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Sunlight-induced mutagenicity of a common sunscreen ingredient

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We have tested the mutagenicity of a UV-B sunscreen ingredient called Padimate-O or octyl dimethyl PABA, which, chemically speaking, is identical to an industrial chemical that generates free radicals when illuminated. It is harmless in the dark but mutagenic in sunlight, attacking DNA directly. A commercial sunscreen containing Padimate-O behaves in the same way. UV-A in sunlight also excites Padimate-O, although less than UV-B. Some related compounds, including a known carcinogen, behave similarly. As mutagens may be carcinogenic, our results suggest that some sunscreens could, while preventing sunburn, contribute to sunlight-related cancers.

Sunscreen; Padimate-O; Octyl dimethyl PABA; Parsol 1789; Mutagenesis; Carcinogenesis

1. INTRODUCTION

In sunlight, UV-B (290–320 nm) causes sunburn and probably causes cancers such as melanoma, which are increasing throughout the world [1,2]. Chemicals which prevent sunburn are thought to reduce carcinogenesis, but any that are excited by sunlight to species such as free radicals, which can damage DNA, could be dangerous. Recognizing this, the Scientific Committee for Cosmetology of the EEC published guidelines in 1982 stating that "Studies of phototoxicity and photosensitization are required for certain ingredients [of cosmetics] where knowledge of the chemical structure indicates a possible hazard. In some instances (as with sun-screening agents) such studies should be performed where the risk could be greater in view of the mode of use" [3]. The common sunscreen Padimate-O falls into this category. Patented in 1968 [4], it closely resembles ethyl 4-dimethyl-aminobenzoic acid (Table I), an industrial photo-initiator [5] known since 1981 to form free radicals on UV-illumination [6]. Padimate-O is not mutagenic in normal Ames tests [7], but the effects of sunlight on this compound are not very clear, and it is still in use, although the amyl ester (Padimate-A, Table I) was withdrawn in the EEC in 1989 for unstated reasons [8]. Here, we use simulated sunlight to test the phototoxicity and photomutagenicity of Padimate esters and some relatives in a simple eukaryote, budding yeast, and we also test directly for damage to DNA.

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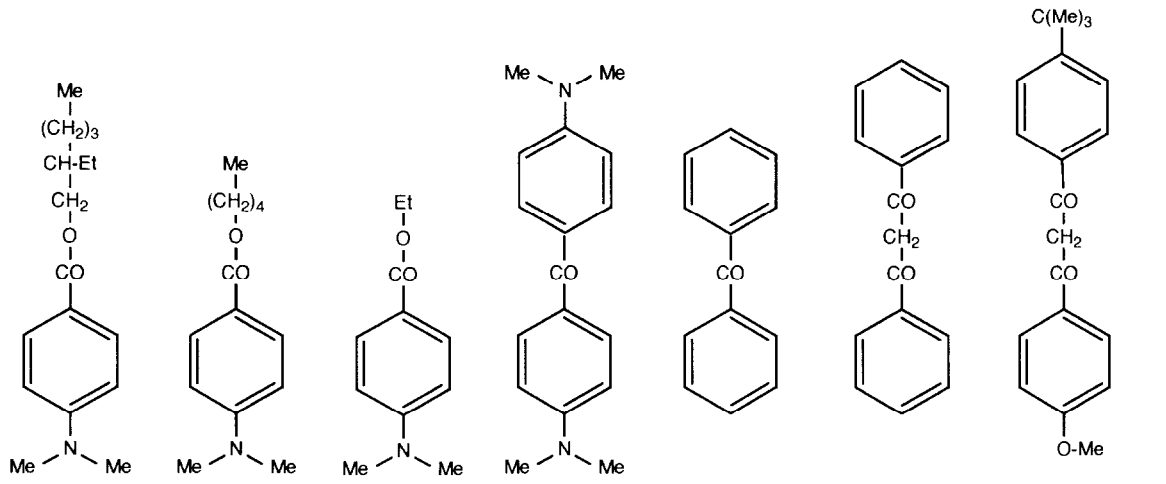
2. EXPERIMENTAL

