PHENOLPHTHALEIN

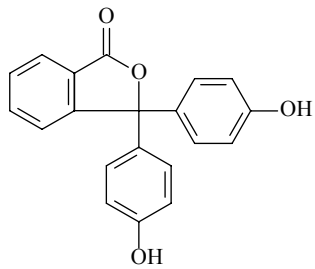
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 77-09-8
Chem. Abstr. Name: 3,3-Bis(4-hydroxyphenyl)-1-(3H)-isobenzofuranone
IUPAC Systematic Name: Phenolphthalein
Synonyms: 3,3-Bis(4-hydroxyphenyl)phthalide; 3,3-bis(*para*-hydroxyphenyl)-phthalide; α-(*para*-hydroxyphenyl)-α-(4-oxo-2,5-cyclohexadien-1-ylidine)-*ortho*-toluic acid

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{20}H_{14}O_4$ Relative molecular mass: 318.33

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White or yellowish-white, triclinic crystals, often twinned (Budavari, 1996)
(*b*) *Melting-point*: 258–262 °C (Budavari, 1996)
(*c*) *Spectroscopy data*: Infrared (prism, [8113; 1471C]; grating [28037]; FT-IR, [1006B]), ultraviolet [2188] and nuclear magnetic resonance (proton, [14709]; ^{13}C, [4455]) spectral data have been reported (Sadtler Research Laboratories, 1980; Pouchert, 1981, 1985)

(*d*) *Solubility*: Practically insoluble in water; soluble in diethyl ether, ethanol and dilute solutions of alkali hydroxides; very slightly soluble in chloroform (Budavari, 1996)

(*e*) *Dissociation constant*: pK_a at 25 °C, 9.7 (Budavari, 1996)

1.1.4 *Technical products and impurities*

Phenolphthalein (white) is available as a 6.5-, 14-, 32.4-, 60-, 65-, 75-, 100-, 120-, 130- and 200-mg tablet, a 60- and 120-mg chewable tablet, a 30-, 65- and 90-mg capsule, a 64.8-mg wafer, a 15-, 50-, 60-, 65-, 66.7- and 200-mg/5 mL and 198 mg/15 mL liquid emulsion and a 117-mg (9%) chocolate square; phenolphthalein (yellow) is available as a 65-, 90-, 95- and 135-mg tablet, an 80-, 90-, 95- and 97.2-mg chewable tablet, a 65- and 130-mg capsule and a 97.2-mg chewing gum. The tablets may also contain aloin, aspartame, bile salts, butylparaben, cascara sagrada, cascara sagrada extract, corn starch, cocoa butter, cocoa paste from cocoa seeds, colourants (D&C Yellow No. 10 aluminium lake, D&C Red No. 28, FD&C Blue No. 1, FD&C Red No. 40, FD&C No. 40 aluminium lake), docusate sodium, dextrates, dibasic calcium phosphate dihydrate, ethyl vanillin, flavours, hydroxypropyl methylcellulose, lactose, leaves of senna, lecithin from soya beans, magnesium stearate, methylene blue, microcrystalline cellulose, oleoresin capsicum, ox bile extract, polydextrose, polyethylene glycol, polysorbate 80, potassium nitrate, povidone, propylene glycol, sodium carbonate (anhydrous), sodium saccharin, sodium starch glycolate, starch, sucrose, titanium dioxide and triacetin. The capsule may also contain dehydrocholic acid, docusate calcium, ethanol, parabens, povidone and sorbitol. The liquid emulsion may also contain agar, benzoic acid, glycerin, liquid paraffin, mineral oil, sodium cyclamate and sorbic acid (Gennaro, 1995; American Hospital Formulary Service, 1997; Canadian Pharmaceutical Association, 1997; Medical Economics Data Production, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998; US Pharmacopeial Convention, 1998).

In the manufacture of phenolphthalein, a stage is reached in which certain by-products formed in the synthesis have not yet been removed, resulting in a product called yellow phenolphthalein. Compounds isolated from one sample of yellow phenolphthalein were: white phenolphthalein, 93%; fluoran, 0.32%; isophenolphthalein, 0.08%; 2-(4-hydroxybenzoyl)benzoic acid, 0.10%. Yellow phenolphthalein was reported to be 2.5 times more active as a laxative in rhesus monkeys than phenolphthalein (Budavari, 1996).

Trade names for phenolphthalein include Alophen Pills, Ap-La-Day, Bonomint, Brooklax, Caolax N.F., Certolax, Cirulaxia, Confetto Falqui, Darmol, Dilsuave, Easylax, Espotabs, Evac-Q-Tabs, Evac-U-Gen, Evac-U-Lax, Ex-Lax, Feen-A-Mint, Figsen, Fletchers Childrens Laxative, Fructines, Fructosan, Lacto-Purga, Laxative Pills, Laxen Busto, Laxettes, Lax-Pills, Lilo, Medilax, Modane, Musilaks, Neo-Prunex, Novopuren, Phenolax, Phenolphthalein Tablets USP 23, Prifinol, Prulet, Purga, Purganol, Purgante, Pürjen Sahap, Reguletts, Sure Lax and Thalinol (National Toxicology Program, 1999;

Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

Trade names for phenolphthalein that have been discontinued include Alophen, Bom-Bon, Confetto, Euchessina, Fructine-Vichy, Koprol, Laxatone, Laxogen, Neopurghes, Phthalin, Prunetta, Purgen, Purgestol, Spulmako-lax and Trilax.

Trade names for multi-ingredient preparations of phenolphthalein include Abfuhrdragees, Agarol, Agoral, Aid-Lax, Alofedina, Alophen, Alsiline, Bicholate, Calcium Docuphen, Caroid, Carter Petites Pilules, Carters, Carters Little Pills, Cholasyn, Colax, Damalax, Dialose Plus, Disolan, Docucal-P, Doulax, Doxidan, Emuliquen Laxante, Evac-Q-Kwik, Ex-Lax Extra Gentle Pills, Ex-Lax Light, Falqui, Fam-Lax, Feen-a-mint Pills, Femilax, Ford Pills, Grains de Vals, Herbalax Forte, Juno Junipah, Kest, Kondremul with Phenolphthalein, Laxa, Laxante Bescansa, Laxante Bescansa Aloico, Laxante Olan, Laxante Salud, Laxarol, Laxo Vian, Le 100 B, Lipograsil, Mackenzies Menthoids, Mahiou, Modane Plus, Mucinum, Nylax, Obstinol, Paragar, Paragol, Petrolagar No. 2, Petrolagar with Phenolphthalein, Phillips Gelcaps, Phillips' Laxative Gelcaps, Phillips' Laxcaps, Phytolax, Pildoras Zeninas, Sanicolax, Takata, Thunas Bilettes, Triolax, Unilax, Vencipon, Veracolate and Vesilax (Royal Pharmaceutical Society of Great Britain, 1999).

