Handbook 3

9-*cis*-Retinoic acid

1. Chemical and Physical Characteristics

1.1 Nomenclature

See General Remarks Section 1.4.

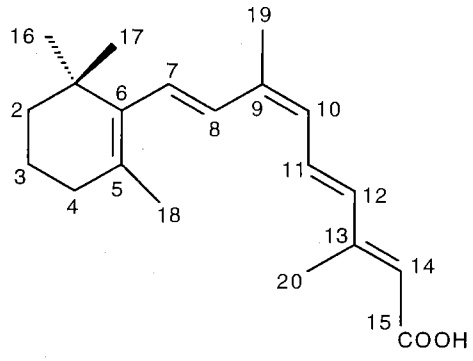
1.2 Name: 9-*cis*-Retinoic acid

Chemical Abstracts Services Registry Number
5300-03-8

IUPAC systematic name
(7*E*,9*Z*,11*E*,13*E*)-9,13-dimethyl-7-(1,1,5-trimethylcyclohex-5-en-6-yl)nona-7,9,11,13-tetraen-15-oic acid (see 1.3), or (2*E*,4*E*,6*Z*,8*E*)-3,7-dimethyl-9-(2,2,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-oic acid

Synonyms
9-*cis*-RA, 9-*cis*-vitamin A acid, 9-*cis*-vitamin A_1 acid, Panretin ®, LGD 1057

1.3 Structural formula



Composition: $C_{20}H_{28}O_2$
Relative molecular mass: 300.45

1.4 Physical and chemical properties

Description
Yellow crystals from ethanol

Melting-point
189–191 °C (Robeson *et al.*, 1955).

Solubility
Soluble in most organic solvents, fats, and oils; sparingly soluble in water.

Spectroscopy
UV and visible: λ_{max} 345 (ethanol), $E^{1\%}_{1\,cm}$ 1230, E_M 36 900 (Robeson *et al.*, 1955; Frickel, 1984; Barua & Furr, 1998)

Nuclear magnetic resonance
^{1}H-NMR ($CDCl_3$, 220 MHz): δ 1.04 (1-CH_3), 1.48 (2-CH_2), 1.64 (3-CH_2), 1.75 (5-CH_3), 2.01 (9-CH_3), 2.04 (4-CH_2), 2.37 (13-CH_3), 5.82 (14-H), 6.09 (10-H), 6.27 (12-H), 6.31 (7-H), 6.67 (8-H), 7.15 (11-H); $J_{7,8}$ (15.7 Hz), $J_{10,11}$ (11.3 Hz), $J_{11,12}$ (14.7 Hz) (Schweiter *et al.*, 1969; Vetter *et al.*, 1971; Frickel, 1984; Barua & Furr, 1998).

^{13}C-NMR ($CDCl_3$ 68 MHz); δ 13.4 (13-CH_3), 18.9 (3-C), 20.5 (9-CH_3), 21.6 (5-CH_3), 28.8 (1,1-CH_3), 32.7 (4-C), 33.9 (1-C), 39.3 (2-C), 119.6 (14-C), 128.1 (10-C), 129.0 (8-C), 129.4 (5-C), 129.6 (7-C, 11-C), 134.7 (12-C), 137.3 (6-C), 137.6 (9-C), 151.2 (13-C), 167.8 (15-C) (Englert, 1975; Frickel, 1984; Barua & Furr, 1998)

Resonance Raman, infrared and mass spectrometry
(Frickel, 1984; Barua & Furr, 1998).

X-Ray analysis
(Frickel, 1984).

Stability
Unstable to light, oxygen and heat. In solution is protected by the presence of antioxidants, such as butylated hydroxytoluene and pyrogallol. A variety of factors influence its stability in tissue culture media. Degradation and isomerization are minimized by storing under an inert gas such as argon, at –20 °C or lower in the dark (Frickel, 1984; Barua & Furr, 1998)

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

2.1 Occurrence

The concentration of 9-*cis*-retinoic acid in the plasma of fasting individuals is < 1 nmol/L. When a 70-kg man ate 140 g of turkey liver containing 0.25 mmol of vitamin A, however, the concentration of 9-*cis*-retinoic acid rose to 9 nmol/L and that of its 9,13-*cis* metabolite to 57 nmol/L within 4 h (Arnhold *et al.*, 1996). The concentrations were 100 pmol/g in mouse kidney and 13 pmol/g in liver, and may well be similar in human tissues (Blaner & Olson, 1994). Thus, the concentration of 9-*cis*-retinoic acid is < 0.1% that of all-*trans*-retinol in human plasma and < 2% that of total vitamin A in the tissues of healthy animals and humans. 9-*cis*-Retinoic acid is present only in traces in plants, if at all. It is therefore a very minor constituent of the diet, and, unlike vitamin A and carotenoids, is not available as a dietary supplement. The one notable exception is the concentration found in human plasma after consumption of liver (Arnhold *et al.*, 1996), possibly via formation from 9-*cis*-retinol, which is known to be present in that organ.

2.2 Production

The synthesis of 9-*cis*-retinoic acid is based on that of the all-*trans* isomer (see Handbook 1, p. 96), with several modifications. Thus, condensation of a 9-*cis*-β-C_{15}-aldehyde with ethyl senecioate in the presence of potassium amide in liquid ammonia gives 9-*cis*-retinoic acid (Mayer & Isler, 1971; Frickel, 1984). Use of a *trans* C_{14} aldehyde in Isler's industrial synthesis of retinol also yields predominantly the 9-*cis* isomer. Photoisomerization of all-*trans*-retinoids in a polar solvent such as acetonitrile yields a mixture of *cis* isomers, in which the 9-*cis* isomer predominates (Frickel, 1984). 9-*cis*-Retinal can also be converted to its acid by mild oxidants (Mayer & Isler, 1971; Frickel, 1984). Newer methods of synthesis for a large number of retinoids have been reviewed (Dawson & Hobbs, 1994).

2.3 Use

Although 9-*cis*-retinoic acid was identified, synthesized and characterized in the 1950s, it received attention as a potential therapeutic agent only after its identification in 1992 as an agonist for the retinoic acid receptor (RAR) and as the putative physiological ligand for the retinoid X receptor (RXR) (Heyman *et al.*, 1992 ; Levin *et al.*, 1992). The use of 9-*cis*-retinoic acid in the treatment of clinical disorders is therefore still in its infancy. The types of cancer that might be affected by treatment with 9-*cis*-retinoic acid are listed in Table 1 (Hong & Itri, 1994; Kelloff *et al.*, 1996; Makishima *et al.*, 1998; Soignet *et al.*, 1998).

Table 1. Types of cancer being considered for treatment with 9-*cis*-retinoic acid in planned or on-going clinical studies

Acute promyelocytic leukaemia
Breast carcinoma
Cervical carcinoma
Colon carcinoma
Kaposi sarcoma
Lung carcinoma
Neuroblastoma
Prostate carcinoma

[a] Modified from Hong & Itri (1994); Kelloff *et al.* (1996); Makishima *et al.* (1998); Soignet *et al.* (1998).

In clinical trials in which the dose was escalated gradually, the maximum tolerated oral dose of 9-*cis*-retinoic acid was found to be approximately 80 mg/m² per day (Rizvi *et al.*, 1998). A topical 0.1% formulation of 9-*cis*-retinoic acid (Panretin®) has been approved for the treatment of Kaposi sarcoma in the United States.

2.4 Human exposure

As indicated above, the total amount of 9-*cis*-retinoic acid in food is very small, probably 10–100 µg/day. 9-*cis*-Retinoic acid is one of the least prevalent of the retinoic acid isomers. Because it is rapidly metabolized in the body and is not stored in the liver or other organs, it does not accumulate over time (Blaner & Olson, 1994). The amount of 9-*cis*-retinoic acid ingested in the diet therefore poses neither benefit nor risk. Exposure to 9-*cis*-retinoic acid is limited, for all practical purposes, to topical and oral treatment of medical disorders. Although the various isomers of retinoic acid, including 9-*cis*-retinoic acid, are

therapeutically effective, many adverse side-effects of therapeutic doses have been reported (Kamm *et al.*, 1984; Armstrong *et al.*, 1994; Nau *et al.*, 1994; Kelloff *et al.*, 1996; see section 7.1).

