Evidence on the Carcinogenicity of Bisphenol A

Carcinogen Identification Committee Meeting

December 14, 2022

Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment, CalEPA

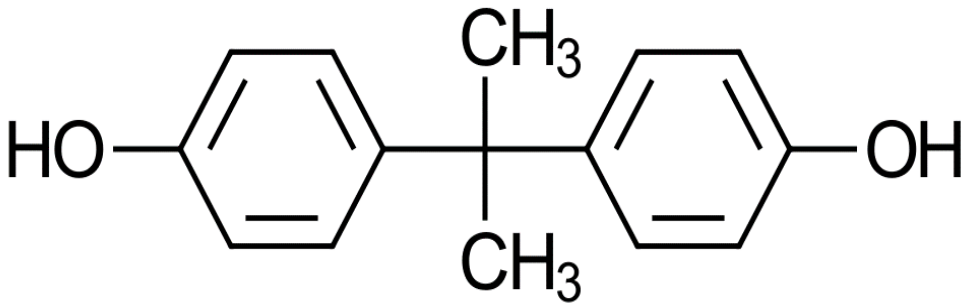
CalEPA
California Environmental Protection Agency

Overview

- Introduction
- Carcinogenicity data on Bisphenol A (BPA)
  - Epidemiologic studies
  - Animal studies
  - Mechanistic data
    - Pharmacokinetics and metabolism
    - Key characteristics (KCs) of carcinogens

Bisphenol A (BPA)

- $C_{15}H_{16}O_2$ (CAS: 80-05-7)



- High production volume chemical with many applications, including
  - polycarbonate plastics
  - epoxy resins
- Ubiquitous presence in the environment
- Exposure pathways: contaminated food and water, ingestion of dust, inhalation, dermal contact, *in utero* transfer, lactation

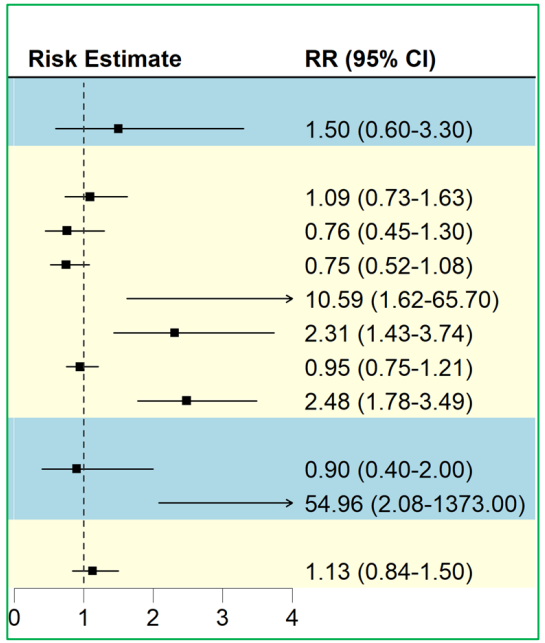
Human Exposure to BPA

- Widespread human exposure across all life stages
  - Biomonitoring studies find BPA in urine, serum, and tissue in the majority of individuals
  - Decreases in BPA detection frequency and levels in recent years in general population samples

- Measurements of BPA through biomonitoring:
  - Reflect short-term exposure (half-life ~6 hours)
  - May not capture high variation in exposure levels

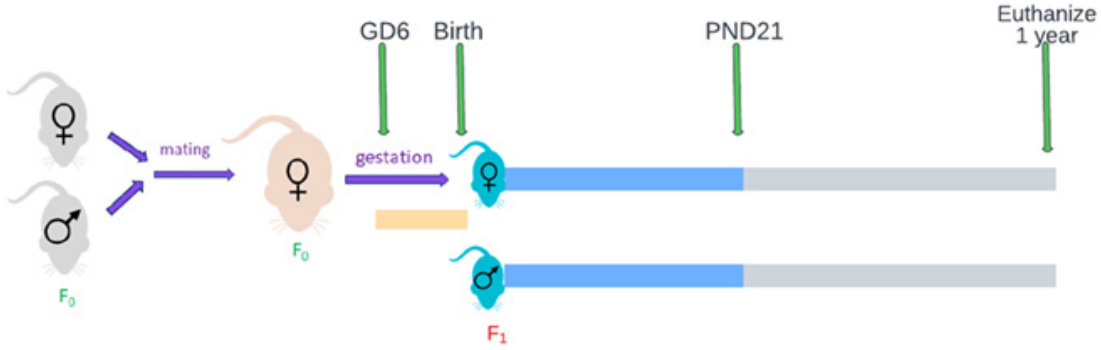
Epidemiologic evidence

Multiple studies on breast, prostate, and thyroid cancers

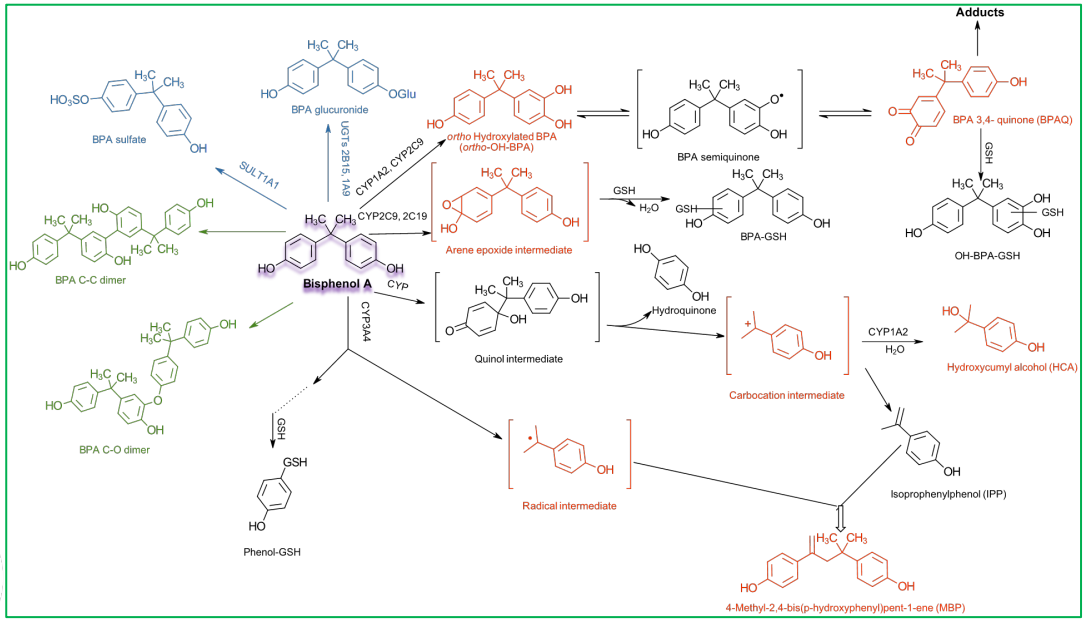


Evidence from animal studies

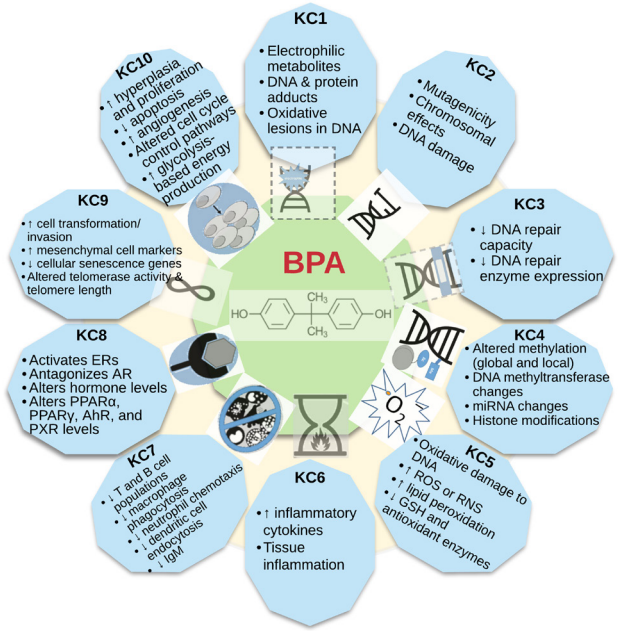
Multiple study designs



Pharmacokinetics and metabolism



Key characteristics of carcinogens



Epidemiologic Studies

Epidemiologic Studies - Overview

Cancer Site	Studies
Breast	13
Prostate	3
Thyroid	2
Lung	2
Bile duct/gallbladder	1
Bone	1
Brain	1
Endometrium	1
Eye	1
Lymphohematopoietic system	1
All cancer mortality	1

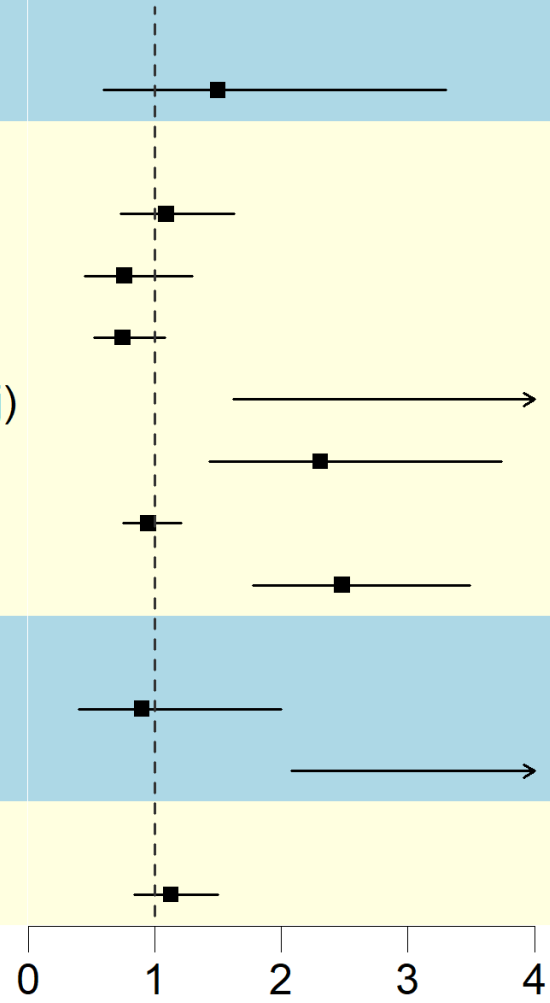
- 51 records identified
- Included all analytical studies, with consideration of:
  - Study quality
  - Direction and magnitude of biases
  - Hill guidance for body of evidence
- Excluded conference abstracts, reviews, studies on uterine leiomyoma

Epidemiologic Studies – Key Issues

- BPA measurement/estimation: long-term exposure may not be represented
  - Measurement error could bias risk estimates towards or away from the null
  - Single time point BPA measurement does not account for highly variable levels
    - Limitation of all the biomonitoring studies
  - Cumulative BPA estimation is also limited
    - Questionnaires: poor correlation with urinary BPA levels
    - Job Exposure Matrices: do not capture widespread exposure from non-occupational sources
- Timing of sample collection: at/after diagnosis
  - Relevant time window not assessed – true causal effects could be missed
  - Reverse causation could not be ruled out
- In cross-sectional studies with cancer outcomes: prevalent cancers may reflect survivor bias, temporality not established

BPA and Breast Cancer

Reference	Exposure Category (ng/ml BPA)	Risk Estimate	RR (95% CI)
JEM			
Aschengrau et al. (1998)	>10 years any BPA		1.50 (0.60-3.30)
Urine			
Trabert et al. (2014)	> 8268 (BPA-G)		1.09 (0.73-1.63)
Morgan et al. (2017)	0.45–1.3 (total)		0.76 (0.45-1.30)
Parada et al. (2019)	3848-411,280 (total)		0.75 (0.52-1.08)
Keshavarz-Maleki et al. (2021)	2.12 (total, not creatinine adj)		10.59 (1.62-65.70)
López-Carrillo et al. (2021)	>12.05 (free)		2.31 (1.43-3.74)
Wu et al. (2021)	>1.87 (total)		0.95 (0.75-1.21)
He et al. (2022)	>1812.6 (total)		2.48 (1.78-3.49)
Tissue			
Reeves et al. (2018)	>0.40 in cases (free)		0.90 (0.40-2.00)
Keshavarz-Maleki et al. (2021)	4.2		54.96 (2.08-1373.00)
Serum			
Salamanca-Fernández et al. (2021)	5.1–68.9 (total)		1.13 (0.84-1.50)



BPA and Thyroid, Prostate Cancer

Tumor site	Reference	Study Design	Exposure Assessment Method	Exposure category or level	RR (95% CI)
Thyroid	Marotta et al. (2019)	Cross-sectional	Serum BPA measured using HPLC/FLD/UV	Exposed to BPA	3.71 (0.67–20.34)
	Zhou et al. (2017)	Cross-sectional	Urinary total BPA measured using HPLC–MS/MS	Urinary BPA >2.84 ng/ml (not adjusted for creatinine)	**3.57 (1.37–9.3)**
Prostate	Salamanca-Fernández et al. (2021)	Case-cohort	Serum BPA analyzed by DLLME & UHPLC-MS/MS	Tertile 3 (5.1–68.9 ng/ml BPA)	1.31 (0.98–1.74)
	Tse et al. (2017)	Case-control	Cumulative BPA index from questionnaire data and lit review	High Cumulative BPA Index	**1.88 (1.24–2.86)**

Carcinogenicity Studies in Animals

Animal Carcinogenicity Studies – Overview

Exposure	Species	Strains	Sex (# of studies)	Route	Study duration
Beginning at or after four weeks of age	Mouse	B6C3F1	**male** (1), **female** (1)	feed	107 weeks
	Rat	F344	**male** (1), **female** (3)	feed, gavage,	12-108 weeks
	Gerbil	Not specified	**male** (2)	drinking water	24-29 weeks
In utero or within the first week of life	Mouse	CD-1, Agouti$^{+/-}$ C57BL/6J:C3H/HeJ	**male** (1), **female** (4)	*s.c.*, *in utero*, *in utero* and via lactation & feed	3-18 months
	Rat	SD (NCTR), F344, SD, Wistar-Furth	**male** (5), **female** (7)	*In utero*, *in utero* and gavage, *in utero* and via lactation	PND50 up to 2 years

Assessing dose-response significance

- Statistical tests are performed using effective number when possible
- One-sided Fisher's exact test for pairwise comparisons
- Exact conditional Cochran-Armitage test for linear trend
  - The test originally derived by Cochran and Armitage relies on a Normal approximation
    - Performs well for large and balanced sample sizes
  - Williams (1988) demonstrated that using the exact conditional distribution of the test statistic improves the accuracy of the test
  - The algorithm used to derive the exact p-value is described in Mehta et al (1992)

Williams DA (1988). Tests for differences between several small proportions. Journal of the Royal Statistical Society. Series C [Applied Statistics] 37(3): 421-434.

