Handbook 4

4-Hydroxyphenylretinamide

1. Chemical and Physical Characteristics

1.1. Nomenclature

See General Remarks, section 1.4.

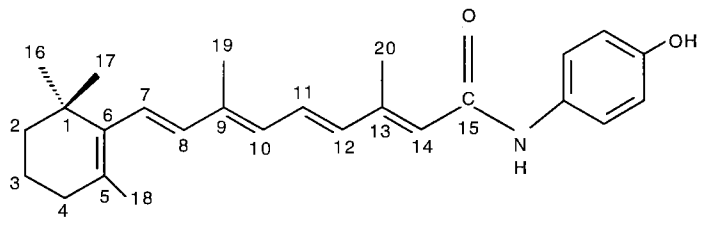
1.2 Name

Chemical Abstracts Services Registry Number
65646-68-6

IUPAC systematic name
N-[4-(all-*E*)-9,13-Dimethyl-7-(1,1,5-trimethylcyclohex-5-en-6-yl)nona-7,9,11,13-tetraen-15-oyl]aminophenol or *N*-[4-(all *E*)-3,7-dimethyl-9-(2,2,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-oyl]aminophenol

Synonyms
Fenretinide, 4-HPR, *N*-(4-hydroxyphenyl)retinamide, hydroxyphenyl-retinamide, *N*-(4-hydroxyphenyl)retinamide, *N*-(4-hydroxyphenyl)-all-*trans*-retinamide

1.3 Structural formula



Composition: $C_{26}H_{33}NO_2$

Relative molecular mass: 391.55

1.4 Physical and chemical properties

Description
Yellow crystals from ethanol or water

Melting-point
173–175 °C (Shealy *et al.*, 1984; Budavari *et al.*, 1996)

Solubility
Soluble in most organic solvents, fats, oils and aqueous micellar solutions; sparingly soluble in water, e.g. 13 nmol/L at pH 6.5 (Li *et al.*, 1996).

Spectroscopy
UV and visible: λ_{max} 362 (methanol), $E^{1\%}_{1\ cm}$ 1225, E_M 47 900 (Budavari *et al.*, 1996; Barua & Furr, 1998). Higher E_M values, i.e. 57 100 and 56 400, have also been reported (Formelli *et al.*, 1996).

Nuclear magnetic resonance:
^{1}H-NMR [$(CD_3)SO_4$, 100 MHz]: δ 1.03 (1,1-CH_3), 1.3–1.8 (2-CH_2, 3-CH_3), 1.70 (5-CH_3), 1.8–2.2 (4-CH_2), 1.99 (9-CH_3), 2.36 (13-CH_3), 6.03 (14-H), 6.1–6.6 (7-H, 8-H, 10-H, 12-H), 6.99 (11-H), 6.6–6.8 and 7.3–7.6 (benzene ring -H), 9.15 (NH), 9.74 (OH) (Coburn *et al.*, 1983; Shealy *et al.*, 1984). Similar spectra with higher resolution were obtained by Barua and Olson (1985).

^{13}H-NMR [$(CD_3)SO_4$, 25.2 MHz]: δ 12.5 (9-CH_3), 13.2 (13-CH_3), 18.8 (3-C), 21.5 (5-CH_3), 28.9 (1,1-CH_3), 32.6 (4-C), 33.8 (1-C), 39.2 (2-C), 123.1 (14-C), 127.4-131.3 (5-C, 7-C, 10-C, 11-C), 136.2 (12-C), 137.1 (8-C), 137.3 (6-C), 137.9 (9-C), 147.4 (13-C), 164.2 (15-C); phenyl ring 115.1 (3′-C, 5′-C), 120.8 (2′-C, 6′-C), 153.3 (1′-C, 4′-C) (Coburn *et al*, 1983; Barua & Furr, 1998)

Resonance Raman
(Barua & Furr, 1998)

Infrared
(Coburn *et al.*, 1983; Shealy *et al.*, 1984; Barua & Furr, 1998)

Mass spectrometry
(Coburn *et al.*, 1983; Barua & Furr, 1998)

X-Ray analysis: (Chrzanowski *et al.*, 1984)

Stability
Unstable to light, oxygen and heat; protected in solution by the presence of antioxidants such as butylated hydroxytoluene and pyrogallol; degradation and isomerization are minimized by storage under an inert gas such as argon at –20 °C or less in the dark (Barua & Furr, 1998)

2. Occurrence, Production, Use, Human Exposure and Analysis

2.1 Occurrence

4-Hydroxyphenylretinamide is a synthetic compound and is available solely for testing in the treatment of some dermatological disorders and neoplasms.

2.2 Production

4-Hydroxyphenylretinamide is synthesized from all-*trans*-retinoic acid, which is converted to all-*trans*-retinoyl chloride by treatment with phosphorus trichloride in dry benzene under nitrogen. Retinoyl chloride in benzene is then added slowly to a cold, stirred solution of 4-aminophenol in dimethyl formamide. After the precipitate of 4-aminophenol hydrochloride has been filtered off, the filtrate is washed with water, concentrated and chilled to yield a crystalline product, which is recrystallized from methanol and water. Use of 13-*cis*-retinoic acid in the same procedure yields the 13-*cis* isomeric product (Shealy *et al.*, 1984). Other variations of this procedure have been used in producing a large number of related amide derivatives (Shealy *et al.*, 1984; Dawson & Hobbs, 1994).

2.3 Use

Clinical chemoprevention trials are under way with 4-hydroxyphenylretinamide as the primary therapeutic agent in tumours of the bladder, breast, cervix, lung, oral cavity, prostate and skin (Hong & Itri, 1994; Kelloff *et al.*, 1994; Formelli *et al.*, 1996). The most frequently used daily dose is 200 mg (Kelloff *et al.*, 1994; Formelli *et al.*, 1996).

2.4 Human exposure

Exposure to 4-hydroxyphenylretinamide is limited for all practical purposes to medical treatment for diseases and disorders. The intestinal absorption of this compound is markedly affected by the composition of the diet, as high-fat diets promote absorption whereas high-carbohydrate diets do not (Doose *et al.*, 1992). The major side-effects seen at the usual daily dose of 200 mg are cutaneous toxicity and occasional cases of night blindness due to a marked reduction in plasma retinol. A three-day drug 'holiday' per month during long-term treatment allows plasma retinol concentrations to recover and minimizes the visual toxicity (Kelloff *et al.*, 1994; Formelli *et al.*, 1996).

2.5 Analysis

4-Hydroxyphenylretinamide in plasma and tissues of treated patients is commonly measured by high-performance liquid chromatography (HPLC; Formelli *et al.*, 1989; Peng *et al.*, 1989; Doose *et al.*, 1992). Blood is collected in heparinized tubes and the plasma or a tissue homogenate is acidified to pH 3–4 and then extracted several times with a suitable volume of an organic solvent such as chloroform or methanol, diethyl ether, dichloromethane, acetonitrile, 2-propanol or ethyl acetate. A known amount of a reference standard, usually all-*trans*-retinyl acetate, is added to the sample to correct for losses during extraction and analysis. Furthermore, an antioxidant such as butylated hydroxytoluene is added at the outset to minimize oxidation of any retinoids present. After the combined extract has been dried with anhydrous sodium sulfate, the solvent is evaporated under yellow light (to avoid isomerization) in nitrogen or argon to dryness. The dried powder is immediately dissolved in the HPLC solvent and injected onto the HPLC column. In some cases, a solid-phase extraction or elution step is introduced to remove contaminants.

4-Hydroxyphenylretinamide is usually detected by measuring the absorption at 362 nm and is quantified by measuring the area of the absorption peak with an integrator. A reversed-phase C_{18} column is usually used for the separation.

A large number of chromatographic systems have been devised for the separation and quantification of retinoic acid and its derivatives (Frolik & Olson, 1984; Furr *et al.*, 1992, 1994; Barua & Furr,

1998; Barua *et al.*, 1999). Suitable procedures for 4-hydroxyphenylretinamide have been defined (Schrader & Sisco, 1987; Formelli *et al.*, 1989; Doose *et al.*, 1992).

3. Metabolism, Kinetics and Genetic Variation

3.1 Humans

3.1.1 Metabolism

The major metabolites of 4-hydroxyphenylretinamide include *N*-(4-methoxy-phenyl)retinamide, 4-hydroxyphenylretinamide-*O*-glucuronide and several other polar retinamides. 4-Hydroxyphenylretinamide is not detectably hydrolysed to all-*trans*-retinoic acid or to other retinoic acid isomers (Formelli *et al.*, 1996).

4-Hydroxyphenylretinamide and *N*-(4-methoxyphenyl)retinamide were found at high concentrations in plasma and breast tissue obtained at surgery from women participating in a trial of 4-hydroxyphenylretinamide for the prevention of contralateral breast cancer (Mehta *et al.*, 1991; see section 3.1.3.). In three human breast carcinoma cell lines and two melanoma cell lines *in vitro*, *N*-(4-methoxyphenyl)retinamide was the major metabolite of 4-hydroxyphenylretinamide, although other, unidentified polar and non-polar retinoids were also detected. The presence of serum in the medium did not affect the retention or metabolism of the compound by the cancer cells. Only cancer cell lines that metabolized 4-hydroxyphenylretinamide to *N*-(4-methoxyphenyl)retinamide were sensitive to the anti-proliferative effect of 4-hydroxyphenylretinamide, and *N*-(4-methoxyphenyl)retinamide did not block cell proliferation, indicating that it is not an active metabolite. The authors suggested that it could serve as an indirect biomarker of the response of cells to 4-hydroxyphenylretinamide (Mehta *et al.*, 1998).

A relevant metabolic effect of 4-hydroxyphenylretinamide is that on the normal transport and metabolism of retinol. In both cancer patients and healthy volunteers, it markedly lowered the plasma concentration of retinol (Formelli *et al.*, 1989; Peng *et al.*, 1989; Dimitrov *et al.*, 1990). This reduction accounts for the impaired night vision associated with administration of 4-hydroxyphenylretinamide. *In vitro* it also inhibited several rodent enzymes (acyl coenzyme A:retinol acyltransferase, lecithin:retinol acyltransferase and retinal reductase) that are thought to be involved in normal retinol metabolism (Ball *et al.*, 1985; Dew *et al.*, 1993). [The Working Group noted that no inhibitory effects of 4-hydroxyphenylretinamide on the human homologues of these enzymes has been reported in the literature and it is unclear whether this observation *in vitro* is directly relevant to patients receiving 4-hydroxyphenylretinamide.]

3.1.2 Kinetics

In three cancer patients given 4-hydroxyphenylretinamide orally at a single dose of 300 mg/m^2, the average half-life in the circulation was 14 h with a mean value for the integrated area under the curve for plasma concentration–time (AUC) of 3.5 mg-h/mL. The half-life of *N*-(4-methoxyphenyl)retinamide is long but variable, ranging from 22 to 54 h, with a mean AUC value of 1.15 mg-h/mL. A rapid, significant reduction in plasma retinol concentration was seen within one to two weeks after treatment (Peng *et al.*, 1989).

Most of the studies of the pharmacokinetics of 4-hydroxyphenylretinamide have been conducted in women with breast cancer in trials of its use for preventing the recurrence of cancer in the contralateral breast (Formelli *et al.*, 1989). The plasma concentrations of both 4-hydroxyphenyretinamide and its methoxy metabolite are linearly related to the dose of 4-hydroxyphenylretinamide. Moreover, like all-*trans*-, 13-*cis*- and 9-*cis*-retinoic acid, 4-hydroxyphenylretinamide markedly reduces plasma retinol concentrations, which is thought to account for the impaired night vision observed in individuals receiving this compound (Formelli *et al.*, 1993). After a five-year treatment, 4-hydroxyphenylretinamide was cleared from the plasma with an average half-life of 27 h, while the rate of elimination of *N*-(4-methoxyphenyl)retinamide was lower. The half-life of 4-hydroxyphenylretinamide was slower after one oral dose (20 h) than after 28 consecutive days (27 h) (Formelli *et al.*, 1989).

3.1.3 Tissue distribution

In the study of Mehta *et al.* (1991), described above, of nine women who had been maintained on 4-hydroxyphenylretinamide at doses of 100, 200 or 300 mg for 15–639 days, the plasma concentrations of the drug were 0.077–1 mmol/L,

while those of *N*-(4-methoxyphenyl)retinamide ranged from not detectable to 0.94 mmol/L, generally correlating well with those of the parent drug. The concentration of 4-hydroxyphenylretinamide in breast tissue was 0.08–6.7 nmol/mg of tissue while that of the methoxy metabolite was 2.6–34 nmol/mg. The breast therefore highly concentrates 4-hydroxyphenylretinamide, and *N*-(4-methoxyphenyl)retinamide even more so. Both compounds were also found at relatively high concentrations in mammary tumour tissue from these women. When the breast tissue from five women with breast cancer was resolved into fat and epithelial cells, 4-hydroxyphenylretinamide tended to be concentrated in the epithelial cells, and the methoxy metabolite tended to be associated with the fat (Mehta *et al.*, 1991).

The concentrations of 4-hydroxyphenylretinamide and *N*-(4-methoxyphenyl)retinamide in plasma, breast tumour, normal breast tissue, breast muscle and breast fat from three patients who had received 4-hydroxyphenylretinamide for 5–252 days are shown in Table 1. The plasma concentrations of both retinoids were substantially lower than those in breast tissue. Their presence in plasma and in nipple discharge indicate that 4-hydroxyphenylretinamide enters breast epithelial cells (Formelli *et al.*, 1993).

3.2 Experimental models

3.2.2 Metabolism

After female rats and mice had been given 4-hydroxyphenylretinamide at 5 mg/kg bw per day intraperitoneally for five days, the tissue concentrations of the drug and its metabolites were assessed in serum, liver, mammary gland and urinary bladder. Of the four metabolites detected, one co-eluted from the reversed-phase HPLC column with the *cis* isomer and a second with the same retention time as *N*-(4-methoxyphenyl)retinamide, a third was tentatively identified as a fatty acyl ester of the parent drug and the fourth remained unidentified. The amounts of each metabolite varied with tissue and species: for instance, the concentration of 4-hydroxyphenylretinamide was significantly lower and that of *N*-(4-methoxyphenyl)retinamide was higher in mouse tissues than in the corresponding tissues of rats, and the *cis* isomer and the putative acyl ester of 4-hydroxyphenylretinamide were detected in rat liver but not mouse liver. Thus, the drug and its metabolites are distributed to the liver, mammary gland and urinary bladder of rats and mice but the distribution is species dependent (Hultin *et al.*, 1986).

Three metabolites of 4-hydroxyphenylretinamide were detected by reversed-phase HPLC in mammary gland extracts from BALB/c mice

Table 1. 4-Hydroxyphenylretinamide and *N*-(4-methoxyphenyl)retinamide concentrations (ng/ml or ng/g) in plasma and breast samples collected 12 h after the last dose, before surgery

	Patient 1: 4-hydroxyphenylretinamide, 200 mg for 7 days				Patient 2: 4-hydroxyphenylretinamide, 300 mg for 5 days				Patient 3: 4-hydroxyphenylretinamide, 100 mg for 168 days + 200 mg for 84 days			
Plasma	216		140		337		400		346		202	
Breast tumour	499	2.3	1024	7.3	567	1.7	1291	3.2	ND		ND	
Breast normal tissue	567	2.6	750	5.4	845	2.5	1024	2.6	473	1.4	6678	33.1
Breast muscle	311	1.4	1079	7.7	626	1.9	1076	2.7	299	0.9	2997	14.8
Breast fat	1776	8.2	1544	11.0	1871	5.5	1376	3.4	1096	3.2	7326	36.2

Reproduced from Formelli *et al.* (1993). ND, not detected

incubated in the presence of insulin, prolactin or steroid hormones (aldosterone, cortisol, progesterone and estradiol) for six days. Two were tentatively identified as 13-*cis*-4-hydroxyphenylretinamide and *N*-(4-methoxyphenyl)retinamide, while the third was not identified. The relative distribution of these metabolites in the organ culture was affected by the hormones added to the culture medium: addition of insulin at 5 mg/mL and prolactin at 5 mg/mL resulted in greater concentrations of *N*-(4-methoxyphenyl)retinamide than in mammary glands treated simultaneously with combinations of steroids. The concentrations of 13-*cis*-4-hydroxyphenylretinamide and of the unidentified metabolite were not markedly affected by inclusion of steroids in the culture medium (Mehta *et al.*, 1988).