2.5 Analysis

9-*cis*-Retinoic acid is commonly measured in plasma and tissues by high-performance liquid chromatography (HPLC; Barua & Furr, 1998). Either 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 and methanol, diethyl ether, dichloromethane, acetonitrile, 2-propanol or ethyl acetate. After the combined extract has been dried with anhydrous sodium sulfate, the solvent is evaporated to dryness under yellow light (to avoid isomerization) in nitrogen or argon. 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.

A reversed-phase C_{18} column is usually used for the separation. It is usually detected by measuring the absorption at 345 nm and quantified by measuring the area under the absorption peak with an integrator. A known amount of a reference standard, usually all-*trans*-retinyl acetate, is added to the tissue, plasma or serum sample to correct for losses during extraction and analysis. An antioxidant such as butylated hydroxytoluene is also added at the outset to minimize oxidation of any retinoids present.

A large number of chromatographic systems has been devised for the separation and quantification of 9-*cis*-retinoic acid (Frolik & Olson, 1984; Furr *et al.*, 1992, 1994; Barua & Furr, 1998; Barua *et al.*, in press). In most reversed-phase HPLC systems, 9-*cis*-retinoic acid is eluted between 13-*cis*-retinoic acid and all-*trans*-retinoic acid.

9-*cis*-Retinoic acid, as its methyl or pentafluorobenzyl ester, can also be separated by gas–liquid or liquid–liquid chromatography and quantified by mass spectrometry. New ionization methods and tandem mass spectrometry have further enhanced the sensitivity and selectivity with which various isomers of retinoic acid can be measured (Barua *et al.*, in press).

3. Metabolism, Kinetics and Genetic Variation

[The Working Group was concerned that there is insufficient experimental evidence to establish whether 9-*cis*-retinoic acid is 'the' or 'a' physiological ligand for the RXR family of receptors. Although there is a considerable body of literature on the formation of 9-*cis*-retinoic acid within cells, tissues and organisms and on its actions in living systems, decisive, unequivocal proof that 9-*cis*-retinoic acid is a physiological form of retinoic acid is lacking. In spite of this uncertainty, the literature on the metabolism, kinetics and tissue distribution of 9-*cis*-retinoic acid is reviewed below without bias, nevertheless referring to it as a 'putative' physiological ligand.]

3.1 Humans

3.1.1 Metabolism

9-*cis*-Retinoic acid was given to healthy men at 20 mg/day for 28 days, and plasma, urine and faeces were collected before treatment and after treatment on days 14 and 28. The major urinary metabolites were 9-*cis*-retinoyl-β-glucuronide and 9-*cis*-4-oxoretinoyl-β-glucuronide. High concentrations of unchanged 9-*cis*-retinoic acid were observed in the faeces. The authors consequently suggested that the substance is poorly absorbed in the gastrointestinal tract. The major metabolites in plasma 2 h after the last dose of 9-*cis*-retinoic acid on day 28 of the study were all-*trans*- and 13-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid and a mixture of 4-oxoretinoic acid isomers (Sass *et al.*, 1995).

A double-blind, placebo-controlled, randomized study was conducted in 40 healthy men given single increasing oral doses of 5, 15, 40, 80 and 150 mg of 9-*cis*-retinoic acid to assess the pharmacokinetics of single doses. The main metabolites in serum were all-*trans*- and 13-*cis*-retinoic acid and all-*trans*- and 9-*cis*-4-oxoretinoic acid. The main metabolite at all doses was 9-*cis*-4-oxoretinoic acid, which was present in blood at concentrations 41–83% of those observed for 9-*cis*-retinoic acid (Weber & Dumont, 1997).

9-*cis*-Retinoic acid was converted to more polar products very slowly by human endothelial cells in culture, whereas the same cells metabolized all-*trans*-retinoic acid rapidly. In contrast, cultured

human hepatocytes metabolized 9-*cis*-retinoic acid faster than they did all-*trans*-retinoic acid (Lansink *et al.*, 1997).

3.1.2 Kinetics

9-*cis*-Retinoic acid was given orally twice daily at doses ranging from 20 to 150 mg/m^2 per day to 22 patients with carcinomas at various organ sites. On day 1 of the study, the time to the peak plasma concentration was 3–4 h at all doses of 9-*cis*-retinoic acid except the lowest and 6 h at the lowest dose. On day 22, the peak plasma concentrations were reached within 2–3.6 h at all doses. After 22 days of administration of 9-*cis*-retinoic acid, the peak concentrations and the values for the integrated area under the curve of plasma concentration–time (AUC) were markedly lower than those calculated for the same patients on day 1. The pharmacokinetics of 9-*cis*-retinoic acid was highly variable between patients, and the parameters overlapped widely between doses. The observed decrease in plasma concentration with increased length of administration also varied, but it was not possible to determine whether the reduction was dose-dependent because of the relatively small number of patients studied (Kurie *et al.*, 1996).

In the study of Weber and Dumont (1997) described above, the pharmacokinetics of 9-*cis*-retinoic acid were linear over the range of doses studied. The peak plasma concentrations were achieved on average within 3–4 h of dosing. The major pathway for elimination was reported to be by metabolism. The average AUC value for the 5-mg dose was 49 ng-h/mL, and that for the 150-mg dose was 1700 ng-h/mL. As has been reported after administration of all-*trans*- and 13-*cis*-retinoic acid to humans, 9-*cis*-retinoic acid induced a dose- or concentration-dependent reduction in plasma retinol concentration, by a maximum of 30% within 24 h after administration; however, the plasma concentration of retinol-binding protein remained unchanged.

3.1.3 Tissue distribution

The only systematic information about the concentrations of 9-*cis*-retinoic acid in human tissues is that reported by Arnhold *et al.* (1996; see Table 3 in General Remarks) and in the studies of pharmacokinetics discussed above. The limited information available suggests that the concentrations are likely to be near the low limits of detection of modern analytical procedures based on HPLC.

3.1.4 Variation within human populations

No information was available about possible differences in the metabolism of 9-*cis*-retinoic acid within human populations.

3.2 Experimental models

3.2.1 Metabolism

9,13-Di-*cis*-retinoic acid was identified by HPLC–mass spectroscopy as a major metabolite of 9-*cis*-retinoic acid in the plasma of female mice given the compound orally at a dose of 50 mg/kg bw. A number of polar metabolites were found, including the β-glucuronides of 9-*cis*-retinoic acid and of 9-*cis*-4-oxoretinoic acid (Tzimas *et al.*, 1994a).

After radiolabelled 9-*cis*-retinoic acid was given orally at 10 or 100 mg/kg bw or intravenously at 10 mg/kg bw to male and female Sprague-Dawley rats, 9-*cis*-4-hydroxy- and 9-*cis*-4-oxoretinoic acid were the major metabolites. 9-*cis*-Retinoic acid also isomerized to 13-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid and all-*trans*-retinoic acid. The amount of volatile radiolabelled products increased with time after dosing, suggesting that β-oxidation of 9-*cis*-retinoic acid might occur. 9-*cis*-13,14 Dihydroretinoic acid was identified by nuclear magnetic resonance spectrometry as a metabolite, and the authors suggested that this represented an initial step in the β-oxidation of 9-*cis*-retinoic acid (Shirley *et al.*, 1996). The proposed oxidative and reductive metabolic pathways for 9-*cis*-retinoic acid in rats are shown in Figure 1.

In pregnant mice and rats given 9-*cis*-retinoic acid as a single oral dose of 100 mg/kg bw, 9-*cis*-retinoyl-β-glucuronide was the major metabolite in plasma and in all the tissues examined, but the concentrations were much larger in mouse than in rat plasma, suggesting species differences in the absorption and metabolism of this compound (Sass *et al.*, 1994).

