Evidence on the Carcinogenicity of Butyl Benzyl Phthalate (BBP)

Jennifer C.Y. Hsieh, Ph.D.
Meng Sun, Ph.D.

Meeting of the Carcinogen Identification Committee
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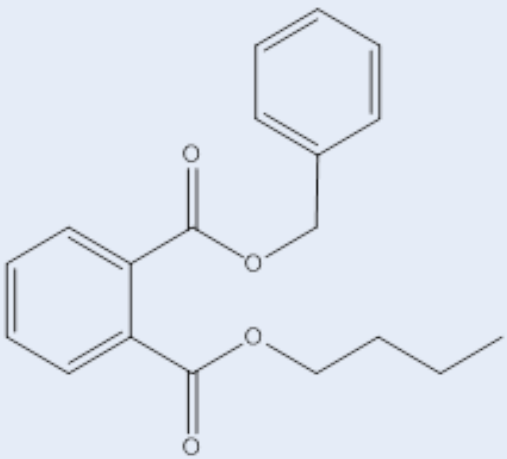
Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Overview

- BBP: use and biomonitoring
- Carcinogenicity Evidence
  - Human epidemiological evidence
  - Carcinogenicity studies in animals
  - Other relevant data
- Possible mechanisms of action
- Reviews by authoritative bodies
- Summary

Butyl Benzyl Phthalate (BBP)



CAS NO: 85-68-7

- **Uses**
  - A plasticizer in polyvinyl chloride (PVC) products, such as flooring tiles and carpet backing
  - Additive in food packaging, medical devices, leather coating, paint, adhesives, ink, etc.
  - Restricted in toys and childcare articles by U.S. and California laws since 2009 (≤ 0.1%)

Biomonitoring Studies

Urinary Biomarker, [MBzP[1]] (µg/L)	U.S./NHANES[2] (2009-2010)				Biomonitoring California (2010-2011)	
	All ages	6-11 years	12-19 years	>20 years	Firefighters (N=101)	Pregnant women (N=89)
Geometric Mean	**6.46**	**11.6**	**10.6**	**5.61**	**8.18**	**7.73**
95% Confidence interval	5.76-7.24	9.51-14.1	8.75-12.9	4.97-6.34	6.56-10.2	5.84-10.2

[1] Monobenzyl Phthalate
[2] National Health and Nutrition Examination Survey

BBP Carcinogenicity Evidence

- Human epidemiological evidence
- Carcinogenicity studies in animals
- Other relevant data

Human Epidemiological Evidence

Two case-control studies of breast cancer and BBP:

- *Aschengrau et al., 1998 (population-based)*
  - No significant associations with breast cancer risk and probable past occupational BBP exposure
  - Limitations:
    - Lack of information on non-occupational exposures
    - Cases included deceased individuals (number/percentage not mentioned)
      - used next-of-kin interviews
- *Lopez-Carillo et al., 2010 (hospital-based)*
  - Significant negative associations with level of urinary MBzP and breast cancer occurrence
  - Limitations: single urine sample was collected after cancer diagnosis

BBP Carcinogenicity Studies in Animals

Species	Strain	Sex	Route	Reference	No. of studies
Rat	F344/N	M, F	feed	NTP[1] (1982)	6
				NTP (1997a)	
				NTP (1997b)	
Mouse	$B6C3F_1$	M, F	feed	NTP (1982)	2
	Strain A	M	i.p. injection	Theiss *et al.* (1977)	1

[1] National Toxicology Program

Carcinogenicity Studies in Rats

- **NTP (1982)**
  - Male and female rat studies
  - Male rat study terminated early
- **NTP (1997a) and NTP (1997b)**
  - Male and female rat studies
  - Carried out at the same time in the same lab
  - NTP (1997a): regular cancer bioassays (every group given ***ad libitum*** feed)
  - NTP (1997b): **weight-matched** groups and **feed restriction** groups

Tumor incidence in female F344 rats, two-year feed study (NTP, 1982)