Trade names for preparations containing phenolphthalein which have been discontinued include Agarbil, Amaro Lassativo, Bilagar, Boldolaxine, Boldolaxine Aloes, Confetti Lassativi, Confetto Complex, Correctol, Crisolax, Dietaid, Dragées 19, Emulsione Lassativa, Flamlax, Lactolaxine, Lax-Lorenz, Laxante Geve, Laxante Richelet, Laxativum, Laxicaps, Medimonth, Ormobyl, Pillole Lassative Aicardi, Pillole Schias, Pluribase, Reolina, Rim and Verecolene Complesso.

1.1.5 *Analysis*

Several international pharmacopoeias specify colorimetric and liquid chromatographic methods for identifying phenolphthalein; visible absorption spectrophotometry and liquid chromatography are used to assay its purity. Phenolphthalein is identified in pharmaceutical preparations by colorimetry and liquid chromatography; liquid chromatography is used to assay for content (British Pharmacopoeial Commission, 1993; US Pharmacopeial Convention, 1994).

Several methods for the analysis of phenolphthalein in various matrices have been reported, which include spectrophotometric, titrimetric, polarographic and chromatographic methods. The chromatographic methods include paper, gas, thin-layer and high-performance liquid chromatography (Al-Shammary *et al.*, 1991).

1.2 Production

Phenolphthalein can be prepared from a mixture of phenol, phthalic anhydride and sulfuric acid which is heated to 120 °C for 10–12 h. The product is extracted with

boiling water, and the residue is dissolved in dilute sodium hydroxide solution, filtered, and precipitated with acid (Gennaro, 1995).

It has been reported that 197 tonnes of phenolphthalein were produced by one US manufacturer in the early 1990s (National Toxicology Program, 1999). Information available in 1999 indicated that phenolphthalein was manufactured and/or formulated in 33 countries (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain; 1999; Swiss Pharmaceutical Society, 1999).

1.3 Use

Phenolphthalein is a stimulant laxative which has been used for the treatment of constipation and for bowel evacuation before investigational procedures or surgery. The laxative effect of phenolphthalein was discovered in 1902, and it has been widely used since that time (Mvros *et al*., 1991). It usually has an effect within 4–8 h after oral administration, generally in tablets or capsules; it is also available as an emulsion with liquid paraffin. It is available without prescription in many countries. The usual oral laxative dose of phenolphthalein (white or yellow) is 30–200 mg daily taken at bedtime for adults and children aged ≥ 12 years (270 mg should not be exceeded); 30–60 mg daily for children aged 6–11 years; and 15–30 mg daily for children aged 2–5 years, given as a single or divided doses. A dose of 260 mg has been used in regimens for bowel evacuation (American Hospital Formulary Service, 1997; Royal Pharmaceutical Society of Great Britain, 1999).

The use of laxatives to relieve constipation and to maintain regularity in bowel habits is common in western cultures. Studies in Australia, the United Kingdom and the USA have found that about 20% of the general population reports regular use of laxatives (Kune, 1993). Two large surveys of the adult population in the USA found that about 10% of adults used some form of laxative at least once a month, that female users outnumbered male users and that the fraction of users increases with age (Everhart *et al*., 1989; Harari *et al*., 1989).

Few studies report the prevalence of use of phenolphthalein laxatives. One study of 424 cases of colon cancer and 414 controls in Washington State, USA, aged 30–62, found that 34% of the control subjects reported constipation requiring treatment (use of a laxative, enema or prunes), 2.7% reported ever having used phenolphthalein laxatives and 1.4% reported having used phenolphthalein laxatives at least 350 times in their lifetimes (Jacobs & White, 1998).

In three populations of 268–813 persons who had undergone endoscopy for colon polyps, two in North Carolina and one in California, USA, comprising approximately equal numbers of cases and controls, 0.8–4.4% of the control subjects had used phenolphthalein laxatives at least once per week. The two groups in North Carolina comprised subjects aged 30–89 years, 58% and 53% of whom were female; the group in California comprised subjects aged 50–74 years of whom 34% were female. The mean ages of the three groups were comparable (59–62 years). Among controls, the

frequent users of phenolphthalein laxatives represented 5.2–30% of all frequent laxative users. In the two studies in North Carolina, 18% of case subjects and 25% of controls reported ever having used phenolphthalein laxatives, and 10% of cases and 7% of controls had used them at least once a month (Longnecker *et al*., 1997). In a study of colorectal cancer in Melbourne, Australia (Kune, 1993), 9.7% of the 723 subjects reported ever having used phenolphthalein laxatives.

Phenolphthalein in a 1% alcoholic solution is also used as a visual indicator in titrations of mineral and organic acids and most alkalis. Phenolphthalein-titrated solutions are colourless at pH < 8.5 and pink to deep-red at pH > 9 (Budavari, 1996).

1.4 Occurrence

Phenolphthalein is not known to occur as a natural product. No data on occupational exposure were available to the Working Group.

1.5 Regulations and guidelines

Phenolphthalein is listed in the Austrian, Belgian, British, Chinese, Czech Republic, Hungarian, Italian, Swiss and US pharmacopoeias (Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

After the publication in 1996 of the results of studies in rodents indicating that phenolphthalein was carcinogenic and genotoxic in several test systems, with damage (loss) of the *p53* tumour suppressor gene (Food & Drug Administration, 1999), many countries moved to restrict over-the-counter sales of phenolphthalein-containing laxatives. Both France and Italy have suspended use of phenolphthalein in prescription and over-the-counter pharmaceutical preparations, and the United Kingdom has changed the status of phenolphthalein from an over-the-counter to prescription agent in pharmaceutical preparations (WHO, 1997; Francesco International, 1998; WHO, 1998). Canada has suspended the sale of all products containing phenolphthalein (Canadian Pharmacists Association, 1999). The German Federal Institute for Drugs and Medical Devices recommended that holders of authorizations to market phenolphthalein-containing laxative products withdraw their products from the market because of the potential toxicological risks. The Japanese Pharmaceutical and Medical Safety Bureau of the Ministry of Health and Welfare issued a statement that laxative products containing phenolphthalein had been voluntarily withdrawn by the manufacturers (WHO, 1998). The Food and Drug Administration (1999) issued a final rule establishing that phenolphthalein is not generally recognized as safe and effective.

2. Studies of Cancer in Humans

Studies of the association between colorectal neoplasia and use of phenolphthalein-containing laxatives are summarized in Table 1.

2.1 Colon cancer

Kune (1993) analysed data on laxative use reported by 685 subjects with colorectal adenocarcinoma diagnosed in 1980–81 in Melbourne, Australia, and 723 controls frequency matched with cases on age and sex. Laxative use throughout adult life was assessed by interview. The relative risk associated with use of commercially produced laxatives was 1.0 (95% confidence interval [CI], 0.86–1.4). Eighty-seven case subjects (13%) and 70 controls (9.7%) reported having used phenolphthalein-containing laxatives (relative risk, 1.4 [95% CI, 0.96–1.9]).

