

# METHOXSALEN PLUS ULTRAVIOLET A RADIATION

Methoxsalen plus ultraviolet A radiation was considered by previous IARC Working Groups in 1980 and 1987 ([IARC, 1980](#), [1987a](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Identification of the agent

*Chem. Abstr. Serv. Reg. No.:* 298-81-7

*Chem. Abstr. Name:* 9-Methoxy-7H-furo[3,2-g][1]benzopyran-7-one

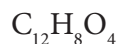
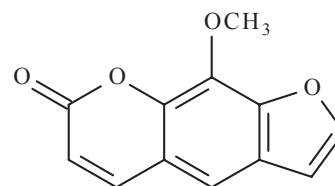
*IUPAC Systematic Name:*

9-Methoxyfuro[3,2-g]chromen-7-one

*Synonyms:* 5-Benzofuranacrylic acid, 6-hydroxy-7-methoxy-,  $\delta$ -lactone; Meladinine; 8-methoxy-6,7-furanocoumarin; 8-methoxypsoralen; 8-methoxy[furano-3',2':6,7-coumarin]; 8-MOP; 8-MP; Oxso-ralen; Puvasoralen; Uvadex

*Description:* Silky needles or long rhombic prisms; odourless with a bitter taste followed by a tingling sensation ([O'Neil, 2006](#))

#### 1.1.1 Structural and molecular formulae, and relative molecular mass



Relative molecular mass: 216.19

### 1.2 Use of the agent

Information for Section 1.2 is taken from [McEvoy \(2007\)](#), [Thomson Healthcare \(2007\)](#), and [Sweetman \(2008\)](#)

Methoxsalen is a psoralen produced naturally by various plants (e.g. celery, parsnips, limes, figs, and others) found in both temperate and tropical regions ([Ashwood-Smith et al., 1985](#); [NTP, 2005](#)). It is also a constituent of the seeds of the *Ammi majus* plant, and of the roots of *Heracleum candicans*. It is a photosensitizer, which markedly increases skin reactivity to long wavelength ultraviolet radiation (UVA: 320–400 nm), an effect used in photochemotherapy or PUVA

[psoralen (P) and high-intensity long wavelength (UVA) irradiation].

### 1.2.1 Indications

#### (a) Psoriasis

Oral methoxsalen is used in conjunction with controlled exposure to UVA radiation for the symptomatic treatment of severe, recalcitrant, disabling psoriasis that is refractory to other forms of therapy, and when the diagnosis has been confirmed by biopsy. Psoralens have been used topically in conjunction with UVA irradiation for the treatment of psoriasis, but the use of topical methoxsalen has largely been abandoned because it produces a greater incidence of adverse effects, and is cosmetically less acceptable than oral psoralens.

#### (b) Cutaneous T-cell lymphoma

Oral methoxsalen is used in conjunction with photopheresis for the palliative treatment of the skin manifestations of cutaneous T-cell lymphoma (e.g. mycosis fungoides, Sézary syndrome).

#### (c) Idiopathic vitiligo

Methoxsalen has been used orally or topically in conjunction with controlled exposure to UVA or sunlight to repigment vitiliginous skin in patients with idiopathic vitiligo. To retain new pigment, periodical treatment with the drug and some form of UVA irradiation is often required.

#### (d) Other uses

Methoxsalen used in conjunction with UVA irradiation has been shown to be effective in the treatment of selected diseases mediated by T cells, rejection after solid organ transplantation, and chronic graft-versus-host disease ([Greinix et al., 2000](#)).

### 1.2.2 Dosage

For the treatment of psoriasis, oral methoxsalen therapy is accompanied by some form of UVA irradiation. Methoxsalen is usually administered with milk or food 1.5–2 hours before exposure to high-intensity UVA light, two or three times weekly. The initial dose of methoxsalen is based on the patient's weight; from 10 mg for a patient weighing less than 30 kg, up to 70 mg for a patient weighing more than 115 kg.

For the treatment of cutaneous T-cell lymphoma, methoxsalen is usually administered orally. Oral methoxsalen may be administered as a single dose with food or in two divided doses approximately 30 minutes apart, to minimize adverse gastrointestinal effects ([McEvoy, 2007](#)).

