Handbook 2

13-*cis*-Retinoic acid

1. Chemical and Physical Characteristics

1.1 Nomenclature

See General Remarks, section 1.4

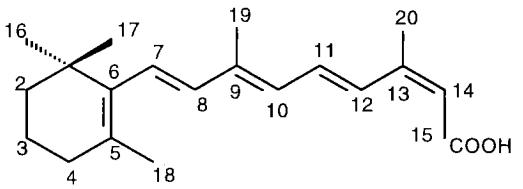
1.2 Name: 13-*cis*-Retinoic acid

Chemical Abstracts Services Registry Number
4759-48-2

IUPAC Systematic name
(7*E*,9*E*,11*E*,13*Z*)-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*Z*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,2,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-oic acid

Synonyms
13*Z*-Retinoic acid, 13-*cis* vitamin A acid, 13-*cis* vitamin A_1 acid, Isotretinoin®, Accutane®, Isotrex®, Roaccutane

1.3 Structural formula



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

1.4 Physical and chemical properties

Description
Reddish-orange plates from isopropyl alcohol

Melting-point
174–175 °C (Budavari *et al.*, 1996).

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

Spectroscopy
UV and visible: λ_{max} 354 (ethanol), $E^{1\%}_{1\ cm}$ 1325, E_M 39 750 (Frickel, 1984; Budavari *et al.*, 1996; Barua & Furr, 1998).

Nuclear magnetic resonance spectroscopy
^{1}H-NMR ($CDCl_3$, 220 MHz): δ 1.03 (1-CH_3), 1.46 (2-CH_2), 1.63 (3-CH_2), 1.72 (5-CH_3), 2.00 (9-CH_3, 4-CH_2), 2.10 (13-CH_3), 5.69 (14-H), 6.17 (8-H), 6.29 (7-H, 10-H), 7.03 (11-H), 7.77 (12-H); $J_{7,8}$ (16 Hz), $J_{10,11}$ (11.5 Hz), $J_{11,12}$ (15 Hz) (Schweiter *et al.*, 1969; Vetter *et al.*, 1971; Frickel, 1984; Barua & Furr, 1998).

^{13}C-NMR ($CDCl_3$, 68 MHz) δ 12.9 (9-CH_3), 19.4 (3-C), 21.1 (13-CH_3), 21.6 (5-CH_3), 29.0 (1,1-CH_3), 33.3 (4-C), 34.4 (1-C), 40.0 (2-C), 115.9 (14-C), 128.9 (7-C), 129.4 (12-C), 130.1 (5-C), 130.3 (10-C), 132.9 (11-C), 137.4 (8-C), 137.9 (6-C), 140.3 (9-C), 153.3 (13-C), 171.4 (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. 13-*cis*-Retinoic acid in solution is protected by the presence of antioxidants, such as butylated hydroxytoluene and pyrogallol. A variety of factors influence the stability of 13-*cis*-retinoic acid in tissue culture media. Degradation and isomerization are minimized by storage under an inert gas such as argon, at ≤ –20 °C in the dark (Frickel, 1984; Barua & Furr, 1998).

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

2.1 Occurrence

The mean concentration of 13-*cis*-retinoic acid in the plasma of fasting individuals is 5.4 nmol/L (Eckhoff & Nau, 1990; Blaner & Olson, 1994). The

mean concentration of a major metabolite, 13-*cis*-4-oxoretinoic acid, is somewhat higher, i.e. 11.7 nmol/L (Eckhoff & Nau, 1990). Most other tissues of the body also contain 13-*cis*-retinoic acid, at concentrations 10 or more times higher than that of plasma (Napoli, 1994). The 13-*cis*-retinoic acid concentration is < 1% that of all-*trans* retinol in human plasma and < 5% that of total vitamin A in the tissues of healthy animals and humans. Since 13-*cis*-retinoic acid is present, if at all, in only traces in plants, it is a very minor constituent of the diet, and consequently contributes very little to the intake of dietary vitamin A. 13-*cis*-Retinoic acid, unlike vitamin A and carotenoids, is not available as a dietary supplement.

2.2 Production

The synthesis of 13-*cis*-retinoic acid is based on that of the all-*trans* isomer (see Handbook 1, p. 96), but with discrete modifications. Thus, condensation of a *trans*-β-C_{15}-aldehyde with ethyl senecioate in the presence of sodium or lithium amide in liquid ammonia gives 13-*cis*-retinoic acid, whereas use of potassium amide yields the all-*trans* isomer (Mayer & Isler, 1971). In several chemical syntheses, the final product is a mixture of the all-*trans* and 13-*cis* isomers of retinoic acid or its esters, which then can be separated (Frickel, 1984). Photoisomerization of all-*trans* retinoids in a nonpolar solvent like hexane yields significant mounts of the 13-*cis* isomer (Frickel, 1984). 13-*cis*-Retinal can also be converted to its acid by mild oxidants (Mayer & Isler, 1971; Frickel, 1984).

2.3 Use

13-*cis*-Retinoic acid is primarily used for treating dermatological disorders (Peck & DiGiovanna, 1994; Vahlquist, 1994) and has also been used to treat several types of human cancer (Hong & Itri, 1994). It was first marketed in the United States in 1982 and in Europe somewhat later, primarily for the treatment of severe nodulocystic acne (Nau *et al.*, 1994).

Skin disorders that have been treated with 13-*cis*-retinoic acid are summarized in Table 1. Although the usual oral doses are 1–2 mg/kg bw per day (Peck & DiGiovanna, 1994; Vahlquist, 1994), such doses often induce adverse side-effects, as discussed in section 7.1. Topical preparations of 13-*cis*-retinoic acid in creams or gels have sometimes been used to treat acne vulgaris, but few other skin disorders (Vahlquist, 1994).

Some precancerous conditions and cancers treated with 13-*cis*-retinoic acid are summarized in Table 2. Oral doses of 0.5–2 mg/kg bw per day have commonly been used, but lower oral doses (5–10 mg/day) have also been employed (Hong & Itri, 1994).

2.4 Human exposure

13-*cis*-Retinoic acid was first introduced into commerce in the United States in 1982, and over the next 60 months 800 000 patients received the drug (Orfanos, 1985; Stern, 1989). Approximately 40% of all 13-cis-retinoic acid prescriptions are written to women, and between 1990 and 1995, the number of prescriptions written to women doubled (Holmes *et al.*, 1998). As already indicated, the amount of retinoic acids in food is very small, probably in the range of 10–100 μg/day. Because 13-*cis*-retinoic acid is rapidly metabolized by the body and is not stored in the liver or other organs, it does not accumulate over time (Blaner & Olson, 1994). As a consequence, exposure to 13-*cis*-retinoic acid is limited, for all practical purposes, to the oral treatment of medical disorders.

Table 1. Some skin disorders treated with 13-*cis*-retinoic acid

Acne vulgaris	Lupus erythematosis
Darier's disease	Nodulocystic acne
Eruptive keratoacanthoma	Pityriasis rubra pilaris
Granuloma annulare	Psoriasis
Hydradenitis suppurativa	Rosacea
Ichthyosis congenita	Scleroderma
Lichen planus	Warts

Modified from Vahlquist (1994) and from Peck and DiGiovanna (1994)

Table 2. Some precancerous conditions and cancers that have been treated with 13-*cis*-retinoic acid

Acute promyelocytic leukaemia
Chronic myelogenous leukaemia
Cutaneous T-cell lymphoma/mycosis fungoides
Skin cancer
Actinic keratosis
Bladder cancer
Cervix cancer
Breast cancer
Head and neck cancer
Oral leukoplakia
Laryngeal papillomatosis
Lung cancer
Myelodysplastic syndrome

Modified from Hong and Itri (1994)

2.5 Analysis

13-*cis*-Retinoic acid in plasma and tissues is commonly measured 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/methanol, diethyl ether, dichloromethane, acetonitrile, 2-propanol or ethyl acetate. After the combined extract with anhydrous sodium sulfate has been dried, 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.

A reversed-phase C_{18} column is usually used for the separation. The compound is usually detected by measuring the absorption at 354 nm, and it is quantified by measuring the area of 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 the retinoids present.

A large number of chromatographic systems have been devised for the separation and quantification of retinoic acid (Frolik & Olson, 1984; Furr *et al.*, 1992, 1994; Barua & Furr, 1998; Barua *et al.*, 1999). In most reversed-phase HPLC systems, 13-*cis*-retinoic acid is eluted before all-*trans*-retinoic acid.

13-*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 13-*cis*-retinoic acid can be measured (Barua *et al.*, 1999).

3. Metabolism, Kinetics and Genetic Variation

It is well accepted that 13-*cis*-retinoic acid is a naturally occurring form of retinoic acid that is normally present in blood and tissues of humans and higher animals (Blaner & Olson, 1994). Since 13-*cis*-retinoic acid is not as active in *trans*-activation assays as the all-*trans*- and 9-*cis*-retinoic acid isomers (Mangelsdorf *et al.*, 1994), it is also generally believed not to be a key retinoic acid for regulating gene transcription. This isomer is, however, effective in inducing retinoid-responsiveness in cells in culture and in animal models when given in large pharmacological doses. 13-*cis*-Retinoic acid is effective in treating human disease, especially dermatological conditions, and it is proposed that many of its effects are mediated by all-*trans*-retinoic acid after isomerization of the 13-*cis* isomer.

Current understanding of the metabolism, plasma transport and tissue distribution of 13-*cis*-retinoic acid in humans and animal models after administration of large doses is reviewed below. More information about the endogenous (physiological) metabolism of 13-*cis*-retinoic acid is given in the General Remarks.

3.1 Humans

3.1.1 Metabolism

It is generally assumed that 13-*cis*-retinoic acid is formed by isomerization of all-*trans*-retinoic acid or possibly by isomerization of 9-*cis*-retinoic acid. As can be seen in Figure 1 of the General Remarks, however, it is theoretically possible that 13-*cis*-retinoic acid is formed in a manner analogous to all-*trans*-retinoic acid, through sequential oxidation of 13-*cis*-retinol and 13-*cis*-retinal. One enzyme that can catalyse the oxidation of 13-*cis*-

retinol to 13-*cis*-retinal has been described (Driessen *et al.*, 1998), but it has not been established that 13-*cis*-retinol or 13-*cis*-retinal is present in significant quantities in human tissues, and it is not presently clear whether 13-*cis*-retinoic acid can be formed through a biosynthetic pathway that does not involve all-*trans*- or 9-*cis*-retinoic acid as the immediate precursor.

13-*cis*-4-Oxoretinoic acid was identified by HPLC and gas chromatography–mass spectrometry as the major circulating metabolite of 13-*cis*-retinoic acid in pooled plasma from patients receiving 20–100 mg/day in the long-term treatment of dermatological disorders. The second most abundant metabolite was all-*trans*-4-oxoretinoic acid. Neither the concentrations of these metabolites nor their distribution relative to that of circulating 13-*cis*- or all-*trans*-retinoic acid was reported (Vane & Bugge, 1981).

The biliary metabolites of 13-*cis*-retinoic acid were investigated in two patients with biliary T-tube drainage after administration of a single oral 80-mg dose of 13-*cis*-[^{14}C]retinoic acid The two patients excreted 23 and 17% of the radiolabel in their bile within four days. HPLC measurements of the extracted bile samples indicated that the β-glucuronide metabolites of 13-*cis*-retinoic acid accounted for about 48% and 44% of the total radioactivity in the bile of the two patients. The two major glucuronide conjugates present were those of 13-*cis*-4-oxoretinoic acid and 13-*cis*-16-hydroxyretinoic acid, with relatively minor amounts of the glucuronide conjugates of 13-*cis*-retinoic acid and 13-*cis*-18-hydroxyretinoic acid (Vane *et al.*, 1990).

13-*cis*-Retinoic acid, 9-*cis*-retinoic acid and all-*trans*-retinol inhibited all-*trans*-retinoic acid 4-hydroxylation by human hepatic microsomes. 13-*cis*- and 9-*cis*-Retinoic acid were competitive inhibitors of all-*trans*-retinoic acid 4-hydroxylation, with K_i:K_m ratios of 3.5 ± 0.8 and 6.3 ± 0.5, respectively. This may suggest that the two *cis*-retinoids are alternative but inferior substrates for the hepatic cytochrome P450 isoforms that mediate the 4-hydroxylation reaction of all-*trans*-retinoic acid (Nadin & Murray, 1996).

13-*cis*-Retinoic acid generated by photo-isomerization of topically administered all-*trans*-retinoic acid may be absorbed percutaneously. After application of a 0.1% cream of all-*trans*-retinoic acid to human cadaver skin *in vitro* with and without exposure to ambient light, 13-*cis*-retinoic acid was detected after 24 h in samples of skin exposed to light but was virtually absent from skin samples maintained in the dark. Since the concentration of 13-*cis*-retinoic acid in the exposed skin sample was similar to that of all-*trans*-retinoic acid, some 13-*cis*-retinoic acid may be absorbed through the skin after photo-isomerization of topically applied all-*trans*-retinoic acid (Lehman & Malany, 1989).

The β-glucuronides of all-*trans*- and 13-*cis*-retinoic acid were found in the circulation of a healthy female volunteer after repeated administration of 13-*cis*-retinoic acid orally at 2.3 μmol/kg bw per day for 27 days. The maximal plasma concentrations of the two β-glucuronides reportedly reached 105 and 13 nmol/L, respectively. The maximal plasma concentration of 13-*cis*-retinoic acid after its oral administration on day 27 was reported to be 90 nmol/L, whereas that of all-*trans*-retinoic acid was about 5.5 nmol/L (Creech Kraft *et al.*, 1991a).

3.1.2 Kinetics

In early work on the pharmacokinetics of 13-*cis*-retinoic acid in humans, phase I and II trials were performed in patients with advanced cancer who received oral doses starting at 0.5 mg/kg bw per day and increasing over four weeks to a maximum of 8 mg/kg bw per day. Although large interindividual differences in peak plasma concentrations were noted, the plasma concentration of 13-*cis*-retinoic acid showed a linear correlation with increasing dose. At the maximal dose, the mean peak plasma concentration was 4 μmol/L. Even after repeated dosing, the drug was rapidly cleared from the plasma. The patients receiving 13-*cis*-retinoic acid had lower plasma retinol concentrations than those of the general population (Kerr *et al.*, 1982).

The pharmacokinetics, blood concentrations and urinary, biliary and faecal excretion of 13-*cis*-[^{14}C]retinoic acid were studied in four healthy male volunteers given a single 80-mg oral dose. About 80% of the dose was recovered as radiolabel in excreta during the study, with approximately equal fractions present in the urine and faeces. A second peak in the blood concentration of radiolabel was observed, suggesting possible enterohepatic circulation of 13-*cis*-retinoic acid. The mean half-life in the blood was 14 h, whereas the corresponding value for the radiolabel was 90 h (Colburn *et al.*, 1985).

Differences in the pharmacokinetics of 13-*cis*-retinoic acid between cancer patients and healthy volunteers have been reported. In these studies, 0.5

mg/kg bw was given orally to four healthy female and four healthy male volunteers and to five patients with cervical carcinoma and 14 male patients with squamous-cell carcinomas of the head and neck. The cancer patients also simultaneously received treatment with interferon-α_{2a} (3 x 10^6 IU three times per week subcutaneously). The mean integrated area under the curve for concentration–time (AUC) for 13-*cis*-retinoic acid was 1.7-fold higher in female patients than in the female volunteers and 1.3 times higher in male patients than in the male volunteers. The maximal blood concentrations of 13-*cis*-retinoic acid in male and female patients were approximately 20% higher than those observed in sex-matched healthy volunteers. Qualitatively similar observations were made for the concentrations of 13-*cis*-4-oxoretinoic acid in the patients with cervical and head-and-neck cancers. The maximal blood concentration of 13-*cis*-4-oxoretinoic acid in female patients was 3.3 times greater than that of the female volunteers and 1.8 times greater in male patients than in the male volunteers. The maximal blood concentrations of 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid and the AUC values for these retinoids were greater for the healthy women than for the healthy men studied. The authors suggested that the higher AUC values of 13-*cis*-retinoic acid observed in the cancer patients may have been due to the treatment with interferon-α_{2a} (Waladkhani & Clemens, 1997).

