NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for PHENOLPHTHALEIN

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NTP Report on Carcinogens Listing for Phenolphthalein

Carcinogenicity

Phenolphthalein is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of increased incidence of malignant and/or combination of malignant and benign tumors in multiple tissue sites and in multiple species. In a two-year B6C3F$_1$ mouse carcinogenicity study, NTP (1996) concluded that phenolphthalein, administered in feed, induced significant increases in the incidence of histiocytic sarcoma and lymphomas of thymic origin in males and females and malignant lymphoma (all types) and benign ovarian sex cord stromal tumors in females. In the corresponding Fischer 344 rat dietary carcinogenicity study, phenolphthalein induced significant increases in the incidence of benign pheochromocytoma of the adrenal medulla in males and females and renal tubule adenoma in males (NTP, 1996). In a 6-month dietary study with female heterozygous p53-deficient transgenic mice, phenolphthalein induced a significant increase in the incidence of malignant lymphoma of thymic origin (Dunnick et al., 1997).

The data available from epidemiology studies on laxative use are limited and not sufficient for evaluating the potential carcinogenicity from exposure to phenolphthalein.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

The malignant thymic lymphomas induced by phenolphthalein in female heterozygous p53-deficient transgenic mice exhibited a loss of the normal p53 allele, suggesting the involvement of a mutagenic mechanism in tumor induction and/or progression (Dunnick et al., 1997).

Phenolphthalein causes enhanced oxygen radical production in *in vitro* systems. *In vivo*, reduction of phenoxyl radicals could allow reformation of phenolphthalein, establishing a futile cycle of oxidation and reduction, thereby generating more free radical species. Thus, phenolphthalein may be a significant source of oxidative stress in physiological systems.

Although negative for mutagenicity and DNA damage in bacteria, phenolphthalein exhibits genetic activity in several *in vitro* and *in vivo* mammalian assays. Phenolphthalein was positive for the induction of chromosomal aberrations in cultured Chinese hamster ovary cells in the presence of metabolic activation and induced *hprt* gene mutations, chromosomal aberrations, and morphological transformation in Syrian hamster embryo cells. Phenolphthalein was also positive for the induction of micronucleated erythrocytes in mice following multiple but not single treatments administered by gavage or dosed feed. Phenolphthalein also induced micronuclei in female heterozygous p53-deficient transgenic mice exposed via dosed feed for 26 weeks. Abnormal sperm were induced in male mice but not male rats treated with phenolphthalein via dosed feed for 13 weeks. Phenolphthalein was negative for Na/K ATPase gene mutations and aneuploidy in Syrian hamster embryo cells.

No data are available that would suggest that the mechanisms thought to account for tumor induction by phenolphthalein in experimental animals would not also operate in humans. Phenolphthalein causes oxidative stress and also demonstrates the capability to alter tumor suppressor gene pathways, which are both mechanisms believed to be involved in human cancer.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

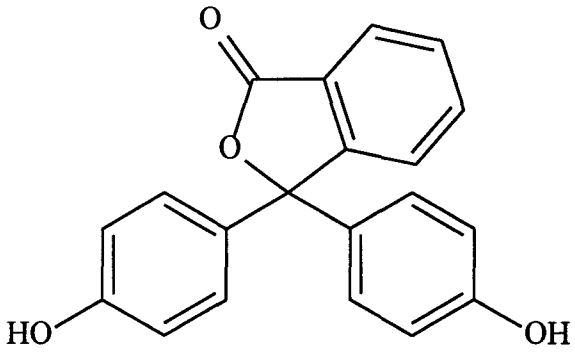
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Phenolphthalein
[77-09-8]



1.1 Chemical Identification

Phenolphthalein ($C_{20}H_{14}O_4$, mol. wt. = 318.33) is also called:

1(3*H*)-Isobenzofuranone, 3,3-bis(4-hydroxyphenyl)- (9CI)
3,3-Bis(4-hydroxyphenyl)-1(3*H*)-isobenzofuranone
3,3-Bis(p-hydroxyphenyl)phthalide
3,3-Bis(4-hydroxyphenyl)phthalide
α-(*p*-Hydroxyphenyl)-α-(4-oxo-2,5-cyclohexadien-1-ylidine)-*o*-toluic acid
α-Di(*p*-hydroxyphenyl)phthalide
Dihydroxyphthalophenone
Phthalide, 3,3,-bis(*p*-hydroxyphenyl)
Phthalimetten®
Purgophen (VAN)
α,4,4′-Trihydroxytriphenylmethane-2-carboxylic acid lactone

Many commercial brand name laxatives have included phenolphthalein in their formulations. They include the following:

Agoral®
Alophen®
Chocolax®
Colax®
Correctol®
Darmol®
Dialose®Plus
Doxidan®
Espotabs®
Euchessina®
Evac-U-Gen®
Evac-Q-Kit®
Evac-Q-Kwik®
Evac-U-Lax®
Evac-Q-Tabs®
Ex-Lax®
Feen-A-Mint Gum®
FemiLax®

Kondremul®
Koprol®
Lactone
LaxCaps®
Laxogen®
Lax-Pills®
Lilo®
Medilax®
Modane®
Phenolax®
Phillips®
Phthlalin®
Prulet®
Purga®
Purgen®
Spulmako-lax®

In September 1997, the U.S. Food and Drug Administration (FDA) proposed reclassification of the use of phenolphthalein in over-the-counter (OTC) laxative products (FDA, 1997). In anticipation of the FDA action, the producers of Correctol® and Feen-a-Mint® brand products replaced phenolphthalein with bisacodyl in January 1996. Bayer's Phillips' GelCaps was voluntarily removed from the market in mid-1997; and Novartis AG, the marketer of Ex-Lax®, announced in late August 1997 that its product would be reformulated, substituting senna for phenolphthalein (Suplee, 1997; Drug Topics, 1997).

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	White or yellow-white; pink to deep red in alkaline solution (pH>9); colorless to pH=8.5	Budavari (1996)
Physical State	Minute, triclinic crystals	Budavari (1996)
Melting Point, ° C	258-262	Budavari (1996)
Specific Gravity	1.299	Budavari (1996)
Dissociation Constant at 25°C (pKa)	9.7	Budavari (1996)
Odor	Odorless	NTP (1996)
Solubility:		
Water at 20°C	Almost insoluble in water.	Budavari (1996)
Organic Solvents	Soluble in alcohol and diethyl ether, very slightly soluble in chloroform. Soluble in dilute solutions of alkali hydroxides and hot solutions of alkali carbonates.	Budavari (1996)

2.0 HUMAN EXPOSURE

2.1 Use

Phenolphthalein in 1% alcoholic solution is used as a visual indicator in titrations of mineral and organic acids and most alkalies. Phenolphthalein-titrated solutions are colorless at pH < 8.5 and pink to deep-red at pH > 9. Phenolphthalein is not suitable, however, for titrating ammonia or alkaloids; carbonate solutions should be boiled before titration because of a high sensitivity to carbon dioxide (Budavari, 1996).