Tumor Site & Type		BBP Dose (ppm)			Trend Test
		0	6,000	12,000	
Hematopoietic System	MNCL	7/44	7/46	18/48*	$p < 0.01$
	Leukemia or Lymphoma	7/44	7/46	19/48*	$p < 0.01$
Liver	Hepatocellular Adenoma or Carcinoma	1/34	1/34	4/38	NS[1]

Fisher pairwise comparison with control: * $p < 0.05$

[1] NS, not significant ($p \geq 0.05$)

Tumor incidence in male F344 rats, two-year study (NTP, 1997a)

Tumor Site & Type		BBP Dose (ppm)				Trend Test
		0	3,000	6,000	12,000	
Pancreas	Acinar Cell Adenoma	3/41	2/40	3/46	10/44* (22.7%)	$p < 0.01$
	Acinar Cell Adenoma or Carcinoma	3/41	2/40	3/46	11/44* (25%)	$p < 0.01$

Fisher pairwise comparison with control: * $p < 0.05$

NTP historical incidence:

- Adenoma: 19/1,191 (1.6%)
- Carcinoma: 0/1,919 (0.0%)

Tumor incidence in female F344 rats, two-year study (NTP, 1997a)

Tumor Site & Type		BBP Dose (ppm)				Trend Test
		0	6,000	12,000	24,000	
Pancreas	Acinar Cell Adenoma	0/37	0/37	0/37	2/35 (5.7%)	NS
Urinary Bladder	Transitional Epithelium Hyperplasia	4/49	0/49	1/49	10/50	$p < 0.01$
	Transitional Epithelium Papilloma	1/48	0/47	0/49	2/49 (4.1%)	NS

NTP historical incidence:

- Acinar cell adenoma: 2/1,194 (0.2%)
- Transitional epithelium papilloma: 4/1,182 (0.3%)

Design of feed restriction studies in F344 rats (NTP, 1997b)

Comparison 1 is NTP (1997a)

Bioassay Protocol	# Animals	Comparison
***Ad libitum*-fed (2-year)**		
Control	50	1
Low dose	50	
Mid dose	50	
High dose	50	2
Weight-matched Control	50	
Feed Restricted (2-year)		
Control	50	3
High dose	50	
Feed Restricted (3-year or 20% survival)		
Control	50	4
High dose	50	

Design of feed restriction studies in F344 rats (NTP, 1997b)

Bioassay Protocol	# Animals	Comparison
***Ad libitum*-fed (2-year)**		
Control	50	1, 2
Low dose	50	1
Mid dose	50	1
High dose	50	1, 2
Weight-matched Control	50	2
Feed Restricted (2-year)		
Control	50	3
High dose	50	3
Feed Restricted (3-year or 20% survival)		
Control	50	4
High dose	50	4

Positive findings in male rats

Design of feed restriction studies in F344 rats (NTP, 1997b)

Bioassay Protocol	# Animals	Comparison
***Ad libitum*-fed (2-year)**		
Control	50	1, 2
Low dose	50	1
Mid dose	50	1
High dose	50	2
Weight-matched Control	50	2
Feed Restricted (2-year)		
Control	50	3
High dose	50	3
Feed Restricted (3-year or 20% survival)		
Control	50	4
High dose	50	4

Positive findings in female rats

Tumor incidence in male F344 rats, two-year study (NTP, 1997b)

Comparison 1 (NTP, 1997a)

Tumor Site & Type		BBP Dose (ppm)		
		Ad Libitum-fed Control	Weight-Matched Control	12,000 ppm
Pancreas	Acinar Cell Adenoma	3/41*	0/44***	10/44
	Acinar Cell Adenoma or Carcinoma	3/41*	1/45**	11/44
Adrenal Medulla	Benign or Malignant Pheochromocytoma	10/44	3/45*	10/45
Hematopoietic System	MNCL	31/50	15/50**	30/50

Fisher pairwise comparison: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Tumor incidence in male F344 rats, two-year study (NTP, 1997b)