The solar simulator consists of a 250-watt ozone-free Xenon lamp (Wotan XBO 250 W) in a housing with a 28 mm diameter silica window and a 2-mm Schott WG 320 filter in front of the window [9]. It was always pre-run for 30 min to allow the output to stabilize [10]. For illumination, yeast in a quartz cuvette in a jacket with a silica window [11] were kept at 28°C (the optimum growth temperature) by circulating water We used two strains of yeast: one removes cyclobutane dimers and 6-4 photoproducts from DNA and is relatively resistant to sunlight; the other cannot ([12] and S McCready, personal communication), and is more sensitive. The genotype of the first strain is: *ade 2 1 can 1.100 SUPQ5 RAD1::LEU2 [psi⁻]*; the repair-deficient strain contains a *rad1* disruption [12,13]. Both require adenine for growth, and mutations at a number of loci, including allosuppressors [13], can remove the requirement for adenine. Cells were grown in the dark in liquid YEPD medium [14], harvested in exponential phase from an overnight culture or in stationary phase from a 48-h culture, washed twice with water and illuminated at 2×10^7 colony-forming units per ml in 20 mM sodium phosphate, pH 7 4, with gentle bubbling with sterile air to keep them suspended. The solubility of Padimate-O was estimated at 50 μM by comparing the peak absorbance (290 nm) of a saturated solution in buffer with the peak absorbance (312 nm) of standard solutions in methanol. To add chemicals, 10 μl of a solution in ethanol was added to 10 ml of yeast suspension; controls received ethanol alone. Double-stranded, end-labelled DNA was synthesized using a USB Sequenase 2 0 kit by annealing a primer to a 7.5 kb M13 mp18 template, allowing limited extension in the presence of [α-^{32}P]dATP and then adding excess unlabelled dXTPs

3. RESULTS AND DISCUSSION

The light in the cuvette (Fig. 1A) mimics the sunlight reaching the basal layer of human epidermis, where cancers form. Both yeast strains, whether exponentially growing or stationary, are unaffected by Padimate-O in the dark (Fig. 1B, curve 1). Both survive 15 min of light alone (curve 2). Stationary cells with the repair system are slightly affected by Padimate-O and light (curve 3), but those without are more sensitive (curve 4). Exponential cells are much more sensitive, especially when

Table I
Structural formulae, chemical and commercial names of compounds discussed in this report



2-ethylhexyl-4-dimethyl-aminobenzoate	amyl-4-dimethyl-aminobenzoate	ethyl-4-dimethyl-aminobenzoate	4,4'-bis (dimethylamino)-benzophenone	benzophenone	dibenzoylmethane	4-*tert*-butyl-4'-methoxy-dibenzoylmethane

Commercial names

Padimate-O	Padimate A	Michler's	ketone	Parsol 1789
Octyl Dimethyl PABA				Parsol A
O-PABA				
Escalol 507	Escalol 506			

For proprietary preparations containing Padimate-O see [32]

they lack the repair system (curves 5 and 6). This suggests that illuminated Padimate-O damages DNA, and is consistent with results from mouse cells [15]. Fig. 2 confirms this suggestion. Illuminated Padimate-O induces mutations (Fig. 2A) and attacks DNA (Fig. 2B). The effects of 50 μM Padimate-O are very similar to those of 500 μM benzophenone, which is well known to attack DNA when illuminated, generating strand breaks and various photoproducts [16,17], although the full range of lesions induced by Padimate-O remains to be established. The effects depend on concentration and require constant illumination (Fig. 3A). 5 μM Padimate-O is less toxic (curve 3) than 50 μM (curve 4B), and if cells are withdrawn from light they survive (curve 4A), suggesting that Padimate-O forms a short-lived reactive species, as expected. A commercial sunscreen has similar effects. We centrifuged an ethanol extract of one declaring Padimate-O (voluntary in the UK [18]), to remove insoluble material (probably zinc or titanium oxide, commonly used as inert, reflective pigments and unlikely to penetrate skin). The supernatant was harmless in the dark but lethal in the light (curve 5).

Sunscreens penetrate skin [19,20], but also attenuate the sunlight passing through it, and could eliminate the potentially damaging effects of Padimate-O. A film of a medium-protection sunscreen containing 3–4% Padimate-O as the sole UV filter has 100% transmittance at wavelengths above 360 nm, 90% at 350 nm, 20% at 320 nm, and 15% at 300 nm [21], removing most but not all UV-B. By using a WG 335 filter, which has 100% transmittance above 360 nm, 85% transmittance at 350 nm, but only 1% at 320 nm, we eliminated essentially all UV-B, simulating a highly protective UV-B sunscreen. Padimate-O is still toxic, although, as expected, it now acts more slowly (Fig. 3B, compare curves 4 and 7), because it absorbs little UV-A, however, as epidermis transmits UV-A quite well (Fig. 1A), sunscreens relying only on UV-B filters may not protect against Padimate-O diffusing through the skin, especially if exposure is increased. This could be important for sunbathers.