3.2.3 Kinetics

After a single intravenous injection of 4-hydroxyphenylretinamide at 5 mg/kg bw to female rats, it was distributed to all the tissues examined (serum, liver, mammary gland and urinary bladder) with the highest concentration in the liver. The distribution continued for 4 h and was followed by first-order elimination kinetics. The half-life of elimination from the liver was 9.4 h, that from serum was 12 h (not significantly different from that in the liver), that from the mammary gland was 44 h and that from the urinary bladder was 9.3 h. In an experiment in which rats and mice were given 4-hydroxyphenylretinamide intraperitoneally at a dose of 5 mg/kg bw per day for five days, the compound was distributed to all tissues, the highest concentrations being reached in the urinary bladder followed by the liver and the mammary gland. The concentrations in the tissues were much greater than those in plasma, indicating that the tissues take up and concentrate 4-hydroxyphenylretinamide from the circulation (Hultin *et al.*, 1986).

Pretreatment of female BDF mice with 4-hydroxyphenylretinamide at 10 mg/kg bw for three days had no effect on its disposition in serum, liver, mammary gland or urinary bladder and had no effect on its pharmacokinetics or that of any of its four metabolites in liver. In contrast, pretreatment of the mice for three days with phenobarbital at 80 mg/kg bw per day had a significant effect on the disposition of 4-hydroxyphenylretinamide in all tissues examined, reduced the AUC values to half those of mice pretreated with the vehicle and significantly reduced the concentrations of the four metabolites in the liver. Thus, although pretreatment with 4-hydroxyphenylretinamide had no effect on its disposition or metabolism, pretreatment with the cytochrome P450 inducer phenobarbital significantly reduced the AUC for 4-hydroxyphenylretinamide in all tissues examined and consequently changed its disposition and metabolism (Hultin *et al.*, 1988).

The distribution of *N*-(4-methoxyphenyl)retinamide in serum, liver, mammary gland, urinary bladder and skin of female BDF mice was assessed after a single oral dose of 10 mg/kg bw. The highest concentrations were found in liver and mammary gland, and the largest AUC value was found for the mammary gland, followed by skin and liver. The elimination half-life of the metabolite was 5.1 h in liver, 5.6 h in serum, 19 h in urinary bladder, 23 h in skin and 27 h in mammary gland. Five metabolites of *N*-(4-methoxyphenyl)retinamide were detected but not identified, and their relative concentrations varied among the tissues. Thus, the metabolism of 4-hydroxyphenylretinamide to *N*-(4-methoxyphenyl)retinamide appears to be only the first of several metabolic steps that give rise to multiple other metabolites (Hultin *et al.*, 1990).

As reported in section 3.2.2, the tissues of female rats and mice receiving 4-hydroxyphenylretinamide intraperitoneally for five days show markedly different distributions and concentrations of metabolites, indicating a difference in the metabolism of this compound in the two species (Hultin *et al.*, 1986).

4. Cancer-preventive Effects

4.1 Humans

4.1.1 Epidemiological studies

No data were available to the Working Group.

4.1.2 Intervention trials

4.1.2.1 Breast

A large, multicentre, randomized, controlled trial of use of 4-hydroxyphenylretinamide for preventing breast cancer was begun in Milan, Italy, in 1987 (De Palo *et al.*, 1997). Patients aged 30–70 who had

been treated surgically for early breast cancer, without axillary lymph node involvement, were randomized to receive either no treatment or 4-hydroxyphenylretinamide at 200 mg/day for five years. The 1496 patients assigned to 4-hydroxyphenylretinamide treatment were instructed to take capsules containing the agent for all but the last three days of each month. Placebo capsules were not given to the 1476 women in the control group. The occurrence of a new, contralateral primary breast tumour was the primary end-point and was assessed by annual mammography and twice yearly clinical examinations. Other events, including new primary tumours in the ipsilateral breast, recurrence of the initial breast tumour or new primary cancers at other sites, were also recorded. Accrual to the study was closed prematurely in 1993, when opinion about use of adjuvant chemotherapy precluded accrual to the untreated control arm of the study. A preliminary report from this study (Decensi *et al.*, 1997a) indicated that there was no difference overall in the incidence of contralateral breast cancer between treated and control women (53 and 54 cases, respectively). The authors noted, however, that there was a statistically significant interaction (p = 0.018) between menopausal status and the effect of treatment. The risk for contralateral cancer appeared to be reduced by treatment in premenopausal women (relative risk = 0.65) and increased in postmenopausal women (relative risk = 1.65). [The Working Group noted that the lack of blinding in the design of the study may complicate interpretation and that there was a significant imbalance in the assignment to study groups, with proportionally fewer premenopausal women assigned to the treatment arm. The Group also noted the difficulty of interpreting statistical analyses of data on subgroups and that the statistically significant interaction between menopausal status and treatment effect was not anticipated.]

4.1.2.2 Ovary

An incidental finding made during the trial described above of use of 4-hydroxyphenylretinamide for preventing breast cancer was the development of ovarian cancer in six women, none of whom were receiving 4-hydroxyphenylretinamide (De Palo *et al.*, 1995a).

4.1.2.3 Prostate

Pienta *et al.* (1997) reported the results of a trial of 4-hydroxyphenylretinamide in 22 patients at risk for adenocarcinoma of the prostate who were given a dose of 100 mg/day for 12 scheduled 25-day cycles, with a three-day break between cycles. No untreated controls were included. Biopsies were performed 6 and 12 months after the start of therapy. Eight patients with no indication of adenocarcinoma in their biopsy samples before the study showed signs of this tumour before or at the time of their 12-month evaluation. This high frequency of cancer and the difficulty in accruing patients led to early closure of the study.

4.1.3 Intermediate end-points

4.1.3.1 Oral cavity

Tradati *et al.* (1994) studied eight patients with diffuse inoperable oral lichen planus or leukoplakia, who received topical applications of 4-hydroxyphenylretinamide twice daily. After one month of therapy, two patients had complete remission and the other six had a greater than 75% response. 4-Hydroxyphenylretinamide was well tolerated, and no local or distant side-effects were observed. [The Working Group noted that oral leukoplakia may regress spontaneously.]

A randomized study of patients with oral leukoplakia was begun in 1988 at the Milan Cancer Institute to evaluate the efficacy of 52 weeks of maintenance therapy with 4-hydroxyphenylretinamide administered systemically at a dose of 200 mg/day after complete laser resection of the lesions. A three-day period with no drug was prescribed at the end of each month to avoid the adverse effect on night blindness of lowering serum retinol concentrations. The most recent report (1993) gave the results for 153 patients, of whom 74 were randomized to 4-hydroxyphenylretinamide and 79 to no intervention (Chiesa *et al.*, 1993; Costa *et al.*, 1994). The proportion of patients with recurrence or new lesions during the trial was similar in the two treatment arms: 13/79 (10 recurrences and three new lesions) in the group receiving 4-hydroxyphenylretinamide and 21/79 (9 recurrences and 12 new lesions) among controls; however, the projected time to treatment failure was reported to be 6% with 4-hydroxyphenylretinamide and 30% for controls. [The Working Group observed differences between the rates of

drop-out of patients in the two groups, which may partially explain the difference in rate of treatment failure, as determined by the crude proportions and the Kaplan-Meier method.]

4.1.3.2 Urinary bladder

Decensi *et al.* (1994a) reported the results of a trial in which 12 patients with superficial urinary bladder cancer were treated with 4-hydroxyphenylretinamide orally at a dose of 200 mg daily and compared with 12 non-randomized, untreated controls. The DNA content and the percent of cells in S or G_2 + M phase were used as the end-points. A trend in recession from aneuploidy to a diploid state and a decrease in the number of cells in S or G_2 + M phase were observed in the patients given 4-hydroxyphenylretinamide; however, few samples were studied (24 for ploidy and 8 for cell-cycle analysis). The proportion of patients with DNA aneuploidy in bladder-washed cells decreased from 8/12 to 6/12 in the group given 4-hydroxyphenylretinamide but increased from 8/12 to 10/12 in the control group. Positive or suspect results were found on cytological examination in 3/12 treated cases before administration of 4-hydroxyphenylretinamide, but all subsequently returned to normal. Two patients in the control group showed progression to invasive cancer. [The Working Group noted that some of these results were reported elsewhere (Costa *et al.*, 1995), with somewhat different data.]

4.1.3.3 Breast

A report from the trial for the prevention of breast cancer in Milan described above was based on data on 149 women who had been enrolled in the treatment arm of the study and had been assessed by mammography (Wolfe categories) before and after four years of treatment (Cassano *et al.*, 1993). No changes in mammographic pattern were observed. [The Working Group noted that no data on mammographic patterns after treatment were included in the report and that there were no data on control women.]

4.2 Experimental models

4.2.1 Cancer and preneoplastic lesions

These studies are summarized in Table 2.

4.2.1.1 Mammary gland

Mouse: Groups of 75–100 female C3H mice with mammary tumour virus were fed a diet supplemented with 4-hydroxyphenylretinamide at 1 mmol/kg of diet; nulliparous mice aged two months were fed the diet for 39 weeks and multiparous mice aged four to six months for up to 14 weeks. At the end of treatment, the animals were killed. The incidence of mammary adenocarcinomas was 85% in nulliparous mice maintained on the control diet, 71% in nulliparous mice fed the 4-hydroxyphenylretinamide-containing diet, 69% in multiparous mice on control diet and 80% in multiparous mice fed the treated diet. The numbers of tumours per animal were reduced from 2.4 in control nulliparous mice to 1.7 in those fed 4-hydroxyphenylretinamide ($p < 0.01$; χ^2 test), but no change in the number of tumours was found in multiparous mice (Welsch *et al.*, 1983).

Rat: Groups of 15–40 female Sprague-Dawley rats, 50 days of age, were given an intravenous injection of *N*-methyl-*N*-nitrosourea (MNU) at 15 or 50 mg/kg bw and a second injection on day 57. Three days later, the rats were fed either a basal diet or one supplemented with 391 mg/kg diet (1 mmol) or 782 mg/kg diet (2 mmol) of 4-hydroxyphenylretinamide until the end of the study 182 days after carcinogen treatment. The incidence of mammary adenocarcinomas was 100% in controls treated with the high dose of MNU, 65% in animals at the high dose of 4-hydroxyphenylretinamide ($p < 0.01$, Fisher's exact test) and 80% in animals at the low dose of 4-hydroxyphenylretinamide (not significant). At the low dose of MNU, the incidence of mammary adenocarcinomas was 30% in rats fed the control diet and 15% and 30% in the groups maintained on diets with the high and low doses of 4-hydroxyphenylretinamide, respectively (not significant). The tumour multiplicity of animals given the high dose of MNU was reduced from 5.2 in controls to 2.3 ($p < 0.01$, Mantel non-parametric test) in animals at the high dose of 4-hydroxyphenylretinamide and to 2.9 ($p < 0.05$) in rats fed the diet low in 4-hydroxyphenylretinamide. At the low dose of MNU, tumour multiplicity was not affected by 4-hydroxyphenylretinamide (Moon *et al.*, 1979). Several other experiments with similar concentrations of 4-hydroxyphenylretinamide and a single

Table 2. Effects of 4-hydroxyphenyl-retinamide on carcinogenesis in animals

Cancer site	Species, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	4-Hydroxyphenyl-retinamide dose (mg/kg diet), route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Mammary gland	C3H mice		Tumour virus	391 (Wayne Lab Chow)	0 d to end						Welsch *et al.* (1983)
	Nulliparous	75–100				85	71	2.4	1.7*	Effective	
	Multiparous	75–100				69	80	1.5	1.7	Ineffective	
Mammary gland	Sprague-Dawley rats, female, 50 d	15–17	MNU (50 mg/kg bw) twice, i.v.	782	+3 d to end	100	65*	5.2	2.3*	Effective	Moon *et al.* (1979)
				391 (Wayne Lab Chow)		100	80	5.2	2.9*	Effective	
		40	MNU (15 mg/kg bw) twice, i.v.	782	+3 d to end	30	15	0.35	0.22	Ineffective	
				391		30	30	0.35	0.35	Ineffective	
Mammary gland	Sprague-Dawley rats, female,120 d	40	MNU (50 mg/kg bw) single, i.v.	782 (AIN-76A)	–60 d to end	68	42*	1.7	1.3	Effective	Moon *et al.* (1992)
Mammary gland	Sprague Dawley rats, female, 50 d	30	MNU (50 mg/kg bw) single, i.v.	391 (Wayne Lab Chow)	–7 d to end	100	100	8.87	7.45*	Effective	McCormick & Moon (1986)
Mammary gland	Sprague-Dawley rats, female, 50 d	25	MNU (50 mg/kg) single i.v.	782 (Wayne Lab Chow)	+7 d to end	100	92	4.82	3.39*	Effective	McCormick *et al.* (1982a)
Mammary gland	Sprague-Dawley rat, female, 21 d	21–30	MNU (25 mg/kg bw) i.v.	782 (NIH-07)	–29 d to 12 wks	86	60	1.3	1.2	Ineffective	Silverman *et al.* (1983)
					–2 d to 12 wks	86	50*	1.3	0.7*	Effective	
					+2 d to 17 wks	86	60	1.3	0.8		
		15	DMBA (10 mg/rat) i.g.	782 (NIH-07)	–21 d to 12 wks	53	60	0.9	0.9	Ineffective	
					+10 d to 12 wks	53	47	0.9	0.8	Ineffective	
Mammary gland	Fischer 344 rat, female, 50 d	25	MNU (45 mg/kg bw) i.v.	782 (AIN 76-A)	–7 d to end	60	76	1.04	1.52	Ineffective	Cohen *et al.* (1994)
					+7 d to end	60	76	1.04	1.92	Ineffective	
				782 (Wayne Lab Chow)	–7 d to end	24	8	0.48	0.20	Ineffective	
					+7 d to end	24	16	0.48	0.32	Ineffective	
				782 (NIH-07)	–7 d to end	44	20*	0.64	0.20*	Effective	

Table 2. (Contd)

Cancer site	Species, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	4-Hydroxyphenyl-retinamide dose/ (mg/kg diet) route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Mammary gland	Sprague-Dawley rats, female, 100 d	30	MNU (50 mg/kg bw) i.v.	782 (Wayne Lab Chow)	–60 d to 0 d –60 d to end	57 57	66 23*	1.4 1.4	2.7 0.3*	Ineffective Effective	Grubbs *et al.* (1990)
Mammary gland	Sprague-Dawley rats, female, 50 d	20	MNU (50 mg/kg bw) i.v.	391 (Wayne Lab Chow)	+7 d to –256 d	64	50	1.1	0.8*	Effective	McCormick *et al.* (1982b)
Mammary gland	Sprague-Dawley rats, female, 50 d	20	DMBA (75 mg/rat) i.g.	293 586	–14 d to 18 wks	70 70	65 30*	1 1	1.1 0.35*	Ineffective Effective	Abou-Issa *et al.* (1988)
Mammary gland	Sprague-Dawley rats, female, 50 d	30	DMBA (15 mg/rat)	90 mg/kg bw/d	0 d to end	84	89	3.65	2.45*	Effective	Bollag & Hartmann (1987)
		22	DMBA (20 mg/rat) i.g.	782 (Wayne Lab Chow)	+7 d to end	91	85*	5.4	3.7*	Effective	McCormick *et al.* (1982a)
Mammary gland	Sprague-Dawley rats, female, 50 d	16	DMBA (15 mg/rat) i.g.	782 391 (AIN-76A)	–7 d to end	88 88	33* 73	2.1 2.1	0.6* 1.4	Effective Ineffective	Abou-Issa *et al.* (1993)
Skin	Sencar mice, female, 3–4 wks	25	DMBA (5 µg/mouse) topically	391 (Wayne Lab Chow)	+ 2 wks to end	4	35	0.08	1.87	Ineffective Tumour enhancing	McCormick *et al.* (1987)
				30 nmol topically	+ 2 wks to end	4	4	0.08	0.04	Ineffective	
Haemato-poietic system (lymphoma)	PIM transgenic mice, 4 wks	30–40	50 mg/kg bw ENU i.p.	98	0 to 20 wks 0 to 35 wks	48 53	20* 43	NR NR	NR NR	Effective Ineffective	McCormick *et al.* (1996)
				196	0 to 20 wks 0 to 35 wks	48 53	37 52	NR NR	NR NR	Ineffective Ineffective	
				391	0 to 20 wks 0 to 35 wks	48 53	20* 40	NR NR	NR NR	Effective Ineffective	
Haemato-poietic system (lymphoma)	AKR/J mice, female, 3–4 wks	10	MCF virus (50 ml)	391 782	– 7 d to end	100 100	60* 50*	NR NR	NR NR	Effective Effective	Chan *et al.* (1997)

