Evidence on the Carcinogenicity of Dibenzanthracenes (DBAs)

Feng C. Tsai, PhD
Jennifer C.Y. Hsieh, PhD
Gwen Osborne, MD, MPH

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Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

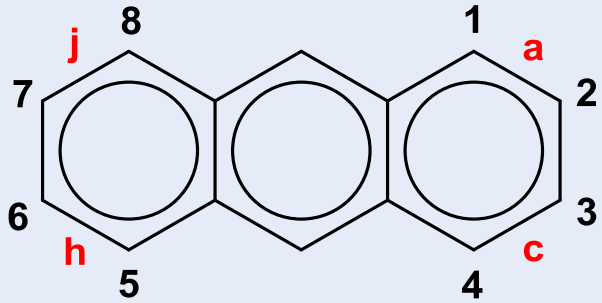
Office of Environmental Health Hazard Assessment

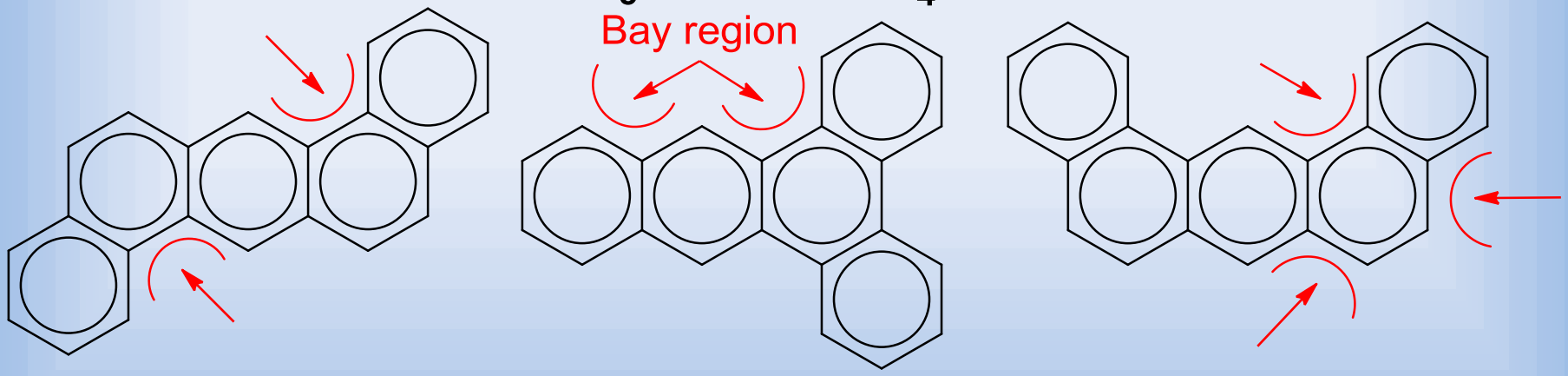
Overview

- Introduction
- Carcinogenicity evidence
  - Animal cancer bioassays
  - Initiation-promotion studies on mouse skin
  - Other relevant data
- Possible carcinogenic mechanisms
- Summary

Dibenzanthracenes (DBAs)

- Chemical formula: $C_{22}H_{14}$ (m.w.: 278.35); lipophilic; five-ring PAHs
- Three isomers; "anthracene" core





DB[*a,h*]A **DB[*a,c*]A** **DB[*a,j*]A**

Reviews by Other Agencies

- **DB[*a,h*]A**
  - Proposition 65 carcinogen (1988)
  - NTP (1981): "*Reasonably anticipated to be a human carcinogen*"
  - IARC (2010): Group 2A carcinogen, "*probably carcinogenic to humans*"
  - US EPA (1994): B2 carcinogen, "*probable human carcinogen*"
- **DB[*a,c*]A and DB[*a,j*]A**
  - IARC (2010): Group 3 chemicals, "*not classifiable as to its carcinogenicity to humans*"

Occurrence and Exposure

- Products of incomplete combustion or pyrolysis
  - Emissions: mobile and stationary sources, cooking, cigarette or marijuana smoke
  - Human exposure: contaminated air (indoor and outdoor), water, soil, food (fresh or cooked)
- Use: research purposes
- Biomonitoring data of DBAs
  - Human (blood, placenta, breast milk)
  - Wildlife (sea turtle eggs)