Unanaesthetized and continuously anaesthetized male Wistar rats housed in metabolic cages were given 9-*cis*-retinoic acid at a single oral dose of 30 mg/kg bw and followed for 72 h. Urine and faeces were collected at 24-, 48- and 72-h intervals. Most of the elimination occurred through the faeces, and about 75% was unchanged 9-*cis*-

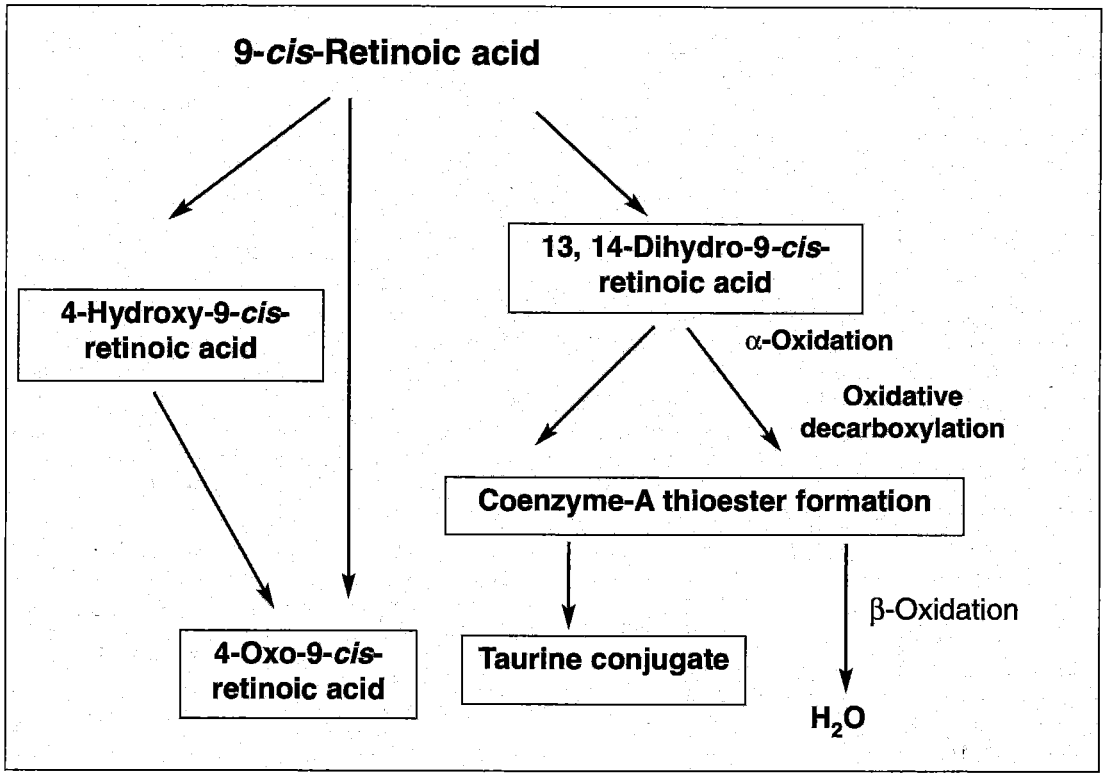


Figure 1. Proposed oxidative and reductive metabolic pathways for 9-*cis*-retinoic acid in rats

retinoic acid. The remainder of the excretion products were all-*trans*- and 13-*cis*-retinoic acid and 9-*cis*-, all-*trans*- and 13-*cis*-4-oxoretinoic acids (Disdier *et al.*, 1996). Unlike Sass *et al.* (1994, 1995), Disdier *et al.* (1996) found that very little 9-*cis*-retinoic acid is eliminated in either the urine or faeces of unanaesthetized rats as the glucuronide conjugate, and only small quantities of glucuronide conjugates were found in anesthetized rats. Disdier *et al.* (1996) suggested that the discrepancy was due to differences in experimental conditions and/or differences between species.

3.2.2 Kinetics

After female mice received 9-*cis*-retinoic acid at 50 mg/kg bw, the concentrations in plasma reached a maximum within 40–60 min and then declined in a mono-exponential manner with an apparent half-life of 64 ± 32 min. The plasma concentration of 9,13-di-*cis*-retinoic acid 90 min after treatment, about 2 µmol/L, was nearly identical to that of 9-*cis*-retinoic acid (Tzimas *et al.*, 1994a). 9,13-Di-*cis*-retinoic acid was also identified as a major circulating metabolite after oral administration of 9-*cis*-retinal to rats and mice (Tzimas *et al.*, 1995).

In nude mice given all-*trans*- or 9-*cis*-retinoic acid at a single oral dose of 10 mg/kg bw, the peak concentration of 9-*cis*-retinoic acid in plasma occurred earlier (15–30 min) than that of all-*trans*-retinoic acid (60–180 min). Both the maximum plasma concentrations and the AUC values were lower for 9-*cis*-retinoic acid than all-*trans*-retinoic acid. In animals given a second dose of either compound two days after the first, the value for the AUC was decreased for all-*trans*-retinoic acid but increased for 9-*cis*-retinoic acid due apparently to the appearance of a second 9-*cis*-retinoic acid peak in the blood 180 min after dosing. The authors speculated that the increase was due to changes in the rate and/or site(s) of uptake or reabsorption of

9-*cis*-retinoic acid from the bile. Treatment with all-*trans*- and 9-*cis*-retinoic acid significantly decreased the concentrations of all-*trans*-retinol in the plasma of nude mice, by 50–60% within 4 h. The reduction was greater after a second dose was given two days after the first and was sustained for at least 48 h (Achkar *et al.*, 1994).

3.2.3 Tissue distribution

No systematic studies of the tissue distribution of 9-*cis*-retinoic acid in animals were available.

3.2.4 Inter-species variation

The metabolic and pharmacokinetic studies summarized above indicate marked species differences in the metabolism of 9-*cis*-retinoic acid in humans and rodents.

4. Cancer-preventive Effects

4.1 Humans

No data were available to the Working Group.

4.2 Experimental models

4.2.1 Cancer and preneoplastic lesions

These studies are summarized in Table 2.

4.2.1.1 Mammary gland

Groups of 24 (control) or 12 (treated) female Sprague-Dawley rats, 50 days of age, were injected intravenously with *N*-methyl-*N*-nitrosourea (MNU) at a dose of 50 mg/kg bw. One week later, the treated animals were given an experimental diet containing 60 or 120 mg/kg 9-*cis*-retinoic acid for 4.5 months. The incidence of mammary adenocarcinomas was 100% in controls and 58% and 25% at the low and high doses of 9-*cis*-retinoic acid ($p < 0.002$ and $p < 0.001$, respectively, Fisher's exact test). The tumour multiplicity was 3.6 for controls and 1.6 and 0.3 at the low and high dose, respectively ($p < 0.002$ in both cases; Mantel non-parametric test). The combination of 9-*cis*-retinoic acid with tamoxifen was more effective than either agent alone (Anzano *et al.*, 1994).

The experiment was repeated with similar results in a later study. 9-*cis*-Retinoic acid in combination with raloxifene was more effective than either agent alone (Anzano *et al.*, 1996).

4.2.1.2 Prostate

Groups of 30–40 male Wistar-Unilever (HsdCpb: WU) rats, seven to eight weeks of age, were treated with cyproterone acetate at a dose of 50 mg/kg bw by gavage for 21 days; then, one day later, with testosterone propionate at three daily doses of 100 mg/kg bw by subcutaneous injection; then, 60 h later, a single injection of 30 mg/kg bw MNU; then, two weeks later, with two Silastic tubing implants containing 40 mg testosterone. Treatment with 9-*cis*-retinoic acid at a dose of 50 or 100 mg/kg diet was initiated one week before MNU administration. The study was continued for 13 months after treatment with the carcinogen. The combined incidence of prostate adenocarcinomas and carcinosarcomas in all accessory sex glands was 79% in controls and 33% and 48% at the low and high doses of 9-*cis*-retinoic acid. The incidence of prostate adenocarcinomas was reduced from 65% in controls to 20% and 18% at the low and high doses of retinoid ($p < 0.01$; Fisher's exact two-sided test) (McCormick *et al.*, 1999).

