GRISEOFULVIN

This substance was considered by previous working groups, in 1975 (IARC, 1976) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

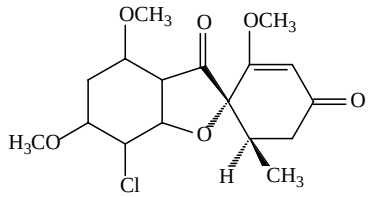
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 126-07-8
Deleted CAS Nos: 8027-03-0; 8055-10-5; 3426-54-8; 11103-62-1; 24659-79-8
Chem. Abstr. Name: (1′*S*,6′*R*)-7-Chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione
IUPAC Systematic Name: 7-Chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione
Synonyms: (2*S*,4′*R*)-7-Chloro-2′,4,6-trimethoxy-4′-methylspiro[benzofuran-2-(3*H*),3′-cyclohexene]-3,6′-dione; (1′*S*-trans)-7-chloro-2′,4,6-trimethoxy-6′-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione; (+)-griseofulvin; (+)-7-chloro-4,6-dimethoxycoumaran-3-one-2-spiro-1′-(2′-methoxy-6′-methylcyclohex-2′-en-4′-one)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{17}H_{17}ClO_6$ Relative molecular mass: 352.77

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White to creamy- or yellowish-white, crystalline powder (Townley, 1979; Royal Pharmaceutical Society of Great Britain, 2000)
(*b*) *Melting-point*: 220 °C (Lide & Milne, 1996)
(*c*) *Spectroscopy data*: Infrared [prism/grating (72624)], ultraviolet (40309), nuclear magnetic resonance [proton (45536)] and mass spectral data have been reported (Sadtler Research Laboratories, 1995; Lide & Milne, 1996).
(*d*) *Solubility*: Very slightly soluble in water (0.2 g/L at 25 °C); sparingly soluble in ethanol and methanol; soluble in acetone, chloroform and dimethylformamide (Townley, 1979; Council of Europe, 1997; US Pharmacopeial Convention, 1999; Royal Pharmaceutical Society of Great Britain, 2000)
(*e*) *Optical rotation*: $[\alpha]_D^{17}$, +376 ° (Lide & Milne, 1996)

1.1.4 Technical products and impurities

To enhance its water solubility and bioavailability in pharmaceutical preparations, griseofulvin is mixed with a non-toxic, water-soluble polymer such as polyvinylpyrrolidone or hydroxypropyl cellulose and spray-dried before treatment with a wetting agent such as sodium lauryl sulfate on benzalkonium chloride. The resulting material is characterized as 'microsize' or 'ultramicrosize' crystals of griseofulvin (Martin & Tsuk, 1982).

The European Pharmacopoeia (Council of Europe, 1997) specifies that the particles of the powder are generally up to 5 μm in maximum dimension, although larger particles, which may occasionally exceed 30 μm, may be present; the *US Pharmacopeia* describes material with a predominance of particles of the order of 4 μm in diameter (US Pharmacopeial Convention, 1998).

Griseofulvin is commercially available as tablets containing 250 or 500 mg microsize or 125, 165, 250 or 330 mg ultramicrosize crystals of griseofulvin, as capsules containing 250 mg microsize griseofulvin and as an oral suspension containing 125 mg/5 mL microsize griseofulvin (Medical Economics Co., 1999a,b; American Hospital Formulary Service, 2000).

The inactive ingredients in griseofulvin tablet formulations may also include calcium stearate, corn starch, colloidal silicon dioxide, lactose monohydrate, magnesium stearate, methylcellulose, methylparaben, polyethylene glycol, titanium dioxide, titanium oxide or wheat gluten. The suspension may also include 0.2% alcohol, dibasic calcium phosphate, docusate sodium, FD&C Red No. 40, FD&C Yellow No. 6, flavours, magnesium aluminium silicate, menthol, propylene glycol, propylparaben, saccharin sodium, simethicone emulsion, sodium alginate or sucrose (Medical Economics Co., 1999c).