Comparison 2

Tumor Site & Type		BBP Dose (ppm)		
		Ad Libitum-fed Control	Weight-Matched Control	12,000 ppm
Pancreas	Acinar Cell Adenoma	3/41*	0/44***	10/44
	Acinar Cell Adenoma or Carcinoma	3/41*	1/45**	11/44
Adrenal Medulla	Benign or Malignant Pheochromocytoma	10/44	3/45*	10/45
Hematopoietic System	MNCL	31/50	15/50**	30/50

Fisher pairwise comparison: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Tumor incidence in male F344 rats, 30-month feed restriction study (NTP, 1997b)

Tumor Site & Type		BBP Dose (ppm)	
		Control	12,000
Pancreas	Acinar Cell Adenoma[1]	0/10	3/13 (23.1%)

[1] The denominators are small because tumors were observed late in the study (Day 897).

NTP historical incidence: 19/1,191 (1.6%)

Tumor incidence in female F344 rats, 3-year feed restriction study (NTP, 1997b)

Tumor Site & Type		BBP Dose (ppm)	
		Control	24,000
Urinary bladder	Transitional Epithelium Hyperplasia	0/50	16/50***
	Transitional Epithelium Carcinoma	0/39	4/45 (8.9%)
	Transitional Epithelium Papilloma or Carcinoma	1/39	6/45 (13.3%)

NTP historical incidence:

- Carcinoma: 0/1,332 (0.0%)
- Papilloma or Carcinoma: 4/1,332 (0.3%)

Co-carcinogenicity Studies in Rats

Strain	Sex	Treatment	Route	Tumor Site Examined	Reference
Sprague -Dawley	F	DMBA + BBP	gavage	mammary	Singletary *et al.* (1997)
F344	M	DMAB + BBP	feed	prostate	Kohno *et al.* (2004)

DMBA: 7, 12-dimethylbenz[a]anthracene
DMAB: 3,2'-dimethyl-4-aminobiphenyl

Carcinogenicity Studies in Mice

- **Two studies in B6C3F$_1$ mice (NTP, 1982)**
  - Two-year feed studies in males and females
  - BBP Doses: 0, 6000, or 12000 ppm
  - No statistically significant increases of any tumors
- **Short-term study in male Strain A mice (Theiss *et al.*, 1977)**
  - 24-week study via i.p. injection
  - BBP Doses: 160, 400, or 800 mg/kg/week
  - Mice were only examined for pulmonary adenomas
  - No increase in number of lung tumors per mouse

Other Relevant Data

- Genotoxicity
- *In vitro* transformation study
- Pharmacokinetics and metabolism
- Effects on breast tumor susceptibility and development
- Effects on cancer-related protein expression in HepG2 cells
- Structure-activity comparisons

In vitro Genotoxicity

- **Mammalian**
  - **Positive**
    - DNA base lesions in a mouse MC3T3-E1 osteoblast cell line and primary calvarial osteoblast cells
    - DNA single strand breaks in human HepG2 cell line
    - DNA-protein crosslinks (DPCs) in rat liver homogenate
  - **Negative**
    - Forward mutations in mouse lymphoma cells
    - Sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells
    - Chromosomal aberrations (CAs) in CHO cells
- **Bacterial**: **negative** in mutation assays

In Vivo Genotoxicity

- **Positive**
  - SCEs and CAs in male $B6C3F_1$ mice
  - DNA-protein crosslinks (DPCs) in mouse hepatic cells
- **Negative**
  - Micronucleus assay in mouse bone marrow
  - Dominant lethality in mouse fetuses

