ETHYLENE GLYCOL, DIETHYLENE GLYCOL, DIOXANE AND RELATED COMPOUNDS

CARCINOGENICITY AND STRUCTURE-ACTIVITY
RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES.
METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

Yin-tak Woo, Ph.D.
Joseph C. Arcos, D.Sc., and
Mary F. Argus, Ph.D.

Preparation for the Chemical Hazard
Identification Branch "Current
Awareness" Program

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5.2.2.5 Phenols and Phenolic Compounds.

5.2.2.5.1 Introduction.

Phenols and phenolic compounds (hereafter collectively called phenolics) represent an important class of compounds that helped to formulate some of the basic concepts of chemical carcinogenesis. Depending on their chemical structure, phenolics may have complete carcinogenic, tumor-initiating, tumor-promoting, or cocarcinogenic activity. Some phenolics inhibit the carcinogenic activity of certain carcinogens, suggesting the possibility of their use as chemoprophylactic agents. Phenolics are ubiquitously present in the environment due to their natural occurrence as well as extensive industrial uses. This section focuses on comercially used phenolics and some naturally occurring simple phenolics; botanical phenolics with complex structures will be discussed in Section 5.3.2.

Owing to their desirable physicochemical properties and biological activities, phenolics have found numerous applications as antioxidants, stabilizers, antibacterials, preservatives, solvents, redox reagents, pharmaceuticals, chemical intermediates, food flavoring agents, etc. Consumer products containing phenolics include processed foods, cosmetics, dyes, drugs, photographic chemicals, pesticides, gasoline, lubricants, adhesives, disinfectants, soaps, paints and paint removers (see also Section 5.2.2.5.5). With an annual production of 2.38 billion lbs. in 1978, phenol ranked 38th in production volume among U.S. chemicals (cited in ref. 1). The annual U.S. consumption of phenolic antioxidants is approximately 1,400, 450 and 230 metric tons for butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate, respectively (2). Other phenolics with production volume exceeding one million lbs. in recent years include bisphenol A, 2,4-dichlorophenol,

pentachlorophenol, cresols, resorcinol and hydroquinone. Eugenol, a widely used food flavor and fragrance additive, had a production volume of 425,000 lbs. in the United States in 1978 (cited in ref. 3). In addition to the above consumer products, human exposure to phenolics may also occur through polluted air or water, tobacco smoke, natural foodstuffs or indirectly via exposure to aromatic compounds which may be hydroxylated to phenolics in the body.

As may be expected from their wide occurrence in consumer products and in the environment, human exposure to some phenolics may be extensive. The potential carcinogenic risk of such exposure does not seem to have attracted much attention until recently. The general lack of high concern may be due to the facts that (a) phenolics (e.g., norepinephrine, tyramine, dopamine, homogentistic acid) are normal constituents of animal and plant tissues where they play a role in metabolic regulation (4), (b) aromatic hydroxylation is generally assumed to be "detoxifying" in nature, and (c) some phenolics may inhibit carcinogenesis (see Section 5.2.2.5.3.4). However, recent mutagenicity studies have indicated that some types of phenolics may be more hazardous than generally assumed. Metabolic studies on benzene (see Appendix I for its carcinogenicity) have implicated polyhydric phenols as the reactive intermediates. This section discusses the carcinogenicity and mutagenicity data on phenolics according to the type of substituents present in the ring, the possible chemical or biochemical mechanisms leading to potential reactive intermediates, and the tumor-promoting, cocarcinogenic and inhibitory activities.

5.2.2.5.2 Physicochemical Properties and Biological Effects.

5.2.2.5.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

The physical and chemical properties of phenolics have been described in several excellent reviews (5-16) and in standard textbooks on organic chemistry (e.g., 17). The physicochemical properties of some widely used simple phenolics are summarized in Table A. The structural formulas of the phenolics discussed in this section are depicted in Table B and D. In general, phenols are more polar, can form stronger hydrogen bonds, and have a greater ability to act as solvents for polar organic molecules, than their corresponding saturated alcohols (e.g., cyclohexanol). The hydroxyl group of phenol (pKa=9.9) is considerably more acidic than that of cyclohexanol (pKa approximately 18). The greater acidity of phenols is attributed to the greater stability of phenoxide anion in which the unshared electron pair is delocalized because of resonance:

[text figure 1]

The acidity of phenols is greatly enhanced by substitution with electron-withdrawing atoms or groups (e.g., $-NO_2$, -Cl), whereas electron-donating substituents (e.g., -R, -OH, -OR, $-NH_2$) tend to have an opposite effect (see Table A). The partition coefficient (log P) of phenols is increased by substitution with halogen, alkyl or nitro group(s) whereas highly polar substituents (such as a hydroxyl group) may lower the log P. Both pKa and log P may affect pharmacokinetic properties and biological effects of phenolics.

Table A. Physicochemical Properties of Phenol and its Derivatives[a]

Compound[b]	m.p. (°C)	b.p. (°C)	Vapor Pressure (mm Hg)	Solubility[c] (gm/100 ml H_2O)	pKa	$\log P_{oct}$[d]
Phenol	40.9	182	1.27 at 41°C	6.6	9.89	1.46
o-Cresol	30.9	191	0.25 at 25°C	2.5	--	1.95
m-Cresol	12.0	203	0.15 at 25°C	2.2	--	1.96
p-Cresol	34.8	202	0.11 at 25°C	1.9	9.82	1.94
2,4-Xylenol	27-28	210	1 at 52.8°C	--	10.6	--
2-Chlorophenol	8.7	175	1 at 12.1°C	<0.1	8.49	2.15
2,4-Dichlorophenol	43-44	210	1 at 53°C	0.45	7.68	3.06
2,4,5-Trichlorophenol	68	245	1 at 72°C	0.12	7.43	3.72
2,4,6-Trichlorophenol	68	246	1 at 76.5°C	0.09	7.42	3.62
2,3,4,6-Tetrachlorophenol	69-70	64 (at 22 mm Hg)	--	0.10	5.38	4.10
Pentachlorophenol	190	309-310	0.12 at 100°C	0.0014 (20°C)	4.96	5.01
2-Nitrophenol	44-45	214-216	1 at 49°c	0.032 (38°C)	7.13	1.77
2,4-Dinitrophenol	114-115	sublimes	--	0.079	4.0	1.51

Table A. Physicochemical Properties of Phenol and its Derivatives[a]
(continued)

Compound[b]	m.p. (°C)	b.p. (°C)	Vapor Pressure (mm Hg)	Solubility[c] (gm/100 ml H_2O)	pKa	$\log P_{oct}$[d]
Picric acid	122-123	300	1 at 195°C	0.67 (100°C)	0.8	2.03
Catechol	105	245.5	volatile in steam	soluble[e]	9.48	--
Resorcinol	111	280	53 at 190°C	58.8	9.44	0.78
Hydroquinone	173-174	285 (at 730 mm Hg)	4 at 150°C	7.0	10.0	--

[a]Summarized from data compiled in Kirk-Othmer Encyclo. Chem. Tech. (3rd ed.) 5, 864 (1979) and 17, 373 (1982); Intern. Agcy. Res. Cancer Monog., 15, 155 (1977); Natl. Inst. Occup. Safety Health (NIOSH) Publ. Nos. 78-133 (Cresol) and 78-155 (Hydroquinone); U.S. Environ. Protection Agcy. (EPA) Publ. Nos. 440/5-80-032 (Chlorinated phenols), 440/5-80-044 (2,4-Dimethylphenol) and 440/5-80-063 (Nitrophenols); "Merck Index" (9th ed.), Merck and Co., Rahway, NJ, 1976; Y. Yasuda, K. Tochikubo Y, Hashicuka et al., J. Med. Chem. 25, 315 (1982); C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley, New York, 1979.

[b]For structural formulas, see Tables B and D.

[c]Unless specified, solubilities are measured at 25°C.

[d]Logarithm of water-octanol partition coefficient.

[e]Soluble in 2.3 parts water

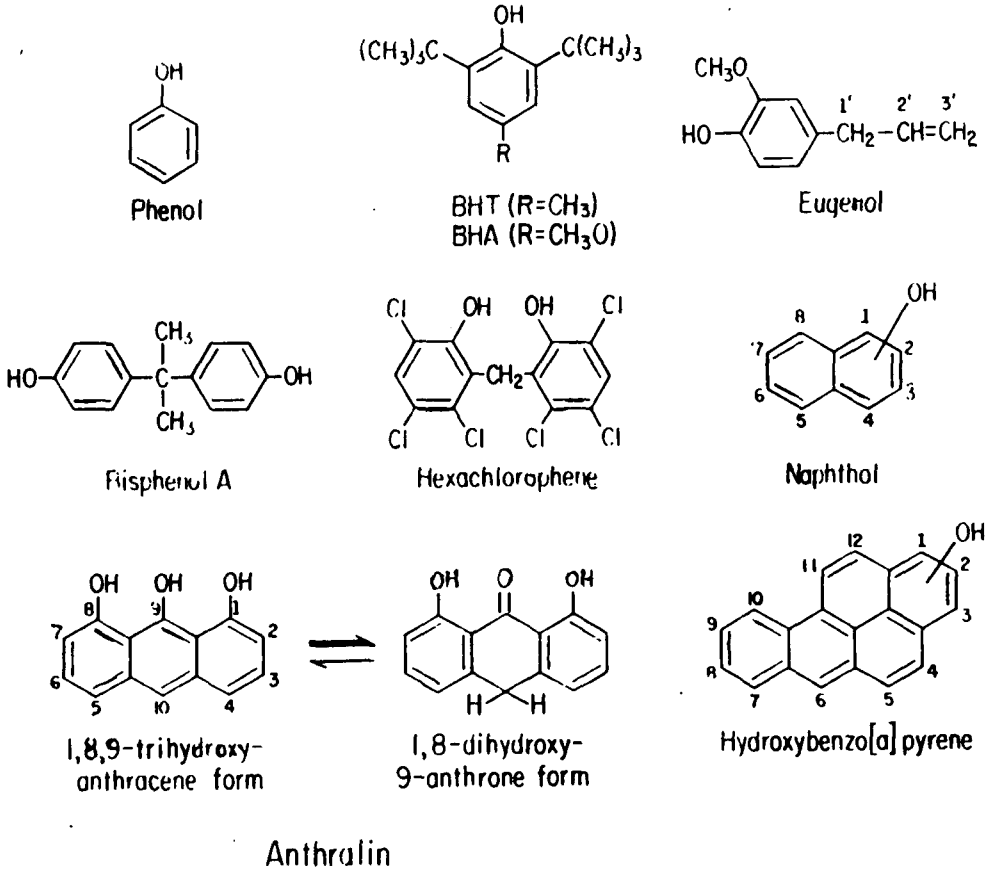
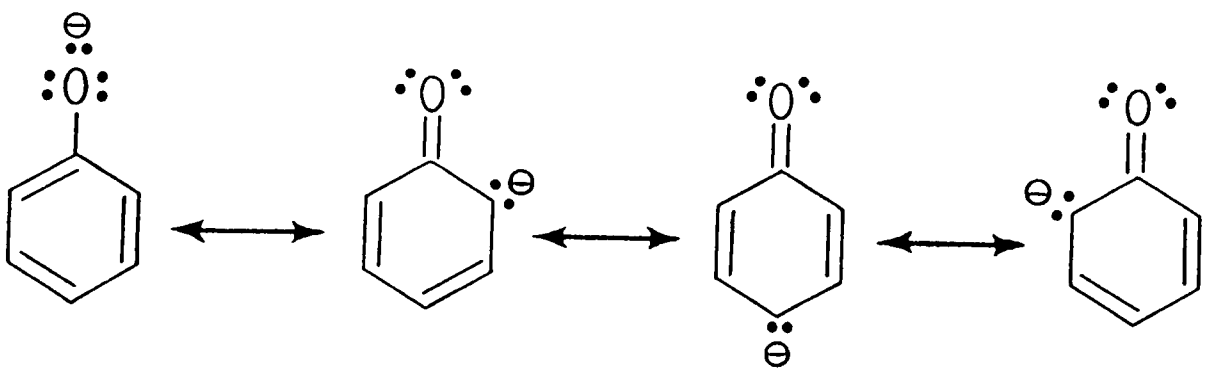


Table B. Structural Formulae of Some Phenolics that have been Tested for Carcinogenicity.