Twelve patients with melanoma and regional node metastases after radical surgery were randomized for combined treatment for three months with recombinant interferon-α_{2a} and oral 13-*cis*-retinoic acid at a dose of 20 or 40 mg/day. The pharmacokinetics of 13-*cis*-retinoic acid and its effects on plasma retinol concentrations were investigated on the first and last days of the treatment schedule. The maximal plasma concentrations of 13-*cis*-retinoic acid were observed 4 h after dosing, with average values of 1.3 and 2.1 µmol/L after the two doses, respectively. The average half-life of 13-*cis*-retinoic acid in the circulation was approximately 30 h. The maximal plasma concentration, the half-life in the circulation and the AUC value over 0–48 h did not change after multiple dosing, whereas the AUC over 48 h for the major blood metabolite, 13-*cis*-4-oxoretinoic acid, increased. Immediately after receipt of the dose of 13-*cis*-retinoic acid, the plasma retinol concentrations began to decline, and they reached a minimum concentration (a reduction of approximately 20%) shortly after the time of the maximal 13-*cis*-retinoic acid concentration, 4–12 h after treatment. After clearance of 13-*cis*-retinoic acid from the circulation, the plasma concentration of retinol returned to baseline (Formelli *et al.*, 1997).

A reduction in plasma retinol concentrations in patients receiving 13-*cis*-retinoic acid at 1 mg/kg bw per day for six months was reported in a further study. Although 13-*cis*-retinoic significantly reduced the plasma concentrations of all-*trans*-retinol in the 13 women enrolled in the study, from 1.9 ± 0.67 µmol/L at baseline to 1.5 ± 0.40 µmol/L after treatment (p = 0.03), the concentrations of the 22 men enrolled in the study did not significantly change (p = 0.43). The reason for this sex difference is unknown (Lippman *et al.*, 1998).

3.1.3. Tissue distribution

No information was available about the tissue distribution of 13-*cis*-retinoic acid in humans after its administration as a drug.

3.1.4 Variations within human populations

Very limited information was available about possible differences in the metabolism of 13-*cis*-retinoic acid in different human populations. As mentioned above, the maximal blood concentration of 13-*cis*-4-oxoretinoic acid in male and female cancer patients treated with interferon-α_{2a} was greater than that in healthy male and female volunteers after an oral dose of 13-*cis*-retinoic acid. The maximal blood concentrations of 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid and the AUC values for these retinoids were also greater in the four healthy women than in the four healthy men studied. Thus disease state, treatment and sex may affect the way in which 13-*cis*-retinoic acid is taken up and metabolized in humans (Waladkhani & Clemens, 1997).

3.2 Experimental models

Most of the information available on the metabolism, plasma transport and tissue distribution of 13-*cis*-retinoic acid is derived from studies in rats and mice, although significant studies have been carried out in dogs and primates.

3.2.1 Metabolism

In rats, 13-*cis*-retinoyl-β-glucuronide is a major metabolite of 13-*cis*-retinoic acid after its administration at pharmacological doses. In bile duct-

cannulated vitamin A-sufficient male rats given large intravenous doses of 4–20 mg/kg bw, analysis of the bile by reversed-phase HPLC showed that treatment was followed by rapid excretion of metabolites. The major bilary metabolite was 13-*cis*-retinoyl-β-glucuronide, and its rate of excretion increased rapidly after injection to reach a maximum 55 min later but then decreased exponentially. After 330 min of collection, biliary excretion of 13-*cis*-retinoyl-β-glucuronide accounted for 35–38% of the dose. The authors concluded that 13-*cis*-retinoyl-β-glucuronide is a major pathway for the metabolism of pharmacological doses of 13-*cis*-retinoic acid in rats and that neither the glucuronidation nor the biliary excretion pathway is saturated after administration of high pharmacological doses of 13-*cis*-retinoic acid (Meloche & Besner, 1986).

3.2.2 Kinetics

The serum concentrations of 13-*cis*-retinoic acid in male BDF_1 mice given 13-*cis*-retinoic acid orally at 10 mg/kg bw were maximal within 15–30 min and then declined in a monoexponential fashion with a half-life of 19 min. The concentrations in liver, lung, small intestine, fat, kidney, brain, heart, spleen, large intestine, muscle, testis and urinary bladder reached their maxima within 15–30 min, then declined exponentially with half-lives of 11–19 min. The liver showed the highest concentration at each time it was examined, followed by fat, lung and kidney. Only a small amount of unmetabolized 13-*cis*-retinoic acid was observed in bile and faeces, and none was found in urine (Kalin *et al.*, 1982).

The pharmacokinetics of three parenteral 13-*cis*-retinoic acid formulations was studied after intraperitoneal administration to rats of 2.5 mg/360 g bw [6.9 mg/kg bw]; the drug was administered as an alkaline solution, suspended in corn oil or as a mixture with polysorbate 80. The alkaline solution was also given intravenously via the tail vein as a control. The mean elimination rate constant, calculated after the intravenous dose, was 0.72 ± 0.088 per h. The peak concentration in plasma and the time to reach this maximum were 14 mg/L and 0.5 h, 22 mg/L and 2 h and 10 mg/L and 1 h for the three formulations, respectively. The AUC values were 35 ± 8.8 mg-h/L for the intravenous dose and 34 ± 10, 62 ± 32 and 26 ± 12 mg-h/L for the intraperitoneal doses of alkaline solution, suspension in oil and mixture with polysorbate 80, respectively (Guchelaar *et al.*, 1992).

The first-pass metabolism of 13-*cis*-retinoic acid in the gut contents, gut wall and liver of dogs was studied after simultaneous administration of 13-*cis*-[^{12}C]retinoic acid intravenously and 13-*cis*-[^{14}C]retinoic acid orally. Blood samples were obtained from the jugular and the portal veins at specified times to quantify the concentrations of the two labelled compounds. In addition, blood, bile, urine and the gastrointestinal contents were analysed for ^{14}C-containing material. The harmonic mean elimination half-life for the simultaneous intravenous and oral administration of 13-*cis*-retinoic acid was approximately 5.5 h. The mean blood clearance after intravenous administration was 5.2 ± 2.4 ml/min per kg bw, and the intrinsic clearance after oral administration was 6.6 ± 3.7 ml/min per kg bw. The average absolute bioavailability of 13-*cis*-retinoic acid in dogs was 21%, indicating an overall first-pass effect of approximately 80%. About 72% of the first-pass effect occurred in the gut lumen, the gut wall and liver making lesser contributions. These results indicate that the low bioavailability of 13-*cis*-retinoic acid in dogs is due mainly to loss of the drug before it reaches the portal circulation, probably because of biological or chemical degradation in the gut lumen before absorption (Cotler *et al.*, 1983).

The effect of the route of administration of 13-*cis*-retinoic acid and its biliary excretion on its pharmacokinetics was examined in dogs given oral, intraportal and intravenous doses of 13-*cis*-retinoic acid at 2.2–5 mg/kg bw before and after bile-duct cannulation. Blood and bile samples were collected and analysed by HPLC. The concentrations of 13-*cis*-retinoic acid in blood were decreased after bile-duct cannulation, and the decreases in the AUC values were greatest after oral dosing, intermediate after intraportal dosing and least after intravenous dosing. 13-*cis*-Retinoic acid was excreted in the bile primarily as the glucuronide. The largest percentage of the dose (27%) was excreted in the bile after intraportal infusion, an intermediate percentage (8.5%) after intravenous dosing and the smallest percentage (3.3%) after oral dosing. These data indicate that biliary excretion affects the blood profile of 13-*cis*-retinoic acid as a function of route of administration and that the differences are probably the result of differences in first-pass clearance. In addition, the apparent bioavailability of 13-*cis*-retinoic acid was 14% in bile-cannulated dogs and

54% in the uncannulated animals, suggesting that enterohepatic recycling of 13-*cis*-retinoic acid may contribute to its oral bioavailability (Cotler *et al.*, 1984).

After a single dose of 13-*cis*-retinoic acid at 100 mg/kg was given to NMRI mice on day 11 of gestation or three doses of 100 mg/kg bw were given 4 h apart, the major plasma metabolite was 13-*cis*-retinoyl-β-glucuronide, followed by 4-oxo metabolites and all-*trans*-retinoic acid. all-*trans*-Retinoic acid was transferred very efficiently to the mouse embryo, whereas the transfer of 13-*cis*-retinoic acid was 10% and that of 13-*cis*-retinoyl-β-glucuronide was 1% that of all-*trans*-retinoic acid. Interestingly, no embryotoxicity was observed after the single oral dose, whereas the multiple doses enhanced the teratogenicity of 13-*cis*-retinoic acid markedly (Creech Kraft *et al.*, 1991b). In a study of the effects of development stage (gestational age) on the transplacental distribution of 13-*cis*- and all-*trans*-retinoic acid and their glucuronides in rats and mice, 13-*cis*-retinoic acid showed more efficient transplacental passage later in development in both rats and mice. The authors suggested that transplacental transfer of 13-*cis*-retinoic acid is enhanced during late organogenesis because of the time of development of the chorioallantoic placenta in these species (Tzimas *et al.*, 1995).

The maternal kinetics and metabolism of 13-*cis*-retinoic acid were examined in cynomolgus monkeys in two studies. all-*trans*-Retinoic acid was eliminated more rapidly than 13-*cis*-retinoic acid, and the elimination rate tended to increase with repeated dosing. Administration of 13-*cis*-retinoic acid resulted primarily in *cis* metabolites, whereas treatment with all-*trans*-retinoic acid resulted primarily in all-*trans* metabolites. The main metabolites observed after treatment with 13-*cis*-retinoic acid at 2 or 10 mg/kg bw per day were 13-*cis*-4-oxoretinoic acid and 13-*cis*-retinoyl-β-glucuronide. Elimination of 13-*cis*-retinoic acid was more rapid in the monkeys than in humans, and the dose of 13-*cis*-retinoic acid required to produce AUC values comparable to those of humans was approximately 10-fold greater (Creech Kraft *et al.* 1991a).

Pregnant cynomolgus monkeys received 13-*cis*-retinoic acid at 2.5 mg/kg bw by nasogastric intubation once a day on days 16–26 of gestation and twice a day on days 27–31. Maternal plasma kinetics was determined after dosing on days 26 and 31, and placental transfer was studied after the last dose on day 31. The plasma half-life was 13 h. The major plasma metabolite was 13-*cis*-4-oxo-retinoic acid. The concentration of all-*trans*-retinoic acid in maternal plasma was 2% that of 13-*cis*-retinoic acid. β-Glucuronides of both all-*trans*- and 13-*cis*-retinoic acid were found at low concentrations. The authors provided extensive characterization of the kinetics of transfer of 13-*cis*-retinoic acid to and its accumulation in the embryos of treated mothers (Hummler *et al.*, 1994).

In detailed multicompartmental studies of pharmacokinetics in female cynomolgus monkeys, all-*trans*- and 13-*cis*-retinoic acid were injected intravenously at a dose of 0.25 or 0.0125 mg/kg bw and all-*trans*-4-oxo- and 13-*cis*-4-oxoretinoic acid at 0.25 mg/kg bw. The elimination half-life was observed to be longer for the *cis* retinoids and was not dose-dependent: 13-*cis*-4-oxoretinoic acid, 837 min > 13-*cis*-retinoic acid, 301 ± 204 min > all-*trans*-retinoic acid, 38 ± 3 min > all-*trans*-4-oxoretinoic acid, 11 ± 2 min. A second plasma peak attributed to enterohepatic circulation were seen only after administration of 13-*cis*-4-oxoretinoic acid. The volume of distribution was greater for 13-*cis*-retinoic acid than for all-*trans*-retinoic acid (Sandberg *et al.*, 1994).

3.2.3 Tissue distribution

The distribution of 13-*cis*-retinoic acid was studied after its intravenous administration at 10 mg/kg bw to male DBA mice by assessing the concentrations in liver, lung, kidney, brain, testis and small intestine at intervals ranging from 5 min to 6 h after administration. At all the intervals studied, the liver took up the greatest concentration of 13-*cis*-retinoic acid, followed by lung, kidney, brain, small intestine and testis. The concentration present in the liver 5 min after administration was the highest in any tissue at any time (13 ± 1.2 µg/g). The concentration present in lung at any time was approximately 50% of that present in liver. In all tissues examined, two phases of elimination were observed (Wang *et al.*, 1980).

The mean tissue concentrations of 13-*cis*-retinoic acid in small intestine, liver, lung, fat, kidney, brain, heart, spleen, large intestine, muscle, testis and urinary bladder were studied after oral administration of 10 mg/kg bw to male BDF_1 mice. In good agreement with the study of Wang *et al.* (1980), the highest concentrations were found in the small intestine and then in the liver, lung and fat, throughout the 120 min of the study. All the tissues examined took up some 13-*cis*-retinoic acid (Kalin *et al.*, 1982).

3.2.4 Inter-species variation

The studies summarized in sections 3.2.2 and 3.2.3 indicate that rats, mice, dogs, monkeys and humans have different patterns of distribution and metabolism of 13-*cis*-retinoic acid. This is most obvious from the patterns of metabolites that are observed when pharmacological doses of 13-*cis*-retinoic acid are administered to animals and humans. Thus, no animal model truly reflects the metabolism or pharmacokinetics in humans.

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 Cancers of the head and neck

The results of a secondary analysis of a randomized clinical trial of 13-*cis*-retinoic acid for the prevention of progression of primary squamous-cell carcinoma of the larynx, pharynx or oral cavity after primary treatment was reported. Patients received either placebo (n = 51) or 13-*cis*-retinoic acid (n = 49). The initial dose of 13-*cis*-retinoic acid was 100 mg/day, but this was later reduced to 50 mg/day because of toxicity. After a median follow-up of 32 months, there was no difference between the two groups with regard to the recurrence of the primary cancer (15 treated patients and 17 placebo patients, p = 0.77); however, there was a statistically significant difference in the number of patients who developed a second primary cancer (two treated patients and 12 on placebo, p = 0.005). All but one of the second primary tumours was in the upper aerodigestive tract (Hong *et al.*, 1990). A further reanalysis (Benner *et al.*, 1994a) after a median follow-up of 4.5 years indicated that the treated patients continued to have significantly fewer second primary tumours: seven (14%) in the 13-*cis*-retinoic acid arm and 16 (31%) in the placebo arm (p = 0.042). When only the second primary tumours that developed in the area of the upper aerodigestive tract or lungs exposed to tobacco were considered, there were only three tumours in the 49 patients treated with 13-*cis*-retinoic acid, whereas there were 13 in 51 patients receiving placebo (p = 0.008). [The Working Group noted that the results of tests of statistical significance are difficult to interpret when the tests are applied to hypotheses that were not specified at the onset of the trial. The Group also noted that these investigators are conducting a much larger multicentre study to test the hypothesis raised by the findings in their earlier report.]

4.1.2.2 Skin

Clinical experience with high doses of 13-*cis*-retinoic acid for the treatment of basal-cell and squamous-cell cancers of the skin (Peck & DiGiovanna, 1994) led to studies of its possible chemopreventive effects.

In a study of five patients with xeroderma pigmentosum and a history of more than two skin cancers per year during the previous two years, the patients were treated with 13-*cis*-retinoic acid at approximately 2 mg/kg bw per day for two years and then followed for an additional year with no therapy. During treatment, 25 tumours were diagnosed (three to nine per patient), which represented an average reduction of 63% from the number before treatment (p = 0.02). After discontinuation of treatment, the tumour occurrence increased by roughly eightfold (Kraemer *et al.*, 1988). [The Working Group noted that the study did not include an untreated control group or a cross-over design. The patients underwent intense surveillance, and the removal of all suspect skin cancers during the month before beginning 13-*cis*-retinoic acid treatment may have increased the number of cancers diagnosed in the pretreatment phase.]

A randomized, placebo-controlled trial of 13-*cis*-retinoic acid was conducted which involved 981 patients who had a history of prior non-melanoma skin cancer but no known inherited predilection to these tumours, such as xeroderma pigmentosum or basal-cell naevus syndrome. The dose of 13-*cis*-retinoic acid, 10 mg/day (roughly 0.15 mg/kg bw per day) was chosen to minimize the toxic side-effects but was substantially lower than those used in other studies of the treatment or prevention of skin cancer. The three-year cumulative incidence of new skin cancers was virtually identical in the treated and placebo groups. There also was no evidence that treatment had an effect in subgroups of patients categorized by sex, age, solar damage and number of prior tumours. The lack of efficacy pertained to both basal-cell and squamous-cell carcinomas (Tangrea *et al.*, 1992a).

