Handbook 5

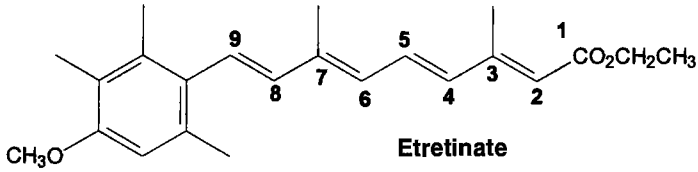
Etretinate

1. Chemical and Physical Characteristics

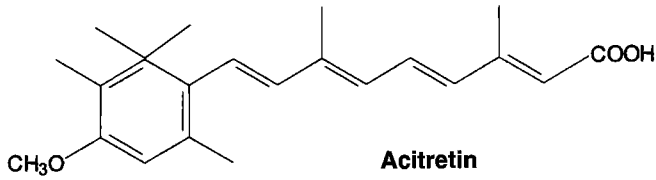
1.1 Nomenclature

Etretinate belongs to the class of synthetic aromatic retinoids in which the lipophilic trimethylcyclohexenyl group of retinoic acid has been replaced by an aromatic ring. In the case of etretinate, the cyclohexenyl group has been replaced by a 4-methoxy-2,3,6-trimethylphenyl group while the all-*trans*-tetraene structure of the retinoic acid side-chain has been retained. In a further departure from the retinoic acid structure, the terminal carboxyl group of etretinate has been derived as an ethyl ester. The free acid form of etretinate is acitretin (Figure 1).

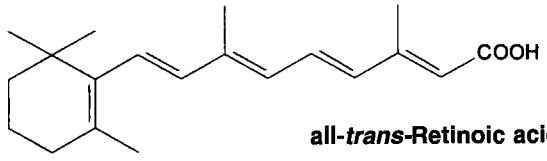
When reference is made to 'etretinate' it is assumed to be the all-*trans* isomer, unlike the retinoic acids. In this nomenclature, the side-chain of etretinate is numbered starting from the carboxylate carbon; but since etretinate is a synthetic derivative of retinoic acid, the retinoid numbering system is often used for etretinate and its derivatives. Application of the numbering system commonly used for retinoic acid to the basic skeleton of etretinate is shown in Figure 2. For example, ethyl 2*Z*, 4*E*, 6*E*, 8*E*-3,7-dimethyl-9-(4-methoxy-2,3,6-trimethylphenyl)nona-2,4,6,8-tetraenoate, a geometric isomer of etretinate, is commonly referred to as 13-*cis*-etretinate (Figure 2). The methyl groups attached to the tetraene side-chain are often referred to as the C-9 and C-13 methyls, in keeping with retinoid nomenclature. The free-acid derivative of etretinate, which is a major metabolite and the biologically active form, is all-*trans*-3,7-dimethyl-9-(4-methoxy-2,3,6-trimethylphenyl)nona-2,4,6,8-tetraenoic acid, commonly known as acitretin or etretin (see Handbook on acitretin; Figure 1).



Etretinate



Acitretin



all-*trans*-Retinoic acid

Figure 1. Structures of all-*trans*-retinoic acid, etretinate and acitretin

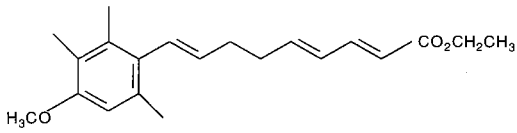
1.2 Name

Chemical Abstracts Services Registry Number
54350-48-0

IUPAC Systematic name
Ethyl all-*trans*-3,7-dimethyl-9-(4-methoxy-2,3,6-trimethylphenyl)nona-2,4,6,8-tetraenoate

Synonyms
Tetison® R010.98359; Tigason

1.3 Structural formula



Composition: $C_{23}H_{30}O_3$
Relative molecular mass: 354

1.4 Physical and chemical properties

Melting-point

104–105 °C (Budavari *et al.*, 1989)

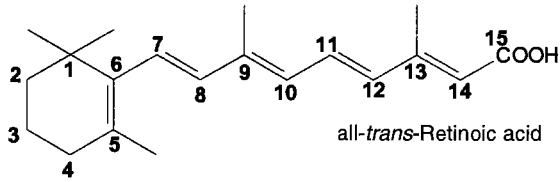
Spectroscopy
UV and visible spectrum: λ_{max} = 351 nm in 1.5% diisopropyl ether in hexane (Englert *et al.*, 1978)

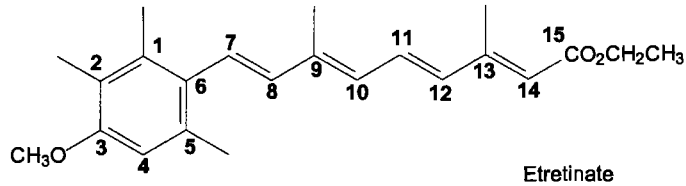
Mass spectrum
(m/e, %): 354 (M^+, 85), 339 (30), 293 (30), 281 (90), 191 (42), 203 (80),163 (86), 150 (100), 265 (38), 251 (53), 201 (55) (Hänni *et al.*, 1977)

^{1}H-NMR ($CDCl_3$, 270 MHz): δ 1.29 (3H, t, *J* = 7.1 Hz), 2.10 (3H, s), 2.15, 2.23 and 2.29 (9H, 3s), 2.36 (3H, s), 3.81 (3H,s), 4.17 (2H, q, *J* = 7.1 Hz), 5.78 (1H, s), 6.19 (1H, d, *J* = 11.4 Hz), 6.24 (1H, d, *J* = 16.3 Hz), 6.31 (1H, d, *J* = 15.1 Hz), 6.60 (1H, s), 6.68 (1H, d, *J*= 16.3 Hz), 7.02 (1H, dd, *J* =11.4, 15.11 Hz) (Englert *et al.*, 1978).

^{13}C NMR ($CDCl_3$, 68 MHz): δ 11.82, 12.86, 13.84, 14.40, 17.36, 21.36, 55.41, 59.50, 110.24, 119.11, 122.84, 128.64, 130.02, 130.41, 130.53, 133.85, 135.82, 135.87, 138.18, 138.95, 152.28, 156.23, 166.85 (Englert *et al.*, 1978)

Geometric isomers
Sixteen possible isomers





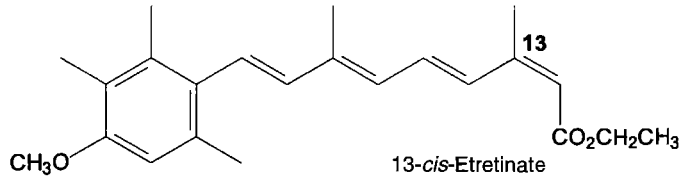


Figure 2. Common numbering scheme for retinoids

1.4.1 *Photochemical properties*

Etretinate is a yellow to greenish-yellow compound with an absorption maximum (λ_{max}) at 351 nm (1.5% diisopropylether in hexane) in the UV and visible spectrum. Because of its conjugated tetraene structure, etretinate can readily undergo photoisomerizaiton reactions when exposed to light, particularly in solution. Irradiation of dilute solutions of etretinate in hexane, benzene or ethanol under an inert atmosphere with a high-pressure xenon lamp gave complex mixtures of products which were shown by mass spectrometric analysis to consist entirely of geometric isomers. The equilibrium concentrations of the main isomers were obtained after 1 h of irradiation and consisted primarily of the 9,13-di-*cis*, 13-*cis*, 11-*cis*, 11,13 di-*cis*, all-*trans*, 9-*cis*, 7,13-di-*cis* and 7-*cis* isomers. Four other uncharacterized isomers were produced in minor amounts (Englert *et al.*, 1978).

1.4.2 *Solubility and interactions* in vivo

Etretinate is a very lipophilic molecule which readily partitions into hydrophobic environments. In humans, etretinate is stored in adipose tissue (Paravicini *et al.*, 1981). More than 99% of that which is found in the circulation is bound to plasma proteins, primarily lipoproteins. Acitretin, the free acid metabolite of etretinate, is bound predominantly to serum albumin in the circulation.

1.4.3 *Relationships between chemical structure and biological activity*

The pharmacological activity of etretinate is probably primarily due to activation of the retinoic acid receptors (RARs), which are ligand-inducible transcription factors belonging to the steroid-thyroid superfamily of nuclear receptors (Evans, 1988). The physiological hormone for the RARs is retinoic acid (Giguère *et al.*, 1987; Petkovich *et al.*, 1987), and extensive studies of structure–activity relationships with retinoid analogues have established that a terminal carboxylic acid group and a lipophilic head group are required for interaction with the RARs (Gale, 1993). Since etretinate is an ethyl ester derivative, it would not be expected to bend the RARs and it would require conversion to its free acid form, acitretin, to activate the RARs. The lipophilic group required for RAR activity is provided by 4-methoxy-2,3,6-trimethylphenyl on etretinate. The planar all-*trans* configuration of the etretinate side-chain appears to be optimal for RAR activity, but 9-*cis*-retinoic acid, the putative physiological ligand for the retinoid X receptors (RXRs), also activates RARs (Heyman *et al.*, 1992). Thus, the 9-*cis* isomer of acitretin may activate RARs.

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

2.1 Occurrence

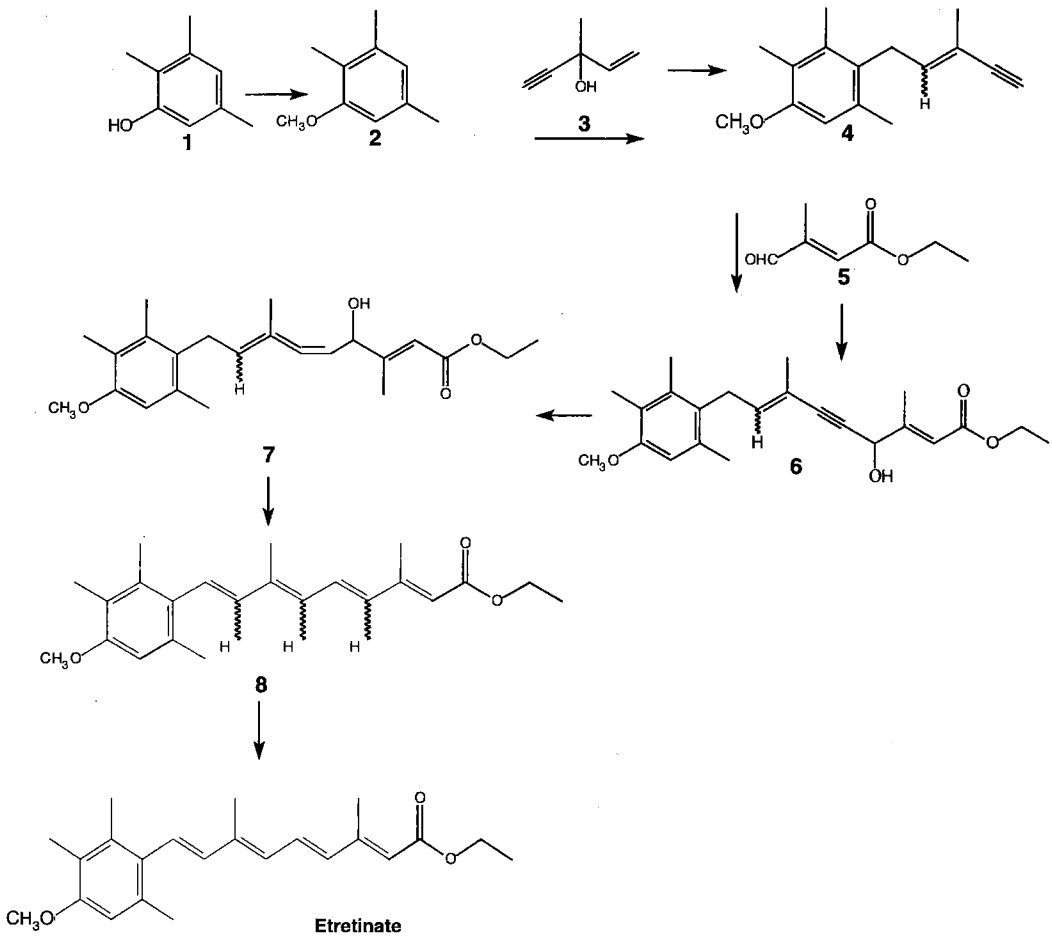
Etretinate is not a naturally occurring compound but can readily be synthesized by a variety of routes.

2.2 Production

The synthesis of etretinate starting from 2,3,5-trimethylphenol, **1**, is outlined in Scheme 1 (Soukup *et al.*, 1989). Compound **1** is first methylated to compound **2** and then subjected to Friedel-Crafts reaction conditions with the alcohol **3** to give a mixture of the terminal alkynes, **4**. Deprotonation of **4** followed by reaction with the aldehyde **5** gave the propargyl alcohol, **6**. Hydrogenation of **6** to give **7** followed by dehydration gave a mixture of geometric isomers, **8**. Isomerization of **8** gave the all-*trans* isomer, etretinate, as the major product with smaller amounts of the 9-*cis* and 13-*cis* isomers.

The chemistry described by Soukup *et al.* (1989) also provides alternative routes to etretinate, as outlined in Scheme 2. The anisole, **2**, can be formylated to give the aldehyde, **9**, which is formally equivalent to β-cyclocitral, the C_{10} unit employed in retinoic acid synthesis. Compound **9** can be used as the starting material in a variety of strategies for elaborating the tetraenoate side-chain of etretinate. Alternatively, the anisole, **2**, can be brominated to the aryl bromide, **10**, which can be reacted with the terminal alkyne, **11**, under Pd(0) catalysed conditions to give the alcohol, **12**, which is formally equivalent to the C_{15} unit used in retinoic acid synthesis. Conversion of **12** to the phosphonium salt, **13**, followed by hydrogenation gave **14**. Wittig reaction of **14** with the aldehyde, **5**, preceded or followed by isomerization, will yield etretinate.