4.2.1.3 Colon

Groups of 30–33 male Fischer 344 rats, eight to nine weeks of age, were injected intraperitoneally with azoxymethane at 15 mg/kg bw weekly for two weeks. The animals were maintained on a AIN76A diet alone or supplemented with 9-*cis*-retinoic acid at 0.1 mmol/kg of diet beginning one week before the first injection of carcinogen until the end of the study 36 weeks later. The incidence of colon adenocarcinomas was 33% in controls and 25% in rats given 9-*cis*-retinoic acid (not significant). 9-*cis*-Retinoic acid reduced the number of adenomas per rat from 3.2 to 2.2 ($p < 0.01$, ANOVA) and reduced the number of aberrant crypt foci per colon from 153 to 97 ($p < 0.01$, ANOVA) (Zheng *et al.*, 1997).

4.2.2 Intermediate biomarkers

9-*cis*-Retinoic acid at 0.1 mmol/kg of diet reduced the incidence of azoxymethane-induced aberrant crypt foci in the colon of rats (for details, see section 4.2.1.3; Zheng *et al.*, 1997).

4.2.3 In-vitro models

4.2.3.1 Cellular studies

The effects of 9-*cis*-retinoic acid have been analysed primarily in established tumour cell lines in monolayer culture, although a few studies were

Table 2. Effects of 9-*cis*-Retinoic acid (9-*cis*-RA) on carcinogenesis in rats

Cancer site	Strain, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose (mg/kg bw). route	9-*cis*-RA dose/route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	9-*cis*-RA	Control	9-*cis*-RA		
Mammary gland	Sprague-Dawley, female, 50 d	24	MNU 50 mg/kg bw, i.v.	60 mg/kg	+ 1 wk to end	100	58*	3.6	1.6*	Effective	Anzano *et al.* (1994)
		12		120 mg/kg		100	25*	3.6	0.3*	Effective	
Mammary gland	Sprague-Dawley, female, 50 d	24 12	MNU 50 mg/kg bw, i.v.	60 mg/kg	+ 1 wk to end	96	75	3.0	1.7	Effective	Anzano *et al.* (1996)
Colon	Fischer, 8–9 weeks	30–33	AOM, 15 mg/kg bw	0.1 mmol/kg diet	–1 wk to 36 wks	33	25	3.3	2.2*	Effective[a]	Zheng *et al.* (1997)
Prostate	Wistar, male, 7–8 weeks	30–40	50 mg/kg bw cyproterone acetate 21 d, 100 mg/kg bw testosterone proprionate s.c. 3 days, 30 mg/kg bw MNU i.v., 40 mg testosterone s.c.	50 mg/kg diet	–1 wk to end	65	20*	NR	NR	Effective	McCormick *et al.* (1999)
						65	18*	NR	NR	Effective	

MNU, *N*'-Methyl-*N*-nitrosourea; i.v., intravenously; AOM, azoxymethane; s.c., subcutaneously; NR, not reported

* Statistically significant (see text)

[a] Effective in reducing aberrant crypt foci and adenomas

carried out with immortalized cells. In general, the effects of 9-*cis*-retinoic acid were similar to those of all-*trans*-retinoic acid in that both inhibited cell proliferation and induced differentiation and apoptosis in some cell lines, perhaps because of their overlapping mechanisms of action. They were also found to have different effects on particular cell systems, perhaps because of their distinct mechanisms of action.

(a) Inhibition of cell proliferation

9-*cis*-Retinoic acid enhanced clonal growth of myeloid and erythroid cells from normal individuals and was more potent than all-*trans*-retinoic acid in stimulating the myeloid cells (Sakashita *et al.*, 1993).

9-*cis*-Retinoic acid, like all-*trans*-retinoic acid, inhibited the growth of the human HPV-16-immortalized ectocervical epithelial cells (Agarwal *et al.*, 1996) and inhibited the proliferation of a large panel of Epstein-Barr virus-immortalized lymphoblastoid cell lines with accumulation of cells in G_0/G_1 and no apparent direct cytotoxicity (Pomponi *et al.*, 1996).

9-*cis*-Retinoic acid inhibited the growth of gastric cancer cell lines without arresting them in G_0/G_1 (Naka *et al.*, 1997). Its inhibitory effects on DNA synthesis in cultured human breast cancer cell lines were equivalent to those of all-*trans*-retinoic acid (Anzano *et al.*, 1994). 9-*cis*-Retinoic acid also inhibited the growth of human breast cancer cells such as T47D under anchorage-dependent and anchorage-independent conditions (Darro *et al.*, 1998). Both 9-*cis*-retinoic acid and all-*trans*-retinoic acid at concentrations ranging from 10 nmol/L to 1 µmol/L inhibited the growth of all-*trans*-retinoic acid-sensitive NB4 cells and of fresh cells from 11 patients with acute promyelocytic leukaemia (Miller *et al.*, 1995). 9-*cis*-Retinoic acid was more potent than all-*trans*-retinoic acid in suppressing the clonal growth of two cell lines and samples from 13 patients with acute myelocytic leukaemia and samples from four patients with acute promyelocytic leukaemia (Sakashita *et al.*, 1993). Samples from three patients with acute myelocytic leukaemia responded to the growth inhibitory effects of 9-*cis*-retinoic acid but were refractory to all-*trans*-retinoic acid (Sakashita *et al.*, 1993). 9-*cis*-Retinoic acid, like all-*trans*-retinoic acid, inhibited the growth in monolayer culture of several cells lines with oestrogen receptors (ERs) but not in those without. The inhibited cells accumulated in G_1. In addition, 9-*cis*-retinoic acid induced down-regulation of ER mRNA and protein and the expression of the oestrogen-responsive genes *PR* and *pS2* in MCF-7 cells (Rubin *et al.*, 1994). The growth of neuroblastoma cells was inhibited by 9-*cis*-retinoic acid in association with suppression of *myc* expression, and 9-*cis*-retinoic acid was 5–10 times more potent than all-*trans*-retinoic acid in this respect (Han *et al.*, 1995). The growth inhibitory effect of 9-*cis*-retinoic acid was reversible in studies in which this was examined, such as in human oral squamous-cell carcinoma cell lines (Giannini *et al.*, 1997).

(b) Modulation of differentiation

9-*cis*-Retinoic acid modulates differentiation in several types of cells. Treatment of human keratinocytes cultured in a submerged culture system with 9-*cis*-retinoic acid for up to five weeks induced a more proliferative phenotype with a longer lifespan than control cultures. The small proline-rich proteins, SPRR1 and SPRR2, were repressed weakly and strongly, respectively; the induction of involucrin was delayed, whereas expression of *Ki67* and of c-*jun* was maintained (Gibbs *et al.*, 1996). 9-*cis*-Retinoic acid induced differentiation in cells from patients with acute promyelocytic leukaemia and acute myeloid leukaemia (Sakashita *et al.*, 1993). Although similar effects on the induction of differentiation in NB4 acute promyelocytic leukaemia cells were observed after treatment with all-*trans*-retinoic acid or 9-*cis*-retinoic acid at 0.1 µmol/L, 9-*cis*-retinoic acid at 0.01 µmol/L was more active than all-*trans*-retinoic acid (Zhu *et al.*, 1995)

9-*cis*-Retinoic acid induced differentiation in cell lines from patients with acute promyelocytic and acute myelogenous leukaemia in primary culture and in HL60 and NB4 cells (Sakashita *et al.*, 1993; Zhu *et al.*, 1995). It is a more potent inducer of differentiation than all-*trans*-retinoic acid in HL60 cells but only at low concentrations in NB4 cells (Zhu *et al.*, 1995). 9-*cis*-Retinoic acid was 5–10 times more potent in inducing neuroblastoma cell differentiation (neurite outgrowth, increased acetylcholinesterase activity) than all-*trans*-retinoic acid (Han *et al.*, 1995). In human head-and-neck squamous carcinoma cells, 9-*cis*-retinoic

acid, like all-*trans*-retinoic acid, suppressed squamous differentiation (decreased the level of keratin K1) and induced RARβ expression (Zou *et al.*, 1999).

(c) Induction of apoptosis

9-*cis*-Retinoic acid induced apoptosis even in cells that did not undergo apoptosis after treatment with all-trans-retinoic acid. It induced apoptosis in some HL-60 sublines even without inducing differentiation, whereas all-trans-retinoic acid could not induce apoptosis unless the cells had first undergone differentiation to mature granulocytes. 9-*cis*-Retinoic acid also induced apoptosis in several human small-cell lung carcinoma cell lines (Güzey *et al.*, 1998), in adult T-cell leukaemia cell lines (Fujimura et al., 1998), in NB4 acute promyelocytic leukaemia cells (Bruel *et al.*, 1995) and in neuroblastoma cell lines (Lovat *et al.*, 1997a).