Mehta CR, Patel N, and Senchaudhuri P (1992). Exact Stratified Linear Rank Tests for Ordered Categorical and Binary Data. Journal of Computational and Graphical Statistics 1(1):21–40.

Tumor Findings in Male Mice: Exposed to BPA Beginning at or after Four Weeks of Age

	Site	Type	Concentration in feed (ppm)			Exact trend test *p*-value
			0	1000	5000	
103-week feed study in male B6C3F1 mice (NTP 1982)	Hematopoietic system	Malignant lymphoma	2/47	8/47*	3/45	NS
		All leukemia [rare]	0/44	1/46	2/45	NS
		Malignant lymphoma and lymphocytic leukemia combined	2/47	9/47*	3/45	NS
	Pituitary	Chromophobe carcinoma [rare]	0/37	0/36	3/42	0.0465

* $p < 0.05$; NS, not significant

Tumor Findings in Rats: Exposed to BPA Beginning at or after Four Weeks of Age

	Site	Type	Concentration in feed (ppm)			Exact trend test *p*-value
			0	1000	2000	
103-week feed study in male F344 rats (NTP 1982)	Hematopoietic system	Leukemia (NOS)	13/50	12/50	23/50*	0.021
	Mammary gland	Fibroadenoma	0/36	0/40	4/34*	0.008
	Testis	Interstitial (Leydig) cell tumor	35/47	48/48***	46/49**	0.0015

	Site	Type	Concentration (mg/kg-day)				Exact trend test *p*-value
			0	50	200	400	
12-week oral study in female F344 rats (Hao et al. 2016)	Pituitary gland	Pituitary tumor	0/10	4/10*	1/10	3/10	NS

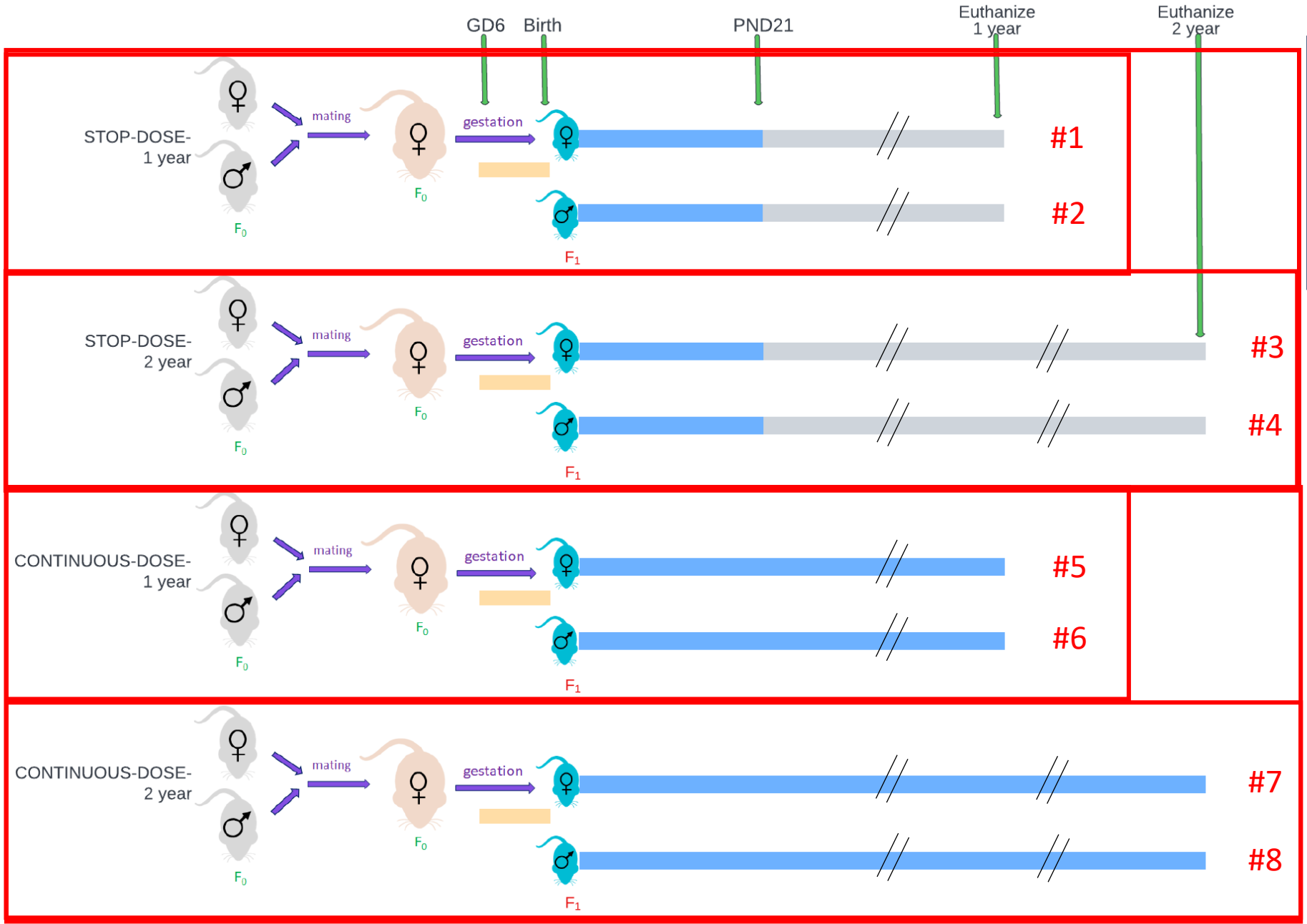
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant; NOS, not otherwise specified

Tumor Findings in Female Mice:
Exposed to BPA Beginning *in utero*, via Lactation, and Post-weaning in Feed Until 10 Months of Age

Female F1 Agouti$^{+/-}$ C57BL/6J:C3H/HeJ mice (Weinhouse et al. 2014)	Site	Type	Concentration (ppm)				Exact trend test *p*-value
			0	5×10^{-5}	0.05	50	
	Liver	Hepatocellular carcinoma	0/9	2/10	1/10	3/9	NS
		Combined hepatocellular adenoma or carcinoma	0/9	2/10	1/10	4/9*	0.0185

* $p < 0.05$; NS, not significant

Overview of the CLARITY-BPA Core Studies Conducted in SD (NCTR) Rats



Gavage daily to dam

Gavage daily

Dose: 0 (vehicle); BPA at 2.5, 25, 250, 2500 or 25000 µg/kg per day

No treatment

Tumor Incidences in Females

CLARITY-BPA Core Studies in SD (NCTR) Rats

Study	Tumor site	Tumor type	Dose (µg/kg-day)						Exact trend test p-value
			0	2.5	25	250	2500	25000	
Stop-dose (*in utero* + 3 weeks) **2-year (#3)**	Mammary gland	Adenoma	1/48	1/44	0/43	3/45	0/47	1/40	NS
		Adeno-carcinoma	3/48	11/44*	5/45	7/48	9/47	5/41	NS
		Combined	4/48	12/44*	5/45	9/48	9/47	6/41	NS
Continuous-dose 1-year (#5)	Uterine	Stromal polyps	1/20	0/20	1/21	0/22	3/20	3/24	$p < 0.05$
Continuous-dose 2-year (#7)	Clitoral gland	Adenoma	0/40	0/38	0/32	0/41	0/33	2/36	$p < 0.05$
		Carcinoma	1/50	1/44	1/43	1/47	4/47	1/45	NS
		Combined	1/50	1/44	1/43	1/47	4/47	3/45	$p < 0.05$

* $p < 0.05$; NS, not significant

Tumor Incidences in Males

CLARITY-BPA Core Studies in SD (NCTR) Rats

Study	Tumor site	Tumor type	Dose (µg/kg-day)						Exact trend test p-value
			0	2.5	25	250	2500	25000	
Stop-dose (*in utero* + 3 weeks) 2-year (#4)	Prostate (dorsal/lateral lobes)	Malignant lymphoma	0/49	0/48	0/48	3/50	2/49	4/45*	$p < 0.01$
	All sites	Malignant lymphoma	1/49	0/48	1/48	3/50	2/49	5/45	$p < 0.01$
	Thyroid gland	C-cell adenoma	0/37	1/31	0/33	0/26	1/34	3/23	$p < 0.05$
Continuous-dose 2-year (#8)	Liver	Hepatocellular carcinoma [Rare]	0/24	0/25	0/24	2/24	1/24	3/19	$p < 0.01$

* $p < 0.05$

Evaluation of Rare Tumors in SD (NCTR) Rats

- Rare tumors were observed
- SD (NCTR) rats were on CLARITY-BPA core study from 2012 to 2015
- Lack of ideal historical control data
- 3 databases used
  - NTP (2008, 2010) (dietary/feed administration, SD (NCTR) rats, 1999 to 2003)
  - Charles River (2013) (oral routes, Crl:CD®(SD)BR rats, 2001 to 2009)
  - NTP (2021) (all routes, Hsd SD rats, 2007 to 2012)
- Each of the 3 databases with its own unique set of limitations
- Rare tumors presented in the HID
  - Less than 1% of tumor incidence in historical control animals in each of the three sets of historical control data
  - With no tumor occurrence in the concurrent controls

Additional Issues Associated with the CLARITY-BPA Core Studies

- Possible exposure of controls to BPA via contamination
  - BPA levels in vehicle and naive control animals were similar to the levels detected in the lowest BPA exposure group
- Insensitive responsiveness of the SD (NCTR) rats
  - Insensitive to known estrogens, such as ethyl estradiol (EE2)
  - Insensitive to known thyroid peroxidase inhibitor, 6-propyl-2-thiouracil (PTU)
- Additional concerns
  - Lack of an unhandled, non-gavaged control group and lack of EE2-treated positive controls in the stop-dose arms

Tumor Findings: By System and Tumor Type

- ***Alimentary system:*** **Hepatocellular tumors** in male SD (NCTR) rats, and female Agouti$^{+/-}$ C57BL/6J:C3H/HeJ mice
- ***Endocrine system***: **Pituitary tumors** in female F344 rats and male B6C3F1 mice; **thyroid C-cell tumors** in male SD (NCTR) rats
- ***Mammary gland*****: Fibroadenoma** in male F344 rats; **adenocarcinoma, and adenoma and adenocarcinoma combined** in female SD (NCTR) rats
- ***Reproductive system:***
  Female**: Clitoral gland tumors & uterine stromal polyps** in SD (NCTR) rats

  Male**: Testicular interstitial (Leydig) cell tumors** in F344 rats
- ***Lymphohematopoietic system*****: Leukemia** in male F344 rats, **lymphoma** in male SD (NCTR) rats and male B6C3F1 mice
- Multiple types of **rare tumors** were observed in several studies in male and female SD (NCTR) rats.

Tumor Findings from Transgenic Animal models

- Female mouse (MMTV-erbB2) mammary tumor models
  - ↓ tumor latency in two studies
  - ↑ tumor multiplicity
  - ↑ tumor volume
  - ↑ lung metastases of mammary tumors
- Mouse model with an estradiol non-responsive mutant ER-α ligand binding domain
  - ↑ “tumor-like outgrowths” (adenocarcinomas) in the flank muscle of female transgenic mice