An alternative synthesis of acitretin, the free acid form of etretinate, is outlined in Scheme 3 (Aurell *et al.*, 1995). The lithium trienediolate generated from the hexa-2,4-dienoic acid, **16**, when

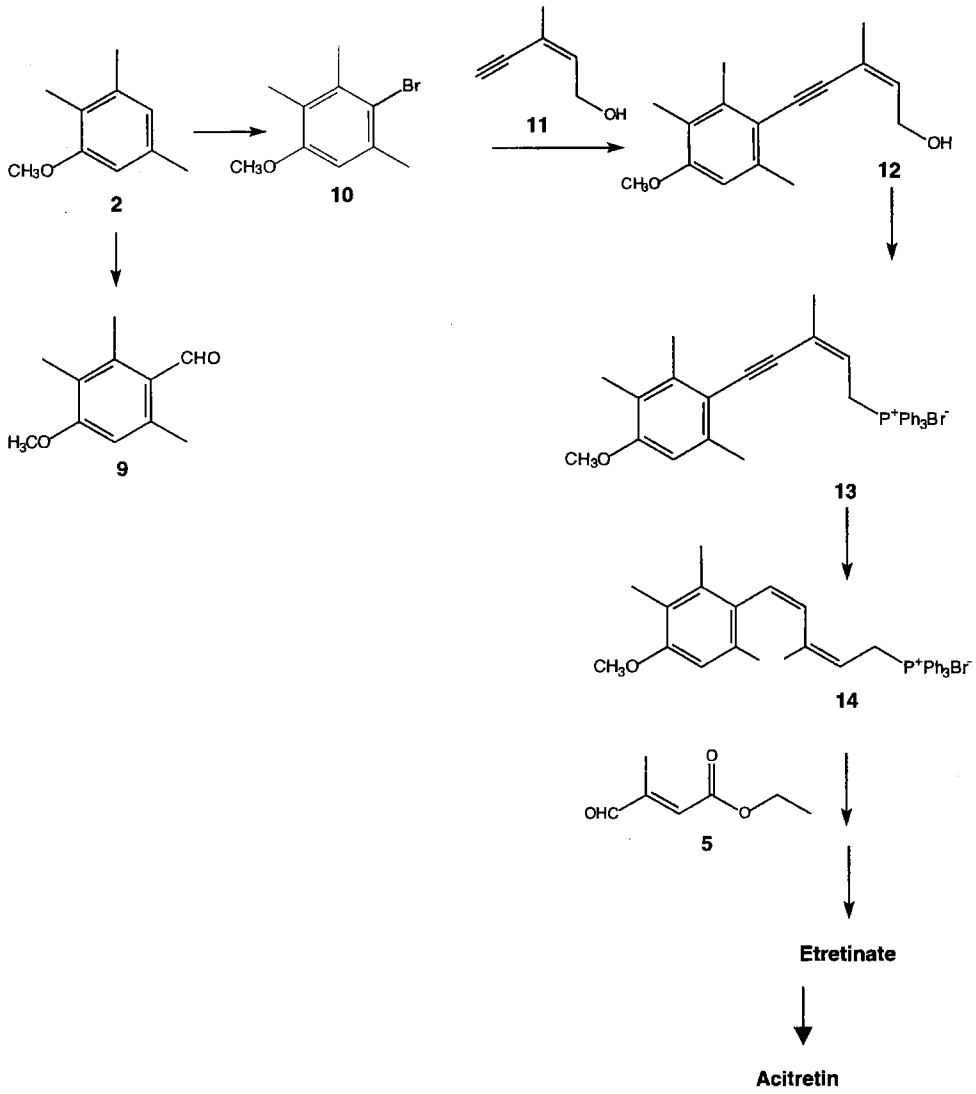


Scheme 1.
Geometric isomers are denoted by wavy lines.

reacted with the ketone, 17, gave the alcohol, 18. Dehydration of 18 followed by isomerization of the resultant mixture of geometric isomers gave acitretin, which can be esterified to etretinate. Synthesis of etretinate, derivatives of etretinate and geometric isomers of these compounds have also been described by Bestmann and Ermann (1984) and Makin *et al.* (1989). The large-scale synthesis of acitretin and various derivatives including etretinate was described by Bollag *et al.* (1978).

2.3 Use

Etretinate was approved by the appropriate regulatory agencies in most countries of the world for use in the treatment of severe recalcitrant psoriasis, including erythrodermic and generalized pustular psoriasis (Ellis & Voorhees, 1987). It is,

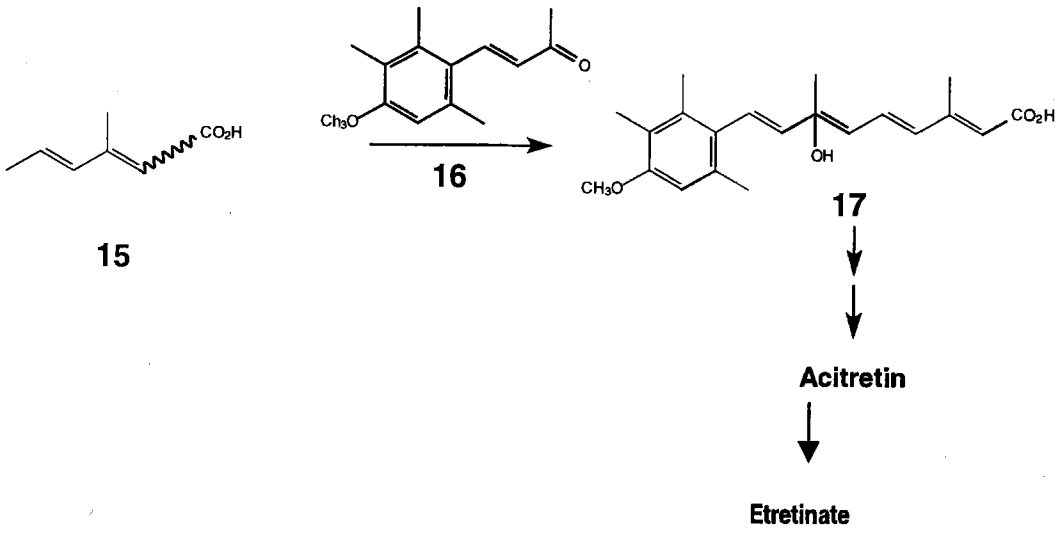


Scheme 2

however, no longer marketed and has been replaced by acitretin wherever the latter has been approved.

2.4 Human exposure

Etretinate has been shown to be effective in the treatment not only of severe recalcitrant psoriasis but also of a variety of other cutaneous disorders of keratinization including Darier disease, lamellar ichthyosis, nonbullous congenital ichthyosiform erythroderma, pityriasis rubra pilaris, epidermolytic hyperkeratosis, keratoderma palmaris et plantaris, X-linked ichthyosis, ichthyosis vulgaris, erythrokeratodermia variabilis and lichen planus (Peck, 1984). Etretinate has also been considered for treatment of certain skin cancers and premalignant conditions, including mycosis fungoides, basal-cell carcinoma, actinic keratoses, keratoacanthoma and epidermodysplasia verruciformis (see section 4.1.3). It has been recommended that



Scheme 3.

etretinate be taken with food to increase its absorption (DiGiovanna *et al.*, 1984).

Etretinate was marketed in 10-mg and 25-mg gelatin capsules for oral administration. Patients with psoriasis usually began with an initial daily dosage of 0.75–1 mg/kg bw given in divided doses (Goldfarb & Ellis, 1998; Paul & Dubertret, 1998). It was recommended that a maximum dose of 1.5 mg/kg bw per day not be exceeded. Maintenance doses of 0.5–0.75 mg/kg bw per day were recommended after an initial response was obtained. A dose of 0.25 mg/kg bw per day was recommended for the initial treatment of erythrodermic psoriasis. Initial responses were observed within 8–16 weeks, but patients were maintained on therapy for up to nine months. Similar doses of etretinate have been used for the treatment of cutaneous disorders of keratinization and cutaneous malignancies and premalignancies (Peck, 1984).

2.5 Analysis

Numerous methods based on high-performance liquid chromatography (HPLC) have been described for the quantitative analysis in plasma of etretinate, acitretin and their geometric isomers. The limit of detection of a reversed-phase HPLC method for analysis of etretinate and acitretin in plasma was 10 ng/ml (Palmskog, 1980), while that of a normal-phase HPLC method for the same two compounds was 4 ng/ml (Paravicini & Busslinger, 1983). An alternative reversed-phase HPLC method for the simultaneous determination of etretinate and acitretin in rat blood required much smaller sample volumes and allowed for serial sampling (Thongnopnua & Zimmerman, 1988). Another normal-phase HPLC method which allowed for the determination of etretinate, acitretin and the 13-*cis* isomer of acitretin, with a detection limit of 3 ng/ml, was used to study the long-term pharmacokinetics of etretinate in patients with psoriasis who had been changed from etretinate to acitretin therapy (De Leenheer *et al.*, 1990). A reversed-phase HPLC method with shorter retention times was also used to detect these three compounds in human plasma (Jakobsen *et al.*, 1987). A programmed-gradient HPLC system for the analysis of etretinate, its metabolites and other retinoids in plasma allowed shortened analysis and better peak shapes (Annesley *et al.*, 1984). Problems of recovery arising from strong binding of retinoids to plasma proteins were addressed by column-switching techniques (Wyss & Bucheli, 1988; Wyss, 1990).

HPLC methods were used to isolate metabolites from the faeces and urine of persons treated with tritium-labelled etretinate. The structures of the metabolites were elucidated by mass spectrometry and ^{1}H-NMR spectroscopy (Hänni *et al.*, 1977). Bile samples collected from patients treated with ^{14}C-labelled etretinate were analysed by HPLC after β-glucuronidase treatment, and the structures of the metabolites were determined by mass spectrometry and ^{1}H-NMR spectroscopy (Vane *et al.*, 1989a). Similarly, HPLC methods were used to isolate metabolites from the blood of persons with psoriasis treated with etretinate, and spectroscopic techniques were used to elucidate their structures (Vane *et al.*, 1989b). Another reversed-phase HPLC method has been developed for the simultaneous assay of etretinate, acitretin and their metabolites in whole perfusate, perfusate plasma, bile and hepatic tissue obtained in a perfused rat liver model (Decker & Zimmerman, 1995).

3. Metabolism, Kinetics and Genetic Variation

Etretinate is a retinoid which requires metabolic conversion to its free acid form, acitretin, in order to exert its biological activity.

3.1 Humans

About 40% of an oral dose of etretinate is bioavailable. The pharmokinetics of etretinate after administration of single doses to healthy volunteers and patients with psoriasis has been studied extensively (Gollnick *et al.*, 1990). The tabulated results of 10 such studies (Larsen, 1994) indicate that the vast majority were undertaken with a dose of 100 mg and the peak plasma concentration was 100–1400 ng/ml. The corresponding concentration of acitretin, recorded 3–6 h after administration of etretinate, was 100–600 ng/ml. The drug can be detected in plasma 30 min after oral intake. The pharmocokinetics of etretinate may be affected by intake of milk or food. No definitive correlation has been demonstrated between the plasma concentration and the therapeutic effects of etretinate.

The pharmocokinetics of etretinate after multiple oral doses has been the subject of at least eight studies, most of which involved patients with psoriasis (Lucek & Colburn, 1985; Larsen, 1994; Orfanos *et al.*, 1997). The mean terminal elimination half-life of etretinate ranged from 1 to 40 days, which was significantly longer than that determined after single doses (4–10 h). The mean terminal half-life was determined to be 25 days for etretinate, 6.5 days for the metabolite acitretin and 16 days for 13-*cis*-acitretin (Larsen, 1994).

Etretinate is an ethyl ester which undergoes extensive hydrolysis in the liver, gut and blood after oral absorption to yield the corresponding acid metabolite, acitretin. Hydrolysis initially takes place in the gut or gut wall. The methoxy group on the aromatic ring is subsequently demethylated, most probably in the liver. Other metabolic transformations include shortening of the side-chain, glucuronidation, hydroxylation of the methoxy group, isomerization to 13-*cis*-acitretin and reduction of one or two double-bonds in the side-chain (Hill & Sani, 1991; Larsen, 1994). In addition to the parent compound, 9-*cis*-acitretin, 13-*cis*-acitretin and three minor metabolites are found in plasma, and various metabolites are excreted in both the bile and the urine. The absorption and metabolism of etretinate have not been correlated with liver damage in patients with psoriasis. After administration of radiolabelled etretinate to healthy subjects, radiolabelled metabolites were detected in urine and faeces for as long as three weeks. The major reason for the observed decrease in the concentration of etretinate in blood after biliary cannulation was reduced absorption due to elimination of solubilizing bile salts in the duodenum (Lucek *et al.*, 1988). In general, there is no correlation between drug dose and rate of elimination.

Etretinate is a highly lipophilic compound which is extensively bound in plasma to lipoproteins. The mean concentrations of serum lipids (triglyceride and cholesterol) increase after treatment with etretinate. The changes in very low-density and low-density lipoprotein suggest that the increase may be due to enhanced synthesis of lipoproteins (Marsden, 1986). Up to about 200 and 130 etretinate molecules can bind to one molecule of low-density lipoprotein and one molecule of high-density lipoprotein, respectively; human serum albumin binds about 10 etretinate molecules (Carrillet *et al.*, 1990). After repetitive dosing, the compound accumulates in fat, liver and adrenals (Rollman *et al.*, 1989).

3.2 Experimental models

After intravenous injection to rats, etretinate is distributed primarily in muscle, skin and particularly in adipose tissue. After 6 h, about 45% of a dose is metabolized to acitretin and about 40% to unidentified metabolites (Eisenhardt & Bickel, 1994). Body fat has a significant effect on etretinate disposition in rats, slowing the systemic clearance (Chien *et al.*, 1992).

In rats, 15% of an oral dose of etretinate is bioavailable. In the bile of rats given etretinate intravenously, the free acid (acitretin) and other conjugated metabolites are present in a conjugated form; one of these lacks the methyl of the methoxy group. Pregnant rats have a lower rate of clearance of etretinate because of a lower rate of formation of acitretin (Hill & Sani, 1991). Etretinate is transferred across the placenta and is secreted in milk (Reiners *et al.*, 1988; Gollnick *et al.*, 1990). In dogs dosed with etretinate, the oral bioavailability is 52%, and the terminal half-life for elimination from plasma is > 300 h (Gollnick *et al.*, 1990).

Etretinate accumulated in adipose tissue in a genetically obese rodent model. At doses up to 30 mg/kg bw, it did not change the mineral composition of bone, despite obvious macroscopic alterations (Krari *et al.*, 1989).

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 Urinary bladder

Alfthan *et al.* (1983) studied the effect of etretinate in the prevention of recurrence of superficial bladder tumours in patients cleared of all visible tumours by electrocoagulation or trans-urethral resection. Fifteen patients were randomized to receive etretinate and 15 to placebo. One given the placebo died of a myocardial infarct within a month, and one receiving etretinate dropped out because of side-effects after four weeks. Etretinate was given to 15 patients at a dose of 50 mg/day for a month, which was reduced to 25 mg/day, and was given to the remaining patients at a dose of 25 mg/day for the duration of therapy. Therapy was continued for 10–26 (mean, 17.6) months. Of the patients given etretinate, six had no recurrences, five had fewer recurrences than before treatment, four had no change, and none had progressive disease. Of those given placebo, two had no recurrences, two had fewer recurrences than before treatment, none had no change and two had progressive disease.

Charbit *et al.* (1983) undertook a double-blind study in a group of 20 patients of the possible effectiveness of etretinate in the prevention of recurrences of superficial tumours of the bladder. Etretinate was given at a dose of 30 mg/day; the 10 controls received a placebo. Treatment was given for an average of 16 months (range, 8–25 months for etretinate and 8–29 months for placebo). The rate of recurrence with etretinate (6/10) was not lower than that with placebo (3/10).