In one cellular system of apoptosis, in which activation of T-cell hybridomas induces a block at G_1/S in the cell cycle and apoptosis, 9-*cis*-retinoic acid inhibited apoptosis by suppressing the expression of Fas ligand (Yang *et al.*, 1995).

4.2.3.2 Antimutagenicity in short-term tests

No published reports were found of studies of the effect of 9-*cis*-retinoic acid on carcinogen- or mutagen-induced genotoxicity either *in vitro* or *in vivo*. The possible effect of this retinoid on cytochrome P450 (CYP) systems was examined in two studies (Table 3). The messenger RNA levels of three CYP isoenzymes were measured in primary rat hepatocytes cultured for 48 h in the presence of 9-*cis*-retinoic acid at 40 µmol/L. An eightfold increase was found for CYP3A ($p < 0.05$), a slight increase for CYPyp1A1 (not significant) and no change for CYPyp1A2 (Jurima-Romet *et al.*, 1997).

In male Sprague-Dawley rats treated with 9-*cis*-retinoic acid at a dose of 30 mg/kg bw per day by gavage for four days, the hepatic levels of CYP2B1/2 and CYP4A increased by over twofold ($p < 0.05$), while that of CYP2E was reduced by 33% ($p < 0.05$) and those of CYP1A2 by 27%, CYP2C11

Table 3. Effects of 9-*cis*-retinoic acid on metabolic activity *in vitro* and *in vivo*

Dose and route	Cells or animals	Investigated effect	Result[a]	LED/HID[b]	Reference
40 µmol/L	Rat hepatocytes	Cytochrome P450 (CYP) mRNA levels			Jurima-Romet *et al.* (1997)
		CYP1A1	–	40 µmol/L	
		CYP1A2	–	40 µmol/L	
		CYPCyp3a1/2	#	40 µmol/L	
30 mg/kg bw per day by gavage for 4 days	Male Sprague-Dawley rats	Liver CYP protein levels		30 mg/kg bw per day by gavage for 4 days	Howell *et al.* (1988)
		CYP2B1/2	#		
		CYP Cyp2C11	–		
		CYP2E	+		
		CYPCyp3A	–		
		CYP4A	#		
		Total CYP concentration	+		
		Effect on retinoid CYP metabolism (glucuronidation)	–		

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

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

by 18% and CYP3A by 4% (all non-significant). When microsomal fractions from 9-*cis*-retinoic acid-treated animals were tested for the ability to metabolize this retinoid *in vitro*, PCYP-mediated metabolism was unchanged and a slight decrease was observed in glucuronidation, although the effect was not significant (Howell *et al.*, 1998).

4.3 Mechanisms of cancer prevention

Some reports and the more extensive information available on all-*trans*-retinoic acid suggest that 9-*cis*-retinoic acid exerts its effect on carcinogenesis at the promotion stage. The mechanisms that could account for the chemopreventive activities of 9-*cis*-retinoic acid are discussed below.

4.3.1 Antagonism of tumour promotion and AP-1 activity

In human bronchial epithelial cells, AP-1 transcriptional activity was reduced markedly by 9-*cis*-retinoic acid (Lee *et al.*, 1996). In a cell line of normal rabbit synovial fibroblasts, 9-*cis*-retinoic acid inhibited the induction of collagenase (metalloproteinase MMP-1) by antagonizing AP-1 at the transcriptional level (Pan *et al.*, 1995). These findings suggest that some of the chemopreventive effects of 9-*cis*-retinoic acid may derive from its antagonistic effects on AP-1.

4.3.2 Inhibition of cell proliferation

9-*cis*-Retinoic acid inhibited the proliferation of several cell lines *in vitro* (see section 4.2.3), including arrestation of some cells in the G_1 phase of the cell cycle (Fujimura *et al.*, 1998). *In vivo*, 9-*cis*-retinoic acid reduced mitotic activity and enhanced apoptosis in adenomas that develop *in vivo* in rats exposed to azoxymethane, and these effects were also considered to be the mechanism by which 9-*cis*-retinoic acid prevented aberrant crypt foci and colon tumours (Zheng *et al.*, 1999). The possible mechanisms of growth inhibition include changes in cell cycle regulatory proteins and modulation of autocrine loops.

4.3.2.1 *Cyclins and cyclin D kinase inhibitors*

Because lesions of human noninvasive breast carcinoma *in situ* overexpress cyclin D, agents that can reduce the level of this cyclin may be useful in chemoprevention. 9-*cis*-Retinoic acid inhibited the levels of expression of cyclins D1 and D3 in human MCF-7, ZR-75 and T-47D breast carcinoma cells *in vitro*, and similar effects were observed in the immortalized HBL-100 and MCF-10A breast cell lines. 9-*cis*-Retinoic acid also suppressed the levels of Cdk2 and Cdk4. These data suggest that 9-*cis*-retinoic acid suppresses cell cycle progression from G_1 to S by reducing cyclin D expression in a variety of breast cell lines *in vitro* (Zhou *et al.*, 1997). In gastric cancer cell lines, 9-*cis*-retinoic acid inhibited growth after a transient increase in the amount of the cyclin-dependent kinase inhibitor, p21/Waf1/Cip1 protein, and also reduced the amount of cdk-7, epidermal growth factor receptor and cyclin D1 proteins. This was followed by a reduction in phosphorylation of the product of the retinoblastoma tumour suppressor gene in sensitive TMK-1 cells but not in resistant MKN-7 cells. These results suggest that the cytostatic effect of 9-*cis*-retinoic acid on gastric cancer cells is mediated through changes in the cell cycle regulatory machinery (Naka *et al.*, 1997).

4.3.2.2 *Modulation of autocrine and paracrine loops*

9-*cis*-Retinoic acid can interfere with autocrine loops, such as that associated with prolactin, which plays an important role in the induction and progression of mammary tumours. 9-*cis*-Retinoic acid down-regulated prolactin receptors in breast cancer cell lines within 1 h, and the maximal effect was achieved within 24 h. It was suggested that this effect on the prolactin signalling pathway is relevant for cancer prevention (Widschwendter *et al.*, 1999). Another growth stimulatory pathway affected by 9-*cis*-retinoic acid is that involving insulin-like growth factor. Treatment of Hs578T breast cancer cells with 9-*cis*-retinoic acid at 100 nmol/L increased the level of insulin-like growth factor binding protein 3 in the conditioned medium. It was suggested that this binding protein contributes to the growth inhibitory effect of 9-*cis*-retinoic acid by reducing the growth stimulatory effect of exogenous insulin-like growth factor-I (Colston *et al.*, 1998). The third example involves the estrogen and ER signalling pathway. 9-*cis*-Retinoic acid inhibited the growth in monolayer culture of several ER-positive, but not ER-negative, cell lines. MCF-7 cells exposed to 9-*cis*-retinoic acid showed a dose-dependent accumulation in G_1. 9-*cis*-Retinoic acid

down-regulated ER mRNA and protein in MCF-7 cells, accompanied by decreased expression of the oestrogen-responsive genes *PR* and *pS2* in MCF-7 cells (Rubin *et al.*, 1994).

4.3.3 Restoration of normal differentiation

The ability of 9-*cis*-retinoic acid to modulate the differentiation of normal and malignant cells might be related to its chemopreventive effects. For example, normal human bronchial epithelial cells often undergo abnormal squamous differentiation in primary culture *in vitro* under certain conditions with exposure to certain growth factors. 9-*cis*-Retinoic acid could restore normal differentiation to such cells as it can inhibit the mRNA expression of the squamous differentiation markers transglutaminase type I, involucrin, keratin 5 and keratin 13 (Lee *et al.*, 1996). 9-*cis*-Retinoic acid also induced differentiation of neuroblastoma (Han *et al.*, 1995; Lovat *et al.*, 1997b) and acute promyelocytic cells (Elstner *et al.*, 1997).