Text-Figure 1

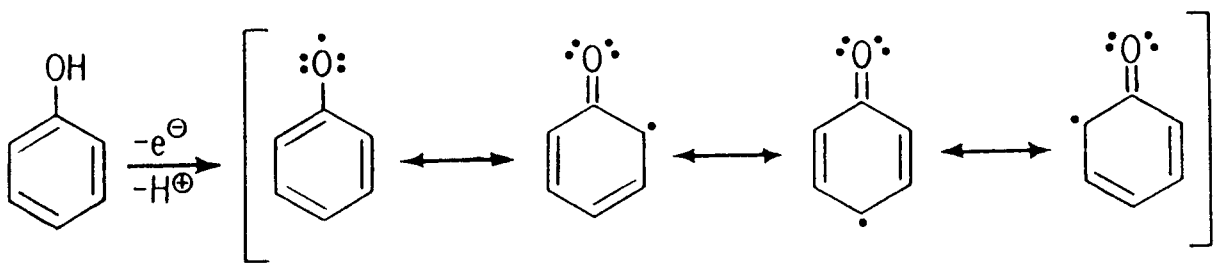
Phenols (and phenoxide anions) are very susceptible to electrophilic substitution. As may be expected from the resonance structures of phenoxide anion, electrophilic agents preferentially attack positions ortho or para to the hydroxyl group. Polyhydric phenols are even more susceptible to electrophilic aromatic substitution, especially if the hydroxyls are meta to one another (e.g., resorcinol, phloroglucinol), in which case, their activating influences reinforce each other.

One-electron oxidation generates from phenols highly reactive phenoxy free radicals, the odd electron of which is delocalized. The positions of the odd electron may be represented by the resonance structures:

[text figure ?]

Depending on the nature and positions of substituents on the aromatic ring, phenoxy radicals may undergo (a) dimerization or coupling reactions yielding dihydroxybiphenyls, hydroxyphenol phenyl ethers or diphenyl peroxides (reversible), or (b) disproportionation reaction yielding phenolic and ketonic products. The stability of phenoxy radicals may be enhanced by substitution with bulky alkyl groups. For example, the radical of 2,4,6-t-butylphenol is reasonably stable in benzene as indicated by the color of the solution and the chemical properties of the solute.

In addition to being more susceptible to electrophilic aromatic substitution than phenols, polyhydric phenols are also more easily oxidized. For 1,2- and 1,4-dihydroxybenzene (e.g., catechol and hydroquinone), 1,2- and 1,4-benzoquinone are the final oxidation products. In fact, quinones and poly-



Text-Figure 2

hydric phenols are often readily interconvertible. It is important to point out that semiquinones are formed during the interconversions if the electrons are transferred one at a time. Semiquinones are reactive electrophilic intermediates; they may also undergo reversible dimerization to form peroxides.

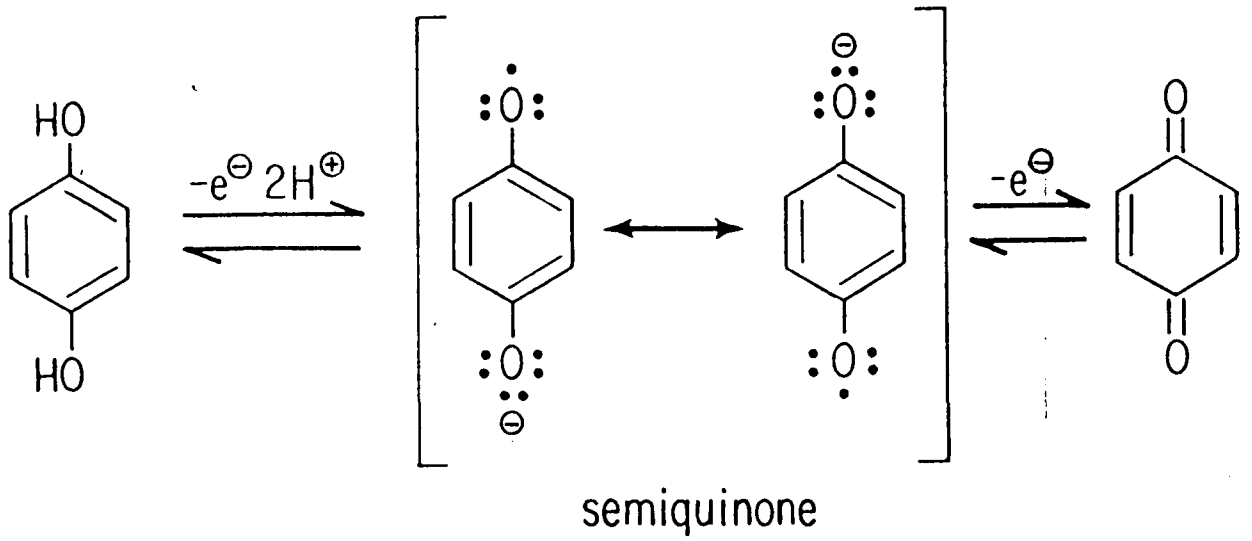
[text figure 3]

In contrast to 1,2- and 1,4-dihydroxybenzene, 1,3-dihydroxybenzene does not give rise to quinone upon oxidation because 1,3-quinone cannot assume an unstrained planar structure. Oxidation of resorcinol yields complex products probably by way of attack at the 4-position, which is activated by being ortho to one hydroxyl and para to the other (17).

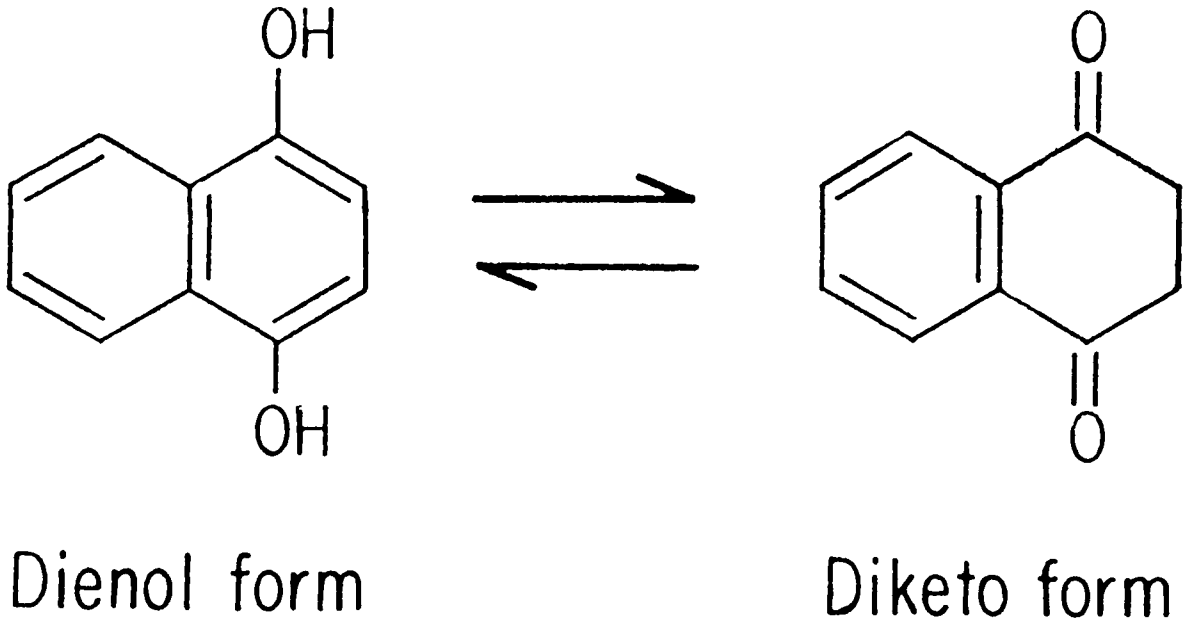
Polycyclic phenolic compounds are generally more reactive and have a greater tendency to tautomerize from enolic to ketonic structure than monocyclic phenolics (6). The naphthols share many reactions in common with resorcinol. 1,4-Naphthaquinol exists in two tautomeric forms:

[text figure 4]

which are stable under ambient temperature conditions and can be isolated. The hydroxy derivatives of anthracene show a greater tendency to exist in the tautomeric keto form. Studies by Segal et al. (18) indicate that anthralin (1,8,9-trihydroxyanthracene) exists predominantly in the ketonic anthrone form (see Table B).



Text-Figure 3



Text-Figure 4

5.2.2.5.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENIC.

Toxic effects. The toxicity of phenolics has been extensively studied because of decades of human exposure to these compounds. The literature is replete with reviews and monographs on the toxicology of phenol (5, 15, 16, 19, 20), chlorophenols (5, 9-11, 14, 16, 21-23), nitrophenols (13, 24), alkylphenols (12, 16, 25), polyhydric phenols (7, 16, 26), phenolic antioxidants (19, 27) and hexachlorophene (28-30). Representative acute toxicity data of some important phenolics are summarized in Table C. As the data in the Table indicate, their toxicity is strongly influenced by the type and position(s) of the ring substituents. In general, substitution with alkyl or alkoxy groups tend to decrease the toxicity, especially with multiple substitution with bulky groups. Chlorophenols are more toxic than phenol itself and toxicity increases with an increase in the extent of chlorination. The mode of toxic action changes from CNS effects (tremors, convulsion) by less chlorinated phenols (which have higher pKa and are therefore largely in undissociated form at body pH) to uncoupling of oxidative phosphorylation by highly chlorinated phenols. Dinitrophenols are considerably more toxic than mononitrophenols. The relative toxicity of the dinitrophenol isomers follows the order: 2,4- > 2,6- > 3,5- > 3,4- > 2,5- > 2,3-isomer (45). This order appears to correlate with the residence time of the isomers in the body (45) and their potency as uncouplers of oxidative phosphorylation (46). The importance of the position of ring substitution on the toxicity of phenols is exemplified by dihydroxybenzenes. Both 1,2-dihydroxybenzene (catechol) and 1,4-dihydroxybenzene (hydroquinone) are more toxic than their 1,3-isomer (resorcinol). It is noteworthy in this connection that both catechol and hydroquinone can be oxidized to quinones whereas resorcinol cannot.

Table C. Acute Toxicity of Phenols and Phenolic Compounds

Compound	Species & Route	LD_{50} (mg/kg)[a]	Reference
Phenol	Mouse, oral	520; 436	(19, 31)
	Rat, oral	530; 650	(7, 32)
	Rat, topical	670	(33)
	Rabbit, topical	850; 1,400	(7, 34)
o-Cresol	Mouse, oral	344	(31)
	Rat, oral	1,470	(31)
	Rabbit, topical	890	(34)
m-Cresol	Mouse, oral	828	(31)
	Rat, oral	2,010	(31)
	Rabbit, topical	2,830	(34)
p-Cresol	Mouse, oral	344	(31)
	Rat, oral	1,460	(31)
	Rabbit, topical	300	(34)
2,4-Xylenol	Mouse, oral	809	(31)
	Rat, oral	3,200	(31)
Thymol	Rat, oral	980	(35)
Butylated hydroxytoluene	Rat, oral	1,700 (M); 1,970 (F)	(36)
Butylated hydroxyanisole	Mouse, oral	2,000	(19)
	Rat, oral	2,200	(19)
Eugenol	Mouse, oral	3,000	(35)
	Rat, oral	2,680	(35)
2-Chlorophenol	Rat, oral	670	(37)
	Rat, i.p.	230	(38)
3-Chlorophenol	Rat, i.p.	335	(38)
4-Chlorophenol	Rat, i.p.	250	(38)
2,4-Dichlorophenol	Rat, oral	580	(37)
	Rat, i.p.	430	(38)
2,4,5-Trichlorophenol	Rat, i.p.	355	(38)
2,4,6-Trichlorophenol	Rat, i.p.	276	(38)
2,3,4,6-Tetrachlorophenol	Mouse, oral	131	(39)
	Mouse, i.p.	82	(39)

Table C. Acute Toxicity of Phenols and Phenolic Compounds (continued)

Compound	Species & Route	LD_{50} (mg/kg)[a]	Reference
Pentachlorophenol	Mouse, oral	74	(39)
	Mouse, i.p.	32	(39)
	Rat, oral	146 (M); 175 (F)	(40)
	Rat, topical	320 (M); 330 (F)	(40)
2-Nitrophenol	Mouse, oral	1,300	(34)
	Rat, oral	2,830	(34)
3-Nitrophenol	Mouse, oral	1,410	(34)
	Rat, oral	930	(34)
4-Nitrophenol	Mouse, oral	470	(34)
	Rat, oral	620	(34)
2,4-Dinitrophenol	Rat, oral	30	(41)
Vanillin	Rat, oral	1,580	(35)
Sodium salicylate	Rat, i.p.	780	(42)
Catechol	Mouse, oral	260	(19)
	Rat, oral	300	(7)
	Rabbit, topical	800	(7)
Resorcinol	Rat, oral	980	(7)
	Rabbit, oral	3,360	(7)
Hydroquinone	Mouse, oral	400	(19)
	Rat, oral	320	(19)
Bisphenol A	Mouse, oral	2,500	(43)
	Rat, oral	4,240	(43)
Hexachlorophene	Rat, oral	66 (M); 56 (F)	(44)

[a]M = male; F = female

Several investigators have attempted to establish a quantitative structure-toxicity relationship of phenols. In a 1970 report, Gubergrits and Kirso (47) derived a modified form of Hammett-Taft equation* which correlates the electron density in the reactive hydroxyl group regions with the toxicity of phenols. Unfortunately, insufficient details of the study were given to allow critical assessment. Stockdale and Selwyn (48), Tollenacre (49), and Motais et al. (50) concur that a correlation exists between the activity of phenols as uncouplers of oxidative phosphorylation and their electronic and hydrophobic bonding properties. Mizutani et al. (51) have delineated the structural requirements of butylated hydroxytoluene (BHT) and related compounds as pulmonary toxicants. The two essential requirements appear to be: (i) a methyl group para to OH, and (ii) ortho-alkyl group(s) which represent steric hindrance against interactions with the OH group. Based on these structure-activity relationships and available metabolic data, they proposed the formation of p-quinone methide (see Section 5.2.2.5.4) as reactive toxic intermediate.