A study to evaluate the efficacy of 13-*cis*-retinoic acid and retinol on the incidence of non-melanoma skin cancer involved 525 subjects with a history of at least four basal-cell carcinomas

and/or cutaneous squamous-cell carcinomas. Subjects were equally assigned at random to receive 13-*cis*-retinoic acid (5–10 mg/day orally), retinol (25 000 units/day orally) or placebo, in a double-blind study design. The time to first occurrence of a new basal-cell and/or cutaneous squamous-cell carcinoma after three years of exposure was the primary end-point used to evaluate efficacy. During the study, 319 basal-cell and 125 cutaneous squamous-cell carcinomas were diagnosed. There was no statistically significant difference in the time to a first new occurrence of either tumour between the group treated with retinoid and that given placebo (Levine *et al.*, 1997).

4.1.2.3 Urinary bladder

A trial was reported in which 13-*cis*-retinoic acid was initially administered at a dose of 0.5 mg/kg bw per day, which was increased to 1 mg/kg bw per day, for the prevention of recurrent early-stage bladder cancer in 20 eligible patients. No control group was included. 13-*cis*-Retinoic acid was toxic, resulting in the dropping out of eight patients from the study before three months and four before six months. Most of the patients had a relapse within one year. The study was terminated because of toxicity and the absence of positive results (Prout & Barton, 1992).

4.1.3 Intermediate end-points

4.1.3.1 Oral cavity

13-*cis*-Retinoic acid has been used in two randomized, placebo-controlled studies on patients with oral premalignant lesions. In an evaluation of treatment of oral leukoplakia with 13-*cis*-retinoic acid in 44 patients, 24 were assigned randomly to treatment at 1–2 mg/kg bw per day for three months, followed by no treatment for six months, and 20 were assigned to placebo. An objective evaluation of the leukoplakias was based on clinical inspection and a requirement for a minimum of four weeks' duration. The size of the lesions was decreased by 50% or more in 16 patients (67%) given 13-*cis*-retinoic acid and two patients (10%) given placebo (p = 0.0002). Histological reversal of the severity of disease was reported in 13 patients (54%) given the retinoid and in two patients (10%) given placebo (p = 0.01). The clinical response correlated with histological response in 56% (9/16) of the patients evaluated. Relapse of leukoplakia occurred in 9/16 patients two to three months after treatment was stopped. The toxic effects of 13-*cis*-retinoic acid were reported to be acceptable in all but two patients and included common retinoid mucocutaneous adverse events and hypertriglyceridaemia. All of the adverse events were reversed by reducing the dose or temporarily discontinuing treatment (Hong *et al.*, 1986).

Four of nine patients treated with 13-*cis*-retinoic acid at 1 mg/kg bw per day for three months showed complete resolution of their oral leukoplakia (Beenken *et al.*, 1994).

When 70 patients were treated with 13-*cis*-retinoic acid at 1.5 mg/kg bw per day for three months, the clinical response rate was 55% (95% confidence interval, 42–67%). The patients were then randomized into two groups, one of which received 13-*cis*-retinoic acid at 0.5 mg/kg bw per day and the other received β-carotene at 64 mg/day, for nine months. The group given 13-*cis*-retinoic acid showed an 8% rate of progression (2/24), whereas those given β-carotene showed a rate of 55% (16/29; $p < 0.001$). The low dose of 13-*cis*-retinoic acid was well tolerated, and no patients dropped out of the trial because of toxic effects (Lippmann *et al.*, 1995a).

Biopsy specimens from patients in the trials described above were evaluated for expression of retinoic acid nuclear receptors and *p53* before and after treatment with 13-*cis*-retinoic acid and compared with those from normal control subjects. In one report (Lotan *et al.*, 1995), all of the normal specimens but only 21/52 (40%) of those from oral premalignant lesions had detectable *RAR-β* mRNA levels at baseline. After three months of treatment with 13-*cis*-retinoic acid, 35/39 (90%) of the oral lesions available for evaluation expressed RAR-β ($p < 0.001$). The authors noted that RAR-β is selectively down-regulated in oral premalignant lesions and that treatment with 13-*cis*-retinoic acid can up-regulate RAR-β in these lesions (Lotan *et al.*, 1995). In a separate report, an inverse relationship was seen between accumulation of *p53* and response to the retinoid (p = 0.006). *p53* accumulation was not modulated by 13-*cis*-retinoic acid (Lippman *et al.*, 1995b). A discrete increase in the micronucleus count in mucosal scrapings of lesions was found when compared with scrapings from mucosa that appeared to be normal at baseline in the 57 patients studied (p = 0.035). A reduction in the micronucleus count was seen after treatment (p = 0.02) (Benner *et al.*, 1994b).

4.1.3.2 Lung

A group of 26 patients with documented cytological abnormalities in sputum samples, ranging from moderate atypical metaplasia to overt carcinoma, were treated with 1–2.5 mg/kg bw per day of 13-*cis*-retinoic acid. No improvement in the degree of atypia was seen. The authors reported alterations in cellular morphology, including increased intracytoplasmic vacuolization, bizarre nuclear shapes, rupture of the nuclear membranes and pyknotic nuclei (Saccomanno *et al.*, 1982).

In a randomized, double-blind, placebo-controlled trial of 13-*cis*-retinoic acid in 86 long-term smokers who had either bronchial dysplasia or a metaplasia index (defined as the number of tissue samples with metaplasia divided by the number of tissue samples counted, multiplied by 100) greater than 15%, the patients initially underwent bronchoscopy, and endobronchial biopsy samples were taken from six sites within the proximate lung field. They were then treated with 13-*cis*-retinoic acid at 1 mg/kg bw per day or placebo for six months. Of the 86 randomized patients, 69 could be assessed after the completion of therapy. There was no evidence of an effect of treatment. The extent of metaplasia had decreased in 19/35 patients (54%) treated with 13-*cis*-retinoic acid and in 20/35 (59%) given placebo (Lee *et al.*, 1994).

4.2 Experimental models

4.2.1 Cancer and preneoplastic lesions

These studies are summarized in Table 3.

4.2.1.1 Skin

Mouse: Groups of 30 female CD-1 mice, eight weeks of age, received skin applications of 200 nmol/L (51.2 µg) of 7,12-dimethylbenz[*a*]-anthracene (DMBA) and were treated topically with 8 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) 14 days after initiation twice weekly for 18 weeks. 13-*cis*-Retinoic acid was given topically at a dose of 34 nmol 1 h before the TPA application. The incidence of papillomas 18 weeks after DMBA initiation was approximately 90% in controls and approximately 50% in mice treated with 13-*cis*-retinoic acid. The multiplicity of tumours was reduced from approximately 10 tumours per mouse to about four by 13-*cis*-retinoic acid (Verma *et al.*, 1979). [The numbers were derived from graphs; no statistics were provided.]

Groups of 20 female Swiss mice, eight weeks of age, were treated topically with 0.8% methylcholanthrene twice weekly for five weeks and once a week for weeks 6–9. One group also received 3 mg/mouse 13-*cis*-retinoic acid topically twice weekly during weeks 3–5 and once a week for weeks 6–9. The animals were killed 23 weeks after the first application of methylcholanthrene. The incidences of papillomas were 89% in controls and 30% in the group given 13-*cis*-retinoic acid [statistics not given]. The incidence of carcinomas was reduced from 26% in controls to 10% in 13-*cis*-retinoic acid-treated mice [statistics not given] (Abdel-Galil *et al.*, 1984).

Female CD-1 mice, eight weeks of age, were initiated with 400 nmol of benzo[*a*]pyrene and one week later stage-I tumour promotion was begun, consisting of twice weekly applications of 3.2 nmol of TPA for two weeks. The animals were then randomized into groups of 20 and stage-II tumour promotion, consisting of twice weekly applications of 8 nmol of TPA for 23 weeks, was begun. Starting on day 1, week 8 or week 23 of stage-II promotion, groups of mice were treated topically with 13-*cis*-retinoic acid, 30 min before TPA application. The control groups were treated with acetone. The experiment was terminated 38 weeks after the start of stage-II promotion. The papilloma yields were four tumours per mouse in the control group and 2.1, 2.9 and 3.3 tumours/mouse in the groups treated from day 1 to week 38, week 8 to week 38 and week 23 to week 38, respectively, with 13-*cis*-retinoic acid during stage-II promotion. The difference in the median number of papillomas per mouse between the group treated from day 1 through week 38 and the control group was statistically significant ($p < 0.05$; one-sided Wilcoxon rank sum test) (Gensler *et al.*, 1986). [The Working Group noted that no data on tumour incidence were given].

Groups of 20 female CD-1 mice, eight weeks of age, received 200 nmol of benzo[*a*]pyrene or 5 µmol of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as initiator and either 17 nmol of TPA or 100 µg of anthralene as promotors for 16 weeks. 13-*cis*-Retinoic acid was applied topically at 34 nmol. The retinoid had no effect on tumour initiation when it was given once 0.5 h before benzo[*a*]pyrene or MNNG and had no effect on tumour promotion when applied 0.5 h before each application of anthralin. The controls had a 70–100% incidence of papillomas, and incidences

Table 3. Effects of 13-*cis*-retinoic acid on carcinogenesis in animals

Cancer site	Species, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose and route	13-*cis*-Retinoic acid dose and route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Skin	CD-1 mice, female, 8 wks	30	0.2 mmol DMBA 8 nmol TPA	34 nmol topical	0 to 18 wk	90	50	10	4	Effective	Verma *et al.* (1979)
Skin	CD-1 mice, female, 8 wks	20	400 nmol B[*a*]P 8 nmol TPA/2x/wk,	17 nmol topical[a]	+2 wk to end	NR	NR	4.0	2.1*	Effective	Gensler *et al.* (1986)
Skin	CD-1 mice, female, 8 wks	20	200 nmol B[*a*]P 17 nmol TPA	34 nmol topical	–0.5 h	80	100	17	21	Ineffective	Gensler & Bowden (1984)
			5 µmol MNNG 17 nmol TPA	34 nmol topical	–0.5 h	100	100	8	7	Ineffective	
			400 nmol B[*a*]P 100 µg anthralene/2x wk	17 nmol topical	+ 1 wk to end	70	80	1.5	1.8	Ineffective	
Skin	CD-1 mice, female, 5–7 wks	30	200 nmol DMBA 444 nmoi anthralene daily/32 wks	1.7 nmoi, topical 17 nmol, topical 170 nmol, topical	+2 wk to end	64	50 55 45	1.52	0.96 1.1 0.6*	Ineffective Ineffective Effective	Dawson *et al.* (1987)
Skin	CD-1 mice, female, 7–9 wks	24	200 nmol DMBA 10 nmol TPA	5 mg/kg diet 50 100 200	–1 to 32 wk	84	84 64 40 25	5.5	4.0[b] 2.7[b] 1.2[b] 0.4[b]	Effective* Effective* Effective* Effective*	Verma *et al.* (1986)
Skin	Swiss mice, female, 8 wks	20	0.8% MCA, 14 applications	3 mg/0.1 mL acetone, topical	+3 to +9 wk	89	30	NR	NR	Effective[c]	Abdel-Galil *et al.* (1984)
Skin	Sencar mice, female, 7–8 wks	25	5 µg DMBA	225 mg/kg diet 30 nmol, topical	+2 wk to end + 2 wk to end	4 4	79* 24*	0.1 0.1	5.9 1.4	Tumour enhancing	McCormick *et al.* (1987)
Urinary bladder	C57BL/6 mice, male, 6–7 wks	20–25	NBHBA 90 mg total 140 mg total i.g., 2 x/wk/6 wks	200 mg/kg diet	+1 wk to end	38 55	5* 32	NR NR	NR NR	Effective Effective	Becci *et al.* (1978)
	Wistar/Lewis rats, female, 7 wks	20–23	1.5 mg MNU, 3 biweekly	120 mg/kg diet 300 mg/kg diet	0 to end	43 43	34 28	NR NR	NR NR	Effective[d] Effective[a]	Squire *et al.* (1977)

Table 3 (Contd)

Cancer site	Species, sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose and route	13-*cis*-Retinoic acid dose and route (basal diet)	Duration in relation to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Urinary bladder (contd)	Fischer 344 rats, female, 21 days	100	2 g/kg diet FANFT (1–10 wks)	120 mg/kg diet 240 mg/kg diet	+ 2 to 50 wk	36 36	42 47	NR NR	NR NR	Ineffective Ineffective	Croft *et al.* (1981)
	Fischer 344 rats, male, 7 wks	30	NBHBA 1200 mg total 1800 mg total 2400 mg total	240 mg/kg diet	1-, 5- and 9 wk delay periods combined	 36 82 94	 17 65 74*	 NR NR	 NR NR	 Effective Effective Effective	Becci *et al.* (1979)
Oesophagus	Sprague-Dawley rats, male, 21 days	40	2 mg/kg bw NMBA Zn-deficient diet	67 mg/kg diet purified diet	0 d to end	76	94	3.04	2.3	Ineffective	Gabrial *et al.* (1982)
Liver	Sprague-Dawley rats, male (110–140 g)	12–24	3'-Me DAB 0.05% in diet	200 mg/kg diet	0 d to 4 wk	90	8	NR	NR	Effective	Daoud & Griffin (1980)
Kidney	Wistar rats, female, 4 wks	40	40 mg/kg bw NDMA i.p.	240 mg/kg diet	2 d to 26 wk	100	100	NR	NR	Ineffective[e]	Hard & Ogiu (1984)
Trachea	Syrian golden hamster, female (152 g)	40–46	MNU, 0.8% for 12 wks, i.t.	120 mg/kg	+1 wk to 6 mth	10	30	NR	NR	Tumour enhancing*	Stinson *et al.* (1981)
Trachea	Syrian, golden hamsters, male, 15 wks	40	MNU (23 times) i.t.(2 x/wk)	172 mg/kg	+1 wk to end	40	55	NR	NR	Ineffective[e]	Yarita *et al.* (1980)
Lung	A/J mice, male, 10 wks	20–40	Urethane i.p. 0.5 mg/kg bw 0.5 mg/kg bw 1.0 mg/kg bw 1.0 mg/kg bw	 150 mg/kg diet 150 mg/kg diet 300 mg/kg diet 300 mg/kg diet	 +2 d to 20 wk +2 d to 20 wk +2 d to 20 wk +2 d to 20 wk	 NR NR NR NR	 NR NR NR NR	 12 12 32 32	 16 16 31 32	 Inefffective Ineffective Ineffective ineffective	 Frasca & Garfinkel (1981)
Salivary glands	Sprague-Dawley rats, male 130–160 g	15	1 mg DMBA injection into gland	100 mg/kg 20 mg/kg AIN76-A diet	0 d to 22 wk	35 35	37 33	NR NR	NR NR	Ineffective	Alam *et al.* (1984)

NR, not reported; DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; B[*a*]P, benzo[*a*]pyrene; MNNG, *N*-methyl-*N*nitro-*N*nitrosoguanidine; MCA, 3-methylcholanthrene; NBHBA, *N*-nitroso-*N*-butyl-*N*-4-hydroxybutylamine; MNU, *N*-methyl-*N*-nitrosourea; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; MBN, *N*-nitrosomethylbenzylamine; 3'-Me DAB, 3'-methyl-4-dimethylaminoazobenzene; NDMA, *N*-nitrosodimethylamine; i.g. intragastrically; i.p., intraperitoneally; i.t., intratracheally; wk, week; d, day

* Statistically significant (see text)

[a] Stage-II tumour promotion

[b] Papillomas ≤ 4 mm diameter

[c] Carcinoma incidence was reduced from 27% in controls to 10% in treated mice.

[d] Based on histological classification of lesions

[e] Kaplan–Meier statistics indicate no protection but an increased risk for dying from tumour.

of 80–100% was seen for all combinations of skin carcinogenesis protocols. Similarly, no effect on tumour multiplicity was found [no statistics given] (Gensler & Bowden, 1984).

Groups of 30 female CD-1 mice, five to seven weeks of age, were treated with 200 nmol of DMBA. Two weeks after initiation, 13-*cis*-retinoic acid was given topically at a dose of 1.7, 17 or 170 nmol twice weekly 1 h before treatment with 444 nmol of anthralene for 32 weeks. At the end of 13-*cis*-retinoic acid treatment, the incidences of skin papillomas were 64% in control mice and 50%, 55% and 45% in the groups at the low, intermediate and high doses of 13-*cis*-retinoic acid. Tumour multiplicity was reduced from 1.5 per mouse in controls to 0.96 at the low dose, 1.1 at the intermediate dose and 0.6 ($p < 0.005$, Student's t test) at the high dose (Dawson *et al.*, 1987).