Phenolphthalein is used in a variety of ingested products and in some scientific applications. Because phenolphthalein is odorless and tasteless, it can be incorporated easily in tablets, powder, and liquid. It has been commonly used as a laxative, available worldwide as an over-the-counter chocolate or gum laxative product. The official dose is 60 mg, but adults usually require 100 to 200 mg (Fingl, 1965). Bedridden patients require 500-mg doses (Sollman, 1957). Dunnick and Hailey (1996) estimated that the low dose used in the 2-year NTP mouse bioassay is about 10 times a human dose of 5 mg/kg on a body surface area basis.

The use of laxatives to relieve constipation and to maintain regularity in bowel habits is widespread in the United States. Two large surveys of the U.S. adult population agreed on the prevalence of laxative use at least once per month (11.5% ca. 1989, 9.7% in 1982-1984) but differed in self-reported constipation (3.4% ca. 1989, 20.3% in 1982-1984). These studies and others agree that female users outnumber male users (e.g., 20.8% of females and 8.0% of males in 1971-1975 and 4.9% in females and 1.3% in males ca. 1989), that the fraction of users increases with age, and that persons who self-report constipation are more likely to use laxatives and stool softeners than persons who do not report constipation (e.g., 22.6% vs. 6.4% in 1982-1984) (Harari et al., 1989 [ca. 1989 data]; Everhart et al., 1989 [1971-1975 and 1982-1984 data]). Differences between the studies may lie in the relative ages of the subjects. In the ca. 1989 population, 47% was younger than 40, whereas in the 1982-1984 study, only 15% of the population was younger than 45.

Few studies report on the prevalence of phenolphthalein laxative use. To judge from the four populations described below, it would appear that no more than 10% of the U.S. population has used phenolphthalein-containing laxatives as often as once per month, but up to 5% may have used them weekly or more often.

One study of 424 cases of invasive adenocarcinoma of the colon and 414 controls in Washington state, ages 30 to 62 years, found that 13.6% of the subjects reported constipation requiring treatment (use of a laxative, enema, or prunes), 4.7% reported ever use of phenolphthalein laxatives, and 3.5% reported use of phenolphthalein laxatives at least 350 times in their lifetimes (Jacobs and White, 1998).

In three U.S. populations of 268 to 813 persons comprising approximately equal numbers of cases of adenomatous colorectal polyps and controls, 0.97 to 5.1% of the subjects used phenolphthalein laxatives at least once per week. The two North Carolina groups included subjects aged 30 to 89 years, 58% and 53% of which were female; the group in Los Angeles, California, included subjects aged 50 to 74 years of which 34% were female. Mean ages of the three groups were comparable (59 to 62 years). The frequent phenolphthalein laxative users represented 8 to 30% of all frequent laxative users. The ever use of phenolphthalein laxatives in the two North Carolina groups was 17.5% and 25%, with 10% and 7% using them at least once per month (Longnecker at al., 1997).

2.2. Producers, Production Process, and Production Volume

The only current U.S. producer of phenolphthalein is Sigma-Aldrich Corporation (SRI International, 1997a). In 1989, two of the U.S. producers of phenolphthalein were Aldrich Chemical Co, Inc., of Milwaukee, WI, and Hill Brothers Chemical Co., of Orange, CA (SRI International, 1989; cited by HSDB, 1997).

Phenolphthalein is produced commercially by condensation of phthalic anhydride with phenol in the presence of sulfuric acid, a dehydrating agent (Martin and Cook, 1961; Budavari, 1996).

The *Chemical Economics Handbook* (1992; cited by NTP, 1996) reported that 250 tons (197 metric tons or Mg) of phenolphthalein was produced annually by Sigma-Aldrich Corporation. Current production is reported to be the same (SRI International, 1997a).

Combined sales of the top 3 phenolphthalein-containing drugs, Correctol® by Schering Plough Health Care, Phillips® by the Bayer Corporation, and Ex-Lax® by Sandoz Pharmaceuticals (currently marketed by Novartis AG), totaled 16.4% of the laxative market in 1989 (Drug Store News, 1990); 23.9% in 1992 (Advertising Age, 1993); and 19.9% in the period July 1993-July 2, 1994 (DeNitto, 1994). The three drugs were still among the top-selling laxatives in 1995 (SRI International, 1997b)

Ex-Lax® was among the top 3 best-selling laxatives in 1996, accounting for about 7% of the brand-name sales. Phenolphthalein was removed from Correctol® in early 1996, and from Phillips GelCaps® and Ex-Lax® in 1997. In the latter 2 cases, the phenolphthalein-containing products were recalled from retail shelves (Suplee, 1997; Drug Topics, 1997).

U. S. FDA (1997) in its September 2 proposal to remove phenolphthalein from the generally recognized as safe and effective category estimated that the number of manufacturers of phenolphthalein-containing laxatives had fallen from the 60 manufacturers in its database to 20 manufacturers by August 1997.

2.3 Environmental Exposure

Exposure is possible through discharges to air and from process units where phenolphthalein is manufactured.

2.4 Occupational Exposure

A National Occupational Exposure Survey (NOES) conducted between 1981-1983 by the National Institute of Occupational Safety and Health (NIOSH) listed 75,243 workers (26% female) as being potentially exposed to phenolphthalein. The number of Health Services employees potentially exposed to phenolphthalein was 20,122 (65% female) (NIOSH, 1990; cited by NTP, 1996).

2.5 Regulations and Criteria

The U.S. Food and Drug Administration proposed on September 2, 1997, to declare all phenolphthalein-containing drug products to be new drugs within the meaning of Section 201(p) of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 321(p)]. In the *Federal Register* notice, which affects 21 CFR Part 310 and 334, FDA proposed to reclassify phenolphthalein from Category I (generally recognized as safe and effective and not misbranded) to Category II (not generally recognized as safe and effective and misbranded) and added it to a list of non-

monograph active ingredients. Phenolphthalein would be added to 21 CFR Section 310.545(a)(12)(iv), the list of stimulant laxatives (U.S. FDA, 1997).

The notice concluded with the statement: "the FDA considers use of phenolphthalein a potential risk to humans. These findings of rodent carcinogenicity and genotoxicity in several test systems indicate that chronic use could lead to damage to the human genome (including p53, which is known to be a tumor suppressor gene) and could increase the risk of malignancy." The FDA invited comments on these findings, and in late 1997 was reviewing written comments received from industry (U.S. FDA, 1997).