In excitation of Padimates, the ester group is unimportant. The key feature is the conjugation of the electron-donating dimethylamino group by the aromatic ring to the electron-withdrawing carbonyl group. It is this 'donor-aromatic-acceptor (DArA) arrangement'

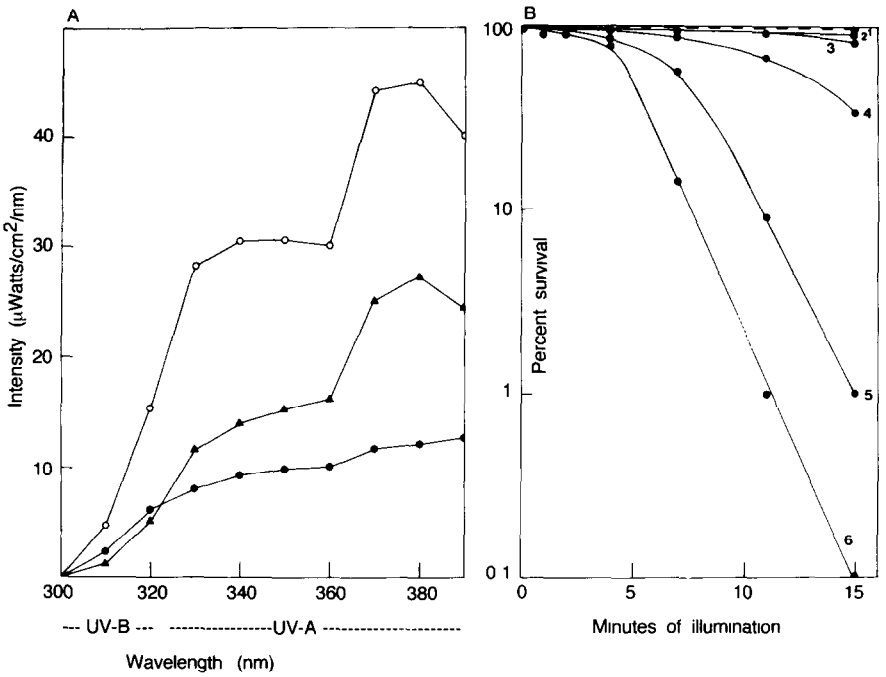


Fig. 1. The light source and the effects of Padimate-O. (A) The spectrum of the light reaching the yeast in the cuvette (●) was defined using a spectroradiometer (Model 752, Optronic Laboratories) and is similar to that passing through human stratum corneum (▲), calculated by multiplying the sunlight spectrum (○) by the transmittance of the stratum corneum [29] The sunlight spectrum was recorded on a bright summer's day (sun angle = 42°) on the roof of the Biochemistry building in Oxford and is very similar to those shown in standard Tables [30]. (B) Effects of Padimate-O. Curve 1 (dashed line), typical effect of incubating exponential or stationary repair-deficient cells with 50 μM Padimate-O in the dark; repair-competent cells behaved in the same way Curve 2, effect of exposing either strain to light alone. Curves 3–6, effect of light plus 50 μM Padimate-O on stationary cells (3,4) and exponential cells (5,6) which possess (3,5) or lack (4,6) the repair system. With stationary cells the results were the same whether Padimate-O was added 20 min before starting illumination or was present throughout their growth, showing that their resistance to Padimate-O is not due to any change in permeability as they age

that allows excited states to form [6] and, as expected, Padimate-O and Padimate-A behave in the same way in our system (Fig. 3B, curves 5 and 7). In a related compound, Michler's ketone, two dimethylamino groups are conjugated to a carbonyl group (Table I), and it has the same effects as the Padimate esters (curve 6). This finding is interesting because Michler's ketone is carcinogenic in rats and mice [22]. Industry now avoids Michler's ketone for that reason, although it is a valuable photo-initiator [5], and prefers ethyl 4-dimethylaminobenzoate (another Padimate ester; Table I), which apparently was assumed to be safe because of its use in skin lotions [23]. Here, Padimate esters have similar effects to Michler's ketone, perhaps indicating a potential hazard. In any case, they damage DNA in sunlight, and it would seem prudent to avoid them, especially as they sometimes contain a mutagenic nitrosamine [24], whether this contaminant constitutes a risk or not [25].