Carcinogenicity Data

- **Human data**: none on the pure DBAs; many on carcinogenic PAH mixtures (*e.g.*, coke oven)
- **Animal cancer bioassays**:
  - **DB[*a,h*]A**: more than 50 bioassays, tested in both sexes of multiple species (*e.g.*, mouse, rat, hamster), and multiple routes (*e.g.*, dermal, oral, *s.c.*, *i.v.*, *i.p.*), induced a variety of tumors (*e.g.*, skin, lung, liver)
  - **DB[*a,c*]A**: nine mouse bioassays (three dermal, five *s.c.* injection and one *i.p*. injection)
  - **DB[*a,j*]A**: two mouse bioassays (one dermal and one *s.c.* injection)

DB[*a,c*]A: Three Dermal Bioassays

- Heidelberger *et al.* (1962):
  - 2X/wk (0.125 mg) for 56 wk on 15 female mice, unspecified strain
- Finzi *et al.* (1968):
  - 2 drops (0.3%) every 2 days for 25 wk on 20 male Swiss mice

DB[*a,c*]A: Dermal Bioassays, continued

- Lijinsky *et al.* (1970):
  - 2X/wk (85 µg) for 65 wk and observed for life on 30 female Swiss mice
  - Skin squamous cell carcinoma or carcinoma/papilloma combined (rare, < 1%)

Skin tumor type	Solvent control	DB[*a,c*]A (total dose: 11 mg)
Papilloma	0/14	1/16 (6%)
Squamous cell carcinoma	0/14	8/16** (50%)
Carcinoma & papilloma	0/14	9/16*** (56%)

** $p < 0.01$; *** $p < 0.001$

DB[*a,c*]A: Five *s.c.* Injection Bioassays

- Heidelberger *et al.* (1962):
  - Two injections of 1.6 mg DB[*a,c*]A to 15 female mice, unspecified strain
  - No tumors observed at 56 wk
- Van Duuren *et al.* (1968):
  - Single injection of 1 mg DB[*a,c*]A to 20 female ICR/Ha Swiss mice
  - No tumors observed at 65 wk
- Kouri *et al.* (1983):
  - Single injection of DB[*a,c*]A (either 150 or 300 µg) to three strains of mice, 30 male/female mice per dose group
  - At 12 months, one skin fibrosarcoma (rare) in both high affinity aryl hydrocarbon receptor (AhR) strains at high dose
  - Tumor incidences for the control groups not reported

DB[*a,c*]A: *i.p.* Injection Bioassay

- Von Tungeln *et al.* (1999): 3 injections (on days 1, 8, 15 of life) to 24 male $B6C3F_1$ mice, observed for 12 months; liver adenoma

Liver tumor	Vehicle controls (DMSO)	DB[*a,c*]A (dose: 111 µg)	Positive controls (6-Nitrochrysene)
Adenoma	2/24 (8%)	9/24* (38%)	5/22 (23%)
Carcinoma	1/24 (4%)	0/24 (0%)	20/22 (91%)

* $p < 0.05$

DB[*a,j*]A: Dermal and *s.c.* Injection Bioassays

Female Swiss mice (Lijinsky *et al.*, 1970)

- **Dermal application:** 2X/wk for 60-81 wk at two doses on 30 mice and observed for life; rare skin tumors (* $p < 0.05$; ** $p < 0.01$)

Skin tumor type	Solvent control	Low-dose DB[*a,j*]A (6.3 mg)	High-dose DB[*a,j*]A (9.4 mg)
Papilloma	0/14 (0%)	2/9 (22%)	2/20 (10%)
Squamous cell carcinoma	0/14* (0%)	2/9 (22%)	6/20* (30%)
Papilloma & carcinoma	0/14** (0%)	4/9* (44%)	8/20** (40%)

- **Single *s.c.* injection:** in 25 mice, observed for life; rare sarcoma

Skin tumor type	Solvent control	DB[*a,j*]A (0.4 mg)
Sarcoma	0/12 (0%)	3/15 (20%)