Mutagenicity. Phenolics have been known for decades to be mitotic spindle poisons in plant tissues. Early studies on the mutagenicity of 15 phenols were reviewed by Dean (52) in 1978. Since then, over 100 phenolics have been tested for mutagenicity in various test systems. The following discussion focuses on studies using the Ames test and mammalian cells with emphasis on structure-activity relationships.

An enormous amount of information on the mutagenicity of phenols (as detected in the Ames test) has become available in the past few years from the

*For a detailed discussion of Hammett equation, see p. 271 of Volume I.

testing of cosmetics ingredients, food additives, water pollutants, tobacco smoke condensates, shale oil products and various environmental samples. The major findings in these studies are summarized in Table D. Unsubstituted phenol (Group A in Table D) is nonmutagenic according to most investigators, although one study (57) indicates that phenol induces frame-shift mutation (strain TA98) following metabolic activation. Alkyl- and alkoxyphenols (Group B in Table D) are generally inactive with the possible exceptions of eugenol and isoeugenol, which have an allyl and a propenyl sidechain, respectively. It is possible that the mutagenicity of these two compounds is associated with their unsaturated sidechains; indeed, the epoxide of eugenol (eugenol-2',3'-oxide) is a direct-acting mutagen (61). None of the chlorophenols (Group C in Table D) tested were found to be mutagenic; however, it is not clear whether the testing of the more highly chlorinated phenols has been adequate because of their limited solubility. Several nitro/aminophenols (Group D in Table D) are mutagenic. These compounds all have at least two positions (one of which ortho to the hydroxyl group) substituted with either nitro or amino groups, or one of each. Since a variety of substituted benzenediamines and nitrobenzenes are mutagenic or carcinogenic (64, 67, 74, 75), the phenolic hydroxyl group appears to play only a modifying role. With one exception, none of the hydroxybenzaldehydes, hydroxyacetophenones and hydroxybenzoic acids (Group E in Table D) were found mutagenic. Rapson et al. (54) listed 3,6-dichloro-2-hydroxybenzaldehyde, but not its 3,5-dichloro-isomer, to be mutagenic. Somewhat conflicting results have been obtained in studies on polyhydric phenols (Group F in Table D). Ben-Gurion (73) found pyrogallol mutagenic in strains TA100 and TA1537, but addition of liver S-9 mix reduced the activity. This finding was supported by the data of Gocke et al. (57) that three polyhydric phenols: resorcinol in TA1535A (+S9) and TA100 (-S9), hydroquinone in TA1535A

Table D. Mutagenicity of Phenols in the Ames Test

Compound	Substituent at Position[a] 2	3	4	5	6	Mutagenicity[b]
(A) Unsubstituted Phenol						
Phenol	H	H	H	H	H	- (53-56) + (57)
(B) Alkylphenols and Alkoxyphenols						
o-, m-, or p-Cresol	CH_3 at 2-, 3-, or 4-position			H	H	- (53, 58)
Xylenols	CH_3 at 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-positions					- (53, 56)
Trimethylphenols	CH_3 at 2,3,5-, 2,4,5-, or 2,4,6-positions					- (53, 56)
2-, 3-, or 4-Ethylphenol	C_2H_5 at 2-, 3-, or 4-position			H	H	- (53, 56)
Thymol	i-C_3H_7	H	H	CH_3	H	- (53)
BHT[c]	t-C_4H_9	H	CH_3	H	t-C_4H_9	- (59, 60)
Guaicol	OCH_3	H	H	H	H	- (53, 54, 58)
4-Hydroxyanisole	H	H	OCH_3	H	H	- (53)
BHA[c]	t-C_4H_9 at 2-/3-position		OCH_3	H	H	- (59, 60)
Eugenol	OCH_3	H	$CH_2CH{=}CH_2$	H	H	- (54, 58, 61) + (53)
Eugenol-2',3'-oxide	OCH_3	H	$CH_2CH{-}CH_2$ O	H	H	+ (61)
Isoeugenol	OCH_3	H	$CH{=}CHCH_3$	H	H	- (58) + (61)
(C) Chlorophenols						
2-, 3-, or 4-Chlorophenol	Cl at 2-, 3-, or 4-position			H	H	- (54)
Dichlorophenols	Cl at 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, or 3,5-positions					- (54, 62)
Trichlorophenols	Cl at 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, or 2,4,6-positions					- (54, 58, 62)

Table D. Mutagenicity of Phenols in the Ames Test (continued)

Compound	Substituent at Position[a] 2	3	4	5	6	Mutagenicity[b]
(C) Chlorophenols (cont'd)						
2,3,4,6-Tetrachlorophenol	Cl	Cl	Cl	H	Cl	- (62)
Pentachlorophenol	Cl	Cl	Cl	Cl	Cl	- (63)[d]
(D) Nitrophenols and Aminophenols						
2- or 4-Nitrophenol	NO_2/H	H	H/NO_2	H	H	- (64, 65)
2-Nitro-3-methylphenol	NO_2	CH_3	H	H	H	- (64)
2,4-Dinitrophenol	NO_2	H	NO_2	H	H	- (64, 66, 67)
m- or p-Aminophenol	H	NH_2/H	H/NH_2	H	H	- (65, 67, 68)
2,4-Diaminophenol	NH_2	H	NH_2	H	H	+ (67)
Acetaminophen	H	H	$NHCOCH_3$	H	H	- (60)
2-Amino-4-nitrophenol	NH_2	H	NO_2	H	H	+ (67, 68)
2-Amino-5-nitrophenol	NH_2	H	H	NO_2	H	+ (64, 68)
2-Nitro-4-aminophenol	NO_2	H	NH_2	H	H	+ (67)
Picric acid	NO_2	H	NO_2	H	NO_2	+ (57) - (64)
4,6-Dinitro-o-cresol	CH_3	H	NO_2	H	NO_2	- (63)[d]
(E) Hydroxybenzaldehydes, Hydroxyacetaphenones, and Hydroxybenzoic Acid						
2-, 3-, or 4-hydroxybenzaldehyde	CHO at 2-, 3-, or 4-position			H	H	- (53, 54)
Vanillin	OCH_3	H	CHO	H	H	- (54)
Syringealdehyde	OCH_3	H	CHO	H	OCH_3	- (54)

Table D. Mutagenicity of Phenols in the Ames Test
(continued)

Compound	Substituent at Position[a] 2	3	4	5	6	Mutagenicity[b]
(E) Hydroxybenzaldehydes, Hydroxyacetaphenones, and Hydroxybenzoic Acid (cont'd)						
3-Chloro-4-hydroxybenzaldehyde	Cl	H	CHO	H	H	- (54)
3,5-Dichloro-2-hydroxybenzaldehyde	CHO	H	Cl	H	Cl	- (54)
3,6-Dichloro-2-hydroxybenzaldehyde	CHO	Cl	H	H	Cl	+ (54)
2-, 3-, or 4-acetophenone	$COCH_3$ at 2-, 3-, or 4-position			H	H	- (53, 54)
Salicylic acid	COOH	H	H	H	H	- (65)
p-Hydroxybenzoic acid	H	H	COOH	H	H	- (54)
Vanillic acid	OCH_3	H	COOH	H	H	- (54)
Syringic acid	OCH_3	H	COOH	H	OCH_3	- (54)
Protocatechuic acid	OH	H	COOH	H	H	- (54)
Propyl gallate	OH	OH	H	$COOC_3H_7$	H	- (59, 60)
(F) Polyhydric Phenols						
Catechol	OH	H	H	H	H	- (53, 69)
Resorcinol	H	OH	H	H	H	- (53, 65, 66, 70, 71)
Hydroquinone	H	H	OH	H	H	- (53, 54) + (57)
Pyrogallol	OH	OH	H	H	H	- (54, 72) ± (53) + (57, 73)
Phloroglucinol	H	OH	H	OH	H	- (54)

Table D. Mutagenicity of Phenols in the Ames Test (continued)

Compound	Substituent at Position[a] 2	3	4	5	6	Mutagenicity[b]
(F) Polyhydric Phenols (cont'd)						
3,4-, 3,5-, 3,6-, or 4,5-Dichlorocatechol	OH	Cl at 3,4-, 3,5-, 3,6- or 4,5-positions				- (53, 58)
3,4,5-Trichlorocatechol	OH	Cl	Cl	Cl	H	- (58)
4-Chlororesorcinol	H	OH	Cl	H	H	- (68)
Chlorohydroquinone	Cl	H	OH	H	H	- (54)
2,5-Dichlorohydroquinone	Cl	H	OH	Cl	H	- (54)

[a]Position 1 of all these compounds is occupied by an OH group.

[b]Mutagenicity: "+" = positive; "±" = equivocal; "-" = negative. Numbers in parentheses are reference numbers.

[c]BHT = butylated hydroxytoluene; BHA = butylated hydroxyanisole.

[d]Metabolic activation system (S9 mix) not included in this study.

(-S9) and pyrogallol in TA1537 (-S9), TA98 and TA100 (±S9), were mutagenic. Florin et al. (53) found pyrogallol to have marginal mutagenic activity. In contrast to these studies, none of these and other polyhydric phenols were found mutagenic by several other investigators (see Table D).

In addition to phenols, a number of hydroxynaphthalenes and hydroxyanthracenes have been tested for mutagenicity. Both 1-naphthol (53, 57, 65, 76) and 2-naphthol (53, 76) are negative in the Ames test. The introduction of nitro groups may confer mutagenic activity. Thus, 1-nitro-2-naphthol (64), 2,4-dinitro-1-naphthol (77) and its 7-sulfono derivative (64) all display mutagenic activity. Brown and coworkers (rev. in ref. 78) have tested the mutagenicity of several hydroxyanthracenes. Anthrone (9-hydroxyanthracene) has been found inactive whereas several polyhydric anthracenes, such as anthralin (1,8,9-trihydroxyanthracene), anthrarobin (3,4-dihydroxyanthracene) and crysarobin (1,8,9-trihydroxy-3-methylanthracene) are all mutagenic in strains TA1537 (a frameshift mutant). Hexachlorophene, a bisphenol, is nonmutagenic (57).

The genotoxic effects of phenolics in plants have prompted many investigators to assess the chromosome-damaging potential of these compounds in mammalian cells. Probst et al. (76) showed that a number of phenolics (such as 4-aminophenol, 4-nitrophenol, 2,4-dinitrophenol, 2,4-dichlorophenol, resorcinol, 1-/2-naphthol, and anthralin) are all inactive in the unscheduled DNA synthesis (UDS) tests. Morimoto and Wolff (79) found that catechol and, to a lesser degree, hydroquinone induce sister chromatid exchange (SCE) in cultured human lymphocytes. Phenol is considerably less active and benzene is inactive. An in vitro study by Bracher et al. (71) showed that resorcinol is inactive in SCE in bone marrow cells of rats. Kawachi (cited in ref. 6) tested six phenolics in several chromosome aberration tests; propyl gallate,

acetaminophen and isobutyl-p-hydroxybenzoate were found active, whereas BHT, BHA, and butyl-p- hydroxybenzoate were inactive. Stich et al. (80) asessed the clastogenic activity of seventeen phenolics in Chinese hamster ovary (CHO) cells by measuring the number of chromatid breaks and exchanges; the most significant finding was that monohydroxylated phenolics (e.g., salicylic acid, p-hydroxybenzoic acid) generally lack clastogenic activity, whereas most dihydroxylated (e.g., catechol, resorcinol, protocatechuic acid) and tri-hydroxylated phenolics (e.g., pyrogallol, phloroglucinol, gallic acid) exhibit a relatively strong chromosome-damaging potential. The clastogenic activity of most of the phenolics is reduced by the addition of liver S-9 mix, but increased by transition metals such as Cu^{++} and Mn^{++}. Comparison of the dose-response data suggests that the relative potency of polyhydric phenols follows the order: catechol > pyrogallol > resorcinol > phloroglucinol. In the micronucleus test (which detects chromosome aberrations in mouse bone marrow cells) by Gocke et al. (57), pyrogallol and hydroquinone were both active whereas phenol, resorcinol, picric acid, 1-naphthol and hexachlorophene were all inactive.

Thus, both the bacterial and mammalian data concur that monohydroxylated phenolics are generally nonmutagenic unless they are ring-substituted with functional groups which are known to confer mutagenic activity. On the other hand, polyhydric phenolics (especially catechol, hydroquinone, pyrogallol) have relatively strong mutagenic or chromosome-damaging potentials.