Groups of 24 female CD-1 mice, seven to nine weeks of age, were treated with 200 nmol of DMBA and 10 nmol of TPA to induce skin papillomas. 13-*cis*-Retinoic acid was included in the basal diet at concentrations of 5–200 mg/kg diet one week before and throughout the 18 weeks of TPA treatment. There were no significant differences in the number of papillomas (all sizes) per group [statistical method not given] or in the percent of animals with tumours [no statistics given]. When only papillomas $\leq$ 4 mm in diameter were counted, the papilloma yields were 4, 2.7, 1.2 and 0.4 in the mice at 5, 50, 100 and 200 mg/kg 13-*cis*-retinoic acid and 5.5 in controls ; the papilloma incidence sin these groups were 84, 64, 40 and 25% respectively, and 84% in controls (p value for tumour multiplicity = 0.0002) [statistical methods and statistics for tumour incidence not given]. In a repeat of the experiment, with 24 female CD1 mice per group, the papilloma multiplicity ($\leq$ 2 mm diameter) was 2.9 in the group receiving 100 mg/kg diet of 13-*cis*-retinoic acid and 4.9 in the controls. In this experiment the mice were allowed to live until carcinomas developed. The carcinoma incidences were 25% in the group receiving 13-*cis*-retinoic acid and 52% in controls [no statistics given]. In another experiment, with 24 female Sencar mice per group, dietary administration of 13-*cis*-retinoic acid at 50 or 100 mg/kg diet had no effect on the incidence or yield of papillomas of all sizes, but when only papillomas $\leq$ 2 mm in diameter were counted, the papilloma yield was 7.2, 5.3 and 2.1 in the control group and the groups on diets containing the two doses of 13-*cis*-retinoic acid [no statistics given] (Verma *et al.*, 1986).

Groups of 25 female Sencar mice, seven to eight weeks of age, were initiated with a single application of 5.0 µg DMBA. Two weeks after initiation, mice received either basal diet or a diet supplemented with 225 mg/kg 13-*cis*-retinoic acid. At 30 weeks after initiation, the control groups had 0.1 papillomas per mouse and a 4% incidence of skin papillomas, whereas mice treated with 13-*cis*-retinoic acid had 5.9 papillomas per mouse and an incidence of 79% ($p < 0.01$, log rank analysis). In the same study, a group of 25 mice was treated with 30 nmol of 13-*cis*-retinoic acid topically twice a week for 28 weeks. The incidence of papillomas was enhanced from 0.1 papillomas per mouse and an incidence of 4% in the controls to 1.4 papillomas per mouse and an incidence of 24% ($p < 0.05$, log rank analysis) in 13-*cis*-retinoic acid-treated animals (McCormick *et al.*, 1987).

4.2.1.2 Urinary bladder

Mouse: Groups of 20–25 male C57BL/6 mice, six to seven weeks of age, were treated intragastrically twice weekly for six weeks with a total dose of 90 or 140 mg *N*-nitroso-*N*-butyl-*N*-4-hydroxybutylamine (NBHBA). One week after the last dose of carcinogen, the mice were given either basal diet or a diet supplemented with 200 mg/kg 13-*cis*-retinoic acid as gelatinized beadlets. All animals were killed six months after the first dose of carcinogen. The incidence of bladder carcinomas was 38% with the low dose of carcinogen and 55% at the high dose; the retinoid treatment reduced the carcinoma incidences to 5% and 32%, respectively. The effect of 13-*cis*-retinoic acid on the low dose of carcinogen was significant ($p < 0.01$; χ^2 analysis) (Becci *et al.*, 1978).

Rat: Groups of 30 male Fischer 344 rats received 100, 150 or 200 mg of NBHBA intragastrically twice a week for six weeks for total doses of 1200, 1800 and 2400 mg. At one, five or nine weeks after the last intubation of NBHBA, the rats were fed either control diet or a diet containing 240 mg/kg 13-*cis*-retinoic acid. The animals were killed one year after the first administration of carcinogen. The incidence and average number of transitional-cell carcinomas was dependent on the dose of carcinogen, and 13-*cis*-retinoic acid reduced the incidences of transitional-cell carcinomas with severe atypia in all groups ($p < 0.01$; Poisson probability distribution) (Becci *et al.*, 1979).

Groups of 20–23 female Wistar/Lewis rats, six to seven weeks of age, were given three doses of 1.5 mg *N*-methyl-*N*-nitrosourea (MNU) by instillation

into the bladder twice a week. 13-*cis*-Retinoic acid was included in their diet as gelatinized beadlets at concentrations of 120 or 300 mg/kg diet either with the first instillation of MNU or one day after the last instillation. All animals were killed nine months after the first MNU treatment. The results for the two groups given 13-*cis*-retinoic acid were combined for statistical analysis. The incidence of bladder tumours was 43% in controls and 34% and 28% at the low and high doses of 13-*cis*-retinoic acid (not statistically significant). When a histological classification was made of the preneoplastic lesions and invasion of epithelial cells was scored, a comparison between the control and 13-*cis*-retinoic acid groups showed statistically significant inhibition of the development of preneoplastic lesions and of cell invasion ($p < 0.05 - < 0.01$, one-sided trend test) (Squire *et al.*, 1977).

Groups of 100 female Fischer 344 rats, 21 days of age, were given 2 g/kg of diet of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide for 10 weeks to induce urinary bladder carcinomas, and were then given 0, 120 or 240 mg/kg of diet of 13-*cis*-retinoic acid or a diet with gelatinized beadlets (extra control group of 20 animals), beginning at week 12 and continuing to the end of the study at 50 weeks. The incidence of urinary bladder transitional-cell carcinomas was 36% in the rats given the gelatinized beadlets and 42% and 47% ($p < 0.05$; exact method for contingency tables) with the low and high doses of 13-*cis*-retinoic acid, whereas the incidence of urinary bladder carcinomas in the controls on the basal diet was 85% ($p < 0.001$ as compared with the controls fed gelatinized beadlets) (Croft *et al.*, 1981).

4.2.1.3 Oesophagus

Groups of 40 male weanling Sprague Dawley rats were given *N*-nitrosomethylbenzylamine intragastrically twice a week for four weeks at a dose of 2 mg/kg bw, in combination with a zinc-restricted diet (7 ppm zinc). 13-*cis*-Retinoic acid was added to the diet at a concentration of 67 mg/kg from the beginning until the end of the study at 29 weeks. The incidence of oesophageal cancers was 76% in control rats and 94% in the 13-*cis*-retinoic acid-treated group. The multiplicity of tumours was 3 in the controls and 2.3 in the 13-*cis*-retinoic acid-treated group [no statistics given] (Gabrial *et al.*, 1982).

4.2.1.4 Liver

Groups of 12–25 male Sprague-Dawley rats weighing 110–140 g bw were given a diet containing 0.05% 3'-methyl-4-dimethylaminoazobenzene for nine weeks. The diet of one group of rats also contained 200 mg/kg 13-*cis*-retinoic acid during the first four weeks of the experiment. The study was terminated at nine weeks. The incidence of liver tumours was 90% in controls and 8% in rats receiving 13-*cis*-retinoic acid (Daoud & Griffin, 1980). [The Working Group noted the short duration of the experiment and that no statistics were given.]

4.2.1.5 Kidney

Groups of 40 female Wistar rats, 21 days of age, received 40 mg/kg bw *N*-nitrosodimethylamine intraperitoneally as a single injection and 13-*cis*-retinoic acid at a concentration of 240 mg/kg diet for 26 weeks. Both the control group and that given 13-*cis*-retinoic acid had a 100% incidence of kidney tumours [no statistics given] (Hard & Ogiu, 1984).

4.2.1.6 Respiratory tract

Groups of 40 male Syrian golden hamsters, 15 weeks of age, received two intratracheal instillations of a 1% solution of MNU each week for 18 or 23 exposures. 13-*cis*-Retinoic acid was given in the diet just after the last injection of carcinogen and continued until the end of the study 56 weeks after the last injection, at a concentration of 128 mg/kg of diet for those animals receiving 18 exposures and 172 mg/kg of diet for those receiving 23 exposures. Neither dietary concentration affected the number of tumour-bearing animals. The incidence of invasive carcinomas was 55% in the controls and 40% in the hamsters that received the dose of 172 mg/kg but the relative risk for dying from tracheal tumours was 1.7 for those animals that received 128 mg/kg and 2 for those that received 172 mg/kg ($p = 0.223$ and 0.043, respectively; χ^2 test of log-likelihood ratio) (Yarita *et al.*, 1981).

Similar results were reported by Stinson *et al.* (1981; see Table 3).

4.2.1.7 Lung

A/J male mice, 10 weeks of age were given a single intraperitoneal injection of urethane at a dose of 0.5 or 1 mg/g bw [500 or 1000 mg/kg bw]. Two days later, groups of 20–40 mice were given a basal diet containing control gelatin beadlets or a diet containing 13-*cis*-retinoic acid at 150 or 300 mg/kg. All animals were killed 20 weeks after urethane injection. The number of pulmonary adenomas per mouse (surface tumours) was 12 and

32 in mice injected with 0.5 and 1 mg/g bw of urethane, respectively, and maintained on control diet and 16 and 16 in the groups injected with the low dose of urethane and maintained on 150 or 300 mg/kg of diet of 13-*cis*-retinoic acid, respectively. The tumour multiplicity was 31 and 33 in the groups injected with the high dose of urethane and maintained on 150 or 300 mg/kg of diet of 13-*cis*-retinoic acid (Frasca & Garfinkel, 1981). [The Working Group noted that the tumour incidence was not given, and no statistical analysis was reported.]

4.2.1.8 Salivary gland

Groups of 14–16 male Sprague-Dawley rats were anaesthetized and dissected to expose their submandibular salivary glands. DMBA (1 mg in 20 µL olive oil) was injected into the glands and the wound was closed. The animals then received either semi-purified AIN 76A diet alone or supplemented with 13-*cis*-retinoic acid at 20 or 100 mg/kg. The study was terminated 22 weeks after initiation. The incidence of malignant salivary gland tumours was 35% in control rats, 37% in rats given the diet containing 20 mg/kg 13-*cis*-retinoic acid and 33% in rats given the diet containing 100 mg/kg (Alam *et al.*, 1984).

4.2.2 Intermediate biomarkers

Mice were given 0.2 µmol of DMBA in 0.2 mL of acetone as an initiator followed by twice weekly applications of 8 nmol of TPA; 13-*cis*-retinoic acid was applied topically 1 h before the TPA application. The mice were killed 4.5 h after the last of seven TPA treatments, and ornithine decarboxylase, which is considered a useful biomarker for experimental skin carcinogenesis, was measured. 13-*cis*-Retinoic acid reduced the activity from 3.5 to 0.04 nmol (Verma *et al.*, 1979).

4.2.3 In-vitro models

The effects of 13-*cis*-retinoic acid were analysed primarily on established tumour cell lines in monolayer culture. A few studies were carried out with immortalized cells.

4.2.3.1 Inhibition of cell proliferation

The inhibitory effects of 13-*cis*-retinoic acid were studied in various cultured cell types. The effects were usually dose-dependent in the concentration range between 1 nmol/L and 1 µmol/L and were time-dependent, being detected within 24–48 h. When changes in the cell cycle were analysed, it was found that 13-*cis*-retinoic acid treatment resulted in cell accumulation in the G_1 phase of the cell cycle. The inhibitory effects were often reversible within a few days after the drug was discontinued.

In most cell lines, the response to 13-*cis*-retinoic acid was similar to the response to all-*trans*-retinoic acid, but in some cells (e.g. head-and-neck carcinoma cell lines) 13-*cis*-retinoic acid and 9-*cis*-retinoic acid were more potent than all-*trans*-retinoic acid in inhibiting cell proliferation (Giannini *et al.*, 1997). In contrast, in other cells (e.g. gastric carcinoma) all-*trans*-retinoic acid was more potent than 13-*cis*-retinoic acid in growth suppression *in vitro* (Jiang *et al.*, 1996).

(a) Immortalized cells

13-*cis*-Retinoic acid, like all-*trans*-retinoic acid, inhibited the growth, increased the adhesiveness and induced tissue transglutaminase in spontaneously immortalized NIH-3T3 mouse fibroblasts (Cai *et al.*, 1991). 13-*cis*-Retinoic acid suppressed the proliferation of HPV-16-immortalized ectocervical epithelial cells (Agarwal *et al.*, 1996) and Epstein-Barr virus-immortalized lymphoblastoid B-cell lines (Dolcetti *et al.*, 1998). In the latter cells, inhibition of proliferation was associated with an increase in P27Kip1 and inhibition of the transition from the G_1 to S phase and appeared to be independent of induction of differentiation or of down-regulation of viral latent antigens. Unexpectedly, retinoid-induced growth arrest appeared to be irreversible at a concentration (1 µmol/L) that might be reached in humans after oral systemic therapy (Dolcetti *et al.*, 1998).

(b) Malignant cells

13-*cis*-Retinoic acid inhibited the growth of head-and-neck cancer cells, and the sensitivity of the cells was associated with the expression of RARβ (Giannini *et al.*, 1997). A similar association was found in renal cancer cell lines (Hoffman *et al.*, 1996). Other cell types whose growth was inhibited by 13-*cis*-retinoic acid include cells from prostate cancer (Dahiya *et al.*, 1994), melanoma (Lotan & Lotan, 1980; Schaber *et al.*, 1994), pancreatic cancer (Jimi *et al.*, 1998), astrocytoma (Rutka *et al.*, 1988), ovarian teratocarcinoma (Taylor *et al.*, 1990), endometrial adenocarcinoma (Carter *et al.*, 1996) and breast carcinoma (Toma *et al.*, 1997). 13-*cis*-Retinoic acid decreased the saturation cell density and mitotic indices of the human teratocarcinoma-derived cell line PA-1 after it reached confluence (Taylor *et al.*, 1990).

Inhibition of anchorage-independent growth of a human lung tumour cell line, A427, in a 28-day assay is used to screen new chemopreventive agents. In this assay, 13-*cis*-retinoic acid caused a 66 ± 16% inhibition at a concentration of 33 µmol/L (Korytynski *et al.*, 1996).

The growth of prostate cancer cells and their potential to form colonies in soft agar was decreased after 13-*cis*-retinoic acid treatment when compared with controls. In nude mice, 13-*cis*-retinoic acid-treated cells produced significantly smaller tumours than untreated cells (Dahiya *et al.*, 1994).

4.2.3.2 Modulation of differentiation

13-*cis*-Retinoic acid was found to enhance cell differentiation in several cell types. It enhanced melanogenesis in melanoma cells (Lotan & Lotan, 1980; Meyskens & Fuller, 1980), regulated the expression of cytokeratin K5, increased *RARβ* mRNA levels in immortalized ectocervical cells (Agarwal *et al.*, 1996) and caused endodermal differentiation in embryonal carcinoma F9 cells (Williams *et al.*, 1987). It was less potent than all-*trans*-retinoic acid but more potent than retinol in inducing differentiation of F9 EC cells. In the human acute promyelocytic leukaemia cell line NB4, 13-*cis*-retinoic acid was less potent than all-*trans*-retinoic acid in inducing granulocytic differentiation (Zhu *et al.*, 1995). [The Working Group noted that this difference in potency *in vitro* may partly explain the low efficacy of 13-*cis*-retinoic acid in inducing complete remission in patients with acute promyelocytic leukaemia.]

13-*cis*-Retinoic acid at 0.01 µmol/L increased the expression of keratins K13, K15 and K19 in human epidermal keratinocytes cultured on a 3T3-feeder layer, which represents an embryonic type of differentiation. The treated cells had decreased expression of the proliferation-associated K16 but an increase in the expression of K6, the other proliferation-associated keratin (Korge *et al.*, 1990).

13-*cis*-Retinoic acid at 10 µmol/L directed a human follicular cell line towards a more normal state of proliferation and differentiation, as evidenced by increases in various parameters of differentiation such as uptake of [^{125}I] and binding of epidermal growth factor and thyroid-stimulating hormone (Van Herle *et al.*, 1990).

Treatment of pancreatic carcinoma cells grown on collagen gels with 13-*cis*-retinoic acid resulted in a change from a fibroblastoid to epithelioid morphology. The effect was inhibited by receptor antagonists (Jimi *et al.*, 1998).