DB[*a,c*]A Initiation-Promotion Studies; study design	**Initiator** (promoter: TPA, unless otherwise specified)	**Result**
ICR/Ha Swiss, 65 weeks (Van Duuren *et al.*, 1968)	DB[*a,c*]A (croton resin)	-
ICR/Ha Swiss, 58 - 60 wk (Van Duuren *et al.*, 1970)	DB[*a,c*]A	+
CD-1, 35 wk (Scribner, 1973)	DB[*a,c*]A	+
SENCAR, 16 wk (Slaga *et al.*, 1980)	DB[*a,c*]A	+/-
	10,11-diol	-
	10,11-diol-12,13-epoxide	-
SENCAR, 28 wk (Scribner and Scribner, 1980)	DB[*a,c*]A	+/- (no control)
CD-1, 68 wk (Chouroulinkov *et al.*, 1983)	DB[*a,c*]A	+/-
	1,2-diol	+
	10,11-diol	+
	3,4-diol	+/-

Example of DB[*a,c*]A Initiation-Promotion Study

- Van Duuren *et al.* (1970)
  - DB[*a,c*]A (1 mg) followed by 56-58 wk TPA in female ICR/Ha Swiss mice
  - Significant increase of papilloma in "DB[*a,c*]A/TPA" compared to "TPA-only"

Treatment		Papilloma incidence	Carcinoma incidence
Initiator	Promoter		
DB[*a,c*]A	TPA	19/50**	4/50
None	TPA	1/20	0/20
DB[*a,c*]A	None	0/20	0/20

** $p < 0.01$

DB[*a,j*]A Initiation-Promotion Studies

Study design	Initiator (promoter: TPA)	Result
SENCAR, 19 weeks (DiGiovanni *et al.*, 1983)	DB[*a,j*]A	+
SENCAR, 22 weeks (Sawyer *et al.*, 1987; 1988)	DB[*a,j*]A	+
	3,4-diol	+
	3,4-diol-1,2-epoxide	+
SENCAR, 16 weeks (Harvey *et al.*, 1988)	DB[*a,j*]A	+
	3,4-diol	+
	3,4-diol-1,2-epoxide	+
SENCAR, 18 weeks (Vulimiri *et al.*, 1999)	DB[*a,j*]A	+

Example of DB[*a,j*]A Initiation-Promotion Study

- Harvey *et al.* (1988)
  - Female SENCAR mice (24 per group)
  - 400 nmol (111 µg) DB[*a,j*]A or its metabolites; TPA (14 wk)

Initiator	Papillomas/mouse	Papilloma incidence
Vehicle (tetrahydrofuran)	0.05	1/24 (5%)
DB[*a,j*]A	0.58	7/24* (29%)
DB[*a,j*]A-3,4-diol	0.70	9/24** (38%)
DB[*a,j*]A-3,4-diol-1,2-epoxide	4.00	22/24*** (92%)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Genotoxicity

- **DB[*a,h*]A**:
  - Positive for bacterial DNA damage and gene mutation
  - Induced unscheduled DNA synthesis (UDS) and gene mutation in mammalian cells *in vitro*
  - Induced sister chromatid exchanges (SCEs) in rodents *in vivo*
- **DB[*a,c*]A and DB[*a,j*]A**:
  - Positive for bacterial DNA damage (DB[*a,c*]A only) and gene mutation
  - Positive in multiple *in vitro* and *in vivo* assays (see next slide)
- **Metabolites of three DBAs**:
  - Genotoxic, *e.g.*, diols or diol epoxides

Genotoxicity	DB[*a,c*]A		DB[*a,j*]A	
	Parent compound	Metabolites	Parent compound	Metabolites
Bacterial gene mutation / DNA damage	+	+	+	+
In vitro				
DNA binding/adducts	+	+	+	+
Unscheduled DNA Synthesis	+	Not tested (NT)	NT	NT
Forward mutation	+	NT	+	NT
Sister chromatid exchange	-	+	NT	NT
In vivo				
DNA binding/adducts (mice)	+	NT	+	+
Oncogene mutation (mice)	+	NT	+	+
Micronucleus (mice)	+	NT	NT	NT
Somatic mutation (*D. melanogaster*)	+	NT	NT	NT