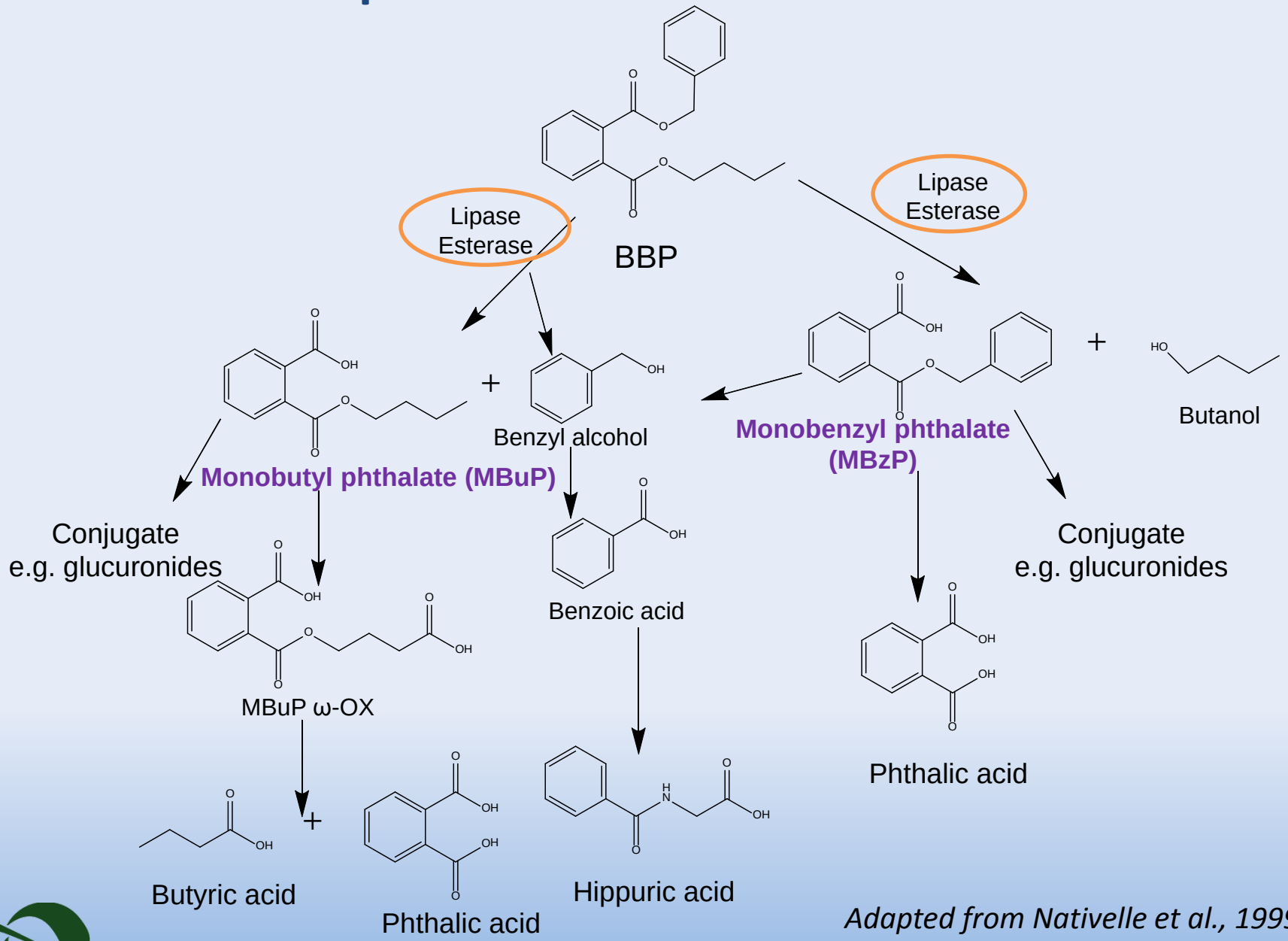
In Vitro Transformation Study

- **Positive** for *in vitro* transformation of Syrian hamster embryo (SHE) cells