4.3.3.1 Induction of apoptosis

The ability of 9-*cis*-retinoic acid to induce apoptosis in a variety of tumour cell lines even without inducing differentiation (Bruel *et al.*, 1995; Nagy *et al.*, 1995; Fujimura *et al.*, 1998) suggests that this effect may occur also in premalignant cells and thereby mediate some of its effects on carcinogenesis. Further support for this conclusion comes from the finding that 9-*cis*-retinoic acid enhanced the apoptotic index in non-involved crypts and in adenomas that developed in azoxymethane-treated rats (Zheng *et al.*, 1999).

4.3.3.2 Increased cell adhesion

9-*cis*-Retinoic acid induced E-cadherin in the human SK-BR-3 breast carcinoma cell line, and it was suggested that this could be a change towards a more normal phenotype (Anzano *et al.*, 1994). Because E-cadherin is not only an adhesion molecule but also functions as a tumour suppressor, its induction by 9-*cis*-retinoic acid could explain some of the chemopreventive effect of the latter.

4.3.3.3 Mechanistic considerations

9-*cis*-Retinoic acid is a pan-RAR, RXR agonist. As such, it can exert its action through RARs, as does all-*trans*-retinoic acid. There is considerable evidence both *in vitro* and in genetic studies that RAR and RXR ligands synergize within the RAR–RXR heterodimer, which is believed to be the major—albeit not the sole—molecular species that mediates retinoid action (Kastner *et al.*, 1995; Lotan *et al.*, 1995; Chambon, 1996). 9-*cis*-Retinoic acid is expected to be a more potent ligand of the RAR–RXR heterodimer and more potent *in vitro* than all-*trans*-retinoic acid owing to its ability to activate both subunits simultaneously. The affinity of 9-*cis*-retinoic acid for RARs is similar to that of all-*trans*-retinoic acid but about 20–50 times greater than the affinity of all-*trans*-retinoic acid for RXRs (Allenby *et al.*, 1993). Since in mammalian cell systems all-*trans*-retinoic acid and 9-*cis*-retinoic acid can be interconverted by unknown enzymatic systems, both isomers may contribute to the pharmacological response elicited when animals are exposed to either compound. In addition to acting through the RAR–RXR heterodimer, 9-*cis*-retinoic acid can act, at least in principle, through RXR homodimers and through a multitude of RXR heterodimers with other nuclear receptors, such as the thyroid hormone, vitamin D, peroxisome proliferator-activated (PPAR) and various so-called orphan receptors (see General Remarks, section 3). There is no firm evidence that a RXR homodimer signalling pathway exists, but the impact of RXR ligands on signalling pathways involving other RXR heterodimers such as, for example, PPAR–RXR (Mukherjee *et al.*, 1997) has to be taken into account when evaluating the biological action of an RXR ligand.

5. Other Beneficial Effects

No reports of well-conducted studies with 9-*cis*-retinoic acid in humans on conditions other than cancer 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.

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

The toxicity of 9-*cis*-retinoic acid is similar to that of other retinoids and mimics the symptoms of hypervitaminosis A. The most frequent effects include headache and adverse changes in the skin and mucous membranes. Commonly reported anomalies in clinical chemistry include hypercalcaemia and lipid abnormalities. Most of the adverse reactions are dose-dependent and reversible.

In a phase-I trial of 9-*cis*-retinoic acid in advanced cancer in which 34 patients received a single daily dose of 5–230 mg/m^2 per day for four weeks, the recommended single daily dose of 9-*cis*-retinoic acid was determined to be 140 mg/m^2 per day (Miller *et al.*, 1996). In another phase-I study, in 22 patients with solid tumours, the subjects received 20–150 mg/m^2 per day in two equal doses, and the recommended dose for continued evaluation was 100 mg/m^2 per day (Kurie *et al.*, 1996).

7.1.1.1 Retinoic acid syndrome

In a clinical study of 9-*cis*-retinoic acid in acute promyelocytic leukaemia, three of 12 patients receiving 30–230 mg/m^2 per day were treated with corticosteroids at high doses for signs suggestive of retinoic acid syndrome (Soignet *et al.*, 1998).

7.1.1.2 Toxicity in the central nervous system and general toxicity

One of the most commonly reported adverse effects of 9-*cis*-retinoic acid is headache, which can range from mild to severe. In 41 healthy men who received a single oral dose of 5–150 mg 9-*cis*-retinoic acid per day, the incidence but not the severity of headache increased with dose, affecting all subjects given doses ≥ 80 mg (Weber & Dumont, 1997). Headache occurred in all of seven subjects with acute promyelocytic leukaemia receiving 30–230 mg/m^2 per day (Miller *et al.*, 1995) and in 15 of 16 patients receiving 50–230 mg/m^2 per day (Soignet *et al.*, 1998). Headache was the most common and often the dose-limiting effect in three studies of 9-*cis*-retinoic acid in patients with cancer (Kurie *et al.*, 1996; Miller *et al.*, 1996; Rizvi *et al.*, 1998). Headaches associated with administration of 9-*cis*-retinoic acid can often be controlled by medication, although unrelenting headache (Rizvi *et al.*, 1998) and migraine (Weber & Dumont, 1997) have been reported.

Other general signs associated with oral administration of 9-*cis*-retinoic acid include fatigue (Kurie *et al.*, 1996; Soignet *et al.*, 1998) and diffuse pain (Miller *et al.*, 1996).

Facial flushing is frequently observed after administration of 9-*cis*-retinoic acid, usually within a few hours, and is sometimes associated with headache (Miller *et al.*, 1995, 1996; Weber & Dumont, 1997; Rizvi *et al.*, 1998; Soignet *et al.*, 1998). Insomnia and changes in mental status have also been reported (Kurie *et al.*, 1996; Aboulafia *et al.*, 1998).

7.1.1.3 Mucocutaneous toxicity

In 41 healthy men given 9-*cis*-retinoic acid at a single oral dose of 5–150 mg, the most common adverse events were cutaneous and consisted primarily of mild xeroderma at doses ≥ 80 mg/day, accompanied by pruritis in one participant (Weber & Dumont, 1997). No significant mucocutaneous reactions were reported in seven patients with acute promyelocytic leukaemia given 30–230 mg/m^2 per day for 3–62 days (Miller *et al.*, 1995). In another study in patients with this disease, dry skin was the second most common adverse effect at doses of 30–230 mg/m^2 per day (Soignet *et al.*, 1998). Cutaneous reactions were also the second most common adverse effects in patients with advanced cancer treated with 9-*cis*-retinoic acid once or twice daily at doses up to 140 mg/m^2 per day. The reactions consisted of grade-1 dry skin and erythema in 10 of 41 patients, and grade-2 peeling of the fingers in one subject (Rizvi *et al.*, 1998). Frequent mucocutaneous reactions of grades 1–2 were seen at doses of 15–230 mg/m^2 per day (Miller *et al.*, 1996), and frequent mucositis was seen at 20–150 mg/m^2 per day (Kurie *et al.*, 1996).

7.1.1.4 Metabolic, nutritional and haematological toxicity

The haematological effects seen after administration of 9-*cis*-retinoic acid appear to be dose-related. No significant change in haematological parameters was reported in five patients with acute promyelocytic leukaemia receiving doses ≤ 140 mg/m^2 per

day (Miller *et al.*, 1995), and no relevant anomalies in serum chemistry were reported in a study of 34 healthy men receiving single doses of 5–150 mg (Weber & Dumont, 1997). Haematological effects occurred in a dose-dependent manner in a study of cancer patients receiving doses of 5–230 mg/m² per day and included grades 1–3 abnormalities in haemoglobin and leukocyte counts and grades 1–2 abnormalities in platelet count. No adverse effects were reported at 5 mg/m² per day, and most of the grades 2 and 3 events occurred at doses ≥ 180 mg/m² per day (Miller *et al.*, 1996).