Tumor Findings from Other Animal Models

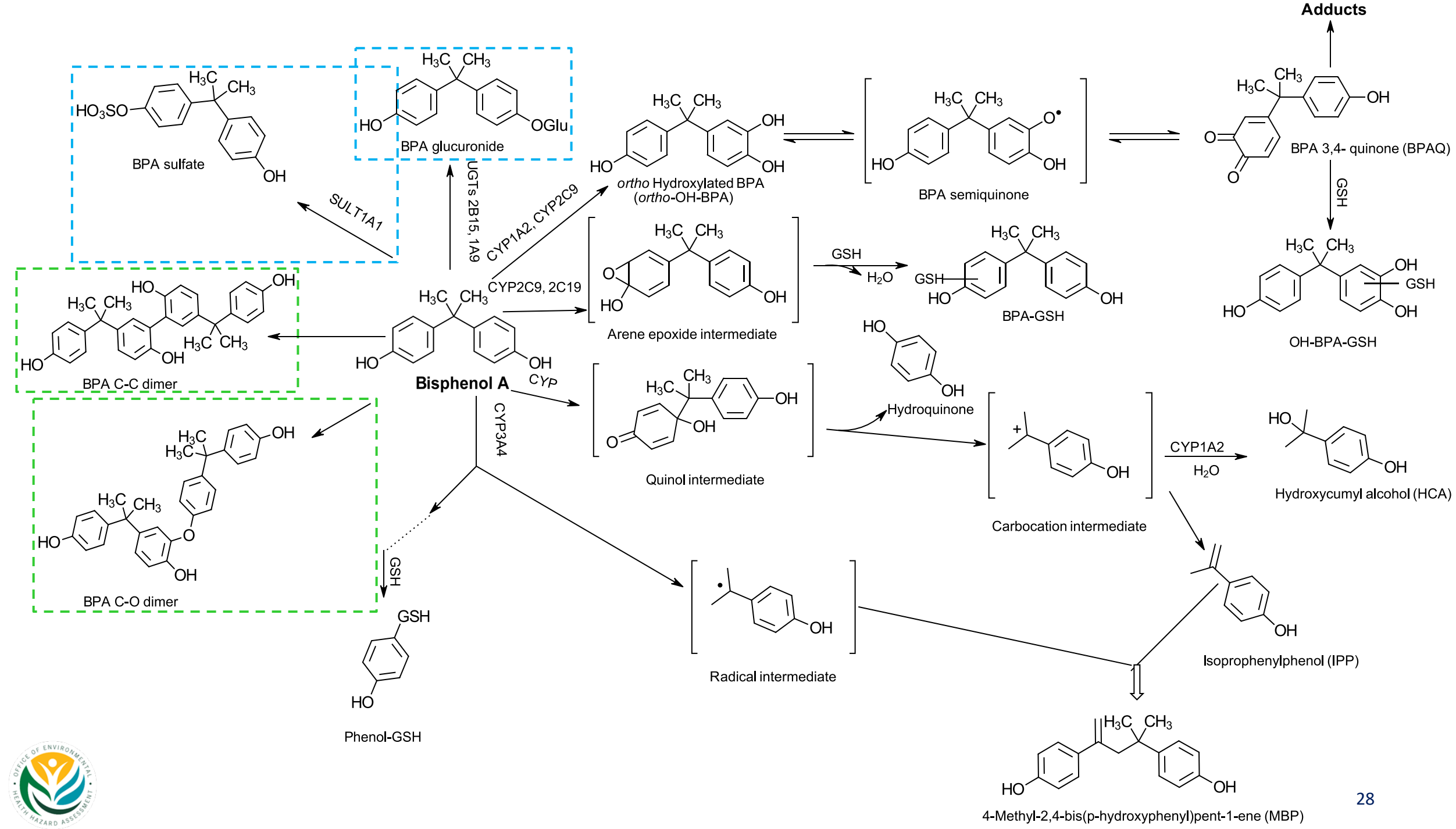
- ***In xenograft, syngeneic, and regenerated organ mouse models***
  - ↑ No. of tumor-bearing mice, mean tumor volume or tumor weight in xenograft models (BPA, xenograft)
  - ↑ growth of established tumors in xenograft models (xenograft, BPA)
  - ↑ tumor volume in syngeneic mouse models
  - ↑ atypical ductal hyperplasia and ductal carcinoma *in situ* in regenerated mammary glands
- ***BPA in combination with other treatments***
  - ↑ mammary tumor incidence and /or multiplicity in female rats, ↓ tumor latency in female rats and mice (BPA, carcinogen)
  - ↑ mammary tumors in female rats (tumor initiator, BPA)
  - ↑ microinvasive carcinoma and PINs of the prostate in male rats (BPA, testosterone & 17β-estradiol)

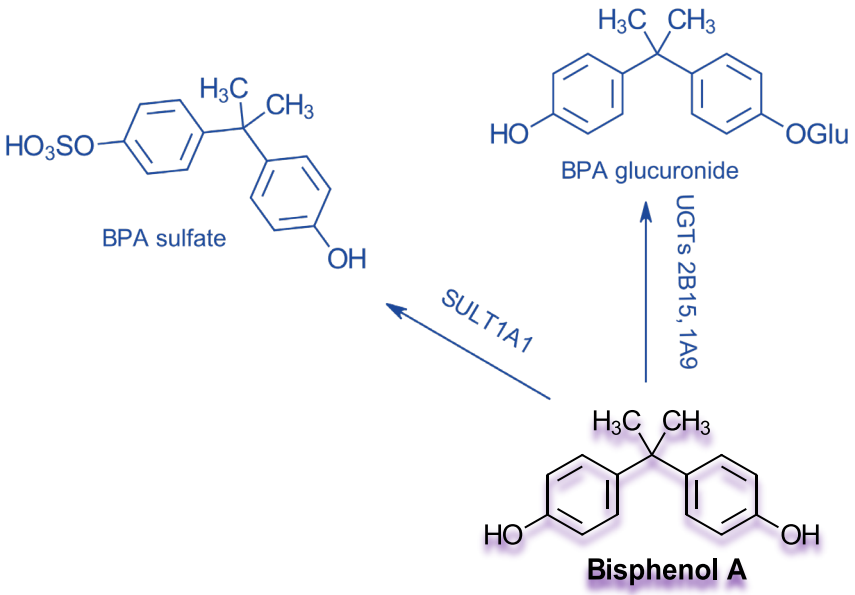
Break for Clarifying Questions from the Carcinogen Identification Committee

Mechanistic considerations and other relevant data

Pharmacokinetics and Metabolism

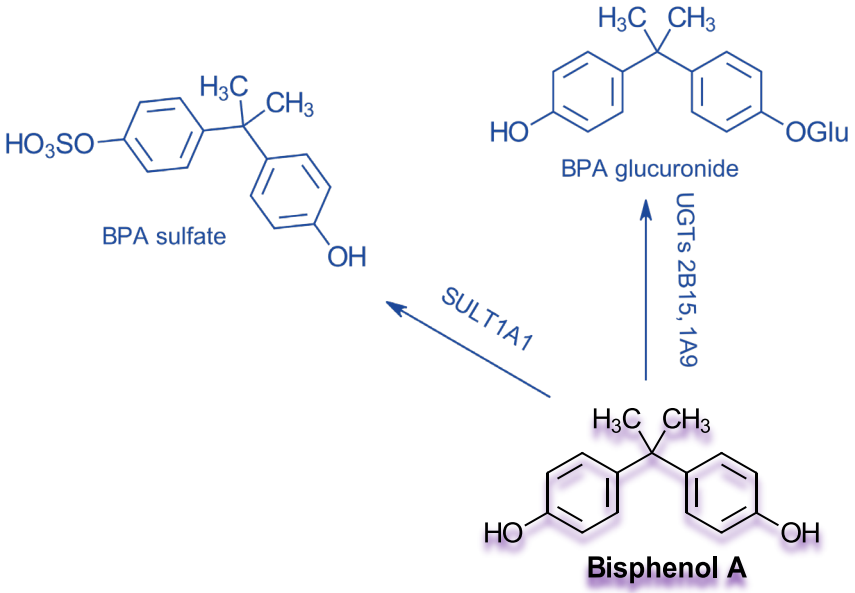
- BPA is rapidly absorbed and widely distributed in humans
  - Crosses blood-brain barrier and placenta
  - Detected in breastmilk, adipose tissues, liver and other organs and body fluids
- Half-lives vary by species and administration route (< 24 hours)
  - Humans by oral route: ~ 6 hours
- In humans, fast excretion mainly via urine (detected in more than 90% of NHANES population)
  - Feces as the main route of excretion in rodents
  - Enterohepatic circulation in rodents, not humans


Adducts
BPA sulfate
BPA glucuronide
SULT1A1
UGTs 2B15, 1A9
CYP1A2, CYP2C9
CYP2C9, 2C19
ortho Hydroxylated BPA
(ortho-OH-BPA)
BPA semiquinone
BPA 3,4- quinone (BPAQ)
GSH
OH-BPA-GSH
BPA C-C dimer
Bisphenol A
CYP
Arene epoxide intermediate
GSH
H2O
BPA-GSH
Hydroquinone
BPA C-O dimer
CYP3A4
Quinol intermediate
Carbocation intermediate
CYP1A2
H2O
Hydroxycumyl alcohol (HCA)
GSH
Phenol-GSH
Radical intermediate
Isoprophenylphenol (IPP)
4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene (MBP)



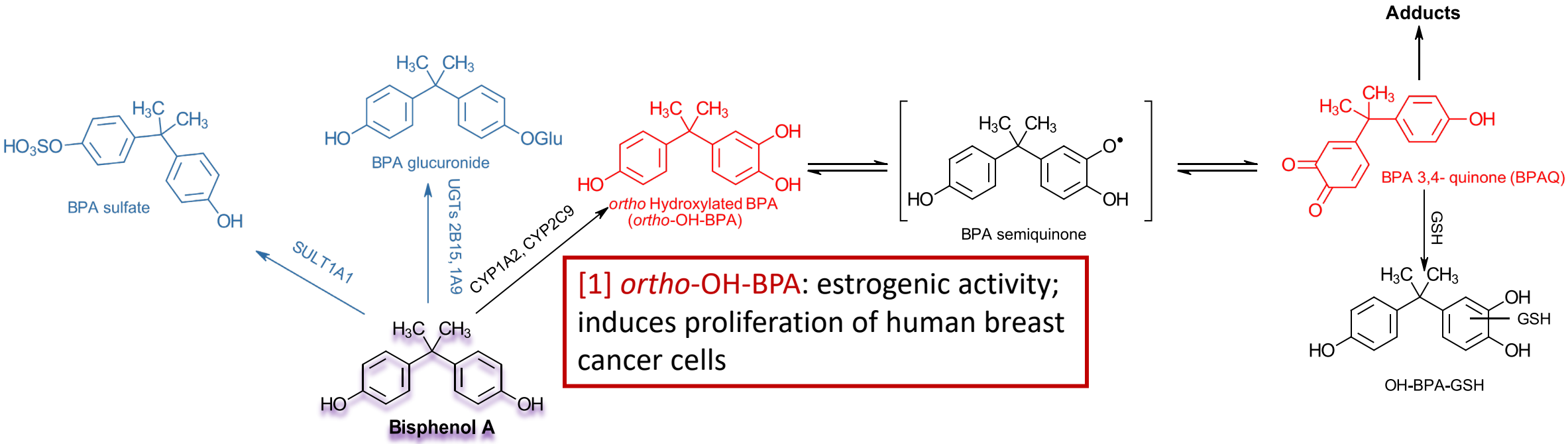
Phase II metabolism:

- Glucuronidation:
  - BPA-G; Primary enzymes include UGT2B15 & UGT1A9
  - ~70% of excreted metabolites (main metabolite in humans and animals)
  - Crosses placenta; subsequent de-conjugation of BPA-G to BPA by fetal β-glucuronidases
- Sulfoconjugation:
  - BPA-S; Primary enzymes include SULT1A1
  - ~20% of excreted metabolites
  - De-conjugation via sulfatases (estrone sulfatase)



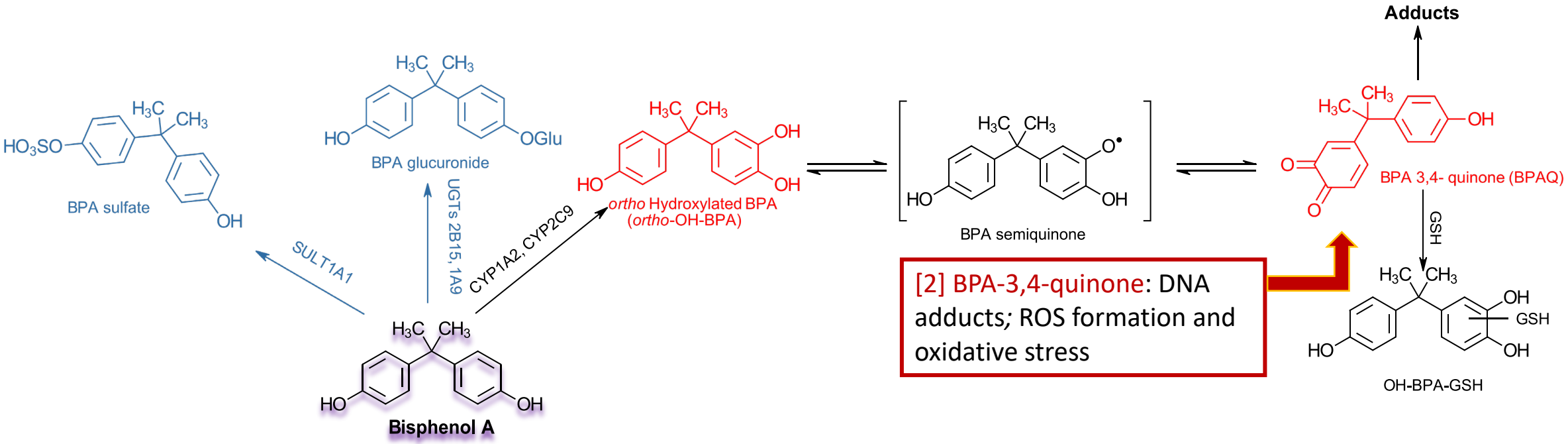
Factors affecting conjugation (section 5.1.4):

- **Genetic polymorphisms**: e.g., UGT2B15*2 leads to significantly decreased glucuronidation
- **De-conjugation reactions**: Estrone sulfatase, fetal β-glucuronidases
- **Co-exposures** to xenobiotics & medications (naproxen, carbamazepine)
- **Disease status**: Reduced glucuronidation (Parkinson's) and sulfation (liver disease; up to 80% reduction)
- **Life stage**: UGT1A1 absent from the fetal liver; UGT2B15 active at reduced levels in human fetus