Pharmacokinetics and Metabolism

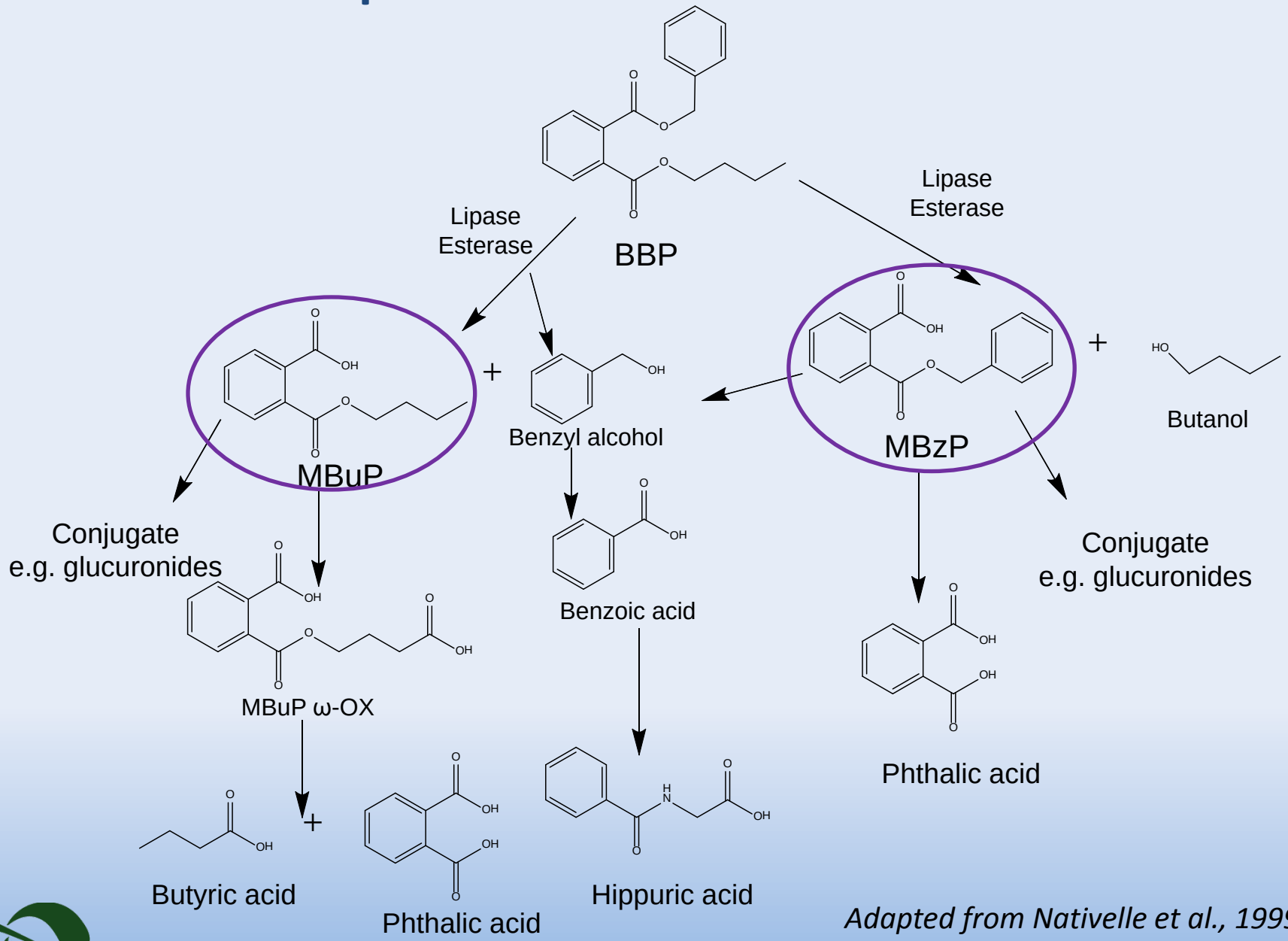
- Similar in both humans and rats
- Rapidly absorbed, distributed, metabolized and eliminated within 24 hours after oral exposure
- Majority of metabolites are excreted in the urine and feces
- No long-term tissue accumulation occurs