Metabolic and nutritional events associated with use of 9-*cis*-retinoic acid include hypercalcaemia, hypercholesterolaemia, hypertriglyceridaemia, hyperbilirubinaemia, increased activities of alkaline phosphatase and aspartate aminotransferase, abnormal serum creatinine and glutamate oxaloacetate transferase activity, haematuria and proteinuria (Miller *et al.*, 1995; Kurie *et al.*, 1996; Miller *et al.*, 1996). In one study, the occurrence of hypertriglyceridaemia was related to dose and time, increasing with protracted use (Miller *et al.*, 1996). Elevated concentrations of triglycerides were present in 6 of 41 patients receiving 9-*cis*-retinoic acid at doses up to 140 mg/m² per day, but no symptomatic hypertriglyceridaemia was seen. The activitity of transaminases was increased in 4 of 41 patients in this study and was dose limiting for one subject at 83 mg/m² per day and for another at 140 mg/m² per day. Dose-limiting hyperbilirubinaemia occurred in one subject receiving 70 mg/m² per day, and grade 4 hypercalcaemia was reported in two patients receiving 50 mg/m² per day; one of these subjects developed renal failure, seizures, sepsis and respiratory failure and ultimately died (Rizvi *et al.*, 1998). In a phase-II clinical trial of 9-*cis*-retinoic acid for AIDS-associated Kaposi sarcoma, the patients received 60 mg/m² per day for two weeks, followed by escalation to 100 mg/m² per day. After three weeks at the higher dose, a 46-year old man developed hypercalcaemia, changes in mental status and renal insufficiency; the symptoms improved within two days of cessation of use of 9-*cis*-retinoic acid (Aboulafia *et al.*, 1998).

Recently identified molecular interactions provide some insight into the mechanism of hyperlipoproteinaemia observed during clinical treatment with retinoids. The lipoprotein levels of 43 patients receiving 9-*cis*-retinoic acid or targretin were compared. Treatment with 9-*cis*-retinoic acid resulted in statistically significant dose- and time-dependent changes from baseline values for plasma triglycerides (increased by 59%), cholesterol (increased by 16%) and high-density lipoprotein cholesterol (decreased by 15%). Treatment with targretin had little effect on these parameters, but the level of apolipoprotein A-1 (apo A-I) tended to be substantially higher in patients taking targretin than in those given 9-*cis*-retinoic acid. In preclinical studies, transcription of the anti-atherogenic apo A-I of the high-density lipoprotein complex was found to be regulated by RXRs. These preliminary data suggested that RXR-selective ligands maintain high-density lipoprotein cholesterol and apo A-I and thus minimize the complications of chronic hyperlipidaemia seen with pan receptor agonists such as 9-*cis*-retinoic acid (Nervi *et al.*, 1997).

7.1.1.5 Musculoskeletal toxicity

Generalized bone pain with a slow onset of hypercalcaemia, eventually requiring medication, was reported in one patient with acute promyelocytic leukaemia receiving 9-*cis*-retinoic acid at 140 mg/m² per day (Miller *et al.*, 1995), and grades 1–3 bone pain occurred in 4 of 18 patients with this disease given 9-*cis*-retinoic acid at doses of 50–140 mg/m² per day (Soignet *et al.*, 1998). Arthralgia and myalgia have also been associated with administration of 9-*cis*-retinoic acid (Kurie *et al.*, 1996; Miller *et al.*, 1996).

7.1.1.6 Gastrointestinal effects

The gastrointestinal effects of 9-*cis*-retinoic acid appear to be dependent on the clinical state of the person taking the drug. Three of 41 healthy male subjects given a single oral dose of 5–150 mg experienced episodic vomiting; in one of these subjects, the vomiting was associated with a migraine headache (Weber & Dumont, 1997). No significant gastrointestinal effects were observed in one study of patients with acute promyelocytic leukaemia receiving 30–230 mg/m² per day (Miller *et al.*, 1995). In another study in this population, grade-1 nausea and vomiting were reported in one subject at 50 mg/m² per day (Soignet *et al.*, 1998). Nausea, vomiting, anorexia and diarrhoea have been reported in cancer patients participating in clinical trials with 9-*cis*-retinoic acid (Kurie *et al.*,

1996; Rizvi *et al.*, 1998). Diarrhoea was one of two dose-limiting effects of 9-*cis*-retinoic acid in one phase-I study, occurring in two patients with colorectal cancer taking 150 mg/m^2 per day.

7.1.1.7 Ocular disorders

As is commonly observed with retinoids, conjunctivitis and blurry vision have been reported during clinical evaluation of 9-*cis*-retinoic acid (Kurie *et al.*, 1996; Rizvi *et al.*, 1998). Ocular toxicity, characterized by detachments of the retinal pigment epithelium and retinal haemorrhage, was dose-limiting in one subject taking 9-*cis*-retinoic acid at 140 mg/m^2 per day (Rizvi *et al.*, 1998).

7.1.1.8 Respiratory effects

Six of 16 patients with lung cancer experienced dyspnoea when taking 9-*cis*-retinoic acid at doses of 50–230 mg/m^2 per day (Miller *et al.*, 1996). Dyspnoea was also reported in a phase-I trial of 9-*cis*-retinoic acid in patients with acute promyelocytic leukaemia given doses of 30–230 mg/m^2 per day (Soignet *et al.*, 1998).

7.1.2 Experimental models

The only toxic effect seen in a number of studies of chemoprevention with 9-*cis*-retinoic acid was weight loss. In athymic nude mice with xenografts of human oral squamous-cell carcinoma, the maximum tolerated oral dose of 9-*cis*-retinoic acid was 60 mg/kg bw, which produced a 4.2% decrease in body weight and mild mucocutaneous irritation after 24 days of treatment on five days per week. The weight loss and mucocutaneous reactions were dose-dependent: no adverse effects were seen at 10 or 30 mg/kg bw per day, while a 10% weight loss and mild-to-moderate mucocutaneous reactions were seen at 100 mg/kg bw per day (Shalinsky *et al.*, 1995). Five doses of 30 mg/kg bw per week were well tolerated in the same model (Shalinsky *et al.*, 1996).

In a model of mammary carcinogenesis induced by MNU, no signs of gross toxicity were observed in female rats fed 60 or 120 mg/kg of diet for 3 or 4.5 months, although some loss of body weight was observed (Anzano *et al.*, 1994). In another model of MNU-induced mammary tumours, rats were given 9-*cis*-retinoic acid intraperitoneally at 100 mg/kg bw per day on days 0, 1, 2, 3 and 9. Transient losses of body weight (up to 15%), alopecia and eye crusting were observed; daily dosing caused the death of some animals (Hsu, 1998). In a model of colon cancer induced by azoxymethane, 9-*cis*-retinoic acid was given to rats at a dose of 30 mg/kg of diet, since transient weight loss was observed after three weeks at 60 mg/kg, and the dose of 300 mg/kg was reported to be toxic [no details provided] (Zheng *et al.*, 1997).

Treatment of nude mice with 9-*cis*-retinoic acid at a single oral dose of 10 mg/kg bw decreased the plasma retinol concentration by 50–60% for at least 48 h; the decrease was greater after a second dose two days later (Achkar *et al.*, 1994).

7.2 Reproductive and developmental effects

7.2.1 Humans

No data were available to the Working Group.

7.2.2 Experimental models

7.2.2.1 Reproductive effects

In vitamin A-deficient mice, 9-*cis*-retinoic acid stimulated the differentiation and proliferation of growth-arrested spermatogonia in the testis (Gaemers *et al.*, 1998).

7.2.2.2 Developmental effects

The teratogenic effects of 9-*cis*-retinoic acid are summarized in Table 4. The teratogenic potency of this compound lies between that of the all-*trans* and 13-*cis* isomers. 9-*cis*-Retinoic acid induces cleft palate and limb defects in mice.

Studies with mice, rats and rabbits have shown a relationship between retinoid structure and the extent of placental transfer. Transfer of 9-*cis*-retinoic acid was intermediate between that of all-*trans*-retinoic acid and 13-*cis*-retinoic acid (Tzimas *et al.*, 1994b; Kochhar *et al.*, 1995), while the embryonic concentrations of 9-*cis*-retinoic acid β-glucuronide were < 5% of their plasma concentrations after administration of 9-*cis*-retinoic acid at mid-gestation (Tzimas *et al.*, 1995). [The Working Group noted that the poor transplacental passage of the β-glucuronide is in accordance with its hydrophilic character and high relative molecular mass.]