4.2.3.3 Induction of apoptosis

13-*cis*-Retinoic acid induced apoptosis in MCF-7 (ER^+) and MDA-MB-231 (ER^-) cell lines but not in ZR-75.1 (ER^+) cells. The apoptotic phenomenon was time-dependent and not related to arrest in a specific phase of the cell cycle. After treatment, the expression of *bcl-2* was reduced in MCF-7, while no treatment-related modifications were observed in ZR-75.1 or MDA-MB-231 (Toma *et al.*, 1997). Induction of apoptosis in the MCF-7 breast carcinoma cell line was observed even at 0.01 µmol/L 13-*cis*-retinoic acid but required six days of incubation (Toma *et al.*, 1998).

4.2.3.4 Antimutagenicity in short-term tests for mutagenicity

(a) Mammalian cells

Few studies have investigated the possibility that 13-*cis*-retinoic acid might play a role in reducing the genotoxic damage induced by mutagens or carcinogens. In three studies of chromosomal alterations in carcinogen-treated mammalian cells involving phytohaemagglutin-stimulated human lymphocytes or lymphoblastoid cultures, the effect of retinoids on the activity of directly acting mutagens was analysed. Mixed results were obtained.

In one study, 13-*cis*-retinoic acid increased the frequencies of sister chromatid exchange and chromosomal breakage induced by diepoxybutane, a DNA cross-linking agent, in lymphocyte cultures from two persons (Auerbach *et al.*, 1984). In another, an anticlastogenic effect of this retinoid was seen against the free radical-generating agent bleomycin in four human lymphoblastoid cell lines and in primary lymphocyte cultures derived from the peripheral blood of 11 subjects. The study design involved preincubation of the cells with a wide range of concentrations of the retinoid (10^{-8} to 10^{-5} mol/L) for 24 h before addition of bleomycin (Trizna *et al.*, 1992, 1993). Addition of 13-*cis*-retinoic acid to lymphocyte cultures from a single normal individual for 1 h before X-irradiation significantly reduced the amount of chromatid damage, but no protective effect was observed when exposure to the retinoid occurred after X-ray treatment. This study also included a group of five patients with xeroderma pigmentosum who were receiving 13-*cis*-retinoic acid. Lymphocyte cultures from the blood of the patients had fewer chromatid breaks and gaps after

X-irradiation than cells from the same patients when they were not receiving retinoids. This radioprotective effect could be transferred to lymphocytes from a normal control by co-cultivating them with plasma from a patient with xeroderma pigmentosum who was receiving 13-*cis*-retinoic acid. The authors concluded that the presence of the retinoid in the plasma of these patients during treatment was responsible for the protection (Sanford *et al.*, 1992).

13-*cis*-Retinoic acid did not significantly modulate the binding of [^{3}H]dimethylbenz[*a*]anthracene to the DNA of primary murine epidermal cells in culture when it was present concurrently with the carcinogen. In contrast, treatment with all-*trans*-retinoic acid, retinol and retinol acetate at the same concentrations was protective against carcinogen–DNA binding (Shoyab, 1981).

When microsomal preparations from uninduced Fischer female rats were incubated with 13-*cis*-retinoic acid in the presence of retinol or benzo[*a*]pyrene, the retinoid produced a significant reduction in the rate of retinol esterification and benzo[*a*]pyrene hydroxylation (Ball & Olson, 1988). In another study, the effect of exposure of primary rat hepatocyte cultures to 13-*cis*-retinoic acid for 48 h was examined on the activities of three cytochrome P450 mRNAs. The levels of P4503A mRNA were increased approximately eightfold ($p < 0.05$), and those of CYP1A1 showed a less than threefold increase (not statistically significant). In contrast, the levels of CYP1A2 mRNA were not altered (Jurima-Romet *et al.*, 1997).

(b) Animals in vivo

Several studies in rodents also considered the effect of treatment with this drug on the activity of enzymes involved in carcinogen metabolism (Table 4). In an early study, female adult Wistar rats were fed diets containing 13-*cis*-retinoic acid at 1 or 5 mg/kg bw per day for 3, 7, 14 or 28 days. Their livers were then removed and the activities of arylhydrocarbon hydroxylase (AHH), aminopyrine-*N*-demethylase and 7-ethoxycoumarin deethylase were assayed. A transitory increase in the activity of aminopyrine-*N*-demethylase occurred after three days, which was decreased to control levels at seven days, and inhibition of AHH activity was seen after 28 days of treatment (Goerz *et al.*, 1984). In a later study, 13-*cis*-retinoic acid was given at a concentration of 6 mg/kg bw per day for 10 or 60 days and the effect on mono-oxygenase enzymes was measured in the liver and skin. After 10 days, a statistically significant induction of aminopyrine-*N*-demethylase was observed in microsomes from livers of retinoid-treated animals. The activities of the other two enzymes, 7-ethoxyresorufin-*O*-deethylase and erythromycin-*N*-demethylase, were significantly reduced in both the liver and skin. In contrast, after 60 days of treatment, there was no significant difference in enzyme activity in liver or skin microsomes. Furthermore, 13-*cis*-retinoic acid reduced the inductive effects of hexachlorobenzene on aminopyrine-*N*-demethylase. Hexachlorobenzene is a potent inducer of several P450 isozymes and has been shown to be carcinogenic in various animal species (Ertürk *et al.*, 1986 ; Goerz *et al.*, 1994).

The effect of 13-*cis*-retinoic acid on the activities of the hepatic and cutaneous mono-oxygenase enzymes AHH, ethoxycoumarin dealkylase and ethoxyresorufin dealkylase was studied in adult male mice. The retinoid decreased the basal activities of all three enzymes in both liver and skin, the effect on ethoxyresorufin dealkylase activity being the most marked (70% inhibition). When the retinoid was given at the same time as the carcinogen 3-methylcholanthrene, the induction of each of these enzymes was suppressed by the retinoid in both tissues (Finnen & Shuster, 1984).

In a large-scale study of the induction of hepatic enzymes by 13-*cis*-retinoic acid in Sprague-Dawley rats, both a dose-range and a time-course analysis were carried out. The hepatic concentrations of AHH were significantly suppressed in animals fed 13-*cis*-retinoic acid, but this treatment increased the cytosolic concentrations of quinone reductase and had no effect on glutathione *S*-transferase. In order to determine the effect of this enzyme alteration on binding of a carcinogen to liver DNA *in vivo*, animals were dosed for seven days with 13-*cis*-retinoic acid at 120 mg/kg bw per day, the dose that gave maximal induction of hepatic quinone reductase and near maximal suppression of AHH. On the eighth day, the animals received an intraperitoneal injection of [^{3}H]benzo[*a*]pyrene. Binding to DNA was reduced to 38% in the liver, 29% in the stomach and 23% in the lung but remained unchanged in the kidney (McCarthy *et al.*, 1987).

The ability of 13-*cis*-retinoic acid to inhibit the induction of micronuclei in bone-marrow polychromatic erythrocytes by a carcinogen has been examined in one study (Table 4). Administration of the retinoid by gavage 2 h before an intraperitoneal

Table 4. Inhibition by 13-*cis*-retinoic acid of genetic and related effects in rodents and in humans *in vivo*

Retinoid tested (dose and administration route)[a]	Carcinogen (dose and administration route)[a]	Animal strain and species	Investigated effect	Result[b]	LED/HID[c]	Reference
13-*cis*-Retinoic acid (1 or 5 mg/kg/day for 3, 7, 14 or 28 days by gavage)	None	Female adult Wistar rats	Mono-oxygenase activities			Goerz *et al.* (1984)
			Hepatic aminopyrine-*N*-demethylase	(#)		
			Hepatic arylhydrocarbon hydroxylase	+		
			Hepatic 7-ethoxycoumarin deethylase	–		
13-*cis*-Retinoic acid (6 mg/kg day orally for 10 and 60 days)	None	Female Wistar rats	Mono-oxygenase enzyme activities		No statistical difference for any enzyme at 60 days	Goerz *et al.* (1994)
			Hepatic aminopyrine-*N*-demethylase	#		
			Hepatic 7-ethoxyresorufin *O*-deethylase	#		
			Hepatic erythromycin-*N*-demethylase	#		
			Cutaneous 7-ethoxy-resorufin-*O*-deethylase	#		
			Cutaneous erythromycin-*N*-demethylase	#		
13-*cis*-Retinoic acid (0.3 µmol/kg bw i.p.)	None	Adult hairless mice	Basal mono-oxygenase enzyme activities			Finnen & Shuster (1984)
			Hepatic arylhydrocarbon hydroxylase	+	By 20%	
			Hepatic ethoxycoumarin dealkylase	+	By 20%	
			Hepatic ethoxyresorufin dealkylase	+	By 70%	
			Cutaneous arylhydrocarbon hydroxylase	+	By 24%	
			Cutaneous ethoxycoumarin dealkylase	+	By 20%	
			Cutaneous ethoxyresorufin dealkylase	+	By 70%	
13-*cis*-Retinoic acid (0.3 µmol/kg bw i.p.)	3-Methyl-cholanthrene (route not given for hepatic assay but was topical for cutaneous; no dose given)	Adult hairless mice	Induced mono-oxygenase enzyme activities			Finnen & Shuster (1984)
			Hepatic arylhydrocarbon hydroxylase	+	By 25%	
			Hepatic ethoxycoumarin dealkylase	+	By 25%	
			Hepatic ethoxyresorufin dealkylase	+	By 75%	
			Cutaneous arylhydro-carbon hydroxylase	+	By 20%	
			Cutaneous ethoxycoumarin dealkylase	+	By 20%	
			Cutaneous ethoxyresorufin dealkylase	+	By 80%	

Table 4. (contd)

Retinoid tested (dose and administration route)[a]	Carcinogen (dose and administration route)[a]	Animal strain and species	Investigated effect	Result[b]	LED/HID[c]	Reference
13-*cis*-Retinoic acid (Dose study: 25–235 mg/kg bw per day for 4 days; time study: 1,2,4, 7 or 14 days at 235 and 600 mg/kg bw per day; both by gavage)	None	Male Sprague-Dawley rats	Hepatic activity Microsomal arylhydrocarbon hydroxylase Cytosolic glutathione-*S*-transferase Cytosolic quinone reductase	 + – #	400 mg/kg bw per day	McCarthy *et al.* (1987)
13-*cis*-Retinoic acid (120 mg/kg bw per day by gavage for 7 days)	Benzo[*a*]pyrene (2 mg/kg bw i.p. on the 8th day)	Male Sprague-Dawley rats	Covalent binding to DNA	+	ID_{38} for liver, ID_{28} for stomach, ID_{23} for lung. No effect for kidney	McCarthy *et al.* (1987)
13-*cis*-Retinoic acid (20–150 mg/kg by single gavage administration 2 h before carcinogen)	Benzo[*a*]pyrene (185 mg/kg bw i.p.)	B6C3F_1 mice	Micronucleus test, bone marrow	+	40 mg/kg bw	Al Dosari *et al.* (1996)
13-*cis*-Retinoic acid (1.5 mg/kg for 3 months followed by either 0.5 mg/kg 13-*cis*-retinoic acid or 30 mg/day β-carotene)	Cigarette smoke (27 current smokers, 9 former smokers, 4 never smokers)	Humans	Micronucleus test, buccal mucosal cells	+		Benner *et al.* (1994b)

i.p., intraperitoneally; ID, dose that inhibited the investigated end-point by x%, as indicated by the authors or calculated from their data

[a] Doses of retinoids and carcinogens and routes of administration are given as reported by the authors.

[b] +, inhibition of the investigated end-point; –, no effect on the investigated end-point, #, enhancement of investigated end-point; (#), weak transient enhancement on end-point

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

injection of benzo[*a*]pyrene significantly reduced the frequency of micronucleus formation in the carcinogen-treated animals (Al Dosari *et al.*, 1996).

4.3 Mechanisms of cancer prevention

The studies summarized in section 4.2.3 suggest that some of the chemopreventive effects of 13-*cis*-retinoic acid may be mediated by increases in the activities of cytochrome P450 enzymes, suppression of carcinogen-induced mono-oxygenases and reduction of genotoxic damage induced by mutagens or carcinogens. Most of the cancer preventive activity of 13-*cis*-retinoic acid appears to occur at the promotion stage.

13-*cis*-Retinoic acid itself does not bind to retinoid receptors, but it can be considered to be a pro-drug of all-*trans*-retinoic acid because it can be isomerized to the *trans* conformation in animal systems. Therefore, 13-*cis*-retinoic acid probably exerts its effects by regulating gene expression after being converted to all-*trans*-retinoic acid, a direct ligand of retinoic acid receptors. Indeed, there is no evidence that 13-*cis*-retinoic acid exerts its effects by a receptor-independent mechanism. The reader is referred to section 4.3 in Handbook 1 for details of the mechanism of action of all-*trans*-retinoic acid.

5. Other Beneficial Effects

13-*cis*-Retinoic Acid is regarded as an effective drug when given systemically in severe forms of acne (see section 2.3).

6. Carcinogenicity

6.1 Humans

No data were available to the Working Group.

6.2 Experimental models

No data on the carcinogenicity of 13-*cis*-retinoic acid were available to the Working Group. In one study in male Sencar mice, a tumour-enhancing effect of 13-*cis*-retinoic acid was observed (McCormick *et al.*, 1987 ; see section 4.2.1.1).

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

At the recommended oral dose of 13-*cis*-retinoic acid, 0.5–20 mg/kg bw as two evenly divided doses, the toxicity experienced by patients is similar to that observed after treatment with other retinoids or high doses of retinol and its esters (IARC, 1998) on vitamin A). Mucocutaneous skin reactions are the most common. Concurrent administration of α-tocopherol (800 IU/day) and 13-*cis*-retinoic acid significantly reduced the mucocutaneous toxicity, liver function abnormalities and hypertriglyceridaemia and increased overall activity in a phase-I clinical trial (Dimery *et al.*, 1997).

7.1.1.1 Mucocutaneous toxicity

(a) Topical administration

In a comparison of the effectiveness and toxicity of topically applied 0.05% 13-*cis*-retinoic acid gel and 0.05% all-*trans*-retinoic acid cream for the treatment of acne vulgaris, the two agents were found to have similar efficacy, but 13-*cis*-retinoic acid was less toxic: 10 of 15 subjects given all-*trans*-retinoic acid complained of stinging, erythema and desquamation during the 12-week treatment, but only 7 of 15 subjects given 13-*cis*-retinoic acid complained of mild skin irritation (Dominguez *et al.*, 1998).

(b) Oral administration

The most frequent side-effects experienced with orally administered 13-*cis*-retinoic acid are mucocutaneous reactions, which are generally dose-dependent. Skin reactions can usually be tolerated without interruption of therapy with the use of emollients and other treatments, and the effects generally resolve upon discontinuation of therapy. Drying of the mucosa of the mouth, nose and eyes is common, and cheilitis occurs in over 90% of patients. Facial dermatitis, rash, increased sensitivity to ultraviolet light, varicella zoster infection, erythema nodosum, erythema multiforme, urticaria, paronychia and median canaliform dystrophy have also been reported (Bigby & Stern, 1988; Dharmagunawardena & Charles-Holmes, 1997; Meigel, 1997). Pyoderma gangrenosum requiring hospitalization occurred in a 17-year-old boy taking 13-*cis*-retinoic acid therapy for acne. Two weeks after beginning therapy at 0.5 mg/kg bw per day, he developed an erythematous papule over the mid-line chest, which became ulcerated and enlarged rapidly even when 13-*cis*-retinoic acid was discontinued. The ulcer healed, with scarring, after treatment with hydrocortisone and prednisolone (Gangaram *et al.*, 1997).