To repigment vitiliginous areas, methoxsalen is usually given in a dose of up to 600 µg/kg (20 mg daily) orally 2–4 hours before measured periods of exposure to UVA radiation twice a week, at least 48 hours apart.

Methoxsalen may also be applied topically in the form of a 1% lotion; occasionally, the lotion may be diluted 10- or 100-fold to avoid adverse reactions.

Methoxsalen is available as 10 mg capsules and liquid-filled capsules for oral administration; it is also available as a 1% lotion for topical administration.

### 1.2.3 Trends in use

No information was available to the Working Group.

## 2. Cancer in Humans

### 2.1 Cohort studies

Cohort studies have been used to examine the association between PUVA treatment and skin cancer. Most studies reviewed in Table 2.1

(available at <http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-19-Table2.1.pdf>) have investigated cohorts of patients with psoriasis, although a few studies also included subgroups of patients treated with PUVA for other skin disorders. In all studies available to the Working Group, most of the psoriatic patients had also been exposed to other antipsoriatic agents, some of which known or suspected to be carcinogenic, including ionizing radiation, arsenics, methotrexate, UVB, and topical tars. Although several of the studies adjusted for such previous or concurrent exposures, missing or incomplete information may have led to insufficient adjustment and consequently, an overestimation of the carcinogenic risk of PUVA. All studies included in this review presented a risk analysis of squamous cell carcinoma of the skin, while only a subgroup of studies evaluated the risk for basal cell carcinoma, and skin melanoma. One study further included selected extracutaneous tumour sites in the analysis. The studies compared the incidence of cancer in the cohorts with expected numbers based on general population rates, and some papers also made internal comparisons, by level of exposure to PUVA.

### 2.1.1 Squamous cell carcinoma of the skin

The relative risk of squamous cell carcinoma of the skin in PUVA-treated patients has been assessed in three large multicentre studies from the United States of America and Sweden, in one countrywide study from Finland, and in four single centre studies from Austria, Scotland, the Netherlands, and the USA.

The cohort studies used a variety of methods, but all found significant increases in the incidence of squamous cell carcinoma of the skin in people treated with PUVA compared to general population rates, with relative risks in the range of 5–10 (Forman *et al.*, 1989; Perkins *et al.*, 1990; Bruynzeel *et al.*, 1991; Chuang *et al.*, 1992; Stern & Laird, 1994; Maier *et al.*, 1996;

Hannuksela-Svahn *et al.*, 1999, 2000; Lindelöf *et al.*, 1999). Much higher relative risks (more than 100-fold) were found in two studies of men (Stern, 1990; Stern *et al.*, 2002).

The studies that undertook analyses by level of exposure to PUVA found dose-related increases in the incidence of squamous cell carcinoma. Dose was assessed by frequency of exposure in one study (Stern & Laird, 1994), and by cumulative dose of UVA in J/cm<sup>3</sup> in three others (Forman *et al.*, 1989; Chuang *et al.*, 1992; Lindelöf *et al.*, 1999).

### 2.1.2 Basal cell carcinoma of the skin

The two multicentre studies from the USA also included data on incident cases of basal cell carcinoma among PUVA-treated patients. Although the study by Forman *et al.* (1989) observed a significantly increased relative risk of basal cell carcinoma, the relative risk was reduced to a non-significant level in the analysis of the subgroup of patients with no previous exposure to ionizing radiation or arsenics, and there was no clear relationship with the standard morbidity and cumulative dosage of UVA. In the multicentre study by Stern and Laird (1994), the risk of basal cell carcinoma in patients with high-intensity exposure to PUVA was increased 1.7-fold (95%CI: 1.1–2.5) compared to that of patients with low-intensity exposure (see Table 2.1 online). The investigators of the Dutch study judged the risk of basal cell carcinoma to be increased 5-fold over that of the general population (Bruynzeel *et al.*, 1991), but there was no correlation between the development of basal cell carcinoma and the cumulative dosage of UVA. [The Working Group noted that the reference rates of basal cell carcinoma applied in the analysis were derived from the routine registration of a regional cancer registry of the country; substantial underreporting of this tumour type is likely, more so than for squamous cell carcinoma, and it may explain the increases in the standard morbidity ratio observed in these

studies, which ascertained cases in the psoriasis cohorts in a manner that differed from the ascertainment in routine cancer registration.]