As described in Section 1.1, many over-the-counter phenolphthalein-containing laxatives were being reformulated in late 1997 without phenolphthalein.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 et seq.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants (HAP) are listed in this part pursuant to section 112(b) of the CAA. Phenolphthalein itself is not an HAP.
	40 CFR 63.100 ff.—Subpart F—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry.	This subpart applies to chemical manufacturing process units that manufacture phenolphthalein (Group III) and are located at a plant site that is a major source as defined in section112(a) of CAA. Owners and operators of sources subject to this subpart shall comply with the requirements of subparts G and H of this part.
	40 CFR 63.110 ff.—Subpart G—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry for Process Vents.	The provisions of this subpart apply to all process vents, storage vessels, transfer racks, and wastewater streams within a source subject to subpart F of this part. Emission standard: Emissions of organic HAPs from phenolphthalein-manufacturing plants shall be controlled to the level represented by a given equation [see 40 CFR 63.112(a)]. Specific process vent and methods and procedures provisions apply.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998, 03/27/75. U.S. Code: 21 U.S.C.321, 331, 351, 352 355, 356, 357, 358, 360, 360b, 360gg-360ss, 371, 374, 379e; 42 U.S.C. 216, 241, 262, 264. 21 CFR 201.60 ff.—Subpart B—Labeling Requirements for Prescription Drugs and/or Insulin. 21 CFR 201.57—Sec. 210.57 Specific requirements on content and format of labeling for human prescription drugs.	On human drug prescriptions, there must be a subsection of the labeling stating whether long-term studies in animals have been performed to evaluate carcinogenic potential, and it must include the species and results. Any precautionary statements on these topics shall also include practical, relevant advice on the significance of these animal findings. If there is evidence from the human data that the drug may be carcinogenic or mutagenic or that it impairs fertility, this information will be contained in the "Warning" section.
	21 CFR 369—PART 369—INTERPRETATIVE STATEMENTS RE: WARNINGS ON DRUGS AND DEVICES FOR OVER-THE-COUNTER SALE. Promulgated: 39 FR 11745 03/29/74. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 371.	Subparts A and B regulate the warning and caution statements included on the labels of drugs and devices for over-the-counter sale.
	21 CFR 369.20—Sec. 369.20 Drugs; recommended warning and caution statements.	Drug preparations containing phenolphthalein should contain, in addition to the general warning, the following statement: "*Caution*—If skin rash appears, do not use this or any other preparation containing phenolphthalein."
O S H A	29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74. U.S. Code: 29 U.S.C. 653, 655, and 657.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
OSHA	29 CFR 1910.1200—Sec. 1910.1200 Promulgated 02/15/89. OSH. Act: Hazard Communication Standard.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training. Labels may be subject to FD & CA requirements.

[a] The regulations in this table have been updated through 62 Federal Register 23394, April 30, 1997.

3.0 HUMAN STUDIES

There are no adequate epidemiology studies specifically addressing the relationship between phenolphthalein use and human cancer. The National Cancer Institute nominated phenolphthalein for study because this was a widely used over-the-counter drug for which there were no adequate toxicity or carcinogenicity studies reported in the literature.

Human carcinogenicity studies reported prior to 1996 are reviewed in NTP (1996, pp. 19-24, see Appendix A). Studies not included in or published after NTP (1996) are summarized below and in **Table 3-1**.

No statistically significant increase in colorectal cancer risk was observed in 1,408 patients (selected from April 1980 to April 1981) of "The Melbourne Colorectal Cancer [Case-Control] Study" for those who used phenolphthalein laxatives (Kune, 1993; cited by NTP, 1996). Controls for this study were randomly selected from the same geographic area and age/sex frequency matched with the clinical cases. Also, in a case-control study of 11,888 California retirement community residents, the association between laxative use and the risk of colorectal cancer was not significant (Wu et al., 1987; cited by NTP, 1996).

Data from three case-control studies were examined to assess the relationship between the use of phenolphthalein-containing laxatives and the occurrence of adenomatous colorectal polyps (Longnecker et al., 1997). The case (730 total subjects) and control (907 total subjects) groups were part of three separate case-control studies conducted in Los Angeles (1991-1993) and North Carolina (1988-1990, 1992-1995). In all studies, subjects were selected from among those patients undergoing an endoscopic procedure. All cases had histologically confirmed adenomatous polyps. Controls in Los Angeles included subjects without polyps of any kind; in North Carolina patients had no adenomatous polyps. Data collected from subjects included frequency of use of phenolphthalein-containing laxatives and other dietary and nondietary factors. The overall prevalence of phenolphthalein-containing laxative use ranged from 1.3% to 4.2%.

The overall adjusted odds ratios for the two North Carolina studies (study-1 OR = 1.0, 0.4-2.2; study-2 OR = 1.1, 0.2 - 5.7) did not suggest that use of phenolphthalein-containing laxatives increased the risk of adenomatous colorectal polyps (Table 3-1), but the estimates had relatively wide confidence intervals because of the generally infrequent use of phenolphthalein containing laxatives. Alternatively, there was a slight association in the Los Angeles study (OR = 1.8, 0.5 - 6.2). However, no increase in risk was identified with increasing frequency of use in the L.A. group. The results were not altered by adjustment for a number of potentially confounding factors including constipation or bowel movement. There was no association in any study with the use of other types of laxatives. The study strengths include the use of data from multiple well-designed case-control studies and adjustment for a number of confounding factors. However, the small number of exposed subjects does not allow for a precise estimate of the relative risk associated with phenolphthalein-containing laxatives (Longnecker et al., 1997).

A retrospective cohort study of 2,277 German patients who underwent colonoscopy was conducted to examine laxative use and melanosis coli as a risk factor for colorectal neoplasms (Nusko et al., 1993). Data on laxative use was obtained from hospital records. A total of 271 patients (11.9% of all patients) reported using laxatives, among whom 15 developed colorectal cancer. Among those patients who did not report using laxatives (n = 2,006), 88 (4.4%) developed colorectal cancer. There was no statistically significant association between laxative use and risk of colorectal cancer [relative risk (RR) = 1.26, 95% confidence interval (CI) = 0.74-2.15, p = 0.485)]. However, there was a statistically significant association between the occurrence of colorectal adenomas and laxative use [RR = 1.72, 95% confidence interval (CI) = 1.46-2.01, p = 0.0001)]. Of the 164 patients (60%) for whom laxative ingredients were known, however, only two used phenolphthalein-containing products.

A recent meta-analysis of 14 case-control studies showed a small but significant association between colorectal cancer and use of cathartics (pooled odds ratio = 1.46 (1.33-1.61); Sonnenberg and Müller, 1993). Specific ingredients were not considered. The authors suggested that the risk may reflect the confounding influence of dietary habits rather than constipation or laxative use.