Proposed Routes of BBP Metabolism



Adapted from Nativelle et al., 1999

Proposed Routes of BBP Metabolism



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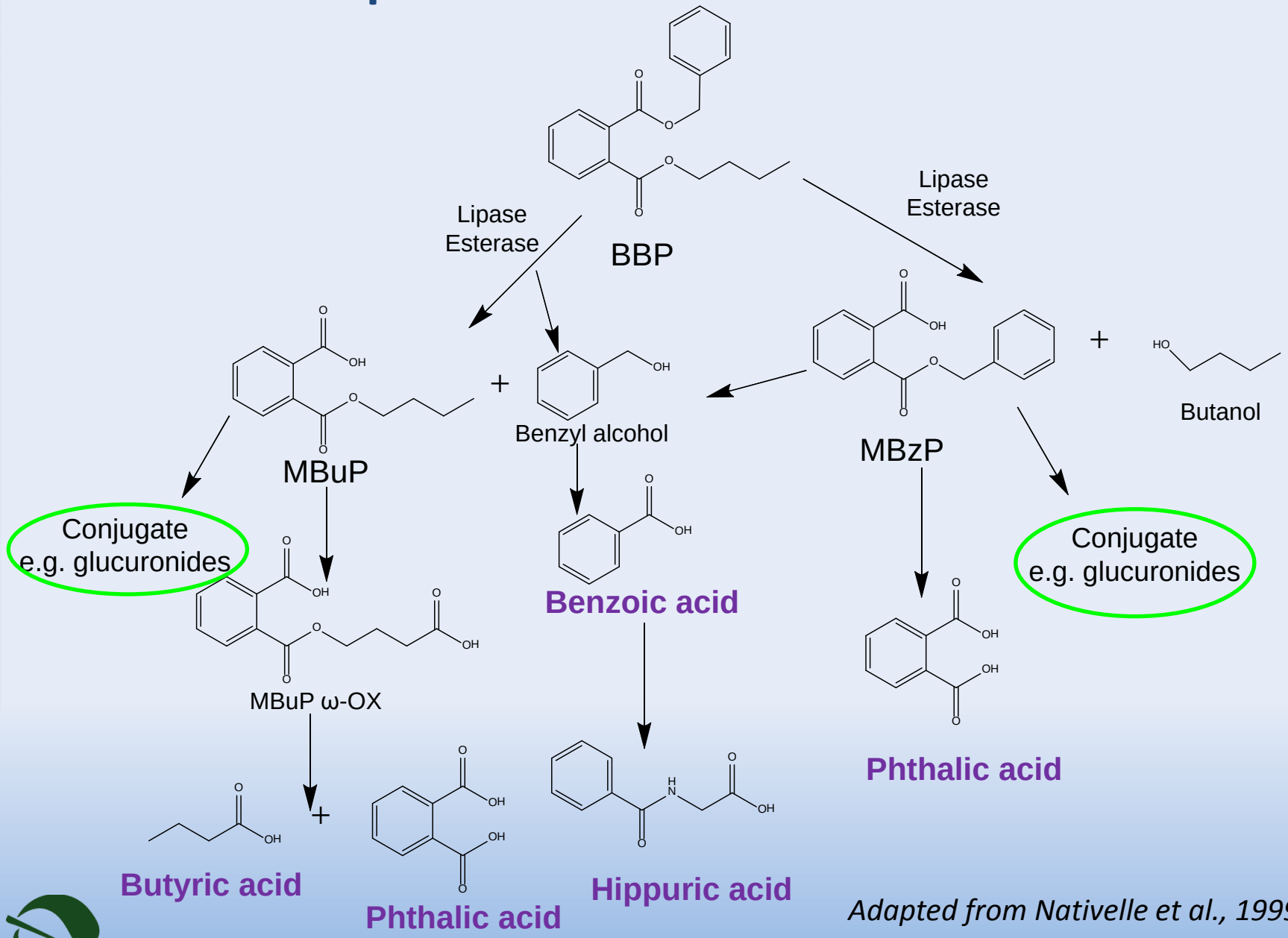
Proposed Routes of BBP Metabolism