### 2.1.3 Malignant melanoma of the skin

In the study by [Lindelöf et al. \(1999\)](#), the standard morbidity ratios were estimated to be slightly, but non-significantly increased. No increase was noted for the subgroup of patients followed for 15 years or more since first treatment with PUVA. In the study from the USA ([Stern et al., 1997](#); [Stern, 2001](#)), patients with at least 200 PUVA treatments were not at significantly higher risk than patients who received less than 200 treatments in multivariate analyses, taking into account other antipsoriatic treatments.

## 3. Cancer in Experimental Animals

### 3.1 Methoxsalen and UVA

Many of the earlier studies on methoxsalen were several short-duration experiments that found no statistically significant increase in the incidence of skin tumours or tumours of internal organs in either sex of mice of various strains when given methoxsalen orally alone or in combination with ultraviolet (250–400 nm) irradiation ([IARC, 1980, 1987a](#)).

Methoxsalen administered in the feed to groups of 40 Swiss mice at 500 ppm for 12 months and exposed to UVA for 2 hours daily for 3 months caused skin tumours in 35% of the mice; when given for 30 minutes daily for 3 months, caused skin tumours in 25% of the mice; and when given for 10 minutes daily for 6 weeks, caused skin tumours in 20% of the mice. No skin tumours were seen in three groups of 40 control mice that received UVA only ([Griffin, 1959](#); [IARC, 1980, 1987a](#)). A second feed study exposed groups of 36 male and 36 female HRA/Skh hairless mice to methoxsalen at 0, 9, 21

or 80 mg/kg body weight/week for 52 weeks, followed by exposure to UVA half an hour after feeding. Surviving animals were kept for study for an additional 28 weeks without treatment. An increased incidence in squamous cell carcinoma was observed in the mid- and high- dose groups in females, and in the high-dose group in males ([Dunnick et al., 1991](#)). Another oral study involved exposing two groups of 16 female Tg.AC mice to methoxsalen in corn oil by gavage at 8 mg/kg body weight for 5 days over a 2-week period for 10 weeks, followed by weekly administration of the chemical for an additional 6 weeks. One exposure group was also exposed to UVA one hour after dosing with methoxsalen. A third group of eight animals served as a control, and were treated with corn oil only followed by UVA exposure. The incidence of skin papillomas in the PUVA group was significantly higher than that of the other two groups ([Chignell et al., 2003](#)).

Methoxsalen has been studied in mice by other routes of administration including skin application or intraperitoneal injection in combination with exposure to UVA. Skin application induced epidermal and dermal skin tumours in five studies. These included carcinomas, squamous cell carcinomas, fibrosarcomas and basal cell carcinomas. Tumours of the eye and ear regions (epidermal fibrosarcomas and squamous carcinomas) were observed in one study following the intraperitoneal administration of methoxsalen to female mice ([IARC, 1980, 1987a](#)).

See [Table 3.1](#).

### 3.2 Methoxsalen alone

Groups of 50 male and 50 female Fischer 344 rats were administered methoxsalen by gavage in corn oil at concentrations of 0, 37.5 or 75 mg/kg 5 days/week for 103 weeks. These animals were not exposed to UVA radiation. Mortality was significantly affected by treatment of the male but not the female rats. In males, Increased incidences of tubular cell adenomas and/or adenocarcinomas