Trade names for griseofulvin include Amudane, B-GF, Biogrisin, Curling factor, Delmofulvina, Dermogine, Fulcin, Fulcine, Fulsan, Fulvicin, Fulvicina, Fulviderm,

Fulvina, Fulvinil, Fulvistatin, Fungivin, Gefulvin, Greosin, Gricin, Grifulin, Grifulvin, Gris-PEG, Grisactin, Griséfuline, griseo von ct, Griseo, Griseoderm, Griseofort, Griseoful, Griseofulvin Capsules USP 23, Griseofulvin Leo, Griseofulvin Oral Suspension USP 23, Griseofulvin Tablets BP 1999, Griseofulvin Tablets USP 23, Griseofulvin Ultra, Griseofulvin Vetag, Griseofulvina, Griseomed, Griseostatin, Grisfulvin, Grisofulvin, Grisol, Grisomicon, Grisovin, Grisovina, Grivate, Grivin, Grizeofulvin, Grysio, Idifulvin, Lamoryl, Likuden, Microcidal, Neo-fulcin, Norofulvin, Polygris, Poncyl, Spirofulvin, Sporostatin, Sulvina, Ultragris, Ultramicrosize Griseofulvin Tablets USP 23, Vetmix Griseofulvin, Viro Griseo M and Walavin (Royal Pharmaceutical Society of Great Britain, 2000; Swiss Pharmaceutical Society, 2000)

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared absorption spectrophotometry with comparison to standards and high-performance liquid chromatography (HPLC) with ultraviolet detection as the methods for identifying griseofulvin; ultraviolet absorption spectrophotometry and HPLC with ultraviolet detection are used to assay its purity. In pharmaceutical preparations, griseofulvin is identified by infrared and ultraviolet absorption spectrophotometry and HPLC with ultraviolet detection; ultraviolet absorption spectrophotometry and HPLC with ultraviolet detection are used to assay for griseofulvin content (British Pharmacopoeia Commission, 1993; Council of Europe, 1997; US Pharmacopeial Convention, 1999).

1.2 Production

Griseofulvin is an antifungal substance typically produced by the growth of certain strains of *Penicillium griseofulvum* (Royal Pharmaceutical Society of Great Britain, 2000). A method for the synthesis of griseofulvin from dimethoxyphenol has been reported (Pirrung *et al*., 1991).

Information available in 2000 indicated that griseofulvin was manufactured by six companies in China, three in Japan and one each in India and the United Kingdom (CIS Information Services, 2000a) and that it was used in the formulation of pharmaceuticals by 44 companies in India, eight companies each in Germany and the United Kingdom, six companies each in Argentina, Japan and the USA, five companies each in Singapore, Switzerland, Taiwan and Thailand, four companies each in China, Indonesia, Italy and Malaysia; three companies each in Australia, Canada, Chile, Ecuador and the Netherlands, two companies each in Brazil, Egypt, Hong Kong, Mexico, New Zealand, Peru, the Philippines, Portugal, South Africa, Spain, Turkey and Viet Nam and one company each in Austria, Finland, Ireland, the Islamic Republic of Iran, Israel, Malta, Norway, Sweden and Venezuela (CIS Information Services, 2000b).

1.3 Use

Griseofulvin is an antibiotic fungistatic drug administered orally in the treatment of dermatophyte and ringworm infections. It is fungistatic against various species of *Microsporum, Epidermophyton* and *Trichophyton in vitro*. It is generally given for infections that involve the scalp, hair, nails and skin (e.g. tinea corporis (ringworm of the body), tinea pedis (athlete's foot), tinea cruris (ringworm of the groin or thigh), tinea barbae (barber's itch), tinea capitis (ringworm of the scalp), tinea unguium (onychomycosis; ringworm of the nails)) and which do not respond to topical treatments; infections of the soles of the feet, the palms of the hands and the nails respond slowly (Medical Economics Co., 1999a,b,c,d; Royal Pharmaceutical Society of Great Britain, 2000).