In a case–control study of the association between colon cancer, constipation and use of phenolphthalein–containing laxatives in Washington State, USA (Jacobs & White, 1998), of 659 potential cases identified, 102 died before being approached and 55 were found to be ineligible. Of the 502 remaining cases, data were obtained from 424. Potentially eligible controls were selected by stratified random sampling of subjects in households identified by random-digit dialling, to approximate the distribution by age, sex and county of residence of the case subjects. Of 549 controls thus identified, data were obtained from 414 subjects. Data on laxative use were obtained by telephone interview, and subjects were also asked to complete a mailed food frequency questionnaire. The reference period was up to two years before diagnosis. Regular use was defined as a total use of more than 90 days. The relative risk for colon cancer associated with up to 349 lifetime uses of phenolphthalein-containing laxatives compared with no regular use was 1.0 (95% CI, 0.3–3.7) after adjustment for fibre as percentage of calories. The relative risk for ≥ 350 lifetime uses was 3.9 (95% CI, 1.5–10). Frequent constipation during the 10 years before the reference date (two years before diagnosis) was associated with an increased risk for colon cancer (4.4; 95% CI, 2.1–8.9). When constipation and commercial laxative use were adjusted for mutually, the association with commercial laxative use was no longer apparent, whereas the association with constipation persisted (2.7; 95% CI, 1.4–5.3). The relative risk associated with use of phenolphthalein-containing laxatives adjusted for constipation was 0.42 (95% CI, 0.10–1.7) for < 350 lifetime uses and 1.4 (95% CI, 0.47–4.3) for > 350 uses. [The Working Group noted the difficulty of excluding possible confounding by indication.]

2.2 Colorectal adenomatous polyps

The association between phenolphthalein-containing laxatives and colorectal adenomatous polyps was investigated in a case–control study in Los Angeles (California,

Table 1. Association between colorectal neoplasia and reported use of phenolphthalein-containing laxatives

Area and period of study (reference)	Source population	Exclusion criteria	Cases (no.)	Controls (no.)	Phenolphthalein-containing laxatives			
					Proportion of cases/controls reporting use (%)	Relative risk (95% CI)		Adjustment for:
Australia, Melbourne, 1980–81 (Kune, 1993)	Resident in Metropolitan Melbourne	Ulcerative colitis, familial polyposis, metachronous colorectal cancer, no data on bowel habits	Histologically confirmed colorectal adenocarcinoma (685)	Population, frequency matched on age and sex (723)	13/9.7	1.4 [0.96–1.9]		NR
USA, Washington State, Seattle Metropolitan area 1985–89 (Jacobs & White, 1998)	White, resident in private household with telephone in King, Pierce or Snohomish counties	History of colon or rectal cancer, polyposis or inflammatory bowel disease; inadequate ability to communicate in English	Incident invasive colon adeno-carcinoma (424)	Population selected by random digit dialling (414)	6.6/2.7	< 350 uses versus no regular use	1.0 (0.3–3.7)	Age, sex and fibre as percentage of calories
						≥ 350 uses versus no regular use	3.9 (1.5–10)	
USA, Los Angeles, 1991–93 (Longnecker *et al.*, 1997)	Subjects undergoing sigmoidoscopy (screening, 45%; minor symptoms, 15%, not stated, 41%); response rate, 83%	Previous bowel cancer or adenoma, bowel surgery, inflammatory bowel disease, polyposis, inability to speak English, non-resident of Los Angeles or Orange County, invasive cancer	Histologically confirmed adenomatous polyps (488)	Subjects undergoing sigmoidoscopy in whom no polyps of any type were found, matched with cases on age, sex, medical facility and period of examination (488)	1.8[a]/0.8[a]	1.8 (0.5–6.2)		Alcohol, smoking, vigorous activity and intake of energy, saturated fat and fruits and vegetables

Table 1 (contd)

Area and period of study (reference)	Source population	Exclusion criteria	Cases (no.)	Controls (no.)	Phenolphthalein-containing laxatives		
					Proportion of cases/controls reporting use (%)	Relative risk (95% CI)	Adjustment for:
USA, North Carolina, 1988–90 (Longnecker *et al.*, 1997)	Subjects undergoing colonoscopy (bleeding, 57%; anaemia, 10%; other, 33%); response rate, 83%	Previous bowel cancer or adenoma, bowel surgery, inflammatory bowel disease, inability to speak English; unsatisfactory bowel preparation, incomplete colonoscopy, colitis	Histologically confirmed adenomatous polyps (*n* = 209) or colorectal cancer (*n* = 27)	Subjects undergoing colonoscopy in whom no colorectal adenomatous polyps were found. Controls not matched to cases (409)	3.8[a]/4.4[a]	1.0 (0.4–2.2)	Age, sex, alcohol, smoking, leisure activity and intake of energy, total fat and fibre from fruits and vegetables
USA, North Carolina, 1992–95 (Longnecker *et al.*, 1997)	Subjects undergoing colonoscopy (bleeding, 35%; anaemia, 8%; follow-up of previous non-adenomatous polyps, 17%; other, 40%): response rate, 45%	Previous bowel cancer or adenoma, bowel surgery, inflammatory bowel disease, inability to speak English; unsatisfactory bowel preparation, incomplete colonoscopy, colitis, invasive cancer	Histologically confirmed adenomatous polyps (142)	Subjects undergoing colonoscopy in whom no colorectal adenomatous polyps were found (169)	2.8[a]/1.8[a]	1.1 (0.2–5.7)	Age, sex, alcohol, smoking, hard physical activity, intake of energy, total fat and fibre from fruits and vegetables

[a] Use ≥ once a week

USA) in the period 1991–93 and in two case–control studies in North Carolina (USA) in 1988–90 and 1992–95 (Longnecker *et al.,* 1997*).* In all three studies, cases and controls were selected from among people undergoing an endoscopic procedure (sigmoidoscopy in Los Angeles, colonoscopy in North Carolina); the cases were those found to have polyps. The main indication for this procedure was screening in the Los Angeles study and bleeding in the North Carolina studies. In the Los Angeles study, data on laxative use were collected by personal interview, and subjects were asked about use of specified agents in the year prior to sigmoidoscopy. The agents specified did not include phenolphthalein-containing laxatives but included 'other laxative preparations' as a category. If the subject reported use of laxatives in this category, the specific preparation was recorded. In North Carolina, subjects were asked over the telephone about the brand of laxative they used most often. For all three studies, the responses to questions about the preparation used were reviewed without knowledge of the subject's case or control status, and laxatives were classified as containing phenolphthalein on the basis of brand. In view of these differences between the studies and differences in the eligibility criteria and matching, the three studies were analysed separately. The relative risk for colorectal polyps associated with use of phenolphthalein-containing laxatives at least once a week was 1.8 (95% CI, 0.5–6.2) in Los Angeles (488 cases, 488 controls), 1.0 (95% CI, 0.4–2.2) in North Carolina in 1988–90 (236 cases, 409 controls) and 1.1 (95% CI, 0.2–5.7) in North Carolina in 1992–95 (142 cases, 169 controls).