**Table 3.1 Studies of cancer in experimental animals exposed to PUVA**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss (F) 100 d <a href="#">Griffin (1959)</a>	Feed 0 or 500 mg/kg methoxsalen for 100 d and exposed to UVA for 2 h daily for 3 mo, 30 min daily for 3 mo, or for 10 min daily for 6 wk 40/group	Skin tumours: 35% (UVA for 2 h/d), 25% (UVA for 30 min/d), and 20% (UVA for 10 min/d) No skin tumours in corresponding control mice that were exposed to UVA only	NR	Wood's type ultraviolet lamp (Black-Ray Model XX15 Long Wave Ultraviolet) [wavelength presumed to be > 320nm]
Mouse, HRA/Skh (M, F) 80 wk <a href="#">Dunnick et al. (1991)</a>	Feed 0, 9, 21 or 80 mg methoxsalen/kg bw/wk 30 min before exposure to 0 or 2 J/cm <sup>2</sup> UVA (320–400 nm) for 5 min Exposure for 52 wk 36/group Animals observed for additional 28 wk without exposure	Skin (squamous cell carcinomas): M–control, 0%; 9 mg/kg bw, 0%; 21 mg/kg bw, 6%; 80 mg/kg bw, 39% F–control, 0%; 9 mg/kg bw, 6%; 21 mg/kg bw, 17%; 80 mg/kg bw, 47%	NR [ <i>P</i> < 0.0001] <sup>a</sup> (high-dose) [ <i>P</i> < 0.05] (mid-dose); [ <i>P</i> < 0.0001] (high-dose)	
Mouse, Tg.AC (F) 17 wk <a href="#">Chignell et al. (2003)</a>	Gavage 0, 8, or 8 mg methoxsalen/kg bw in corn oil 5×/2 wk for 10 wk followed by once/wk for 6 wk. The corn oil control and one dosed group also exposed to UVA (2 J/cm <sup>2</sup> ) 1 h after dosing 8, 16, 16; (all 20-wk-old)	Skin (papillomas): 11/16 (UVA), 13/14 (PUVA), 4/8 (methoxsalen only) Mean of maximum tumours/tumour-bearing animal: 7.4 (PUVA) vs 1.5 (UVA alone) ( <i>P</i> < 0.01, Mann–Whitney U-test) Mean maximum tumours/animal: 6.9 (PUVA) vs 1.1 for other groups ( <i>P</i> < 0.01, Mann–Whitney U-test)	<i>P</i> < 0.05 <sup>a</sup> for PUVA vs methoxsalen only	
Mouse, SKH:hairless (sex NR) 30 wk <a href="#">Forbes &amp; Urbach (1975)</a>	Skin painting Daily skin applications of 40 µL of either methanol or 0.01% methoxsalen in methanol 30–60 min before a 10-min whole-body UVA exposure (300–400 nm) on 5 d/wk for 30 and 14 wk, respectively 30/group	Skin tumours: 15/30 at 14 wk for the PUVA group 12/24 at 30 wk for the methanol + UVA group	NR	Most of the observed skin tumours developed at the site of application were squamous cell carcinomas (≈90%). Study was poorly reported Age NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, SKH:hairless (sex NR) 38 wk <a href="#">Forbes et al., (1976)</a>	Skin painting Skin applications of 40 µL of either methanol or 0.1% methoxsalen in methanol before a 2-h whole body UVA exposure (300–400 nm) on 5 d/wk for 30 and 14 wk, respectively 24/group	Skin tumours: 50% at 20 wk for the PUVA group 50% at 27 wk in the methanol + UVA group ( $P < 0.01$ )	From 18 to 28 wk, the numbers of tumour-bearing animals and of tumours per animal were significantly higher in the PUVA group than in the methanol + UVA group ( $P < 0.05$ and $P < 0.01$ , respectively)	In both groups, ≈45/50 histologically analysed tumours were squamous cell carcinomas Age NR
Mouse, SKH:hairless-1 and HRS/J/AN1 (F) 90 wk <a href="#">Grube et al. (1977)</a>	Skin painting Skin applications of 250 µg methoxsalen in ethanol 60 min before whole body UVA exposure to either 300–400 nm, 320–400 nm or 365 nm on 5 d/wk for 24 wk 20–25 animals per strain	SKH:hairless-1, respective to the 3 wavelengths: Squamous cell or basal cell carcinomas of the skin–17/20, 15/19, and 8/19 Fibrosarcomas–6/20, 5/19, and 2/19 HRS/J/An1, respective to the 3 wavelengths: Squamous cell or basal cell carcinomas of the skin–0/25, 4/23, and 9/23 Fibrosarcomas–5/25, 1/23, and 4/23	NR	Lack of information on controls and inadequate reporting of experimental details
Mouse, Swiss (F) 60 wk <a href="#">Santamaria et al., (1979)</a>	Skin painting Skin applications of 0 ( $n = 5$ /group) or 5 ( $n = 25$ /group) µg of methoxsalen in 0.05 mL ethanol 60 min before UVA exposure (300–400 nm) twice/wk for 15, 30, 45 or 60 min respectively for 60 wk 8 groups	Subcutaneous malignant tumours: 43% incidence in treated animals from all four groups combined 15% incidence in the four control groups of mice exposed to UVA alone	NR	Tumours reported to be mammary adenocarcinomas, skin carcinomas and carcino-mixo-sarcomas. Inadequate reporting of use of controls and of survival times. The pooling of various experimental groups and tumour types make the study difficult to evaluate Age unspecified



Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss (F) 11 mo <a href="#">Griffin et al. (1958)</a>	Intraperitoneal 0.4 mg methoxsalen 6×/wk for 10 mo with exposure to UVA for either 30 min daily for 3 mo, or for 10 min daily for 6 wk 20/group Two control groups ( <i>n</i> = 20) only exposed to UVA	Epidermal tumours: > 50% in mice exposed to UVA for 30 min/d 100% in mice exposed to UVA for 10 min/d No tumours in corresponding controls	NR	Wood's type ultraviolet lamp (Black-Ray Model XX15 Long Wave Ultraviolet; wavelength > 320 nm). The tumours reported were fibrosarcomas and squamous cell carcinomas of the ears and the eye region
Mouse, SKH: hairless-1 (F) 40 wk <a href="#">Nagayo et al. (1983)</a>	Skin painting Daily skin applications of 40 µL of either methanol or 0.1% methoxsalen in methanol before a 15-min whole-body UVA exposure (320–420 nm) 5 d/wk for 40 wk 20/group	Skin tumours: Methanol + UVA, 0/18; PUVA, 20/20	[ <i>P</i> < 0.0001] <sup>a</sup>	6/58 skin tumours were squamous cell carcinomas

<sup>a</sup> Fisher Exact test

bw, body weight ; d, day or days; F, female; M, male; min, minute or minutes; mo, month or months; PUVA, methoxsalen with UVA; vs, versus; wk, week or weeks

**Table 3.2 Studies of cancer in experimental animals exposed to methoxsalen**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 103 wk <a href="#">NTP (1989)</a>	Gavage 0, 37.5 or 75 mg methoxsalen/kg bw in corn oil 5 ×/wk for 103 wk 50/group	<b>Males</b> Kidney tumours: Tubular cell adenomas or adenocarcinomas–1/50 (control), 12/50 (low), 11/49 (high) Tubular cell adenocarcinomas–0/50 (control), 1/50 (low), 3/49 (high) Zymbal gland: Carcinomas or squamous cell carcinomas–1/50, 7/50, 4/49 Fibromas, subcutaneous–1/50, 5/50, 7/49 Lung: Alveolar/bronchiolar adenomas–4/50, 9/50, 9/49	$P < 0.001$ (Life Table)     $P = 0.008$ for low dose (Life Table)  $P = 0.004$ (Life Table)  $P = 0.015$ (Life Table)	Study was negative in females

bw, body weight; F, female; M, male; wk, week or weeks

of the kidney and carcinomas of the Zymbal gland were observed. This was also true for subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung. There were no significant increased incidences of neoplasms observed in dosed female rats ([NTP, 1989](#)).

See [Table 3.2](#).

## 4. Other Relevant Data

### 4.1 Absorption, distribution, metabolism and excretion

#### 4.1.1 Humans

Methoxsalen is rapidly absorbed after oral treatment, with peak plasma concentrations reached after 2–6 hours, 50% as unchanged drug ([Schalla et al., 1976](#); [Gazith & Schaefer, 1977](#); [Steiner et al., 1977](#); [Busch et al., 1978](#)). Following topical application, methoxsalen penetrates rapidly into the epidermis and dermis, and the

high concentrations reached remain constant over a period of 16 hours ([Kammerau et al., 1976](#)).