Table 2. (Contd)

Cancer site	Species, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	4-hydroxyphenyl-retinamide dose/ (mg/kg diet) route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Prostate	Lobund-Wistar male rats, 3 months	20	MNU (30 mg/kg bw) i.v., after 1 wk TP (45 mg)	391 (L-485 (Tek-Lad))	+7 months to end (14 months)	88	21*	NR	NR	Effective	Pollard *et al.* (1991)
Prostate	WU, male rats, 7–8 wks	40	8 mg cyproterone acetate, daily/20 d i.p. 100 mg/kg TP, s.c. 50 mg MNU/kg i.v. s.c. implants of testosterone in silastic tubes	391 (Wayne Lab Chow)	+ 1 d to end (450 d)	45	59	NR	NR	Ineffective	McCormick *et al.* (1998)
Prostate	ACI rats, male, 21–25 months	44	None Spontaneous	78	NR	43.2	27.5	NR	NR	Ineffective	Ohshima *et al.* (1985)
Lung	A/J mice, female, 6 wks	30	NNK (2 mg per mouse), i.p	1556 783 (AIN-76-A1)	–7 d to 52 wks –7 d to 52 wks	73[b] 73[b]	50[b] 57[b]	16.4[a] 16.4[a]	15.4[a] 18.7[a]	Ineffective	Conaway *et al.* (1998)
Urinary bladder	BDF male mice, 4–56 wks	70	NBHBA (7.5 mg) i.g. 8 wkly	156 313 (AIN-76A)	–7 d to end	39 41	46 42	NR	NR	Ineffective Ineffective	Moon *et al.* (1994a)
Urinary bladder	BDF male mice, 4–5 wks	99	NBHBA (7.5 mg) i.g. 8 wkly	1.5 mmol/kg diet (Wayne Lab Chow)	0 to end	35	21*	NR	NR	Effective	Moon *et al.* (1982)
Colon	Fischer 344 male rats, 8 wks	25–33	AOM (15 mg/kg bw) Twice wkly, i.p.	391 782 (AIN-76A)	–1 to 36 wks	33	13* 12*	0.47 0.47	0.1* 0.08*	Effective Effective	Zheng *et al.* (1997)

MNU, *N*-methyl-*N*-nitrosourea; i.v., intravenously; d, days, DMBA, 7,12-dimethylbenz[*a*]anthracene; i.g., intragastrically; ENU, *N*-ethyl-*N*-nitrosourea; i.p., intraperitoneally; NR, not reported; TP, testosterone propionate; s.c., subcutaneously; NNK, 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NBHBA, *N*-nitroso-*N*-butyl-N-4-hydroxybutylamine; AOM, azoxymethane

[a] Adenomas and adenocarcinomas combined

[b] Adenocarcinomas only

* Statistically significant (see text); effective, either incidence or multiplicity

intravenous dose of MNU at 50 mg/kg bw consistently showed the effectiveness of 4-hydroxyphenylretinamide at a concentration of 2 mmol/kg diet (McCormick *et al.*, 1982a; McCormick & Moon, 1986; Moon *et al.*, 1989; Ratko *et al.*, 1989; Moon & Mehta, 1990).

Groups of 40 female Sprague-Dawley rats, 60 days of age, received semipurified AIN-76A diet or the diet supplemented with 4-hydroxyphenylretinamide at 2 mmol/kg ; 60 days later, they were given a single intravenous injection of MNU at 50 mg/kg bw. The animals were killed 180 days after the carcinogen treatment. The incidence of mammary adenocarcinomas was 68% in controls and 42% in those given 4-hydroxyphenylretinamide ($p < 0.05$ χ^2 test). The multiplicity of tumours was unaffected (Moon *et al.*, 1992).

Groups of 21–30 female Sprague-Dawley rats received MNU at 25 mg/kg bw into the tail vein at 50 days of age and were given NIH-07 basal diet or NIH-07 diet containing 782 mg/kg diet 4-hydroxyphenylretinamide beginning at 21, 48 or 60 days of age. The diet was continued until 12 weeks after the carcinogen treatment in all groups except in a group that received 4-hydroxyphenylretinamide from 52 weeks of age until 17 weeks after exposure to the carcinogen. The incidence of mammary adenocarcinomas 28 weeks after exposure to the carcinogen was statistically significant inhibited only in the group that received 4-hydroxyphenylretinamide two days before the injection of MNU, the incidence being reduced to 50% as compared with 86% in controls ($p < 0.05$; Wilcoxon test). The tumour multiplicity was reduced from 1.3 to 0.7 tumours per rat. In a similar experiment within the same study with 7,12 dimethylbenz[*a*]anthracene (DMBA) as the carcinogen, groups of 15 rats were treated intragastrically with a single dose of 10 mg in sesame oil at 50 days of age, and dietary treatment with 4-hydroxyphenylretinamide was begun 21 or 60 days afterwards. 4-Hydroxyphenylretinamide showed no inhibitory effect in this study (Silverman *et al.*, 1983).

Groups of 25 female Fischer 344 rats were given MNU intravenously at a dose of 45 mg/kg bw at 50 days of age. The groups then received either Wayne lab chow or NIH-07 or AIN-76A semipurified diets containing 4-hydroxyphenylretinamide at 2 mmol/kg diet starting either seven days before or seven days after the carcinogen until the end of the study 25 weeks after administration of MNU. The mammary carcinoma incidence was 60% in control rats maintained on AIN-76A diet, 24% in those on Wayne lab chow and 44% in those on NIH-07 diet. Treatment with 4-hydroxyphenylretinamide had a significant effect only in animals on NIH-07 diet supplemented seven days before carcinogen treatment, the incidence being decreased from 44% to 20% and the tumour multiplicity from 0.64 to 0.20 ($p < 0.05$: Student's *t* test) (Cohen *et al.*, 1994).

Groups of 30 female Sprague-Dawley rats received an intravenous injection of MNU at 50 mg/kg bw at 100 days of age. The animals received Wayne lab chow alone or supplemented with 4-hydroxyphenylretinamide at 2 mmol/kg diet starting either 60 days before the carcinogen treatment or before, during and after the carcinogen treatment. The animals were killed 180 days after injection of the carcinogen. The incidence of mammary adenocarcinomas was increased from 57% in controls to 66% in the group given 4-hydroxyphenylretinamide before the carcinogen, and the tumour multiplicity was increased from 1.4 to 2.7 (not significant). In animals given 4-hydroxyphenylretinamide for the duration of the study, the adenocarcinoma incidence was reduced from 57% in controls to 23% in treated rats ($p < 0.05$; Fisher exact test) and the tumour multiplicity was reduced from 1.4 to 0.3 tumours per rat ($p < 0.05$; Wilcoxon rank test; Grubbs *et al.*, 1990).

Groups of 25 female Sprague-Dawley rats, 50 days of age, were treated by oral gavage with a single dose of 20 mg DMBA; seven days later, they received Wayne lab chow alone or supplemented with 4-hydroxyphenylretinamide at 2 mmol/kg for 180 days. Mammary tumours (fibroadenomas and carcinomas combined) developed in 91% of controls and 85% of the 4-hydroxyphenylretina-mide-treated rats ($p< 0.05$; log rank analysis), and the tumour multiplicity was reduced from 5.4 to 3.7 ($p < 0.05$; Student's *t* test; McCormick *et al.*, 1982a).

Groups of 20 female Sprague-Dawley rats received a semipurified AIN-76A diet alone or supplemented with 4-hydroxyphenylretinamide at 0.75 or 1.5 mmol/kg of diet and 14 days later, when they were 50 days of age, were given a single dose of 75 mg DMBA by gavage. The incidence of mammary adenocarcinomas 18 weeks later was 70% in controls, 65% in rats given the diet con-

taining the low dose of 4-hydroxyphenylretinamide and 30% in rats given the high dose ($p < 0.05$; Student's *t* test). The tumour multiplicity was 1.0 in controls, 1.1 at the low dose of 4-hydroxy-phenylretinamide and 0.4 at the high dose (Abou-Issa *et al.*, 1988). Similar results were reported by Bollag and Hartman (1987), McCormick *et al.* (1982a) and Abou-Issa *et al.* (1993; see Table 2). [The Working Group noted that the statistical test used is not appropriate for the data analysed.]

4.2.1.2 Skin

Groups of 25 female Sencar mice, seven to eight weeks of age, received topical applications of 5 µg DMBA in acetone and, starting two weeks later, 4-hydroxyphenylretinamide either at 30 nmol in acetone topically twice a week or at 1 mmol/kg of diet for 30 weeks. DMBA by itself resulted in an incidence of skin papillomas of only 4%. Topical treatment of DMBA-initiated skin with 4-hydroxyphenylretinamide did not affect papilloma development, but dietary treatment increased the incidence of skin papillomas to 35%. The tumour multiplicity was increased from 0.1 per mouse in controls to 1.9 with 4-hydroxyphenylretinamide ($p < 0.01$; log rank analyses ; McCormick *et al.*, 1987).

4.2.1.3 Lymphoma

Groups of 30–40 male PIM transgenic mice, which are highly susceptible to the induction of T-cell lymphomas owing to overexpression of the *pim-1* oncogene, were given an intraperitoneal injection of *N*-ethyl-*N*-nitrosourea (ENU) at 50 mg/kg bw and immediately afterwards maintained on diets containing 4-hydroxyphenylretinamide at a concentration of 98, 196 or 391 mg/kg of diet. The lymphoma incidence was determined at 20 and at 35 weeks. The incidence 20 weeks after the carcinogen injection was 48% in controls and 20% at the low and high doses of 4-hydroxyphenylretinamide ($p < 0.05$; Fisher's exact test). At 35 weeks, the incidence of lymphomas was 53% in controls and 43, 52 and 40% at the low, intermediate and high doses of 4-hydroxyphenylretinamide, respectively (not significant). The rate of survival at 35 weeks was 53% in control mice and 78% in those at the high dose of 4-hydroxyphenylretinamide ($p < 0.01$; log rank analysis). The rate of survival was also increased at the low and intermediate dietary concentrations, but not statistically significantly so (McCormick *et al.*, 1996).

Groups of 10 female AKR/J mice, two to three weeks of age, were maintained on lab chow diet alone or supplemented with 4-hydroxyphenylretinamide at 391 or 782 mg/kg of diet and one week later received an injection of 50 µl of a solution containing 1 x 10^5 plaque-forming units per ml of mink cell focus-forming virus into the thymus area. The animals were killed 20 weeks after the virus injection. The incidence of lymphoma was 100% in controls, 60% at the low dose of 4-hydroxyphenylretinamide and 50% at the high dose ($p < 0.01$; Student's *t* test; Chan *et al.*, 1997). [The Working Group noted that the statistical analysis and the presentation of the results were difficult to interpret.]

4.2.1.4 Prostate

Groups of 20 male Lobund-Wistar rats, three months of age, were given a single intravenous injection of MNU at 30 mg/kg bw and then received a Silastic tube implant containing 45 mg of testosterone propionate, which was replaced every two months. Seven months after the MNU injection, 4-hydroxyphenylretinamide was added to the basal diet at 1 mmol/kg. The animals were killed 14 months after the carcinogen injection. The incidence of prostate tumours was 88% in the control rats and 21% in those given 4-hydroxyphenylretinamide ($p < 0.001$; statistical method not given; Pollard *et al.*, 1991).

Groups of 40 male WU (HsdCpb:WU) rats, seven to eight weeks of age, received intraperitoneal injections of 8 mg of cyproterone acetate daily for 20 days; one day later, they received a single subcutaneous injection of testosterone propionate at 100 mg/kg bw, and 60 h after the testosterone treatment, they were given an intravenous injection of MNU at 50 mg/kg bw. They then received subcutaneous implants of Silastic tubes filled with testosterone, which were replaced every 90 days. One day after the injection of MNU, one group received 4-hydroxyphenylretinamide at 1 mmol/kg diet (391 mg/kg) for the duration of the experiment, which was terminated 450 days after the carcinogen treatment. The incidence of prostate adenocarcinomas was 45% in controls and 59% with 4-hydroxyphenylretinamide, and the incidence of

accessory gland tumours was 67% in controls and 62% with 4-hydroxyphenylretinamide (McCormick *et al.*, 1998).

Groups of 44 ACI/segHapBR retired breeder rats, 21–25 months of age, received either basal diet or diet supplemented with 4-hydroxyphenylretinamide at 783 mg/kg diet for 54 weeks. The incidence of prostate tumours was 43% in control rats and 28% in those given 4-hydroxyphenylretinamide (not significant; Ohshima *et al.*, 1985).

4.2.1.5 Lung

Groups of 30 female A/J mice, five weeks old, were maintained on an AIN-76A diet alone or supplemented with 4-hydroxyphenylretinamide at 2 or 4 mmol/kg diet and one week later were given an intraperitoneal injection of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at 2 mg per mouse. At 52 weeks, when the animals were killed, the incidence of adenocarcinoma was 73% in control mice, 50% in the group given the high dose of 4-hydroxyphenylretinamide and 57% in the group given the low dose (not significant); the incidence of adenoma was 27%, 43% and 43% and the tumour multiplicity was 17, 15 and 19 in the three groups, respectively (not significant). The body weights were reduced by > 30% in the group given 4-hydroxyphenylretinamide at 4 mmol/kg of diet (Conaway *et al.*, 1998). [The Working Group noted that the marked weight loss could have affected the tumour incidence.]

4.2.1.6 Urinary bladder

Groups of 99 male C57BL/6 x DBA/2F$_1$ (BDF) mice, five to six weeks of age, were given one intragastric instillation of 7.5 mg of *N*-nitroso-*N*-butyl-*N*-4-hydroxybutylamine (NBHBA) each week for eight weeks. The animals were fed either basal diet or diet supplemented with 4-hydroxyphenylretinamide at 1.5 mmol/kg of diet for seven months after the first dose of NBHBA. The incidence of bladder carcinomas was 35% in control mice and 21% in those given 4-hydroxyphenylretinamide ($p < 0.05$; χ^2 test) (Moon *et al.*, 1982).

Groups of 70 male BDF mice, five to seven weeks of age, were maintained on an AIN-76A diet alone or supplemented with 4-hydroxyphenylretinamide at 156 or 313 mg/kg and one week later were given 7.5 mg of NBHBA by intragastric intubation each week for eight weeks. At 26 weeks, when the animals were killed, the incidence of bladder carcinomas was 39% and 41% in the two control groups, 46% at the low dose of 4-hydroxyphenylretinamide and 42% at the high dose (not significant; Moon *et al.*, 1994a).

4.2.1.7 Colon

Groups of 25–33 male Fischer 344 rats, seven to eight weeks of age, were maintained on a AIN-76A diet alone or supplemented with 4-hydroxyphenylretinamide at 391 or 782 mg/kg diet and one week later received intraperitoneal injections of azoxymethane at 15 mg/kg bw weekly for two weeks. At 36 weeks after the carcinogen treatment, the incidence of colon adenocarcinoma was 33% in controls, 13% at the low dose of 4-hydroxyphenylretinamide and 12% at the high dose ($p < 0.05$; ANOVA followed by Tukey test). The multiplicity of adenocarcinomas was 0.47 in control rats and 0.1 at the low dose and 0.08 at the high dose of 4-hydroxyphenylretinamide ($p < 0.01$; ANOVA; Zheng *et al.*, 1997).