[The Working Group noted the low statistical power of these studies to detect associations, resulting from the low prevalence of use of phenolphthalein-containing laxatives.]

3. Studies of Cancer in Experimental Animals

Oral administration

Mouse

Groups of 50 male and 50 female B6C3F$_1$ mice, six to seven weeks of age, were given diets containing phenolphthalein (purity, 99.9%) at a concentration of 3000, 6000 or 12 000 mg/kg for two years, equivalent to 0, 300, 600 or 1200 mg/kg bw in males and 0, 400, 800 or 1500 mg/kg bw in females. Only females treated with the highest dose had a significantly decreased rate of survival when compared with controls. The plasma concentrations of total phenolphthalein were similar at all doses. As shown in Table 2, the incidence of histiocytic sarcoma (principally in the liver but also at other sites) was significantly greater in males and females at the two higher doses than in controls. The incidence of malignant lymphoma (all types) was significantly increased in all groups of treated females, but not in males. The incidence of lymphoma of thymic origin was significantly increased in all groups of exposed

Table 2. Incidences of lesions in mice fed diets containing phenolphthalein

Sex	Dose (mg/kg diet)	No. examined	Numbers of animals with lesions					
			Histiocytic sarcoma[a]	Atypical thymic aplasia	Lymphoma of thymic origin[b]	Malignant lymphoma[c]	Ovarian hyperplasia	Benign sex cord/stromal tumour
Male	0	50	1	0	0	6		
	3000	50	3	3	4	8		
	6000	50	11**	7**	7**	12		
	12 000	49	12**	7**	2	8		
Female	0	50	0	0	1	15	4	0
	3000	50	2	7**	9**	28**	11*[d]	7**
	6000	50	7**	6**	10**	33**	10	6*
	12 000	50	7**	5**	7*	25*	17**	5*

From Dunnick & Hailey (1996); National Toxicology Program (1996); *$p < 0.05$; **$p < 0.01$, logistic regression

[a] Historical range: males, 0–2%; females, 0–4%

[b] Includes lymphomas of 'primary' or 'probable' thymic origin

[c] Includes all lymphomas

[d] 49 animals examined

females and in males at 6000 ppm. As shown in Table 2, the incidence of benign ovarian sex-cord stromal tumours was significantly increased in treated females; the mean historical incidence of all ovarian luteomas was 0.4% (Dunnick & Hailey, 1996; National Toxicology Program, 1996).

Groups of 20 female *p53*$^{+/-}$ heterozygous mice, 7–10 weeks of age, received diets containing phenolphthalein at a concentration of 0 (control), 200, 375, 750, 3000 or 12 000 mg/kg for 26 weeks, equivalent to average daily doses of phenolphthalein of 0, 43, 84, 174, 689 or 2375 mg/kg bw per day. The two lowest concentrations delivered doses of phenolphthalein that were approximately 0.5–1.5 times the recommended human dose based on a mg/m^2 body surface area comparison. The incidence of malignant lymphoma of the thymus was significantly increased in heterozygous *p53*-deficient female mice given the two higher doses. Atypical thymic hyperplasia, seen in 3/20 animals at 750 mg/kg, 3/20 at 3000 mg/kg and 5/20 at 12 000 ppm, was considered to represent proliferative change preceding lymphoma. The incidence of atypical hyperplasia or malignant lymphoma was increased in animals at 750 ppm. The incidence of malignant lymphomas was significantly increased at the two highest doses (0/19 in controls and 1/20, 0/20, 2/20, 17/20 ($p < 0.01$) and 14/20 ($p < 0.01$) at the five doses, respectively). Loss of the *p53* wild-type allele was found in 2/2 thymic lymphomas from animals at 750 mg/kg, 13/13 at 200 mg/kg and 6/6 at 12 000 mg/kg (Dunnick *et al.*, 1997).

In a study published as an abstract, *p53*$^{+/-}$ knock-out mice [age not specified] were given phenolphthalein [purity not specified] for 26 weeks by gavage at a dose of 800 or 2400 mg/kg bw per day [number of treatments per week not specified] or in the diet at 2400 mg/kg bw per day [dietary concentration not specified]. [Details of the control groups were not reported.] The experiment was terminated at 26 weeks. The incidences of thymic lymphomas were 3/15, 4/15 and 12/15 in males and 5/15, 8/15 and 14/15 in females receiving 800 (by gavage), 2400 (by gavage) and 2400 (in the diet) mg/kg bw, respectively (Furst *et al*., 1999).

Rat

Groups of 50 male and 50 female Fischer 344 rats, seven weeks of age, were given diets containing phenolphthalein (purity, 99.9%) at a concentration of 0, 12 000, 25 000 or 50 000 mg/kg for two years, equivalent to 0, 500, 1000 or 2000 mg/kg bw for males and 0, 500, 1000 or 2500 mg/kg bw for females. As in the mice, the total plasma concentrations of phenolphthalein did not increase with increasing dose. The survival rate in all groups of treated animals was similar to that of controls. As shown in Table 3, the incidence of benign phaeochromocytoma of the adrenal medulla was significantly increased in all treated male groups, and most were bilateral. The incidence of malignant phaeochromocytoma was not increased by treatment at any dose. The incidence of benign phaeochromocytoma was also increased in female rats given the highest dose, but the incidences of bilateral tumours and malignant phaeochromocytoma were not increased in

Table 3. Incidences of lesions in the adrenal medulla in Fischer 344 rats fed diets containing phenolphthalein

Sex	Dose (mg/kg diet)	No. examined	Numbers of animals with lesions		
			Hyperplasia	Phaeochromocytoma	
				Benign[a]	Benign and malignant[b]
Male	0	50	13	17	18
	12 500	50	22*	34**	35**
	25 000	50	18	34**	35**
	50 000	50	23*	34**	35**
Female	0	50	10	3	3
	12 500	50	18	11*	12*
	25 000	50	15	9	10*
	50 000	50	11	2	2

From Dunnick & Hailey (1996); National Toxicology Program (1996); *$p < 0.05$; ** $p < 0.01$, logistic regression

[a] Historical range: males, 10–63% (mean, 31%); females, 0–8% (mean, 4%)