Morphologic Changes

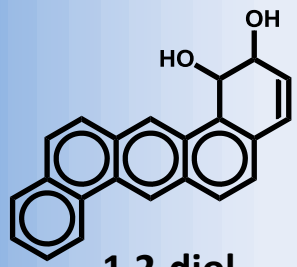
- *In vitro* cell transformation studies
  - DB[*a,h*]A: Positive
  - DB[*a,c*]A: Positive in three of four studies using hamster embryo cells
  - DB[*a,j*]A: Not studied
- *In vivo* preneoplastic changes in *s.c.* transplanted tracheas (rats)
  - DB[*a,c*]A: Positive (epithelial hyperplasia, squamous metaplasia) following exposure via intratracheal implant (Topping *et al*., 1978)

Pharmacokinetics and Metabolism

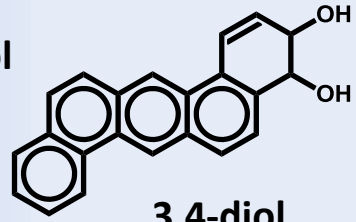
- Most ADME data from DB[*a,h*]A
- Absorbed slowly following dermal application and *s.c.* injection; absorbed within hours following gavage
- Rapidly distributed within the body (*e.g.*, GI, liver)
- Multiple enzyme-mediated metabolic pathways: CYP450s and epoxide hydrolase (EH)
- Similar metabolites identified for three isomers: diols and diol epoxides
- Eliminated within days, mainly in feces and urine