4.2.1.8 Combinations of 4-hydroxyphenylretinamide with other putative preventive agents

4-Hydroxyphenylretinamide has been evaluated for tumour-preventive activity in combination with other agents in models of experimental carcinogenesis (Moon *et al.*, 1994b). For example, combinations with tamoxifen (Ratko *et al.*, 1989), ovariectomy (McCormick *et al.*, 1982a) or calcium glucarate (Abou-Issa *et al.*, 1993) were more effective than the single agents alone.

4.2.2 Intermediate biomarkers

In two models of liver carcinogenesis in rats, with *N*-nitrosodiethylamine or a choline-deficient diet, 4-hydroxyphenylretinamide significantly decreased the size and number of glutathione *S*-transferase placental-form-positive foci, considered to be preneoplastic lesions, and the concentrations of 8-hydroxyguanine in the liver, an indicator of oxidative damage (Tamura *et al.*, 1997).

4.2.3 In-vitro models

4.2.3.1 Inhibition of cell transformation

Several studies have shown that 4-hydroxyphenylretinamide can act as a preventive agent at early stages of the carcinogenesis process. It was highly active in suppressing 3-methylcholanthrene-

induced neoplastic transformation in the C3H/10T1/2 clone 8 mouse fibroblast line (Bertram, 1980), and it suppressed the transformation of rat tracheal epithelial cells induced by benzo[*a*]pyrene (Steele *et al.*, 1990). In a model of mammary carcinogenesis *in vitro* in cultures of the whole mammary organ from female BALB/c mice, *N*-nitrosodiethylamine induced transformation of mammary cells, resulting in nodule-like alveolar lesions, which are analogous to the precancerous hyperplastic alveolar nodules seen in mouse mammary gland *in vivo*. When 4-hydroxyphenylretinamide was added to the organ culture medium six days after the carcinogen, it suppressed the incidence of nodule-like alveolar lesions by 61%. The authors concluded that 4-hydroxyphenylretinamide can inhibit expression of the transformed phenotype at the promotional level (Chatterjee & Banerjee , 1982).

4.2.3.2 Inhibition of cell proliferation

4-Hydroxyphenylretinamide at concentrations below 1 µmol/L rarely affected the growth of cells of various types, whereas growth was inhibited at that dose. DNA synthesis was decreased by 51% in asynchronously growing PC3 prostate carcinoma cells or cells synchronized with serum deprivation when treated with 4-hydroxyphenylretinamide at 1 µmol/L. The reduction in proliferation rate was associated with accumulation of cells in the G_0/G_1 phase of the cell cycle (Igawa *et al.*, 1994; Roberson *et al.*, 1997). The inhibition is associated with suppression of c-*myc* gene expression (Igawa *et al.*, 1994). In other prostate carcinoma cells, however, 4-hydroxyphenylretinamide did not cause accumulation in G_1 (Sun *et al.*, 1999a). Similarly, 4-hydroxyphenylretinamide inhibited the growth of oesophageal squamous carcinoma cell lines without inducing arrest of cell growth in the G_1 phase nor apoptosis, but it caused down-regulation of epidermal growth factor receptors (Muller *et al.*, 1997).

4.2.3.3 Induction of cellular differentiation

Many retinoids are active in several models of cellular differentiation such as murine EC F9 cells, human HL-60 cells and neuroblastoma cells, but 4-hydroxyphenylretinamide was ineffective in most of these systems. No differentiation was induced in HL-60 cells (Delia *et al.*, 1993) or neuroblastoma cells (Ponzoni *et al.*, 1995), but 4-hydroxyphenylretinamide induced primitive endodermal differentiation when added at a concentration of 1 µmol/L, a suboptimal concentration for induction of apoptosis (Clifford *et al.*, 1999).

4.2.3.4 Induction of apoptosis

Numerous studies over the past six years have highlighted induction of apoptosis as a major effect of 4-hydroxyphenylretinamide on tumour cells *in vitro*. This retinoid induced apoptosis in malignant haematopoietic (Delia *et al.*, 1993), neuroblastoma (Di Vinci, 1994; Mariotti *et al.*, 1994; Ponzoni *et al.*, 1995), cervical (Oridate *et al.*, 1995), breast (Swisshelm *et al.*, 1994; Sheikh *et al.*, 1994; Pellegrini *et al.*, 1995; Sheikh *et al.*, 1995), ovarian (Supino *et al.*, 1996; Sabichi *et al.*, 1998), head-and-neck (Oridate *et al.*, 1996; Sun *et al.*, 1999b), small-cell lung (Kalemkerian *et al.*, 1995), non-small-cell lung (Zou *et al.*, 1998; Sun *et al.*, 1999b) and prostate (Igawa *et al.*, 1994; Hsieh & Wu, 1997; Roberson *et al.*, 1997; Sun *et al.*, 1999a) cancer cell lines. It is noteworthy that many of the cell lines that are sensitive to 4-hydroxyphenylretinamide were resistant to doses of all-*trans*-retinoic acid or 9-*cis*-retinoic acid up to 10 µmol/L.

The induction of apoptosis often requires doses of 4-hydroxyphenylretinamide > 1 µmol/L and even as much as 10 µmol/L (Lotan, 1995; Supino *et al.*, 1996). [The Working Group noted that because the reported plasma concentration of 4-hydroxyphenylretinamide in patients treated with 200 mg/day is 1 µmol/L or less, the concentration in the target tissues may not be sufficient to induce apoptosis.]

4.2.3.5 Antimutagenicity in short-term tests

Little information is available on the effect of 4-hydroxyphenylretinamide on carcinogen-induced genotoxicity (Table 3). In a single study, it did not affect the ability of DMBA to induce sister chromatid exchange in mammary gland organ cultures during the 24-h exposure (Manoharan & Banerjee, 1985). [The Working Group noted that this study was limited to a single dose of both carcinogen and retinoid and the main focus of the study was on the effect of β-carotene and not 4-hydroxyphenylretinamide.] A second study showed a protective effect of 4-hydroxyphenylretinamide against the induction by bleomycin, which generates free radicals, of chromosomal breakage in two

Table 3. Effects of 4-hydroxyphenylretinamide on genetic and related effects *in vitro* and *in vivo*

Dose of retinoid	Genotoxic agent (dose)	Cells or animal	Investigated effect	Result[a]	LED/HID[b]	Reference
1 µmol/L	DMBA (7–8 µmol/L)	Mouse mammary cells	Sister chromatid exchange	–	1 µmol/L	Manorharan & Banerjee (1985)
1 pmol/L – 1 µmol/L (preincubation for 24 h)	Bleomycin (0.004 U/ml)	Two human lymphoblastoid cell lines	Chromosomal breakage	–	1 µmol/L	Trizna *et al.* (1993)
40 µmol/L	None	Rat hepatocytes	Cytochrome P450 (CYP) RNA levels			Jurima-Romet *et al.* (1997)
			CYP1A1	–	40 µmol/L	
			CYP1A2	–	40 µmol/L	
			CYP3a1/2	#	40 µmol/L	
800 mg/kg bw per day by gavage for 4 days	None	Male Sprague-Dawley rats	Hepatic levels of activity		800 mg/kg bw per day for 4 days	McCarthy *et al.* (1987)
			Arylhydrocarbon hydroxylase	+		
			Glutathione *S*-transferase	#		
			Quinone reductase	–		
600 mg/kg bw per day by gavage for 7 days	Benzo[*a*]pyrene (2 mg/kg bw i.p. on 8th day)	Male Sprague-Dawley rats	Covalent binding to DNA *in vivo*	+	600 mg/kg bw per day for 7 days (ID_{40} for liver, ID_{21} for stomach, ID_{11} for lung. No effect on kidney)	McCarthy *et al.* (1987)

DMBA, 7,12-dimethylbenz[*a*]anthracene; i.p., intaperitoneally; inhibitory dose

[a] +, inhibition of the investigated end-point; – no effect on investigated end-point; #, enhancement of investigated end-point

[b] LED, lowest effective dose that inhibits the investigated effect; HID, highest ineffective dose

human lymphoblastoid cell lines. In this study, the cells were preincubated with the retinoid for 24 h before addition of bleomycin (Trizna *et al.*, 1993).

Although there is no information on the effect of this retinoid on carcinogen metabolism *in vitro*, it may affect the activity of some metabolic enzymes. In primary rat hepatocyte cultures treated for 48 h with 4-hydroxyphenylretinamide, it affected the messenger RNA levels of cytochrome P450s (CYPs), the effects depending on the cytochrome being studied. The level of CYP3A RNA was increased approximately eightfold by the treatment ($p < 0.05$), whereas those of CYP1A1 and CYP1A2 were not affected (Jurima-Romet *et al.*, 1997). 4-Hydroxyphenylretinamide may also affect the enzyme activity *in vivo* (Table 3).

4.3 Mechanisms of cancer prevention

4.3.1 Inhibition of early stages

Several reports described above indicate that 4-hydroxyphenylretinamide can alter the activity of carcinogen-metabolizing enzymes, the level of expression of certain CYPs and the binding of benzo[*a*]pyrene to DNA in several tissues. Thus, it

may act at the very early stages of carcinogenesis. It inhibited transformation when added after a carcinogen at concentrations that did not inhibit proliferation, indicating activity after the initiation phase of carcinogenesis.

4.3.2 Inhibition of cell proliferation

4.3.2.1 Modulation of proteins regulating cell cycles

4-Hydroxyphenylretinamide down-regulates cyclin D1, $p34^{cdc2}$ and cdk4 expression and Rb phosphorylation and increases ceramide synthesis in HL-60 cells (DiPietrantonio *et al.*, 1996, 1998). It also down-regulated proliferating cell nuclear antigen, cyclins D and E, $p34^{cdc2}$, p53 and Rb in the androgen-independent prostate cancer cell line JCA-1 (Hsieh *et al.*, 1995). Up-regulation of the Rb protein was observed in the breast cancer cell lines MCF-7 and T-47D (Kazmi *et al.*, 1996), and down-regulation of c-*myc* was demonstrated in PC3 prostate cancer cells (Igawa *et al.*, 1994). Increased expression of the cdk inhibitor p21/WAF1/Cip1 was observed in several prostate cancer cell lines (Sun *et al.*, 1999a).

4.3.2.2 Modulation of autocrine and paracrine loops

Several studies have shown that 4-hydroxyphenylretinamide can modulate components of growth factor and receptor signalling pathways that enhance or suppress growth stimulatory signals. Treatment of oesophageal carcinoma cells resulted in down-regulation of the c-erb-B1 epidermal growth factor receptor (Muller *et al.*, 1997), and similar results were obtained with breast cancer cells (Pellegrini *et al.*, 1995). Down-regulation of *c-erb-B2* (*HER-2/neu*) mRNA and protein in breast carcinoma cells has also been described (Pellegrini *et al.*, 1995; Grunt *et al.*, 1998). Other families of factor and receptor systems were also modulated. Insulin-like growth factor (IGF) signalling was abrogated in several cancer cell types as a result of down-regulation of IGF-I-like protein in the medium, a reduction in IGF binding proteins 4 and 5, a decrease in type-I IGF receptor mRNA and IGF-I binding to breast cancer cells (Favoni *et al.*, 1998). Up-regulation of mac25, a putative member of the tumour suppressing IGF-BP family, in senescing mammary epithelial cells has also been reported (Swisshelm *et al.*, 1995). In contrast, 4-hydroxyphenylretinamide increased the activity of the negative growth regulator TGFβ1 and TGFβ receptor type II in prostate carcinoma cells (Roberson *et al.*, 1997).

4-Hydroxyphenylretinamide decreased the expression of androgen receptors in an androgen-dependent prostate carcinoma cell line, LNCaP (Hsieh & Wu, 1997).

4.3.3 Restoration of normal differentiation

4-Hydroxyphenylretinamide rarely induces differentiation of cancer cells, perhaps because it induces apoptosis within a relatively short time (see below). For example, in the neuroblastoma cell line (Ponzoni *et al.*, 1995) and in HL-60 cells, a classical retinoid-differentiated cell line, no differentiation was observed after treatment with 4-hydroxyphenylretinamide. This retinoid potentiated differentiation of HL-60 cells induced by all-*trans*-retinoic acid by suppressing the catabolism and enhancing retinoylation of the latter compound (Takahashi *et al.*, 1995; Taimi & Breitman, 1997).

The differentiation into primitive endoderm seen with all-*trans*-retinoic acid has, however, been detected in murine F9 EC cells treated with 4-hydroxyphenylretinamide at 1 μmol/L, a suboptimal concentration for induction of apoptosis (Clifford *et al.*, 1999). Therefore, some differentiation may occur *in vivo*, where the plasma concentration is at about this value.

4.3.4 Inhibition of prostaglandin production

4-Hydroxyphenylretinamide inhibited tumour promoter-induced cyclooxygenase-2 expression in human colon adenocarcinoma cells (Aliprandis *et al.*, 1997).

4.3.5 Induction of apoptosis

Since 4-hydroxyphenylretinamide induces apoptosis in a large number of tumour cell types, including those that are resistant to all-*trans*-retinoic acid (see above), it is reasonable to suggest that it acts through a mechanism that is either independent of retinoid receptors or includes, in addition to receptor activation, some other effects that trigger apoptosis.

4.3.6 Decreased cell adhesion

4-Hydroxyphenylretinamide abrogated neuroblastoma cell adhesion by down-modulation of integrin receptors such as integrin β 1, which may

have resulted in detachment from the matrix and triggered programmed cell death (Rozzo *et al.*, 1997).

4.3.7 Molecular mechanisms

4.3.7.1 Retinoid receptor pathway

The biological activities of the natural retinoids are thought to be mediated by two classes of nuclear retinoid receptor: the RARs and the RXRs (see General remarks, section 3). There is no convincing evidence that 4-hydroxyphenylretinamide can bind to these receptors, but it was reported to compete with radiolabelled all-*trans*-retinoic acid for binding to a crude nuclear extract with 15% of the potency of unlabelled all-*trans*-retinoic acid (Sani *et al.*, 1995), and weak binding to retinoid receptors has been reported (Sheikh *et al.*, 1995). Doses of 4-hydroxyphenylretinamide > 1 mol/L were required for 50% inhibition of the binding of ^{3}H-4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoic acid to recombinant RARs (Sheikh *et al.*, 1995). Nonetheless, 4-hydroxyphenylretinamide activated the transcription of RARβ, which is a classical retinoid-regulated gene with a DR5 retinoic acid response element in its 5′ flanking region. 4-Hydroxyphenylretinamide activated the transcription of a reporter gene driven by RARβ RARE through co-transfected RARγ better than through RARβ and RARα (Fanjul *et al.*, 1996; Kazmi *et al.*, 1996). In addition, it activated a reporter gene via endogenous retinoid receptors (Sun *et al.*, 1999b) and induced the expression of RARβ in senescing mammary epithelial cells *in vitro* (Swisshelm *et al.*, 1995). These findings are a clear indication that, in some cells, 4-hydroxyphenylretinamide can activate RARs and some of their target genes. The status of RARβ may affect the response, as indicated by several reports of the greater sensitivity to 4-hydroxyphenylretinamide-induced apoptosis of ovarian cancer cells expressing transfected RARβ (Sabichi *et al.*, 1998; Pergolizzi *et al.*, 1999). In LNCaP prostate carcinoma cells, however, this retinoid suppressed RARβ mRNA levels yet induced apoptosis (Sun *et al.*, 1999a), and it does not induce apoptosis in some cells in which all-*trans*-retinoic acid induces RARβ (Zou *et al.*, 1998). Therefore, the presence of RARβ may not be necessary for induction of apoptosis by 4-hydroxyphenylretinamide.

4.3.7.2 Increased generation of reactive oxygen species

4-Hydroxyphenylretinamide can increase the generation of reactive oxygen species immediately after its addition to cultured leukaemic cells (Delia *et al.*, 1997) and cervical carcinoma cells (Oridate *et al.*, 1997). This increase is important for induction of apoptosis, since antioxidants can block apoptosis in these cell lines (Delia *et al.*, 1993, 1997; Oridate *et al.*, 1997; Sun *et al.*, 1999a). Since the concentrations of 4-hydroxyphenylretinamide that are required to induce apoptosis are rather high, it is difficult to block its effect with receptor antagonists at a high molar ratio. When such experiments were performed, however, no effective antagonism was observed (Sun *et al.*, 1999a). In five cell lines from head-and-neck tumours, five from lung cancers and three from prostate cancers, 4-hydroxyphenylretinamide induced generation of reactive oxygen species in only the three that were somewhat more sensitive (Sun *et al.*, 1999a,b). Thus, an additional, unknown mechanism besides RAR activation and induction of reactive oxygen species may mediate 4-hydroxyphenylretinamide-induced apoptosis.