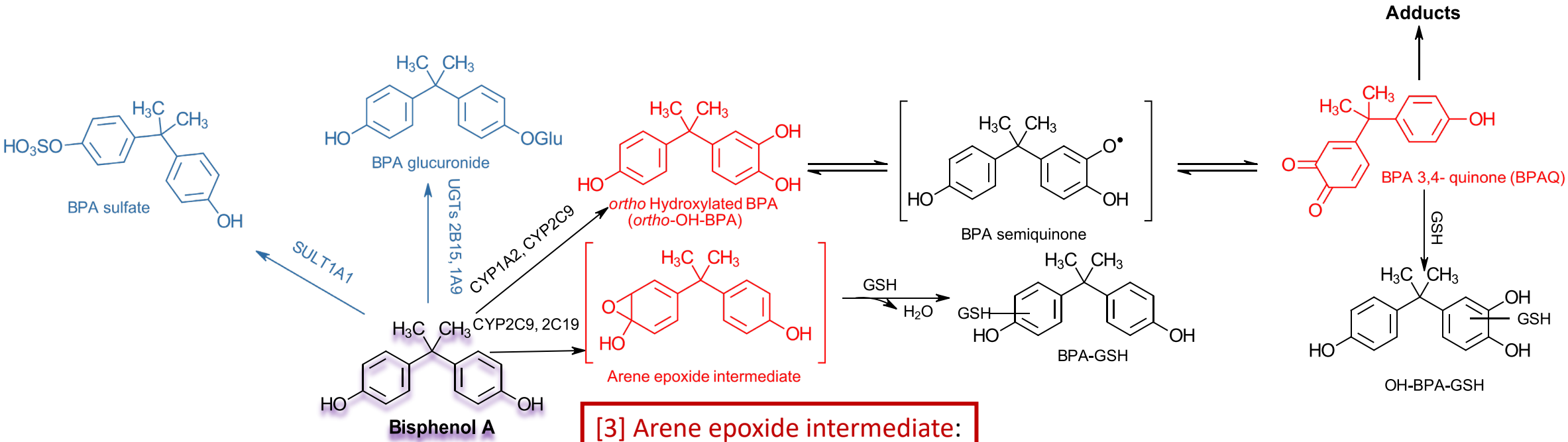


[1] *ortho*-OH-BPA: estrogenic activity; induces proliferation of human breast cancer cells