Table 3-1. Human Studies of Effects of Exposure to Phenolphthalein

Design	Population Group	Exposure	Effects	Potential Confounders/Effects	Comments	Reference
Case-controlled	**Cases:** 730 men and women with histologically confirmed adenomatous polyps; identified at three designated medical facilities: Los Angeles (L.A.), North Carolina 1 and 2 (NC1, NC2); individuals were excluded if evidence of previous bowel cancer, adenoma, bowel surgery, inflammatory bowel disease, polyposis, or could not speak English **Controls:** 907 men and women total; at L.A. had no polyps of any type and were individually matched to cases by age, sex, medical facility, and period of exam; at NC1 and NC2 had not adenomatous polyps and were not matched to cases	**Estimation:** Laxative use, dietary variables, and other nondietary variables collected by personal interview (L.A.) or by telephone (NC1 and NC2) **Duration:** L.A.: 1991-93; NC1: 1988-90; NC2: 1992-1995 **Response Rate (%):** L.A.-83; NC1-83; NC2-45 **Categories:** Laxative with/without phenolphthalein used at least once/week; laxatives classified as containing phenolphthalein if one of 19 brands reported	**Evaluation:** Calculation of Odds Ratio (OR) to assess relation between use of laxatives and risk of adenoma; logistic regression models used to adjust ratios for potentially confounding factors **OR (95% CI):** For use of phenolphthalein-containing laxatives once a week or more: L.A.: 1.3 (0.9-1.9) NC1: 1.0 (0.5-1.7) NC2: 0.9 (0.4-1.8)	OR adjusted a priori L.A.: alcohol, smoking, vigorous activity, intake of energy, saturated fat, fruits, and vegetables NC1 and NC2: age, sex, alcohol, smoking, intake of energy, total fat, fiber from fruits and vegetables NC1: also adjusted for 'leisure activity' NC2: also adjusted for 'hard physical activity' Additional adjustment for race, body mass index, use of nonsteroidal anti-inflammatory agents Adjustment of OR had no significant effect on the relation between laxative use and risk of adenoma	Prevalence of phenolphthalein-laxative use was less than 5%; the low prevalence probably accounted for wide confidence intervals	Longnecker et al. (1997)
Retrospective Cohort	2,277 patients (1,193 males and 1,084 females) diagnosed by colonoscopy. Results of colonoscopies classified as: normal, polyps, carcinomas, chronic inflammatory bowel disease, diverticulitis, operative anastomases (postresectional state), and melanosis coli	271 patients had a history of laxative use. Ingredients of laxatives used were known for 164 patients. Only two reported use of phenolphthalein-containing products	**Evaluation:** Calculation of Relative Risk (RR) to assess relation between use of laxatives and colorectal adenomas/carcinomas: **RR(95% CI)** of colorectal adenomas in patients using laxatives: 1.72 (1.43-2.01), p = 0.0001 **RR (95% CI)** of colorectal carcinomas in patients using laxatives: Not significant at p = 0.485	The authors noted that the following information was difficult to obtain: sufficient information about the intake of laxatives, and duration and frequency of laxative intake. Therefore, a causal relationship between laxative use and colorectal tumors was not clarified exactly in this study.	Number of individuals using phenolphthalein-containing laxatives was very low in this study (see exposure).	Nusko et al. (1993)

Table 3-1. Human Studies of Effects of Exposure to Phenolphthalein (Continued)

Design	Population Group	Exposure	Effects	Potential Confounders/Effects	Comments	Reference
Meta-analysis of 14 previously published case-control studies	11 of the 14 case-control studies contained information about laxative use prior to the onset of colorectal cancer. **Exposed:** 4,413 individuals reported use of cathartics **Controls:** 22,258 individuals reported no use of cathartics	In the 11 studies, cathartics were categorized under the following specifications of use: cascara, senna, Beecham's pills, salts, liquid paraffin (mineral oil), other cathartics, > weekly and constantly, laxatives >1/week for <10 years and more, suppositories and enemas 1-6/week, stool-bulk additives, ever used laxatives regularly, laxatives, chronic use of laxatives, laxatives >1/week, suppository and enemas 1-6/week, laxatives daily or weekly, self-reported commercial laxative use, and use of laxatives regularly/often	**Evaluation:** Pooled odds ratios of the colorectal cancer risk among exposed and unexposed subjects. **OR (95% CI)** for use of cathartics: 1.46 (1.33-1.61)	Multivariate analysis showed that low intake of fiber, vegetables, and vitamin C-containing foods and a high intake of fat have an important confounding effect.	The article did not provide ingredients in the cathartic agents. The percentage of these compounds containing phenolphthalein is unknown.	Sonnenberg and Müller (1993)

CI = confidence interval

4.0 EXPERIMENTAL CARCINOGENICITY

Dietary exposure to phenolphthalein for two years induced a significant increase in multiple types of tumors in male and female rats and mice (NTP, 1996, pp. 39-79, see Appendix A). The results of these studies are summarized below. More recently, dietary exposure to phenolphthalein for 6 months induced a significant increase in the incidence of malignant lymphoma of the thymus in female heterozygous p53-deficient transgenic mice (TSG-p53™) (Dunnick et al., 1997; see summary below and **Table 4-1**).

4.1 Rats

In the NTP two-year bioassay, male and female Fischer 344 rats were administered 12000, 25000, or 50000 ppm (37.7, 78.450, or 157.08 mmol/kg) phenolphthalein in the diet (NTP, 1996). Survival of all groups of exposed males and females was similar to that of controls.

The incidence of benign pheochromocytoma of the adrenal medulla was significantly increased in all dosed male groups. Most pheochromocytomas in dosed males were bilateral. Malignant pheochromocytoma was not affected. The incidence of benign pheochromocytoma was also increased in female rats exposed to 12000 ppm. Bilateral tumors and malignant pheochromocytoma were not affected in dosed females.

The incidence of renal tubule adenoma (single and step sections combined) was significantly increased in all dosed male groups. A few renal tubule carcinomas were also observed in dosed males. In females, one renal tubule adenoma was observed in the 50000-ppm dose group, but an extended evaluation failed to reveal additional tumors and this adenoma was not considered treatment-related.

4.2 Mice

In the NTP two-year bioassay, male and female $B6C3F_1$ mice were administered 3000, 6000, or 12000 ppm (9.4, 18.85, or 37.7 mmol/kg) phenolphthalein in the diet (NTP, 1996). Females treated with 12000 ppm had a significantly decreased rate of survival compared to controls.

The incidence of histiocytic sarcoma was significantly greater in 6000- and 12000-ppm males and females as compared to controls. The incidence of malignant lymphoma (all types) was significantly increased in exposed females, but not in males. The incidence of lymphoma of a thymic origin was significantly increased in exposed females and in 6000-ppm males. The incidence of benign ovarian sex-cord stromal tumors was significantly increased in exposed females. The incidence of hepatocellular adenoma or carcinoma was significantly reduced in exposed females and in 6000- and 12000-ppm males.