Methoxsalen is extensively metabolized, and no unchanged drug is excreted in the urine ([Schalla et al., 1976](#); [Busch et al., 1978](#)). Approximately 80% of a dose is excreted in the urine within 8 hours as hydroxylated and glucuronide derivatives ([Pathak et al., 1974](#)).

#### 4.1.2 Experimental systems

After oral administration of  $^{14}\text{C}$ -methoxsalen to dogs and rats, absorption is rapid, with peak plasma radioactivity levels occurring within 2 hours after dosing in rats, and within 30 minutes in dogs ([Busch et al., 1978](#)). After oral and intravenous administration of  $^{14}\text{C}$ -methoxsalen to rats, radioactivity is found in the liver, kidneys, and the cortic part of the adrenal gland. Skin concentrations of radioactivity are comparable with blood levels and are similar in albino and pigmented rats; ultraviolet light increases the subcutaneous concentrations of radioactivity ([Wulf & Hart, 1979](#)).



After oral administration to rats,  $^{14}\text{C}$ -methoxsalen is excreted in trace amounts together with eight polar metabolites, some of them conjugated (Busch *et al.*, 1978).

After intravenous administration of  $^{14}\text{C}$ -methoxsalen to dogs, radioactivity disappears rapidly from plasma, although small levels of radioactivity are observed to persist for 5 weeks after administration, suggesting that the persistent plasma radioactivity is due to a metabolite bound to plasma protein. Elimination occurs in both urine and bile; 45% of the dose appearing in the urine, and 40% in the faeces within 72 hours of administration. Methoxsalen is extensively metabolized, and less than 2% of the drug is excreted unchanged in the urine. Two metabolites resulting from the opening of the furan ring are 7-hydroxy-8-methoxy-2-oxo-2*H*-1-benzopyran-6-acetic acid, and  $\alpha$ ,7-dihydroxy-8-methoxy-2-oxo-2*H*-1-benzopyran-6-acetic acid, and one resulting from the opening of the pyrone ring is an unknown conjugate of (*Z*)-3-(6-hydroxy-7-methoxybenzofuran-5-yl)-2-propenoic acid (Kolis *et al.*, 1979).

## 4.2 Mechanisms of carcinogenesis

### 4.2.1 Genotoxic effect

Evidence from a large number of studies indicates that PUVA causes DNA damage in a variety of prokaryotic and eukaryotic cells. The events mentioned in the previous *IARC Monograph* included the induction of chromosomal aberrations, sister chromatid exchange, mutations, DNA damage, and DNA crosslinks in human cells *in vitro*. The treatment was also reported to transform mouse C3H10T1/2 cells, and to induce chromosomal aberrations, micronuclei, sister chromatid exchange, mutation, unscheduled DNA synthesis, and DNA crosslinks in rodent cells in culture. In addition, mitotic recombination and mutation were found in fungi, and mutation and DNA damage in bacteria. The

treatment was also reported to induce sister chromatid exchange in epithelial cells of cheek pouches of hamsters treated *in vivo*; by contrast, negative results were mentioned for the induction of sister chromatid exchange in patients treated with PUVA (IARC, 1987b).

In the absence of UVA light, methoxsalen was found to induce mutation in bacteria, but the evidence was considered inconclusive with respect to chromosomal aberrations and sister chromatid exchange in human cells *in vitro*, gene mutation and DNA damage in rodent cells *in vitro*, and mutation in yeast (IARC, 1987b).

### 4.2.2 DNA adduct formation

The major photochemical reactions of methoxsalen in the presence of DNA were described several decades ago (Cole, 1971), and are briefly summarized below.