Pedersen *et al.* (1984) studied patients with non-invasive bladder tumours who had experienced at least two recurrences in the previous 18 months, which had been surgically removed. Forty-seven patients were randomized to receive etretinate at 50 mg/day and 49 to receive placebo. During the first four months, 11 patients given etretinate withdrew because of side-effects, and one died of intercurrent disease. Four patients given placebo also withdrew, and two died of intercurrent disease. At four months, cystoscopy was repeated, and recurrent tumours were resected. One patient with invasive cancer who had been given placebo was taken off the study. By eight months, six further patients given etretinate had stopped treatment because of toxic effects, but four could be evaluated and cystoscopy was repeated. There was no difference in the recurrence rate: nine of 33 evaluable patients given etretinate and 15 of 40 given placebo were free of tumour.

Studer *et al.* (1984) studied 86 patients with recurrent superficial bladder tumours treated by trans-urethral resection in a double-blind randomized trial. Etretinate was initially given at a dose of 50 mg/day, but this was reduced to 25 mg/day in 90% of the cases because of side-effects. At the time of the report, 25 patients had been followed for two or more years, 40 were still under observation and 21 had been withdrawn from the trial. Of the latter, three given etretinate had died of causes unrelated to treatment, and treatment of two patients given etretinate and nine on placebo had been stopped because of progression of the disease.

There were fewer recurrences at 3, 12 and 24 months in the group given etretinate than in the group given placebo, but the differences were not statistically significant. There was, however, a statistically significantly lower frequency of multifocal recurrences in patients given etretinate. [The Working Group noted that the design of the study and the methods of statistical analysis were not clearly described.]

A subsequent report on this study (Studer *et al.*, 1995) provided additional information on outcomes. Data on 42 patients given placebo and 37 treated with etretinate showed no difference in the proportions who had a recurrence after randomization (74 and 70%, respectively) or in the time to first recurrence. The authors reported, however, that there was a statistically significantly longer time between subsequent recurrences among treated patients in comparison with those given the placebo. They also noted that more patients on placebo were withdrawn from the study because of apparent treatment failure, and that this may have biased the results against treatment with etretinate. [The Working Group noted that the statistical approach described in this report was unconventional and did not include a 'time-to-failure' component.]

Yoshida *et al.* (1986) studied 174 patients with superficial bladder tumours that had been treated by trans-urethral resection. Ninety-four subjects were randomized to receive etretinate at 10 mg/day and 80 to receive no treatment. Patients were examined for recurrence every three months. Nine of the patients in the group receiving etretinate and eight controls dropped out of the study. The recurrence rate over the two-year observation period was 18% in the group given etretinate and 38% in the controls ($p < 0.1$; Kaplan-Meier). Etretinate was reported to have reduced the rate of recurrence of multiple tumours and tumours smaller than 1 cm. [The Working Group noted that patients were randomized in this trial by the envelope method, which allows breaking of randomization and that the control group did not receive a placebo.]

Hirao *et al.* (1987) included 130 patients with superficial bladder cancer treated by trans-urethral electroresection in a randomized study in which a number of agents administered intravesically or orally were evaluated. Twenty patients who received the agents orally were allocated to receive etretinate at 10 mg/day for two years. The tumours of five patients given etretinate and eight of 27 controls recurred. The actuarial non-recurrence rate at 48 months was 69% in the group given etretinate and 58% in controls (not significant).

4.1.2.2 Head-and-neck cancers

Bolla *et al.* (1994) studied the effect of etretinate on the development of second primary tumours in 316 patients who had been treated for squamous-cell carcinoma of the head and neck. Therapy with etretinate consisted of a loading dose of 50 mg/day for the first month, followed by 25 mg/day for up to 24 months. Randomly allocated controls received a placebo by the same schedule. Treatment began no later than 15 days after surgery or the initiation of radiotherapy. Over a median 41-month follow-up period, there were no differences between the groups in overall survival or disease-free survival. Second primary tumours occurred in 28 patients given etretinate and 29 given placebo.

In a subsequent report (Bolla *et al.*, 1996), based on a mean follow-up of 65 months, 42 new primary tumours were found in the etretinate-treated group and 40 in the placebo group (not significant).

4.1.3 Intermediary end-points

4.1.3.1 Lung

Gouveia *et al.* (1982) determined by means of bronchoscopy and bronchial biopsy the degree of metaplasia in 70 volunteers who had smoked at least 15 pack-years. The 34 who had an index of metaplasia > 15% were given a six-month course of etretinate at 25 mg/day. No control group was included. At six months, a second bronchoscopy with repeat bronchial biopsies was performed. Of 11 subjects who had completed the therapy at the time of this preliminary communication and who had continued to smoke, two showed a reduction in the index of metaplasia from that before treatment.

As an extension of this study, Misset *et al.* (1986) reported on 40 heavy smokers who had received six months' treatment with etretinate at 25 mg/day. The majority of patients who had continued to smoke had a reduced index of metaplasia, but eight appeared to have increased scores.

Arnold *et al.* (1992) evaluated the effect of etretinate on the presence of bronchial atypia in the sputum of 150 current smokers with at least a

15-pack-year history of smoking, mild bronchial atypia in at least two samples of sputum or moderate or severe atypia in one sample. The subjects were selected from a pool of 2223 potential participants attending one of two clinics in Ontario, Canada. Eligible subjects were randomized to receive 25 mg/day etretinate orally or an identical placebo, for six months. The pre-treatment distributions of the characteristics of the 75 subjects allocated to etretinate and the 75 allocated to placebo were almost identical, although the only two subjects with severe dysplasia were allocated to receive etretinate. Compliance with the intervention was good in both groups. In a comparison of the pre- and post-treatment distribution of atypia, an overall reduction was seen in each group; the final distributions were virtually identical, approximately 20 subjects in each group having no atypia.

4.1.3.2 Oral cavity

Koch (1981) studied the effects of two dose regimens of etretinate in 48 patients with leukoplakia, 12 of whom were heavy smokers. There was no control group. One group of 21 patients received etretinate at 75 mg/day orally for six weeks, and a second group of 24 patients received 50 mg/day orally plus a 0.1% paste locally. After termination of therapy, five patients in the first group and seven in the second showed complete remission, while 10 and 13 in the two groups, respectively, showed partial regression of their lesions. There were no cases of progression. Relapses occurred after completion of therapy. [The Working Group noted that the long-term results tabulated 24 months after completion of therapy were difficult to interpret.]

4.1.3.3 Skin

The most frequently studied intermediary end-point for skin neoplasia is actinic keratosis, which is difficult to follow-up longitudinally and may regress spontaneously. Retinoid therapy produces mucocutaneous changes, which may make it easier for patients and investigators to identify the treated or control status of subjects and may also complicate recognition of neoplastic skin lesions.

Moriarty *et al.* (1982) included 50 patients with actinic keratosis in a double-blind cross-over trial to compare the effects of etretinate at 75 mg/day with a placebo. After the first phase of two months, five of 22 evaluable patients who had received etretinate showed complete remission and 14 partial remission of their lesions, whereas the corresponding numbers in the 23 patients given placebo were none and one, respectively. In the second phase, after cross-over, five of 22 evaluable patients who had received etretinate showed complete remission and 13 had partial remission of their lesions. The placebo group consisted of 22 patients, of whom 3 dropped out. Of the remaining 19, one had complete remission and none had partial remission. The authors stated that the results were so conclusive that statistical analysis was not required. [The Working Group noted that the findings in the placebo group during the second phase of the study, after cross-over, are difficult to interpret but suggest that there was very rapid recurrence of lesions after cessation of treatment with etretinate.]

Grupper and Berretti (1983) treated 80 patients with premalignant or malignant skin conditions with etretinate at 1 mg/kg bw per day initially, followed by a progressive reduction to 0.75 mg/kg bw and then 0.5 mg/kg bw per day or less for up to five months. There was no control group. Twenty-two of 26 patients given etretinate who initially had multiple actinic keratoses and all six patients who had keratoacanthomas showed complete clearance. The remaining patients, who had squamous- or basal-cell carcinomas, had poorer responses. After cessation of therapy, 16 of those who initially had actinic keratoses and one of those who had keratoacanthomas showed relapse of their lesions.

Watson (1986) included 15 patients with severe multiple actinic keratoses in an eight-month double-blind cross-over trial. Etretinate was given at a dose of 1 mg/kg bw per day for two weeks for a maximum dose of 75 mg, and slightly lower doses were given subsequently, depending on the side-effects. The end-points evaluated were the numbers of lesions on representative involved areas and the size of the largest lesions. During the first four months, improvement was seen in eight of nine patients who received etretinate and one of six on placebo, while none of the patients on etretinate and five on placebo showed worsening of their lesions. During the second four-month cross-over phase, the lesions of all six patients given

etretinate and one on placebo improved; only one patient given placebo showed worsening. The apparent protective effect in the second four-month period may have been due to prolonged retention of etretinate in the tissues. [The Working Group noted that no formal statistical analysis of the results was reported.]

4.2 Experimental models

4.2.1 Cancer and preneoplastic lesions

These studies are summarized in Table 1.

4.2.1.1 Skin

Mouse: Three groups of 15–20 inbred, albino, hairless (Skh-hr1) female mice, 10–12 weeks of age, were exposed to ultraviolet radiation (UVR) on five consecutive days each week for 12 weeks. The UVR fluence used was initially 0.53 J/cm^2 but was raised to 1.6 J/cm^2 after 12 weeks, when it was maintained at that rate for a maximum of 25 weeks but the frequency reduced to twice weekly. The three groups received 0, 120 or 600 µg of etretinate by gavage in 0.1 ml of peanut oil three times weekly beginning two weeks before the start of UVR and continuing until death. Etretinate did not statistically significantly modulate skin tumour development in terms of time to onset of tumours, total tumour yield or the type of tumours produced (Kelly *et al.*, 1989).

Female Swiss albino mice weighing 20–22 g were shaved and received two applications of 150 µg of 7,12-dimethylbenz[*a*]anthracene (DMBA) in 0.2 ml acetone onto the skin with an interval of 14 days between applications. Three weeks after DMBA treatment, 0.5 mg of croton oil was applied twice weekly for three to eight months. Treatment with etretinate was begun when the average diameter of the papillomas was 3 mm. At that time, groups of four mice were treated either intraperitoneally or intragastrically with etretinate dissolved in arachis oil and administered once weekly (12.5–400 mg/kg bw) or daily (5–40 mg/kg bw) for two weeks. Control mice received the vehicle alone. The sum of the diameters of the papillomas per animal was reduced from 26 mm in controls to 17 mm at the dose of 12.5 mg/kg bw given intraperitoneally and to 6.6 mm at the dose of 200 mg/kg bw dose given intraperitoneally ($p < 0.05$, Student's t test). Intragastric treatment with 25–400 mg/kg bw etretinate produced similar results. The intermediate doses resulted in papillomas of intermediate size. Intraperitoneal injection of etretinate thus appeared to be more effective than oral administration and daily treatment more effective than weekly administration in regressing established papillomas (Bollag, 1974).

Groups of 30 female Charles River CD-1 mice, seven to nine weeks of age, were treated once with 0.2 µmol of DMBA in acetone for tumour initiation and with 8 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) two times per week for tumour promotion for 20 weeks. Etretinate was applied topically at a dose of 140 nmol 1 h before each application of TPA. The development of papillomas was checked weekly by visual observation. The incidence of papillomas at 20 weeks was about 90% in acetone-treated controls and about 40% in those treated with etretinate, and the tumour multiplicity was about 10 papillomas per mouse in controls and about 1.5 papillomas per mouse in those given etretinate. [The numbers were estimated from graphs; no statistics were given.] (Verma *et al.*, 1979).

Rabbit: Both auricles of 21 domestic rabbits [strain and sex not specified] were painted with 1% DMBA in petrolatum on five days per week, and 11 of the animals were also treated with 30 mg (later reduced to 20 mg) etretinate (8–10 mg/kg bw) by gavage on five days per week. Two animals served as untreated controls. After eight or nine weeks of treatment, six of the seven surviving controls developed a total of 25 keratoacanthoma-like tumours, while no tumours developed in the seven surviving treated animals [no statistics given] (Mahrle & Berger, 1982).

4.2.1.2 Oesophagus

Rat: A total of 38 male and 38 female Sprague-Dawley rats, 100 days of age, were divided in three experimental groups (eight controls of each sex and two groups of 15 male and 15 female treated animals), and all were given weekly subcutaneous injections of *N*-nitrosomethylbenzylamine at a dose of 2.5 mg/kg bw as a 0.1% aqueous solution for 15 weeks. The treated groups received etretinate in the diet throughout the study at concentrations of 0, 30 or 100 mg/kg of diet, the last dose being reduced to 60 mg/kg of diet after six weeks. The animals were maintained on their respective diets for life and were killed when moribund.

Table 1. Effects of etretinate on carcinogenesis in experimental animals

Cancer site	Species sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	Etretinate dose, route	Treatment relative to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Skin	Mouse, SKh-hr 1, female	20	UVR, 0.53–1.6 J/cm^2 in 12 wks, 5 d/wk followed by 1.6 J/cm^2 for maximum 13 wks, twice per wk	120 and 600 µg 3 times per wk by gavage	– 2 wk to end	70/25[a] 70/25[a]	59/32[a] 56/41[a]	5.7 5.7	5.8 7.6	Not effective	Kelly *et al.* (1989)
Skin	Mouse, Swiss female	4	DMBA, 150 µg twice and croton oil two wks after DMBA twice per wk	Once per wk by gavage or i.p. at 12.5–400 mg/kg bw or daily at 5–40 mg/kg bw for 2 wks	After papilloma developed	NR	NR	NR	NR	Effective in reducing papilloma size	Bollag (1974)
Skin	Mouse, CD-1, female	30	DMBA, 0.2 µmole once and TPA, 8 nmole twice per wk for 20 wks	140 nmole twice per wk for 20 wks	+ 2 wk – 20 wk	90	40	10	1.5	Effective	Verma *et al.* (1979)
Skin	Rabbit, domestic [strain and sex not specified]	7 (survivors)	DMBA (1% in petrolatum), 5 d per wk	30–20 mg (8–10 mg/kg bw), gavage, 5 d per wk	d 0 – wk 9	86	0	3.6[b]	0	Effective	Mahrle & Berger (1982)
Oesophagus	Rat, Sprague-Dawley, males and females	8/sex (controls) 15/sex (treated)	NMBA, 2.5 mg/kg bw per wk for 15 wks	30 mg/kg diet, for life	d 0 to end	69	67	NR	NR	Ineffective	Schmähl & Habs (1981)
				60 mg/kg diet, for life		69	57*	NR	NR	Effective	

Table 1 (contd)