It is not clear how a small structural variation such as isomerization at C-13 and/or C-9 results in such drastic differences in the degree of placental transfer, because several physicochemical parameters of the retinoic acid isomers, such as relative

Table 4. Teratogenic effects of 9-*cis*-retinoic acid

Species	Dose (mg/kg bw)	Effects	Reference
Mouse	25; GD 11 100; GD 11	Cleft palate Limb defects	Kochhar *et al.* (1995)
Chick	Soaked bead implant, stage 20	Pattern duplication in wing	Thaller *et al.* (1993)
Xenopus embryo	Stages 8–18	Pattern formation in embryo	Creech Kraft *et al.* (1994)
Rat	Microinjection into cultured embryos on GD 10	Branchial arch and some somite defects	Creech Kraft & Juchau (1993)

GD, gestation day

molecular mass, pK_a and lipophilicity, are very similar or identical (Tzimas *et al.*, 1994b). In contrast, these retinoids display marked differences in their binding to embryonic cellular retinoic acid-binding proteins (CRABPs): whereas all-*trans*-retinoic acid is a high-affinity ligand of CRABP I and II, 13-*cis*-retinoic acid and 9-*cis*-retinoic acid bind to them with much lower affinity, if at all (Siegenthaler & Saurat, 1989; Allenby *et al.*, 1993; Fiorella *et al.*, 1993; Horst *et al.*, 1995).

7.3 Genetic and related effects

7.3.1 Humans

No data were available to the Working Group.

7.3.2 Experimental models

No data were available to the Working Group.

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

9-*cis*-Retinoic acid is synthesized from 9-*cis*-retinol by oxidation of the C-15 alcohol group to a carboxylic acid. Like all members of the vitamin A family, 9-*cis*-retinoic acid is lipophilic, sensitive to light, heat and oxygen and readily isomerized to a mixture of *cis* and *trans* isomers. Because of its acidic nature, it is slightly more soluble in water than retinol or retinal, but still poorly so. 9-*cis*-Retinoic acid has characteristic absorption spectra in the ultraviolet and visible, infrared and resonance Raman portions of the electromagnetic spectrum owing to its tetraene structure.

9-*cis*-Retinoic acid and its 4-oxo metabolite are present in blood and tissues of animal species in smaller amounts than retinol or retinyl ester and are not present in plant tissues. Human exposure occurs during treatment with topical or oral preparations for medical purposes.

9-*cis*-Retinoic acid has been used to treat acute promyelocytic leukaemia, and a topical formulation is approved for the treatment of Kaposi sarcoma. The maximal oral dose used in clinical studies of cancer is 100–150 mg/m^2 per day.

9-*cis*-Retinoic acid is usually separated by high-performance liquid chromatography and detected by its absorption at 345 nm. After chemical formation of a suitable ester, it can also be separated and detected by gas–liquid chromatography and can be quantified by mass spectrometry.

8.2 Metabolism and kinetics

Although 9-*cis*-retinoic acid is a potent ligand for retinoid X receptors, the mechanism for its endogenous presence in cells has not been established unequivocally. Three metabolic pathways have been proposed: (i) sulfhydryl groups in small molecules like glutathione and in proteins can catalyse the interconversion of 9-*cis*- and all-*trans*-retinoic acid; (ii) enzymes that can oxidize 9-*cis*-

retinol and 9-*cis*-retinal have been identified, suggesting that 9-*cis*-retinoic acid may be synthesized from 9-*cis*-retinol; and (iii) 9-*cis*-retinoic acid can be generated by cleavage of dietary 9-*cis*-β-carotene. Both 4-oxo- and glucuronide metabolites of 9-*cis*-retinoic acid have been identified in studies of pharmacokinetics in humans and animals.

8.3 Cancer-preventive effects

8.3.1 Humans

No data were available to the Working Group.

8.3.2 Experimental models

The preventive efficacy of 9-*cis*-retinoic acid was evaluated in two studies of carcinogen-induced mammary carcinogenesis, one on prostate carcinogenesis and one on colon carcinogenesis, in rats. 9-*cis*-Retinoic acid prevented mammary and prostate tumours but not colon tumours; however, it reduced the numbers of aberrant crypt foci and adenomas in the colon.

In general, the effects of 9-*cis*-retinoic acid *in vitro* were similar to those of all-*trans*-retinoic acid, in that both inhibited cell proliferation and induced differentiation and apoptosis in some cell lines; however, the 9-*cis* isomer was more potent than the all-*trans* isomer in several cell systems. 9-*cis*-Retinoic acid caused growth inhibition in normal, immortalized and malignant cell lines, often but not always in G_0 or G_1. Induction of differentiation and apoptosis were seen in several types of cells. The cells that were sensitive to 9-*cis*-retinoic acid responded to concentrations that are achieved in plasma with standard pharmacological doses *in vivo*.

The potential ability of 9-*cis*-retinoic acid to inhibit carcinogen-induced genotoxicity has not been studied *in vitro* or *in vivo*; however, two studies suggest that it could affect damage induced in DNA by a carcinogen by altering some cytochrome P450 isozymes both *in vitro* and *in vivo*.

8.3.3 Mechanisms of cancer prevention

9-*cis*-Retinoic acid appears to suppress cell proliferation and increase differentiation and apoptosis. The mechanisms by which proliferation can be inhibited involve antagonism of AP-1, decreased concentrations of cyclins and increased amounts of cyclin-dependent kinase inhibitor and intervention in growth-stimulating signalling pathways. Induction of apoptosis and differentiation also appear to contribute to the putative cancer-preventive effect of 9-*cis*-retinoic acid.

8.4 Other beneficial effects

No data were available to the Working Group.

8.5 Carcinogenicity

No data were available to the Working Group.

8.6 Other toxic effects

8.6.1 Humans

The toxicity of 9-*cis*-retinoic acid is similar to that of other retinoids and may result in symptoms similar to those of hypervitaminosis A. The most frequent signs and symptoms include headache and adverse skin and mucous membrane reactions. Most of the adverse reactions are dose-dependent and are reversible when therapy is discontinued. Symptoms of 'retinoic acid syndrome', a potentially life-threatening condition, have been observed during oral therapy with 9-*cis*-retinoic acid. The haematological effects that occur with administration of 9-*cis*-retinoic acid are reduced haemoglobin, leukocyte and platelet counts. The reported metabolic and nutritional effects include hypercalcaemia, hypercholesterolaemia, hypertriglyceridaemia, hyperbilirubinaemia, elevated alkaline phosphatase and aspartate aminotransferase activity, abnormal serum creatinine and glutamate oxaloacetate transferase activity, haematuria and proteinuria. The gastrointestinal effects of 9-*cis*-retinoic acid appear to be dependent on the clinical state of the person taking the drug and can limit the dose that can be given in certain instances, such as in the treatment of patients with colorectal cancer. No studies were available on the reproductive or developmental effects of 9-*cis*-retinoic acid or on its genotoxicity in humans.

8.6.2 Experimental models

Administration of 9-*cis*-retinoic acid to athymic nude mice decreased body weight and caused mucocutaneous reactions, alopecia and eye crusting.

No data were available on the effects of 9-*cis*-retinoic acid on reproductive parameters in animals. Orally administered 9-*cis*-retinoic acid is teratogenic in mice.

9. Recommendations for research

9.1 General recommendations for 9-*cis*-retinoic acid and other retinoids

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

9.2 Recommendations specific to 9-*cis*-retinoic acid

1. Clarify whether 9-*cis*-retinoic acid is a physiologically significant ligand in cell differentiation and its role in cancer chemoprevention.

2. Study in more detail the effects and mechanisms of action of 9-*cis*-retinoic acid in humans and in animal models.

10. Evaluation

10.1 Cancer-preventive activity

10.1.1 Humans

There is *inadequate evidence* that 9-*cis*-retinoic acid has cancer-preventive activity in humans.

10.1.2 Experimental animals

There is *limited evidence* that 9-*cis*-retinoic acid has cancer-preventive activity in experimental animals. This evaluation is based on the observation of inhibitory effects in two studies of mammary carcinogenesis and one of prostate carcinogenesis in rats.

10.2 Overall evaluation

There are no data on the cancer preventive activity of 9-*cis*-retinoic acid in humans. 9-*cis*-Retinoic acid is a known teratogen in mice.

11. References

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