Biologically active and reactive metabolites are shown in red color

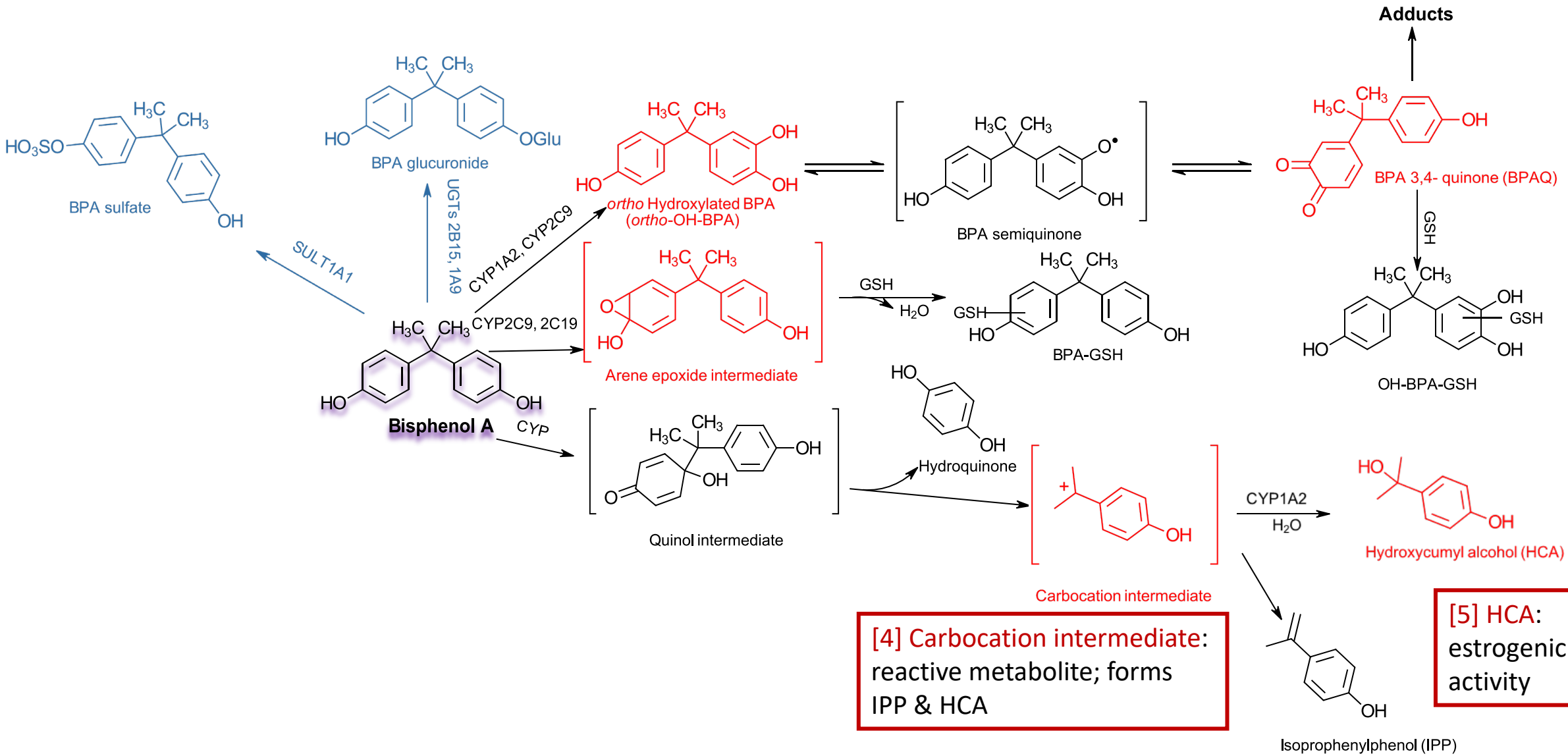


Biologically active and reactive metabolites are shown in red color

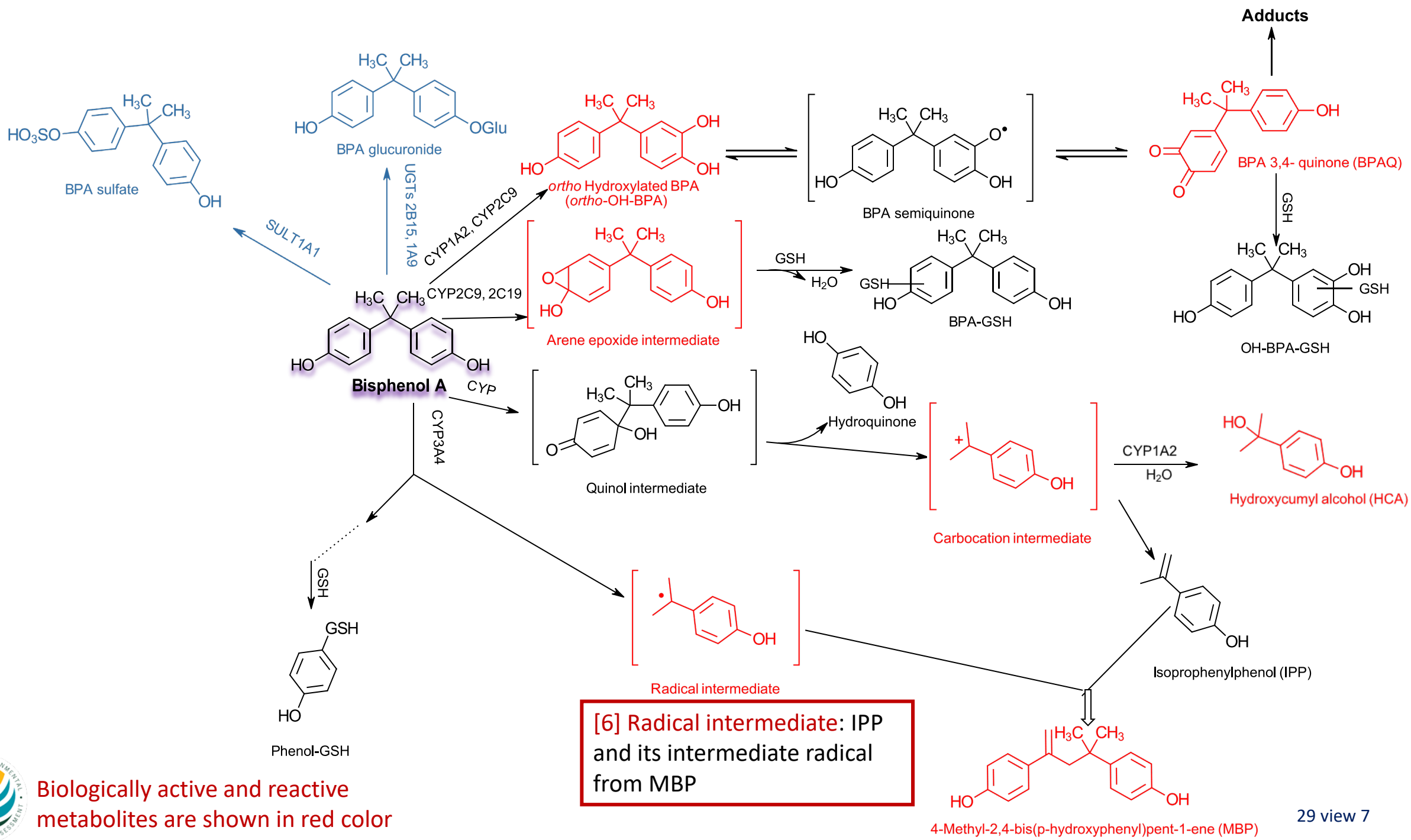


[3] Arene epoxide intermediate: reactive metabolite

Biologically active and reactive metabolites are shown in red color

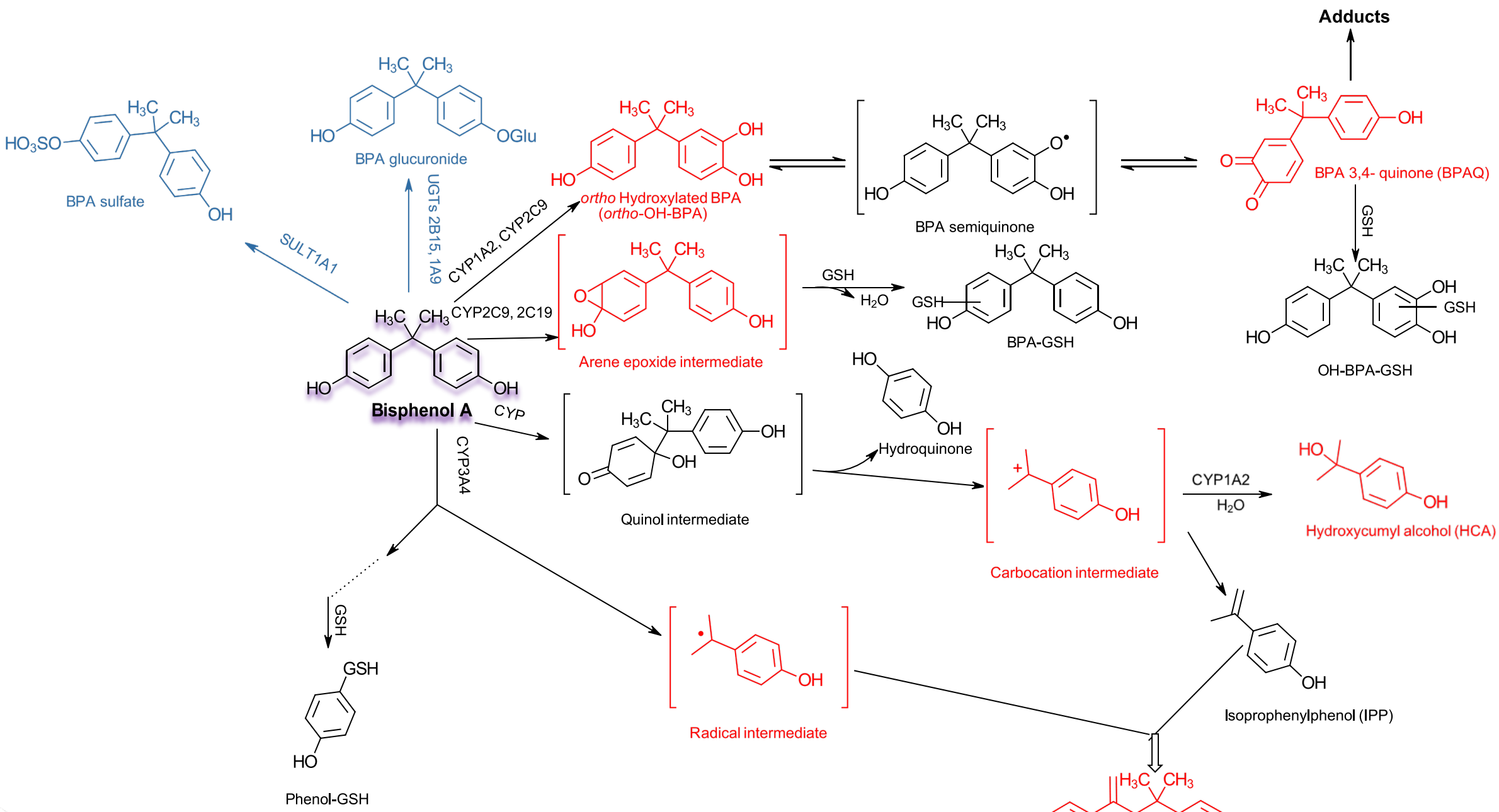


Biologically active and reactive metabolites are shown in red color



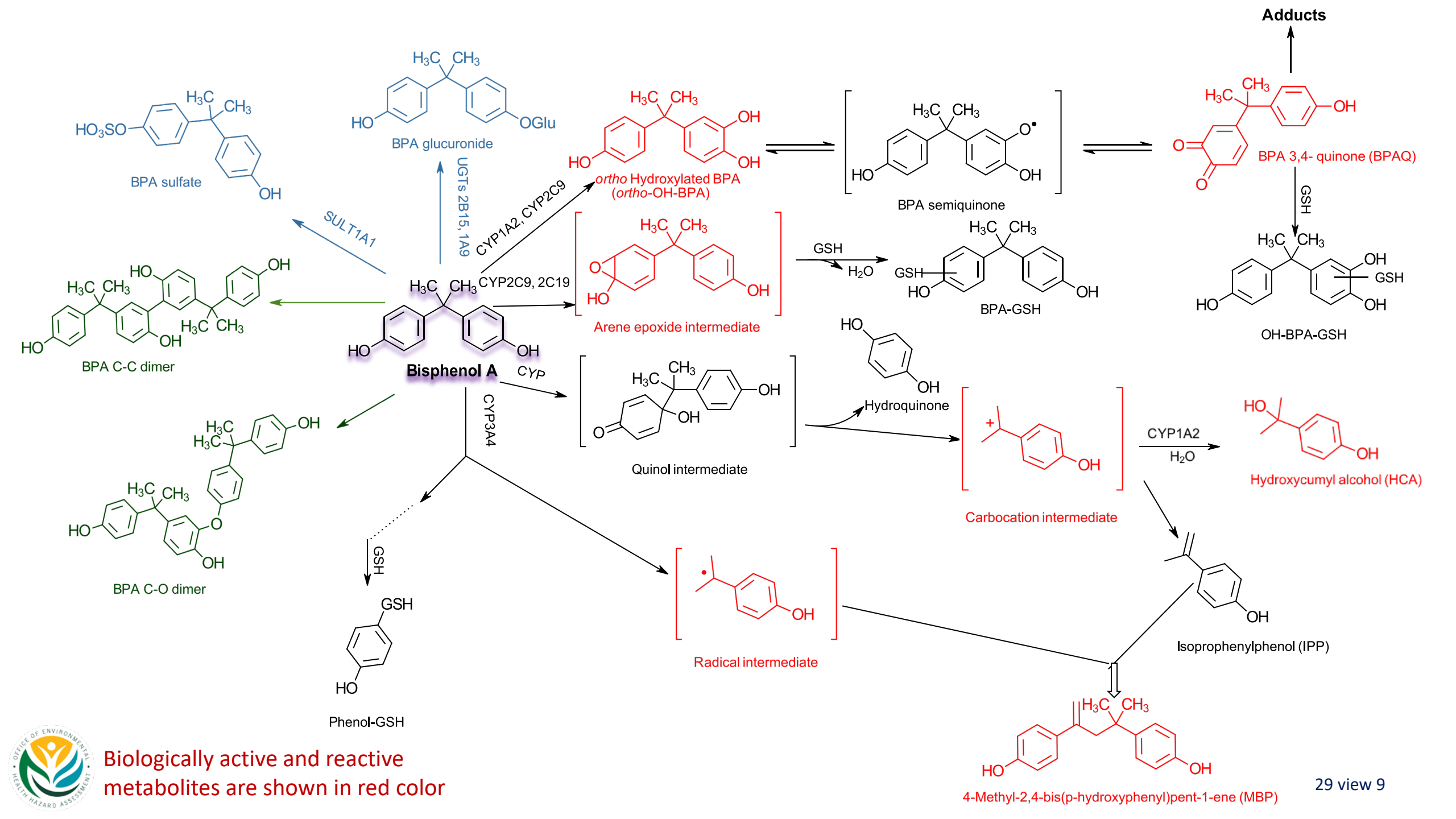
[6] Radical intermediate: IPP and its intermediate radical from MBP

Biologically active and reactive metabolites are shown in red color



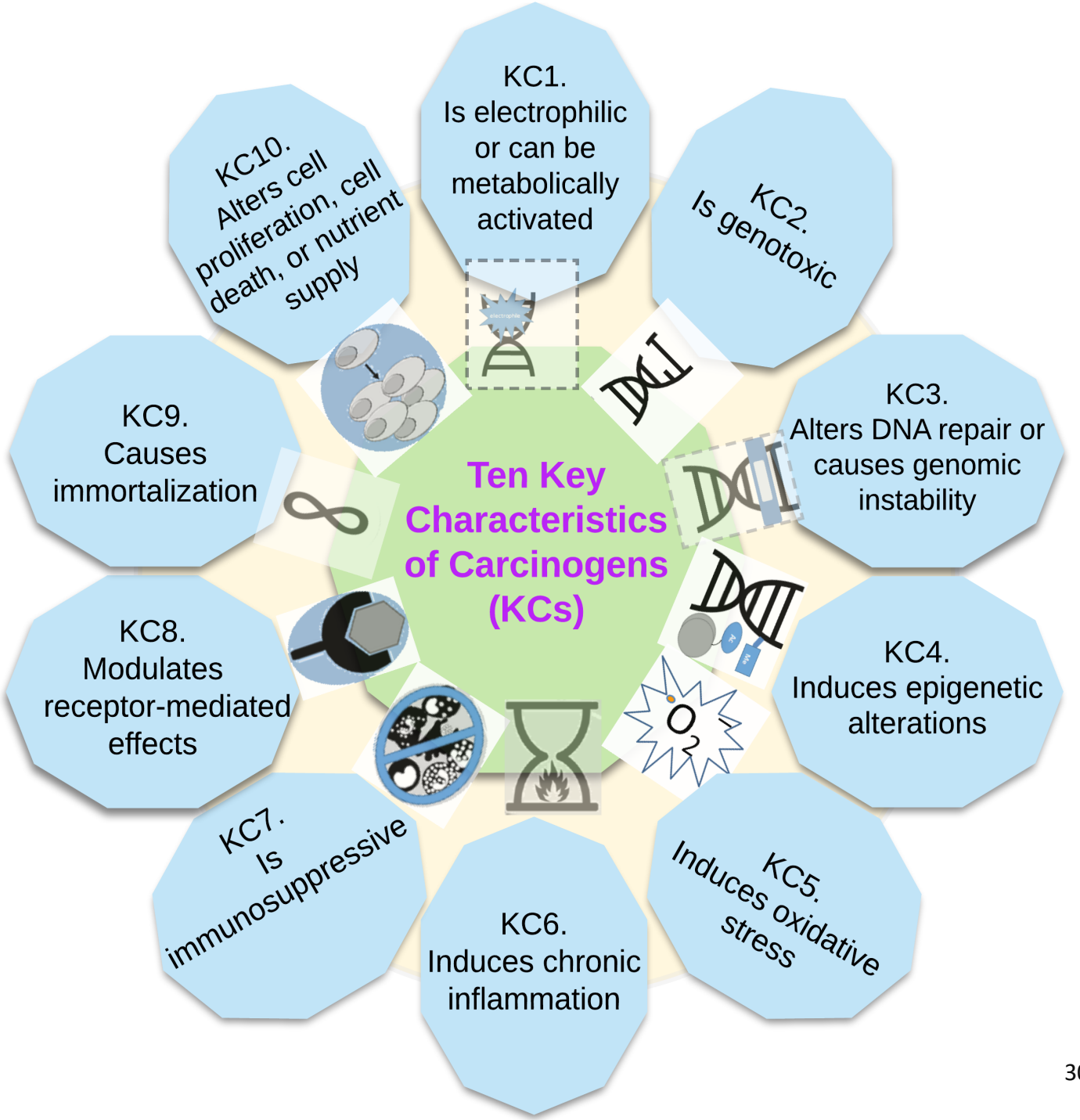
Biologically active and reactive metabolites are shown in red color

[7] MBP: estrogenic activity; induces proliferation of human breast cancer cells



Biologically active and reactive metabolites are shown in red color

Key Characteristics of Carcinogens



Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications. See also Preamble to the IARC monographs (IARC 2019).

KC 8: Modulates Receptor-mediated Effects

Effects on Estrogen Receptors (ERs)

- Classical ER-mediated effects, *e.g. in vitro* and *in vivo* estrogenicity (Chapin et al. 2008)
  - BPA modulates ER-mediated effects through several ER subtypes
- Non-canonical ER activities, *e.g.* the rapid onset of extranuclear responses, the low-dose effects and the non-monotonic dose-responses (↑ female rat mammary tumor in CLARITY-BPA core study #3)
  - For example, BPA affects membrane-associated estrogen receptors, G-protein coupled estrogen receptor, and estrogen-related receptor gamma
  - BPA can induce epigenetic changes to regulate the expression of ERα and cancer-related ER target genes

Section 5.3.8 and Appendix J

KC 8: Modulates Receptor-mediated Effects (cont'd)

Effects on progesterone receptor

- ↑ PR expression in human and mammalian *in vitro* studies

Effects on androgen receptor

- Exhibited antiandrogenic activity in human and mammalian *in vitro* studies

Effects on thyroid hormone receptors

- Antagonized TRβ activity in human *in vitro* studies

Effects on other nuclear receptors

- Altered expression or activity of PPARα, PPARγ, AhR, and PXR

Section 5.3.8 and Appendix J

KC 8: Modulates Receptor-mediated Effects (cont'd)

Effects on hormone levels

- Estradiol: positive correlations in some human observational studies in specific populations
- Testosterone: positive association in women and girls with PCOS
  - ↓ testosterone levels in male mice
- Prolactin: positive associations in human occupational studies
  - ↑ prolactin levels in rats
- No consistent associations with progesterone or thyroid hormones

Section 5.3.8 and Appendix J

KC 10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

- ↑ cell proliferation in human cell lines (normal, immortalized, and cancer cells) *in vitro*
- ↑ hyperplasia and cell proliferation in multiple organs in multiple strains of rats and mice *in vivo*
- ↓ apoptosis, ↑ anti-apoptotic proteins (*e.g.* Bcl-2), ↓ pro-apoptotic proteins (*e.g.* BAX, caspases) in human cancer cell lines
- Altered signaling pathways related to cell cycle control (*e.g.* ↑ cyclins, CDKs and PCNA, ↓ p21 and p53) in human cancer cell lines
- ↑ angiogenesis in human HUVEC cells and ↑ pro-angiogenesis gene expression (*e.g.* VEGF) in human cells (normal and cancer)
- ↑ glycolysis-based energy production in human cancer cell lines

Section 5.3.10 and Appendix K

KC 1: Is Electrophilic or Can Be Metabolically Activated

- Multiple electrophilic and reactive metabolites
  - BPA-3,4-quinone (BPAQ)
  - Arene epoxide intermediate
  - IPP radical, which forms MBP
  - Unidentified electrophilic compound leading to BPA dimer
- Oxidative lesions in DNA (8-hydroxydeoxyguanosine; 8-OHdG) (KC2, KC5)
- Formation of DNA adducts *in vivo* and *in vitro*, and cell-free systems
- Binds to cysteine residues to form protein adducts (rat *in vivo* and in a cell-free system)

Section 5.3.1

KC 2: Is Genotoxic

Mutations

- ↑ in human embryo-derived fibroblasts and HEK 293T cells *in vitro*
- ↑ in dominant lethal mutation rate in male rats *in vivo*
- No effects in bacteria, yeast, or *Drosophila*

Chromosomal effects

- ↑ in MN, CA, and various types of chromosomal abnormalities in *in vitro* studies (human and animal cells) and *in vivo* animal studies
- ↑ in CA in plants in 3 studies; ↑ in microtubule abnormalities in acellular systems in 2 studies

Section 5.3.2 and Appendix F

KC 2: Is Genotoxic (cont'd)

DNA damage

- Positive associations between urinary or serum levels of BPA and 8-OHdG (> 10 human observational studies)
- Positive associations between urinary BPA levels and sperm DNA fragmentation (2 human observational studies)
- ↑ DNA adduct formation, DNA strand breaks, oxidative damage to DNA, and γ-H2AX in multiple experimental systems
- ↑ DNA damage-control protein expression in 2 types of human cells *in vitro* and in an earthworm *in vivo* study

Section 5.3.2 and Appendix F

KC 5: Induces Oxidative Stress

- ↑ oxidative damage to DNA (8-OHdG)
  - 13 of 19 human observational studies
  - 3 of 3 rodent *in vivo* studies
- ↑ reactive oxygen or nitrogen species in more than 100 human *in vitro* and rodent *in vivo* and *in vitro* studies
  - Dose- or concentration-dependent increases in some studies
- ↑ lipid peroxidation (8-isopropane or malondialdehyde) in human observational studies, human *in vitro* and rodent *in vivo* studies
- ↓ GSH or antioxidant enzyme activities or levels in rodent *in vivo* and *in vitro* studies

Section 5.3.5 and Appendix H

KC 3: Alters DNA Repair or Causes Genomic Instability

DNA repair capacity

- ↓ repair of DNA damage in human cells *in vitro* and rodent cells *in vitro*

DNA repair genes

- ↓ MyH, TP53 expression in human cells *in vitro*
- ↓ *mlh1* expression in *Drosophila melanogaster* (1 study)

Section 5.3.3

KC 4: Induces Epigenetic Alterations

Epigenetic findings in human observational studies and human cells *in vitro,* as well as animals *in vivo* and animal cells *in vitro*

- Altered methylation of regions associated with specific genes
  - *E.g.*, promoter hypermethylation of CAPS2 and TNFRSF25 in human cord blood
- Global methylation changes
  - *E.g.*, LINE-1 methylation in human cord blood
- miRNA changes
  - *E.g.*, altered expression of cancer-related miRNAs in human cells *in vitro*
- Histone modifications
  - *E.g.*, altered regulation of mRNA expression of HDACs and HATs in human cells *in vitro*

Section 5.3.4 and Appendix G

KC 6: Induces Chronic Inflammation

Human observational studies

- Positive associations with C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) (8 cross-sectional studies)
- Positive association with interleukin-6 (IL-6) (8 cross-sectional, 1 cohort study)
- No significant association with IL-1β, IL-10, TNF-α, or CRP (2 cohort studies)

Animal studies

- Chronic inflammation with longer-term BPA exposure (many studies)
  - Histopathology in many tissues
  - Significant increases in pro-inflammatory biomarkers including IL-1β, IL-6, TNF-α
- Negative association between BPA exposure and inflammatory biomarkers (2 studies)