In a phase-III study of the efficacy of 13-*cis*-retinoic acid at 0.15 mg/kg bw per day for three years in the chemoprevention of new basal-cell carcinoma, the prevalence of mucocutaneous reactions was significantly higher in the treated group (70% versus 35%) (Tangrea *et al.*, 1992b, 1993). In a phase-I/II study of the use of 13-*cis*-retinoic acid to prevent acute leukaemia, the starting dose of 1 mg/kg bw twice daily for one month was escalated by 0.5 mg/kg bw per day at monthly intervals. The highest dose achieved was 2 mg/kg bw per day because of significant toxicity, including severely dry skin, cheilitis, conjunctivitis and epistaxis (Greenberg *et al.*, 1985). In a phase-II investigation of the use of 13-*cis*-retinoic acid for regression of aggressive laryngeal papillomatosis, a dose of 1–2 mg/kg bw per day was used for 5–20 months. The reported effects include cheilosis, dry skin, balanitis, conjunctivitis and epistaxis (Alberts *et al.*, 1986). A phase-II study of its use in mycosis fungoides began with a dose of 2 mg/kg bw per day; however, since most subjects developed dryness of the skin and mucous membranes, subsequent patients started at 1 mg/kg bw per day (Kessler *et al.*, 1987). A dose of 1 mg/kg bw per day for six months was used in a phase-II double-blinded study in chronic smokers found to have squamous metaplasia and/or dysplasia in bronchial biopsies. The symptoms that were more frequent in the treated group were cheilitis, skin dryness, conjunctivitis, hypertriglyceridaemia, arthralgia and rash (Lee *et al.*, 1994). In a phase-II trial in patients with oral leukoplakia, an initiation phase of 1.5 mg/kg bw per day for three months

was followed by a maintenance phase of 0.5 mg/kg bw per day for nine months. Sixty-eight subjects completed one month of induction, most reporting at least low-grade toxicity; 23 subjects experienced grade 3 or 4 toxicity during the induction phase. The effects seen were typical of those associated with orally administered 13-*cis*-retinoic acid and included dry skin, cheilitis, conjunctivitis and triglyceridaemia. No effects on the liver were observed at either the induction or maintenance dose (Lippman *et al.*, 1993).

7.1.1.2 Ocular toxicity

Dry eyes and conjunctivitis are common side-effects of 13-*cis*-retinoic acid. Development of cataracts, keratoconus, keratitis and blepharitis have also been reported, and headaches, a common effect of retinoid therapy, are sometimes accompanied by visual disturbances (Bigby & Stern, 1988). Retinoic acid analogues may be incorporated into the rod photoreceptor elements during the continuous process of outer disc shedding and renewal, and this may contribute to reported disturbances of night vision (Lerman, 1992). Adverse ocular reactions do not usually require discontinuation of treatment since they are often reversible; however, persistent dry eye syndrome and evidence of unresolved lens abnormalities have been reported in some subjects even after cessation of therapy (Brown & Grattan, 1989; Lerman, 1992; Meigel, 1997).

The ocular effects of 13-*cis*-retinoic acid were examined in 55 patients undergoing treatment [dose not given] for severe nodular acne. The adverse effects were decreased tear-film break-up time, increased blepharitis and a large increase in colonization of *Staphylococcus aureus*. All of the ocular complications manifested within four weeks of initiation and were fully reversible upon discontinuation of treatment (Egger *et al.*, 1995).

7.1.1.3 Musculoskeletal toxicity

In children and adolescents given prolonged courses (16–87 months) of treatment at doses up to 4 mg/kg per day, musculoskeletal complaints (myalgia, arthralgia), vertebral hyperostoses with diffuse fractures and hyperostoses in the greater and lesser trochanters and at fascial insertions can develop (Pittsley & Yoder, 1983). Most of the changes are dose-dependent (Ellis *et al.*, 1985) and subtle, with small hyperostoses at insertions of the spinal ligaments, the plantar fascia, clavicle or Achilles tendon (Carey *et al.*, 1988). The toxicity seen in adolescents (Erhardt & Harangi, 1997) is similar to that in adults (Kilcoyne *et al.*, 1986). The upper trunk, middle and lower part of the thoracic and lumbar spine, the appendicular skeleton and the muscles of the legs can be involved (Shalita *et al.*, 1988). In some of these patients, the consequences are more severe, beginning with accumulations of small osteophytes along the anterior cervical and thoracic vertebrae, followed by ossification of the anterior and posterior longitudinal ligaments, leading to spinal cord compression (Pittsley & Yoder, 1983; Pennes *et al.*, 1984, 1985; Kilcoyne *et al.*, 1986; Carey *et al.*, 1988; Pennes *et al.*, 1988; Scuderi *et al.*, 1993). Of even greater concern is the frank skeletal toxicity in the long bones, manifest by radiodense metaphyseal bands, growth arrest (Marini *et al.*, 1988), relative narrowing of the diaphyses in femora and tibiae and premature epiphyseal closure (Milstone *et al.*, 1982; Valentic & Barr, 1985; Lawson & McGuire, 1987). Patients as young as three years have developed signs of early osteoporosis (Lawson & McGuire, 1987), and skeletal toxicity can become evident in as little as five weeks (Novick *et al.*, 1984).

Rheumatological symptoms are common in adults treated with 13-*cis*-retinoic acid, although these effects are generally subclinical (reviewed by Kaplan & Haettich, 1991). The rheumatoid symptoms include hyperostosis of the spine and appendicular bone, abnormalities of calcium metabolism, arthritis and musculoskeletal pain. Of the rheumatoid symptoms, hyperostosis is the most common and occurs mainly with protracted treatment and high doses. The incidence may exceed 80% after a few years of administration (Kaplan & Haettich, 1991). Achilles tenosynovitis, myalgia and arthralgia are also associated with oral administration of 13-*cis*-retinoic acid (Bigby & Stern, 1988).

In order to evaluate the chronic effect of 13-*cis*-retinoic acid, 10 subjects were given 0.15 mg/kg bw per day for nine months. One subject discontinued because of joint pain, and myalgia and/or arthralgia occurred in 30% of the participants (Edwards *et al.*, 1986). In a phase-II pilot study of patients with leukoplakia, the subjects started at a dose of 1 mg/kg bw per day until a clinical response was observed and then received 0.25 mg/kg bw per day indefinitely. Of the 15 subjects enrolled in the study, two withdrew because of persistent bone pain and photosensitivity. Of the nine subjects who completed therapy, 22% experienced bone pain (Beenken *et al.*, 1994).

The influence of 13-*cis*-retinoic acid on bone density was investigated in 15 male patients receiving a six-month treatment with average initial dose of 0.9 mg/kg bw per day, reduced every four weeks to an average final dose of 0.4 mg/kg bw per day (Kocijancic, 1995). Short-term treatment with low doses of 13-*cis*-retinoic acid thus has no clinically important effects on bone density. A similar conclusion was reached in a study of nine subjects treated with a six-month course of 13-*cis*-retinoic acid (average initial dose, 0.7 mg/kg bw adjusted to a maintenance dose of 0.9 mg/kg bw per day after 1–3 months), although transient inhibitory effects on markers of bone turnover were observed (Kindmark *et al.*, 1998).

7.1.1.4 Metabolic and haematological disorders

Approximately 25% of patients treated with 13-*cis*-retinoic acid for acne have disturbances of lipid metabolism, manifesting as hyperlipidaemia with or without hypercholesterolaemia. Rarely, acute complications develop secondary to hyperglyceridaemia, such as pancreatitis (Meigel, 1997). Haematopoietic and lymphatic complications have been reported, including leukopenia and anaemia (Bigby & Stern, 1988).

In a phase-I/II study of the use of 13-*cis*-retinoic acid in myelodysplastic syndrome, a starting dose of 1 mg/kg bw per day was escalated by 0.5 mg/kg bw per day at monthly intervals. The dose-limiting toxic effect in this study was thrombocytopenia, an unexpected observation (Greenberg *et al.*, 1985). In another study, the same population was given 20–125 mg/m^2 per day for 21 days. Hepatotoxicity (hyperbilirubinaemia and elevated serum alanine transaminase activity) was dose-limiting. The most common adverse effects were hyperkeratosis, cheilosis and haematological responses, and the concentration of serum lipids was increased at 80 mg/m^2 per day (Gold *et al.*, 1983). In a phase-III study of the efficacy of 13-*cis*-retinoic acid on the prevention of new basal-cell carcinoma, a dose of 0.15 mg/kg bw per day for three years increased serum triglycerides in 7% of subjects (2% with placebo) (Tangrea *et al.*, 1992b, 1993). In studies of the efficacy of orally administered 13-*cis*-retinoic acid in the prevention of skin cancer, the doses ranged from 5–10 mg per day to 2 mg/kg bw per day. Two of seven subjects given the highest dose were able to complete two years of therapy. Increased liver function and triglycerides were observed in some subjects; the liver function returned to normal during therapy, although one subject was required to withdraw from treatment because of persistent abnormalities in tests for liver function. The toxicity and abnormal clinical chemistry parameters were less severe when a dose of 0.5 mg/kg bw per day was used (Kraemer *et al.*, 1992). With 5–10 mg per day for three years, approximately 1% of subjects experienced clinical adverse reactions, consisting primarily of elevated serum cholesterol concentration or liver enzyme activity (Moon *et al.*, 1997).

In a phase-II clinical trial of the use of interferon-α (5 x 10^6 U/m^3) in combination with 13-*cis*-retinoic acid (1 mg/kg bw per day) in 13 patients with metastatic melanoma, all patients experienced elevated serum cholesterol and tryglyceride concentrations, in addition to fatigue, myalgia, anorexia, stomatitis and cheilitis. Seven patients required 50% reductions in the 13-*cis*-retinoic acid dose because of hypertriglyceridaemia, fatigue, severe stomatitis with anorexia and weight loss (Rosenthal & Oratz, 1998). The same combination was investigated in a phase-II trial in patients with recurrent squamous-cell cervical carcinoma. The starting doses were 1 mg/kg bw per day 13-*cis*-retinoic acid and 6 x 10^6 U/day recombinant interferon-α_{2a}. Thirty- four patients were evaluable for toxicity. Four patients developed grade 3 or higher anaemia, 13 developed grades 1–3 leukopenia (median nadir of leukocytes, 2800), three developed grade 3 neutropenia and two developed grade 3 thrombocytopenia. Other severe events included gastro-intestinal toxicity of grade 3 or higher and grade 3 neurological toxicity (Look *et al.*, 1998). The combination of interferon-α, interleukin-2 and 13-*cis*-retinoic acid was studied in patients with metastatic renal-cell carcinoma. Severe hyperlipidaemia was observed in a small fraction of patients, which was attributed to the 13-*cis*-retinoic acid (1 mg/kg bw per day) and appeared to be cumulative with successive cycles (Stadler *et al.*, 1998). Hypercalcaemia was the dose-limiting toxic effect of 13-*cis*-retinoic acid in 39 children who had received a bone-marrow transplant, 23% of whom developed grades 1–3 hypercalcaemia (Villablanca *et al.*, 1993).

Mild increases in the activity of aminotransferases were reported in 5–35% of patients receiving 0.5–3 mg/kg bw per day for three months, but these were reversible on discontinuation of therapy. Pharmacokinetic and clinical data suggested that 13-*cis*-retinoic acid is not concentrated in the liver, and there is little evidence that it causes significant hepatotoxicity (Fallon &

Boyer, 1990). This conclusion may not apply to all patient populations, however; in a phase-II trial in which 13-*cis*-retinoic acid was given at 1 mg/kg bw per day to people with HIV-associated Kaposi sarcoma, hepatotoxicity was observed in 27% (Bower *et al.*, 1997).

7.1.1.5 Effects on the central nervous system

Headache, often described as severe and unrelenting, decreased hearing acuity, dizziness, oculogyric crisis and personality disorder have been associated with oral administration of 13-*cis*-retinoic acid for dermatological conditions. Fixation of the eyes in one direction, facial spasm and loss of speech developed in one patient after two weeks. Loss of libido, impotence and insomnia were reported by one subject. Evidence of pseudotumour cerebri has been found in subjects complaining of headaches (Bigby & Stern, 1988). Reports of severe depression, psychosis and suicides prompted the United States Food and Drug Administration to warn that oral use of 13-*cis*-retinoic acid for treatment of acne may cause uncommon psychotic disorders. It was noted that such problems might be more common among patients likely to take the drug (Josefson, 1998; Nightingale, 1998)

7.1.1.6 Urogenital toxicity

Renal impairment developed in one 34-year-old man after treatment for acne with 40 mg/day of 13-*cis*-retinoic acid for two months. He developed a moderate inflammatory syndrome, elevated creatinine concentration, proteinuria and haematuria with no indication of infection or abnormal immunological responses. With hydration and discontinuation of 13-*cis*-retinoic acid, the creatinine concentration returned to normal within seven days and the proteinuria, haematuria and inflammatory syndrome resolved within three days (Pavese *et al.*, 1997). Several cases of urethritis have been associated with temporary administration of 13-*cis*-retinoic acid (Edwards & Sonnex, 1997).

7.1.1.7 Other toxicity

In a phase-II trial of 13-*cis*-retinoic acid in combination with interleukin-2 and interferon-α in patients with metastatic renal carcinoma, 13-*cis*-retinoic acid was administered at 1 mg/kg bw per day. Eleven patients required 15 dose reductions because of toxicity attributed to 13-*cis*-retinoic acid. Flu-like symptoms (fever, chill, rigor, myalgia, arthralgia) developed in all patients and were considered severe in 21% of the patients during the first cycle. These symptoms tended to abate over time and with additional cycles, with the exception of fatigue, which worsened with prolonged therapy (Stadler *et al.*, 1998).

7.1.2 Experimental models

7.1.2.1 Acute and short-term toxicity

The LD_{50} of 13-*cis*-retinoic acid in mice and rats treated orally was 3400 and 4000 mg/kg bw, respectively; the LD_{50} was approximately 2000 mg/kg bw in rabbits (Kamm, 1982). In studies to find the maximum tolerated dose of 13-*cis*-retinoic acid in athymic nude mice before conducting studies of tumour suppression, no symptoms of hypervitaminosis A were seen over the dose range of 10–100 mg/kg bw per day, although body weight decreased by about 7%. A dose of 120 mg/kg was chosen for further evaluation (Shalinsky *et al.*, 1995). During examination of the effect of 13-*cis*-retinoic acid on MNU-induced tumours in Sprague-Dawley rats, oral doses of 25–200 mg/kg bw per day were administered for four weeks. Body-weight loss, alopecia and eye crusting were found in all animals, and 50% of those at the high dose died (Hsu, 1998).

Rats received 10–50 mg/kg bw per day in the diet for 90 days and dogs received 120 mg/kg bw by capsule for seven weeks. The dose-related effects included decreased food consumption and body-weight gain, erythema, alopecia, mucosal changes, elevated serum alkaline phosphatase and transaminase activities, increased liver weight and decreased testicular weight. The effects seen only in rats included long bone fractures and elevated serum triglyceride concentrations; those seen only in dogs were apparent joint pain and decreased spermatogenesis (Kamm, 1982; Kamm *et al.*, 1984). In an independent study, 4–40 mg/kg bw per day given to rats orally for 12 weeks caused significant dose-related decreases in plasma albumin concentration and increased haemoglobin and alkaline phosphatase activity. The changes in albumin and haemoglobin were greater in female than in male animals. Only one bone fracture and no histological changes occurred (Hixson *et al.*, 1979). In mice given 60–400 mg/kg bw per day orally for 21 days, the main dose-related effects included bone fractures, dermal or epidermal inflammation and hyperkeratosis. Alkaline phosphatase activity was increased only at 80, 160 and 320 mg/kg bw per day. The bone fractures were not always accompa-

nied by increases in alkaline phosphatase activity (Hixson & Denine, 1978).

7.1.2.2 Ocular toxicity

The effect of long-term systemic use of 13-*cis*-retinoic acid on the eyelids was investigated in female New Zealand rabbits treated orally for 60 days with 2 mg/kg bw per day 13-*cis*-retinoic acid. All 20 treated animals showed mild clinical signs of blepharoconjunctivitis, including hyperaemia of the eyelid margins and tearing. Histopathological examination revealed marked degenerative changes in the meibomian glands of both upper and lower eyelids. The acini of the meibomian glands showed a decreased number of basaloid epithelial cells, and remnants of degenerated and necrotic acinar cells, and secretory debris were noted in the lumina of the affected glands. There was no evidence of an inflammatory reaction in the affected glands or the eyelid tissue surrounding them, and there was no evidence of significant periacinar fibrosis (Kremer *et al.*, 1994).

7.1.2.3 Long-term toxicity

13-*cis*-Retinoic acid was administered to rats at doses of 2, 18 or 32 mg/kg bw per day to rats in the diet for two years, after an initial 13-week period at 1 mg/kg bw per day to avoid fractures in young, growing animals. The dose-related effects included increased mortality rate and decreased food consumption and body-weight gain. Decreased haemoglobin and haematocrit and elevated serum triglyceride concentrations were observed at the two highest doses. Increased alkaline phosphatase activity and increased liver and kidney weights were observed in all treated groups. The non-neoplastic histological changes that occurred at increased incidences in animals at the two highest doses included fibrosis and inflammation of the myocardium, arterial calcification, focal tissue calcification and focal osteolysis of the bone (Kamm, 1982; Kamm *et al.*, 1984).