Because griseofulvin has some vasodilatatory activity, its use has resulted in some improvement in a small number of patients with Raynaud's disease and angina pectoris. Because it is structurally similar to colchicine and shares its activity as a metaphase inhibitor, griseofulvin has been used in the treatment of gout (American Hospital Formulary Service, 2000).

The dosage of griseofulvin varies depending on whether the drug is administered as a microsize or ultramicrosize preparation. In addition, the recommended doses of ultramicrosize griseofulvin vary slightly depending on the manufacturer and the formulation of the drug. Therapy with griseofulvin is generally maintained for at least 2–4 weeks for the treatment of tinea corporis; at least 4–12 weeks for the treatment of tinea capitis; 4–8 weeks for tinea pedis; and from 4–6 months to 1 year or longer for tinea unguium (American Hospital Formulary Service, 2000).

The usual adult dose of ultramicrosize griseofulvin for the treatment of tinea corporis, tinea cruris or tinea capitis is 330–375 mg/day in single or divided doses, depending on the manufacturer and formulation of the drug; the usual adult dose of ultramicrosize griseofulvin for the treatment of infections that are more difficult to eradicate, such as tinea pedis and tinea unguium, is 660–750 mg/day, depending on the manufacturer and formulation. The usual adult dose of microsize griseofulvin for the treatment of tinea corporis, tinea cruris, or tinea capitis is 500 mg/day and 1 g daily for the treatment of infections that are more difficult to eradicate, such as tinea pedis and tinea unguium (Gennaro, 1995; American Hospital Formulary Service, 2000; Royal Pharmaceutical Society of Great Britain, 2000).

The usual dose of ultramicrosize griseofulvin for children > 2 years of age is approximately 7.3 mg/kg bw per day, although doses up to 10–15 mg/kg bw daily have been used. The manufacturers suggest that children weighing approximately 14–23 kg can receive 82.5–165 mg of ultramicrosize griseofulvin daily and those weighing > 23 kg can receive 165–330 mg/day. Alternatively, the manufacturers suggest that children weighing 16–27 kg can receive 125–187.5 mg of ultramicrosize griseofulvin daily and those weighing > 27 kg can receive 187.5–375 mg daily. For the treatment of tinea capitis and tinea corporis, the American Academy of Pediatrics recommends

that children receive ultramicrosize griseofulvin at a single daily dose of 5–10 mg/kg bw (maximum dose, 750 mg). The usual paediatric dose of microsize griseofulvin is 10–11 mg/kg bw per day, although doses up to 20–25 mg/kg bw per day have been used. The manufacturers suggest that children weighing approximately 14–23 kg can receive 125–250 mg microsize griseofulvin daily and that children weighing > 23 kg can receive 250–500 mg daily. Alternatively, some clinicians suggest that children be given microsize griseofulvin at a dose of 300 mg/m^2 daily. The American Academy of Pediatrics recommends that children receive microsize griseofulvin at a daily dose of 10–20 mg/kg bw (maximum dose, 1 g) given in up to two divided doses (Gennaro, 1995; American Hospital Formulary Service, 2000; Royal Pharmaceutical Society of Great Britain, 2000).

When preparations, available in some countries, containing ultramicrocrystalline or ultramicrosize griseofulvin are used, the doses are reduced by one-third to one-half of the recommended doses of microcrystalline or microsize griseofulvin. Griseofulvin is probably best given with or after meals (Royal Pharmaceutical Society of Great Britain, 2000).

The duration of treatment depends on the thickness of the keratin layer: 2–6 weeks for infections of the hair and skin, up to 6 months for infections of the fingernails and 12 months or more for infections of the toenails (Royal Pharmaceutical Society of Great Britain, 2000).