5. Other Beneficial Effects

No data were available to the Working Group.

6. Carcinogenicity

6.1 Humans

No data were available to the Working Group.

6.2 Experimental models

No data were available to the Working Group. In a study of single-stage skin carcinogenesis, 4-hydroxyphenylretinamide enhanced development of DMBA-induced papillomas (see section 4.2.1).

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

Unlike many other retinoids, 4-hydroxyphenylretinamide is usually well tolerated, and its clinical toxicity is considered to be 'mild and reversible' (Modiano *et al.*, 1990a). Intervention trials of 4-hydroxyphenylretinamide, including its clinical

toxicology, have been reviewed (Cobleigh, 1994; Costa *et al.*, 1994; De Palo *et al.*, 1995b; Veronesi *et al.*, 1996).

7.1.1.1 Ocular toxicity

In the earliest therapeutic trials, 4-hydroxyphenylretinamide was generally well tolerated, although some patients discontinued treatment with the drug due to night blindness and other side-effects, including increased concentrations of triglycerides and mucocutaneous complaints (Garewal *et al.*, 1989; Modiano *et al.*, 1989). The adverse events resolved upon cessation of treatment and were presumably related to the high doses administered (up to 800 mg/day) (Kaiser-Kupfer *et al.*, 1986). The frequent occurrence of visual abnormalities in these early trials led to the design of new dosing regimens and treatment intervals.

In 37 patients with advanced cancers who were treated with 300–400 mg/day of 4-hydroxyphenylretinamide for 13–300 days, 10% reported decreased night vision. One patient had electroretinogram changes, with a significant decrease in the amplitude for scotopic (dark-adapted or rod-mediated) vision after one month of treatment. All of the cases of night blindness resolved when treatment was discontinued (Modiano *et al.*, 1990b).

4-Hydroxyphenylretinamide was administered in a phase-I study to 100 surgically treated breast cancer patients at doses of 100, 200 and 300 mg/day for an initial six months and then at 200 mg per day for six months thereafter. One of the patients receiving 300 mg daily experienced impaired night vision after six months of treatment (Costa *et al.*, 1989).

Night blindness after administration of 4-hydroxyphenylretinamide is a consequence of interference with the formation of the retinol-binding protein and transthyretin complex (Berni & Formelli, 1992) and reductions in circulating retinol and retinol-binding protein. These effects can occur within hours after the first dose (Formelli *et al.*, 1989). The consequence is a reduction in retinal photoreceptor sensitivity (Decensi *et al.*, 1997b) and delay in rod–cone timing, which leads to an increased absolute luminance threshold (Caruso *et al.*, 1998). Of 14 men given 4-hydroxyphenylretinamide at 100 mg/day for up to one year, 13 had normal age-matched electroretinographic responses for the first six months; two subjects showed a gradual decline in rod-mediated a-wave amplitude, which returned to normal after treatment had ceased (Krzeminski *et al.*, 1996).

In order to overcome these effects, a three-day drug 'holiday' per month was instituted (Rotmensz *et al.*, 1991), to allow recovery of serum retinol concentrations and preservation of the ability to adapt to darkness (Formelli *et al.*, 1987). When this interrupted dosing regimen was used in an Italian phase-I trial extended to 30 months, the incidence of ophthalmic disturbances was 4% (Decensi *et al.*, 1997b). Although daily administration of 200 mg of 4-hydroxyphenylretinamide reduced the plasma concentrations of retinol and retinol-binding protein in all treated patients by an average of 71% 24 h after each dose, the plasma concentrations increased in all patients after the three-day interruption, some returning to baseline concentrations (Formelli *et al.*, 1993).

In a clinical trial for the prevention of bladder cancer, the main toxic effects were decreased dark adaptation and abnormal electroretinograms. The dose regimen used — 200 mg/day with a three-day holiday per month for two years — did produce night blindness but this was considered not to be severe enough to reduce or end treatment. Night blindness was reported in approximately 20% of the subjects assigned to 4-hydroxyphenylretinamide and 2% of the untreated control group in both years of the study. After the end of treatment, all of the side-effects disappeared (Decensi *et al.*, 1997c).

In a phase-III clinical trial for the prevention of breast cancer, 1432 evaluable patients were treated daily with 200 mg of 4-hydroxyphenylretinamide with a three-day drug holiday each month. Mild and moderately diminished dark adaptation occurred at plasma retinol concentrations of 160 and 100 ng/ml, respectively, but only half of the subjects reported symptoms (Decensi *et al.*, 1993, 1994b). A constant 65% reduction in mean retinol concentration was seen during the five years of treatment (Formelli *et al.*, 1993; Veronesi *et al.*, 1996). In more complete reviews of the long-term effects of 4-hydroxyphenylretinamide on visual and retinal function (Mariani *et al.*, 1996; Decensi *et al.*, 1997b), the cumulative incidence of visual complaints, including loss of dark adaptation, was reported to have reached nearly 20% at five years,

with more frequent occurrence at the start of treatment. Multivariate analysis of these data suggested an interaction between the age of the patients and the duration of treatment in predicting an impaired electroretinogram response resulting from treatment with 4-hydroxyphenylretinamide. The most recent review of ocular toxicity in another trial of breast cancer patients indicates that the incidence of complaints of night blindness in patients treated with 4-hydroxyphenylretinamide at 200 mg/day for four months is not cumulative, is proportional to dose and returns to normal after drug withdrawal (Caruso *et al.*, 1998).

The evaluation of 4-hydroxyphenylretinamide in the prevention of cancers at several other sites has also been associated with reduced retinol concentrations with and without ocular toxicity. In one study, 4-hydroxyphenylretinamide was applied topically at 100 mg twice daily for three months to patients with facial actinic keratoses. It was not absorbed into the circulation when given by this route (Moglia *et al.*, 1996). In another study, reported only as an abstract, oral treatment with 4-hydroxyphenylretinamide at 200, 300 or 400 mg/day was continued for three months in patients with more than 15 actinic keratoses, with either a two-day/week or a three-day/month drug holiday. Reversible symptomatic night blindness developed in two patients on 400 mg/day, and one patient at 200 mg/day had asymptomatic electroretinogram abnormalities (Sridhara *et al.*, 1997).

7.1.1.2 Other ophthalmic effects

Other ocular effects reported in chemoprevention trials with 4-hydroxyphenylretinamide are more reminiscent of the standard mucocutaneous toxicity seen with other synthetic retinoids. In one study, the incidence of visual disturbances was compared with that of other ophthalmic signs, which included ocular dryness, lachrymation, conjunctivitis and photophobia. The cumulative incidence of these complaints was 8% at five years, and they were not associated with a reduction in plasma retinol concentration as in the effect on dark adaptation but rather with the age of the patient. This suggests that the underlying mechanism is different for the direct effect on retinal function by retinol depletion and the effects on the conjunctival and lachrymal apparatus (Mariani *et al.*, 1996).

7.1.1.3 Dermatological effects

Cutaneous toxicity has been seen in clinical trials less frequently with 4-hydroxyphenylretinamide than with other synthetic retinoids, although patients in the early trials with high doses often discontinued the drug because of erythema and rash. A widespread, painful morbilliform skin eruption was reported in a patient with basal-cell carcinoma being treated with 800 mg/day of this retinoid (Gross *et al.*, 1991), and similar cutaneous side-effects were noted with 600 mg/day in the treatment of psoriasis (Kingston *et al.*, 1986).

In the Italian trial of 4-hydroxyphenylretinamide at 200 mg/day for breast cancer, dermatological complaints were the commonest symptoms reported and included pruritis, skin dryness and cheilitis. Two patients out of 53 experienced peeling of the palms and soles, and six patients reported alopecia, five had nail fragmentation, two had xerosis and one each had pruritis and urticaria (Rotmensz *et al.*, 1991).

4-Hydroxyphenylretinamide has been used in two trials for the treatment of oral leukoplakia. Topical application at 100 mg/day to patients with oral leukoplakia and lichen planus produced no local mucocutaneous side-effects (Tradati *et al.*, 1994). In 115 patients who had undergone laser resection of leukoplakic lesions and had been prescribed oral maintenance therapy with 4-hydroxyphenylretinamide at 200 mg/day or placebo for one year with a three-day drug holiday per month, the toxicity was mild with no evidence of night blindness. Twenty of the 39 patients taking the drug finished the year without interruption, four required dose reduction or interruption of treatment due to dermal toxicity including skin dryness and dermatitis, and one patient refused to continue the study because of dermatitis (Chiesa *et al.*, 1992).

7.1.1.4 Metabolic and biochemical effects

Clinical treatment with retinoids has been associated with hepatotoxicity and hyperlipoproteinaemia. Seven of 101 patients who had undergone surgery for breast cancer and were treated with 4-hydroxyphenylretinamide at 100–300 mg/day for six months had liver enzyme activities that were two to four times above baseline, but no serious permanent liver toxicity (Costa *et al.*, 1989).

In a phase-II trial for bladder cancer, not only ophthalmic disturbances but also increased plasma triglyceride concentrations and minor dermatological complaints were recorded. No significant difference in biochemical toxicity was observed between treated and untreated patients during the first two years of treatment, but a statistically significantly higher incidence of grade-1 hypertriglyceridaemia was observed in patients receiving 4-hydroxyphenylretinamide at completion of follow-up in the third year. This study suggests a possible relationship between treatment and delayed low-grade hypertriglyceridaemia (Decensi *et al.*, 1997c).

In an initial trial of 4-hydroxyphenylretinamide at 200 mg/day in the treatment of oral leukoplakia, three of 39 patients had to have dose reductions or interruption of treatment because of increased plasma concentrations of triglycerides or bilirubin, and two patients were dropped from the study because they had elevated concentrations of triglycerides or abnormal results in tests for liver function. The relationship of these abnormal findings in clinical chemistry to treatment was uncertain because several of these patients had other intercurrent diseases (Chiesa *et al.*, 1992).

7.1.1.5 Other toxic effects

In studies of short-term treatment with 4-hydroxyphenylretinamide in the interval between diagnosis and radical prostatectomy in patients with prostate carcinoma, the serum retinol concentrations were lower (1400 nmol/L) in the treated group than in those given placebo (2600 nmol/L), as in other clinical trials (Thaller *et al.*, 1996).

In eight patients with inoperable oral lichen planus or leukoplakia, topical application of 4-hydroxyphenylretinamide at 200 mg/day twice daily for one month did not elicit any sign of local or systemic toxicity (Tradati *et al.*, 1994). In 20 patients with actinic keratoses given 4-hydroxyphenylretinamide at 200 mg/day topically, twice daily for three months, no local or systemic toxicity was observed. Two of the 20 subjects refused to complete the study for cosmetic reasons, as 4-hydroxyphenylretinamide caused yellow blotches on their faces. After twice daily topical applications to the lesions on the face, the circulating concentrations of 4-hydroxyphenylretinamide were below the analytical limit of detection (Moglia *et al.*, 1996).

7.1.2 Experimental models

The Working Group was aware of studies of the short-term and long-term toxicity and carcinogenicity of 4-hydroxyphenylretinamide in rats, conducted by the pharmaceutical company that produces this drug. The results have not been published.

As part of a study of chemopreventive efficacy, 4-hydroxyphenylretinamide did not reduce body-weight gain in rats fed the compound at 2 mmol/kg of diet (0.1 mmol/kg bw per day) for six months. The livers of these rats were essentially normal, and there was no effect on total liver retinoid concentration. The oestrus cycles after four months were normal (Moon *et al.*, 1979). The lack of an effect of dietary 4-hydroxyphenylretinamide on final body weight was confirmed in another study, in which, however, the serum triglyceride and cholesterol concentrations were increased by 760% ($p < 0.01$) and 114% ($p < 0.01$), respectively, after 22 weeks. The liver weights were also significantly increased, although the concentrations of triglyceride and cholesterol in the liver were not affected (Radcliffe, 1983).

7.2 Reproductive and developmental effects

7.2.1 Humans

No reports of adverse effects on male or female reproductive function or of developmental toxicity were found in the open literature. The parent drug and its 4-methoxyphenyl metabolite cross the human placenta. After 20–27 months of treatment at 200 mg/day orally, 4-hydroxyphenylretinamide and *N*-(4-methoxyphenyl)retinamide were measured in the plasma, placenta and embryos of two women who conceived while receiving the drug and elected to abort. The plasma concentrations of 4-hydroxyphenylretinamide (5–26 ng/ml) and *N*-(4-methoxyphenyl)retinamide (49–87 ng/ml) reflected the 10–60-day interruptions of dosing in these two cases. In one patient who had had nearly two months' interruption, the concentrations of the two compounds in the placenta and embryo were at or near the limit of analytical detection (15 ng/g), whereas the concentrations in the placenta and embryo (25 and 75 ng/g, respectively)

Table 4. Teratogenic effects of 4-hydroxyphenylretinamide

Species	Dose (mg/kg bw)	Effects	Reference
Rat	300 or 600	Resorptions; cardiac vessel defects	Turton *et al.* (1992)
Rat	125 or 800 GD 6–15	Hydrocephaly, microphthalmia	Kenel *et al.* (1988)
Rabbit	125 or 800 GD 6–18	Dome-shaped head, delay in skull bone ossification, microphthalmia	Kenel *et al.* (1988)

GD, gestation day

of the second case were nearly identical to those in maternal plasma. The embryos were not autopsied (Formelli *et al.*, 1998).

7.2.2 Experimental models

4-Hydroxyphenylretinamide has low teratogenic potency in rats and rabbits (Table 4), indicating that an acidic terminal group is necessary for the exertion of strong teratogenicity, probably via binding to retinoid receptors. In rats, 4-hydroxyphenylretinamide crosses the placenta readily, and the fetal concentrations are approximately one half of corresponding maternal values (Kenel *et al.*, 1988; Table 4).

7.3 Genetic and related effects

7.3.1 Humans

No data were available to the Working Group.

7.3.2 Experimental models

4-Hydroxyphenylretinamide was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537 or TA1538, either in the presence or absence of exogenous metabolic activation over a wide range of doses (0.5–400 mg/plate; Paulson *et al.*, 1985; Table 5), nor did it alter the mutation frequency at the *Tk* locus in L5178Y mouse lymphoma cells, either in the presence or absence of exogenous metabolic activation from rat liver (Paulson *et al.*, 1985). No increase in sister chromatid exchange frequency was found in mouse mammary cells in organ culture exposed to 4-hydroxyphenylretinamide, although the study was limited to a single dose of 1 mmol/L in the absence of exogenous activation (Manoharan & Banerjee, 1985).

4-Hydroxyphenylretinamide did not affect the chromosomal structure or number in bone-marrow cells of Crl:COBS(WI)BR rats receiving a single oral dose of 7000 mg/kg bw followed by sampling at 4, 16 and 24 h. Nor was any effect observed when rats were treated with 50, 200 or 800 mg/kg bw day for five days (Paulson *et al.*, 1985).

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

4-Hydroxyphenylretinamide is a synthetic compound formed from all-*trans*-retinoic acid and 4-aminophenol. Because it contains retinoic acid, it is sensitive to light, heat and oxygen. As it is lipophilic, it is freely soluble in oils and non-polar solvents but poorly soluble in water. It has characteristic absorption spectra in the ultraviolet and visible, infrared and resonance Raman portions of the electromagnetic spectrum.

4-Hydroxyphenylretinamide and its methoxy metabolite are found in plasma after administration of the parent compound. 4-Hydroxyphenylretinamide is used mainly at a daily dose of 200 mg (0.51 mmol) in the treatment of several types of malignant and premalignant lesions.

4-Hydroxyphenylretinamide, like other retinoids, is analysed mainly by high-performance liquid chromatography and is quantified on the basis of its absorbance at 362 nm.