Cancer site	Species sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	Etretinate dose, route	Treatment relative to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Forestomach	Mouse, C57BL, female	56–68	DMBA, 25 mg/kg bw by gavage; TPA, 10 mg/kg bw by gavage every wk until day 253	0.17 0.51 1.53 mg/kg bw	d 7 to end	62 62 62	56 48 35*	NR NR NR	NR NR NR	Not effective Not effectve Effective	Wagner *et al.* (1983)
Colon	Rat, BD-6, male	25–30	DMH, 20 mg/kg bw s.c. for 20 wks	50 mg/kg bw	d 0 to end	92	71*	1.8	1.65	Effective	Hadjiolov & Grueva (1986)
Lung	Rat, Wistar, male	48 (control) 46 (treated)	Plutonium dioxide (nose-only inhalation) d –15, 5 mg benzo[*a*]pyrene, haematite, intratracheal, d 0	25 mg/kg bw by gavage per wk	d 0 to end	72	63	NR	NR	No effect	Nolibe *et al.* (1983)
Lung	Mouse, CD-1, female	137 (control) 44 treated	SQ 18506, 1 mg/kg bw by gavage	75 mg/kg bw by gavage	– 18 h	1	11*	NR	NR	Tumour enhancing effect	Dunsford *et al.* (1984)
Urinary bladder	Rat, Fischer 344, male	15 (control) 18–29 treated	NBHBA, 0.025% in drinking-water for 8 wks	50 mg/kg diet	– 8 wk to d 0 d 0 to wk 8 + 8 wk to +16 wk – 8 wk to end	100 100 100 100	17* 56* 55* 77*	NR NR NR NR	NR NR NR NR	Effective Effective Effective Effective	Murasaki *et al.* (1980)
				100 mg/kg diet	– 8 wk to d 0 d 0 to wk 8 +8 wk to +16 wk	100 100 100	41* 31* 40*	NR NR NR	NR NR NR	Effective Effective Effective	Murasaki *et al.* (1980)

Table 1 (contd)

Cancer site	Species sex, age at carcinogen treatment	No. of animals per group	Carcinogen dose, route	Etretinate dose, route	Treatment relative to carcinogen	Incidence		Multiplicity		Efficacy	Reference
						Control	Treated	Control	Treated		
Haemato-poetic system (leukaemia)	Rat, Long-Evans, males and females	29–48 males and females/ group	DMBA, i.v. 10–30 mg/kg bw, 4 pulse doses to offspring	20 mg/kg bw by gavage, daily, for life	d 0 to end	90	85	NR	NR	Ineffective	Berger & Schmähl (1986)
Skin	Rabbits, domestic Japanese white [sex not specified]	6	0.1 ml Shope papilloma-virus (SPV) 10^{-1} (1000 ID_{50}) or SPV 10^{-2} (100 ID_{50}) per site (2–4 sites)	200 mg/kg im twice per wk [total no. not specified]	After	NR	NR	NR	NR	Effective[a]	Ito (1981)
Connective tissue	Hamster, Syrian golden [sex not specified]	32–56 controls 32–76 treated	Rous sarcoma virus (Schmidt-Ruppin), s.c., inoculation at 1 day of age	100 mg/kg, bw i.p. 3 wks of age and wkly thereafter	+3 wks to end	56 75	5 0	NR NR	NR	Effective	Frankel *et al.* (1980)
	Chicken [not further specified]	20	Rous sarcoma virus (Bryan), inoculation via wing web at 5 d of age	50 mg/kg bw 25 mg/kg bw 25 mg/kg bw	d 0 d +5 d +18	100	5	NR	NR	Effective	Frankel *et al.* (1980)

UVR, ultraviolet radiation; NR, not reported; DMBA, 7,12-dimethylbenz[*a*]anthracene; i.p., intraperitoneal; TPA, 12-*O*-tetradecanoylphorbol 13-acetate NMBA, *N*-nitrosomethybenzylamine; DMH, dimethylhydrazine; s.c., subcutaneous; NBHBA, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; SQ 18506, 5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole; i.m., intramuscular; wk, week

[a] % animals with papillomas/% animals with carcinomas

[b] 1.8 tumours per auricle

[c] inhibition of tumour growth rate

* Statistically significant (see text)

Squamous-cell carcinomas of the oesophagus developed in 11/16 controls, 20/30 animals in the group receiving etretinate at 30 mg/kg and 17/30 in the group receiving 60 mg/kg. The tumour incidence in the group given the low dose of etretinate was not significantly different from the age-standardized expected frequency in controls, but the tumour incidence in the group given the high dose was significantly reduced ($p < 0.025$, χ^2 test (Schmähl & Habs, 1981).

4.2.1.3 Forestomach

Mouse: A total of 277 female C57BL mice, about 12 weeks of age, were divided into five groups and treated by gavage with a single dose of 25 mg/kg bw DMBA. On day 7, 10 mg/kg bw TPA were given orally and repeated every week until termination of the study on day 253, and etretinate was administered in the diet at doses of 0.17, 0.51 or 1.53 mg/kg bw. A fourth group given 4.59 mg/kg bw was excluded from evaluation because of severe toxic effects. The incidence of tumours (papilloma or carcinoma of the forestomach) was 62% in controls, whereas only 35% of animals given the high dose of etretinate developed forestomach tumours ($p < 0.001$; Peto's trend test involving all groups) (Wagner *et al.*, 1983). [The Working Group noted marked weight loss and reduced survival in the group given 1.53 mg/kg bw etretinate and that papillomas and carcinomas were counted together.]

4.2.1.4 Colon

Rat: Groups of 25 (controls) and 30 (treated) male BD-6 rats weighing 160 g [age not specified] were treated subcutaneously with dimethylhydrazine at a dose of 20 mg/kg bw weekly for 20 weeks. The treated group was simultaneously injected intramuscularly with 50 mg/kg bw etretinate weekly for 20 weeks. The animals were killed after 48 weeks. Adenocarcinomas of the colon were found in 20/28 rats treated with etretinate and 23/25 controls ($p < 0.05$, Student's t test), although the average number of tumours per rat was similar (Hadjiolov & Grueva, 1986).

4.2.1.5 Lung

Mouse: Groups of 137 controls and 44 treated female CD-1 mice, six weeks of age, received the antiparasitic nitrovinylfuran SQ 18506 (*trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole), which has been shown to be carcinogenic in rodents, at a dose of 1 mg/kg bw by gastric intubation. The compound was homogenized in 25% glycerol and administered twice daily for five days. Three such treatments were given at intervals of four weeks. Etretinate at 75 mg/kg bw was given by gavage 18 h before the first dose of SQ 18506 in each course of treatment. The cancers observed most commonly were squamous-cell carcinomas of the forestomach, lymphomas, myeloid leukaemias and sarcomas. Etretinate had no effect on the incidence of these tumours, but lung adenocarcinomas occurred in 5/44 animals given etretinate and 1/137 controls ($p < 0.05$, Fisher-Irwin exact test) (Dunsford *et al.*, 1984).

Rat: Two groups of 48 (controls) and 46 (treated) male Wistar rats, eight weeks of age, were submitted to nose-only inhalation of plutonium dioxide 15 days before intratracheal instillations of benzo[*a*]pyrene and haematite (5 mg each) in 0.2 ml saline. Etretinate was given to the rats by gavage at a dose of 25 mg/kg bw weekly from day 0 for life. The incidence of squamous-cell carcinomas of the lung in the carcinogen-exposed rats (72%) was not significantly modified by long-term administration of etretinate (63%) [No statistical methods were described.] (Nolibe *et al.*, 1983).

4.2.1.6 Urinary bladder

Rat: Five groups of 15–29 male Fischer 344 rats [age not specified] were given 0.025% *N*-nitrosobutyl-*N*-(4-hydroxylbutyl)amine (NBHBA) in the drinking-water for eight weeks, and etretinate was administered in the diet at a concentration of 50 mg/kg. The effects of etretinate on NBHBA-induced bladder carcinogenesis were evaluated when given eight weeks before, for eight weeks during or for eight weeks after carcinogen administration and when given continuously throughout the experiment. Treatment with NBHBA alone resulted in the induction of bladder papillomas in all rats (15/15), whereas the incidence of papillomas was decreased in animals given etretinate before (5/29), during (10/18) or after (12/22) NBHBA ($p < 0.01$; statistical test not given) and in those given etretinate continuously (77%; $p < 0.05$). The group given etretinate before NBHBA also had a significantly decreased incidence of

bladder carcinomas, from 8/15 in controls to 6/29 in etretinate-treated animals ($p < 0.05$ [test not given], while the other groups given etretinate showed no significantly lower incidence of carcinomas (Murasaki *et al.*, 1980).

In a second, similar study, four groups of 15–26 male Fischer 344 rats were given etretinate at 100 mg/kg diet for eight weeks before, during or after treatment with NBHBA. Treatment with NBHBA alone induced papillomas in all rats, while the incidence was significantly lower in groups given etretinate before (9/22; $p < 0.001$), during (8/26; $p < 0.001$) or after (10/25; $p < 0.001$) NBHBA [statistical methods not given in detail]. Carcinomas developed in 8/15 rats in the group treated with NBHBA alone and in 2/22 ($p < 0.01$) given etretinate before NBHBA, 0/26 ($p < 0.001$) given etretinate with NBHBA and 2/25 ($p < 0.01$) given etretinate after NBHBA (Murasaki *et al.*, 1980).

4.2.1.7 Leukaemia

Rat: Five groups of 29–48 male and female Long-Evans rat pups received DMBA intravenously at doses of 30, 10, 20 and 10 mg/kg bw on days 27, 42, 57 and 70 of life, respectively, and etretinate daily by gavage at a dose of 5 or 20 mg/kg bw for life on the day after the first, second, third or fourth DMBA injection. Etretinate did not reduce the incidence of leukaemia or mammary tumours (Berger & Schmähl, 1986).

4.2.1.8 Virus-induced tumours

(a) Shope papilloma virus

A group of 12 domestic Japanese white rabbits [sex not specified] developed 23 papillomas 12–14 days after inoculation with Shope papilloma virus onto the clipped and shaved skin. Six rabbits bearing 12 papillomas were selected and injected intramuscularly with 200 mg/kg bw etretinate twice weekly [total numbers of treatments not specified]. The remaining six rabbits, bearing 11 papillomas, served as controls. After two weeks, the tumours in the controls continued to grow, whereas those in treated animals showed marked growth retardation, and about 60% of all the tumours regressed completely during the 4–10 weeks after cessation of treatment. Once regression had occurred, there was no regrowth [no statistics given] (Ito, 1981).

(b) Rous sarcoma virus

Golden Syrian hamsters [sex unspecified] were inoculated subcutaneously with Rous sarcoma virus (Schmidt-Ruppin) at one day of age. Three weeks later, 56 control hamsters received peanut oil alone and 32 animals received etretinate at 100 mg/kg bw suspended in peanut oil intraperitoneally each week. After 130 days, no tumours were observed in the etretinate-treated hamsters, whereas large sarcomas occurred at the injection site in 42/56 control animals. In a second experiment, 4/76 etretinate-treated hamsters and 18/32 controls developed tumours [no statistics given] (Frankel *et al.*, 1980).

Forty chickens [not further specified] were inoculated in the wing web with Rous sarcoma virus (Bryan) at five days of age; 20 of these were simultaneously treated intramuscularly with etretinate at 50 mg/kg bw in peanut oil and 20 with peanut oil alone, followed by 25 mg/kg bw etretinate or peanut oil 5 and 18 days later. After 41 days of observation, 1/20 chickens given etretinate developed palpable tumours at the injection site, whereas large invasive sarcomas developed in all control chickens [no statistics given] (Frankel *et al.*, 1980).

4.2.2 Intermediate biomarkers

No data were available to the Working Group.

4.2.3 Cellular studies

4.2.3.1 In vitro

Etretinate is highly lipophilic and is insoluble in water. In most of the tests described below it was supplied to the cells dissolved in dimethylsulfoxide (DMSO) or ethanol, and the final concentration of the solvent was usually added to control samples. When the cells were exposed for more than two days, the medium was changed every two or three days.

(a) Effect on cell proliferation (see Table 2)

The antiproliferative effects of etretinate have been investigated *in vitro* in cancer cells and in normal epidermal cells. Although etretinate is de-esterified to acitretin, the presence of acitretin in the culture medium or in the cells was not assessed in any of the studies. In studies of antiproliferative effects, etretinate was generally less effective than acitretin, all-*trans*-retinoic acid and 13-*cis*-retinoic acid.

Table 2. Effects of etretinate on cell proliferation, differentiation and tumour promotion *in vitro*

Cell line; end-point	Vehicle	Concentration (mol/L) and exposure	Response	Comments	Reference
Cell proliferation					
Human melanoma; colony-forming ability	DMSO	10^{-9} to 10^{-5} for 1 h	Decreased AIG in 4/6	No dose–response relationship	Meyskens & Salmon (1979)
Murine and human melanoma cell lines; proliferation	Methanol	10^{-11} to 10^{-5} for 7 days	Decreased proliferation in the murine cell line, no effect in the two human cell lines		Gaukroger *et al.* (1985)
Murine melanoma cell line; proliferation and melanogenesis	Ethanol	10^{-5} for 6 days	Decreased proliferation, 2–3-fold increase in melanogenesis	Too high single concentration, less active than all-*trans*-retinoic acid	Lauharanta *et al.* (1985)
Epstein-Barr virus-transformed lymphoblastoid cell lines; proliferation and differentiation	DMSO	3×10^{-7} to 10^{-5} for 3, 5, 7, 10 days	Decreased proliferation	Less active than all-*trans*-retinoic acid	Pomponi *et al.* (1996)
Normal human epidermal cells; proliferation	Ethanol	10^{-7} to 10^{-4} for 1–2 weeks	25–30% decrease in proliferation	No dose–response relationship, less active than all-*trans*-retinoic acid and 13-*cis*-retinoic acid	Hashimoto *et al.* (1985)
Normal pig epidermal cells; TdR incorporation	DMSO	10^{-7} to 10^{-4} for 24 h	No effect on proliferation	Less active than acitretin	Hashimoto *et al.* (1990)
Normal neonatal murine epidermal cells; DNA synthesis	DMSO	10^{-8} to 10^{-4} for 4 days	Decreased proliferation in fast-growing (high Ca^{++}) cells, increased proliferation in slow-growing (low Ca^{++}) cells	Less active than acitretin	Tong *et al.* (1988)
Normal human keratinocytes; proliferation	DMSO	10^{-12} to 10^{-6} for 48 h	10–15% increase in proliferation. No effect on proliferation in cells grown in growth factor-deficient medium	Effect depended on culture conditions	Zhang *et al.* (1994)
Normal human endothelial cells from skin vesseis; proliferation and differentiation (immunocytochemical differentiation)	DMSO	10^{-8} to 10^{-5} for 6 days	No effect on proliferation or differentiation	Less active than acitretin	Imcke *et al.* (1991)
Normal chick vascular smooth-muscle cells: cell proliferation and differentiation (elastin synthesis)	Not reported	10^{-9} to 10^{-5} for 48 h	Slightly decreased proliferation Increased differentiation	Less active than all-*trans*-retinoic acid	Hayashi *et al.* (1995a)

Table 2. (contd)