Although griseofulvin is usually given systemically, beneficial responses in fungal skin infections have been reported with some topical formulations (Royal Pharmaceutical Society of Great Britain, 2000).

Griseofulvin is also used as a veterinary antifungal drug (US Pharmacopeial Convention, 1998; Budavari, 2000; Food and Drug Administration, 2000).

1.4 Occurrence

1.4.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 2000), about 1700 pharmacists in the USA were potentially exposed to griseofulvin.

1.4.2 *Environmental occurrence*

No data were available to the Working Group.

1.5 Regulations and guidelines

Griseofulvin is listed in the pharmacopoeias of China, the Czech Republic, France, Germany, Italy, Japan, Poland, the United Kingdom and the USA and in the

European and International pharmacopoeias (British Pharmacopeia Convention, 1993; Society of Japanese Pharmacopoeia, 1996; Royal Pharmaceutical Society of Great Britain, 2000; Swiss Pharmaceutical Society, 2000; Vidal, 2000). It is also registered for human use in Ireland, Norway, Portugal, Spain and Sweden (Instituto Nacional de Farmacia e do Medicamento, 2000; Irish Medicines Board, 2000; Medical Products Agency, 2000; Norwegian Medicinal Depot, 2000; Spanish Medicines Agency, 2000).

2. Studies of Cancer in Humans

2.1 Case report

A 48-year-old woman with a history of gastric ulcers was admitted to a dermatological clinic in Essen, Germany, with tinea affecting the skin and nails of the feet and left hand. She was treated orally with a total dose of 31 g of griseofulvin over a period of about 1.5 months. Analysis of the patient's peripheral blood did not show alterations suggestive of haematological disorders, and there was no apparent splenomegaly. Seven months later, when the woman was hospitalized after a minor traffic accident, analysis of the blood strongly suggested chronic granulocytic leukaemia. The patient was subsequently followed and treated for this condition (König *et al*., 1969/70).

2.2 Cohort studies

Griseofulvin was included in a hypothesis-generating cohort study designed to screen a large number (215) of drugs for possible carcinogenicity, which covered more than 140 000 subscribers enrolled between July 1969 and August 1973 in a prepaid medical care programme in northern California (USA). Computer records of persons to whom at least one drug prescription has been dispensed were linked to the cancer records of hospitals covered by the medical care programme and the regional cancer registry. The observed numbers of cancers were compared with those expected, standardized for age and sex, for the entire cohort. Three publications summarized the findings for follow-up periods of up to 7 years (Friedman & Ury, 1980), 9 years (Friedman & Ury, 1983) and 15 years (Selby *et al*., 1989). Griseofulvin was included only in the two most recent reports. In the 9-year follow-up, an excess of thyroid cancer was reported among 744 griseofulvin users (two observed cases versus 0.2 expected; $p < 0.05$), while no excess was seen for cancers at all sites (23 observed cases versus 22.7 expected). In the 15-year follow-up, no results were reported for griseofulvin, implying that no significant association was observed for any of the 56 cancer sites considered. [The Working Group noted, as did the authors, that, since some 12 000 comparisons were made in this hypothesis-generating study, the associations should be verified independently. Data on duration of use were not provided.]

3. Studies of Cancer in Experimental Animals

Griseofulvin has been evaluated previously (IARC, 1976). One new report has become available (Rustia & Shubik, 1978), and a selection of the most relevant studies from the previous monograph were re-analysed.

3.1 Oral administration

Mouse: Groups of male and female Charles River mice, 5–6 weeks of age, were fed diets containing 1% (w/w) griseofulvin of various particle sizes (regular, microcrystalline and milled) with specific surface areas of 0.41, 1.3 and 1.52 m^2/g, respectively, for 12–16 months. 'Hepatomas' developed in 4/8 male and 0/9 female mice fed regular-size griseofulvin, 4/4 male and 4/5 female mice fed microcrystalline griseofulvin and 1/1 male and 3/3 female mice treated with milled griseofulvin. No tumours occurred in four male or four female controls (De Matteis *et al*., 1966).