Table 5. Genetic and related effects of 4-hydroxyphenylretinamide in short-term tests *in vitro* and *in vivo*

Test system	Result[a] Without S9	With S9	HID[b]	Reference
Salmonella typhimurium TA100, reverse mutation	–	–	400 µg/plate	Paulson *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	–	–	400 µg/plate	Paulson *et al.* (1985)
Salmonella typhimurium TA1537, reverse mutation	–	–	400 µg/plate	Paulson *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	–	–	400 µg/plate	Paulson *et al.* (1985)
Salmonella typhimurium TA98, reverse mutation	–	–	400 µg/plate	Paulson *et al.* (1985)
Mouse mammary organ cultures, sister chromatid exchange	–	–	1 µmol/L	Manoharan & Banerjee (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	–	–	10 µg/ml (–S9) 150 µg/ml (+S9)	Paulson *et al.* (1985)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	0 0	– –	7000 mg/kg bw by gavage Single dose of 800 mg/kg bw for 5 days	Paulson *et al.* (1985)

[a] –, No effect on the investigated end-point; 0, not tested; S9, exogenous metabolic system

[b] HID, highest ineffective dose

8.2 Metabolism and kinetics

The main metabolites of 4-hydroxyphenylretinamide include all-*trans*-*N*-(4-methoxyphenyl)-retinamide, 4-hydroxyphenylretinamide-*O*-glucuronide and several other polar retinamides. 4-Hydroxyphenylretinamide given at clinically relevant doses is not detectably hydrolysed to all-*trans*-retinoic acid or other retinoic acid isomers. In women with breast cancer who have received 4-hydroxyphenylretinamide for five years, the drug is cleared from the plasma with a half-life of 24 h, which is much slower than the half-lives of all-*trans*- and 9-*cis*-retinoic acid. 4-Hydroxy-phenylretinamide accumulates in target tissues such as the breast. Studies in animal models suggests that it is both distributed and metabolized differently in mice and rats, and it is unclear whether either of these species can be used as a true model for humans.

8.3 Cancer-preventive effects

8.3.1 Humans

In a preliminary report of a large randomized trial of use of 4-hydroxyphenylretinamide, equivocal results were obtained with regard to the development of new contralateral tumours among women previously treated for early breast cancer. There were fewer new cancers among treated pre-menopausal women but more cancers among treated postmenopausal women. A

decrease in the risk for ovarian cancer was reported among all treated women in this trial.

Two studies, only one of which was randomized, of intermediate end-points suggested an effect of 4-hydroxyphenylretinamide against oral leukoplakia. A possible effect on ploidy in urinary bladder cells has also been described.

8.3.2 Experimental models

The chemopreventive efficacy of 4-hydroxyphenylretinamide has been evaluated in animal models of mammary gland, prostate, lung, skin, urinary bladder and colon carcinogenesis and lymphomagenesis. It was effective in reducing the tumour incidence or multiplicity in 11 of 12 studies of mammary carcinogenesis in mice or rats. The results depended on the experimental conditions, including the strain and age of the animals, their diet and the dose of both carcinogen and retinoid. It was effective in one study in a model of urinary bladder carcinogenesis in mice and ineffective in another, and it was effective in one study of prostate carcinogenesis but not in two others. It was ineffective in one study of lung carcinogenesis in mice. It was effective in one study of carcinogenesis of the colon and in two studies of lymphomagenesis in mice. In one study in mice, 4-hydroxyphenylretinamide was ineffective or enhanced skin tumour development.

Studies *in vitro* suggest that 4-hydroxyphenylretinamide can affect carcinogenesis at several levels: it inhibited the transformation of cultured cells and of tissue in organ culture; it inhibited the proliferation of a variety of tumour cell lines and, rarely, induced apoptosis. It induced differentiation only rarely.

There are insufficient data to conclude whether 4-hydroxyphenylretinamide can reduce the genotoxic effects of carcinogens *in vitro* or *in vivo*. Indications that it alters the metabolism of carcinogens and thus affects DNA damage are provided by a study showing alterations to cytochrome P450 mRNA levels in cell cultures exposed to the retinoid, and a study in which phase I and phase II enzymes were shown to be altered in the livers of animals fed this compound. The altered metabolism was associated *in vivo* with a reduction in the binding to tissue DNA of a carcinogen known to be metabolized by these enzymes.

8.3.3 Mechanisms of cancer prevention

Few reports indicate any activity of 4-hydroxyphenylretinamide at the initiation stage of carcinogenesis, and most suggest it acts on promotion. The mechanisms that may account for the cancer-preventive effects of this retinoid appear to be associated with its ability to inhibit cell proliferation by increasing the amount of a cyclin-dependent kinase inhibitor and to down-regulate cyclin D1 and enhancing apoptosis. Its limited effects on differentiation raise doubts as to whether this is a mechanism for cancer prevention. The high doses required to affect apoptosis raise questions about the relevance *in vivo* of the effects on apoptosis seen *in vitro*.

8.4 Other beneficial effects

4-Hydroxyphenylretinamide was not effective in the treatment of several skin disorders or of rheumatoid arthritis.

8.5 Carcinogenicity

8.5.1 Humans

No data were available to the Working Group.

8.5.2 Experimental models

No data were available to the Working Group.

8.6 Other toxic effects

8.6.1 Humans

4-Hydroxyphenylretinamide has been safely administered in chemoprevention trials at 200 mg per day for prolonged periods with no significant toxicity. Night blindness and hypertriglyceridaemia are the most common side-effects of treatment and may necessitate discontinuation of treatment in some patients.

No reports were available on the reproductive, developmental or genotoxic effects of 4-hydroxyphenylretinamide in humans.

8.6.2 Experimental models

In rats, long term administration of 4-hydroxyphenylretinamide increased the serum concentrations of triglycerides and cholesterol.

4-Hydroxyphenylretinamide is transferred extensively to the rat conceptus, but its embryotoxicity and teratogenic potency are much lower that those of all-*trans*-retinoic acid, probably

because it lacks a terminal acidic group. The few available studies show no genotoxic effects *in vitro* or *in vivo*.

9. Recommendations for Research

9.1 General recommendations for 4-hydroxyphenylretinamide and other retinoids

See section 9 of the Handbook on all-*trans*-retinoic acid.

9.2 Recommendations specific to 4-hydroxyphenylretinamide

1. Define the efficacy, extent and mechanism of action of 4-hydroxyphenylretinamide in the prevention of human breast and ovarian cancer.

2. Determine the mechanism of action of 4-hydroxyphenylretinamide in cancer chemoprevention.

10. Evaluation

10.1 Cancer-preventive activity

10.1.1 Humans

There is *inadequate evidence* that 4-hydroxyphenylretinamide has cancer-preventive activity in humans.

10.1.2 Experimental animals

There is *sufficient evidence* that 4-hydroxyphenylretinamide has cancer-preventive activity in experimental animals. This evaluation is based on the observation of inhibitory effects in models of mammary carcinogenesis in mice and rats and its effectiveness in a limited number of studies against prostate and colon carcinogenesis and lymphomagenesis.

10.2 Overall evaluation

There is inadequate evidence that 4-hydroxyphenylretinamide has cancer-preventive activity in humans, but there is sufficient evidence that 4-hydroxyphenylretinamide has cancer-preventive activity in experimental animals, supported by data from cellular systems *in vitro* and information on the mechanisms of the cancer-preventive effects. 4-Hydroxyphenylretinamide does not have significant toxicity in humans treated at the usual dose. Therefore, 4-hydroxyphenylretinamide shows promise as a cancer-preventive agent in humans.

11. References

Abou-Issa, H.M., Duruibe, V.A., Minton, J.P., Larroya, S., Dwivedi, C. & Webb, T.E. (1988) Putative metabolites derived from dietary combinations of calcium glucarate and *N*-(4-hydroxyphenyl)retinamide act synergistically to inhibit the induction of rat mammary tumors by 7,12-dimethylbenz[*a*]anthracene. *Proc. Natl Acad. Sci. USA*, **85**, 4181–4184

Abou-Issa, H., Curley, R.W.J., Panigot, M.J., Wilcox, K.A. & Webb, T.E. (1993) *In vivo* use of N-(4-hydroxyphenyl retinamide)-O-glucuronide as a breast cancer chemopreventive agent. *Anticancer Res.*, **13**, 1431–1436

Aliprandis, E.T., Merritt, G., Prada, F., Rigas, B. & Kashfi, K. (1997) Fenretinide inhibits phorbol ester-induced cyclooxygenase-2 expression in human colon adenocarcinoma cells. *Biochem. Soc. Trans.*, **25**, 457S–457S

Ball, M.D., Furr, H.C. & Olson, J.A. (1985) Enhancement of acyl coenzyme A:Retinol acyltransferase in rat liver and mammary tumor tissue by retinyl acetate and its competitive inhibition by *N*-(4-hydroxyphenyl) retinamide. *Biochem. Biophys. Res. Commun.*, **128**, 7–11

Barua, A.B. & Furr, H.C. (1998) Properties of retinoids: Structure, handling, and preparation. In: Redfern, C.P.F., ed., *Retinoid Protocols*. Totowa, NJ, Humana Press, pp. 3–28

Barua, A.B. & Olson, J.A. (1985) Preparation of retinamides by use of retinoyl fluoride. *J. Lipid Res.*, **26**, 258–262

Barua, A.B., Furr, H.C., Olson, J.A. & van Breemen, R.B. (1999) Vitamin A and carotenoids. In: DeLeenheer, A., Lambert, W. & van Bocxlaer, J., eds, *Modern Chromatographic Analysis of the Vitamins*. 3rd Ed. *New York, Marcel Dekker* (in press)

Berni, R. & Formelli, F. (1992) In vitro interaction of fenretinide with plasma retinol-binding protein and its functional consequences. *FEBS Lett.*, **308**, 43–45

Bertram, J.S. (1980) Structure–activity relationships among various retinoids and their ability to inhibit neoplastic transformation and to increase cell adhesion in the C3H/10T1/2 CL8 cell line. *Cancer Res.*, **40**, 3141–3146

Bollag, W. & Hartmann, H.R. (1987) Inhibition of rat mammary carcinogenesis by an arotinoid without a

polar end group (Ro 15-0778). *Eur. J. Cancer Clin. Oncol.*, **23**, 131–135

Budavari, S., O'Neil, M.J., Smith, A., Heckelman, P.E., & Kinneary, J.F., eds (1996) *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*, 12th Ed. Whitehouse Station, NJ, Merck & Co.

Caruso, R.C., Zujewski, J., Iwata, F., Podgor, M.J., Conley, B.A., Ayres, L.M. & Kaiser-Kupfer, M.I. (1998) Effects of fenretinide (4-HPR) on dark adaptation. *Arch. Ophthalmol.*, **116**, 759–763

Cassano, E., Coopmans de Yoldi, G., Ferranti, C., Costa, A., Mascotti, G., De Palo, G. & Veronesi, U. (1993) Mammographic patterns in breast cancer chemoprevention with fenretinide (4-HPR). *Eur. J. Cancer*, **29A**, 2161–2163

Chan, L.N., Zhang, S., Cloyd, M. & Chan, T.S. (1997) *N*-(4-Hydroxyphenyl)retinamide prevents development of T-lymphomas in AKR/J mice. *Anticancer Res.*, **17**, 499–503

Chatterjee, M. & Banerjee, M.R. (1982) N-Nitrosodiethylamine-induced nodule-like alveolar lesion and its prevention by a retinoid in BALB/c mouse mammary glands in the whole organ in culture. *Carcinogenesis*, **3**, 801–804

Chiesa, F., Tradati, N., Marazza, M., Rossi, N., Boracchi, P., Mariani, L., Clerici, M., Formelli, F., Barzan, L., Carrassi, A., Pastorini, A., Camerini, T., Giardini, R., Zurrida, S., Minn, F.L., Costa, A., De Palo, G. & Veronesi, U. (1992) Prevention of local relapses and new localisations of oral leukoplakias with the synthetic retinoid fenretinide (4-HPR). Preliminary results. *Eur. J. Cancer B. Oral Oncol.*, **28B**, 97–102

Chiesa, F., Tradati, N., Marazza, M., Rossi, N., Boracchi, P., Mariani, L., Formelli, F., Giardini, R., Costa, A., De Palo, G. & Veronesi, U. (1993) Fenretinide (4-HPR) in chemoprevention of oral leukoplakia. *J. Cell Biochem.*, **17F** (Suppl.), 255–261

Chrzanowski, F.A., Fegely, B.J., Sisco, W.R. & Newton, M.P. (1984) Analysis of *N*-(4-hydroxyphenyl)retinamide polymorphic forms by X-ray powder diffraction. *J. Pharm. Sci.*, **73**, 1448–1450

Clifford, J.L., Menter, D.G., Wang, M., Lotan, R. & Lippman, S.M. (1999) Retinoid receptor-dependent and -independent effects of *N*-(4-hydroxyphenyl)-retinamide in F9 embryonal carcinoma cells. *Cancer Res.*, **59**, 14–18

Cobleigh, M.A. (1994) Breast cancer and fenretinide, an analogue of vitamin A. *Leukemia*, **8** (Suppl. 3), S59–S63

Coburn, W.C., Jr, Thorpe, M.C., Shealy, Y.F., Kirk, M.C., Frye, J.L. & O'Dell, C.A. (1983) Spectroscopic characterization of 13-*cis* and all-*trans*-retinamide. *J. Chem. Eng. Data*, **28**, 422–428

Cohen, L.A., Epstein, M., Saa-Pabon, V., Meschter, C. & Zang, E. (1994) Interactions between 4-HPR and diet in NMU-induced mammary tumorigenesis. *Nutr. Cancer*, **21**, 271–283

Conaway, C.C., Jiao, D., Kelloff, G.J., Steele, V.E., Rivenson, A. & Chung, F.L. (1998) Chemopreventive potential of fumaric acid, *N*-acetylcysteine, *N*-(4-hydroxyphenyl)retinamide and β-carotene for tobacco-nitrosamine-induced lung tumors in A/J mice. *Cancer Lett.*, **124**, 85–93

Costa, A., Malone, W., Perloff, M., Buranelli, F., Campa, T., Dossena, G., Magni, A., Pizzichetta, M., Andreoli, C., Del Vecchio, M., Formelli, F. & Barbieri, A. (1989) Tolerability of the synthetic retinoid Fenretinide® (HPR). *Eur. J. Cancer Clin. Oncol.*, **25**, 805–808

Costa, A., Formelli, F., Chiesa, F., Decensi, A., De Palo, G. & Veronesi, U. (1994) Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide. *Cancer Res.*, **54**, 2032s–2037s

Costa, A., De Palo, G., Decensi, A., Formelli, F., Chiesa, F., Nava, M., Camerini, T., Marubini, E. & Veronesi, U. (1995) Retinoids in cancer chemoprevention. Clinical trials with the synthetic analogue fenretinide. *Ann. N.Y. Acad. Sci.*, **768**, 148–162

Dawson, M.I. & Hobbs, P.D. (1994) The synthetic chemistry of retinoids. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine*, 2nd Ed. New York, Raven Press, pp. 5–178

Decensi, A., Formelli, F., Torrisi, R. & Costa, A. (1993) Breast cancer chemoprevention: Studies with 4-HPR alone and in combination with tamoxifen using circulating growth factors as potential surrogate endpoints. *J. Cell Biochem.*, **17G** (Suppl.), 226–233

Decensi, A., Bruno, S., Costantini, M., Torrisi, R., Curotto, A., Gatteschi, B., Nicolo, G., Polizzi, A., Perloff, M., Malone, W.F. & Bruzzi, P. (1994a) Phase IIa study of fenretinide in superficial bladder cancer, using DNA flow cytometry as an intermediate end point. *J. Natl Cancer Inst.*, **86**, 138–140

Decensi, A., Torrisi, R., Polizzi, A., Gesi, R., Brezzo, V., Rolando, M., Rondanina, G., Orengo, M.A., Formelli, F. & Costa, A. (1994b) Effect of the synthetic retinoid fenretinide on dark adaptation and the ocular surface. *J. Natl Cancer Inst.*, **86**, 105–110