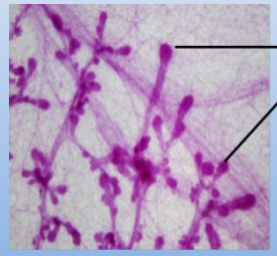


Adapted from Nativelle et al., 1999

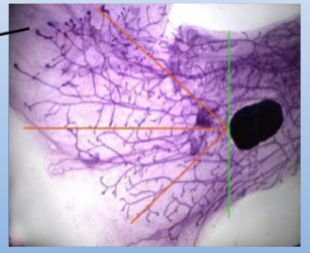
BBP Effects on Mammary Development

***In utero* and early-life exposure in SD rats** (Moral *et al.,* 2007; 2011)

- Altered cancer-related gene expression in mammary gland of female offspring
  - Neonatal/prepubertal exposure: ↑ genes involved in proliferation, communication, signal transduction
  - In utero exposure: ↓ genes involved in differentiation, lactation, immune response, apoptosis
- Increased cell proliferation index of gland structures, e.g. terminal duct (TD) and terminal end bud (TEB)
- Altered mammary gland morphology, e.g. ↑ number of TEB
- Potentially elevates breast cancer susceptibility



TEB



Effects of BBP on Multiple Stages of Neoplastic Transformation in Human Breast Cell Lines

Tumor Progression

Normal	↑Proliferation ↓ Apoptosis			Angiogenesis			Epithelial-mesenchymal transition			Invasion and migration			Metastasis		
	Gene expression	√	√	Gene expression	√	√	Gene expression	√		Gene expression	√	√	Gene expression	√	
	In vitro	√	√	*In vitro*	√	√	*In vitro*	√		*In vitro*	√	√	*In vitro*		
	*In vivo**			*In vitro/ in vivo#*	√	√	*In vivo**			*In vivo**			*In vivo**	√	

√ (epithelial stem cells): Data were collected from BBP-treated R2d cells (Hsieh *et al*., 2012a,b)
√ (cancer cells): Data were collected from BBP-treated MCF-7 or MDA-MB-231 cells (Hsieh *et al*., 2012a,b,c & Kim *et al*., 2011 *etc*.)
In vivo*: Cells were xenografted into athymic nude mice, then mice were treated with BBP.
***In vitro/in vivo#*:** BBP-treated cells were mixed with matrigel and injected into nude mice.

Effects of BBP on Protein Expression in Human Liver Cancer Cell Line

- Proteomic analysis in HepG2 cells (Choi *et al.,* 2010): Alteration of proteins involved in tumor progression, metastasis, oxidative stress *(Table 21)*

Functions	Change /Gene symbol
Tumor progression & metastasis	↓DEK
	↑Gelsolin
	↓ITIH2
	↑RKIP
	↑Rho-GDI
Oxidative stress	↓Cystatin C

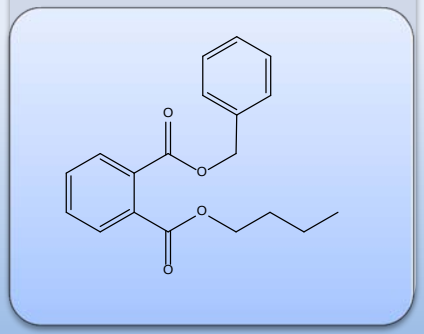
Structurally Related Phthalates: Common Tumor Sites

Chemical	MNCL		Pancreas		Renal and Bladder		Liver		Testicular interstitial cell		Uterus	
	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats
BBP		M F		M[1]		F[3]						
DEHP		M		M[1]			M F	M F		M		
DINP		M F	F[2]	M[2]		M[4]	M F	M F		M		F[5]

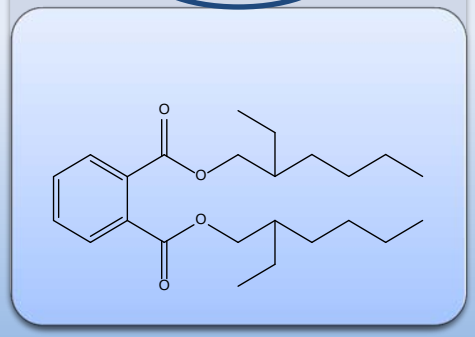
[1]Pancreatic acinar cell adenoma or carcinoma; [2]Pancreatic islet cell carcinoma; [3]Bladder transitional epithelium hyperplasia, papilloma and carcinoma; [4]Renal transitional epithelial cell carcinoma and renal tubular carcinoma; [5]Endometrial adenocarcinoma