The heterozygous p53-deficient (+/-) mouse model has been proposed for rapid identification of carcinogenic responses of mutagenic chemicals (Tennant et al., 1995). To further characterize the mechanisms of phenolphthalein-induced carcinogenesis, a NIEHS study was conducted in the heterozygous p53-deficient (+/-) mouse (Dunnick et al., 1997). The p53 (+/-) mouse has a null mutation introduced into one p53 gene by homologous recombination in murine embryonic stem cells. The insertion of the *neo* cassette into the Trp53 locus resulted in a deletion of a 450-base-pair fragment containing 106 nucleotides of exon 5, and about 350 nucleotides of intron 4 that inactivated gene function (Donehower et al., 1992).

In the NIEHS study, p53 (+/-) female mice received phenolphthalein in the diet at 200, 375, 750, 3000, or 12000 ppm. The average daily dietary consumption of phenolphthalein was estimated as 43, 84, 174, 689, or 2375 mg/kg body weight/day [or 129, 252, 522, 2867 mg/m^2 body surface area/day (Freirich et al., 1966)]. Human exposure is approximately 5 mg/kg/day or 185 mg/m^2 body surface area/day under expected conditions of use (Dunnick and Hailey, 1996). The two lowest levels in the NIEHS transgenic study delivered phenolphthalein exposures that were approximately 0.5 to 1.5 times the recommended human exposure levels based on a mg/m^2 body surface area comparison.

In this study, the incidence of malignant lymphoma from the thymus was significantly increased in heterozygous p5-deficient female mice given 3000 or 12000 ppm (9.4 or 37.7 mmol/kg) phenolphthalein in the diet continuously for 26 weeks but not in mice given lower doses of 200, 375, or 750 ppm (0.6, 1.2, 2.4 mmol/kg diet). The incidence observed in the 0 to 12000-ppm dose groups was 0/19, 1/20, 1/20, 5/20, 20/20, and 19/20 (Dunnick et al., 1997).

Table 4-1. Experimental Carcinogenicity of Phenolphthalein Published Post NTP (1996)

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose Route	Duration of Exposure	Results/Comments	Reference
Mice							
p53 transgenic mice	Five groups of 20 females	20 females	>99% pure article; administered diets ± 10% of scheduled dose	200, 375, 750, 3000, or 12000 ppm (mg/kg diet) (0.6, 1.2, 2.4, 9.4, or 37.7 mmol/kg diet) Estimated as 43, 84, 174, 689, or 2375 mg/kg bw and 129, 252, 522, 2867, or 7128 mg/m^2 body surface area per day	26 weeks	The incidence of malignant lymphoma of the thymus was increased at the 3000- and 12000-ppm dose levels (0/19, 1/20, 1/20, 5/20, 20/20, and 19/20). The 200 and 375-ppm dose levels delivered phenolphthalein exposures approximately 0.5 to 1.5 times the estimated human exposure levels (4 mg/kg/day) based on body surface area comparisons.	Dunnick at al. (1997)

5.0 GENOTOXICITY

Studies of the genotoxic effects of phenolphthalein have been reviewed and summarized by NTP (1996, p. 70, see Appendix A). Studies published after or not included in NTP (1996) are summarized below and in **Table 5-1**.

5.1 Genotoxicity Studies Summarized in NTP (1996)

Phenolphthalein was negative in the *Bacillus subtilis* rec assay for DNA damage in the absence of S9 metabolic activation. Similar results were reported for phenolphthalein's inability to induce *his* gene mutations in *Salmonella typhimurium*. A negative response was obtained in strains TA1535, TA1537, TA1538, TA98, and TA100 both with and without S9 activation using either the plate incorporation or preincubation methods.

In vivo, micronuclei were induced by phenolphthalein in male and female B6C3F1 mouse peripheral blood polychromatic (PCE) and normochromatic (NCE) erythrocytes via dosed feed for 13 weeks. Abnormal sperm and low sperm density were induced in male B6C3F1 mice but not F344 rats treated with phenolphthalein via dosed feed for 13 weeks.

5.2 Genotoxicity Studies Published Post NTP (1996)

Phenolphthalein induced a positive increase in *hprt* but not Na^+/K^+ ATPase gene mutations or aneuploidy in Syrian hamster embryo (SHE) cells exposed for 48 hours (Tsutsui et al., 1997).

A highly significant, reproducible increase in chromosomal aberrations was reported in phenolphthalein-exposed Chinese hamster ovary cells in the presence but not the absence of S9 activation (Witt et al., 1995). Phenolphthalein also induced a positive increase in chromosomal aberrations but not aneuploidy in SHE cells exposed for 6 (aberrations) or 48 (aneuploidy) hours (Tsutsui et al., 1997).

Similarly, a dose-dependent increase in morphological transformation was induced by phenolphthalein in SHE cells exposed for 48 hours (Tsutsui et al., 1997).

In vivo, phenolphthalein induced an equivocal increase in DNA single-strand breaks in the peripheral blood leukocytes of female p53-deficient transgenic mice (C57Bl/6 background) following dosed-feed exposure for 26 weeks and sampling at days 39, 92, 137, and 183 (Tice et al., 1998). No increase was observed in the livers of treated mice sampled upon termination of exposure.

Micronuclei were induced by phenolphthalein in male and female B6C3F1 mouse peripheral blood polychromatic (PCE) and normochromatic (NCE) erythrocytes via a variety of exposures including gavage for 2 to 3 days, or dosed feed for 4 to 14 days (Witt et al., 1995). Similarly, phenolphthalein induced micronuclei in the blood PCE of CD-1 mice after 14 weeks of continuous feed treatment (Witt et al., 1995). Micronuclei were also induced in the bone marrow of male B6C3F1 mice treated by gavage for 3 days or feed for 4 to 14 days (Witt et al., 1995). No significant increases in micronuclei were observed in mouse bone marrow after only 2 days of gavage treatment or 3 days of feed treatment (Witt et al., 1995). A positive response was also observed in the peripheral blood PCE and NCE of female p53-deficient transgenic mice treated via dosed feed for 26 weeks and sampled at days 39, 92, 137, and 183 (Tice et al., 1998). Kinetochore analysis at the top dose showed that micronuclei were induced primarily from whole chromosomes rather than from breaks resulting in chromosomal fragments. The

lowest effective dose (LED) for the induction of micronucleated erythrocytes in female heterozygous p53-deficient mice by phenolphthalein was 200 ppm.. Based on surface area, the amount of phenolphthalein ingested at this dose (i.e., ~30 mg/kg/day = ~90 mg/m^2 body surface) is within the dose range anticipated for human consumption (~5 mg/kg/day or ~185 mg/m^2 body surface area) (Dunnick and Hailey, 1996).