Decensi, A., Costa, A., De Palo, G., Formelli, F., Marubini, E., Mariani, L., Fontana, V. & Veronesi, U. (1997a)

Retinoid–menopause interactions in a breast cancer prevention trial. *Proc. Am. Assoc. Cancer Res.*, **38**, 529–529

Decensi, A., Fontana, V., Fioretto, M., Rondanina, G., Torrisi, R., Orengo, M.A. & Costa, A. (1997b) Long-term effects of fenretinide on retinal function. *Eur. J. Cancer*, **33**, 80–84

Decensi, A., Torrisi, R., Bruno, S., Curotto, A., Malcangi, B., Nicolo, G., Bruttini, G.P., Costantini, M., Rondanina, G., Baglietto, L., Gatteschi, B., Varaldo, M. & Bruzzi, P. (1997c) Randomized trial of fenretinide in superficial bladder cancer using DNA flow cytometry as an intermediate endpoint. *Proc. Am. Soc. Clin. Oncol.*, **16**, 541A–541A

Delia, D., Aiello, A., Lombardi, L., Pelicci, P.G., Grignani, F., Formelli, F., Ménard, S., Costa, A., Veronesi, U. & Pierotti, M.A. (1993) *N*-(4-Hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. *Cancer Res.*, **53**, 6036–6041

Delia, D., Aiello, A., Meroni, L., Nicolini, M., Reed, J.C. & Pierotti, M.A. (1997) Role of antioxidants and intracellular free radicals in retinamide-induced cell death. *Carcinogenesis*, **18**, 943–948

De Palo, G., Veronesi, U., Camerini, T., Formelli, F., Mascotti, G., Boni, C., Fosser, V., Del Vecchio, M., Campa, T., Costa, A. & Marubini, E. (1995a) Can fenretinide protect women against ovarian cancer? *J. Natl Cancer Inst.*, **87**, 146–147

De Palo, G., Veronesi, U., Marubini, E., Camerini, T., Chiesa, F., Nava, M., Formelli, F., Del Vecchio, M., Costa, A., Boracchi, P. & Mariani, L. (1995b) Controlled clinical trials with fenretinide in breast cancer, basal cell carcinoma and oral leukoplakia. *J. Cell Biochem.*, **22** (Suppl.), 11–17

De Palo, G., Camerini, T., Marubini, E., Costa, A., Formelli, F., Del Vecchio, M., Mariani, L., Miceli, R., Mascotti, G., Magni, A., Campa, T., Di Mauro, M.G., Attili, A., Maltoni, C., Del Turco, M.R., Decensi, A., D'Aiuto, G. & Veronesi, U. (1997) Chemoprevention trial of contralateral breast cancer with fenretinide. Rationale, design, methodology, organization, data management, statistics and accrual. *Tumori*, **83**, 884–894

Dew, S.E., Wardlaw, S.A. & Ong, D.E. (1993) Effects of pharmacological retinoids on several vitamin A-metabolizing enzymes. *Cancer Res.*, **53**, 2965–2969

Dimitrov, N.V., Meyer, C.J., Perloff, M., Ruppenthal, M.M., Phillipich, M.J., Gilliland, D., Malone, W. & Minn, F.L. (1990) Alteration of retinol-binding-protein concentrations by the synthetic retinoid fenretinide in healthy human subjects. *Am. J. Clin. Nutr.*, **51**, 1082–1087

DiPietrantonio, A., Hsieh, T.C. & Wu, J.M. (1996) Differential effects of retinoic acid (RA) and *N*-(4-hydroxyphenyl)retinamide (4-HPR) on cell growth, induction of differentiation, and changes in $p34^{cdc2}$, Bcl-2, and actin expression in the human promyelocytic HL-60 leukemic cells. *Biochem. Biophys. Res. Commun.*, **224**, 837–842

DiPietrantonio, A.M., Hsieh, T.C., Olson, S.C. & Wu, J.M. (1998) Regulation of G1/S transition and induction of apoptosis in HL-60 leukemia cells by fenretinide (4HPR). *Int. J. Cancer*, **78**, 53–61

Di Vinci, A., Geido, E., Infusini, E. & Giaretti, W. (1994) Neuroblastoma cell apoptosis induced by the synthetic retinoid N-(4-hydroxyphenyl)retinamide. *Int. J. Cancer*, **59**, 422–426

Doose, D.R., Minn, F.L., Stellar, S. & Nayak, R.K. (1992) Effects of meals and meal composition on the bioavailability of fenretinide. *J. Clin. Pharmacol.*, **32**, 1089–1095

Fanjul, A.N., Delia, D., Pierotti, M.A., Rideout, D., Qiu, J. & Pfahl, M. (1996) 4-Hydroxyphenyl retinamide is a highly selective activator of retinoid receptors. *J. Biol. Chem.*, **271**, 22441–22446

Favoni, R.E., de Cupis, A., Bruno, S., Yee, D., Ferrera, A., Pirani, P., Costa, A. & Decensi, A. (1998) Modulation of the insulin-like growth factor-I system by *N*-(4-hydroxyphenyl)retinamide in human breast cancer cell lines. *Br. J. Cancer*, **77**, 2138–2147

Formelli, F., Carsana, R. & Costa, A. (1987) *N*-(4-Hydroxyphenyl)retinamide (4-HPR) lowers plasma retinol levels in rats. *Med. Sci. Res.*, **15**, 843–844

Formelli, F., Carsana, R., Costa, A., Buranelli, F., Campa, T., Dossena, G., Magni, A. & Pizzichetta, M. (1989) Plasma retinol level reduction by the synthetic retinoid fenretinide: A one year follow-up study of breast cancer patients. *Cancer Res.*, **49**, 6149–6152

Formelli, F., Clerici, M., Campa, T., Di Mauro, M.G., Magni, A., Mascotti, G., Moglia, D., De Palo, G., Costa, A. & Veronesi, U. (1993) Five-year administration of fenretinide: Pharmacokinetics and effects on plasma retinol concentrations. *J. Clin. Oncol.*, **11**, 2036–2042

Formelli, F., Barua, A.B. & Olson, J.A. (1996) Bioactivities of N-(4-hydroxyphenyl)retinamide and retinoyl β-glucuronide. *FASEB J.*, **10**, 1014–1024

Formelli, F., De Palo, G., Costa, A. & Veronesi, U. (1998) Human transplacental passage of the retinoid fenretinide (4HPR). *Eur. J. Cancer*, **34**, 428–429

Frolik, C.A. & Olson, J.A. (1984) Extraction, separation, and chemical analysis of retinoids. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids.* New York, Academic Press, pp. 181–233

Furr, H.C., Barua, A.B. & Olson, J.A. (1992) Retinoids and carotenoids. In: De Leenheer, A.P., Lambert, W.E. & Nelis, H.J., eds, *Modern Chromatographic Analysis of Vitamins,* 2nd Ed. New York, Marcel Dekker Inc., pp. 1–71

Furr, H.C., Barua, A.B. & Olson, J.A. (1994) Analytical methods. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine,* 2nd Ed. New York, Raven Press, pp. 179–209

Garewal, H.S., List, A., Meyskens, F., Buzaid, A., Greenberg, B. & Katakkar, S. (1989) Phase II trial of fenretinide [*N*-(4-hydroxyphenyl) retinamide] in myelodysplasia: Possible retinoid-induced disease acceleration. *Leuk. Res.,* **13**, 339–343

Gross, E.G., Peck, G.L. & DiGiovanna, J.J. (1991) Adverse reaction to fenretinide, a synthetic retinoid. *Arch. Dermatol.,* **127**, 1849–1850

Grubbs, C.J., Eto, I., Juliana, M.M., Hardin, J.M. & Whitaker, L.M. (1990) Effect of retinyl acetate and 4-hydroxyphenylretinamide on initiation of chemically-induced mammary tumors. *Anticancer Res.,* **10**, 661–666

Grunt, T.W., Dittrich, E., Offterdinger, M., Schneider, S.M., Dittrich, C. & Huber, H. (1998) Effects of retinoic acid and fenretinide on the c-*erb*B-2 expression, growth and cisplatin sensitivity of breast cancer cells. *Br. J. Cancer,* **78**, 79–87

Hong, W.K. & Itri, L.M. (1994) Retinoids and human cancer. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine,* 2nd Ed. New York, Raven Press, pp. 597–630

Hsieh, T.C. & Wu, J.M. (1997) Effects of fenretinide (4-HPR) on prostate LNCaP cell growth, apoptosis, and prostate-specific gene expression. *Prostate,* **33**, 97–104

Hsieh, T.C., Ng, C. & Wu, J.M. (1995) The synthetic retinoid *N*-(4-hydroxyphenyl)retinamide (4-HPR) exerts antiproliferative and apoptosis-inducing effects in the androgen-independent human prostatic JCA-1 cells. *Biochem. Mol. Biol. Int.,* **37**, 499–506

Hultin, T.A., May, C.M. & Moon, R.C. (1986) *N*-(4-Hydroxyphenyl)-all-*trans*-retinamide pharmacokinetics in female rats and mice. *Drug Metab. Dispos.,* **14**, 714–717

Hultin, T.A., McCormick, D.L., May, C.M. & Moon, R.C. (1988) Effects of pretreatment with the retinoid *N*-(4-hydroxyphenyl)-all-*trans*-retinamide and phenobarbital on the disposition and metabolism of *N*-(4-hydroxyphenyl)-all-*trans*-retinamide in mice. *Drug Metab. Dispos.,* **16**, 783–788

Hultin, T.A., Filla, M.S. & McCormick, D.L. (1990) Distribution and metabolism of the retinoid, *N*-(4-methoxyphenyl)-all-*trans*-retinamide, the major meta-bolite of *N*-(4-hydroxyphenyl)-all-*trans*-retinamide, in female mice. *Drug Metab. Dispos.,* **18**, 175–179

Igawa, M., Tanabe, T., Chodak, G.W. & Rukstalis, D.B. (1994) *N*-(4-Hydroxyphenyl)retinamide induces cell cycle specific growth inhibition in PC3 cells. *Prostate,* **24**, 299–305

Jurima-Romet, M., Neigh, S. & Casley, W.L. (1997) Induction of cytochrome P450 3A by retinoids in rat hepatocyte culture. *Hum. Exp. Toxicol.,* **16**, 198–203

Kaiser-Kupfer, M.I., Peck, G.L., Caruso, R.C., Jaffe, M.J., DiGiovanna, J.J. & Gross, E.G. (1986) Abnormal retinal function associated with fenretinide, a synthetic retinoid. *Arch. Ophthalmol.,* **104**, 69–70

Kalemkerian, G.P., Slusher, R., Ramalingam, S., Gadgeel, S. & Mabry, M. (1995) Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines. *J. Natl Cancer Inst.,* **87**, 1674–1680

Kazmi, S.M., Plante, R.K., Visconti, V. & Lau, C.Y. (1996) Comparison of *N*-(4-hydroxyphenyl)retinamide and all-*trans*-retinoic acid in the regulation of retinoid receptor-mediated gene expression in human breast cancer cell lines. *Cancer Res.,* **56**, 1056–1062

Kelloff, G.J., Crowell, J.A., Boone, C.W., Steele, V.E., Lubet, R.A., Greenwald, P., Alberts, D.S., Covey, J.M., Doody, L.A., Knapp, G.G., Nayfield, S.G., Parkinson, D.R., Prasad, K., Prorok, P.C., Sausville, E.A. & Sigman, C.C. (1994) Clinical development plan: *N*-(4-Hydroxyphenyl)retinamide. *J. Cell Biochem.,* **20** (Suppl.), 176–196

Kenel, M.F., Krayer, J.H., Merz, E.A. & Pritchard, J.F. (1988) Teratogenicity of *N*-(4-hydroxyphenyl)-all-*trans*-retinamide in rats and rabbits. *Teratog. Carcinog. Mutag.,* **8**, 1–11

Kingston, T.P., Lowe, N.J., Winston, J. & Heckenlively, J. (1986) Visual and cutaneous toxicity which occurs during N-(4-hydroxyphenyl)retinamide therapy for psoriasis. *Clin. Exp. Dermatol.,* **11**, 624–627

Krzeminski, R., Zwas, F., Esper, P. & Pienta, K. (1996) Electroretinographic findings in subjects after administration of fenretinide. *Doc. Ophthalmol.,* **91**, 299–309

Li, C.Y., Zimmerman, C.L. & Wiedmann, T.S. (1996) Solubilization of retinoids by bile salt/phospholipid aggregates. *Pharm. Res.*, **13**, 907–913

Lotan, R. (1995) Retinoids and apoptosis: Implications for cancer chemoprevention and therapy. *J. Natl Cancer Inst.*, **87**, 1655–1657

Manoharan, K. & Banerjee, M.R. (1985) β-Carotene reduces sister chromatid exchanges induced by chemical carcinogens in mouse mammary cells in organ culture. *Cell Biol. Int. Rep.*, **9**, 783–789

Mariani, L., Formelli, F., De Palo, G., Manzari, A., Camerini, T., Campa, T., Di Mauro, M.G., Crippa, A., Delle Grottaglie, M., Del Vecchio, M., Marubini, E., Costa, A. & Veronesi, U. (1996) Chemoprevention of breast cancer with fenretinide (4-HPR): Study of long-term visual and ophthalmologic tolerability. *Tumori*, **82**, 444–449

Mariotti, A., Marcora, E., Bunone, G., Costa, A., Veronesi, U., Pierotti, M.A. & Della Valle, G. (1994) N-(4-Hydroxyphenyl)retinamide: A potent inducer of apoptosis in human neuroblastoma cells. *J. Natl Cancer Inst.*, **86**, 1245–1247

McCarthy, D.J., Lindamood, C., III & Hill, D.L. (1987) Effects of retinoids on metabolizing enzymes and on binding of benzo(a)pyrene to rat tissue DNA. *Cancer Res.*, **47**, 5014–5020

McCormick, D.L. & Moon, R.C. (1986) Retinoid–tamoxifen interaction in mammary cancer chemoprevention. *Carcinogenesis*, **7**, 193–196

McCormick, D.L., Mehta, R.G., Thompson, C.A., Dinger, N., Caldwell, J.A. & Moon, R.C. (1982a) Enhanced inhibition of mammary carcinogenesis by combined treatment with *N*-(4-hydroxyphenyl)retinamide and ovariectomy. *Cancer Res.*, **42**, 508–512

McCormick, D.L., Becci, P.J. & Moon, R.C. (1982b) Inhibition of mammary and urinary bladder carcinogenesis by a retinoid and a maleic anhydride–divinyl ether copolymer (MVE-2).*Carcinogenesis*, **3**, 1473–1476

McCormick, D.L., Bagg, B.J. & Hultin, T.A. (1987) Comparative activity of dietary or topical exposure to three retinoids in the promotion of skin tumor induction in mice. *Cancer Res.*, **47**, 5989–5993

McCormick, D.L., Johnson, W.D., Rao, K.V., Bowman-Gram, T., Steele, V.E., Lubet, R.A. & Kelloff, G.J. (1996) Comparative activity of *N*-(4-hydroxyphenyl)-all-*trans*-retinamide and α-difluoromethylornithine as inhibitors of lymphoma induction in PIM transgenic mice. *Carcinogenesis*, **17**, 2513–2517

McCormick, D.L., Rao, K.V., Dooley, L., Steele, V.E., Lubet, R.A., Kelloff, G.J. & Bosland, M.C. (1998) Influence of *N*-methyl-*N*-nitrosourea, testosterone, and *N*-(4-hydroxyphenyl)-all-*trans*-retinamide on prostate cancer induction in Wistar-Unilever rats. *Cancer Res.*, **58**, 3282–3288

Mehta, R.G., Hultin, T.A. & Moon, R.C. (1988) Metabolism of the chemopreventive retinoid *N*-(4-hydroxyphenyl)retinamide by mammary gland in organ culture. *Biochem. J.*, **256**, 579–584

Mehta, R.G., Moon, R.C., Hawthorne, M., Formelli, F. & Costa, A. (1991) Distribution of fenretinide in the mammary gland of breast cancer patients. *Eur. J. Cancer*, **27**, 138–141