In a 55-week study in dogs, the animals were started at 3, 20 or 120 mg/kg bw per day by capsule. Within four weeks, the dogs at the highest dose had severe weight loss and debilitation, and dosing was resumed only eight weeks later on a cycle of 60 mg/kg bw per day for six weeks and no treatment for two weeks. The clinical signs of toxicity observed during the treatment cycle included severe weight loss, skin changes, ophthalmic changes (e.g. corneal ulcers and corneal opacity), decreased haemoglobin and haematocrit and increased serum alkaline phosphatase activity; most of the signs diminished during the rest periods. The ocular changes tended to revert to normal with discontinuation of treatment but did not completely clear during the observation periods. The microscopic changes included fibrosis and focal calcification in the myocardium and aorta, increased liver weight, testicular atrophy with spermatogenic arrest and lymph node oedema. Although increased liver weight and testicular atrophy with spermatogenic arrest were also observed at the intermediate dose, no clinical or histological signs of toxicity were found at the lowest dose (Arky, 1998).

7.2 Reproductive and developmental effects

7.2.1 Humans

7.2.1.1 Reproductive effects

No evidence was found of changes in serum testosterone, follicle-stimulating hormone or luteinizing hormone or in testicular size, sperm count or morphological apperance in patients receiving a therapeutic course of 13-*cis*-retinoic acid (Peck *et al.*, 1979, 1982; Török & Kasa, 1985). In 20 men aged 15–42 years with acne, two of whom had normozoospermia and 18 had oligospermia or other abnormal sperm parameters, given 0.2–2 mg/kg bw per day for three months (followed by drug withdrawal or 0.5 mg/kg per day for the next three months), no statistically significant changes in total sperm output, motility or morphology were observed. In those patients with pre-existing sperm abnormalities, the motility and morphology improved but returned to pre-treatment levels three months after withdrawal of the drug. The authors attributed the improvements in semen quality to the anti-inflammatory properties of 13-*cis*-retinoic acid at therapeutic doses (Schill *et al.*, 1981). Among the adverse events associated with therapeutic use of 13-*cis*-retinoic acid in men are isolated reports of impotence, gynaecomastia, ejaculatory failure and local inflammation. Case reports of male reproductive tract toxicity constitute no more than 1% of all drug side-effects (Coleman & MacDonald, 1994).

Abnormal or irregular menses (less frequent or lighter bleeding, 'skipped' or 'late' menstrual periods or menorrhagia) associated with use of 13-*cis*-retinoic acid have been reported only infrequently (Christmas, 1988; Cox, 1988) but can occur in 2–11% (Bruno *et al.*, 1984) to, in one report, all of five healthy women given the drug (Edwards *et al.*, 1986). These reports are inconsistent in that a

greater incidence of such complaints occurred in patients given lower doses (0.1–0.22 or 0.11–0.14 mg/kg bw per day) (Bruno *et al.*, 1984; Edwards *et al.*, 1986) than in those given higher doses (0.75–1.21 mg/kg bw per day) (Bruno *et al.*, 1984).

In general, urinary and circulating steroids were not clinically altered in women given 13-*cis*-retinoic acid orally for up to 20 weeks (Lookingbill *et al.*, 1988; Matsuoka *et al.*, 1989; Rademaker *et al.*, 1991).

Among 10 women aged 19–29 who were maintained on 0.5 mg/kg bw per day, there was no evidence for systemic interference or interaction between 13-*cis*-retinoic acid and orally administered laevonorgestrel and ethinyloestradiol. The authors therefore considered that 13-*cis*-retinoic acid at 1 mg/kg bw per day would probably not interfere with the actions of oral contraceptive steroids (Orme *et al.*, 1984a,b).

7.2.1.2 Developmental effects

In June 1983, the first United Stated Food and Drug Administration alert on 13-*cis*-retinoic acid-induced teratogenicity was issued, after two spontaneous abortions occurred in a prospective cohort study. Within the next two weeks, the first three cases of 13-*cis*-retinoic acid embryopathy had been identified (Rosa, 1992). Between 1982 and 1984, 120 000 women of childbearing potential in the USA were treated. Thirty-five pregnancies associated with 13-*cis*-retinoic acid treatment were reported to the Administration, 29 of which resulted in spontaneous abortion or infants with birth defects, while six infants were normal (Stern *et al.*, 1984; Orfanos, 1985).

While seven adverse pregnancy outcomes in which the father had been exposed were reported up to 1992, the pattern of congenital malformation was diverse. These cases are considered chance associations, particularly since maternal use of the father's prescription was not always excluded (Rosa, 1992). Since 13-*cis*-retinoic acid is not genotoxic and since the preclinical studies showed no sign of developmental toxicity after paternal exposure alone (reviewed by Howard & Willhite, 1986), the human risk for teratogenic effects due to transmission of 13-*cis*-retinoic acid in semen or by other means is considered negligible.

The symptoms of 13-*cis*-retinoic acid embryopathy in humans and animals are remarkably consistent (Braun *et al.*, 1984; Howard & Willhite, 1986; Willhite *et al.*, 1986; Rosa, 1992; Newman *et al.*, 1993). The neuropsychological deficits induced by 13-*cis*-retinoic acid in rodents are also reflected in human experience (Adams, 1993). From a case series of 61 infants who had been exposed to 13-*cis*-retinoic acid, Lynberg *et al.* (1990) calculated a rate of 25% for major defects (excluding neuropsychological delay or deficit) and an overall relative risk of 7.1 (Khoury *et al.*, 1991). If the risk for spontaneous and elective abortion and for developmental delay and mental retardation is excluded, the mean prospective risk for major craniofacial, cardiac, thymic and central nervous system terata is 21–26% [95% confidence interval, 11–58] (Koebert *et al.*, 1993; Lammer *et al.*, 1985; Dai *et al.*, 1992). These estimates do not account for under-diagnosis and under-reporting of identified cases (Ayme *et al.*, 1988; Rosa, 1992). As of 1993, 94 abnormal pregnancy outcomes had been confirmed, which did not include embryos that were spontaneously aborted (absolute risk, 40%), delivered prematurely (absolute risk, 16%) (Lammer, 1988), terminated by elective abortion or were not reported to the Food and Drug Administration (Schardein, 1993). Of women exposed to 13-*cis*-retinoic acid, 64% elect to have an abortion (Anon., 1986). For all courses of treatment, Chan *et al.* (1995) estimated that one elective abortion occurred for every 319 prescriptions for 13-*cis*-retinoic acid that were filled.

The period of exposure considered to incur the highest risk is two to five weeks after conception (Rosa, 1992). Even brief exposure (three days) during the first month of gestation is sufficient to induce terata (Hersh *et al.*, 1985), but not all mothers who have taken the drug before and during the most sensitive stages of embryogenesis have given birth to affected infants (Kassis *et al.*, 1985). If exposure is stopped at least 2 to > 60 days before conception, the risks for malformation (5%) or spontaneous abortion (9.1%) are not significantly different from those for women of reproductive age in the general population (Dai *et al.*, 1989). At least two cases of functional neurological deficit have been reported, however, with limited exposure until after completion of the first trimester (Rosa, 1992). The guidelines recommend that contraception be continued for at least one month after drug withdrawal (Teratology Society, 1991).

The structural terata observed in infants born to mothers given 0.5–1.5 mg/kg bw per day arise primarily in those organs and tissues derived from the cranial neural crest and branchial apparatus (Benke, 1984; Fernhoff & Lammer, 1984; Rosa,

1984; Coberly *et al.*, 1996). Rudimentary pinnae, microtia or anotia (Lott *et al.*, 1984; Hill, 1984; Tremblay *et al.*, 1985; Jahn & Ganti, 1987; Lynberg *et al.*, 1990), congenital deafness (due to a vestigal tympanic membrane and/or malformation of the middle and inner ear), conotruncal cardiac defects (double outlet right ventricle, atrial and ventricular septal defects), tetralogy of Fallot (pulmonary stenosis, ventricular septal defect, overriding aorta, right ventricular hypertrophy), preductal aortic coarctation and related aorticopulmonary septation abnormalities (interrupted/hypoplastic ascending aorta, patent ductus arteriosus, aortic/pulmonary stenosis; dysplastic pulmonary arch), ectopic, hypoplastic or aplastic thymic defects, retinal and optic nerve anomalies (evident in strabismus, nystagmus or eyes that cannot follow; abnormal visual-evoked potential), cleft palate, micrognathia, mandibular asymmetry, Dandy–Walker malformation of the central nervous system (deficient or absent vermis, cerebellar agenesis or hypoplasia, absent aqueduct, dysplastic or malformed inferior medullary olive and/or pontine nuclei, dilated ventricle, focal cortical agenesis), skull malformations (hypertelorism, depressed nasal bridge, maxillary hypoplasia) and facial nerve paralysis constitute the constellation of terata (Lammer, 1988; Lynberg *et al.*, 1990). Elevation of the tentorium cerebelli and the prominent occiput arise from cystic dilatation of the fourth ventricle (Lammer & Armstrong, 1992). Cleft palate, hydrocephalus (usually secondary to aqueductal stenosis), microcephaly, lissencephaly, holoprosencephaly, microphthalmia and malformations of the axial (occult spina bifida) and appendicular skeleton (absent clavicle, synostosis, oligodactyly, camptodactyly) are rather less common (de la Cruz *et al.*, 1984; McBride, 1985; Robertson & MacLeod, 1985; Lammer, 1988; Rizzo *et al.*, 1991; Rosa, 1992). Autopsy of four neonates who died at weeks 14–156 showed membranous atresia of the external auditory canals and Mondini–Alexander defect (flattened cochlea with fewer turns than normal, near complete absence of cochlear neurons, abnormally large utricle and saccule) and malformation of the external ear (Siebert & Lammer, 1990 ; Burk & Willhite, 1992; Coberly *et al.*, 1996). An evaluation of 61 cases of 13-*cis*-retinoic acid-exposed infants with birth defects showed that 70% had some type of ear malformation and 50% had some kind of ear defect combined with cardiovascular and/or central nervous system defect (Lynberg *et al.*, 1990; Khoury *et al.*, 1991). Those infants who survive can develop congestive heart failure, acute respiratory distress and seizures (Jahn & Ganti, 1987) and become cyanotic. They may have difficulty in feeding because of an impaired ability to suck (Hill, 1984; Westerman *et al.*, 1994). Clinical signs of impaired or abnormal neurological development include hypotonia, hypertonia and cranial nerve pareses (Lammer & Armstrong, 1992).

Longitudinal follow-up of children at five years of age who had been exposed to 13-*cis*-retinoic acid *in utero* showed that at least 20% were mentally retarded and 43–47% were of substandard intelligence. All children with an intelligence quotient (IQ) < 71 had structural terata, and the majority had malformations of the central nervous system; structural terata were found in 40% of children with an IQ of 71–85 and 10% of those with an IQ of 86–115. None of the children with an IQ > 116 (the smallest percentage of the population) had malformations of the central nervous system. Many of these children had considerable motor deficits and were unable to walk or sit. Having one or more major congenital malformations of the brain was always associated with borderline to frank mental retardation in children who otherwise showed no structural terata or malformation. Among those with a normal IQ, 16% had an uncommon non-verbal learning disability manifest in difficulties with spatial cognition (e.g. inability to understand shapes and forms and trouble reading a map). These problems were often evidenced by an inability to form complete sentences or to articulate complete thoughts (Adams & Lammer, 1991, 1993).

Despite a vigorous education programme aimed at patients and their physicians (Marwick, 1984) and requiring informed consent before prescription of the drug to pregnant women (Zarowny, 1984; Stern, 1989), cases of 13-*cis*-retinoic acid embryopathy persist (Rappaport & Knapp, 1989; Pilorget *et al.*, 1995; Holmes *et al.*, 1998). Mothers as young as 15 to women in their early 40s (average age, 25 ± 6.7 years) were affected; 31% of the mothers were adolescents, 77% were aware that the drug was teratogenic and most (86%) were high-school graduates (Adams & Lammer, 1993; Pastuszak *et al.*, 1994), yet 38% still used no means of contraception and 8% had ceased contraception while on the drug (Pastuszak *et al.*, 1994). Some 87% of the affected pregnancies occurred after a prescription by a dermatologist.

Early reports showed that 50% of the female patients practised no contraception and 33% were pregnant at the initiation of therapy (Strauss *et al.*, 1988). Between 1989 and 1993, 3.4 pregnancies occurred for every 1000 courses of treatment in the USA, and the rate of elective abortion was 2.3 for every 1000 courses of treatment (Mitchell *et al.*, 1995). The fact that 33–35% of all female patients capable of bearing children did not use any contraceptive methods while on the drug is a consistent finding (Lammer *et al.*, 1985; Hogan *et al.*, 1988). Patient compliance with contraception during therapy with 13-*cis*-retinoic acid has thus been problematic, despite required routine labelling, printed patient brochures, patient checklists and pregnancy testing before, during and after treatment, signature of a consent form acknowledging their instructions, suggestions for two simultaneous forms of contraception and viewing an instructional videotape (Pastuszak *et al.*, 1994; Moskop *et al.*, 1997). Moskop *et al.* (1997) concluded:

"The opportunity for benefit and the responsibility to prevent harm are inseparably linked in the use of 13-*cis*-retinoic acid — if patients are to reap the benefits of this drug, they must also share with the drug's manufacturer and its physician-prescribers, in the responsibility to prevent its harms."

The developmental and clinical toxicology of oral 13-*cis*-retinoic acid in paediatric patients has been summarized (DiGiovanna & Peck, 1983).

7.2.2 Experimental models

7.2.2.1 *Reproductive effects*

Rats treated with 13-*cis*-retinoic acid at 30 mg/kg bw daily for eight weeks did not show effects on spermatogenesis (Kuhlwein & Schütte 1985). In rats fed 10, 25 or 50 mg/kg bw 13-*cis*-retinoic acid daily for 13 weeks, however, there was a dose-dependent reduction in testicular weight and histological evidence of decreased spermatogenesis. Similar effects were found in dogs given 120 mg/kg bw daily for seven weeks (Kamm, 1982).

7.2.2.2 *Developmental effects*

Numerous studies have shown that embryos of every animal species are susceptible to the embryotoxic effects of excess 13-*cis*-retinoic acid (Table 5). The types of terata resemble those seen with all-*trans*-retinoic acid (see Handbook 1, section 7.2.1).

The bioavailability and potential developmental toxicity of 13-*cis*-retinoic acid after topical application were evaluated. [The Working Group noted that experiments with animals are difficult to interpret because potentially teratogenic doses may ulcerate or otherwise damage their skin. Studies in humans and animals suggest low bioavailability and low teratogenicity of topical 13-*cis*-retinoic acid (Chen *et al.*, 1997).

The teratogenic potency of 13-*cis*-retinoic acid has marked interspecies variation: the lowest teratogenic doses of this retinoid in mice and rats are one order of magnitude higher than those in rabbits and cynomolgus monkeys (see Table 5) and > 100-fold higher than those in humans (see section 7.2.1). In mice and rats, the lowest teratogenic doses are much higher than the corresponding doses of the all-*trans*-isomer. In contrast, in rabbits and cynomolgus monkeys, the differences between the two retinoids are not as marked, and 13-*cis*-retinoic acid appears to be a more potent teratogen than all-*trans*-retinoic acid in monkeys (Hummler *et al.*, 1990; Korte *et al.*, 1993).

The plasma elimination rate of 13-*cis*-retinoic acid shows much more pronounced interspecies variation than that of all-*trans*-retinoic acid (Nau, 1990, 1995). The plasma half-life of 13-*cis*-retinoic acid was comparable to that of all-*trans*-retinoic acid only in mice and rats, whereas it was one order of magnitude higher in rabbits, monkeys and humans (reviewed by Nau, 1995).