Section 5.3.6 and Appendix I

KC 7: Is Immunosuppressive

- Effects on T cell and B cell cellularity or proliferation
  - ↓ T and B cell cellularity or proliferation in human cells *in vitro*, rodents *in vivo*, rodents *in vitro,* and fish
- Effects on neutrophils
  - ↓ chemotactic capacity in human cells *in vitro* and mice
- Effects on macrophages
  - ↓ phagocytosis in human cells *in vitro*, rats, mice, and fish
  - ↓ macrophage populations in mice (1 study)
  - ↓ macrophage proliferation in fish (1 study)

KC 7: Is Immunosuppressive (cont'd)

- Effects on dendritic cells
  - ↓ endocytotic capacity in human cells *in vitro* (1 study)
  - ↓ dendritic cells in rats (1 study)
- Effects on natural killer cells
  - ↓ percentage of splenocytes that were NK cells in mice (1 study)
- Effects on IgM levels
  - ↓ IgM levels in mice and fish

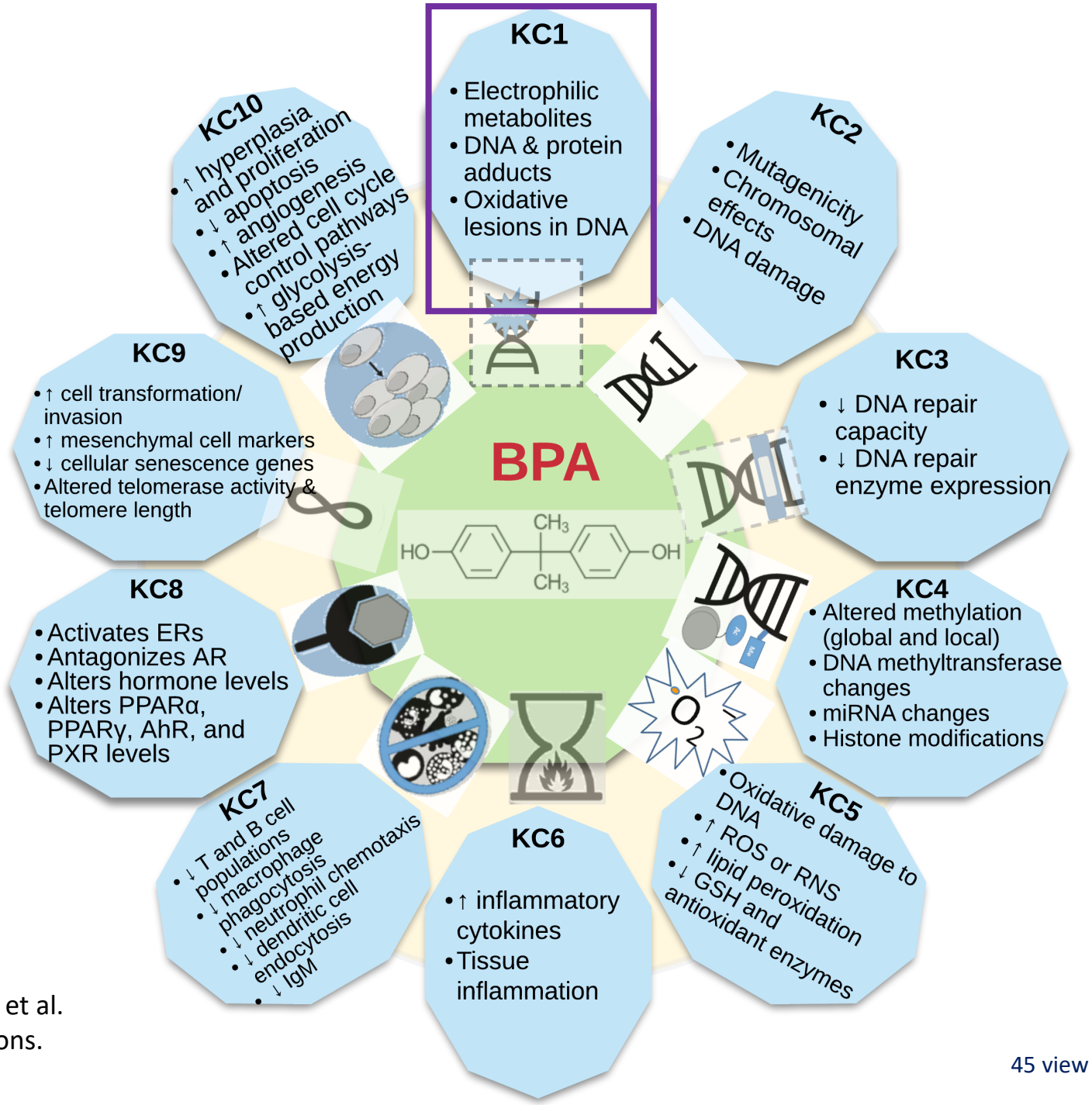
KC 9: Causes Immortalization

- Cell transformation
  - ↑ transformation frequency in Syrian hamster embryo cells
- Cell invasion
  - ↑ in Matrigel invasion assays of multiple types of primary human cells *in vitro*
- Epithelial-Mesenchymal-Transition markers
  - ↑ vimentin, fibronectin, snail, and MMP-9 expression in human cells *in vitro*
  - ↓ E-cadherin expression in human cells *in vitro*
  - No change in slug expression in human cells *in vitro*
- Cellular senescence markers
  - ↓ p21 expression in human cells *in vitro* (1 study)
- Telomerase expression, activity, or telomere length
  - ↓ telomere length in women (1 study)
  - Altered telomerase expression or activity in human cells *in vitro*

Section 5.3.9

Key Characteristics of Carcinogens: BPA

KC1
- Electrophilic metabolites
- DNA & protein adducts
- Oxidative lesions in DNA