Table 5-1. Summary of Phenolphthalein Genotoxicity Studies Published Post NTP (1996)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response -/+ Activation	Comments	Reference
5.2 Mammalian Systems *in vitro*							
Syrian hamster embryo (SHE) cells	*hprt* and Na^+/K^+ ATPase gene mutations	-	>98%	10, 20, and 40 µM for 48 h	positive (*hprt*), negative (ATPase)	Treatment induced a dose-dependent increase in mutant frequency (LED = 20 µM) at the *hprt* locus only.	Tsutsui et al. (1997)
Chinese hamster ovary (CHO) cells	chromosomal aberrations	-/+	NG in source used	11, 23, 30, 40, and 50 µg/mL for 16 h -S9 and 62 +S9	negative/ positive	Highly significant increases observed in two trials with S9 activation (LED = 40 µg/mL).	Witt et al.. (1995)
Syrian hamster embryo (SHE) cells	chromosome aberrations and aneuploidy	-	>98%	10, 20, and 40 µM for 6 h (aberrations) or 48 h (aneuploidy)	positive (aberrations), negative (aneuploidy)	A statistically significant level of chromosomal aberrations was induced at the highest dose only (40 µM) with no corresponding aneuploidy at any dose.	Tsutsui et al. (1997)
Syrian hamster embryo (SHE) cells	morphological transformation	-	>98%	10, 20, and 40 µM for 48 h	positive	Treatment induced a dose-dependent increase in percent transformation (LED = 10 µM).	Tsutsui et al. (1997)
5.2 Mammalian Systems *in vivo*							
Female heterozygous p53-deficient transgenic mice (C57B1/6 background) 20/dose	DNA single-strand breaks in peripheral blood leukocytes and liver cells (single cell gel assay)	n.a.	>99%	200, 375, 750, 3000, and 12,000 ppm in feed, continuously for 26 wk	equivocal (blood), negative (liver)	Blood smears were prepared on days 39, 92, 137, and 183 of treatment; liver, at termination. Significant increase in DNA damage was observed in blood leukocytes on day 39 and 137 but not days 92 and 183, making results inconclusive.	Tice et al. (1998)

Table 5-1. Summary of Phenolphthalein Genotoxicity Studies Published Post NTP (1996) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response -/+ Activation	Comments	Reference
male B6C3F$_1$ mice	micronuclei induction in bone marrow and peripheral blood erythrocytes	n.a.	NG in source used	1000-4000 mg/kg/d gavage for 2-3 d (single or multiple injections per day); 4000 and 6000 mg/kg/d feed for 3-14 d	positive in both tissues	Positive response in blood and bone marrow PCE (LED = 6000 mg/kg). Negative response was observed in 2-day single injection gavage and 3-day dosed feed treatments.	Witt et al. (1995)
male CD-1 mice	micronuclei induction in peripheral blood erythrocytes	n.a.	NG in source used	120-3500 mg/kg/d via feed for 14 wk	positive	Positive response in peripheral blood PCE (LED = 120 mg/kg/day).	Witt et al.. (1995)
female heterozygous p53-deficient transgenic mice (C57B1/6 background) 20/dose	micronuclei induction in peripheral blood erythrocytes	n.a.	>99%	200, 375, 750, 3000, and 12,000 ppm in feed, continuously for 26 wk	positive	Blood smears were prepared on days 39, 92, 137, and 183 of treatment. At each sample time a highly significant level of micronucleated NCE (LED = 200 ppm) and PCE (LED = 375 ppm) was observed. Micronuclei were induced predominantly from numerical chromosomal damage (kinetochore-positive micronuclei were present at the top dose tested).	Tice et al. (1998)

HID = highest ineffective dose; LED = lowest effective dose; n.a. = not applicable; NG = not given

6.0 OTHER RELEVANT STUDIES

6.1 Absorption, Distribution, Metabolism, and Excretion

6.1.1 Absorption and Metabolism

Phenolphthalein is absorbed in the intestine (Visek et al., 1956; AHFS, 1995; cited by NTP, 1996) and is almost completely converted to its glucuronide during extensive first pass metabolism in the intestinal epithelium and liver (Parker et al., 1980; cited by NTP, 1996) via uridine diphosphate glucuronosyltransferase (UDPGT) (Sund and Hillestad, 1982; cited by NTP, 1996). In the guinea pig, small amounts of sulfate conjugate metabolites have been detected in isolated mucosal sheets originating in the jejunum and colon (Sund and Lauterbach, 1986; cited by NTP, 1996).

Six hours after female Wistar rats were given [^{3}H]phenolphthalein, analysis of the systemic circulation showed that all of the radioactivity was associated with the glucuronide conjugate (Colburn et al., 1979; cited by NTP, 1996). Five to six hours after i.v. administration, absorption of [^{3}H]phenolphthalein from the intestine coincided with a secondary peak in blood radioactivity that followed hydrolysis by bacterial β-glucuronidase of [^{3}H]phenolphthalein glucuronide excreted in the bile. Enterohepatic recirculation is rate-limited by the hydrolysis of phenolphthalein glucuronide to aglycone (Bergan et al., 1982; cited by NTP, 1996).

The extent of enterohepatic recirculation of phenolphthalein was examined in rats after surgical cannulation of the bile duct (Parker et al., 1980; cited by NTP, 1996). Within 24 hours, 95% of 25 mg [^{3}H]phenolphthalein/kg administered intraperitoneally (i.p.) to female Wistar rats was recovered as glucuronide in the bile, with 0.2% recovery from the urine. In rats that were not surgically altered, 86% of the same dose was recovered in the feces, with little glucuronide, and 10% was recovered in the urine, primarily as the glucuronide. In female Wistar rats with biliary fistulae, 100% of the dose was eliminated in the bile, with 98% in the glucuronide form (Millburn et al., 1967; cited by NTP, 1996).

In studies of male Sprague-Dawley CR-1 strain rats whose femoral vein, artery, and bile duct were cannulated, i.v. administration of 3, 30, or 60 mg (9, 94, or 190 μmol) phenolphthalein led to elimination of 99.5% of the dose in the bile as the glucuronide. When the same rats were given 3, 30, or 100 mg (6, 59, or 195 μmol) phenolphthalein glucuronide by i.v. administration, no phenolphthalein was detected in the bile (Mehendale, 1990; cited by NTP, 1996).

Within 72 hours of oral administration of 4.8 mg [^{14}C]phenolphthalein/kg to female dogs (unspecified breed), 51% of the radioactivity was excreted in the feces and 36% was eliminated in the urine. After an i.v. dose, 54% in feces and 37% in urine were found. When the same dogs were bile-duct cannulated, an oral dose led to 31% radioactivity in feces, 38% in urine, and 22% in bile. An i.v. dose resulted in 11% elimination in feces, 35% in urine, and 43% in bile (Visek et al., 1956; cited by NTP, 1996).