In the study published since the previous evaluation, groups of 30–40 male and 30–40 female Swiss mice, 7 weeks of age, were fed diets containing 0.1, 0.3, 1.5 or 3% (w/w) griseofulvin [purity unspecified] for life. The diets with the three higher concentrations were given daily for alternate 5-week periods (5 weeks on, 5 weeks off). A group of 100 male and 100 female controls received basal diet. The study was terminated at 120 weeks. A dose-related decrease in survival rate was seen in the treated groups, and 0/98, 1/38, 2/25, 20/29 and 15/18 males and 0/98, 0/38, 0/28, 15/28 and 20/23 females developed 'hepatomas' at the concentrations of 0 (control), 0.1, 0.3, 1.5 and 3%, respectively (Rustia & Shubik, 1978). [The Working Group noted that some of the tumours were described as 'less differentiated trabecular', which would be considered carcinomas under current histological criteria.]

Rat: In the study published since the previous evaluation, groups of 30 male and 30 female Wistar rats, 7 weeks of age, were fed diets containing 0.2, 1 or 2% (w/w) griseofulvin [purity unspecified] for life. Treatment was given daily for alternate 5-week periods (5 weeks on, 5 weeks off). A group of 100 male and 100 female controls received basal diet. The survival rate of treated animals was slightly higher than that of controls up to the end of the study of 160 weeks. Follicular-cell adenomas and carcinomas of the thyroid (both follicular and papillary) were found in 1/98, 4/30, 11/30 and 16/30 males and 2/99, 2/30, 8/30 and 7/30 females given the diets containing 0 (control), 0.2, 1 and 2% griseofulvin, respectively. The increases at the two higher doses were statistically significant ($p < 0.001$) (Rustia & Shubik, 1978).

Hamster: In the study published since the previous evaluation, groups of 30 male and 30 female Syrian hamsters, 7 weeks of age, were fed diets containing 0.3, 1.5 or 3% (w/w) griseofulvin [purity unspecified] for life. A group of 49 male and 49 female controls received basal diet. The study was terminated at 120 weeks, when the survival rate of treated animals was similar to that of controls. Most female hamsters

had died by week 90. No increase in tumour incidence was observed (Rustia & Shubik, 1978).

3.2 Subcutaneous administration

Mouse: Random-bred infant Swiss (ICR/Ha) mice were injected subcutaneously with suspensions of griseofulvin. Doses of griseofulvin in excess of 0.25 mg on day 1 of life produced acute toxicity. After administration of 0.5, 0.5, 1.0 and 1.0 mg on days 1, 7, 14 and 21 of age, respectively (total dose, 3 mg), a higher incidence of 'hepatomas' was found in male mice alive at 49 weeks (7/16; 44%) than in solvent controls (4/48, 8%). No liver tumours were found in females (Epstein *et al*., 1966). [The Working Group noted the inadequate survival in this study.]

3.3 Administration with known carcinogens

Mouse: Groups of female Swiss mice, 6 weeks of age, were given topical applications of 240 μg benzo[*a*]pyrene followed by acetone, griseofulvin, croton oil or croton oil preceded 4 or 24 h earlier by griseofulvin. Griseofulvin had no promoting activity in skin carcinogenesis when given alone but reduced the skin tumour promoting activity of croton oil (Vesselinovitch & Mihailovich, 1968).

4. Other Data Relevant to an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The plasma–concentration time curve was bi-exponential in five male volunteers given 90–180 mg of griseofulvin intravenously, with a half-time of 0.7–1.7 h for the first exponent and 9.5–21 h for the second. Absorption was found to occur up to 30 h after oral ingestion of 500 mg griseofulvin, with 27–72.5% of the dose absorbed (Rowland *et al.*, 1968). Particle size, fat intake, dissolution rate, formulation and dosage all affected the degree of griseofulvin absorption (Lin & Symchowicz, 1975).