Embryonic concentrations of 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid were one order of magnitude lower than the plasma concentrations after dosing of the maternal animals with 13-*cis*-retinoic acid. The embryonic concentrations of 13-*cis*-retinoic acid were < 5% of the plasma concentrations after administration to mice, rats and rabbits at mid-gestation; higher embryo:maternal plasma concentration ratios for 13-*cis*-retinoic acid were observed at later gestational stages in rats and mice (Tzimas *et al.*, 1995). The hypothesis that the relatively extensive placental transfer of 13-*cis*-retinoic acid to the monkey embryo, as compared with the rodent embryo at mid-gestation, is related to the type of placenta (Hummler *et al.*, 1994) was addressed by comparing the placental transfer of 13-*cis*-retinoic acid in mice and rats at gestational ages at which the chorioallantoic placenta is either starting to differentiate (day 11 of gestation for mice and day 12 for rats) or is well established (day 14 for mice and day 16 for rats) (Tzimas *et al.*,

Table 5. Teratogenic effects of 13-*cis*-retinoic acid

Species	Dose (mg/kg bw)	Effects	Reference
Hamster	37.5; GD 8–11	Cranio-facial effect	Eckhoff & Willhite (1997)
Rabbit	15; GD 8–11	Resorptions, teratogenicity	Tembe *et al.* (1996)
Rabbit	10; GD 6–18	Low teratogenic response	Tzimas *et al.* (1994)
CF-1 mouse	100–400; GD 11–13	Delayed ossification, cleft palate, reduced by phenobarbital pretreatment	Yuschak & Gautieri (1993)
Cynomolgus monkey	2.5; GD 10–25	Heart defects	Hummler *et al.* (1990, 1994)
	2 x 2.5; GD 26, 27	External ears, thymus aplasia	
Rat	75; GD 8–10	Craniofacial defects; tail defects, spina bifida	Collins *et al.* (1994)
Cynomolgus monkey	2.5; DG 10–20	Craniofacial defects; ear and heart defects	Korte *et al.* (1993)
	2 x 2.5; GD 21–24		
NMRI mouse	10; GD 11	2% cleft palate	Creech Kraft *et al.* (1989)
ICR mouse	100, 150; GD 11	Cleft palate and limb defects, multiple dosing more effective; 4-oxo metabolite more teratogenic than parent drug	Kochhar & Penner (1987); Kochhar *et al.* (1996)
NMRI mouse	> 100	Cleft palate and limb defects	Creech Kraft *et al.* (1987)

GD, gestation day

1995). With advancing gestation, there was a twofold higher embryo:maternal plasma concentration ratio of 13-*cis*-retinoic acid in both rats and mice. The finding supports the hypothesis that the extensive placental transfer of 13-*cis*-retinoic acid and 13-*cis*-4-oxo-retinoic acid in cynomolgus monkeys can be accounted for by the presence of a functional chorioallantoic placenta on day 31 of gestation (Hummler *et al.*, 1994).

The teratogenicity induced by 13-*cis*-retinoic acid may be due at least in part to the action of the all-*trans*-isomer (Creech Kraft *et al.*, 1987, 1991b; Soprano *et al.*, 1994). The situation is, however, different in rats, rabbits and cynomolgus monkeys. In rats and rabbits, the embryonic concentrations of all-*trans*-retinoic acid after administration of teratogenic doses of 13-*cis*-retinoic acid were comparable to the endogenous concentrations. In monkeys, all-*trans*-retinoic acid may contribute to the teratogenicity of 13-*cis*-retinoic acid, because a significant increase in all-*trans*-retinoic acid over endogenous concentrations was observed after 13-*cis*-retinoic acid treatment (Tzimas *et al.*, 1996). Thus, embryonic exposure to all-*trans*-retinoic acid is not a prerequisite for 13-*cis*-retinoic acid-induced teratogenicity in all instances.

These data indicate that much higher doses of 13-*cis*-retinoic acid are required to elicit teratogenic effects in mice and rats ('insensitive' species) than in rabbits, monkeys and humans ('sensitive' species). This is partly due to the greater degree of detoxification of 13-*cis*-retinoic acid via β-glucuronidation and the more rapid elimination of the drug in mice and rats than in the other species. In addition, the placental transfer of 13-*cis*-retinoic acid and 13-*cis*-4-oxo-retinoic acid is more extensive in monkeys (Hummler *et al.*, 1994) than in mice, rats and rabbits (Tzimas *et al.*, 1995).

7.3 Genetic and related effects

7.3.1 Humans

During an intervention trial involving patients with oral premalignant lesions, the micronucleus frequencies in mucosal scrapings of the lesions and in normal-appearing mucosa were evaluated in patients receiving 13-*cis*-retinoic acid at 1.5 mg/kg bw. The treatment was continued for three months and followed by maintenance at 0.5 mg/kg bw or treatment with β-carotene at 30 mg/day. Most of these patients were cigarette smokers (27 of 40 were current smokers). The micronucleus frequencies were significantly reduced in both lesions and normal mucosa after the three-month treatment, and this effect was maintained during the following nine months by supplementation with either 13-*cis*-retinoic acid or β-carotene (Benner *et al.*, 1994b).

7.3.2 Experimental models

7.3.2.1 In vitro

The genotoxicity of 13-*cis*-retinoic acid in cultured cells has been examined in three studies, all on alterations of chromosomes. Mixed results were obtained (Table 6). 13-*cis*-Retinoic acid induced a dose-dependent increase in the frequency of sister chromatid exchange in human diploid fibroblasts, but it had no effect on V79 Chinese hamster cells. The authors attributed these findings to a lack of a measurable mono-oxygenase activity in the V79 cells. In support of this hypothesis, the retinoid-induced increase in sister chromatid exchange frequency was shown to be prevented by the addition of an inhibitor of P448-dependent mono-oxygenase, α-naphthoflavone, to the fibroblasts (Tetzner *et al.*, 1980). An increase in sister chromatid exchange frequency was also seen in human lymphocyte cultures treated with the retinoid, although the chromosomal aberration frequencies were unchanged (Auerbach *et al.*, 1984). The effect of this retinoid on sister chromatid exchange and chromosomal aberration frequencies in human embryonic palatal mesenchymal cells was examined in the presence and absence of a microsomal activation system. The authors suggested that there was a slight decrease in sister chromatid exchange frequency in the absence of exogenous metabolic activation, but this effect was not statistically significant. No change was observed in chromosomal aberration frequency (Watanabe & Pratt, 1991).

7.3.2.2. In vivo

No data were available to the Working Group.

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

13-*cis*-Retinoic acid is derived from 13-*cis*-retinol by oxidation of the C-15 alcohol group to a carboxylic acid or by isomerization of all-*trans*-retinoic acid. Like all members of the vitamin A family, 13-*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. Because of its conjugated tetraene structure, 13-*cis*-retinoic acid has characteristic absorption spectra in the ultraviolet and visible, infrared and resonance Raman portions of the electromagnetic spectrum.

13-*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 essentially absent from plant tissues. Human exposure occurs during treatment with oral preparations and, to a much smaller extent, with topical ointments for medical or cosmetic purposes.

13-*cis*-Retinoic acid has been used to treat dermatological disorders and several forms of cancer. The efficacious oral doses are 0.5–2 mg/kg of body weight per day.

13-*cis*-Retinoic acid is usually separated by high-performance liquid chromatography and detected by its absorption at 354 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

13-*cis*-Retinoic acid is present normally in blood and is widely distributed in tissues at concentrations similar to those of all-*trans*-retinoic acid. Sulfhydryl groups, both those present in small dialysable molecules like glutathione and those present as amino-acid residues in proteins, can catalyse interconversion of all-*trans*-retinoic acid and the 13-*cis*-isomer. Since 13-*cis*-retinoic acid does not bind to retinoic acid receptors, it is generally assumed that it acts as a precursor for all-*trans*-retinoic acid. Pharmacokinetic studies indicate that 13-*cis*-retinoic acid has a much longer elimination half-life in most species than all-*trans*-retinoic acid.

Table 6. Genetic and related effects of 13-*cis*-retinoic acid in short-term tests *in vitro*

Test system	Result[a]		LED/HID[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human diploid fibroblasts	+	0	0.5 µg/ml	Tetzner *et al.* (1980)
Sister chromatid exchange, V79 Chinese hamster fibroblasts	–	0	8 µg/ml	Tetzner *et al.* (1980)
Sister chromatid exchange, human lymphocytes	+	0	25 µmol/L	Auerbach *et al.* (1984)
Sister chromatid exchange, human embryonic palatal mesenchymal cells	–	–	100 µmol/L (–S9) 200 µmol/L (+S9)	Watanabe & Pratt (1991)
Chromosomal aberrations, human lymphocytes	–	0	50 µmol/L	Auerbach *et al.* (1984)
Chromosomal aberrations, human embryonic palatal mesenchymal cells	–	–	250 µmol/L (–S9) 200 µmol/L (+S9)	Watanabe & Pratt (1991)

[a] Result: +, positive; – considered to be negative; 0, not tested

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

8.3 Cancer-preventive effects

8.3.1 Humans

Secondary analyses of the results of one randomized trial of use of 13-*cis*-retinoic acid as adjuvant therapy for cancers of the head and neck indicated a statistically significant reduction in the incidence of second primary tumours of the upper aerodigestive tract. A study of use of 13-*cis*-retinoic acid at high doses and in a group at inherited high risk, with no controls, suggested that this compound is effective in preventing basal- and squamous-cell cancers of the skin. Two randomized controlled trials among patients at lower risk and involving lower (and better tolerated) doses of 13-*cis*-retinoic acid have shown no evidence of preventive efficacy.

High doses of 13-*cis*-retinoic acid were shown to be effective against oral leukoplakia in two randomized trials, one with controls receiving placebo and the other with persons receiving β-carotene. One controlled trial showed no effect of 13-*cis*-retinoic acid in reducing cytological changes in the bronchi. Studies of molecular markers suggested that 13-*cis*-retinoic acid increases expression of human retinoic acid receptor β, but the relevance of these findings to cancer-preventive activity is unclear.

A single intervention study showed a decrease in micronucleus formation in cells of the buccal cavity in patients, some of whom were smokers, who had been treated with 13-*cis*-retinoic acid for 12 months.

8.3.2 Experimental models

The preventive efficacy of 13-*cis*-retinoic acid has been evaluated in two-stage skin carcinogenesis models in mice and in urinary bladder carcinogenesis models in mice and rats. 13-*cis*-Retinoic acid was effective in most studies with both models. It was ineffective in models of tracheal, salivary gland, oesophageal and renal carcinogenesis.

In vitro, 13-*cis*-retinoic acid inhibited proliferation in numerous cell lines, few of which were immortalized and most of which were established tumour cell lines. 13-*cis*-Retinoic acid inhibited growth in both monolayers of adherent cell

cultures and in semi-solid medium (anchorage-independent growth). 13-*cis*-Retinoic acid also induced differentiation in transformed cells and triggered apoptosis in a few cell lines. In most cell lines, the response to 13-*cis*-retinoic acid was similar to that to all-*trans*-retinoic acid.

The ability of 13-*cis*-retinoic acid to inhibit genetic and related effects in cell cultures has been evaluated in a limited number of studies, and these have yielded mixed results. In two studies, a reduction in the frequency of chromosomal damage was seen in human lymphocytes exposed to radical-generating agents (bleomycin and X-irradiation) when they were pretreated with 13-*cis*-retinoic acid; in contrast, a third study showed an increase in the frequency of diepoxybutane-induced sister chromatid exchange and chromosomal damage in human lymphocytes treated concurrently with the mutagen and the retinoid.

Orally administered 13-*cis*-retinoic acid inhibited the induction of micronucleated cells in the bone marrow of animals treated with benzo[*a*]pyrene and reduced the binding of this carcinogen to DNA in the liver, stomach and lung, but not the kidney. Although the mechanism of this protective effect is unknown, several studies showed a significant alteration in microsomal enzyme activity in both liver and skin of mammals treated with 13-*cis*-retinoic acid.

8.3.3 Mechanisms of cancer prevention

13-*cis*-Retinoic acid is readily isomerized to all-*trans*-retinoic acid which may explain most, if not all, of its actions.

8.4 Other beneficial effects

Treatment with 13-*cis*-retinoic acid is of benefit to patients suffering from a wide variety of dermatological disorders.

8.5 Carcinogenicity

No data were available to the Working Group.

8.6 Other toxic effects

8.6.1 Humans

The toxicity of 13-*cis*-retinoic acid is similar to that seen in hypervitaminosis A; most of the adverse reactions are dose-dependent and reversible, although some persist after discontinuation of therapy. The doses of 13-*cis*-retinoic acid used for chemoprevention are generally within the range of doses recommended for the treatment of acne; therefore, the toxicity seen in chemoprevention trials is essentially the same as that seen with use of 13-*cis*-retinoic acid for dermatological indications. The symptoms affect the skin and mucous membranes and the musculoskeletal system, including dry skin, cheilitis, conjunctivitis and arthralgia. Skin reactions can usually be tolerated without interruption of therapy by the use of emollients and other treatments, and the effects generally resolve after therapy is discontinued. The most common abnormalities seen in laboratory examinations include hypertriglyceridaemia, hypercholesterolaemia, decreased serum concentrations of high-density lipoproteins and elevated activity of serum liver enzymes. Rarely, acute complications develop secondary to these abnormalities. Headache, often described as severe, impaired hearing, dizziness and psychological disorders have been associated with oral administration of 13-*cis*-retinoic acid.

13-*cis*-Retinoic acid is a confirmed human teratogen. The potential developmental toxicity associated with maternal therapy with this retinoid depends on the dose, the stage of gestation or the age of the patient, the duration of treatment and the route of administration. Dose-dependent desquamation of the testicular germinal epithelium to the point of necrosis has been observed in preclinical studies with 13-*cis*-retinoic acid, but such changes have not been observed in patients given the standard therapeutic dose.

There are no reports of genotoxic activity of 13-*cis*-retinoic acid in humans.

8.6.2 Experimental models

In short-term studies of toxicity in rats and dogs, dose-related effects were seen in both species, including decreased food consumption and body-weight gain, erythema, alopecia, mucosal changes, elevated serum alkaline phosphatase and transaminase activities and increased liver weight. Long-bone fractures and elevated serum triglyceride concentrations were seen in rats, while apparent joint pain was seen in dogs.

The dose-related effects seen in long-term studies of toxicity in rats included increased mortality rates and decreased food consumption and body-weight gain. Decreased haemoglobin concentration and haematocrit and elevated serum triglyceride concentrations were observed at higher doses, and increased alkaline phosphatase activity and increased liver and kidney weights were observed in all treated groups. Fibrosis and

inflammation of the myocardium, arterial calcification, focal tissue calcification and focal osteolysis were seen at higher doses. In dogs, a dose of 120 mg/kg bw per day led to severe weight loss and debilitation within four weeks. With a dose cycle of 60 mg/kg bw per day for six weeks and no treatment for two weeks, toxic clinical effects observed during the treatment cycle included severe weight loss and skin changes, decreased haemoglobin concentration and haematocrit and increased serum alkaline phosphatase activity. Fibrosis and focal calcification in the myocardium and aorta and increased liver weight and lymph node oedema were observed at higher doses.

Long-term oral administration of 13-*cis*-retinoic acid can induce testicular toxicity in animals and interfere with spermatogenesis. 13-*cis*-Retinoic acid is an even more potent teratogen than all-*trans*-retinoic acid in monkeys, because of its relatively long half-life, metabolism to an active metabolite (13-*cis*-4-oxoretinoic acid) and efficient placental transfer.

The capacity of 13-*cis*-retinoic acid to induce chromosomal damage and sister chromatid exchange in cultured mammalian cells was examined in three studies. No change in the frequency of chromosomal aberrations was seen, but the results for sister chromatid exchange were contradictory, two showing an increase and the third showing no effect.

9. Recommendations for Research

9.1 General recommendations for 13-*cis*-retinoic acid and other retinoids

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

9.2 Recommendations specific to 13-*cis*-retinoic acid

None.

10. Evaluation

10.1 Cancer-preventive activity

10.1.1 Humans

There is *limited evidence* that 13-*cis*-retinoic acid has cancer-preventive activity in humans. This evaluation is based on its effectiveness against oral leukoplakia and preliminary evidence for prevention of second primary cancers of the aerodigestive tract.

10.1.2 Experimental animals

There is *limited evidence* that 13-*cis*-retinoic acid has cancer-preventive activity in experimental animals. This evaluation is based on the observation of inhibitory effects in most but not all studies with models of skin and urinary bladder carcinogenesis.

10.2 Overall evaluation

13-*cis*-Retinoic acid probably has cancer-preventive activity in humans, but it has a relatively low therapeutic ratio of efficacy to toxicity and is an established human teratogen. 13-*cis*-Retinoic acid is of value for treating a variety of dermatological disorders.

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