[b] Historical range: females, 2–12%; mean, 5%

females. As seen in Table 4, the incidence of renal tubular adenoma (single and step sections combined) was also significantly increased in all treated male groups, and a few renal tubular carcinomas were also observed. In females, one renal tubular adenoma was observed at the highest dose (Dunnick & Hailey, 1996; National Toxicology Program, 1996). [The Working Group noted the high doses administered.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The absorption of phenolphthalein in humans has been estimated to be 15% of an oral dose (American Hospital Formulary Service, 1995). The absorbed compound is excreted primarily in the urine as phenolic-hydroxyglucuronide or sulfate conjugates. Some conjugated compound is also excreted in the faeces via the bile, and the resulting enterohepatic recirculation probably contributes to prolongation of the laxative effect (Hardman *et al*., 1996), a hypothesis supported by the observation that phenolphthalein is ineffective as a laxative in patients suffering from obstructive jaundice or

Table 4. Incidences of renal tubular lesions in male Fischer 344 rats fed diets containing phenolphthalein

Numbers of animals	Dose (mg/kg diet)			
	0	12 500	25 000	50 000
Examined	50	50	50	50
Original sections				
With hyperplasia	0	6**	7**	2
With adenoma	0	4	2	6*
With carcinoma	0	1	1	2
With adenoma and carcinoma[a]	0	5*	3	7**
Step sections				
With hyperplasia	3	23**	29**	27**
With adenoma	1	7*	15**	11**
With carcinoma	0	0	1	0
With adenoma and carcinoma[a]	1	7*	15**	11**
Combined (original and step sections)				
With hyperplasia	3	25**	29**	27**
With adenoma	1	10**	15**	15**
With carcinoma	0	1	2	2
With adenoma and carcinoma[a]	1	10**	16**	16**

From Dunnick & Hailey (1996); National Toxicology Program (1996); * $p < 0.05$; ** $p < 0.01$, logistic regression test

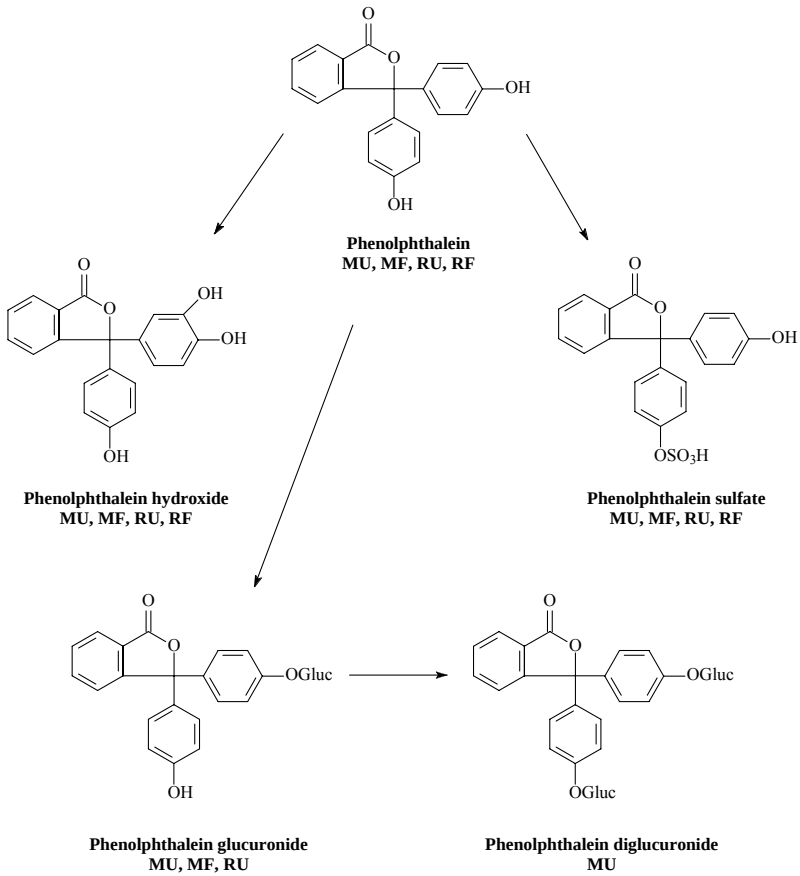
[a] Historical range, 0–6% (mean, 0.9%)

in experimental animals with ligated common bile ducts (Steigmann *et al*., 1938). Small doses of phenolphthalein (30–60 mg) are excreted by humans entirely as conjugated metabolites in urine or faeces, while larger doses (300 mg) result in excretion of both the free and conjugated drug (Williams, 1959). Use of phenolphthalein by women during breast-feeding may cause diarrhoea in their infants (Tyson *et al*., 1937).

4.1.2 *Experimental systems*

Phenolphthalein is absorbed in the intestine (Visek *et al*., 1956) and is almost completely converted to its glucuronide during extensive first-pass metabolism in the intestinal epithelium and liver (Parker *et al*., 1980) via uridine diphosphate glucuronosyltransferase (UDPGT) in rodents and dogs (Sund & Hillestad, 1982; National Toxicology Program, 1996). In guinea-pigs, small amounts of sulfate-conjugated metabolites have been detected in isolated mucosal sheets originating in the jejunum and colon (Sund & Lauterbach, 1986). Faecal excretion is the major route of elimination of phenolphthalein in rats, while in mice both urinary and faecal elimination are important. The metabolites

Figure 1. Metabolism of [^{14}C]phenolphthalein in Fischer 344 rats and B6C3F$_1$ mice



From Griffin *et al.* (1998)
Gluc, glucuronide; MU, mouse urine; MF, mouse faeces; RU, rat urine; RF, rat faeces

identified in urine and faeces are phenolphthalein glucuronide, phenolphthalein sulfate and phenolphthalein hydroxide (Griffin *et al*., 1998; see Figure 1).

Six hours after an intravenous injection of [^{3}H]phenolphthalein to female Wistar rats, analysis of the systemic circulation showed that all of the radiolabel was associated with the glucuronide conjugate (Colburn *et al*., 1979). Enterohepatic recirculation is limited by the rate of hydrolysis of phenolphthalein glucuronide to aglycone by intestinal bacterial β-glucuronidase (Bergan *et al*., 1982; National Toxicology Program, 1996).

The extent of enterohepatic recirculation of phenolphthalein was examined in rats with cannulated bile ducts. Within 24 h, 95% of a dose of 25 mg/kg bw [^{3}H]phenolphthalein administered intraperitoneally to female Wistar rats was recovered as glucuronide in the bile, with 0.2% in the urine. In rats without cannulated bile ducts, 86% of the same

dose was recovered in the faeces, with little glucuronide, and 10% was recovered in the urine, primarily as the glucuronide (Millburn *et al*., 1967; Parker *et al*., 1980).

In male Sprague-Dawley CR-1 strain rats with cannulated femoral veins, femoral arteries and bile ducts given an intravenous dose of 3, 30 or 60 mg phenolphthalein, 99.5% of the dose was eliminated in the bile as the glucuronide. When the same rats were given 3, 30 or 100 mg phenolphthalein glucuronide by intravenous administration, no phenolphthalein was detected in the bile (Mehendale, 1990).