In humans, 6-desmethylgriseofulvin was the major urinary metabolite after oral administration of griseofulvin, with 48% of an oral dose recovered as the free form and 37.4% as the glucuronide conjugate. Very little was excreted as unmetabolized griseofulvin, and only 2% appeared as the glucuronide of 4-desmethylgriseofulvin (Lin *et al.*, 1973a).

4.1.2 *Experimental systems*

Oral administration of [^{3}H]griseofulvin (9.2 μCi; dose not specified) to Sprague-Dawley rats [sex not specified] resulted in excretion of 18.2% of the radiolabel, 82% of which was during the first 48 h. A similar excretion pattern was seen after topical administration of griseofulvin, 17.6% appearing in the urine (Nimni *et al.*, 1990).

In bile-cannulated male CD rats, 77% of an intravenous dose of 7.5 mg/kg bw [^{14}C]griseofulvin appeared in the bile and 12% in the urine. In contrast, in male New Zealand rabbits, only 11% of the dose was found in the bile and 78% in the urine. In the urine of intact and cannulated rats, two major metabolites were present, the 4- and 6-desmethyl derivatives of griseofulvin. Rabbit urine contained 6-desmethylgriseofulvin as the predominant metabolite. In rats, most of the 4-desmethylgriseofulvin appeared as the glucuronide conjugate, whereas 6-desmethylgriseofulvin occurred only in its free form (Symchowicz *et al.*, 1967).

In mice given griseofulvin orally, 34% appeared in the urine as 4-desmethylgriseofulvin and its glucuronide and 23% as free 6-desmethylgriseofulvin (Lin *et al.*, 1972).

4.1.3 *Comparison of animals and humans*

In mice and rats, 4-desmethylgriseofulvin glucuronide and unconjugated 6-desmethylgriseofulvin are the major metabolites of griseofulvin, but in humans (as in rabbits), 6-desmethylgriseofulvin, in both its conjugated and unconjugated forms, is the major metabolite.

4.2 Toxic effects

4.2.1 *Humans*

Griseofulvin therapy can disturb porphyrin metabolism in humans (Knasmüller *et al.*, 1997). A clear indication of a porphyrinogenic effect was found in a study of 84 patients, of whom 52 were receiving griseofulvin at a dose of 0.5 g three times daily for 1 month, followed by 0.5 g twice daily for 23 months. Forty-two of the patients had completed their course or were completing it during the study and had therefore been off treatment for 2–80 weeks. The total faecal porphyrin concentrations of patients currently receiving the drug were more than 2.5-fold higher than those of untreated controls, and those of patients who had finished their course were more than twofold higher (Rimington *et al.*, 1963).

4.2.2 *Experimental systems*

(*a*) *Effects on thyroid function*

Administration of griseofulvin by gavage at a dose of 100 or 2000 mg/kg bw per day to groups of 10 male and 10 female Wistar rats for 30 days resulted in a significant

reduction in serum thyroxine concentrations in males at both doses. In females, a clear effect was restricted to the higher dose. Serum triiodothyronine concentrations were reduced only in the females at 2000 mg/kg bw per day. Serum thyroid-stimulating hormone concentrations were increased in both males and females at the highest dose, paralleled by a pronounced increase in thyroid gland weight (again only at the highest dose). Histopathological examination indicated the presence of follicles with high prismatic epithelial cells, but no hyperplastic changes (Sandow, J. & Rechberg, W. cited by Knasmüller *et al.*, 1997).