Some Metabolites of DBAs

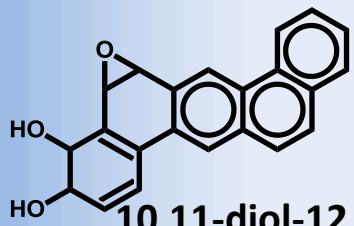
DB[*a,h*]A



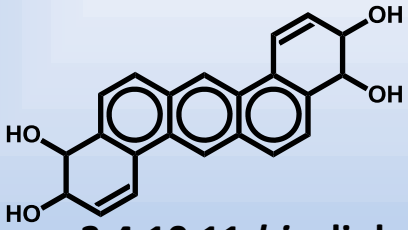
1,2-diol



3,4-diol

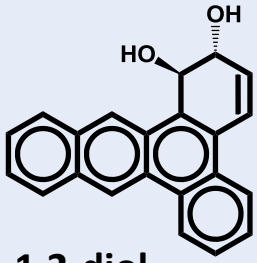


10,11-diol-12,13-epoxide

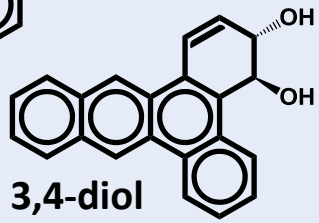


3,4:10,11-*bis*-diol

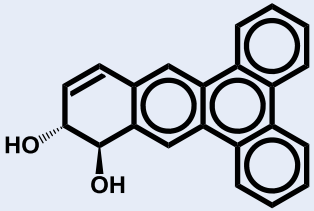
DB[*a,c*]A



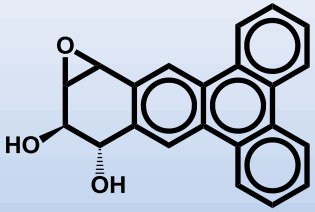
1,2-diol



3,4-diol

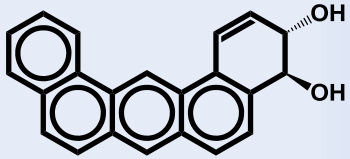


10,11-diol

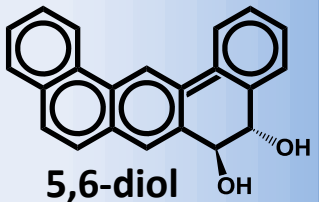


10,11-diol-12,13-epoxide

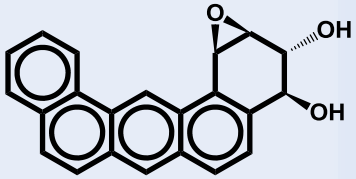
DB[*a,j*]A



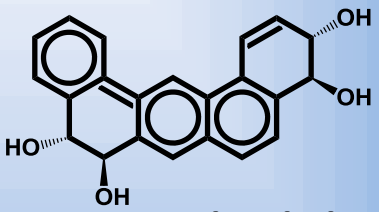
3,4-diol



5,6-diol

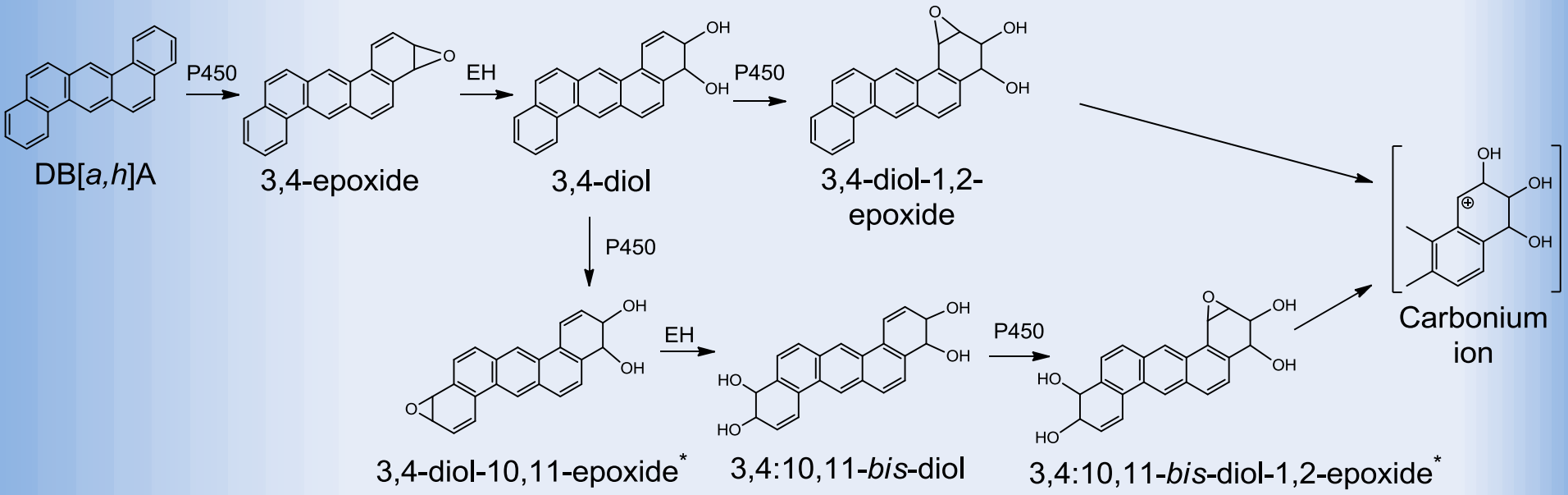


3,4-diol-1,2-epoxide



3,4:8,9-*bis*-diol

Some Metabolic Pathways of DB[*a,h*]A

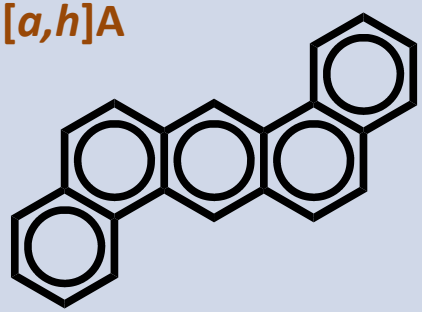
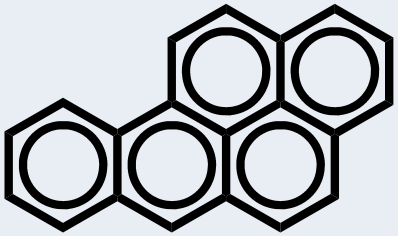
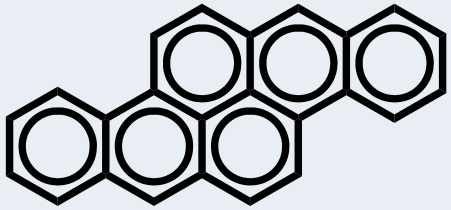
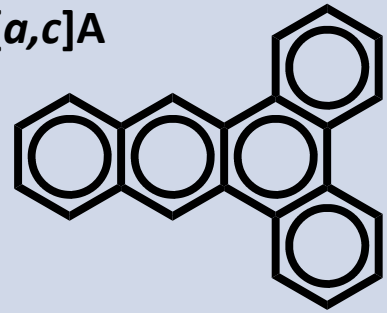
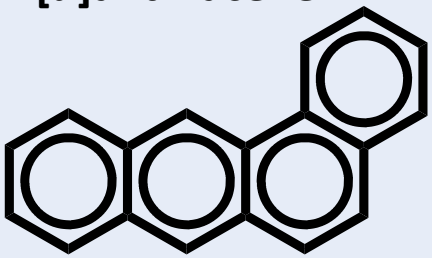
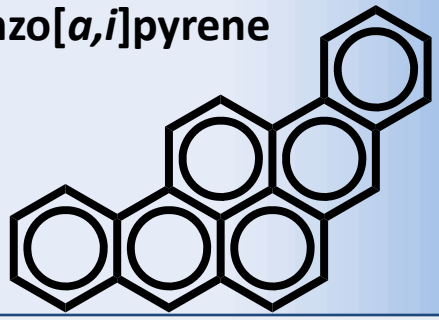
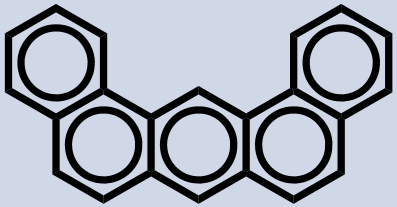
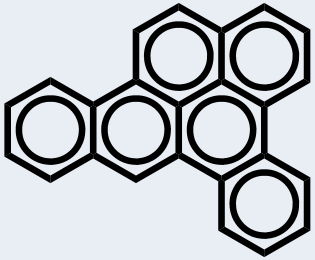
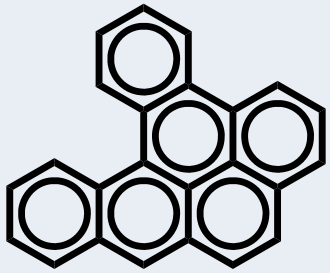


* proposed metabolites

Structure-Activity Comparisons

- Six structurally related non-substituted PAHs (four to six aromatic rings):