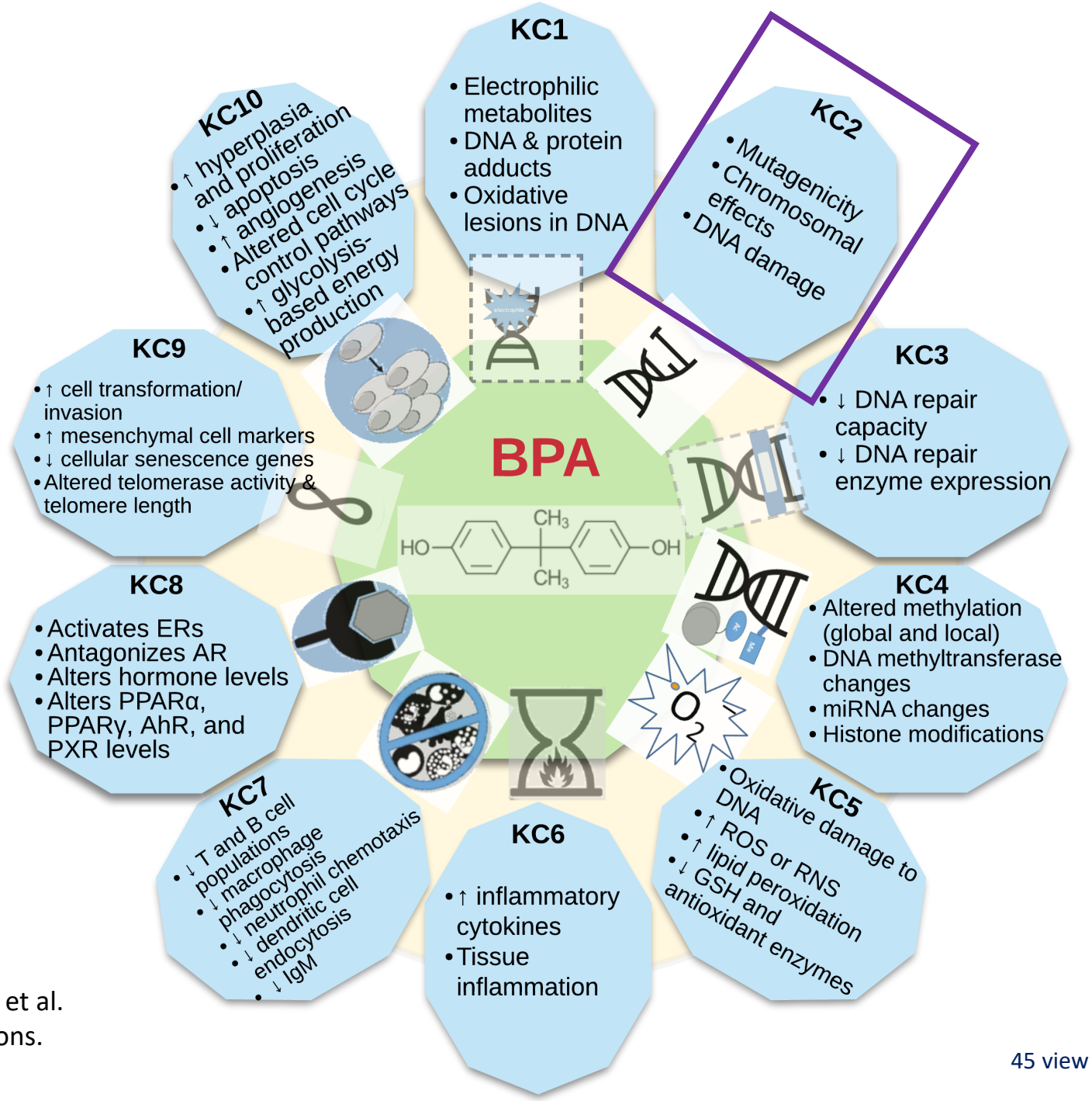


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC2

- Mutagenicity
- Chromosomal effects
- DNA damage

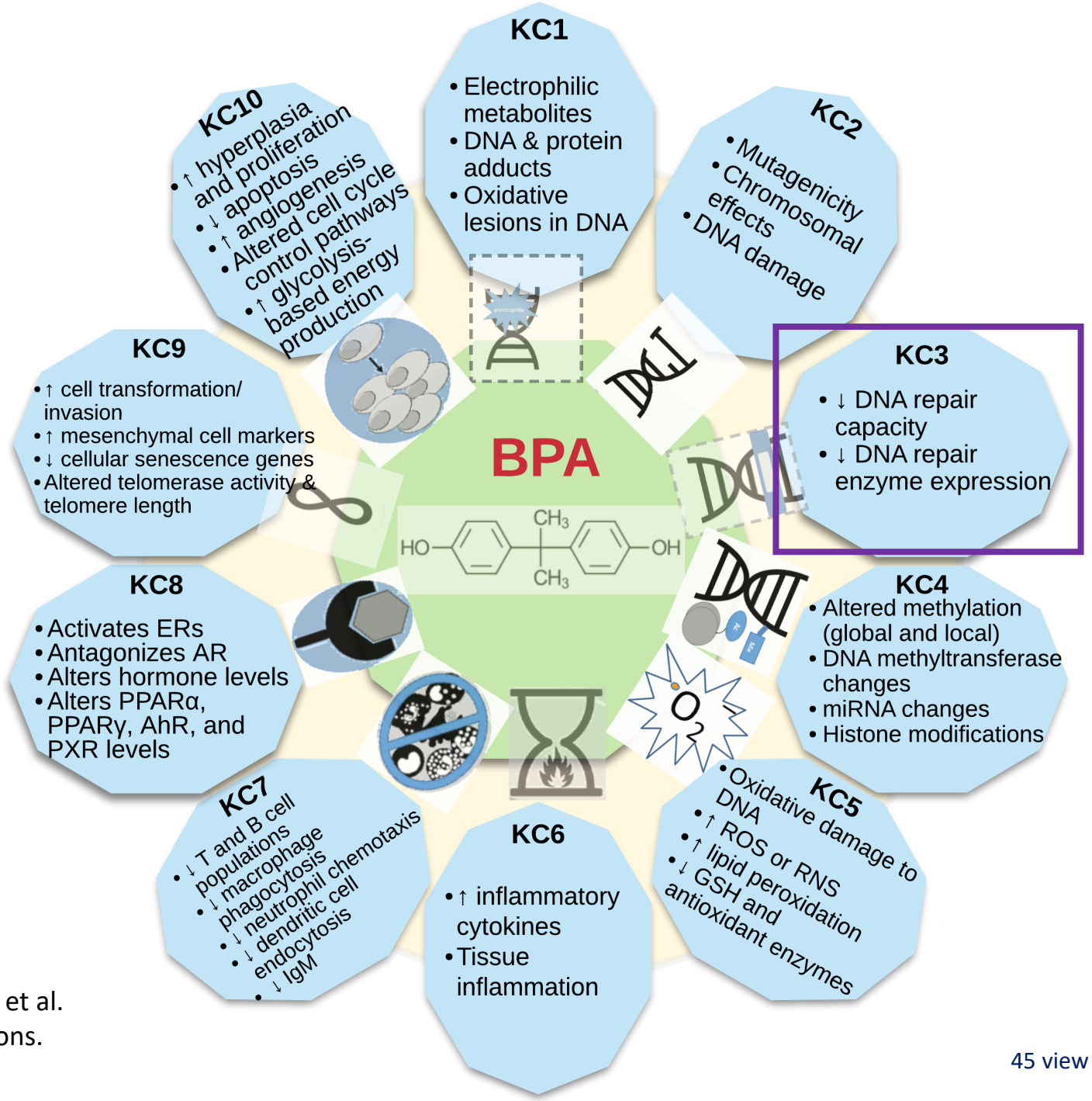


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC3

- ↓ DNA repair capacity
- ↓ DNA repair enzyme expression

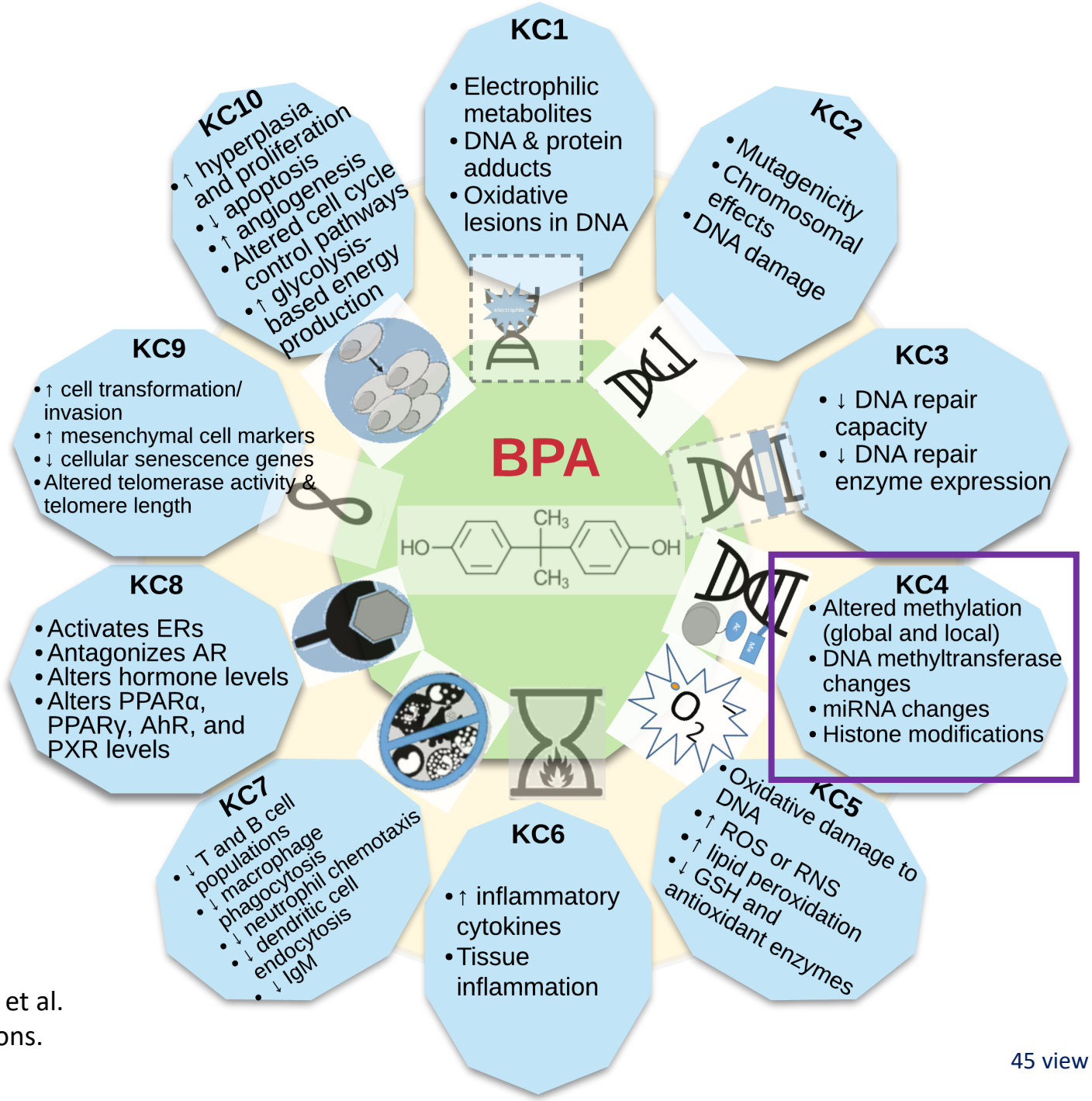


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC4

- Altered methylation (global and local)
- DNA methyltransferase changes
- miRNA changes
- Histone modifications

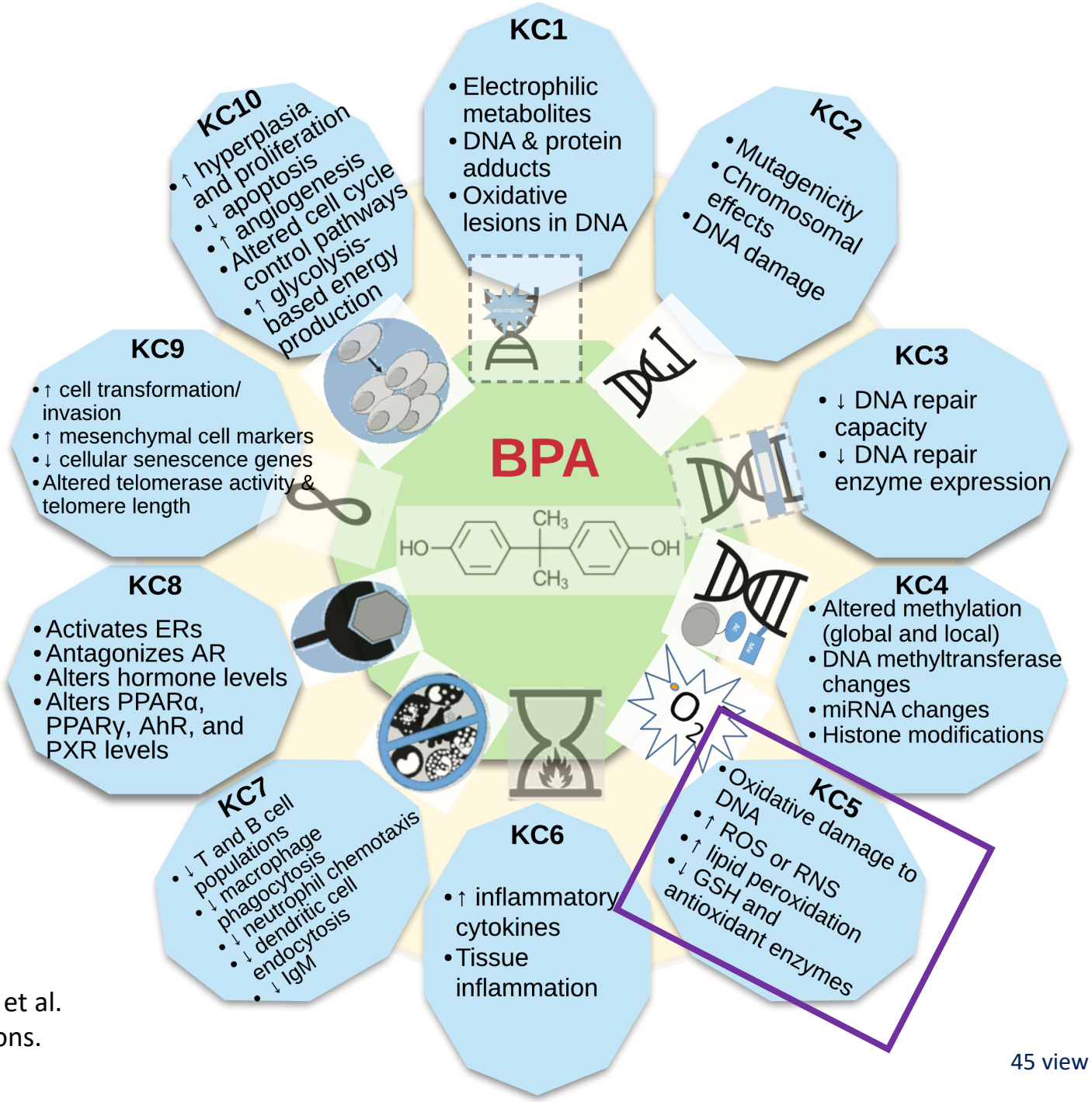


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC5

- Oxidative damage to DNA
- ↑ ROS or RNS
- ↑ lipid peroxidation
- ↓ GSH and antioxidant enzymes

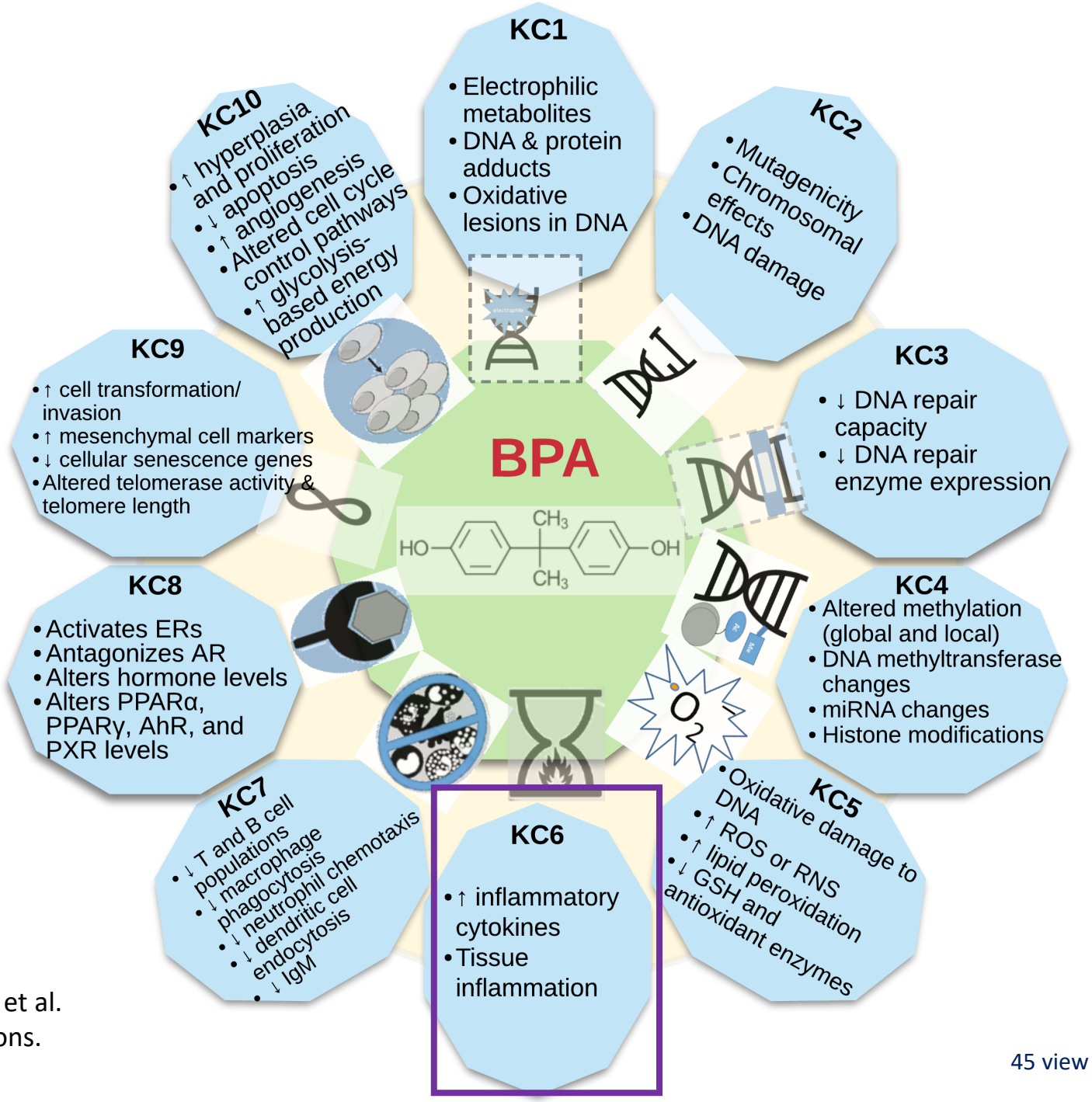


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC6

- ↑ inflammatory cytokines
- Tissue inflammation

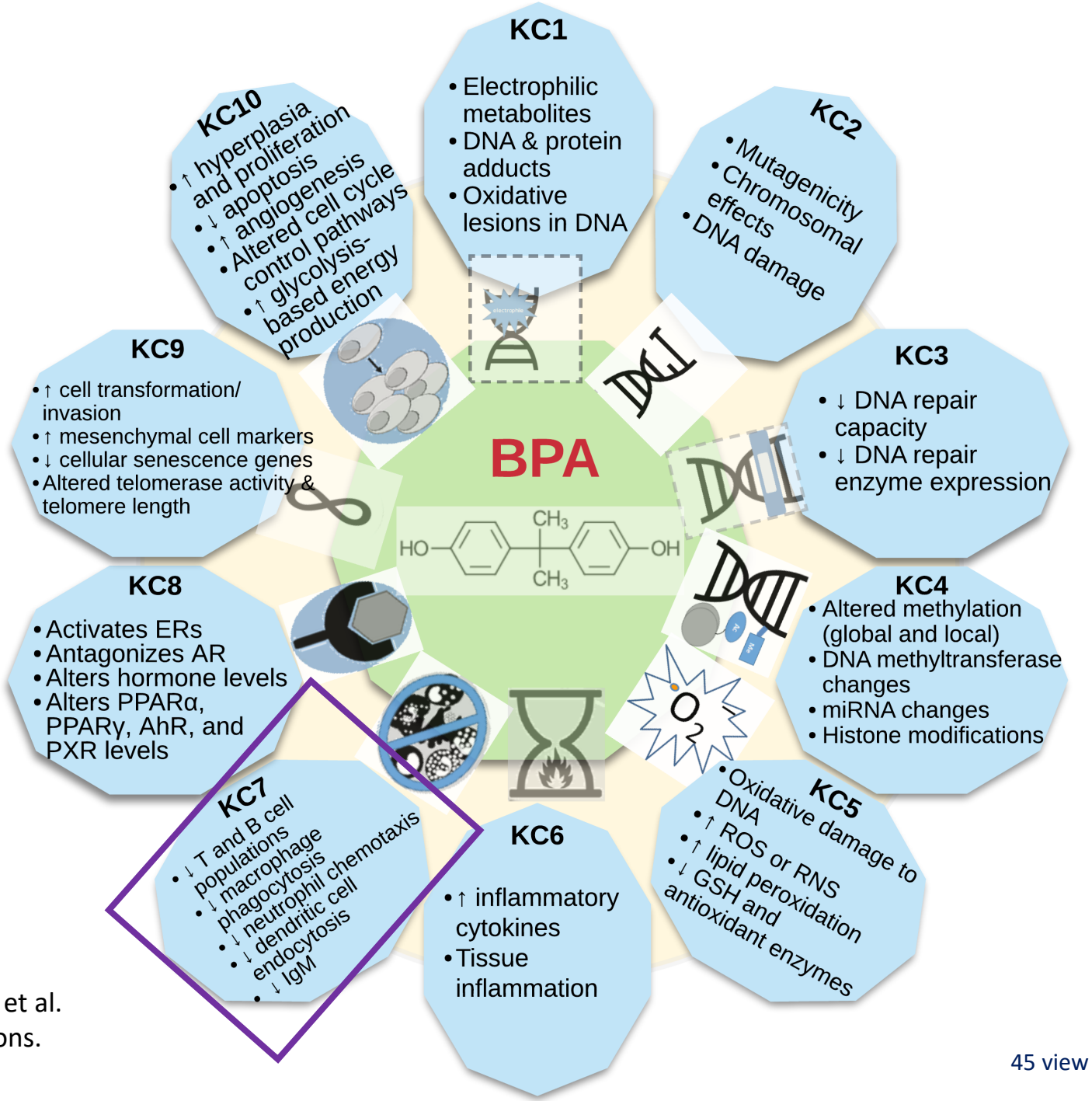


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC7

- ↓ T and B cell populations
- ↓ macrophage phagocytosis
- ↓ neutrophil chemotaxis
- ↓ dendritic cell endocytosis
- ↓ IgM