Teratogenicity. A number of phenolics have been tested for teratogenicity; among these, hexachlorophene and salicylates are active in some species. The safety of the use of hexachlorophene was first questioned since the discovery in 1971 of its toxic effects on neonatal rat brain. Oakley and Shepard (81), Gaines et al. (44), and Kennedy et al. (82, 83) demonstrated

subsequently that hexachlorophene causes malformations (angulated ribs, cleft palates, micro- and anophthalmia) in the offspring of rats given high (maternally toxic) doses of the compound in the diet or by gavage. Teratogenic effects were also observed in rats exposed to the compound via vaginal treatment (84). In rabbits, oral administration of hexachlorophene led to a relatively low incidence of rib malformation (82) whereas mice appeared to be resistant to the teratogenic effects of the compound (85). Recently, retrospective epidemiological studies performed on employees of six Swedish hospitals suggested that severe congenital malformations occurred more frequently in infants (25/460) born to mothers who used hexachlorophene soaps during at least the first trimester of pregnancy than those (0/233) born to similarly employed mothers who did not use hexachlorophene soaps (86). It is important to note that hexachlorophene is synthesized from 2,4,5-trichlorophenol, the same chemical that in the manufacture of 2,4,5-trichlorophenoxyacetic acid is known to give rise to chlorinated dibenzodioxins.

Sodium salicylate has been shown to cause gross anomalies (rib anomalies) in mice (87) and rats (88). Minor and Becker (88) compared the teratogenic potential of sodium salicylate (active), sodium benzoate (active) and phenol (inactive) and concluded that the teratogenicity is associated with carboxyl moiety, while the hydroxyl group plays a contributory but not obligatory role. It should be noted, however, that p-hydroxybenzoic acid was not teratogenic in mice (87). Goldman and Yakovac (89) reported that the teratogenicity of salicylate is probably unrelated to its activity as an uncoupler of oxidative phosphorylation because 2,4-dinitrophenol, a well known uncoupler, is not teratogenic in rats. Four other phenolics have been found to have no significant teratogenic effects: the food additives, butylated hydroxytoluene (BHT) and hydroxyanisole (BHA) in mice and rats (90), and monkeys (91); the wood

preservative, pentachlorophenol in rats (92); and the herbicide, dinitro-o-cresol in mice (93).

5.2.2.5.3 Carcinogenicity and Structure-Activity Relationships.

5.2.2.5.3.1 OVERVIEW. Over fifty phenolics have been tested for carcinogenicity. Many of these studies are, however, probably of insufficient quality to provide unequivocal results. Available data suggest that most monohydric monocyclic phenols are either inactive or, at most, weakly carcinogenic. Phenol itself is inactive by oral administration but induces skin tumors in mice after repeated applications of high (irritating) doses. Comparative studies by Boutwell and Bosch (94) showed that ring substitution with chlorine (at the 2-position) or methyl groups yields compounds with skin carcinogenicity comparable to that of phenol; substitution with bulky alkyl group or electron-withdrawing substituents ($-NO_2$, -CHO, -COOH) at 2- or 4-position abolishes the carcinogenicity of phenol.

2,4,6-Trichlorophenol and 4-amino-2-nitrophenol are, at the time of this writing, the only two simple phenolics that have been unequivocally shown to be carcinogenic in rodents after oral administration. The former induces lymphomas or leukemias in male F344 rats and liver tumors in $B6C3F_1$ mice while the latter is carcinogenic toward the urinary bladder of F344 rats. There is some suggestive evidence that butylated hydroxytoluene (BHT) and eugenol may be carcinogenic in some strains of mice.

Bacterial and mammalian data suggest that polyhydric phenols may have relatively high mutagenic potential (see Section 5.2.2.5.2.2). Four polyhydric phenols (catechol, resorcinol, hydroquinone and pyrogallol) have, thus far, been tested for carcinogenicity on the skin of mice and rabbits and found

to be generally inactive. There is some evidence that oral administration of hydroquinone may increase the incidence of renal tumors; it also increases the incidence of bladder tumors after *in situ* pellet implantation. Two bisphenols (bisphenol A and hexachlorophene) have been adequately tested and consistently found to be inactive.

Among the polycyclic phenols, anthralin (1,8,9-trihydroxyanthracene) is generally inactive as a complete skin carcinogen but may induce lymphomas in one strain (ICR) of mice. 2-Hydroxybenzo[a]pyrene, a phenolic derivative of benzo[a]pyrene, has recently been shown to be an exceptionally strong skin carcinogen; thus, the generally held assumption that phenolic metabolites of polycyclic aromatic hydrocarbons are "detoxified" metabolites has no universal validity.

An interesting study of the potential carcinogenicity of several phenolic and related compounds in two tumor-prone hybrid tobacco plants (*Nicotinia* hybrids) has been conducted by Andersen (95). Pyrogallol, resorcinol and 3-hydroxyanthranilic (3-hydroxy-2-aminobenzoic) acid induced tumors in the plants at the seedling stage, whereas phenol and catechol were inactive. On the molar basis, pyrogallol was the most carcinogenic. Acetylation of the hydroxyl groups (yielding pyrogallol triacetate) or introduction of a carboxyl group to the ring (yielding gallic acid) completely abolishes the carcinogenicity of pyrogallol to the plants. The author suggested that, except for 3-hydroxyanthranilic acid, the structural requirement for carcinogenicity toward the *Nicotiniama* hybrids is the presence of at least two hydroxyl groups positioned *meta* to each other.

5.2.2.5.3.2 SIMPLE (MONOCYCLIC) PHENOLICS. Close to forty simple phenolics have been tested for carcinogenicity. The major findings of these

studies are summarized in Table E according to the type of substituents present in the aromatic ring.

Phenol. Boutwell and coworkers (94, 96) reported first that mice topically treated with phenol for long periods of time developed skin tumors. The finding was confirmed by Salaman and Glendenning (97). The carcinogenicity of phenol is, however, very weak; an ulcerative concentration (20%) of phenol is needed to elicit a weak carcinogenic action (97). Boutwell and Bosch (94) noted that during the first 6 weeks of phenol (10 or 20% solution in benzene or dioxane) treatment many of the mice bore wounds and showed reparative hyperplasia. Most of the skin tumors were papillomas. In the experiments of Van Duuren and Goldschmidt (98), in the 50 ICR/Ha Swiss mice treated topically with 3 mg phenol for 368 days only one mouse developed a skin papilloma, so that the compound was not considered carcinogenic by the authors.

Phenol has also been tested in rodents by oral administration. Unpublished Food and Drug Administration data (19) indicate that 17/36 rats survived over 2 years on a diet containing 0.25, 0.5 or 1.0% phenol. Histopathological examination of the 6 rats that survived the highest dose did not reveal any significant change. The U.S. National Cancer Institute (1) has completed in 1980 a bioassay testing of phenol. Groups of 50 F344 rats and 50 $B6C3F_1$ mice were given drinking water containing 2,500 or 5,000 ppm of the compound for 103 weeks. An increased incidence of leukemia or lymphomas was detected in male rats (low dose group only); however, it could not be established whether the increased incidence was acutally due to phenol treatment. Thus, it was concluded that, under the conditions of this bioassay, phenol was not carcinogenic in either species of rodents.

Table E. Carcinogenicity of Phenol and its Substituted Derivatives

Compound[a]	Species and Strain	Route	Principal Organ Affected	Reference
(A) Unsubstituted Phenol				
Phenol	Mouse, albino	topical	Skin	(94, 96)
	Mouse, "S"	topical	Skin	(97)
	Mouse, ICR/Ha	topical	None	(98)
	Mouse, $B6C3F_1$	oral	None	(1)
	Rat, —	oral	None	(FDA data, cited in ref. 19)
	Rat, F344	oral	None	(1)
(B) Alkylphenols and Alkoxyphenols				
2,4-, 2,5-, 2,6-, 3,4- or 3,5-Xylenol	Mouse, albino	topical	Skin	(94)
Butylated hydroxytoluene (BHT)	Mouse, A/He	i.p.	None[b]	(99)
	Mouse, BALB/c	oral	Lung	(100)
	Mouse, CF-1	oral	Lung	(Brooks et al., cited in ref. 101)
	Mouse, $B6C3F_1$	oral	None	(102)
	Rat, Wistar	oral	None	(36)
	Rat, CD SPF	oral	None[c]	(103)
	Fat, F344	oral	None	(102)
Thymol	Mouse, A/He	i.p.	None[b]	(99)
o-Phenylphenol	Mouse, $B6C3F_1$ or $B6AKF_1$	oral or s.c.	None[d]	(104, 105)
p-Phenylphenol	Mouse, $B6C3F_1$ or $B6AKF_1$	oral	None[d]	(104, 105)
	Mouse, $B6C3F_1$	s.c.	Hematopoietic system	(104)
Guaiacol	Mouse, —	implantation	None	(106)
4-Hydroxyanisole	Mouse, Swiss	topical	None	(107)
	Rabbit, New Zealand	topical	None	(107)
Butylated hydroxyanisole (BHA)	Mouse, C3H/Anf	topical or s.c.	None	(108)
	Mouse, A/He	i.p.	None[b]	(99)
	Mouse, CD-1	oral	None	(109)
	Rat, —	oral	None	(110)
	Dog, —	oral	None (15-mo study)	(111)

Table E. Carcinogenicity of Phenol and its Substituted Derivatives (continued)

Compound[a]	Species and Strain	Route	Principal Organ Affected	Reference
(B) Alkylphenols and Alkoxyphenols (cont'd)				
Eugenol	Mouse, ICR/Ha Swiss	topical	None	(98)
	Mouse, $B6C3F_1$	oral	Liver (equivocal)	(3)
	Rat, F344	oral	None	(3)
(C) Chlorophenols				
2-Chlorophenol	Mouse, albino	topical	Skin (papillomas)	(94)
2,4,5-Trichlorophenol	Mouse, $B6C3F_1$ or $B6AKF_1$	s.c.	None[d]	(104)
2,4,6-Trichlorophenol	Mouse, $B6C3F_1$	oral	Liver, hematopoietic system[d]	(104, 105)
	Mouse, $B6AKF_1$	oral	None[d]	(104)
	Mouse, $B6C3F_1$ or $B6AKF_1$	s.c.	None[d]	(104)
	Mouse, $B6C3F_1$	oral	Liver	(112)
	Rat, F344	oral	Hematopoietic system	(112)
2,3,4,6-Tetrachlorophenol	Mouse, $B6C3F_1$	s.c.	Hematopoietic system[d]	(104)
Pentachlorophenol	Mouse, $B6C3F_1$ or $B6AKF_1$	oral	None[d]	(104, 105)
	Mouse, $B6C3F_1$	s.c.	Liver[d]	(104)
	Rat, Sprague-Dawley	oral	None	(92)
(D) Nitrophenols and Aminophenols				
o-Hydroxyacetanilide	Rat, —	oral	Pituitary gland	(113a)
2-Amino-4,5-xylenol	Mouse, —	implantation	Urinary bladder	(113b)
4-Dimethylamino-3,5-xylenol	Mouse, $B6C3F_1$ or $B6AKF_1$	oral or s.c.	None[d]	(104, 105)

Table E. Carcinogenicity of Phenol and its Substituted Derivatives (continued)

Compound[a]	Species and Strain	Route	Principal Organ Affected	Reference
(D) Nitrophenols and Aminophenols (cont'd)				
4-Amino-2-nitrophenol	Mouse, $B6C3F_1$	oral	None	(114)
	Rat, F344	oral	Urinary bladder	(114)
2-sec-Butyl-4,6-dinitrophenol	Mouse, $B6C3F_1$ or $B6AKF_1$	oral or s.c.	None[d]	(104, 105)
(E) Hydrobenzaldehydes and Hydroxybenzoic Acids				
Vanillin (3-methoxy-4-hydroxy benzaldehyde)	Mouse, A/He	i.p.	None[b]	(99)
3-Ethoxy-4-hydroxybenzaldehyde	Mouse, A/He	i.p.	None[b]	(99)
Salicylic acid	Mouse, —	implantation[e]	None	(106)
Methyl salicylate	Mouse, A/He	i.p.	None[b]	(99)
Butyl-p-hydroxybenzoate	Mouse, $B6C3F_1$ or ICR	oral	None	(60)
	Rat, various strains	oral	None	(60)
Isobutyl-p-hydroxybenzoate	Mouse, $B6C3F_1$ or ICR	oral	None	(60)
Propyl gallate	Mouse, A/He	i.p.	None[b]	(99)
	Rat, —	oral	None	(19)
(F) Polyhydric Phenols				
Catechol (pyrocatechol)	Mouse, —	implantation[e]	None[f]	(106)
	Mouse, ICR/Ha	topical	Skin (?)[g]	(98)
	Rat, —	oral	None	(FDA data, cited in ref. 19)
Resorcinol	Mouse, Swiss	topical	None	(107, 115)
	Mouse, ICR/Ha	topical	None	(98)
	Rabbit, New Zealand	topical	None	(107)

Table E. Carcinogenicity of Phenol and its Substituted Derivatives (continued)

Compound[a]	Species and Strain	Route	Principal Organ Affected	Reference
(F) Polyhydric Phenols (cont'd)				
Hydroquinone	Mouse, —	implantation[e]	Urinary bladder	(106)
	Mouse, ICR/Ha	topical	None	(98)
	Rat, —	oral	Kidney (?)[h]	(FDA data, cited in ref. 19)
Pyrogallol	Mouse, Swiss	topical	None	(107, 115)
	Mouse, ICR/Ha	topical	None	(98)
	Rabbit, New Zealand	topical	None	(107)
(G) Bisphenols				
Bisphenol A	Mouse, $G6C3F_1$	oral	None	(116)
	Rat, F344	oral	None	(116)
Hexachlorophene	Mouse, Swiss	topical	None	(107, 115)
	Mouse, 57Bl or XVII/G	oral	None	(117)
	Mouse, XVII/G (neonatal)	s.c.	None	(117)
	Mouse, XVII/G	lactational	None	(117)
	Mouse, $(C57xC3H)F_1$	transplacental	None	(117)
	Rat, Sprague-Dawley	oral	None	(117)
	Rat, F344	oral	None	(102)
	Rabbit, New Zealand	topical	None	(107)

[a]See Tables B and C for structural formulae.