  - ❖At least three aromatic rings (linear configuration), and at least one bay region structure, tested in animals
  - Genotoxic (*in vivo* and *in vitro* assays) and carcinogenic
  - Form diol or diol epoxide metabolites that are genotoxic and carcinogenic
  - Proposition 65 and IARC carcinogens (Group 1, 2A or 2B; except DB[*a,e*]P)

DBAs and Structurally-Related PAHs

DB[*a,h*]A 	Benzo[*a*]pyrene 	Dibenzo[*a,h*]pyrene 
DB[*a,c*]A 	Benz[*a*]anthracene 	Dibenzo[*a,i*]pyrene 
DB[*a,j*]A 	Dibenzo[*a,e*]pyrene 	Dibenzo[*a,l*]pyrene 

Structure-Activity Comparisons

Chemical	Genotoxicity		Skin tumor initiator		Target tumor site (Mouse)		
	Parent	Metabolites	Parent	Metabolites	Skin	Liver	Lung
DB[*a,h*]A	+	+	+	+	+	+	+[1,2]
DB[*a,c*]A	+	+	+	+	+	+	
DB[*a,j*]A	+	+	+	+	+		
B[*a*]P	+	+	+	+	+	+	+[1,2]
B[*a*]A	+	+	+	+	+	+	+
DB[*a,h*]P	+	+	+	+	+	+	+[2]
DB[*a,i*]P	+	+	+	+	+	+	+
DB[*a,l*]P	+	+	+	+	+	+	+
DB[*a,e*]P	+	NT	+	NT	+	NT	NT

[1] Also positive in rat; [2] Also positive in hamster

QSAR Models

- Publicly available models:
  - VEGA: platform containing CAESAR and ToxTree
  - Lazar
  - QSAR Toolbox
- Published model results that correlate electronic properties with carcinogenicity:
  - Barone *et al.* (1996)
  - Vijayalakshmi and Suresh (2008)