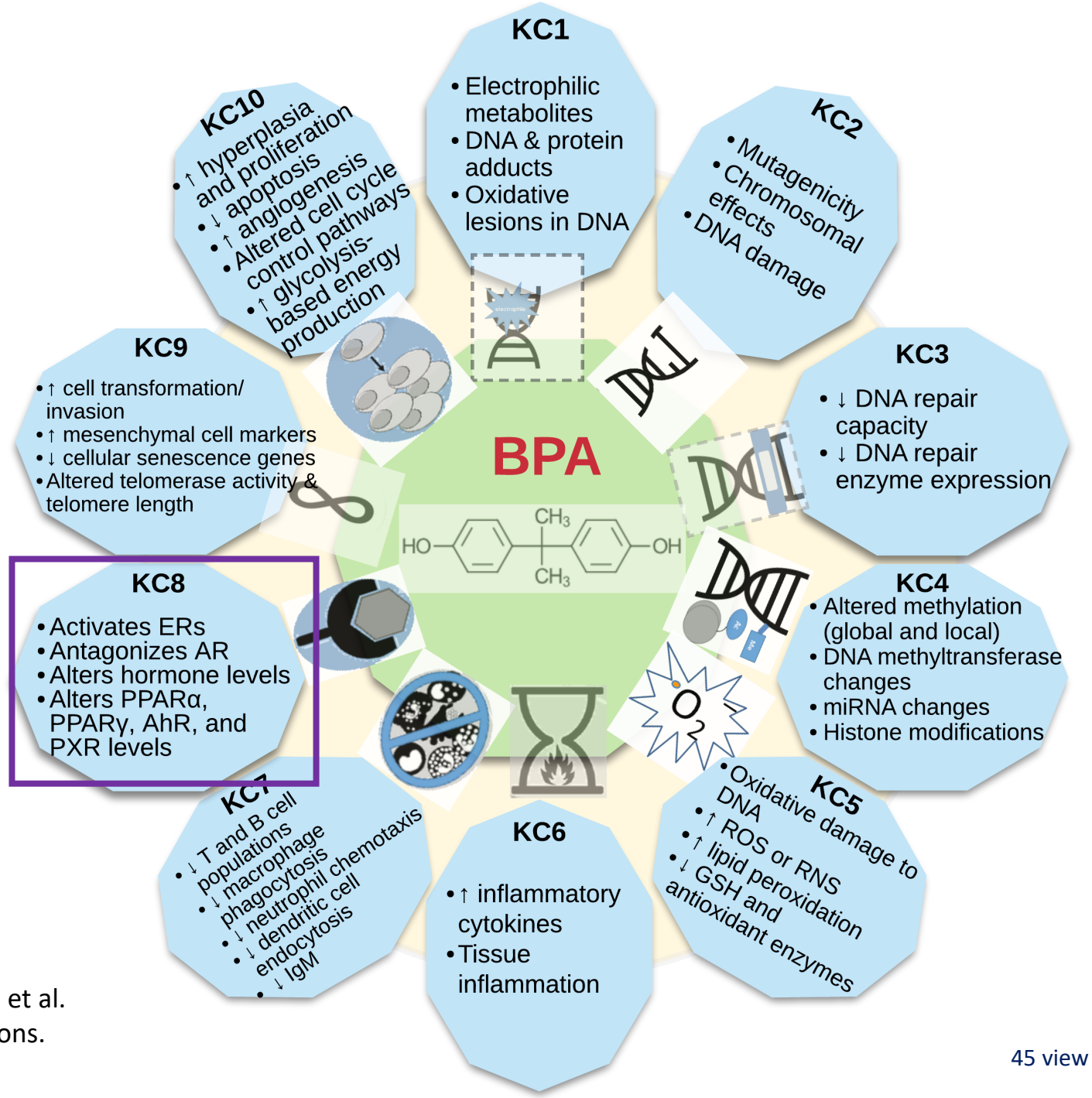


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC8

- Activates ERs
- Antagonizes AR
- Alters hormone levels
- Alters PPARα, PPARγ, AhR, and PXR levels

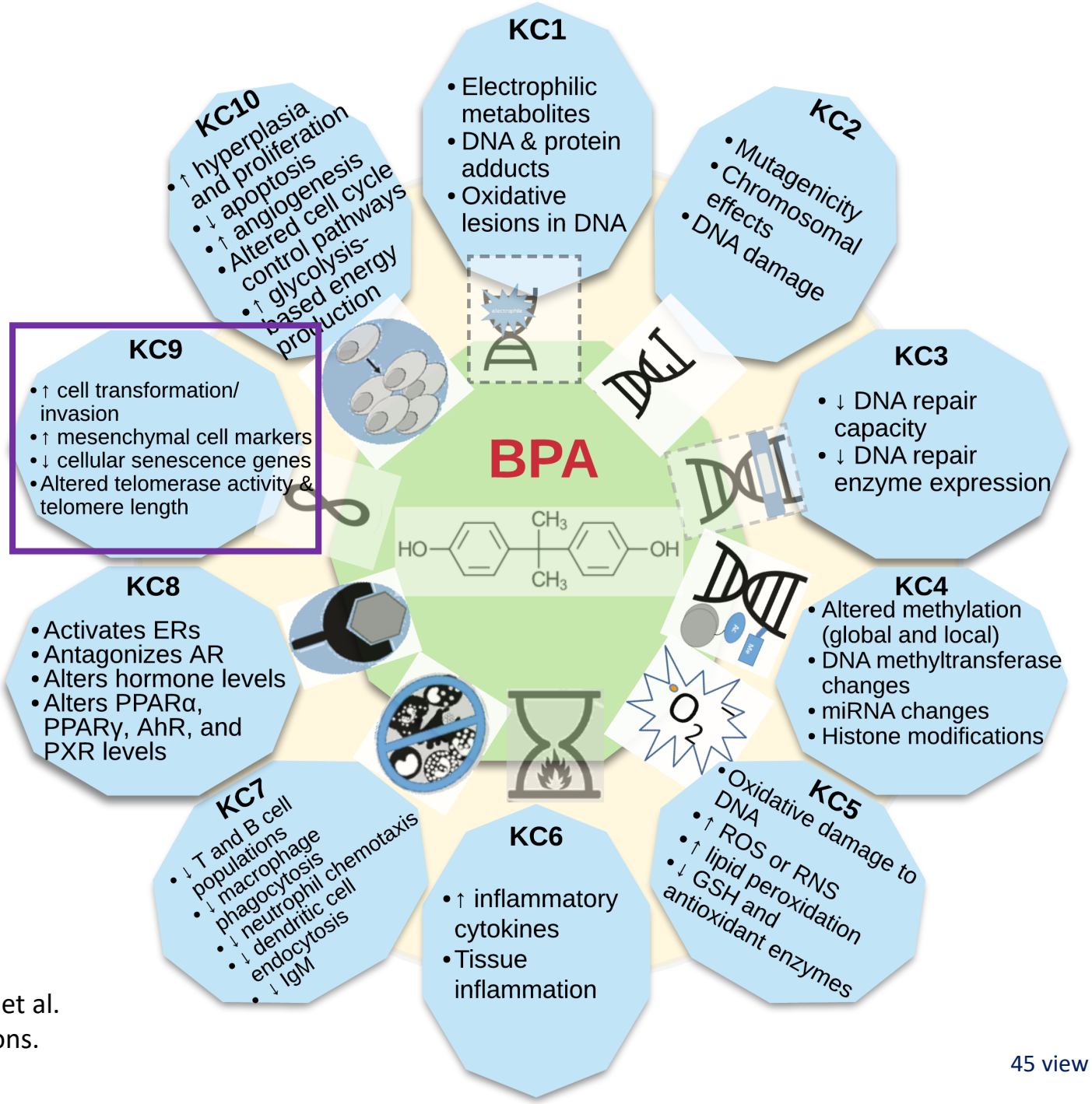


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC9

- ↑ cell transformation/ invasion
- ↑ mesenchymal cell markers
- ↓ cellular senescence genes
- Altered telomerase activity & telomere length

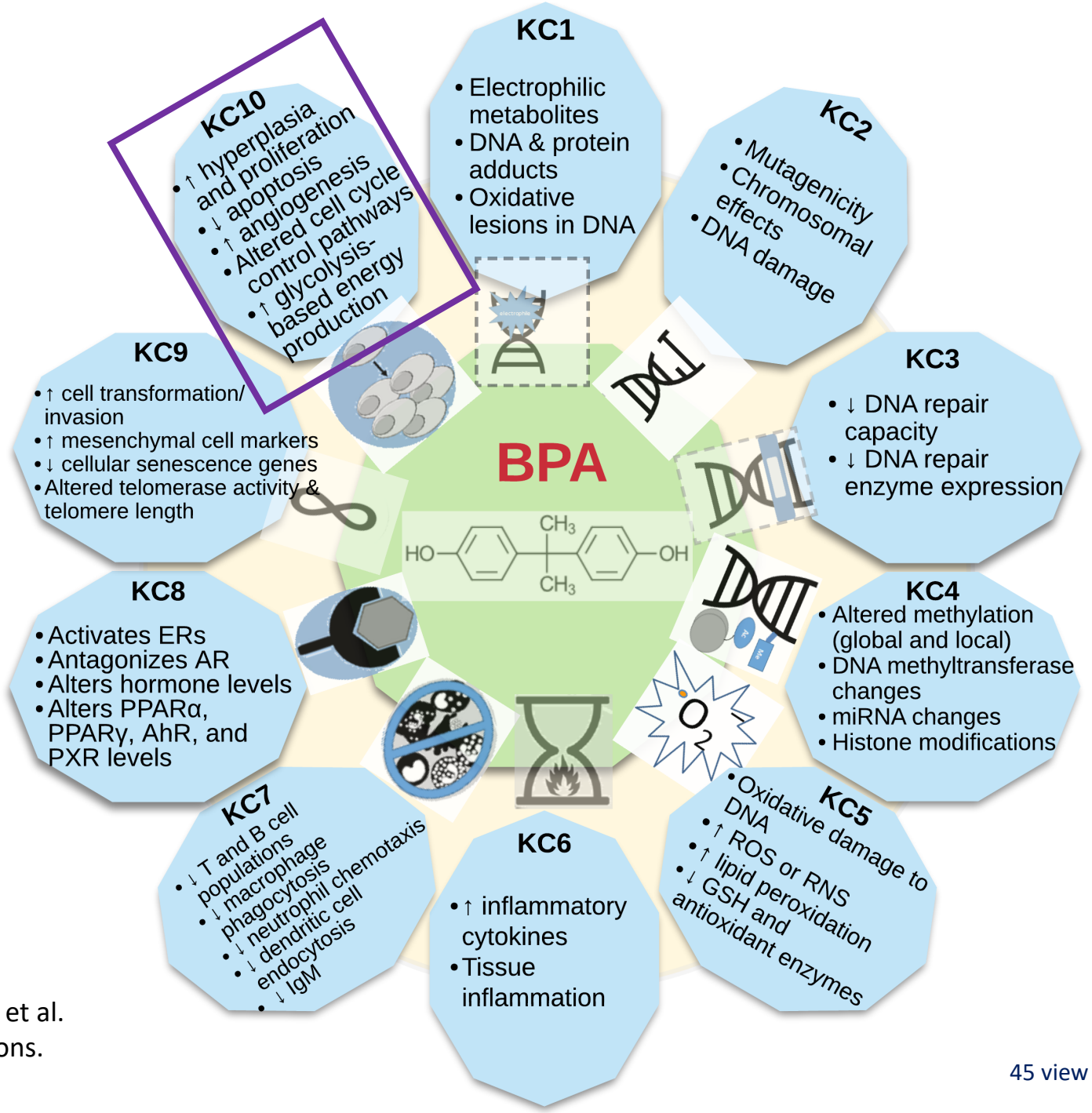


Images of the KCs are adapted from Guyton et al. (2018) & Smith et al. (2020) with modifications.

Key Characteristics of Carcinogens: BPA

KC10

- ↑ hyperplasia and proliferation
- ↓ apoptosis
- ↑ angiogenesis
- Altered cell cycle control pathways
- ↑ glycolysis-based energy production



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