[b]Pulmonary adenoma assay (24-week study),

[c]One rat developed a mammary adenocarcinoma.

[d]Preliminary screening study.

[e]Bladder implantation with cholesterol pellets (25-week study).

[f]Erroneously reported to have a significant carcinogenic effect by an IARC study group.

[g]One mouse developed a squamous carcinoma.

[h]Suggestive evidence of increase in incidence of renal tumors over spontaneous incidence.

Alkylphenols and alkoxyphenols. This group includes a number of commercially important antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Five isomers of xylenol (dimethylphenol) were tested for carcinogenicity by Boutwell and Bosch (94). The compounds were applied as 10% solutions in benzene to mouse skin twice weekly for 20 weeks. At the end of treatment, skin papillomas were noted in each group with the incidences for the 5 isomers as follows: 3,5- (55%) $\geq$ 2,4- (50%) > 2,4- (31%) $\geq$ 2,5- (24%) > 2,6- (8%). Skin carcinomas appeared at 28 weeks with incidences: 14% for 3,4- and for 3,5-; 12% for 2,4-; 8% for 2,5-; and 0% for the 2,6-isomer.

The potential carcinogenicity of butylated hydroxytoluene (BHT) was the subject of intensive investigations because of its wide use as a food additive. Deichmann et al. (36) were probably the first to report the lack of carcinogenicity of BHT in the rat. Groups of 15 rats of each sex administered 0.2, 0.5, 0.8 or 1.0% BHT in the diet for 24 months showed no signs of pathological changes. Ulland et al. (103) studied the effect of BHT on carcinogenesis induced by a number of known carcinogens in Charles River CD SPF rats. In the control group given BHT (6,600 ppm in diet) alone, none of 10 male rats developed tumors after 37 weeks but one of 10 female rats developed a mammary adenocarcinoma during 44 weeks of the study. The authors considered BHT noncarcinogenic because of the high spontaneous mammary tumor incidence in female rats of this strain. The lack of carcinogenicity of BHT in the rat has been further confirmed in a NCI bioassay study (102). No tumors occurred in F344 rats exposed to diets containing either 3,000 or 6,000 ppm BHT for 105 weeks.

There appears to be some evidence that BHT may have some carcinogenic activity in certain strains of mice. Clapp et al. (100) reported that BALB/c

mice given diets containing 0.75% BHT for 16 months had significantly higher incidence (63.6%) of lung tumors (mainly papillary adenomas) than that (24%) in controls. This finding was supported by Brooks et al. (cited in ref. 101) who observed a significnat, dose-dependent increase in lung tumors in CF-1 mice fed BHT for 100 weeks. The incidences were 53.2%, 73.8% and 75% for mice fed 1,000, 2,500 and 5,000 ppm, respectively, compared to 46.8% for controls.

In contrast to the above studies, a 1978 NCI bioassay (102) failed to show a significant carcinogenic effect of BHT in $B6C3F_1$ mice maintained on diets containing either 3,000 or 6,000 ppm for 107 or 108 weeks. Stoner et al. (99) found BHT inactive in pulmonary adenoma assay in strain A/He mice receiving a total dose of as much as 6 g/kg BHT intraperitoneally in a 24-week period.

Like BHT, butylated hydroxyanisole (BHA) has received great attention because of its extensive use as an antioxidant food stabilizer. Wilder and Kraybill (110) were probably the first to report a chronic toxicity study of BHA in the rat. Groups of 15 or more newly weaned rats were maintained on diets containing 0.05, 0.5, 1.0 or 2.0% BHA for 21-22 months. No significant histopathological changes attributable to BHA were observed. In at least 3 strains of mice, BHA was also consistently noncarcinogenic by several routes. In the study of Hodge et al. (108), groups of 100 strain C3H/Anf mice (50 of each sex) were either given single subcutaneous injections (10 mg/mouse) or weekly skin applications (0.1 mg) of BHA; no evidence of tumors was noted up to 519 days. Brown (109) showed that BHA had no carcinogenic effects in two strains (CD-1 and C3H) of mice; in fact, high levels (0.5%) of BHA appeared to decrease slightly the "spontaneous" incidence of liver tumors in C3H mice. Moreover, Stoner et al. (99) found BHA inactive in pulmonary adenoma assay using A/He mice. A 15-month study in dogs also produced no evidence for the carcinogenicity of BHA (111).

Eugenol, a widely used food additive for flavor and fragrance, has been tested in a U.S. National Toxicology Program carcinogenesis bioassay at two dose levels. A preliminary report of the study (3) showed that the compound significantly increased the incidence of hepatocellular adenomas or carcinomas in $B6C3F_1$ mice of the low-dose group but not the high-dose group; the results were judged to be equivocal. In F344 rats eugenol was clearly not carcinogenic. Also, a preliminary study (Swanson, Miller and Miller, unpublished data cited in ref. 61) in preweaning mice failed to provide evidence for carcinogenicity.

Of the other phenolics in Group (B) of Table E, thymol (99), o-phenylphenol (104, 105), guaiacol (106) and 4-hydroxyanisole (107) are all noncarcinogenic in various screening studies. Only p-phenylphenol increased the incidence of reticulum cell sarcomas in male $B6C3F_1$ mice after a single subcutaneous administration of 1 g/kg; however, it was inactive by oral administration (104, 105).

Chlorophenols. Several chlorinated derivatives of phenol are carcinogenic (Group (C) in Table E). Repeated skin application of a 20% solution of 2-chlorophenol (in dioxane) produced skin papillomas in 46% of albino mice with an average of 0.64 papilloma/mouse after 12 weeks. For comparison, a 20% solution of phenol led to skin papillomas in 63% of the animals with an average of 0.94 papilloma/mouse (94). A preliminary screening study in 1968 of four higher chlorophenols in $B6C3F_1$ and $B6AKF_1$ mice under various conditions of administration (104, 105) gave the following results. 2,4,5-Trichlorophenol was inactive after a single subcutaneous injection of 1 g/kg. 2,4,6-Trichlorophenol caused an increase in the incidence of hepatomas and reticulum cell sarcomas in $B6C3F_1$ mice receiving a diet containing 260 ppm of the compound. 2,3,4,6-Tetrachlorophenol appeared to increase the incidence of

reticulum cell sarcoma in male $B6C3F_1$ mice that received a single subcutaneous dose of 100 mg/kg. Pentachlorophenol (Dowcide-7) was inactive by oral administration; however, a single subcutaneous administration of 46.4 mg/kg enhanced the incidence of hepatomas in male $B6C3F_1$ mice. Pentachlorophenol was also inactive in Sprague-Dawley rats fed the technical grade compound at daily dose levels of 1, 3, 10 and 30 mg/kg body weight for 22-24 months (92).

The carcinogenicity of 2,4,6-trichlorophenol has been confirmed in 1979 in a NCI bioassay study (112). Significant increases in liver tumors were found in $B6C3F_1$ mice of both sexes fed diets containing 5,000 and 10,000 ppm (for males) and 5,214 and 10,428 ppm (for females) of the compound, for 105 weeks. The incidences of hepatocellular carcinomas or adenomas were 4/20 (control), 32/49 (low dose), 39/47 (high dose) for males and 1/20 (control), 12/50 (low dose), 24/48 (high dose) for females. 2,4,6-Trichlorophenol (5,000 or 10,000 ppm in diets for 107 or 108 weeks) was also carcinogenic in male F344 rats inducing lymphomas or leukemias (control 4/20, low dose 25/50, high dose 29/50).

Nitrophenols and aminophenols. As previously discussed in Section 5.1.2.1 (Vol. IIB, p. 6), certain derivatives of aminophenols (e.g., o-hydroxyacetanilide, 2-amino-4,5-xylenol) are carcinogenic (see Group (D) of Table E). It does not appear, however, that the phenolic hydroxyl group per se is a structural requirement for carcinogenicity. For example, whereas p-ethoxyacetanilide (phenacetin) is active (see Vol. IIB, p. 6), p-hydroxyacetanilide (acetaminophen) appears to be inactive. A preliminary NCI carcinogenesis screening study included two amino/nitrophenols (4-dimethylamino-3,5-xylenol and 2-sec-butyl-4,6-dinitrophenol); both compounds did not display any carcinogenic activity. The NCI has recently conducted a bioassay of 4-amino-2-nitrophenol (1,250 or 2,500 ppm in diet for 103 weeks) in F344

rats and $B6C3F_1$ mice (114). The compound was inactive in mice but carcinogenic in male (and possibly also in female) rats inducing transitional-cell carcinomas of the urinary bladder. It is well documented that many disubstituted amino/nitrobenzenes are carcinogenic/mutagenic (e.g., 74, 75). 2-Amino-4-nitrophenol and 2-amino-5-nitrophenol are currently being tested by NCI/NTP and a Japanese team (60) is re-evaluating the possible carcinogenic potential of acetaminophen.

Hydroxybenzaldehydes and hydroxybenzoic acids. None of the compounds in Group (E) of Table E appears to be carcinogenic; however, the results on vanillin, 3-ethoxy-4-hydroxybenzaldehyde, methyl salicyclate and salicyclic acid cannot be considered conclusive because of the preliminary nature of the studies (pulmonary adenoma assay or bladder implantation). Butyl and isobutyl p-hydroxybenzoates (60) and propyl gallate (rev. in ref. 19) have been tested in long-term studies up to dietary levels of 0.6%, 1.2% and 5%, respectively; no evidence of carcinogenicity was found.

Polyhydric phenols. Chemical structural considerations (Section 5.2.2.5.2.1), mutagenicity data (Section 5.2.2.5.2.2) and carcinogenicity studies in plants (Section 5.2.2.5.3.1) suggest that this group of compounds may be more hazardous than hitherto assumed: only limited carcinogenicity data (Group (F) of Table E) are available at the time of this writing. Catechol was inactive in bladder implantation study (106), induced only 1 skin carcinoma among 50 mice after repeated topical applications for 368 days (98), and apparently did not induce any tumor in a chronic feeding study up to a dietary level of 1% (cited in ref. 19).

Resorcinol and pyrogallol have only been tested by topical route. Both were inactive in Swiss mice (107, 115), ICR/Ha Swiss mice (98) and New Zealand

rabbits (107). Several hair-dye formulations, which contain resorcinol (0.4%), were also noncarcinogenic in mice after weekly or biweekly topical applications for 18 months (118).

Hydroquinone is inactive as a complete skin carcinogen in ICR/Ha Swiss mice (98), but active as a skin tumor initiator in mice (with croton oil as promotor) (119). In the bladder implantation assay, hydroquinone significantly increased the incidence of bladder tumors in mice (106). It cannot be ascertained, however, whether the above effect was due to the complete carcinogenicity or tumor-promoting activity of hydroquinone. Chronic oral toxicity study of hydroquinone by the U.S. Food and Drug Administration (cited in ref. 19) indicates increase in incidence of chronic gastrointestinal ulceration and kidney tumors over spontaneous incidence. A variety of polyhydric phenols (hexylresorcinol, hydroquinone, propyl gallate and resorcinol) are being tested or have been selected for carcinogenesis bioassay in the U.S. Naitonal Toxicology Program at the time of this writing.