Some sunscreens lack the dimethylamino group but do contain a carbonyl group. One example is Parsol 1789, a common UV-A filter functionally similar to dibenzoylmethane, which in turn is related to benzophenone (Table I). Dibenzoylmethane becomes toxic when illuminated (Fig. 3B, curve 3), although less so than Padimate-O (curve 6). Simple derivatives of dibenzoylmethane, such as Parsol 1789, may behave in the same way, either before or after metabolism to dibenzoylmethane.

Most studies on Padimate esters involve scoring tumour formation in hairless mice. They indicate that, for a given total exposure to light, sunscreens based on Padimate-O reduce tumourigenesis [26,27], however, the consequences of increasing the exposure (as in sunbathing) are less certain. For example, Kligman et al. [26] found that the dose of UV-B required for 50% of control mice to develop tumours was 11 j/cm^2. The dose needed for 50% of mice treated with a sunscreen containing 2% Padimate-O to develop tumours was 17 j/cm^2, although the time required for tumours to appear was increased. One difficulty in these experiments is to distinguish between the protective effect of the surface film of Padimate-O and the direct effects of any contact of the Padimate-O with dividing cells. Our own experiments emphasize the importance of making this distinction, and suggest that any UV light which passes through a sunscreen and reaches dividing cells is likely