Methoxsalen undergoes DNA intercalation, with a preference for 5'-TpA sites (Tessman *et al.*, 1985), and subsequently alkylates DNA upon photo-activation. The major reaction mechanism involves [2+2] cycloaddition to the 5,6-double bond of a thymidine upon absorption of the first photon, which generates two types of cyclobutane mono-adducts, depending on whether the addition occurs at the 4',5'-double bond of the furan ring or the 3,4-double bond of the pyrone ring of the psoralen. The absorption of a second photon by the furan mono-adduct then leads to cycloaddition of the 5,6-double bond of the pyrone to a flanking thymidine in the complementary strand, generating an interstrand crosslink (Johnston & Hearst, 1981). Other types of photoproducts have been reported, including adducts to the sugar moiety of deoxyadenosine (Cadet *et al.*, 1988).

### 4.2.3 Mutagenic effects

The mutagenic effects of PUVA in mammalian cells have been extensively investigated.

Treatment of Chinese hamster ovary cells with PUVA (for analysis of the mutation spectrum at the adenine phosphoribosyl transferase (*Aprt*) locus) suggests that bi-adducts are likely to be the major PUVA-induced pre-mutagenic lesions in mammalian cells (Sage *et al.*, 1993). Likewise, the spectrum of mutations induced at the hypoxanthine (guanine) phosphoribosyl transferase (*HPRT*) gene of human fibroblasts exposed to a split-dose protocol (two UVA doses separated in time, the first one producing mainly mono-adducts) indicates that most of the mutations are observed at crosslinked sites; however, a significant level of mutation induction is also detected after the first dose of UVA, where a higher proportion of mono-adducts would be expected (Yang *et al.*, 1994). This study suggests that both mono-adducts and crosslinks have mutagenic properties.

There has also been extensive use of the *supF* gene for the analysis of PUVA-induced mutations. In one representative study, the spectrum of mutations was characterized after treatment of fibroblasts from transgenic mice containing chromosomally integrated lambda phage with the *supF* gene as a mutation reporter gene (Gunther *et al.*, 1995); a significant feature of the mutation spectrum was the predominant occurrence of mutations at 5'TpA sites, in concordance with the sequence specificity observed *in vitro* in reactions with isolated DNA (Sage & Bredberg, 1991).

Whereas the formation of psoralen photo-products at 5'ApT:5'TpA sites is closely correlated with the *Tp53* mutation spectra obtained *in vitro* and in studies of PUVA treatment of animals (Nataraj *et al.*, 1996; Besaratinia & Pfeifer, 2004; Lambertini *et al.*, 2005), such correlations do not hold for *TP53* mutations in PUVA-related human squamous cell carcinoma (Nataraj *et al.*, 1997; Wang *et al.*, 1997; Gasparro *et al.*, 1998; Monti *et al.*, 2000). PUVA-induced DNA adducts have recently been shown to be substrates for the base-excision repair pathway

in human cells (Couvé-Privat *et al.*, 2007) but the efficiency of the repair mechanism remains to be established. The peculiarity of the *TP53* mutation spectrum in PUVA-induced human squamous cell carcinoma has been interpreted as indicating that other types of DNA damage may contribute to the carcinogenicity and mutagenicity of PUVA. In addition to the direct interaction of photo-activated methoxsalen with DNA, reactive oxygen species (including singlet oxygen and superoxide) have been suggested as having a role in PUVA-induced cytotoxicity (Foote, 1991; Liu *et al.*, 1999). The formation of increased levels of the 8-hydroxy-2'-deoxyguanosine biomarker following PUVA treatment of either calf thymus DNA or cultured human epidermoid carcinoma cells (compared with those obtained after treatment with methoxsalen or UVA alone) (Liu *et al.*, 1999) is consistent with this interpretation.

### 4.3 Synthesis

Methoxsalen in combination with UVA is carcinogenic via a genotoxic mechanism that involves photo-activation.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of methoxsalen plus with UVA radiation. Methoxsalen in combination with UVA radiation causes cancer of the skin (squamous cell carcinoma).

There is *sufficient evidence* in experimental animals for the carcinogenicity of methoxsalen plus UVA radiation.

There is *limited evidence* in experimental animals for the carcinogenicity of methoxsalen.

Methoxsalen plus UVA radiation is *carcinogenic to humans* (Group 1).

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