Bisphenols. Two bisphenols have been tested for carcinogenicity (Group (G) of Table E). Bisphenol A (4,4'-isopropylidenediphenol), a widely used intermediate in the manufacture of phenolic resins, displays no convincing evidence of carcinogenicity in F344 rats and $B6C3F_1$ mice at dose levels of 1,000 or 2,000 ppm for rats of either sex, 1,000 or 5,000 ppm for male mice, and 5,000 or 10,000 ppm for female mice (116). Only a marginally significant increase in the incidence of leukemias in male rats and in the combined incidence of lymphomas and leukemias in mice have been noted, which may be construed as representing borderline activity toward the hematopoietic system. Hexachlorophene (2,2'-methylene-bis-3,4,6-trichlorophenol), a commonly used antibacterial agent (prior to 1972), was found noncarcinogenic in several bioassays (102, 107, 117).

5.2.2.5.3.3 POLYNUCLEAR PHENOLICS. Few polynuclear phenolics have been tested for carcinogenicity probably because of the pervasive belief that phenolic derivatives are "detoxified" metabolites of aromatic amines and hydrocarbons. Although 2-amino-1-naphthol was, at one time, considered to be the proximate carcinogen of 2-naphthylamine, subsequent studies did not substantiate this view (Section 5.1.4.2.1 in Vol. IIB) and it is now generally accepted that N-hydroxylation is the common activating mechanism for virtually all aromatic amines.

The carcinogenic potential of anthralin (dithranol, 1,8,9-trihydroxy-anthracene, or 1,8-dihydroxy-9-anthrone) has been explored because of its use for the treatment of psoriasis and chronic dermatoses (120, 121). Anthralin was consistently found to be a tumor-promotor (see Section 5.2.2.5.3.4); it appears to have no complete carcinogenic activity or is at most marginally carcinogenic in a few strains of mice (18, 98, 122). The only evidence for the complete carcinogenicity of anthralin was obtained by Yasuhira (123, 124). Topical applications of anthralin (0.033 or 0.1% solution in acetone) alone to two groups of young ICR mice led to the induction of malignant lymphomas in 6/40 (15%) and 4/20 (20%) of the animals. No tumors were noted in groups given urethan alone, croton oil alone or urethan + croton oil. In two groups of ICR mice given urethan + anthralin, the incidence of lymphomas was 21/40 (52%) and 7/20 (35%) indicating strong synergism between anthralin and urethan or enhancement of the carcinogenic effect of anthralin by urethan.

As mentioned above, phenolic derivatives of aromatic hydrocarbons were generally regarded to be "detoxified" metabolites. However, a 1977 study by Wislocki et al. (125) showed that, among the 12 possible isomeric phenols derived from benzo[a]pyrene, 2-hydroxybenzo[a]pyrene (2-OH-BP) is a strong carcinogen comparable in potency to that of benzo[a]pyrene. Topical applica-

tion of 0.4 umoles of 2-OH-BP to the skin of C57BL/6J mice once every 2 weeks led to induction of squamous cell carcinomas in 100% of the animals after 53 weeks of treatment. Tested under similar conditions, 11-OH-BP was a much weaker carcinogen, inducing skin tumors in only 14% of the mice; all the other 10 isomers were inactive (125, 126). It is interesting to note that application of 2-OH-BP or 9-OH-BP was followed by marked epidermal hyperplasia resembling that caused by typical tumor promotors; the other 10 isomers were either less active or completely inactive in inducing hyperplasia (127). It is possible that the strong carcinogenicity of 2-OH-BP is due in part to its strong promotor-like activity and that 9-OH-BP probably lacks tumor-initiating activity and is, therefore, not a complete carcinogen. Following a simple assumption, the exceptional carcinogenicity of 2-OH-BP could be due to the fact that hydroxyl groups in the 2- and 11-positions of trans-9,10-dihydroxy-7,8-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene may enhance the chemical reactivity of the oxirane ring. However, Wislocki et al. (125) cautioned that diol-epoxide derivatives cannot be assumed to be the ultimate carcinogenic form of 2-OH-BP or 11-OH-BP, because the metabolic fate of BP phenols is expectably different from that of BP.

5.2.2.5.3.4 PHENOLICS AS MODIFIERS OF CHEMICAL CARCINOGENESIS. Depending on the chemical structure, phenolics may enhance or inhibit the carcinogenic action of a variety of chemical agents. The enhancing effect may be mediated by mechanisms such as tumor-promotion, cocarcinogenesis, or modification of pharmacokinetic parameters (absorption, metabolism, excretion) to provide a "favorable" condition for carcinogenesis. Some phenolics inhibit carcinogenesis and this is brought about by influencing pharmacokinetic parameters in an "unfavorable" way, by trapping potentially carcinogenic reactive intermediates, or by both mechanisms. Detailed discussion of the phenolics as

modifiers of chemical carcinogenesis will be covered in Section 6, Vol. IV; a bird's eye view of some key studies and structure-activity relationships is presented here.

Phenolics as promotors. It is well documented that phenolics are tumorigenesis promotors. Boutwell and Bosch (94, 128) tested the promoting activity of over forty simple phenols using 7,12-dimethylbenz[a]anthracene as the initiator. Phenol itself is a relatively weak promotor. Following initiation, twice weekly applications with 2.5 mg phenol elicited approximately the same promoting action as 0.125 mg croton oil. Structure-activity studies indicate that a free phenolic hydroxyl is required for promoting activity, since both the methyl ether of phenol (anisole) and acyl derivative (phenylacetate) are inactive. Ring substiution with halogen atom(s) or methyl group(s) yield compounds with comparable activity except that most highly substituted compounds (e.g., pentachloro-, 2,4,6-trichloro- and 2,3,5-trimethylphenol) are inactive. Replacement of a methyl group by a higher alkyl group tends to reduce activity. Among polyhydric phenols, catechol, hydroquinone and pyrogallol are inactive while the data on resorcinol are equivocal. Phenols substituted with electronegative substituents, such as $-NO_2$, -CHO, -COOH, are inactive. Monohydric naphthols (1- or 2-naphthol) are only slightly active; introduction of a second phenolic hydroxyl, as in the 1,5-, 2,6-, 1,7- or 2,7-naphthols abolishes activity. Anthralin (1,8,9-trihydroxyanthracene or 1,8-dihydroxy-9-anthrone) has been consistently found to be a skin tumor promotor (see Section 5.2.2.5.3.3). Structure-activity studies by Van Duuren and associates (rev. in ref. 129) showed that the intactness of 1-, 8-, and 9-substituents as well as the central ring was needed for promoting activity. Esterification of the 1- and 8-positions, opening of the central ring or introduction of an additional hydroxyl group to the 10-position all

resulted in the loss of activity. Among the twelve phenolic isomers of benzo[a]pyrene, only 2- and 9-hydroxy derivatives display strong "promotor-like activity" (inducing epidermal hyperplasia); all the other isomers are either weak or inactive in this regard (127).

Phenolics as cocarcinogens.* Van Duuren and Goldschmidt (98) have tested the cocarcinogenic activity of several phenolics in an attempt to evaluate their role in tobacco carcinogenesis. When administered together with benzo[a]pyrene (BP), catechol and pyrogallol enhanced the carcinogenicity of BP remarkably. In contrast, phenol, eugenol, resorcinol and hydroquinone partially inhibited the carcinogenicity of BP. The authors suggested that vicinal phenolic functions may be a structural requirement of simple phenolics as cocarcinogens. Interestingly, both catechol and pyrogallol are inactive as promotors in the typical two-stage carcinogenesis assay, suggesting that the mechanism of cocarcinogenesis differs from the mechanism of promotor action. The potent cocarcinogenicity of catechol is supported by a recent study of Hecht *et al.* (130) showing catechol as a major component of several smoke condensate subfractions that display cocarcinogenic activity.

Phenolics as tumor inhibitors. The fact that a number of phenolic antioxidants (*e.g.*, BHA, BHT) inhibit the carcinogenic effects of some carcinogens created an intense interest toward the search among the phenolics for possible chemoprophylactic agents against chemical carcinogenesis. Wattenberg and associates (131a, 131b) have made significant contributions in this area. Structure-activity studies on twenty-two phenolics using benzo[a]pyrene as the

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*A "cocarcinogen" is defined as an agent that enhances the potency of a carcinogen when administered *simultaneously* and *repeatedly* with that carcinogen. A cocarcinogen is not carcinogenic by itself. Cocarcinogens are not necessarily tumor-promotors and vice versa.

carcinogen and the forestomach as the carcinogenicity target tissue showed that: (a) substitution in position para to the OH group of the unsubstituted phenol increases the inhibitory activity in this order: $OCH_3 > SCH_3 > H$, (b) among BHA-related compounds, the position of the t-butyl group with respect to the OH is of considerable importance; the most active inhibitor is 3-t-butyl-4-hydroxyanisole, (c) three or more substitutions on the phenol ring render the molecule inactive as an inhibitor, (d) phenols with intercyclic bond(s) are generally inactive. The mechanism of inhibitory action of phenolics is not clearly understood; reduction of activating pathways (132) as well as direct (free radical trapping) or indirect (induction of "protective" enzymes such as GSH S-transferase, NADPH-quinone reductase) enhancement of detoxifying pathways (133-135) have been suggested as possible mechanisms.

5.2.2.5.4 Metabolism and Mechanism of Action.

Phenol readily penetrates the mammalian body through virtually any route. After absorption, most of the phenol is either oxidized to CO_2 and water (and traces of catechol and hydroquinone) or excreted as "free" or "conjugated" (with sulfuric, glucuronic or other acids) phenol in the urine. It is well documented that phenol is a major metabolite of benzene (see Appendix I of this volume). Greenlee et al. (136) proposed that the toxic effects of benzene may be associated with the further metabolism of phenol to hydroquinone and 1,2,4-benzenetriol (via catechol) which may then be readily oxidized to highly reactive semiquinone and/or quinone metabolites and bind covalently to cellular macromolecules. This view is substantiated by the finding of Tunek et al. (137) that upon microsomal activation, phenol binds covalently to microsomal proteins much more efficiently than benzene itself. It is interesting to point out, however, that phenol does not seem to bind to microsomal RNA to any significant extent.

The major metabolic pathway of BHT (see Figure 1) involves the oxidation of the methyl group to yield 2,6-di-t-butyl-4-hydromethylphenol (II), 3,5-di-t-butyl-4-hydroxybenzaldehyde (III) and 3,5-di-t-butyl-4-hydroxybenzoic acid (IV); this pathway is generally believed to be "detoxifying" in nature (138). Small amounts of 1,2-bis-(3,5-di-t-butyl-4-hydroxyphenyl)ethane (V) and its corresponding quinone, 3,5,3',5'-tetra-t-butylstilbene-4,4'-quinone (VI) were also detected (see 139 and refs. therein) suggesting possible coupling reaction of BHT free radicals. Shaw and Chen (139) identified 4-hydroxy-4-methyl-2,6-di-t-butylcyclo-2,5-hexadienone (VIII) and an in vitro metabolite of BHT and proposed the formation of the corresponding 4-hydroperoxy compound (VII) as an intermediate. Recently, Takahashi and Hiraga (140) found that BHT may be oxidized in vivo to a p-quinone methide, 2,6-di-t-butyl-4-methylene-2,5-cyclohexadienone (IX); quinone methides of similar structures are known to be potent alkylating agents (141). The free radical, the 4-hydroperoxy and the quinone methide derivatives of BHT are all electrophilic reactive intermediates capable of attacking nucleophilic sites in cellular macromolecules. Indeed, covalent binding of BHT to lung, liver and kidney microsomes has been demonstrated (139, 142, 143). There is some evidence that the covalent binding may be related to its pulmonary toxicity in mice (143); it remains to be elucidated whether the binding is related to the possible carcinogenicity of BHT seen in some strains of mice.

The presence of the allylic side chain in eugenol suggests that is may be metabolically activated by epoxidation. Swanson et al. (61) showed that the 2',3'-oxide of eugenol is a direct-acting mutagen in the Ames test while eugenol itself is not. Evidence that the epoxidation of allylic side chain does indeed occur in vivo has been obtained by Solheim and Scheline (144) using methyl ether derivatives of eugenol.