6.1.2 Distribution

Studies done in dogs and mice using [^{14}C]phenolphthalein showed the radioactivity at levels parallel to the concentration in the blood in a wide and even distribution. Other experiments showed less than 0.03% of the dose in the liver and gall bladder and no radiolabel in the blood of newborn puppies, following administration of a 4.8 mg/kg (0.015 mmol/kg) oral

dose to the mother 50 hours prior to her giving birth. This result led investigators to suggest extremely limited passage across the placenta (Visek et al., 1956; cited by NTP, 1996).

6.1.3 Excretion

Bile, urine, feces, and milk are all routes of excretion for phenolphthalein (Visek et al., 1956; AHFS, 1995; both cited by NTP, 1996). Seventy-two hours after administration of a radioactive oral dose to dogs, 50% was recovered in the feces and 36% in the urine. Studies of mice reported 56% of an oral dose recovered from the urine within 48 hours and an additional 38% recovered from the feces. When an i.v. dose was given, 30% was recovered from the urine and 68% from the feces (Visek et al., 1956; cited by NTP, 1996). Some phenolphthalein is excreted into the bile, and the prolonged cathartic effect may be due to the ensuing enterohepatic recirculation (Gilman et al., 1980; cited by HSDB, 1997). Pre-treatment with hepatic-microsomal-enzyme inducers increased biliary excretion of metabolites in rats, but a dose of phenolphthalein followed by enzyme inhibitors decreased it. Dosing with metabolites yielded no effects (Chemical Society, 1972; cited by HSDB, 1997).

6.2 Pharmacokinetics

The systemic blood concentration-time profile of phenolphthalein for the 24 hours following single i.v. bolus injection was described by a classical compartmental pharmacokinetics model, which indicated a long half-life (Colburn et al., 1979; cited by HSDB, 1997). There are indications that this long half-life is an artifact of enterohepatic recirculation.

Whole-body autoradiography studies of male BOM:NMRI mice showed high levels of radioactivity in the stomach, gall bladder, and small intestine 10 to 20 minutes after administration of an intragastric dose of 1 mL/kg [^{14}C]phenolphthalein (10 μCi/100g) (Sund et al., 1986; cited by NTP, 1996). As evidenced by the presence of radioactivity in peripheral organs (including the kidney, liver, and skin), the compound was absorbed from the gastrointestinal tract and moved through the intestinal tract. After 2 hours, it arrived at the large intestine; 4 hours after administration, maximum radioactivity was observed in the rectum. Two days after administration, no radiolabel was detected.

6.3 Structure-Activity Relationships

6.3.1 Diphenylmethane Structural Analogs

Phenolphthalein and several structurally related chemicals (e.g. bisacodyl.) have been used as laxatives (Sund, 1983).

Phenolphthalein is a derivative of diphenylmethane (Binder, 1977; cited by NTP, 1996). Other compounds containing the diphenylmethane substructure include bisacodyl [603-50-9], picosulfate [10040-45-6], oxyphenisatin [125-13-3] and its salts (oxyphenisitin diacetate [115-33-3], oxyphenisatin disulfide [37811-54-4]), *p,p'*-diaminodiphenylmethane (DDPM) [101-77-9], and phenol red [143-74-8]. Bisacodyl (used in laxatives) induces both calculi and epithelial proliferative lesions (including transitional cell carcinoma) in the urinary bladder of rats (Toyoda et al., 1993, 1994). Geboes et al. (1993) stated that bisacodyl and picosulfate (used in laxatives) have no major influence in colonic and ileal epithelial cell proliferation and should not be regarded as a tumor-promoting substances. Yang et al. (1993) also found in one study that bisacodyl did not promote early precancerous lesions in colonic epithelial cells of rats.

Oxyphenisatin and its salts (used in laxatives) are known to induce liver damage (Dietrichson et al., 1976; Delchier et al., 1979; Sund, 1983; and Homberg et al., 1985).

Phenol red (a widely used pH indicator) has shown growth factor and estrogen effects in cancer cell lines (Devleeschouwer et al., 1992; Welshons et al., 1988; Glover et al., 1988; Bindal and Katzenellenbogen, 1988). In contrast, another study found that phenol red had no effect on the growth of normal human, mouse, and rat mammary cells (Richards et al., 1988).

The compound *p, p'*-diaminodiphenylmethane (DDPM) (used as an epoxy resin hardener) was found to be carcinogenic (Hirose et al., 1986), but since it is an aromatic amine and carcinogenic effects would be expected, it is not considered further here.

6.3.2 Alkylating Substructure

The probability that opening of the 5-atom lactone ring in phenolphthalein would produce an alkylating carbocation was noted by Benigni et al. (1996) and Huff et al. (1996).

6.3.3 Physical-Chemical Properties

The log P (log of the octanol-water partition coefficient) of phenolphthalein is 0.95, indicating it is a hydrophobic compound that is retained in tissues. Additionally K_e (an electrophilicity parameter) has been calculated for phenolphthalein as 3.597, which is considered high. High K_e values seem to indicate a directly acting carcinogen's general chemical reactivity, its tendency to undergo a reductive metabolism, or its ability to attack DNA (Benigni et al., 1996).

7.0 MECHANISMS OF CARCINOGENESIS

7.1 Ovarian Cancer

Phenolphthalein exposure caused an increased incidence of ovarian neoplasms in mice as discussed in section 4 (NTP, 1996). These neoplasms were of sex-cord stromal origin and did not follow the normal path of ovarian tumorigenesis, which generally starts with the destruction and/or loss of oocytes and ends in gonadotropin-stimulated tumor growth (NTP, 1996; Dunnick and Hailey, 1996). Instead, phenolphthalein might stimulate cellular proliferation in the ovary in part through its competitive binding to the estrogen receptor (NTP, 1996; Ravdin et al., 1987; cited by Dunnick and Hailey, 1996). Estradiol can stimulate proliferation in ovarian cells (Rao et al., 1978; cited by NTP, 1996) and it is possible that phenolphthalein could mimic this behavior.

7.2 Radical Formation

If phenolphthalein is converted to a quinoid, it can induce free radical formation (NTP, 1996). Two phenolic substituents are present in phenolphthalein and neither has substituents *ortho* to the hydroxyl group. Thus, phenolphthalein should be readily oxidized to phenoxyl free radicals. In electron paramagnetic resonance (EPR) studies done on horseradish peroxidase (HRP) oxidation of the compound, a primary phenolphthalein phenoxyl radical was observed in addition to a larger signal that was likely due to polymeric free radical species. In addition to detection of the phenoxyl radicals, indirect results of the presence of a phenoxyl radical such as production of superoxide, glutathione thiyl, and ascorbate radicals, have been detected by EPR (Sipe et al., 1997). Further evidence for the existence of phenoxyl radicals comes from the observation that phenolphthalein stimulates intestinal prostaglandin formation (Beubler and Juan,

1978a, 1978b; Capasso et al., 1985, 1988; all cited by Sipe et al., 1997). Markey et al. (1987; cited by Sipe et al., 1997) found that the ability of compounds to act as peroxide substrates correlates directly with their ability to stimulate prostaglandin formation. Thus the prostaglandin-stimulating action of phenolphthalein is indirect evidence that it is metabolized to a phenoxyl free radical (Sipe et al., 1997).