Studies in dogs and mice given [^{14}C]phenolphthalein showed that the radiolabel is evenly distributed throughout the body. In newborn pups of bitches given 4.8 mg/kg bw orally 50 h before whelping, < 0.03% of the dose was found in the liver and gall-bladder and none in the blood, indicating extremely limited passage across the placenta (Visek *et al*., 1956).

Phenolphthalein is excreted in bile, urine, faeces and milk. In mice, 56% of an oral dose was recovered from the urine within 48 h and an additional 38% from the faeces. When an intravenous dose was given, 30% was recovered from the urine and 68% from the faeces (Visek *et al*., 1956). Some phenolphthalein is excreted into the bile, and the prolonged cathartic effect may be due to the ensuing enterohepatic recirculation (Hardman *et al*., 1996). Pre-treatment with hepatic microsomal enzyme inducers increased biliary excretion of metabolites in rats, but post-treatment with enzyme inhibitors decreased it (National Toxicology Program, 1996).

Within 72 h of oral administration of 4.8 mg/kg bw [^{14}C]phenolphthalein to mongrel bitches, 51% of the radiolabel was excreted in the faeces and 36% in the urine. After an intravenous dose, 54% was found in the faeces and 37% in the urine. When the same animals received a cannula in the bile-duct and were given an oral dose, 31% of the radiolabel was found in faeces, 38% in urine and 22% in bile. After an intravenous dose, 11% was eliminated in faeces, 35% in urine and 43% in bile (Visek *et al*., 1956).

The profile of systemic blood concentration–time for phenolphthalein during 24 h after a single intravenous bolus injection was described by a classical compartmental pharmacokinetics model, with evidence of enterohepatic recirculation (Colburn *et al*., 1979).

In the two-year bioassays of the National Toxicology Program (1996), the concentrations of total phenolphthalein in plasma were 100–200 μg/mL.

Whole-body autoradiography of male BOM:NMRI mice showed high concentrations of radiolabel in the stomach, gall-bladder and small intestine 1 h after administration of an intragastric dose of 1 mL/100 g bw [^{14}C]phenolphthalein (10 μCi/100g) [10 mL/kg bw or 3.2 mg/kg bw]. As evidenced by the presence of radiolabel in peripheral organs (including the kidney, liver and skin), the compound was absorbed. After 2 h, it had arrived in the large intestine, and 4 h after administration, maximum radiolabel was observed in the rectum. Two days after administration, no radiolabel was detected (Sund *et al*., 1986).

4.2 Toxic effects

4.2.1 *Humans*

Until the mid-1990s, phenolphthalein was regarded as non-toxic and safe for consumption, although therapeutic oral doses occasionally produced abdominal discomfort, diarrhoea, nausea, decreased blood pressure and faintness (American Hospital Formulary Service, 1995). Serious side-effects were reported in cases of habitual phenolphthalein consumption under conditions of abuse (Cooke, 1977; Pietrusko, 1977).

The main target organ for the toxic effects of phenolphthalein is reported to be the intestine. Indiscriminate use of phenolphthalein results in chronic constipation and laxative dependence, loss of normal bowel function and bowel irritation. Habitual use for several years may cause a 'cathartic colon', i.e. a poorly functioning colon with atonic dilatation, especially on the right side, resulting in extensive retention of the bowel contents. The clinical condition, which resembles chronic ulcerative colitis both radiologically and pathologically, involves thinning of the intestinal wall and loss of the normal mucosal pattern of the terminal ileum (Cummings, 1974; Cummings *et al*., 1974; Cooke, 1977; Pietrusko, 1977; American Hospital Formulary Service, 1995).

Anecdotal cases of long-term use or overdose of phenolphthalein have been associated with abdominal pain, diarrhoea, vomiting, electrolyte imbalance (hypokalaemia, hypocalcaemia and/or metabolic acidosis or alkalosis), dehydration, malabsorption, protein-losing gastroenteropathy, steatorrhoea, anorexia, weight loss, polydipsia, polyuria, cardiac arrhythmia, muscle weakness, prostration and histopathological lesions (Heizer *et al.*, 1968; Velentzas & Ikkos, 1971; Cummings, 1974; LaRusso & McGill, 1975; Pohl & Lowe, 1978; American Hospital Formulary Service, 1995). Kidney, muscle and central nervous system disturbances are thought to be due to electrolyte imbalance. Loss of intestinal sodium and water stimulates compensatory renin production and secondary aldosteronism, leading to sodium conservation and potassium loss by the kidney. The hypokalaemia contributes to renal insufficiency and is sometimes associated with rhabdomyolysis (Copeland, 1994).

Abuse of phenolphthalein-containing laxatives has been associated with gastrointestinal bleeding, iron-deficient anaemia (Weiss & Wood, 1982), acute pancreatitis (Lambrianides & Rosin, 1984) and multiple organ damage in cases of massive overdose, including fulminant hepatic failure and disseminated intravascular coagulation (Sidhu *et al.*, 1989).

Allergy to phenolphthalein is often manifested as cutaneous inflammatory reactions or fixed drug eruptions, i.e. solitary or multiple, well-defined, erythematous macules that may progress to vesicles and/or bullae. These lesions characteristically recur in the same location with each subsequent dose of phenolphthalein and generally leave residual hyperpigmentation that increases in intensity with each exposure; numerous melanin-containing dermal macrophages have been found in pigmented areas (Wyatt *et al*., 1972; Davies, 1985; Stroud & Rosio, 1987; Zanolli *et al.*, 1993). In extreme cases, recurrences have involved progressively more severe lesions characterized as bullous erythema

multiforme, with focal haemorrhage and necrosis and perivascular lymphocytic infiltration (Shelley *et al.*, 1972) and, in one case report, toxic epidermal necrolysis (Kar *et al.*, 1986).

A review of 204 cases of phenolphthalein ingestion in children aged five years and younger reported to the Pittsburgh Poison Center (USA) over a 30-month period indicated that ingestion of ≤ 1 g was associated with a minimal risk of developing dehydration due to excessive diarrhoea and resulting fluid loss (Mrvos *et al.*, 1991). Despite the profile of low acute toxicity documented in this study, cases of fatal poisoning of children have been reported; symptoms of pulmonary and cerebral oedema, multiple organ effects and encephalitis were attributed to hypersensitivity reactions (Cleves, 1932; Kendall, 1954; Sarcinelli *et al.*, 1970). Repeated administration of phenolphthalein-containing laxatives to children has led to serious illness and multiple hospitalizations (Sugar *et al.*, 1991; Ayass *et al.*, 1993).