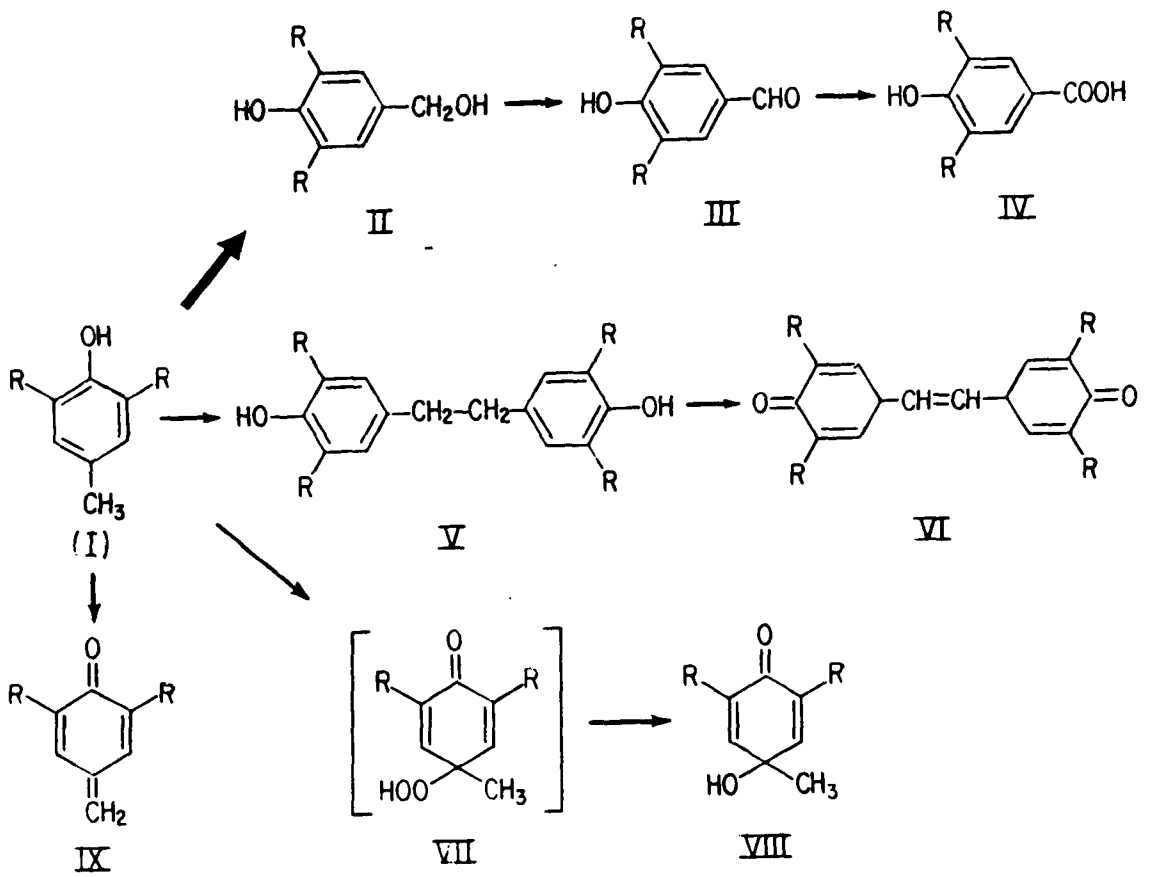


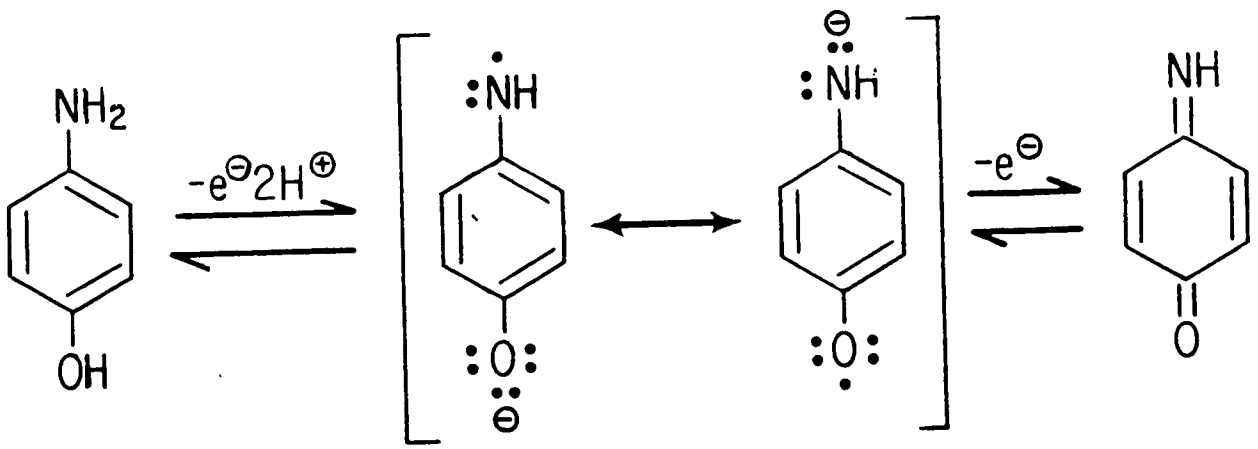
Fig. 1. Metabolic Pathways of Butylated Hydroxytoluene (BHT).

A comparison of *in vivo* covalent binding of bromobenzene and its metabolite, *p*-bromophenol, has recently been conducted by Monks *et al.* (145). *p*-Bromophenol binds significantly to liver proteins and to a lesser extent to kidney proteins. However, the level of binding of *p*-bromophenol is substantially lower than that of bromobenzene. This led the authors to conclude that the toxicity of bromobenzene is unrelated to the metabolism of *p*-bromophenol.

Extensive investigation on the biotransformation of pentachlorophenol (PCP) have been carried out (*rev.* in ref. 14). Human and animal studies indicate that PCP is excreted mainly in the urine, as such or as its glucuronide (146). The principal metabolites are tetrachloro-*p*-hydroquinone (146, 147). Tetrachloro-*p*-hydroquinone is also the major urinary metabolite (35%) of 2,3,5,6-tetrachlorophenol in the rat. The other two tetrachlorophenol isomers (2,3,4,5- and 2,3,4,6-) are excreted mainly unchanged together with small amounts of tetrachloro-*p*-hydroquinone (39). The toxicological consequence of biotransformation of chlorophenols to chloroquinones is not clearly understood. By analogy to phenol and hydroquinone, such biotransformation may represent bioactivation.

Little information is available on the metabolic activation of aminophenols. It is interesting to point out that aminophenols have properties similar to polyhydric phenols; for example, *p*-aminophenol may be oxidized to quinonimine. In analogy to the oxidation of hydroquinone to quinone, it is

[*text figure* 5]



Text-Figure 5

conceivable that semiquinone-type of reactive intermediate may also be formed by one-electron oxidation of aminophenols with amino group para or ortho to the hydroxyl group.

The above brief summary serves to illustrate that electrophilic reactive intermediates (free radicals, epoxides, peroxides, semiquinone, quinone methides) may be generated from phenolics. It remains to be elucidated whether these intermediates represent potential proximate carcinogens. A systematic structure-activity relationship analysis of the effect of substituents on the stability (stable enough to reach targets) and reactivity (active enough to react with targets) of these intermediates may help to identify compounds with high carcinogenic potential. It is important to point out that despite demonstration of covalent binding of phenolics to proteins, clear evidence of covalent binding to DNA or RNA is still lacking. However, Ts'o et al. (148) have pointed out that reactions of free radicals with DNA may not necessarily lead to covalent carcinogen-DNA adducts, but to other types of damage such as strand scission and cross-linking. In this respect, it should be noted that a number of phenolics have been shown to cause chromosome damage through the activated oxygen species ($^{\bullet}OH$, $^{\bullet}O_2^{\ominus}$, H_2O_2) generated during the metabolism. Besides the above genotoxic mechanisms, phenolics may also conceivably promote or initiate carcinogenesis by epigenetic mechanisms. Thus, the well known effects of some phenolics as protein-denaturants or inhibitors of energy metabolism may be related to carcinogenesis by high doses of phenolics. Two recent findings, induction of endogenous tumor virus gene expression by hexachlorophene (149) and suppression of immune response by technical grade (but not pure) pentachlorophenol (150) are suggestive of possible mechanisms of carcinogenesis.

5.2.2.5.5 Environmental Significance.

Phenolics are ubiquitously present in the environment. Human exposure may occur via inhalation, ingestion or skin absorption of the phenolics themselves or of aromatic compounds which may be hydroxylated in the body to yield phenolic metabolites. The environmental occurrence of and exposure to phenolics have been extensively reviewed (9-15, 21, 23, 151).

Phenolics in air and tobacco smoke. Pentachlorophenol (PCP) has been detected in the air (average 0.26-1.89 $\mu g/m^3$; max. 15 $\mu g/m^3$) of a wood treatment plant in Idaho (152). The occurrence of PCP appears not to be limited to occupational setting. Cautreals et al. (153) reported that air samples collected in a residential city area of Antwerp, Belgium contained 5.7-7.8 ng/m^3 PCP. For comparison, air samples collected from a relatively remote area in Bolivia, South America contained only 0.25-0.93 ng/m^3. In addition to PCP, phenol and cresols have also been detected as pollutants in urban air (154). Lao et al. (155) reported the presence of 4-nitrophenol in an urban air sample. Nojima et al. (156) detected the formation of nitrophenols and nitrocresols after photochemical reaction of nitrogen monoxide with benzene and toluene. It seems likely that humans may be exposed to nitrophenols in areas where severe photochemical smog exists.

Tobacco smoke contains a variety of phenolics. Catechol, the most abundant phenolic in cigarette smoke, is believed to be formed during the incomplete combustion of tobacco flavonoids such as rutin, quercetin and chlorogenic acid (157). Hoffmann and Wynder (154) reported that the mainstream smoke of a 85 mm filterless popular U.S. cigarette contained an average of 300 μg catechol, 100 μg phenol, 72 μg cresols and 20 μg 2,4/2,5-dimethylphenol. Schlotzhauer et al. (158) identified catechol, resorcinol, hydroquinone and

their alkyl-substituted derivatives, vanillin and its derivatives, dimethoxyphenols, phenylphenols and naphthols in an acidic fraction of cigarette smoke condensate. These phenolics (especially dihydroxyphenols) are believed to contribute to the carcinogenic effects of cigarette smoke by acting as promotors or cocarcinogens (98, 130, 154).

Phenolics in drinking water. A number of phenolics have been detected in the finished drinking water (FDW) of several cities. Buhler *et al.* (159) reported the occurrence of PCP (0.06 ppb) and hexachlorophene (0.01 ppb) in FDW of Corvallis, Oregon; analysis of incoming raw river water sample showed the presence of 0.17 ppb PCP and 0.03 ppb hexachlorophene. Phenolics were suspected to be present in FDW of Ames, Iowa because of its taste and odor; however, an analysis by Burnham *et al.* (160) did not substantiate this view. [This study was incorrectly cited to show a positive finding of 0.2 ppm 4-nitrophenol in an EPA Water Quality Criteria Document (13)]. Deinzer *et al.* (161) detected 2,4,6-trichlorophenol in FDW of Cincinnati, Ohio. Shackelford and Keith (162) listed hexachlorophene as being present in two samples of FDW. During an accidental spillage of phenol in July of 1974, a massive contamination of well water in a rural area of southern Wisconsin occurred (163). An outbreak of human poisoning ensued. Analyses of a well nearest the spill site revealed peak phenol concentrations between 15 and 126 mg/l. The forecast is that the contamination of the well water in the area will persist for years. Numerous other phenolics have been detected in plant effluents, pulp and paper mill effluents, agricultural run-off, etc; these phenolics could be present in FDW if these effluents contaminated the raw water sources.

Phenolics in foodstuffs. A variety of phenolics are present in foodstuffs due to natural occurrence, intentional use as food additive, or inadvertent contamination. Fruits, vegetables (164-167), and beverages such as

coffee (168) and tea (167, 169) are relatively rich in phenolics of natural origin. Kaiser (169) identified phenol, cresols and dimethylphenols in black fermented tea; he further showed that brewed tea may promote the skin carcinogenic activity of benzo[a]pyrene. Many phenolics are widely used as food additives. The most notable among these are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG). These compounds are used as antioxidants to prevent the rancidity of foods which contain fats, by terminating chain reactions involving free radicals responsible for the oxidative degradation of unsaturated fats. The estimated annual consumption in the United States is 1,400, 450 and 230 metric tons for BHT, BHA and PG, respectively. In 1974, the Food and Agriculture Organization of United Nations (27) temporarily adopted an ADI (acceptable daily intake) of 0-0.5 mg/kg body weight for BHA, BHT, or BHA and BHT combined, and 0-0.2 mg/kg body weight for PG and related gallates. The U.S. Food and Drug Administration (101) proposed to restrict the use of BHT in 1977 due to its possible carcinognicity in some strains of mice. Other important food additives are eugenol and vanillin, used to enhance food flavor and fragrance. The average maximum use levels of eugenol in beverages, ice cream, gelatins and chewing gums range from 1.4 to 500 ppm with levels as high as 2,000 ppm used in processed meat products (170). Inadvertent contamination of foodstuffs may occur as a result of direct contamination or degradation of pesticides. For example, Murray et al. (171) detected PCP levels of 2.4-8.3 ng/g (average 5.3 ng/g) in oysters from Galveston Bay, Texas. Johnson and Manske (172) found PCP in 13/240 food samples collected in 20 U.S. cities; the concentration ranged from 0.01 to 0.04 mg/kg. Phenolics that have been detected in food as a result of degradation of pesticide residues include PCP, 2,4,5-/2,4,6-trichlorophenol (from hexachlorobenzene), 2,4-dichlorophenol (from 2,4-D, 2,4,5-T, Silvex or

Nemacide) and mononitrophenols (from parathion, fluoridifen or nitrofen). Smoked meat products also contain phenolics probably due to their formation during incomplete combustion of hardwood sawdust used to generate the smoke (173).