QSAR Predictions of Carcinogenicity

QSAR Model	DB[*a,c*]A	DB[*a,j*]A
VEGA ToxTree	+	+
VEGA CAESAR	+	+
Lazar	+	+
OECD QSAR Toolbox	+	+
Vijayalakshmi & Suresh (2008)	+	+
Barone *et al.* (1996)	+	±[1]

[1] Prediction was not clear

Possible Carcinogenic Mechanisms

- **Genotoxic mechanisms**
- Receptor mediated mechanisms: **aryl hydrocarbon (AhR),** estrogen (ER), androgen receptor (AR)
- Immune suppression related mechanisms
- Cell growth related mechanisms

Genotoxic Mechanisms

- Metabolic activation to DNA reactive species
  - **Carbonium ions**: converted from diol epoxides (e.g., bay-region hypothesis)
  - **Radical cations**: generated from one-electron oxidation reactions catalyzed by peroxidases or CYP450s
  - ***Ortho & para*-Quinones**: directly reactive; can also produce reactive oxygen species (ROS)

Chemical	Genotoxic	Skin tumor initiator	Carcinogenic
DB[*a,h*]A	+	+	+
1,2-diol	+	-	-
3,4-diol	+	+	+
5,6-diol	+	-	-
3,4-diol-1,2-epoxide	+	+	+
DB[*a,c*]A	+	+	+
1,2-diol	+	+	NT
3,4-diol	+	+	NT
10,11-diol	+	+	NT
10,11-diol-12,13-epoxide	+	-	NT
DB[*a,j*]A	+	+	+
3,4-diol	+	+	NT
5,6-diol	+	NT	NT
1,2-diol-3,4-epoxide	+	NT	NT
3,4-diol-1,2-epoxide	+	+	NT
3,4:10,11-*bis*-diol	+	-	NT

Ah Receptor-Mediated Mechanisms

- *Studies of DBAs*
  - Enzyme induction (*e.g.*, CYP 1A1 / 1A2 / 1B1)
  - Enhanced DNA adduct formation
  - Enhanced skin tumor induction

- *Studies of other carcinogenic PAHs*
  - Alteration of oncogenes / tumor suppressor genes
  - Cross-talk with other nuclear receptors, *e.g.*, ER
  - Immune suppression

Possible Carcinogenic Mechanisms

Chemical	Genotoxicity	AhR-mediated	Cell cycle, proliferation / apoptosis	Immune suppression
DB[*a,c*]A	X	X	X	X
DB[*a,j*]A	X	X	x	NT
DB[*a,h*]A	X	X	X	X
B[*a*]P	X	X	X	X

NT: not tested

Summary: Animal Bioassays

- **DB[*a,c*]A**:
  - Significant increase of skin squamous cell carcinoma and carcinoma/papilloma combined in female Swiss mice by dermal application
  - Significant increase of liver adenoma at 12 months in male B6C3F$_1$ mice, following neonatal *i.p.* injection
  - Skin tumor initiator (parent and metabolites)
- **DB[*a,j*]A**:
  - Significant increase of skin squamous cell carcinoma and carcinoma/papilloma combined, with dose-dependent trend in female Swiss mice by dermal application
  - Rare skin sarcomas in female Swiss mice by *s.c.* injection (3/15 vs. 0/12)
  - Skin tumor initiator (parent and metabolites)
- **DB[*a,h*]A**:
  - Tumors induced in multiple species, by multiple routes

Summary: Other Relevant Data

- All three DBA isomers and their metabolites are genotoxic (positive in bacteria, *in vivo* and *in vitro* assays, DNA adduct formation)
- DB[*a,h*]A and DB[*a,c*]A induce cell transformation *in vitro*
- DB[*a,c*]A induces preneoplastic morphologic change *in vivo* in *s.c.* transplanted rat tracheas
- All three DBA isomers activate AhR-mediated pathways
- All three DBA isomers share strong structure-activity similarities with six comparison PAH carcinogens
- DB[*a,c*]A and DB[*a,j*]A carcinogenicity supported by QSAR model predictions