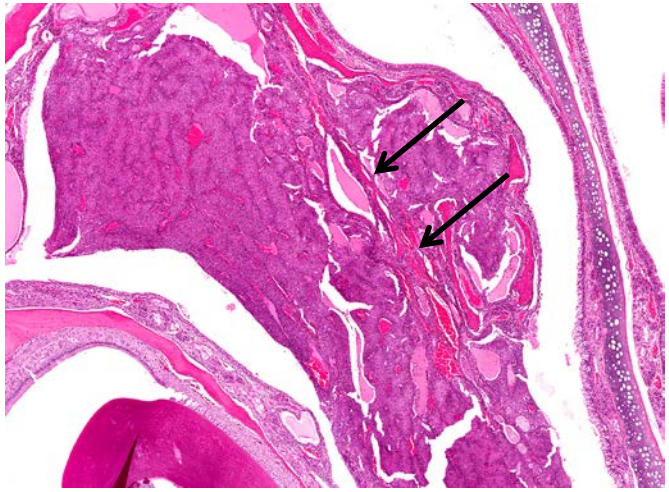


Thickened, irregular trabeculae with loss of the normal hepatic architecture and cells displaying pleomorphism and anisokaryosis
(variation in nuclear size among cells)

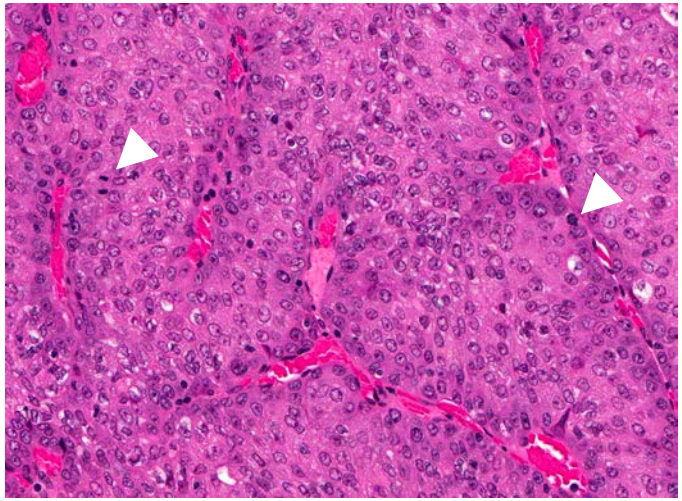
DMPT Chronic Study – Nose -Transitional Epithelial Carcinoma from a High Dose Male Rat



Carcinoma arising from the transitional epithelium on the turbinate in Level I nasal section (H&E, 0.9X).



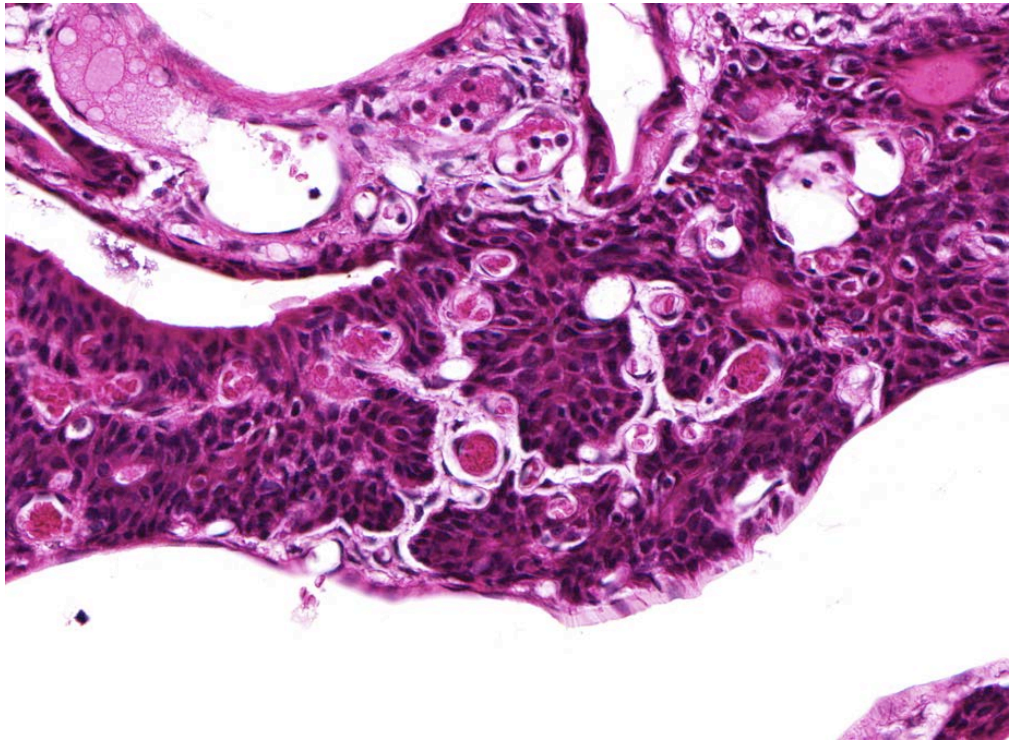
The tumor is invading through the bone of the turbinate (arrows) (H&E, 4X).



Higher magnification shows cells in division (arrowheads) (H&E, 40X).

DMPT Chronic Study – Transitional Epithelial Adenoma from a High Dose Male Rat

Adenoma arising from the transitional epithelium on the lateral wall in Level I nasal section (H&E, 0.9X).



In contrast to the carcinoma seen previously, there is no invasion into the underlying tissue and the cells are more differentiated (H&E, 40X).

DMPT Conclusions - Rats

- Male F344/N rat
  - Clear evidence of carcinogenic activity
    - Hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined)
    - Nasal cavity neoplasms (primarily nasal cavity transitional epithelium adenoma)
      - Thyroid gland follicular cell neoplasms may have been related to treatment
- Female F344/N rat
  - Clear evidence of carcinogenic activity
    - Hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined)
      - Nasal cavity transitional epithelium adenomas were considered to be related to treatment

DMPT Conclusions - Mice

- Male B6C3F1/N mice
  - Clear evidence of carcinogenic activity
    - Hepatocellular adenoma (multiple), hepatocellular carcinoma, and hepatoblastoma
- Female B6C3F1/N mice
  - Clear evidence of carcinogenic activity
    - Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma
    - Alveolar/bronchiolar neoplasms (primarily adenoma)
      - Forestomach squamous cell papillomas were considered to be related to treatment

DMPT conclusions – nonneoplastic effects

- **Liver and Nasal cavity** in male and female rats and mice
- **Spleen and/or Bone marrow** in male and female rats and female mice
- **Kidney** in male and female rats
- **Lung** in female mice
- **Forestomach** in male rats and female mice
- **Mesenteric lymph nod**e in male rats and female mice
- **Olfactory lobe** in male and female mice.
- **Hematologic toxicity** and increases in methemoglobin levels in male and female rats and mice (as measured at 3 months)