Phenolics as drugs or in toiletry products. Phenolics which have been widely used as drugs are salicylates and acetaminophen (as analgesics and antipyretics), anthralin (for treatment of psoriasis and chronic dermatoses), thymol (as anthelmintic or antiseptic) and phenol (antiseptic, analgesic for terminally ill cancer patients), etc. Hexachlorophene was used as a surgical scrubbing agent, in bathing newborns to prevent skin infection and in many toiletry products until 1972 when discovery of neurotoxicity in infants led to the restriction of its use in the United States (174). Phenolics such as 4-amino-2-nitrophenol, resorcinol, hydroquinone, pyrogallol, 1-naphthol, 4-hydroxyanisole are present in cosmetics and toiletry products, such as hair dyes, depigmentation agents, soap and perfumes (57, 107).

The magnitude of carcinogenicity risk of human exposure to phenolics is relatively unknown. No cancer epidemiology study for specific phenolics has thus far been conducted. There is some evidence that humans exposed to chlorophenoxy type herbicides (which contain chlorophenols) may incur an increased risk for malignant lymphoma (175-177); however, it is not known whether the increased risk can acutally be attributed to chlorophenols. One physician (178) noted an unusual coincidence of the induction of bladder cancer in two patients who were occupationally exposed to cresol and creosote; thus far, no similar cases has been reported. Phenolics are present in hair dyeing chemicals, which are suspected to be potentially hazardous to exposed women (68). As pointed out in Section 5.2.2.5.3, a variety of phenolics are being tested for carcinogenicity at the time of this writing.

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Notes Added After Completion of Section 5.2.2.5

Close to 40 phenolic compounds have been tested for mutagenicity using the Ames *Salmonella* test in recent studies carried out for the U.S. National Toxicology Program (1-4). Among these, only *o*-aminophenol (1), *m*-aminophenol (3), and 2,4-diaminophenol (1) were reported to give positive results. The positive findings with *o*-aminophenol and *m*-aminophenol are at variance with the results of a number of previous studies (5, 6; *see also* Table LX) indicating the lack of mutagenicity of all three isomeric forms of aminophenol in the Ames test. *o*-Phenylphenol was listed as positive in one publication (1) but the accompanying data provide no support for the conclusion; the compound was listed as a nonmutagen in a more recent publication (3). Most of the 33 compounds that yielded negative data and two that gave equivocal results have been previously shown to be nonmutagenic (*see* Table LX). Negative compounds which do not appear to have been previously tested include hydroquinone monomethyl ether (1), *o*-benzyl-*p*-chlorophenol, *o*-*sec*-butylphenol, tetrabromobisphenol A (2), *p*-*tert*-pentylphenol (3), and propyl gallate (4). Sodium *o*-phenylphenate (the sodium salt of *o*-phenylphenol), a bladder carcinogen, is not mutagenic in the Ames test (7). Several phenolic compounds exhibit comutagenic activity. In the presence of sufficient amount of S9 mix, catechol substantially enhances the mutagenic activity of benzo[a]pyrene and of 4-nitroquinoline-1-oxide toward *Salmonella typhimurium* (8). Similarly, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and, to a lesser extent, propyl gallate increases the mutagenicity of aflatoxin B_1 (9). The mechanism of the enhancement is not known. A number of phenolic compounds enhance the mutagenicity of other compounds by inducing their activating enzymes. Pretreatment of mice with 2(3)-*tert*-butyl-4-hydroxyanisole results in a 5-fold increase in the ability of liver microsomes to activate

aflatoxin B_1 to a mutagen (10) whereas 2,3,4,5-tetrachlorophenol enhances the ability of rat liver microsomes to activate benzo[a]pyrene (11).

Among phenolic compounds tested for teratogenic activity, o-phenylphenol (12), 2,4-dichlorophenol (13), chlororesorcinol (14), pyrogallol (14), and m-aminophenol (15) are all inactive in the rat. In each of the above studies, several dose levels were used with the highest dose level being sufficiently high to cause some degree of maternal or fetal toxicity. o-Aminophenol and p-aminophenol are teratogenic in the Syrian golden hamster whereas the data on m-aminophenol were equivocal (16). In this study, the compounds were administered to rats intraperitoneally, intravenously or orally on day 8 of gestation at doses of 100, 150 or 200 mg/kg; the fetuses were examined on day 13 of gestation. The most frequently observed malformations were encaphalocele and limb, tail and eye defects. The teratogenic effects were observed at doses which did not produce any apparent maternal toxicity. The authors suggested the formation of a reactive quiononeimine metabolite as a proximate (or ultimate) teratogenic intermediate. This view was supported by the finding that p-benzoquinoneimine exhibited similar teratogenic effects as p-aminophenol in the hamster.

The carcinogenicity of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has been further tested in view of their widespread use as food additives. Two groups of Japanese investigators confirmed that BHT is not carcinogenic in Wistar rats (17) or $B6C3F_1$ mice (18) after long-term feeding. In agreement with previous studies using mice as the test species, BHA has also been reported to be noncarcinogenic in $B6C3F_1$ mice (Yokoro, *cited in* ref. 19). However, there is strong evidence to indicate that BHA is carcinogenic toward the forestomach of F344 rats and Syrian golden hamsters. Ito *et al.* (19) fed groups of F344 rats diets containing 0.5 or 2.0% BHA for 2

years. In the high dose group, virtually all the rats developed papillomas in the forestomach; 18 of 52 male rats and 15 of 51 female rats also had squamous cell carcinomas in the forestomach. In the low dose group, the only significant change was an increase in the incidence of hyperplasia of the forestomach. None of the control animals exhibited similar pathological changes. A preliminary 24-week study (20) on Syrian golden hamsters suggested that this species may be even more susceptible to BHA-induced forestomach carcinogenesis; 17 of 17 hamsters maintained on diets containing 1.0 or 2.0% BHA developed forestomach papillomas within 24 weeks. In view of these new findings, the potential health hazard of widespread use of BHA should be urgently reevaluated. Both BHT and BHA have been shown to be either an inhibitor or a promotor of carcinogenesis depending on the target organ, the type of carcinogen (initiator) and the temporal sequence of administration. Adding to the existing body of literature on the ambivalence of BHA and BHT (see Section 5.2.2.5.3.4; see also review in ref. 21), Imaida et al. (22) showed that both BHA and BHT enhance 4-hydroxybutylbutylnitrosamine-induced bladder carcinogenesis in rats. Maeura et al. (23) found BHT to be a promotor of 2-acetylaminofluorene-induced bladder carcinogenesis in rats but an inhibitor of hepatocarcinogenesis by the same compound. Prophylactic effects against methylazoxymethanol-induced colon carcinogenesis (24), benzo[a]pyrene-induced pulmonary carcinogenesis (25) in mice have been demonstrated by BHA and an inhibition of dimethylbenz[a]anthracene-induced mammary carcinogenesis by BHT has been reported (26).

o-Phenylphenol (OPP) and its sodium salt (sodium o-phenylphenate, OPP-Na), widely-used broad spectrum fungicides used in protection of various edible crops, have recently been tested for carcinogenic activity. Hiraga and Fujii (27) reported that OPP-Na was a notably active carcinogen in male F344

rats inducing tumors of the urinary system within a relatively short latency period. In a subchronic 13-week study, 9 of 10 male rats fed 2% OPP-Na in the diet were found to have urinary bladder tumors with five of these tumors diagnosed as transitional cell carcinomas. Female rats appeared to be substantially less susceptible; only 2 of the 10 female rats fed 4% OPP-Na developed bladder papillomas within this time period. A subsequent 91-week study showed that the carcinogenic effect of OPP-Na was dose-dependent; the incidences of transitional cell carcinomas of the urinary bladder, renal papilla or pelvis in male rats fed 0.125, 0.25, 0.5, 1.0, 2.0 or 4.0% OPP-Na were 0, 0, 5, 35, 95 and 85%, respectively. In a preliminary communication, Fujii and Hiraga (28; and K. Hiraga, personal communication) reported that dietary administration of OPP to F344 rats for 2 years also induced urinary bladder tumors. The carcinogenic potency of OPP appeared to be less than that of OPP-Na. A carcinogenesis bioassay of OPP by the U.S. National Toxicology Program was near completion at the time of this writing. In a 36-week study designed to test the tumor-promoting activity of dietary administration of 2% OPP and OPP-Na, Fukushima et al. (29) found that OPP-Na induced urinary bladder tumors in 8 of 44 F344 rats whereas OPP had no significant effect within this time period. When administered after tumor initiation with a known bladder carcinogen, 4-hydroxybutylbutylnitrosamine, OPP-Na exhibited potent promoting activity whereas OPP had little or no promoting activity. The molecular basis for this apparent considerable difference between the free phenol and its sodium salt is not clear. It may be related to the easier generation, from the sodium salt, of phenolate (phenoxide) anion, which is stabilized by resonant limit structures involving an extended conjugated system through the unsubstituted phenyl ring. These structures would facilitate the generation of semiquinone-type reactive intermediates (see below).

Two chlorinated phenols, 2-chlorophenol and pentachlorophenol, have no significant carcinogenic activity in Sprague-Dawley rats after prenatal (throughout the gestation period) and postnatal (from weaning to up to 24 months of age) exposure to 5, 50 or 500 ppm (2-chlorophenol in drinking water or pentachlorophenol in feed) (30; and J.H. Exon, personal communication). In contrast to the lack of complete carcinogenic activity, both 2-chlorophenol and, to a lesser extent, pentachlorophenol exhibited promoting or cocarcinogenic activity enhancing the carcinogenic action of the transplacental carcinogen, N-ethyl-N-nitrosourea. The enhancing effect of 2-chlorophenol was evident even at the low dose of 5 ppm administered either prenatally or postnatally.

A carcinogenesis bioassay of propyl gallate, a polyhydric phenolic compound, has recently been completed by the U.S. National Toxicology Program (4). Groups of 50 F344/N rats and 50 $B6C3F_1$ mice of each sex were maintained on diets containing 6,000 or 12,000 ppm propyl gallate for 103 weeks. There were higher incidences of preputial gland tumors, islet-cell tumors of the pancreas and pheochromocytomas of the adrenal gland in male rats in the low dose group, as well as an astrocytoma and a glioma (two rare types of brain tumors) in two low-dose females. None of these tumors were found in the high-dose group, however. Thus, under the conditions of this bioassay, propyl gallate was concluded to be not carcinogenic in F344/N rats or $B6C3F_1$ mice of either sex.

The *in vitro* metabolism of 3-*t*-butyl-4-hydroxyanisole has been studied by Rahimtula (31). 3-*t*-Butyl-4-hydroxyanisole is oxidized to a variety of metabolites which include formaldehyde, a dimeric product, polar metabolites, as well as a reactive intermediate(s) that binds irreversibly to proteins. The authors proposed that 3-*t*-butyl-4-hydroxyanisole is oxidized predominantly via

one electron oxidation to yield reactive free radical(s) which dimerizes, undergoes further metabolism or binds to protein. The study underscores the importance of peroxidases in the metabolism of 3-*t*-butyl-4-hydroxyanisole; the *in vivo* significance of these findings remains to be investigated.

The possible molecular mechanisms involved in toxicity and bladder carcinogenesis by *o*-phenylphenol (OPP) or its sodium salt (OPP-Na) have been investigated by Reitz *et al*. (32). Both compounds appear to have little or no genotoxic activity as demonstrated by the lack of mutagenicity in the Ames test and the lack of *in vivo* covalent binding of OPP or OPP-Na to rat bladder DNA. The metabolism of OPP or OPP-Na was shown to be dose-dependent. At doses below 50 mg/kg, conjugation to polar metabolites (glucuronide or sulfate conjugates) was the predominant reaction. At doses above 500 mg/kg, there was evidence of oxidative formation of 2,5-dihydroxybiphenyl. It was postulated that the reactive intermediates (*e.g.*, semiquinone) generated by this oxidative pathway may be associated with the toxic or carcinogenic action induced by high concentrations of OPP or OPP-Na.

A number of investigators have recently presented evidence of the alteration of immune functions of animals treated chronically with phenolic compounds. Immunosuppression has been implicated as a possible mechanism of carcinogenic or tumor-promoting action of phenolic compounds; however, the evidence for such an association does not appear to be very strong or consistent. LaVie and LaVie (33) reported that *o*-phenylphenol was immunosuppressive in mice; this finding could not be confirmed by Luster *et al*. (34). Kerkvliet *et al*. (35) demonstrated that chronic treatment of mice with technical grade pentachlorophenol suppressed the immune functions and increased the susceptibility of the animals to tumor cell challenge. Mixed findings were reported for purified pentachlorophenol which was not immunosuppressive in mice (35),

reduced humoral and cell-mediated immune functions but enhanced macrophage activity in rats (36), and suppressed humoral immunity and T-cell responses to mitogens in chickens (37). 2-Chlorophenol, an active tumorigenesis promotor, was not immunosuppressive in the rat (36).

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