(*b*) *Effects on liver*

Feeding mice a diet containing 1% griseofulvin for 5–8 days resulted in liver enlargement, porphyria and hypercholesterolaemia (De Matteis, 1966). The accumulation of protoporphyrin in mouse liver is due to decreased conversion of protoporphyrin to haem caused by inhibition of mitochondrial ferrochelatase. After feeding of 1% griseofulvin to mice, only 25% of the initial ferrochelatase activity in the liver was present after 3 days. Concomitantly, hepatic 5-aminolaevulinate synthetase activity was enhanced 6.6-fold. The effects seen in rats were much less pronounced (De Matteis & Gibbs, 1975). Griseofulvin-induced accumulation of porphyrins in mouse liver was followed by cell damage and necrotic and inflammatory processes (Gschnait *et al.*, 1975). A green pigment that inhibited ferrochelatase was isolated from the livers of mice treated with griseofulvin, which had chromatographic characteristics identical to those of *N*-methyl protoporphyrin (Holley *et al.*, 1990). In a detailed study of the dose–response relationship of the porphyrinogenic action of griseofulvin given to mice [strain not specified] at 0.1, 0.5 or 1.0% in the diet for 38–450 days, serum proto- and cycloprophyrins as well as liver protoporphyrin and liver weight were clearly increased at the two highest feed concentrations (Shimoyama & Nonaka, 1987).

Administration of a diet containing 2.5% griseofulvin to three random-bred albino mice [sex not specified] for up to 194 days resulted in accumulation of hyalin (Mallory) bodies in hepatocytes (Denk *et al.*, 1975).

Feeding a diet containing 0.5% griseofulvin for 10 days to partially hepatectomized male Sprague-Dawley rats resulted in a 26% stimulation of liver-weight gain over the regenerative response in hepatectomized controls (Gershbein & Pedroso, 1985).

Protoporphyria was also induced in CF1 mice by topical application of griseofulvin (dose not given) every other day for up to 52 days (Polo *et al.*, 1997).

(*c*) *Other effects*

Griseofulvin has anti-mitotic properties, which were shown to be associated with binding to tubulin both in a cell-free system and in intact cells in culture. It was therefore concluded that it interfered with the normal polymerization of microtubule protein (Weber *et al.*, 1976; Wehland *et al.*, 1977). Griseofulvin was shown to interact directly with the tubulin dimer (Sloboda *et al.*, 1982). Microtubules have been suggested to play a role in thyroid secretion through an effect on colloid endocytosis

(Williams & Wolff, 1970). Contraction of microtubules is important in the process of fusion of colloid droplets and lysosomal bodies in follicular cells essential for thyroid-stimulating hormone-stimulated release of thyroxine and triiodothyronine from colloid and subsequent diffusion into the circulation (Capen, 2000).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Fourteen volunteers were given 2 g of griseofulvin daily for 3 months. No changes in semen quality (motility or morphology) were detected. In eight men examined, no changes in the histological appearance of the testes were seen (MacLeod & Nelson, 1959).

Although there have been a few case reports, no adequate epidemiological studies on the teratogenic potential of griseofulvin in humans were available to the Working Group.

4.3.2 *Experimental systems*

High doses of griseofulvin (200–2000 mg/kg bw) given to mice simultaneously with human chorionic gonadotropin to induce ovulation caused mitotic arrest of the oocytes in metaphase I. The arrested cells could overcome the division block and form zygotes that were often polyploid. When griseofulvin was given 2 h after human chorionic gonadotropin, cell division was less affected, but the frequency of hyperploid cells was substantially increased. These effects were due to an action of the drug on the spindle apparatus (Knasmüller *et al*., 1997).

High incidences of skeletal defects were reported in rats, mice, cats and dogs after administration of griseofulvin (summarized by Schardein, 1993). Some of these studies are reviewed below.

When female rats were given griseofulvin (microsize particles) orally at a dose of 125, 250, 750, 1250 or 1500 mg/kg bw per day on days 6–15 of gestation, malformations were observed in the offspring of dams at doses ≥ 250 mg/kg bw per day, and survival was decreased. The malformations included tail anomalies, anophthalmia, anal atresia and exencephaly (Klein & Beall, 1972). Slonitskaya (1969) made similar observations in rats given an oral dose of 50 or 500 mg/kg bw per day on days 11–14 of gestation.