Cell line; end-point	Vehicle	Concentration (mol/L) and exposure	Response	Comments	Reference
Cell differentiation					
Hamster trachea; reversal of metaplasia	DMSO	10^{-11} to 10^{-6} for 10 days	Reversal of metaplasia	Less active than all-*trans*-retinoic acid	Newton *et al.* (1980)
Human myelomonocytic cell lines (HL60, U937); cell viability and differentiation (NBT reduction)	DMSO	10^{-6} for 4 or 6 days	No effect on viability or differentiation	all-*trans*-Retinoic acid was active	Chomienne *et al.* (1986)
HL-60; differentiation (morphology, NBT reduction)	DMSO	10^{-6} for 5 days	No effect on differentiation	all-*trans*-Retinoic acid induced differentiation	Ladoux *et al.* (1987)
Bone-marrow mononuclear cells from myelodysplastic patients; differentiation (morphology and immunophenotyping)	Ethanol	10^{-6} for 6 days	No effect on differentiation	13-*cis*-Retinoic acid induced differentiation	Hast *et al.* (1986)
Human teratocarcinoma (PA-1)	Not reported	10^{-8} to 10^{-6} for 4 days	Contact inhibition of growth, changes in cell morphology, altered composition of intracellular and membrane proteins, increased intercellular communication, reduced activity of alkaline phosphatase	As active as all-*trans*-retinoic acid	Taylor *et al.* (1990)
Normal human epidermal cells; keratin expression and envelope formation	Not reported	10^{-8} to 10^{-5} for 9 days	Increased keratin K14: K16 ratio expression, decreased envelope formation	Less active than acitretin	West *et al.* (1992)
Normal human dermal and epidermal cells; surface area, differentiation (involucrin and CRABP II mRNA levels)	DMSO	10^{-6} for 2 weeks	Decreased epidermal surface area, decreased CRABP II and mRNA levels		Sanquer *et al.* (1993)
Normal pig skin explant cultures; growth and keratin formation	DMSO	10^{-7} to 10^{-5} for 4 days	Slightly increased epidermal outgrowth, no effect on keratin formation	Less active than acitretin	Aoyagi *et al.* (1981a,b)

Table 2. (contd)

Cell line; end-point	Vehicle	Concentration (mol/L) and exposure	Response	Comments	Reference
Fetal mouse lung and neonatal rat tracheas exposed to 3,4-benzopyrene and cigarette smoke condensate; mitotic activity, differentiation	Not reported	5.6×10^{-6} for 12–14 days with the carcinogen or for 4 days after the carcinogen	Decreased hyperproliferation, restoration of secretory and ciliary function		Lasnitzki & Bollag (1982)
Human keratinocytes exposed to TCDD; differentiation (CLE)	DMSO	10^{-11} to 10^{-6} separately and simultaneously with\	Increased CLE formation at low concentrations followed by decreased CLE formation at 10^{-6} acid and retinol	Dose–response related decrease in differentiation by all-*trans*-retinoic	Berkers *et al.* (1995)

TdR, ^{3}H-thymidine; DMSO, dimethylsulfoxide; AIG, anchorage-independent growth; CRABP, cellular retinoic acid binding protein; TCDD, 2,3,7,8-tetrachlorodibenzo-*para*-dioxin; CLE, cross-linked envelope; NBT, nitroblue tetrazolium test

Etretinate reduced the colony-forming ability in soft agar of four out of six samples of fresh human melanoma cells obtained at biopsy from patients. The inhibitory effect was seen at a low concentration of the drug (10^{-9} mol/L) and did not increase at higher concentrations. The cells were exposed for only 1 h (Meyskens & Salmon, 1979). [The Working Group noted that the assay is for toxicity and that the short exposure excluded the detection of cytostatic effects.]

Etretinate inhibited the growth of a murine melanoma (PG19) cell line but affected the growth of two human melanoma cell lines only minimally when tested at concentrations of 10^{-11}–10^{-5} mol/L for seven days (Gaukroger *et al.*, 1985). Another murine melanoma (S91) cell line was sensitive to the antiproliferative effect of etretinate at a concentration of 10^{-5} mol/L for six days. Etretinate-treated cells were conspicuously flatter and more spread out than untreated cells. The effect on proliferation was reversible, as usually occurs with retinoids, and was associated with a two- to three-fold increase in melanogenesis. Etretinate was less active than all-*trans*-retinoic acid in inhibiting proliferation and in inducing melanogenesis (Lauharanta *et al.*, 1985). The proliferation of rat bladder carcinoma cells was not affected by etretinate even at a concentration of 10^{-4} mol/L for 1 h (Fujita & Yoshida, 1984). [The Working Group noted the very high concentration used.] In Epstein-Barr virus-transformed lymphoblastoid cell lines, etretinate inhibited cell proliferation in a dose-dependent manner without inducing differentiation. It was markedly less efficacious than all-*trans*-retinoic acid (Pomponi *et al.*, 1996).

The antiproliferative activity of etretinate has also been investigated in untransformed cells, which were mostly epithelial. When human epidermal cells obtained from foreskins and grown as primary cultures were treated with etretinate for one week, a slight reduction in proliferation was seen which was not dose-dependent. A reduction in cell area, perhaps due to interference with cell-to-cell or cell-to-substrate attachment, was also observed. In this system, etretinate was less potent than all-*trans*-retinoic acid and 13-*cis*-retinoic acid (Hashimoto *et al.*, 1985). In pig epidermal cells, etretinate, in contrast to acitretin, had no effect on cell proliferation when tested for 24 h (Hashimoto *et al.*, 1990).

The state of proliferation of keratinocytes can influence the action of etretinate. Different rates of

proliferation of neonatal murine epidermal keratinocytes were obtained by growing cells in media with high or low concentrations of Ca^{++}. Etretinate caused dose-dependent inhibition of DNA synthesis in rapidly growing cells cultured in a medium with a high concentration of Ca^{++} but stimulated DNA synthesis in slowly growing cells in a medium with a low concentration of Ca^{++} (Tong *et al.*, 1988). Acitretin had effects similar to those of etretinate but was more effective. Etretinate tested for 48 h in normal human keratinocytes caused growth promotion when the cells were grown in keratinocyte-complete growth medium but had no effect when the cells were grown in growth factor-deficient medium (Zhang *et al.*, 1994).

Unlike acitretin, etretinate did not inhibit the proliferation or the differentiation of primary cultures of endothelial cells obtained from small vessels and capillaries of human skin (Imcke *et al.*, 1991). In contrast, treatment with etretinate for 48 h inhibited the proliferation of vascular smooth-muscle cells from chick embryos by 30–40%, and the inhibition was associated with stimulation of elastin synthesis. Etretinate was less effective than all-*trans*-retinoic acid (Hayashi *et al.*, 1995a).

(b) Effects on cell differentiation (see Table 2)

Explants of trachea from vitamin A-deficient hamsters have been used to measure the effects of retinoids on the squamous metaplasia that usually results when this tissue is cultured in the absence of vitamin A. Etretinate dissolved in DMSO reversed the metaplasia, with a median effective dose of 2×10^{-8} mol/L when applied over 10 days. The median effective dose of all-*trans*-retinoic acid under these conditions was 3×10^{-11} mol/L, indicating that etretinate was significantly less potent. Over 90% of the control cultures showed metaplasia (Newton *et al.*, 1980).

Etretinate did not affect differentiation of lymphoid cells. In the human myelomonocytic cell lines HL-60 and U937, often used to test the efficacy of differentiating agents, all-*trans*-retinoic acid and 13-*cis*-retinoic acid induced differentiation but etretinate at 10^{-6} mol/L for four or six days was completely inactive in both these cells and in fresh human leukaemic blasts (Chomienne *et al.*, 1986; Ladoux *et al.*, 1987). Similarly, etretinate was inactive in bone-marrow mononuclear cells from patients with myelodysplastic syndrome (Hast *et al.*, 1986). In contrast, in a teratocarcinoma-derived cell line (PA-1), etretinate induced differentiation in terms of morphology, cytoskeletal organization, intercellular communication and cell surface glycoprotein expression, at concentrations that had no antiproliferative effect. In this system, etretinate was as active as all-*trans*-retinoic acid (Taylor *et al.*, 1990).

Etretinate can modify the differentiation profile of keratinocytes. In epidermal cells isolated from skin biopsies, etretinate decreased the relative amount of keratin 16, with a consequent marked increase in the ratio of keratin 14 to keratin 16. It was less active than acitretin in altering this ratio but was more potent than acitretin in inhibiting envelope formation (West *et al.*, 1992). Differentiation of human epidermal cells can be accomplished by growing them on a collagen gel at the air–liquid interphase. Etretinate decreased the epidermal surface area of cultures exposed to air to a similar degree as all-*trans*-retinoic acid, whereas it had no effect in submerged cultures. The effect was associated with down-regulation of the mRNA of cellular retinoic acid-binding protein (CRABP) II and of involucrin, a precursor of the cross-linked envelope (Sanquer *et al.*, 1993). In explant cultures obtained from pig dorsal skin, etretinate slightly stimulated epidermal outgrowth without affecting keratin formation. In the same system, acitretin significantly stimulated epidermal outgrowth (Aoyagi *et al.*, 1981a,b).

(c) Effects on carcinogen-induced neoplastic transformation and on abnormal differentiation (see Table 2)

Exposure of rodent bronchial and tracheal epithelium grown in organ culture to 3,4-benzopyrene or to cigarette smoke condensate leads to hyperplasia. Moreover, the secretory epithelium in the bronchial epithelium is inhibited, and the number of goblet cells in the trachea is reduced and the cilia become clumped. Addition of etretinate for 12–14 days with the carcinogen or for four days after the carcinogen inhibited or reversed these effects (Lasnitzki & Bollag, 1982).

Altered keratinocyte differentiation is induced in humans by dioxins. In primary human keratinocyte cultures from neonatal or adult foreskin, differentiation measured as incorporation of

^{35}S-methionine-labelled proteins into the cross-linked envelopes, was increased by 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). Etretinate added simultaneously with TCDD at a low concentration (10^{-10} mol/L) induced a significant increase in the formation of cross-linked envelopes but a decrease at a higher concentration (10^{-6} mol/L). In the same system, all-*trans*-retinoic acid antagonized the induction of differentiation by TCDD in a dose-dependent manner (Berkers *et al.*, 1995).

(d) Effects on immune function (see Table 3)

Etretinate, unlike 13-*cis*-retinoic acid, had no effect on DNA synthesis or on cell morphology when added for a short time (5 h) to guinea-pig and human lymphoid cell cultures (Nordlind & Thyberg, 1983). In experiments in which human lymphocyte proliferation was induced by various mitogens, etretinate inhibited only phytohaemagglutinin-induced proliferation (Dupuy *et al.*, 1989). Etretinate at an extremely high concentration (10^{-4} mol/L) for three days inhibited the proliferation of human peripheral blood mononuclear cells and their mitogen response to phytohaemagglutinin (Chaidaroglou *et al.*, 1998). [The Working Group noted the extremely high concentration used.] Incubation of lectin-induced rat thymocytes with etretinate for three days caused a dose-dependent inhibition of proliferation even at a concentrations as low as 1×10^{-7} mol/L. In the same experiments, etretinate did not inhibit production of interleukin (IL)-1 by rat peritoneal macrophages or IL-1-dependent events in T-cell activation, whereas it suppressed IL-2-stimulated T-cell proliferation (Stosic-Grujicic & Simic, 1992). Etretinate did not inhibit the generation of O_2 or production of H_2O_2 in zymosan-stimulated polymorphonuclear leukocytes, although, unlike acitretin and all-*trans*-retinoic acid, it slightly stimulated generation of hydroxy radicals ($OH^{\bullet}$) (Yoshioka *et al.*, 1986). The effects of etretinate on the production of cytokines *in vitro* appear to depend on the cell type and culture conditions. Etretinate caused a dose-dependent increase in the production of IL-1 in murine keratinocytes (Tokura *et al.*, 1992), but it did not affect unstimulated or tumour necrotizing factor-α-stimulated IL-1α or IL-8 secretion in normal human keratinocytes. Nevertheless, it inhibited the secretion of these two cytokines when it was induced by phytohaemagglutinin (Zhang *et al.*, 1994). Etretinate had no effect on IL-1α-induced IL-6 production by human lung fibroblasts (Zitnik *et al.*, 1994).

4.2.3.2 Antimutagenicity in short-term tests

In Chinese hamster V79 cells exposed to mitomycin-C at 0.03 µg/ml for 3 h, exposure for 24 h to etretinate at doses of 1–4 µg/ml did not alter the induction of sister chromatid exchange by mitomycin-C (Siranni *et al.*, 1981).

4.3 Mechanisms of cancer prevention

As etretinate is converted to acitretin, the mechanisms of action described in the Handbook on that compound apply.

4.3.1 Effects on cell differentiation

Most studies of the mechanisms of action of etretinate have addressed the mechanisms that might account for its effects on differentiation in epidermal cells. Etretinate can alter the course of epidermal differentiation and the concomitant expression of differentiation-specific epidermal proteins. After topical treatment of hairless rhino mice, etretinate induced the synthesis of keratins K6, K16 and K17 and suppressed filaggrin in the epidermis, whereas it reduced proteolysis of keratins in the stratum corneum (Eichner *et al.*, 1992). In epidermal cells isolated from skin biopsy samples and cultured *in vitro*, the decrease in the relative amount of K16 induced by etretinate markedly increased the ratio of K14 to K16. A similar increase in the ratio of K14 to K16 was found in epidermal lesions of etretinate-treated patients (West *et al.*, 1992). In skin biopsy samples from patients with psoriasis vulgaris, etretinate reduced keratinocyte hyperplasia and, unexpectedly, enhanced keratinocyte differentiation, as evidenced by increased filaggrin production,increased numbers and size of keratohyalin granules, greater abundance of keratin filaments and increased secretion of intercellular lipids (Gottlieb *et al.*, 1996). In human dermis and epidermis grown in culture and induced to undergo differentiation by being lifted into the air, etretinate down-regulated the expression of involucrin mRNA (Sanquer *et al.*, 1993). In one patient with keratoderma striatum, the filagrin pattern returned to normal during etretinate therapy, whereas the altered involucrin pattern was not affected. The tonofibrils and keratohyalin granules were reduced in number and size

Table 3. Effects of etretinate on immune function *in vitro*

Test system; end-point	Vehicle	Concentration (mol/L) and exposure	Response	Comments	Reference
Guinea-pig and human lymphoid cells; DNA synthesis, cell morphology	Acetone	1.4×10^{-6}, 1.4×10^{-5} for 5 h	No effect		Nordlind & Thyberg (1983)
Human lymphocyte mitogenic response to PHA, MLR and MECLR; cell viability	DMSO	10^{-8} to 10^{-5} for 4–6 days	Inhibition of mitogenic response to PHA; no consistent response	Less active than acitretin	Dupuy *et al.* (1989)
Human peripheral blood mononuclear cells; mitogenic response to PHA and TPA, TdR incorporation	DMSO	10^{-6} to 10^{-4} for 3 days	No effect on mitogenic response to PHA; inhibition of mitogenic response to TPA at high concentration and stimulation at lower concentration		Chaidaroglou *et al.* (1998)
PHA- and ConA-activated rat thymocytes, and macrophages; TdR incorporation, cytokine production	DMSO	2×10^{-8} to 2×10^{-4} for 3 days	Inhibition of proliferation No effect on IL-1 production		Stosic-Grujicic & Simic (1992)
Zymosan-stimulated polymorphonuclear leukocytes; generation of reactive oxygen species	50% DMSO, 50% ethanol	2×10^{-6} to 2×10^{-4} during stimulation	No effect on O_2 or H_2O_2 production; slight stimulation of generation of $OH^{\bullet}$	Acitretin and all-*trans*-retinoic acid reduced generation of $OH^{\bullet}$	Yoshioka *et al.* (1986)
Murine epidermal keratinocytes; IL-1 production	DMSO	8×10^{-9} to 8×10^{-6} for 1–3 days	Stimulation (2.5-fold) of IL-1 activity, no effect on cell proliferation	Less active than all-*trans*-retinoic acid	Tokura *et al.* (1992)
Normal human keratinocytes: IL-1α-induced IL-8 production	DMSO	10^{-12} to 10^{-6} for 48 h	No effect on unstimulated or TNFα-stimulated secretion; inhibition of PHA-induced secretion		Zhang *et al.* (1994)
Normal human lung fibroblasts; IL-6 production	Not reported	10^{-6} for 48 h	No effect on IL-1α-induced IL-6 production		Zitnik *et al.* (1994)