DEHP: Diethyl hexyl phthalate **(Prop.65, IARC 2B & USEPA B2)**; **DINP**: Diisononyl phthalate (OEHHA, 2013)

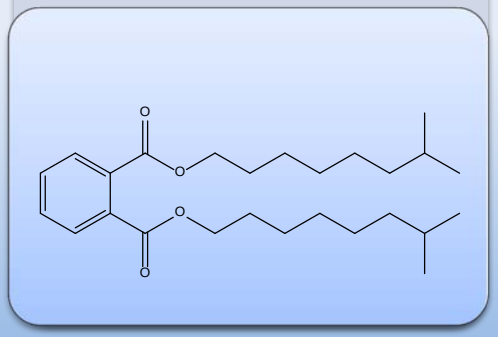
BBP



DEHP



DINP



Structurally Related Phthalates: Common Effects

Chemical	Genotoxicity			In vitro cell transformation	PPARα & γ	ER	AhR	PXR	CAR	GJIC inhibition	Anti-androgenic & anti-steroidogenesis
	DNA damage	Gene mutations	Chromosomal damage								
BBP	+	-	+	+	+	+	+	+	NE	NE	+
DEHP	+	+	+	+	+	+	+	+	+	+	+
DINP	NE	-	-	+/-[1]	+	+	-	+	+	+	+

NE: Not evaluated. PPAR: Peroxisome proliferator activated receptor. ER: Estrogen receptor. AhR: Aryl hydrocarbon receptor. PXR: Pregnane X receptor. CAR: Constitutive androstane receptor. (These receptors are present in the target tumor sites in BBP-treated animals) GJIC: Gap junction intercellular communication. [1]One of nine assays positive.

Possible Mechanisms of Action

- Genotoxicity (DNA and chromosomal damage)
- Aryl hydrocarbon receptor (AhR)-mediated
  - ↑tumor progression in MDA-MB-231 cells
- Estrogen receptor (ER)-mediated
  - ↑cell proliferation in human breast cell lines
  - ↑angiogenesis in MCF-7 derived cells
  - ↑epithelial-mesenchymal transition in R2d cells
- Peroxisome proliferator activated receptor (PPARα & γ) -mediated
- Anti-androgenic and anti-steroidogenesis
- Epigenetic mechanisms

Reviews by Authoritative Bodies

- U.S. EPA Classified in Group C, “possible human carcinogen” in 1993
  - BBP is currently under re-assessment by U.S. EPA
- IARC
  - Classified in Group 3, “not classifiable as to its carcinogenicity to humans” in 1999
    - Inadequate evidence in humans
    - Limited evidence in experimental animals
- BBP has not been classified as to its carcinogenicity by U.S. FDA or NIOSH.

Summary of Evidence

Human epidemiological evidence

- Two case-control studies of breast cancer, both with limitations in study design. No positive associations.

Animal carcinogenesis data

- Statistically significant increases in tumor incidence:
  - Mononuclear cell leukemia in male and female rats
  - Adrenal medulla tumors in male rats
  - Pancreatic acinar cell tumors in male rats (rare)
- Tumor incidence increase not statistically significant, but tumor types considered to be rare:
  - Bladder transitional epithelium papilloma and carcinoma in female rats (rare), with hyperplasia
  - Pancreatic acinar cell tumors in male and female rats (rare)

Summary of Evidence (Cont.)

Other Relevant Data

- Multiple positive genotoxicity studies in mammalian cells
- Morphological transformation of SHE cells *in vitro*
- Alter expression of carcinogenicity associated genes and proteins
- Induce multiple stages of neoplastic transformation in human breast cell lines
- Common tumor sites with DINP and DEHP