4.2.2 *Experimental systems*

Fischer 344/N rats and B6C3F_1 mice were given an NIH 07 diet containing phenolphthalein at a concentration of 0, 3000, 6000, 12 000, 25 000 or 50 000 mg/kg *ad libitum* for 13 weeks, equivalent to intakes of 0, 200, 400, 800, 1600 or 3500 mg/kg bw for rats, 500, 1000, 2000, 4100 or 9000 mg/kg bw for male mice and 600, 1200, 2400, 5000 or 10 500 mg/kg bw for female mice. Phenolphthalein did not appear to be toxic in rats, and no laxative effect was observed. Rats at the two higher doses showed slightly lower weight gain. Treated rats showed increased relative (to body weight) kidney weights (males only) and elevated absolute and relative liver weights at concentrations of 12 000–50 000 ppm. Female rats showed no effect on body-weight gain, but those receiving concentrations of 6000–50 000 mg/kg had elevated liver weights. The primary treatment-related findings in mice involved the reproductive and haematopoietic systems. The haematopoietic changes included bone-marrow hypoplasia (at 12 000–50 000 mg/kg) and increased splenic haematopoiesis (males only; 25 000 and 50 000 mg/kg) (National Toxicology Program, 1996).

In female mice [strain not specified] fed 5, 25 or 50 mg/kg bw phenolphthalein per day orally for 135 days, no toxic manifestations or evidence of histopathological changes were found in the liver, kidney or gastrointestinal tract (Visek *et al.*, 1956).

Phenolphthalein at doses of 25 and 50 μg/mL was cytotoxic in cultured Chang liver cells, causing decreased cell growth and increased anaerobic glycolysis, i.e. increased glucose consumption and lactate production (Nishikawa, 1981).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Phenolphthalein is a partial oestrogen in immature rat uteri. Doses of 1–10 mg given subcutaneously twice daily for two days to female Wistar rats weighing 35–40 g induced a dose-related increase in uterine weight, but the maximum increase was only about half of that induced by oestradiol. Phenolphthalein was shown to bind to the oestrogen receptor and was a competitive antagonist to oestradiol (Nieto *et al*., 1990).

In a study reported in an abstract, exposure of female B6C3F_1 mice to 1895 mg/kg bw phenolphthalein orally [method not stated] daily for 30 or 60 days caused no changes in weight gain, oestrous cycles or the numbers of oocyte-containing follicles of any class (primordial, primary, growing or antral), or any detectable pathological change in ovarian cells (Hoyer *et al*., 1997).

Using a continuous breeding protocol, Chapin *et al*. (1997a) administered phenolphthalein in the feed of Swiss CD-1 mice at a concentration of 0.1, 0.7 or 3.0% w/v, to provide estimated intakes of 0.15, 1.0 and 4.5 g/kg bw per day (National Toxicology Program, 1996; Chapin *et al*., 1997b). Pairs of 40 control and 20 treated mice were housed together and allowed to produce up to five litters, the last of which was reared and their reproductive performance measured. Significant reproductive toxicity was observed at the intermediate and high doses. At the intermediate dose, the proportions of pairs producing one to five litters were 100, 89, 84, 68 and 36%, the percentages producing second to fifth litters being significantly smaller than in controls. The decrease at the high dose was more severe, only 5% of pairs producing a fifth litter. Overall, the mean number of litters per pair was reduced by 24 and 50% at the intermediate and high doses, and the number of pups per litter decreased by 58–59%. The final litters were reared on the same diets as the parents. Up to 70% of the pups at the high dose died within four days of birth. Cross-over breeding of animals at the intermediate dose with controls showed that the fertility of the females was affected, the litter sizes being reduced to half. Breeding of the F_1 offspring at the intermediate dose with controls showed that treatment halved the number of litters and the litter size. The survival of the F_2 pups was not affected. Examination of F_0 males at the intermediate dose showed a reduction in testis weight by 36% and in the epididymal sperm count by 30%, and seminiferous tubular degeneration was seen in 9 of 10 treated males. The oestrous cycles and ovarian histology of females at this dose were not affected. Very similar results were found in the F_1 adults at termination. No adverse effects were observed at the low dose.

After 13 weeks of exposure to the same doses as used in the studies of toxicity, there was no evidence of reproductive toxicity in female B6C3F_1 mice or male or female Fischer 344/N rats. Lower epididymal weights and lower sperm density (number of sperm/g of crude epididymal tissue) were observed in male mice at 12 000, 25 000 and 50 000 mg/kg (National Toxicology Program, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

The results of these studies are summarized in Table 5.

Phenolphthalein was not mutagenic in several assays in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 in the presence or absence of exogenous metabolic activation. It did not induce DNA damage in DNA repair-deficient strains of *Bacillus subtilis*.

Phenolphthalein did not induce sister chromatid exchange in Chinese hamster ovary cells in the presence or absence of exogenous metabolic activation, but it induced a dose-related response in chromosomal aberrations in these cells only in the presence of exogenous metabolic activation.

In experiments in which a number of end-points were studied in Syrian hamster embryo cells (a mixed population of cell types that retain some endogenous metabolizing enzymic activity, including oxidation and peroxidation), phenolphthalein induced chromosomal aberrations and *Hprt* mutations, but not ouabain mutations or aneuploidy. No evidence was found for adduct formation in DNA of these cells. The data for micronuclei failed to reach statistical significance ($p = 0.057$). Phenolphthalein caused cellular transformation in the same cell line, indicating that it is metabolized appropriately in this system.

Phenolphthalein increased the incidence of micronucleated erythrocytes in male and female B6C3F$_1$ mice and in male Swiss CD-1 mice. [The Working Group noted that the doses were significantly higher than those to which humans would be exposed.]

Tice *et al*. (1998) studied the effects of phenolphthalein at various concentrations in the diet of transgenic female mice heterozygous for the *p53* gene, over a six-month period. They found significant increases in the frequency of micronucleated erythrocytes, most of which appeared to arise from whole chromosomes rather than chromosomal damage; these were observed at doses comparable to those to which humans are exposed. Inconclusive evidence was found for DNA damage in blood leukocytes, and there was no evidence for DNA damage, apoptosis or necrosis in liver parenchymal cells.

In phenolphthalein-induced thymic lymphomas in B6C3F$_1$ mice, p53 protein accumulated in most tumour cell nuclei, but detectable p53 protein was not seen in control thymuses in this model (Dunnick *et al*., 1997). Other studies have shown that accumulation of p53 protein results from *p53* gene alterations (Hegi *et al*., 1993).