PHA, phytohaemagglutinin; MLR, mixed lymphocyte reaction; MECLR, mixed epidermal cell lymphocyte reaction; DMSO, dimethylsulfoxide; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; ConA, concanavalin A; TdR, ^{13}H-thymidine; IL, interleukin; TNFα, tumour necrotizing factor α

(Fartasch *et al.*, 1990). Etretinate may also cause changes in keratinocyte membranes, since the lectin binding of keratinocytes from1 the skin of guinea-pigs treated with etretinate changed after treatment (Nomura *et al.*, 1994). Galactose incorporation into epidermal glycoproteins was increased in explant cultures from pig ear skin treated with etretinate (King & Pope, 1984). Slight effects of etretinate on extracellular collagen and collagenase synthesis or activity have been reported. In cultured human skin fibroblasts, etretinate only slightly inhibited collagenase expression (Bauer *et al.*, 1983), and it did not affect type IV collagenolytic activity in human melanoma cells (Oikarinen & Salo, 1986). In one patient with lichen sclerosis and atro-phisms, clinical improvement seen after treament with etretinate was not accompanied by enhanced collagen synthesis (Niinimaki *et al.*, 1989). In contrast, etretinate inhibited TGFβ1-stimulated type-1 collagen production by normal human lung fibroblasts in culture (Redlich *et al.*, 1995).

The content of steroid receptors may positively correlate with the degree of mammary tumour differentiation. Mammary tumours from dogs treated with etretinate showed an increased cytoplasmic oestrogen receptor content, which may suggest that etretinate induced differentiating effects in the mammary gland cells (Cappelletti *et al.*, 1988).

4.3.2 Inhibition of cell proliferation and oncogene expression

The enzyme ornithine decarboxylase is induced during proliferation. Etretinate completely inhibited induction by TPA of ornithine decarboxylase mRNA in a simian virus 40-transformed human keratinocyte cell line (Xue *et al.*, 1996). It also markedly inhibited Epstein-Barr virus induction by croton oil and *n*-butyrate in Raji infected cells (Zeng *et al.*, 1981). The immunoreactivity of p53 in premalignant and malignant lesions indicates that expression of this oncogene plays a role in skin cancer; however, etretinate did not affect the expression of *p53* in premalignant and malignant cutaneous lesions of kidney transplant recipients (Gibson *et al.*, 1997).

4.3.3 Effects on immune function and cytokine production

Although etretinate, unlike retinyl palmitate and 13-*cis*-retinoic acid, did not enhance the cell-mediated immune response of mice to sheep red blood cells (Athanassiades, 1981), clinical studies indicate that it can activate T cells. In patients with psoriasis or other dermatoses, etretinate treatment at 1 mg/kg bw per day for 28 days significantly increased the reactions to recall antigens (Fulton *et al.*, 1982). In another study in patients with psoriasis, etretinate at 1 mg/kg bw per day for two months stimulated the number of peripheral T lymphocytes, which was lower than that of control subjects (David *et al.*, 1990). The cytotoxic activity of neutrophils, which before therapy was greater in patients with psoriasis than in control subjects, was decreased by etretinate treatment at 1 mg/kg bw for 28 days (Ellis *et al.*, 1985a). Etretinate also decreased the migration of neutrophils from the bloodstream to human skin (Dubertret *et al.*, 1982). It did not affect generation of the leukocyte chemotactic factor leukotriene B_4 from rat polymorphonuclear leukocytes stimulated by calcium ionophores (Bray, 1984), but an inverse relationship was found between the dose of etretinate and the number of intraepidermal infiltrating polymorphonuclear leukocytes after epicutaneous application of leukotriene B_4 (Lammers & van de Kerkhof, 1987). Natural killer cell activity has been found to be decreased in most patients with psoriasis, and etretinate increased the number and activity of these cells in several studies (Jansén *et al.*, 1985; McKerrow *et al.*, 1988; Majewski *et al.*, 1989). In one case the increase was transient, in that the natural killer cell activity rose during the first two months of treatment but returned thereafter to the starting value (Jansén *et al.*, 1985). Etretinate abolished the manifestation of chronic graft-versus-host disease in semi-allogenic recipient rats (Stosic-Grujicic *et al.*, 1996). In patients with psoriasis, etretinate had no effect on the elevated serum IL-1 concentrations, but it caused a substantial decrease in serum tumour necrotizing factor-α and interferon concentrations (Shiohara *et al.*, 1992).

4.3.4 Effects on angiogenesis

Etretinate inhibited angiogenesis induced by a human epidermoid cancer cell line, although it was less effective than acitretin. When the same effect was tested in a non-tumorigenic cell line, etretinate had a stimulatory effect (Rudnicka *et al.*, 1991).

5. Other Beneficial Effects

Etretinate has been shown to be of benefit to patients with psoriasis and cutaneous disorders of keratinization. Infants treated with etretinate at doses of up to 1.3 mg per day for up to six weeks have had no clear adverse reactions. The drug is efficacious in the treatment of congenital lamellar ichthyosis (Collin *et al.*, 1989; Rogers & Scarf, 1989; Lawlor & Peiris, 1985a,b; Ward & Jones, 1989; Nayar & Chin, 1992).

Harlequin fetus is the most severe manifestation of autosomal recessive nonbullous congenital icthyosis erythroderma, which is a distinct autosomal recessive ichthyosis or the phenotypic expression of several genotypes. Left untreated, infants with this syndrome almost always die shortly after birth, following sepsis and excessive loss of fluid, electrolyte and protein through the thick, inelastic, water-permeable skin. With constant nursing care, maintenance of body temperature, control of infection and initial treatment with etretinate at 2.5 mg/day from birth through to the age of six weeks and then 1.5 mg/day to the age of 10 months, these infants can survive, and development of motor and vocabulary skills can be consistent with the norm by 6–24 months of age (Collin *et al.*, 1989; Lawlor & Peiris, 1985b). These infants may also have renal, thymic, thyroid and pulmonary malformations and develop pulmonary infection, respiratory difficulty and feeding problems and die despite administration of etretinate (Waisman *et al.*, 1989). While long-term follow-up of these children has not been reported, they appear to tolerate this protocol without the typical signs of retinoid intoxication.

6. Carcinogenicity

6.1 Humans

No epidemiological studies were available to the Working Group.

Isolated case reports of neoplasms occurring after treatment with etretinate have been published. One report described a case of malignant B-cell lymphoma and one of Hodgkin disease (Woll *et al.*, 1987); a second reported one case of Hodgkin disease, one malignant teratoma of the testis and a squamous-cell carcinoma of the anus (Harrison, 1987), and a third was of a case of Hodgkin disease (Desablens *et al.*, 1989).

6.2 Experimental models

No studies were available to the Working Group in which the effects of etretinate on cancer development in the absence of carcinogen treatment were evaluated; however, etretinate enhanced lung carcinogenesis (see section 4.2.1).

7. Other Toxic Effects

7.1 Adverse effects

7.1.1 Humans

Several authoritative reviews have appeared on the clinical toxicology of etretinate (Orfanos, 1980; Peck, 1981; Lauharanta, 1982; Ward *et al.*, 1983; Lippman *et al.*, 1987; Orfanos *et al.*, 1987). The longest experience with the toxicity of synthetic retinoids is associated with their therapeutic use in dermatological practice, as many patients have been treated continuously with etretinate for 15–20 years (Kamm, 1982). The usual initial dose is 0.5–1 mg/kg bw per day for two to four weeks. After 8–16 weeks, the maintenance dose is 0.25–0.75 mg/kg per day. The maximum daily dose is 75–100 mg/day, and the lowest effective dose is 10 mg/day.

The toxic effects observed are summarized in Table 4 (Halioua & Saurat, 1990).

(a) Dermatological toxicity

The standard mucocutaneous symptoms associated with treatment with etretinate at 0.25–1 mg/kg bw per day orally are dose-dependent and vary widely in frequency and severity. Cheilitis is seen in the majority of patients but this symptom rapidly resolves upon discontinuation of the drug. Epistaxis can result from drying of nasal membranes. An erythematous rash may develop in up to 50% of patients treated for psoriasis. An exaggerated healing response typical of the effects of retinoids on granulation tissue may result at the sites of wounds or healing lesions (Lauharanta, 1980; Ward *et al.*, 1983; Ellis & Voorhees, 1987). There was an isolated report of fatal epidermal necrolysis associated with treatment with this drug (McIvor, 1992).

Alopecia occurred in nine of 56 patients given 40–75 mg/day (0.7–0.9 mg/kg bw per day) for more

Table 4. Toxic effects reported with standard long-term use of etretinate

Body system	Adverse effect
Mucocutaneous	Xerosis, skin fragility, mucous membrane dryness, dermatitis, exuberant granulation tissue, photosensitivity, changes in hair and nails
Skeletal	Vertebral abnormalities, diffuse idiopathic hyperostosis, osteophytes on vertebral bodies, calcification of anterior spinal ligament and other tendons, extraspinal involvement, osseous hyperostosis
Hepatic	Elevated liver enzyme activity, severe hepatotoxic damage
Biochemical and haematological	Hypertriglyceridaemia, hypercholesterolaemia, elevated creatinine kinase activity, anaemia, monocytosis, lymphoma
Ocular	Blepharitis, conjunctivitis, night blindness
Neurological	Headache, intracranial hypertension, depression, otitis externa, earache
Muscular	Myalgia, muscle weakness, myopathy
Other	Sexual dysfunction (male and female), menstrual changes, gynaecomastia, fatigue, nausea, renal oedema

From Halioua & Saurat (1990)

than four weeks (Lauharanta, 1982). When the dose was increased to 0.9–1.5 mg/kg bw per day (Hönigsmann *et al.*, 1978; Orfanos *et al.*, 1978; Binazzi & Cicilioni, 1979), alopecia affected 21–29% of patients (Goerz & Orfanos, 1978; Lassus, 1980). Women seem to be preferentially affected (Foged & Jacobsen, 1982). Alopecia becomes evident within five to six weeks of continuous dosing (Mahrle *et al.*, 1979), and it is the principal reason given for discontinuation of treatment (Goerz & Orfanos, 1978; Lassus, 1980; Lauharanta, 1982). The condition resolves within one to two months after drug withdrawal (Mahrle *et al.*, 1979; Lauharanta, 1982). Some patients given the drug at 25–50 mg/day have reversible, dose-dependent hair kinking, which generally begins 3–12 months after initiation of oral therapy (Graham *et al.*, 1985). A case of increased curliness of scalp hair (pili torti) was reported in a 15-year-old girl given 0.3 mg/kg bw day (Hays & Camisa, 1985); similar reports of patients with previously straight hair are anecdotal (Ellis & Voorhees, 1987).

Fingernail growth increased and the nails became dystrophic, thin and clear and were shed in 2/10 patients given etretinate for 12 weeks (Galosi *et al.*, 1985). The nails returned to normal after cessation of treatment (Ferguson *et al.*, 1983). In about 10% of patients given a therapeutic course at 0.25–1 mg/kg bw per day, a mucin-like material (thought to be synthesized by keratinocytes undergoing mucous metaplasia) accumulates in the skin, leading to a shiny, smooth texture (Ellis & Voorhees, 1987).

Palmoplantar desquamation, scaling and alopecia were observed more commonly among patients given etretinate at 0.25–1 mg/kg per day than in those receiving 13-*cis*-retinoic acid at 0.5–1 mg/kg bw per day (Cunningham & Ehmann, 1983). Granulation about the toes, groin and nails can affect about 15% of etretinate-treated patients (Campbell *et al.*, 1983; Hodak *et al.*, 1984; Wolska *et al.*, 1985). In up to 50% of patients, a reversible 'retinoid dermatitis' can develop (Crivellato, 1982; Molin *et al.*, 1985; Ellis & Voorhees, 1987). Bone and joint pain occurs in 25–50% of treated patients, and older patients appear to be more severely affected. Fewer than 10% of patients experience myalgia, but affected individuals voluntarily restrict regular exercise or strenuous physical activity while taking the drug (Orfanos *et al.*, 1987).

Cheilitis is the most frequent side-effect in etretinate-treated patients (Table 5). In 10% of patients so affected, this progresses to rhagades (Lauharanta, 1982). Etretinate less frequently induces skin fragility (Neild *et al.*, 1985), dry

Table 5. Incidences of mucocutaneous side effects with standard oral etretinate therapy

Condition	Incidence (%)
Cheilitis	42–100
Dry mouth	21–95
Dry nasal mucosa	21–87.5
Epistaxis, petechiae	5
Facial dermatitis	5–7
Palmoplantar desquamation	17–40
Desquamation	23–94
Skin thinning	6–93
Skin fragility	25
Xerosis	20–30
Dermatitis	5
Alopecia	3–69
Conjunctivitis or ocular irritation	5–50
Pruritus	15–25

Summarized from Windhorst & Nigra (1982); Cunningham & Ehmann (1983); Ward *et al.* (1983); Orfanos *et al.* (1987)

nose and mouth, paronychia, bruise, dermatitis, epistaxis, xerosis, pruritus and conjunctivitis (see Table 5 and references cited therein).

(b) Skeletal toxicity

Long-term treatment with etretinate was often associated with skeletal changes, including demineralization of bones and extraosseous calcification (Kaplan & Haettich, 1991). These changes often occurred in the vertebrae, more often in the cervical rather than the thoracic or lumbar spine. Extraspinal tendon and ligament calcification most often affected the ankle, pelvis, knee, shoulder and then elbow joints. In one clinical trial, 38 of 45 patients with psoriasis or other disorders of keratinization treated with an average dose of 0.8 mg/kg for a mean of 60 months had radiographic evidence of extraspinal tendon and ligament calcification. Of 38 patients given etretinate for an average of five years, 32 had radiographic evidence of extraspinal ligament and tendon calcification (DiGiovanna *et al.*, 1986). Vertebral disk degeneration, osteoporosis, spinal hyperostosis with calcification of the spinal ligaments and periosteal thickening occurred in a substantial fraction of adults receiving oral etretinate at doses as low as 25–50 mg/day for years (Burge & Ryan, 1985; Ellis *et al.*, 1985b; Logan, 1987; Halkier-Sorensen & Andresen, 1989).