Mehta, R.R., Hawthorne, M.E., Graves, J.M. & Mehta, R.G. (1998) Metabolism of *N*-[4-hydroxyphenyl]retinamide (4-HPR) to *N*-[4-methoxyphenyl]retinamide (4-MPR) may serve as a biomarker for its efficacy against human breast cancer and melanoma cells. *Eur. J. Cancer*, **34**, 902–907

Modiano, M., Pond, G., Holdsworth, M., Plezia, P. & Alberts, D. (1989) Phase I and pharmacokinetic (PK) study of selective intraarterial chemoembolization (CE) with angiostat collagen, CDDP, mitomycin, adriamycin for metastatic colorectal carcinoma to the liver. The University of Arizona experience. *Clin. Res.*, **37**, 143A–143A

Modiano, M.R., Dalton, W.S., Lippman, S.M., Joffe, L., Booth, A.R. & Meyskens, F.L.J. (1990a) Ocular toxic effects of fenretinide. *J. Natl Cancer Inst.*, **82**, 1063–1063

Modiano, M.R., Dalton, W.S., Lippman, S.M., Joffe, L., Booth, A.R. & Meyskens, F.L.J. (1990b) Phase II study of fenretinide (N-[4-hydroxyphenyl]retinamide) in advanced breast cancer and melanoma. *Invest. New Drugs*, **8**, 317–319

Moglia, D., Formelli, F., Baliva, G., Bono, A., Accetturi, M., Nava, M. & De Palo, G. (1996) Effects of topical treatment with fenretinide (4-HPR) and plasma vitamin A levels in patients with actinic keratoses. *Cancer Lett.*, **110**, 87–91

Moon, R.C. & Mehta, R.G. (1990) Cancer chemoprevention by retinoids: Animal models. *Methods Enzymol.*, **190**, 395–406

Moon, R.C., Thompson, H.J., Becci, P.J., Grubbs, C.J., Gander, R.J., Newton, D.L., Smith, J.M., Phillips, S.L., Henderson, W.R., Mullen, L.T., Brown, C.C. & Sporn, M.B. (1979) *N*-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. *Cancer Res.*, **39**, 1339–1346

Moon, R.C., McCormick, D.L., Becci, P.J., Shealy, Y.F., Frickel, F., Paust, J. & Sporn, M.B. (1982) Influence of 15 retinoic acid amides on urinary bladder carcinogenesis in the mouse. *Carcinogenesis*, **3**, 1469–1472

Moon, R.C., Pritchard, J.F., Mehta, R.G., Nomides, C.T., Thomas, C.F. & Dinger, N.M. (1989) Suppression of rat mammary cancer development by *N*-(4-hydroxyphenyl)retinamide (4-HPR) following surgical removal of first palpable tumor. *Carcinogenesis*, **10**, 1645–1649

Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F. & Sigman, C.C. (1992) Chemoprevention of MNU-induced mammary tumors in the mature rat by 4-HPR and tamoxifen. *Anticancer Res.*, **12**, 1147–1153

Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F. & Sigman, C.C. (1994a) Chemoprevention of OH-BBN-induced bladder cancer in mice by oltipraz, alone and in combination with 4-HPR and DFMO. *Anticancer Res.*, **14**, 5–11

Moon, R.C., Mehta, R.G. & Rao, K.V.N. (1994b) Retinoids and cancer in experimental animals. In: Sporn, M.B., Roberts, A.B. & Goodman, D.S., eds, *The Retinoids: Biology, Chemistry, and Medicine*, 2nd Ed. New York, Raven Press, pp. 573–595

Muller, A., Nakagawa, H. & Rustgi, A.K. (1997) Retinoic acid and N-(4-hydroxyphenyl)retinamide suppress growth of esophageal squamous carcinoma cell lines. *Cancer Lett.*, **113**, 95–101

Ohshima, M., Ward, J.M. & Wenk, M.L. (1985) Preventive and enhancing effects of retinoids on the development of naturally occurring tumors of skin, prostate gland, and endocrine pancreas in aged male ACI/segHapBR rats. *J. Natl Cancer Inst.*, **74**, 517–524

Oridate, N., Lotan, D., Mitchell, M.F., Hong, W.K. & Lotan, R. (1995) Induction of apoptosis by retinoids in human cervical carcinoma cell lines. *Int. J. Oncol.*, **7**, 433–441

Oridate, N., Lotan, D., Xu, X.C., Hong, W.K. & Lotan, R. (1996) Differential induction of apoptosis by all-*trans*-retinoic acid and *N*-(4-hydroxyphenyl)retinamide in human head and neck squamous cell carcinoma cell lines. *Clin. Cancer Res.*, **2**, 855–863

Oridate, N., Suzuki, S., Higuchi, M., Mitchell, M.F., Hong, W.K. & Lotan, R. (1997) Involvement of reactive oxygen species in *N*-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells. *J. Natl Cancer Inst.*, **89**, 1191–1198

Paulson, J.D., Oldham, J.W., Preston, R.F. & Newman, D. (1985) Lack of genotoxicity of the cancer chemopreventive agent *N*-(4-hydroxyphenyl)retinamide. *Fundam. Appl. Toxicol.*, **5**, 144–150

Pellegrini, R., Mariotti, A., Tagliabue, E., Bressan, R., Bunone, G., Coradini, D., Della Valle, G., Formelli, F., Cleris, L., Radice, P., Pierotti, M.A., Colnaghi, M.I. & Ménard, S. (1995) Modulation of markers associated with tumor aggressiveness in human breast cancer cell lines by *N*-(4-hydroxyphenyl)retinamide. *Cell Growth Differ.*, **6**, 863–869

Peng, Y.M., Dalton, W.S., Alberts, D.S., Xu, M.J., Lim, H. & Meyskens, F.L.J. (1989) Pharmacokinetics of *N*-4-hydroxyphenylretinamide and the effect of its oral administration on plasma retinol concentrations in cancer patients. *Int. J. Cancer*, **43**, 22–26

Pergolizzi, R., Appierto, V., Crosti, M., Cavadini, E., Cleris, L., Guffanti, A. & Formelli, F. (1999) Role of retinoic acid receptor overexpression in sensitivity to fenretinide and tumorigenicity of human ovarian carcinoma cells. *Int. J. Cancer*, **81**, 829–834

Pienta, K.J., Esper, P.S., Zwas, F., Krzeminski, R. & Flaherty, L.E. (1997) Phase II chemoprevention trial of oral fenretinide in patients at risk for adenocarcinoma of the prostate. *Am. J. Clin. Oncol.*, **20**, 36–39

Pollard, M., Luckert, P.H. & Sporn, M.B. (1991) Prevention of primary prostate cancer in Lobund-Wistar rats by *N*-(4-hydroxyphenyl)retinamide. *Cancer Res.*, **51**, 3610–3611

Ponzoni, M., Bocca, P., Chiesa, V., Decensi, A., Pistoia, V., Raffaghello, L., Rozzo, C. & Montaldo, P.G. (1995) Differential effects of *N*-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: Apoptosis *versus* differentiation. *Cancer Res.*, **55**, 853–861

Radcliffe, J.D. (1983) Effect of N-4-hydroxyphenyl retinamide on lipid metabolism in rats. *Nutr. Rep. Int.*, **28**, 799–803

Ratko, T.A., Detrisac, C.J., Dinger, N.M., Thomas, C.F., Kelloff, G.J. & Moon, R.C. (1989) Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. *Cancer Res.*, **49**, 4472–4476

Roberson, K.M., Penland, S.N., Padilla, G.M., Selvan, R.S., Kim, C.S., Fine, R.L. & Robertson, C.N. (1997) Fenretinide: Induction of apoptosis and endogenous transforming growth factor β in PC-3 prostate cancer cells. *Cell Growth Differ.*, 8, 101–111

Rotmensz, N., De Palo, G., Formelli, F., Costa, A., Marubini, E., Campa, T., Crippa, A., Danesini, G.M., Delle Grottaglie, M., Di Mauro, M.G., Filiberti, A., Gallazzi, M., Guzzon, A., Magni, A., Malone, W., Mariani, L., Palvarini, M., Perloff, M., Pizzichetta, M.

& Veronesi, U. (1991) Long-term tolerability of fenretinide (4-HPR) in breast cancer patients. *Eur. J. Cancer,* **27**, 1127–1131

Rozzo, C., Chiesa, V., Caridi, G., Pagnan, G. & Ponzoni, M. (1997) Induction of apoptosis in human neuroblastoma cells by abrogation of integrin-mediated cell adhesion. *Int. J. Cancer,* **70**, 688–698

Sabichi, A.L., Hendricks, D.T., Bober, M.A. & Birrer, M.J. (1998) Retinoic acid receptor β expression and growth inhibition of gynecologic cancer cells by the synthetic retinoid *N*-(4-hydroxyphenyl)retinamide. *J. Natl Cancer Inst.,* **90**, 597–605

Sani, B.P., Shealy, Y.F. & Hill, D.L. (1995) N-(4-Hydroxyphenyl)retinamide: Interactions with retinoid-binding proteins/receptors. *Carcinogenesis,* **16**, 2531–2534

Schrader, P.A. & Sisco, W.R. (1987) High-performance liquid chromatographic analysis of fenretinide in rodent feed. *J. Chromatogr.,* **408**, 430–434

Shealy, Y.F., Frye, J.L., O'Dell, C.A., Thorpe, M.C., Kirk, M.C., Coburn, W.C., Jr & Sporn, M.B. (1984) Synthesis and properties of some 13-*cis*- and all-*trans*-retinamides. *J. Pharm. Sci.,* **73**, 745–751

Sheikh, M.S., Li, X.S., Chen, J.C., Shao, Z.M., Ordonez, J.V. & Fontana, J.A. (1994) Mechanisms of regulation of WAF1/Cip1 gene expression in human breast carcinoma: Role of p53-dependent and independent signal transduction pathways. *Oncogene,* **9**, 3407–3415

Sheikh, M.S., Shao, Z.M., Li, X.S., Ordonez, J.V., Conley, B.A., Wu, S., Dawson, M.I., Han, Q.X., Chao, W.R., Quick, T., Niles, R.M. & Fontana, J.A. (1995) *N*-(4-Hydroxyphenyl)retinamide (4-HPR)-mediated biological actions involve retinoid receptor-independent pathways in human breast carcinoma. *Carcinogenesis,* **16**, 2477–2486

Silverman, J., Katayama, S., Radok, P., Levenstein, M.J. & Weisburger, J.H. (1983) Effect of short-term administration of *N*-(4-hydroxyphenyl)-all-*trans*-retinamide on chemically induced mammary tumors. *Nutr. Cancer,* **4**, 186–191

Sridhara, R., Peck, G., Wu, S., Edwards, P., Crowell, J., Fontana, J. & Conley, B. (1997) Pharmacokinetics (PK) and pharmacodynamics (PD) of fenretinide (4-HPR) in patients (pts) treated in a skin cancer prevention trial. *Proc. Am. Soc. Clin. Oncol.,* **16**, 540A–540A

Steele, V.E., Kelloff, G.J., Wilkinson, B.P. & Arnold, J.T. (1990) Inhibition of transformation in cultured rat tracheal epithelial cells by potential chemopreventive agents. *Cancer Res.,* **50**, 2068–2074

Sun, S.Y., Yue, P. & Lotan, R. (1999a) Induction of apoptosis by *N*-(4-hydroxyphenyl)retinamide and its association with reactive oxygen species, nuclear retinoic acid receptors, and apoptosis-related genes in human prostate carcinoma cells. *Mol. Pharmacol.,* **55**, 403–410

Sun, S.Y., Kurie, J.M., Yue, P., Dawson, M.I., Shroot, B., Chandraratna, R.A., Hong, W.K. & Lotan, R. (1999b) Differential responses of normal, premalignant, and malignant human bronchial epithelial cells to receptor-selective retinoids. *Clin. Cancer Res.,* **5**, 431–437

Supino, R., Crosti, M., Clerici, M., Warlters, A., Cleris, L., Zunino, F. & Formelli, F. (1996) Induction of apoptosis by fenretinide (4HPR) in human ovarian carcinoma cells and its association with retinoic acid receptor expression. *Int. J. Cancer,* **65**, 491–497

Swisshelm, K., Ryan, K., Lee, X., Tsou, H.C., Peacocke, M. & Sager, R. (1994) Down-regulation of retinoic acid receptor beta in mammary carcinoma cell lines and its up-regulation in senescing normal mammary epithelial cells. *Cell Growth Differ.,* **5**, 133–141

Swisshelm, K., Ryan, K., Tsuchiya, K. & Sager, R. (1995) Enhanced expression of an insulin growth factor-like binding protein (mac25) in senescent human mammary epithelial cells and induced expression with retinoic acid. *Proc. Natl Acad. Sci. USA,* **92**, 4472–4476

Taimi, M. & Breitman, T.R. (1997) *N*-4-Hydroxyphenylretinamide enhances retinoic acid-induced differentiation and retinoylation of proteins in the human acute promyelocytic leukemia cell line, NB4, by a mechanism that may involve inhibition of retinoic acid catabolism. *Biochem. Biophys. Res. Commun.,* **232**, 432–436

Takahashi, N., Sausville, E.A. & Breitman, T.R. (1995) *N*-(4-Hydroxyphenyl)retinamide (Fenretinide) in combination with retinoic acid enhances differentiation and retinoylation of proteins. *Clin. Cancer Res.,* **1**, 637–642

Tamura, K., Nakae, D., Horiguchi, K., Akai, H., Kobayashi, Y., Andoh, N., Satoh, H., Denda, A., Tsujiuchi, T., Yoshiji, H. & Konishi, Y. (1997) Inhibition by *N*-(4-hydroxyphenyl)retinamide and all-*trans*-retinoic acid of exogenous and endogenous development of putative preneoplastic, glutathione *S*-transferase placental form-positive lesions in the livers of rats. *Carcinogenesis,* **18**, 2133–2141

Thaller, C., Eichele, G., Slawin, K., Thompson, T.C. & Kadmon, D. (1996) Retinoid alterations induced by fenretinide therapy in prostate cancer. *Proc. Am. Assoc. Cancer Res.,* **37**, 243–243

Tradati, N., Chiesa, F., Rossi, N., Grigolato, R., Formelli, F., Costa, A. & De Palo, G. (1994) Successful topical treatment of oral lichen planus and leukoplakias with fenretinide (4-HPR). *Cancer Lett.,* **76**, 109–111

Trizna, Z., Benner, S.E., Shirley, L., Furlong, C. & Hong, W.K. (1993) *N*-(4-Hydroxyphenyl)retinamide is anticlastogenic in human lymphoblastoid cell lines. *Anticancer Res.*, **13**, 355–356

Turton, J.A., Willars, G.B., Haselden, J.N., Ward, S.J., Steele, C.E. & Hicks, R.M. (1992) Comparative teratogenicity of nine retinoids in the rat. *Int. J. Exp. Pathol.*, **73**, 551–563

Veronesi, U., De Palo, G., Costa, A., Formelli, F. & Decensi, A. (1996) Chemoprevention of breast cancer with fenretinide. In: Hakama, M., Beral, V., Buiatti, E., Faivre, J. & Parkin, D.M., eds, *Chemoprevention in Cancer Control* (IARC Scientific Publications No. 136). Lyon, International Agency for Research on Cancer, pp. 87–94

Welsch, C.W., DeHoog, J.V. & Moon, R.C. (1983) Inhibition of mammary tumorigenesis in nulliparous C3H mice by chronic feeding of the synthetic retinoid, N-(4-hydroxyphenyl)retinamide. *Carcinogenesis*, **4**, 1185–1187

Zheng, Y., Kramer, P.M., Olson, G., Lubet, R.A., Steele, V.E., Kelloff, G.J. & Pereira, M.A. (1997) Prevention by retinoids of azoxymethane-induced tumors and aberrant crypt foci and their modulation of cell proliferation in the colon of rats. *Carcinogenesis*, **18**, 2119–2125

Zou, C.P., Kurie, J.M., Lotan, D., Zou, C.C., Hong, W.K. & Lotan, R. (1998) Higher potency of *N*-(4-hydroxyphenyl)retinamide than all-*trans*-retinoic acid in induction of apoptosis in non-small cell lung cancer cell lines. *Clin. Cancer Res.*, **4**, 1345–1355