In *p53*$^{+/-}$ heterozygous mice, phenolphthalein induced atypical hyperplasia and malignant lymphomas of thymic origin within six months in 0% of controls, 5% of animals at 200 mg/kg, 5% at 375 mg/kg, 25% at 750 mg/kg, 100% at 3000 mg/kg and

Table 5. Genetic and related effects of phenolphthalein

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtilis rec strains, differential toxicity	–	–	1000[c]	Kada *et al*. (1972)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	320 µg/plate	Bonin *et al*. (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	333 µg/plate	Mortelmans *et al*. (1986)
Gene mutation, Syrian hamster embryo cells, oubain resistance *in vitro*	–	NT	12.8	Tsutsui *et al*. (1997)
Gene mutation, Syrian hamster embryo cells *in vitro*, *Hprt* locus	+	NT	6.4	Tsutsui *et al*. (1997)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	50	National Toxicology Program (1995)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	–	NT	25	Gibson *et al*. (1997)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	+	40	Witt *et al*. (1995)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	12.8	Tsutsui *et al*. (1997)
Aneuploidy, Syrian hamster embryo cells *in vitro*	–	NT	12.8	Tsutsui *et al.* (1997)
Binding (covalent) to DNA, Syrian hamster embryo cells *in vitro*	–	NT	12.8	Tsutsui *et al.* (1997)
Cell transformation, Syrian hamster embryo cells *in vitro*	+	NT	20	Kerckaert *et al*. (1996)
Cell transformation, Syrian hamster embryo cells *in vitro*	+	NT	3.2	Tsutsui *et al*. (1997)
Micronucleus formation, AHH-1 *Tk*$^{+/-}$ human lymphoblastoid cells *in vitro*	–	NT	10	Bishop *et al*. (1998)
Micronucleus formation, MCL-5 human lymphoblastoid cells *in vitro*	+	NT	0.5	Bishop *et al*. (1998)
DNA damage in blood leukocytes, transgenic female TSG-*p53* mice (comet assay) *in vivo*	?		2074; in diet, 6 mo	Tice *et al*. (1998)

Table 5 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, peripheral blood erythrocytes, male and female B6C3F$_1$ mice *in vivo*	+		~1000[d]; in diet, 13 wk	Dietz *et al*. (1992)
Micronucleus formation, peripheral blood erythrocytes, male Swiss CD-1 mice *in vivo*	+		120; in diet, 14 wk	Witt *et al*. (1995)
Micronucleus formation, peripheral blood erythrocytes, transgenic female TSG-*p53* mice *in vivo*	+		37; in diet, 6 mo	Tice *et al*. (1998)

[a] +, positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; d, day; mo, month; wk, week
[c] Absorbed onto paper disc
[d] LED represents the average dose in male and female mice for the formation of normochromatic erythrocytes.

95% of animals at 12 000 mg/kg. Two of two thymic lymphomas examined from animals at 750 mg/kg, 13/13 from those at 3000 mg/kg and 6/6 from those at 12 000 mg/kg had lost the remaining *p53* wild-type allele (Dunnick *et al*., 1997). No spontaneous thymic lymphomas were found in control mice in these studies, but in other studies in $p53^{+/-}$ mice of spontaneous tumours (which may occur in mice after one year of age), only 55% showed loss of the remaining functional *p53* allele (Harvey *et al*., 1993). The presence of functional p53 protein is essential for normal cell growth. When this protein is absent, as is the case in phenolphthalein-induced thymic lymphomas, regulation of cell cycle electrophoresis is lost and malignant progression may be enhanced.

4.5 Mechanistic considerations

Analogy with related biphenolic compounds suggests that phenolphthalein has oestrogenic activity; however, studies with MCF-7 human breast cancer cells in tissue culture (Ravdin *et al*., 1987) and in rat uterus *in vivo* (Nieto *et al*., 1990) suggested only a weak oestrogenic response. Tsutsui *et al*. (1997) used the nuclease P1 enhancement version of the ^{32}P-postlabelling assay to investigate whether (and what type of) DNA adducts were responsible for the morphological transformation induced by phenolphthalein. Although they found no adducts, they recognized the possible limitations of the techniques and suggested that small DNA adducts formed by free radicals could be involved in the effects. Sipe *et al*. (1997) showed free radical metabolism of phenolphthalein by peroxidases *in vitro*.

The observation of Witt *et al*. (1995) that chromosomal damage in Chinese hamster ovary cells occurred only when exogenous metabolic activation was added suggests that some as yet unidentified metabolite is responsible for these effects. Bishop *et al*. (1998) also interpreted differences in the micronucleus response between the two human lymphoblastoid cell lines, MCL-5 and AHH-1 $TK^{+/-}$, as being likely to reflect the importance of a metabolite in chromosome-damaging effects.

Tice *et al*. (1998) suggested that numerical chromosomal loss is responsible for the enhanced incidence of thymic tumours seen after treatment with phenolphthalein in *p53* heterozygous mice. Dunnick *et al*. (1997) noted that these tumours uniformly showed loss of heterozygosity for the *p53* allele rather than point mutations, suggesting either chromosome loss or deletions of large chromosomal segments.

The ability to detect micronuclei but not mutations at the *TK* locus in AHH cells may indicate that cells containing phenolphthalein-induced lesions are susceptible to apoptosis (Bishop *et al*., 1998).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Phenolphthalein has been widely used as a laxative for nearly a century. Generally available without prescription, it is now being withdrawn from the market in many countries because of recent toxicological concern. Phenolphthalein has also long been used in the laboratory as an indicator in acid–base titrations.

5.2 Human carcinogenicity data

In the few available studies, there was no consistent association between the occurrence of colon cancer or adenomatous colorectal polyps and use of phenolphthalein-containing laxatives. Cancers at other sites have not been studied.

5.3 Animal carcinogenicity data

Phenolphthalein was tested for carcinogenicity by oral administration in two experiments in mice and in one experiment in rats. In one experiment in mice, it induced histiocytic sarcomas and lymphomas in both males and females and benign ovarian tumours in females. In an experiment in mice lacking one allele of the *p53* tumour suppressor gene, it increased the incidence of lymphomas. This result was confirmed in a separate study reported as an abstract. It induced benign renal tumours in male rats and benign phaeochromocytomas in males and females.

5.4 Other relevant data

Phenolphthalein is absorbed in the small bowel and is conjugated in the liver to form phenolphthalein glucuronide, which is eliminated in the bile. As it passes through the small intestine, it is partially deconjugated and reabsorbed.

Phenolphthalein and its glucuronide enhance oxygen radical production and cause oxidative damage *in vitro*. Phenolphthalein has also been shown to have low oestrogenic activity in some model systems. Phenolphthalein induced micronucleated erythrocytes in mice given multiple but not single treatments by gavage or in feed. Abnormal spermatozoa were induced in male mice but not male rats treated with phenolphthalein in the feed for 13 weeks. The malignant thymic lymphomas induced by phenolphthalein in female heterozygous *p53*-deficient mice showed loss of the normal *p53* allele.

Phenolphthalein induced chromosomal aberrations, *Hprt* gene mutations and morphological transformation but not aneuploidy or ouabain-resistant mutations or sister chromatid exchange in cultured mammalian cells. It did not induce gene mutations in bacteria.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of phenolphthalein.

There is *sufficient evidence* in experimental animals for the carcinogenicity of phenolphthalein.

Overall evaluation

Phenolphthalein is *possibly carcinogenic to humans (Group 2B).*

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