While brief therapy with etretinate in children usually results in no clinically discernible skeletal toxicity, prolonged treatment at > 1 mg/kg bw per day has resulted in thinning of the long bones, focal osteoporosis, fracture after minor trauma and premature epiphyseal closure (Rosinska *et al.*, 1988; Lowe & David, 1998). Children given etretinate at doses up to 2.5 mg/kg bw per day for five or six years experienced premature closure of the epiphyseal plate (Prendiville *et al.*, 1986). While some cases of etretinate-induced skeletal toxicity progress with prolonged therapy (Sillevis-Smitt & de Mari, 1984), others fail to show clinically detectable evidence of such changes (Brun & Baran, 1986), particularly when the dose is reduced (< 1 mg/kg bw per day) promptly when the patient's condition improves (Tamayo & Ruiz-Maldonado, 1981; Traupe & Happle, 1985; Paige *et al.*, 1992). When the dose is maintained at 0.2–0.5 mg/kg bw per day or at no more than 1 mg/kg bw per day, the margin of safety is increased. No skeletal toxicity was seen in infants started on treatment as young as two weeks, in children as old as 11 years and in young adults given the drug continuously for one month to 11 years (Glover *et al.*, 1987; Rosinska *et al.*, 1988; Paige *et al.*, 1992).

The highest dose reported to have no skeletal toxicity was 25–50 mg/day in an eight-year-old child who had been treated for six years (Presbury, 1984), and a dose of 2 mg/kg bw per day for 36–42 months was tolerated without clinically evident detrimental effects (Tamayo & Ruiz-Maldonado, 1981). No significant bone abnormalities were found on physical examination, technetium bone scans and X-rays of cervical, thoracic and lumbar vertebrae of patients as young as six years who were maintained on standard long-term (6–100 months) therapy with etretinate (Mills & Marks, 1993). Genetic profile, physical activity and level of psychological stimulation confound direct evaluation of delayed or deficient development in affected individuals given higher doses for 16–42 months from as early as seven months of age (Tamayo & Ruiz-Maldonado, 1980, 1981). Since etretinate can have developmental toxicity in children, it has

been proposed that its prescription be limited to those with severe recalcitrant disease that seriously impairs their quality of life (Shelnitz *et al.*, 1987).

(c) Hepatotoxicity

The principal danger associated with standard therapy with etretinate is the onset of chronic, aggressive hepatitis (Fredriksson & Pettersson, 1978; Glazer *et al.*, 1982; van Voorst Vader *et al.*, 1984), which can progress to toxic centrilobular necrosis (Thune & Mork, 1980). In general, the clinical signs of liver involvement begin within the first four weeks of treatment (Roenigk *et al.*, 1985; Roenigk, 1989). While 90% of patients treated with etretinate do not develop histologically or clinically significant hepatotoxicity (Foged *et al.*, 1984; Glazer *et al.*, 1984), about 1% develop frank hepatitis. Elevated activities of serum aspartate and alanine aminotransferases occur in 18 and 23%, respectively, of patients receiving etretinate orally (Fontan *et al.*, 1983; Kaplan *et al.*, 1983). Hyperbilirubinaemia was reported in patients on etretinate, but only after more than four years of therapy (Ott, 1981). The increased activities of liver enzymes returned to pretreatment values upon cessation of treatment (Mahrle *et al.*, 1979; Lauharanta, 1982).

Prior exposure to methotrexate for psoriasis at a total administered dose of 480–11 860 mg increased the risk for hepatotoxicity; in about 10% of these patients, the disease progressed from fibrosis to cirrhosis. No significant dmage to the liver was found during etretinate therapy (Roenigk *et al.*, 1985).

Approximately 20–30% of all patients receiving standard therapy with etretinate develop temporary abnormalities in liver function that are reversible when the dose is reduced or the drug is withdrawn. Etretinate may be more hepatotoxic than other synthetic retinoids because of its higher concentration in the liver. Of 652 patients treated in clinical trials, 10 had clinical or histological hepatitis that was considered to be possibly or probably related to treatment. The liver function in eight of these patients returned to normal after discontinuation of etretinate (Gollnick, 1981). Rare cases of severe hepatotoxic damage have been reported, which appear to result from hypersensitivity to the drug (Sanchez *et al.*, 1993).

(d) Metabolic and biochemical effects

The induction of hyperlipidaemia is one of the most common side-effects of retinoid therapy in general, and the increased risk for atherosclerosis associated with long-term treatment is of considerable concern. Administration of etretinate results in elevated serum concentrations of cholesterol (> 300 mg/dl) and triglycerides (> 250 mg/dl) in 25–50% of all patients (Michaëlsson *et al.*, 1981; Ellis *et al.*, 1982; Gollnick & Orfanos, 1985). Of 652 patients on etretinate, 16% developed hypercholesterolaemia and 45% developed hypertriglyceridaemia (Gollnick, 1981). The hyperlipidaemia is attributable to increased production of very-low-density lipoprotein. Large increases in triglyceride concentrations are reported to occur only in individuals with pre-existing hypertriglyceridaemia, but smaller increases in the concentrations of triglycerides, cholesterol and apoprotein B and decreases in those of high-density lipoprotein and cholesterol are very common (Marsden, 1989).

Patients with predisposing factors such as obesity, alcohol abuse, diabetes and smoking habits appear to have a higher incidence of hyperlipidaemia when treated with etretinate. Although the exact mechanism of retinoid-induced hyperlipidaemia is unknown, it may increase the absorption of dietary lipids, increase chylomicron production or reduce its clearance, increase the synthesis of triglycerides and cholesterol in the liver and increase the synthesis or decrease the catabolism of low-density lipoproteins (Ellis *et al.*, 1982; Marsden, 1989). Other less frequent biochemical effects seen with etretinate therapy include the development of pseudoporphyria (McDonagh & Harrington, 1989) and elevated creatinine kinase activity. Some studies have also shown an effect of etretinate on the therapeutic efficacy of concomitant medications, such as warfarin (Ostlere *et al.*, 1991) and oral contraceptives (Berbis *et al.*, 1987).

(e) Ocular, central nervous system and other toxicity

The ocular manifestations seen with etretinate therapy include both conjunctival symptoms and rare retinal dysfunction associated with depletion of retinol (Weber *et al.*, 1988). Effects on the central nervous system such as headache and impaired vision may be associated with benign intracranial

hypertension induced by etretinate (Peck, 1982; Bonnetblanc *et al.*, 1983; Viraben *et al.*, 1985). Depression has been associated with use of etretinate at 75–100 mg/day, particularly among people who consume copious quantities of ethanol, but is less frequent when the dose of etretinate is reduced to < 50 mg/day (Logan, 1987; Henderson & Highet, 1989). Muscle damage (Hodak *et al.*, 1987) and renal impairment (Cribier *et al.*, 1992) have also been described.

Photosensitivity is usually not a result of exposure to etretinate, and the threshold for erythema can actually be increased (Ippen *et al.*, 1978; Mahrle *et al.*, 1982). Sensitivity to light can be increased in a minority of patients (Collins *et al.*, 1986), and photophobia developed in two of 23 patients given etretinate at 75–100 mg/day for three weeks (Ippen *et al.*, 1978). Cases of night blindness, iritis, subcapsular cataract, corneal erosion, reductions in visual activity with blurring and retinal haemorrhage have been recorded among patients receiving etretinate orally (Viraben *et al.*, 1985).

Cases of gynaecomastia have also been reported (Carmichael & Paul, 1989). Inflammation of the external ear canal due to accumulation of excess cerumen and associated pain can occur but are seldom reported in the literature (Kramer, 1982; Juhlin, 1983).

7.1.2 Experimental studies

The toxicity of etretinate was studied in rats at doses up to 20 mg/kg bw per day and in dogs at doses up to 30 mg/kg bw per day during the initial development of this drug as an anti-psoriatic agent. The most striking manifestation of toxicity in rodents was bone fractures, but this osteolytic response was not seen in dogs. Other dose-related changes in animals treated with etretinate included alopecia, erythema, reductions in body weight and food consumption, stiffness and alterations in gait, haematological changes and changes in serum chemistry and testicular atrophy with evidence of reduced spermatogenesis (reviewed by Kamm, 1982).

The ocular toxicity of etretinate was examined in rabbits, which showed degenerative changes in the meibomian gland (Kremer *et al.*, 1994).

7.2 Reproductive and developmental effects

7.2.1 Humans

7.2.1.1 *Reproductive effects*

While cases of dysmenorrhoea (Halkier-Sorensen, 1987), male impotence (Halkier-Sorensen, 1988; Krause, 1988; Reynolds, 1991) and loss of libido (Halkier-Sorensen, 1987) in patients treated with etretinate have been reported, these are isolated, and the extent to which etretinate contributed to these observations is not clear (Krause, 1988). The changes resolved without sequelae after withdrawal of the drug. There is no evidence that etretinate interferes with the actions of oral contraceptives or progesterone in women given etretinate orally at 0.7–1 mg/kg bw per day (Berbis *et al.*, 1987).

In 23 men given etretinate at 25–75 mg/day for three months, there was no effect on sperm count, total sperm output or morphology or on spermatogenesis (Schill *et al.*, 1981; Török, 1984; Török *et al.*, 1987). Morphological examination of semen from 11 men showed no change in absolute or progressive motility, and the wife of one patient conceived during treatment and gave birth to a healthy baby (Török, 1984).

7.2.1.2 *Developmental effects*

The first cases of teratogenicity associated with exposure to etretinate before or during pregnancy were recognized in 1983 (Happle *et al.*, 1984), and seven infants with congenital defects and one fetal death had been recorded by 1985 (reviewed by Rosa, 1992). By 1986, eight infants afflicted by etretinate embryopathy had been identified (Kietzmann *et al.*, 1986; Orfanos *et al.*, 1987), and by 1988, 22 cases occurring after continuous exposure during pregnancy had been reported (Rosa, 1992). Between 1976 (when etretinate was first introduced in the United Kingdom) and December 1990, 83 pregnancies, including 12 in the USA, in which the women reported having been exposed to etretinate had been reported; in 16 of these, the infant was normal at birth, and in 47 the pregnancy was terminated. Malformations occurred after daily exposure to 0.4–1.5 mg/kg bw (Rosa *et al.*, 1986). Far fewer pregnancies and elected abortions (Chan *et al.*, 1995) have been reported in association with use of etretinate than with 13-*cis*-retinoic acid, probably because it is indicated for

an older population than that in which 13-*cis*-retinoic acid is used (Lehucher Ceyrac *et al.*, 1992).

The terata arising after exposure to etretinate include meningomyelocoele, malformation of the chondrocranium and viscerocranium, the tetralogy of Fallot, ventricular septal defect, anophthalmia, malformations of the hip, ankle and forearm, syndactyly, short digits, imperforate anus and genitourinary disorders such as absent penis or meatus urinarius (Happle *et al.*, 1984; Lambert *et al.*, 1988; Martinez-Tallo *et al.*, 1989; Geiger *et al.*, 1994). Only one of 23 newborns with a history of exposure to etretinate *in utero* showed even borderline microtia (Rosa, 1992), whereas this occurs in 70% of infants with 13-*cis*-retinoic acid-associated embryopathy (Lynberg *et al.*, 1990). Digit and limb malformations (unilateral hypoplasia; Grote *et al.*, 1985) occurred in 31% of these infants and in 2% of those with equivalent exposure to 13-*cis*-retinoic acid. Cardiac terata were far less prevalent in infants exposed to etretinate (16%) than in those exposed to 13-*cis*-retinoic acid (42%).

While fetuses conceived shortly after cessation of therapy with 13-*cis*-retinoic acid are not at increased risk for developmental toxicity (Dai *et al.*, 1992), congenital malformations have been recorded among aborted fetuses (Happle *et al.*, 1984; Lambert *et al.*, 1988; Verloes *et al.*, 1990) and among infants born to mothers who had discontinued therapy with etretinate 4–12 months before conception (Grote *et al.*, 1985; Lammer, 1988; Geiger *et al.*, 1994). By 1988, 11 such cases had been reported (Rosa, 1992). Cardiovascular (abnormal vena cava, atrial septal defect) and renal (horseshoe kidney) terata were reported in a 23-week-old fetus aborted by a mother who had discontinued use of etretinate at 0.5 mg/kg bw per day seven to eight months before conception (Verloes *et al.*, 1990).

Between 1976 and 1991, 123 women reported pregnancies within two years of cessation of etretinate use. Of these, 34% (42) either aborted spontaneously or elected termination; malformations were seen in 10. The true extent of the problem is unknown, however, and calculation of risk is compromised by failure to report spontaneous abortions accurately, failure to examine and report abortuses that have been exposed to etretinate before or during pregnancy and failure to delineate and to separate those infants with terata that may be due to factors other than etretinate. For example, one infant with malformations not typical of retinoid embryopathy (premature with inguinal hernia) was included in one summary (Mitchell, 1992); another case (Lammer, 1988) has been questioned in that the defects resembled those of the CHARGE syndrome (coloboma, heart defects, choanal atresia, retardation, genital and ear anomalies), which is of genetic origin (Blake & Wyse, 1988).

Not all mothers who conceive after discontinuing use of etretinate gave birth to infants with congenital malformations, even when exposure continued into the first trimester (Cordero *et al.*, 1981; Ruther & Kietzmann, 1984; Jäger *et al.*, 1985; Vahlquist & Rollman, 1990). By 1988, six normal pregnancies had been reported after use of etretinate had been stopped one to nine months before conception (Rosa, 1992). Given the delay between direct exposure to etretinate and abnormal pregnancy outcome and identification of inherited syndromes of terata with features in common with etretinate embryopathy, debate on the case reports has been vigorous (Blake & Wyse, 1988; Greaves, 1988).

In view of the elimination half-time of about 100 days (DiGiovanna *et al.*, 1989) and the fact that 99% of the body burden of etretinate is removed within seven elimination half-times, the calculated period of theoretical excess risk is less than 700 days before conception (Geiger *et al.*, 1994). The precise duration of the increased teratogenic risk after cessation of use of etretinate is unknown, but it is generally recommended that post-therapy contraception be continued for at least two years after treatment (Rinck *et al.*, 1989). Clinical experience has demonstrated that the risk for embryonic death associated with exposure to etretinate is greatest during the first three weeks of pregnancy, and the risk for teratogenic effects is highest when exposure occurs during weeks 4–8 of pregnancy. When exposure occurs after that time or ceases two years before conception, the risk for major malformations is close to that expected for the general population (Geiger *et al.*, 1994).

7.2.2 *Experimental models*

In animals, etretinate is converted to its acid metabolites, acitretin and 13-*cis*-acitretin (Löfberg *et al.*, 1990; Bouvy *et al.*, 1992), which are assumed

to be the proximate teratogens since etretinate has no activity in various assays *in vitro* (see below).