As described in section 6.2, phenolphthalein is rapidly metabolized to its glucuronide. In the studies of Sipe et al. (1997), the same qualitative oxidation reactions seen with phenolphthalein were seen with its glucuronide. While the glucuronide was substantially less active than its parent compound, its high concentration in the body could mean that it is the primary source of phenoxyl radicals *in vivo* (Sipe et al., 1997).

Reduction of phenoxyl radicals *in vivo* can be accomplished by reduced glutathione , NADH, or ascorbate and could allow reformation of phenolphthalein, establishing a cycle of oxidation and reduction. This futile metabolism of both phenolphthalein and phenolphthalein glucuronide can stimulate oxidation of ascorbate, glutathione, and NAD(P)H followed by reaction of the newly produced free radicals with oxygen, resulting in production of superoxide (Sipe et al., 1997). Superoxide is converted to hydrogen peroxide and molecular oxygen in the presence of superoxide dismutase (SOD). Hydrogen peroxide can be a source of hydroxyl radical in the presence of reduced iron, and the highly reactive hydroxyl radical has been found to react with DNA, yielding a variety of products (Mouret et al., 1991; cited by Sipe et al., 1997).

7.3 Genetic Toxicity

Results of investigations of the mutagenicity of phenolphthalein provide evidence of its genotoxicity. For example, studies on mice exposed to phenolphthalein have shown significant increases in micronucleated PCEs and NCEs (Tice et al., 1998; Dietz et al., 1992). Doses greater than or equal to 2,000 mg/kg per day for at least two days induced micronuclei in erythrocytes, which could be detected in either bone marrow or blood. Doses of 120 mg/day when given over a longer period of time (14 weeks) were also highly effective in inducing micronucleated erythrocytes (Witt et al., 1995).

Tice et al. (1998) found that in female transgenic heterozygous p53-deficient mice evaluated under chronic exposure conditions, the induced micronuclei contained predominantly whole chromosomes. The lowest effective dose inducing a genotoxic response, micronucleated NCE, was 200 ppm. In this *in vivo* test system, phenolphthalein caused genetic damage at doses comparable to human therapeutic doses on a mg/m^2 basis. U.S. FDA (1997) stated that low doses were about 15 times the human exposure.

The malignant thymic lymphomas induced by phenolphthalein in female heterozygous p53-deficient transgenic mice exhibited a loss of the normal p53 allele, suggesting the involvement of a mutagenic mechanism in tumor induction and/or progression (Tice et al., 1998).

7.4 Thymic Lymphoma and Estrogenic Effects

NTP has studied approximately 400 chemicals in long-term bioassays in F344 rats and $B6C3F_1$ mice. Only three chemicals have been found to cause thymic lymphomas in mice, including phenolphthalein, butadiene, and ddC (2′,3′-dideoxycytidine). There is no indication that any of the estrogenic chemicals studied caused thymic lymphomas, e.g. DDT (NCI, 1978) or zearalenone (NTP, 1982). Phenolphthalein also caused thymic lymphomas in the p53-deficient

mouse, and in all tumors examined there was loss of the wild p53 allele. The wild p53 allele remains in many of the spontaneous tumors in the p53-deficient mice (Harvey et al., 1993; cited by Dunnick et al., 1997). These studies show that the mechanism for tumor formation in the phenolphthalein-induced thymic lymphomas in the p53-deficient mice probably involves the loss of p53 function (Dunnick et al., 1997).

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APPENDIX A

Excerpt from the NTP Technical Report
Toxicology and Carcinogenesis Studies of Phenolphthalein
in F344/N Rats and B6C3F$_1$ Mice (Feed Studies),
pp. 4-38, 71-91, November 1996

APPENDIX B

Description of Online Searches for Phenolphthalein

DESCRIPTION OF ONLINE SEARCHES FOR PHENOLPHTHALEIN

Searches were limited to 1995 [the year before the NTP bioassay (NTP, 1996) which has an extensive literature review] through July 1997.

Online searches for phenolphthalein [CASRN 77-09-8] were performed in databases on the systems of STN International, DIALOG, NLM's TOXNET, and the Chemical Information Systems from 1980 to date. Toxicology information was sought in the EMIC, EMICBACK, RTECS, TOXLINE, CANCERLIT, EMBASE, BIOSIS, and MEDLINE (name and CASRNs combined with terms of metabolism and the MESH heading for all neoplasms). Occupational safety and health information was obtained from NIOSHTIC. HSDB provided a general review. The Chemical Abstracts Service Registry file and SANSS provided chemical identification information.

Market information, including production, shipments, sales and consumption, labor use, and workers by type was sought in PROMT (The Predicasts Overview of Markets and Technology) and The Chemical Economics Handbook database.

Structural analogs were also searched including, picosulfate [10040-45-6], oxyphenisatin [125-13-3], phenolphthalein diacetate [115-33-3], phenolphthalein disulfate [37811-54-4]. sulisatin [54935-03-4], deacetylbisacodyl [603-41-8], phenol red [143-74-8], bisacodyl [603-50-9].

Regulatory information was obtained from the latest quarterly update of the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to the titles 21 (FDA), 29 (OSHA), and 40 (EPA) regulations. Updates on FDA regulatory actions were sought in the Federal Register full text database and in DIOGENES as well as at the FDA World Wide Web site on the Internet.

Also, the review of 1200 life sciences journals was accomplished using Current Contents on Diskette® for current awareness.

APPENDIX C

Report on Carcinogens (RoC), 9th Edition
Review Summary

Report on Carcinogens (RoC), 9th Edition
Review Summary

Phenolphthalein

NOMINATION
Review based on results of an NTP Bioassay of Phenolphthalein (1996), reporting clear evidence of carcinogenicity in 3 of 4 experimental animal groups.

DISCUSSION
Phenolphthalein is used as a laboratory reagent and acid-base indicator and has been used as a cathartic drug in over-the-counter laxative preparations. Experimental animal cancer results indicate clear evidence of benign and malignant tumor formation at multiple tissue sites in multiple species. Phenolphthalein causes oxidative stress and also demonstrates the capability to alter p53 pathways; both are considered common mechanisms involved in human cancers. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as a reasonably anticipated human carcinogen	9 yes/1 no
NTP EC Working Group (RG2)	list as a reasonably anticipated human carcinogen	7 yes/0 no/1 a*
NTP Board RoC Subcommittee	list as a reasonably anticipated human carcinogen	6 yes/0 no

*a-abstentions

Public Comments Received
A total of 3 public comments were received, all providing comments on the content of the background document prepared for the review of this nomination.