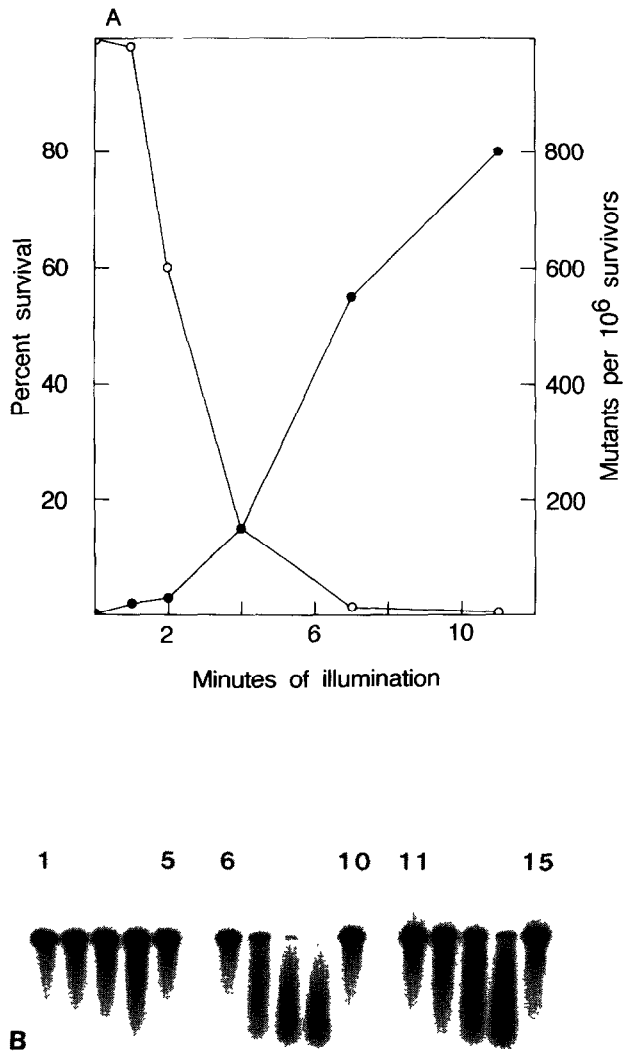


Fig 2. DNA damage induced by Padimate-O (A) Induction of mutations. Exponential, repair-deficient cells were illuminated in 50 μM Padimate-O. Survival (○) was scored as in Fig. 1, and mutants (●) by counting the colonies that grew after plating undiluted samples on medium lacking adenine. Light alone and Padimate-O in the dark generated no mutants. (B) Chemical damage to DNA. 2 μg of 7.5 kb double-stranded, end-labelled DNA in 30 μl was irradiated through a WG 320 filter in the presence or absence of saturating Padimate-O (50 μM) or benzophenone (500 μM) after first concentrating the light about 1,000 times with a quartz lens to partly compensate for the greatly reduced target size compared to the complete yeast genome Samples were analyzed on a 0.7% alkaline agarose gel, thus detecting both strand breaks and cold alkali-labile sites (principally Dewar isomers of 6-4 photoproducts [31]) Lanes 1–5, effect of irradiation alone for 0, 20, 40 and 60 min and of incubating in the dark for 60 min; lanes 6–10, effect of 500 μM benzophenone with irradiation for 0, 20, 40 and 60 min or incubation in the dark for 60 min; lanes 11–15, effect of 50 μM Padimate-O with irradiation for 0, 20, 40 and 60 min or incubation in the dark for 60 min.

to be particularly damaging if Padimate-O has entered those cells. It remains to be seen whether they are relevant to the paradox that sunscreens do not seem to prevent melanoma or basal cell carcinoma in humans, and to suggestions that sunscreens might encourage, rather than prevent, sunlight-related cancers [28].

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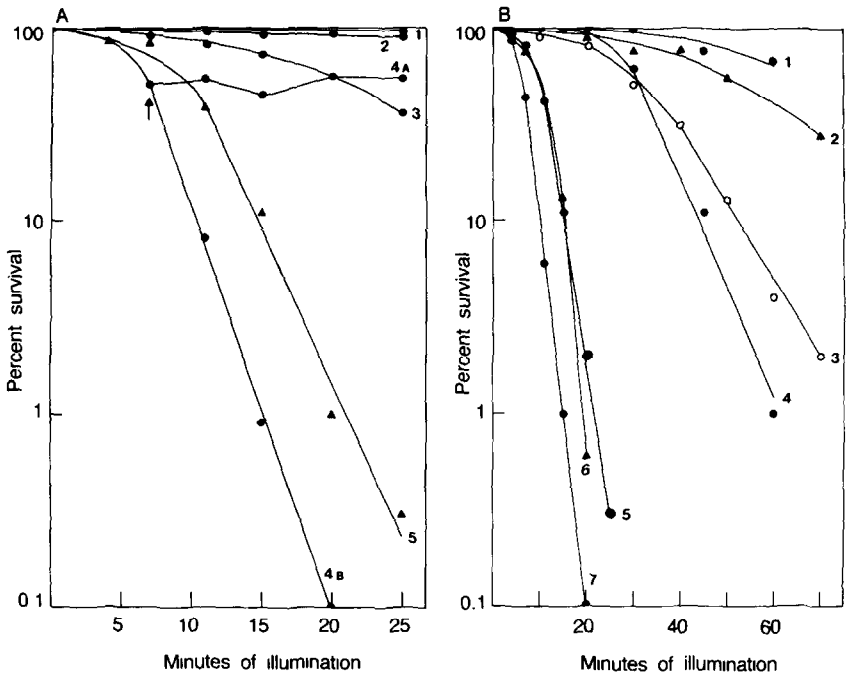

Fig. 3. Effects of Padimate-O, a commercial sunscreen and related compounds in relation to conditions of exposure. (A) Dependence of Padimate-O on concentration and light and effects of a sunscreen. Exponential, repair-deficient cells were treated as in Fig. 1. Curve 1, 50 μM Padimate-O in the dark. Curve 2, light alone. Curve 3, illumination in 5 μM Padimate-O. In curve 4, cells were illuminated in 50 μM Padimate-O for 7 min (arrowed), at which point half were withdrawn and incubated in the dark (4A) while illumination of the rest continued (4B). Curve 5, illumination after adding a sunscreen. 330 mg of a sunscreen containing 4% Padimate-O were made up to 1.025 ml with ethanol, giving approximately 50 mM Padimate-O. After centrifuging, 5 μl of supernatant was added to 5 ml of yeast. Results with the extract in the dark were indistinguishable from curve 1. (B) Delayed effects of Padimate-O after eliminating UV-B, and effects of Padimate-A, Michler's ketone and dibenzoylmethane. Exponential, repair-deficient cells were illuminated through a 2-mm WG 335 filter either alone (curve 1) or in 50 μM Padimate-O (curve 4). Cells were also illuminated with normal simulated sunlight either alone (curve 2), or with 50 μM dibenzoylmethane (curve 3), 50 μM Padimate-A (curve 5), 50 μM Michler's ketone (curve 6) or 50 μM Padimate-O (curve 7).

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