7.2.2.1 Reproductive effects

Male rats became infertile after receiving daily doses of etretinate at 5 or 25 mg/kg bw for four weeks. Animals at the low dose were normal, but many effects were seen at the high dose: some of the rats died, and the rest had bone fractures in the paws, smudges around the eyes, decreased spontaneous activity and decreased body weight and they were infertile, as seen by a decrease in serum testosterone concentration, a decrease in sperm motility and number, abnormal sperm shape, atrophy of the seminiferous tubules and Leydig cells, necrosis of spermatocytes and atrophy of the seminal vesicle and prostatic epithelium. They also showed atrophy of acidophils but an increased number of gonadotrophs in the pituitary gland (Hayashi *et al.*, 1995b). Guinea-pigs exposed to 25 mg/kg bw for six weeks showed similar effects on the testes: decreased spermatogenic activity, lack of mature sperm and reduced diameters of the seminiferous tubules (Tsambaos *et al.*, 1980).

7.2.2.2 Developmental effects

Administration of etretinate to hamsters on day 8 of gestation at a dose of 44 mg/kg bw resulted in 100% abnormal fetuses, while administration of 22 mg/kg bw resulted in 88% abnormal fetuses. The median effective dose for abnormalities was 5.7 mg/kg bw, indicating that it is nearly twice as potent as all-*trans*-retinoic acid. The defects observed included shortening and oligodactyly of the limbs, exencephaly, encephalocoele, spina bifida, exophthalmos, clefting and agnathia, fused and hooked ribs and aplastic or hypoplastic tail (Williams *et al.*, 1984).

In mice, administration of etretinate on day 11 of gestation produced cleft palate and shortening of the long bones of the limb. The number of abnormal fetuses increased with increasing dose: 25 mg/kg bw resulted in 52% abnormal embryos, while 100 mg/kg bw resulted in 98% abnormal embryos. The median effective dose for abnormalities was 26 mg/kg bw, which was similar to that of all-*trans*-retinoic acid. Earlier administration resulted in much more severe abnormalities (Reiners *et al.*, 1988; Kochhar *et al.*, 1989). In mice given a dose of 50 mg/kg bw on day 8.25 of gestation, microcephaly, exophthalmos, smaller zygomatic arches leading to a narrower and shorter facial skeleton, persistence of Meckel's cartilage, absent pinna and spina bifida were observed. The offspring had smaller brains with a hypoplastic cerebellum. Examination of embryos after 24 h revealed extensive cell death in the rhombomeres and rhombic lip (which makes the cerebellum) and in the neural crest (which makes the cranial skeleton). As the branchial arches were also smaller, many of the abnormalities can be attributed to the induction of cell death in specific embryonic populations (Alles & Sulik, 1992).

Administration of etretinate on day 9 of gestation at a dose of 60 mg/kg bw resulted in 100% abnormal embryos, but the abnormalities were concentrated at the caudal end of the embryo rather than in the head (Mesrobian *et al.*, 1994). No spina bifida or limb abnormalities were seen; rather, 100% had imperforate anus and tail abnormalities and a high rate of urethral atresia. Surprisingly, excessive cell death was not observed, in contrast to that seen after administration on day 8.25 (Alles & Sulik, 1992). In a study of the development of anorectal malformations after the same administration regimen (60 mg/kg bw on day 9), there was decreased cell proliferation in the cloacal membrane and either excessive apoptosis or lack of apoptosis, depending on the region of study. Thus, the cellular dynamics of this region of the embryo were severely altered (Kubota *et al.*, 1998).

Etretinate was given to mice at 10 or 25 mg/kg bw on day 6, 7 or 8 of gestation. It was not teratogenic when given on day 6 or 7, and day 8 was considered to be the earliest susceptible time for the embryos (Agnish *et al.*, 1990).

In a more refined method, to avoid giving single large doses of etretinate on particular days of gestation, a low dose is infused continually into the stomach of pregnant mice. Infusion of a dose of 2.5 mg/kg bw on days 8–15 of development resulted in a very high frequency of limb defects, cleft palate, micrognathia, phalangeal defects and tail defects (Löfberg *et al.*, 1990). Similar results were obtained after daily administration of etretinate to mouse embryos over the period of gestation of 7–17 days; the lowest teratogenic dose was 4 mg/kg bw (Kistler, 1987).

Visceral and cardiac heterotaxy were induced by administration of a dose of 15 mg/kg bw on day

7. The heart defects were severe and included transposition of the great arteries, aortic arch abnormalities and complete atrial defect (Kim *et al.*, 1995).

A similar pattern of abnormalities is seen in rats. At doses of 5–10 mg/kg bw, exencephaly and spina bifida were seen after administration on days 7–8 of gestation, craniofacial malformations in 100% of embryos when given on days 8.5–9, and digital and tail malformations when given on days 9.5–11 (Granström & Kullaa-Mikkonen, 1990a; Granström *et al.*, 1990, 1991). The lowest teratogenic dose for rat embryos was reported to be 8 mg/kg bw (Kistler, 1987); no teratogenesis was seen with doses of 1, 3 or 6 mg/kg bw, but typical teratogenic effects occurred at 10, 15 and 25 mg/kg bw (Agnish *et al.*, 1990). The craniofacial malformations induced by etretinate included micrognathia, displaced and smaller Meckel's cartilage, facial and palatal clefts, reduced nasal width, flattened face and reduced number of cranial neural crest cells. Other abnormalities that have been reported include an abnormal hypophysis, hypoplasia of the submandibular salivary glands and tooth aplasia. In similar studies of craniofacial malformations induced by a dose of 10 mg/kg bw on day 8.5 of gestation, microtia and meningocoele were observed with cranial displacement of the otocyst, which is a classical sign of retinoid teratogenicity. The non-cranial malformations reported include omphalocoele and cardiac and renal malformations (Granström *et al.*, 1991; Jacobsson & Granström, 1997). The craniofacial malformations could be prevented by prior administration of vitamin B_6 (Jacobsson & Granström 1996). At the same dose (10 mg/kg bw) on days 8.5–10.5 of gestation, the characteristic craniofacial defects noted above were accompanied by auricular displacement or absent outer ears, skin tags between the auricle and the mouth, ossicles of the inner ear that were small, altered in position or fused, shorter cochlea with fewer turns and hypoplastic stapedial artery and facial nerve (Granström, 1990; Granstrom *et al.*, 1991; Jacobsson & Granström, 1997). Defective fusion of the nasal processes, resulting in fistulas and clefts and missing or reduced nasal cartilages, has also been reported (Granström & Kullaa-Mikkonen, 1990b).

In these studies of craniofacial defects in rats, etretinate was found to be four times as teratogenic as all-*trans*-retinoic acid. In a comparative study of several retinoids, however, the opposite was found to be the case: all-*trans*-retinoic acid was more teratogenic than etretinate. In this study, the craniofacial defects were accompanied by thymus abnormalities, urogenital defects including hydronephrosis, dilated ureter, genital agenesis, undescended testis and cardiac vessel defects. As in mice, imperforate anus and caudal agenesis were also reported in the rat embryos (Turton *et al.*, 1992).

7.2.2.3 In vitro

In striking contrast to the results described above, etretinate is virtually inactive *in vitro*. It did not inhibit chondrogenesis in mouse limb bud cultures, whereas the activity of acitretin was similar to that of all-*trans*-retinoic acid (Kistler, 1987; Reiners *et al.*, 1988; Kochhar *et al.*, 1989). Indeed, when esterases were added to the culture medium, etretinate was activated (Kochhar *et al.*, 1989). Etretinate also had no effect in whole-embryo cultures of rats at the maximum soluble concentration (Steele *et al.*, 1987). [The Working Group concluded that since etretinate is metabolized to acitretin, which itself is active *in vivo*, acitretin is the proximate teratogen.]

7.3 Genetic and related effects

Etretinate at a concentration of 1–50 mg/ml had no effect on sister chromatid exchange frequency in exponentially growing Chinese hamster V79 cells (Sirianni *et al.*, 1981).

8. Summary of Data

8.1 Chemistry, occurrence and human exposure

Etretinate [ethyl all-*trans*-3,7-dimethyl-9-(4-methoxy-2,3,6-trimetriphenyl)nona-2,4,6,8-tetraenoate) is a synthetic retinoid of the aromatic class which is structurally related to all-*trans*-retinoic acid. Because of its conjugated tetraene structure, etretinate has characteristic absorption in the ultraviolet and visible spectra and readily undergoes photoisomerization in solution to multiple geometric isomers. Etretinate is a highly lipophilic molecule which is readily partitioned into hydrophobic compartments, including human adipose tissue.

Etretinate is a purely synthetic compound which can readily be prepared by various routes. Human exposure occurs entirely during treatment with formulations for oral administration, primarily for dermatological indications. Etretinate has been replaced by acitretin in most countries. The recommended doses were 0.25–1.5 mg/kg bw per day.

A variety of normal-phase and reversed-phase high-performance liquid chromatographic methods is available for the detection and quantification of etretinate and its geometric isomers.

8.2 Metabolism and kinetics

Etretinate must be metabolized to its free acid form in order to exert biological activity. The pharmacokinetics of etretinate has been studied extensively, particularly in patients with psoriasis. In both humans and experimental animals, the tissue distribution of etretinate is influenced primarily by its lipophilicity.

8.3 Cancer-preventive effects

8.3.1 Humans

Etretinate was evaluated in six randomized trials for its efficacy in preventing the recurrence of superficial tumours of the urinary bladder. None showed unequivocal evidence of an effect of treatment; efficacy was suggested in analyses of some end-points. Controls receiving placebo were used in three of these trials, whereas the controls in the largest study were untreated.

Etretinate was not effective in preventing second primary tumours in subjects with head-and-neck tumours when compared with those given placebo.

In two reports of the same study without a separate control group, etretinate was reported to reduce an index of metaplasia in bronchial biopsy samples from heavy smokers. In a randomized trial involving 150 subjects, however, etretinate showed no efficacy in reducing atypia in sputum samples when compared with placebo.

In one study with no controls in which etretinate was given orally at a high dose or orally at a moderate dose plus topical application as a paste, regression of leukoplakia of the mouth was reported, more notably when topical application was added.

In one study with no controls, oral treatment with etretinate appeared to reduce the severity of actinic keratotic and keratocanthoma lesions of the skin. In two double-blind cross-over trials involving patients with actinic keratosis, improvement in terms of the number and size of lesions was reported in patients treated orally with etretinate, although no statistical analysis was reported for either trial.

8.3.2 Experimental models

The cancer-preventive efficacy of etretinate has been assessed in mouse, rat and rabbit models and in virus-induced tumours. It was ineffective in a skin tumorigenesis model in mice but effective in a similar model in rabbits. In one study in mice, etretinate reduced the size of skin papillomas. It was effective in various models of digestive tract carcinogenesis in mice and rats.

In single studies, etretinate was ineffective in preventing either leukaemia or lung tumours but was effective in preventing urinary bladder carcinogenesis in rats. It was effective in three studies in models of benign tumours induced in mice by Shope papilloma virus and malignant tumours induced by Rous sarcoma virus.

In some experimental models, etretinate enhanced the tumorigenic effects of carcinogens.

Etretinate has been shown to modify differentiation in several models *in vitro*: in hamsters, squamous metaplasia induced by vitamin A deficiency was reversed. Since metaplasia is considered to be a potential precursor of neoplasia, this activity of etretinate is considered to be significant. In respiratory tracts exposed to carcinogens, it inhibited loss of mucus secretion and ciliary action. In many studies with human and animal keratinocytes, etretinate causes changes in differentiation similar to those seen after treatment with all-*trans*-retinoic acid, and in one study it modified the toxic effects of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. In contrast to all-*trans*-retinoic acid, it did not induce differentiation in promyelocytic leukaemic cell lines. It inhibited proliferation in murine and human melanoma cell lines, in lymphoblastoid lines and in normal keratinocytes. Because of differences in the experimental protocols, it is not clear whether etretinate is selectively active against tumour cells. In all cases, it was less active than all-*trans*-retinoic acid. Etretinate has been studied in many models of immune function *in vitro*, but no consistent responses were reported.

8.3.3 *Mechanisms of cancer prevention*

The active form of etretinate is acitretin. There have been no detailed studies of the mechanism of action of etretinate. Its ability to inhibit the induction of ornithine decarboxylase in keratinocytes after treatment with phytohaemagglutinin suggests that, like all-*trans*-retinoic acid, it acts in the promotional phase of carcinogenesis.

8.4 Other beneficial effects

Etretinate was demonstrated to be of benefit to patients with psoriasis or congenital lamellar ichthyosis.

8.5 Carcinogenic effects

8.5.1 *Human studies*

Only case reports were available to the Working Group.

8.5.2 *Experimental models*

No data were available to the Working Group; however, in one study of prevention, etretinate enhanced lung carcinogenesis in rats.

8.6 Other toxic effects

8.6.1 *Humans*

Long-term treatment with etretinate for dermatological disorders may result in several toxic effects, including mucocutaneous, skeletal, hepatic, ocular, neurological (headache) and neuromuscular complications, and abnormal concentrations of serum lipids. Children given etretinate can experience progressive skeletal toxicity.

Etretinate is a confirmed human teratogen. In view of its long elimination half-life of 100 days, pregnancy should be avoided for at least two years after exposure. Terata arising from exposure include meningomyelocoele, malformations of the chrondrocranium and viscerocranium, the tetralogy of Fallot, ventricular septal defect, anophthalmia, malformations of the hip, ankle and forearm, syndactyly, short digits, imperforate anus and genitourinary disorders. Malformations occur after daily exposure to 0.4–1.5 mg/kg bw. Clinical experience shows that the risk of death of embryos exposed to etretinate is greatest when exposure occurs during the first three weeks of pregnancy. The teratogenic risk is highest when exposure occurs during weeks 4–8 of pregnancy.

8.6.2 *Experimental models*

Studies of toxicity in experimental animals reflect the spectrum of effects observed in human beings. It causes sterility in males after long-term administration at high doses. It was not active in two tests for teratogenicity *in vitro*, but it is metabolized *in vivo* to acitretin, which is a potent teratogen, and therefore produced the typical retinoid embryopathy of the central nervous system, craniofacial region, limbs, heart, genitourinary tract and tail. In general, etretinate appears to be more teratogenic than all-*trans*-retinoic acid in experimental animals.

Etretinate did not induce sister chromatid exchange in hamster cells in a single study.

9. Recommendations for Research

9.1 General recommendations for etretinate and other retinoids

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

9.2 Recommendations specific to etretinate

None.

10. Evaluation

10.1 Cancer-preventive activity

10.1.1 *Humans*

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

10.1.2 *Experimental animals*

There is *limited evidence* that etretinate has cancer-preventive activity in experimental animals. This evaluation is based on the observation of inhibitory effects in single studies with models of skin, digestive tract and urinary bladder carcinogenesis and in three models of virus-induced tumours.

10.2 Overall evaluation

There is *inadequate evidence* for the cancer-preventive activity of etretinate in humans and *limited evidence* in experimental animals. Etretinate is toxic and a confirmed human teratogen, and is no longer available for use in most countries.

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