Administration of micronized griseofulvin dissolved in polyethylene glycol 300 to rats at a dose of 50, 250 or 500 mg/kg bw per day [route not stated but probably oral] on days 6–15 of gestation caused a dose-related reduction in pup birth weight and in the number of live pups, while the incidence of resorptions was increased. A variety of severe vertebral and rib malformations were reported at the two higher doses [abstract only, numbers of malformations and of animals involved not specified] (Steelman & Kocsis, 1978).

A review of a number of case reports and a small study of four cats treated with griseofulvin for ring worm suggested that griseofulvin may be teratogenic in cats, causing a variety of defects in the central nervous system, the eye and soft tissue (Scott *et al*., 1975).

4.4 Effects on enzyme induction/inhibition and gene expression

4.4.1 *Humans*

Griseofulvin affects microsomal enzymes in humans (Lapina *et al.*, 1989; Hammond & Strobel, 1990).

4.4.2 *Experimental systems*

Six mice [strain not specified] fed a diet containing 1% griseofulvin for 5–8 days had a hexobarbital sleeping time that was 55% that of 12 controls (De Matteis, 1966). Feeding of griseofulvin to mice led to hypertrophy of the endoplasmic reticulum, but no enhancement of the total cytochrome P450 (CYP) content, so that the CYP content per milligram of microsomal protein was decreased (Lin *et al.*, 1973b).

Administration of griseofulvin induced a 126-fold increase in CYP2A5 mRNA and a 10-fold increase in 7-hydroxylation of coumarin in the liver of DBA/2 mice; in C57BL/6 mice, the increases were ninefold and sevenfold, respectively (Salonpää *et al.*, 1995).

Feeding male BALB/c mice a diet containing 2% griseofulvin for 3 weeks resulted in a more than fourfold increase in liver cytosolic glutathione *S*-transferase activity (Vincent *et al.*, 1989).

Male Swiss albino mice given a diet containing 2.5% griseofulvin for 12 days showed a 50% reduction in total CYP content and a twofold increase in cytochrome b_5 (Denk *et al*., 1977). When the same dose was given for 10 days to male CD_1 mice, similar effects were seen (Cantoni *et al*., 1983). Denk *et al*. (1977) also showed that NADH and NADPH ferricyanide reductase activities (expressed per mg microsomal protein) were increased by 30% and 90%, as were NADH and NADPH cytochrome c reductase activities (by 150% and 275%). Decreases of 25–40% in CYP (expressed per mg microsomal protein) were seen in hepatic microsomal preparations of hyperplastic nodules that were induced by administration of a diet containing 2.5% griseofulvin for at least 6 months. These nodules also had increased activity of NADH and NADPH-cytochrome c reductase (4-fold and 1.4-fold, respectively), NADPH-ferricyanide reductase (1.5-fold) and stearoyl coenzyme A desaturase (nearly twofold). However, NADPH-supported lipid peroxidation was decreased (58%) (Denk *et al*., 1980). Other studies in which male Swiss mice were given a diet containing 2.5% griseofulvin for 6–8 months followed by a standard diet for an additional 2 months showed induction of liver nodules, and the surrounding tissue had higher 5-aminolaevulinate synthase activity than the nodules (Denk *et al*., 1981). The changes were more marked in control

liver than in the nodules. The authors were further able to show that, with the same dose, route of administration and mouse strain, increased transglutaminase activity occurred from day 14 of treatment, continuing to day 79. The activity returned to normal when the griseofulvin-containing diet was replaced by a normal diet. Neoplastic nodules in the same livers showed similar increases in transglutaminase activity (Denk *et al*., 1984).

Increased 5-aminolaevulinate synthetase (six- to sevenfold) was found when a diet containing 1% griseofulvin was given to mice [strain not specified] for 3 days. Rats receiving the same treatment had more than a twofold increase in the activity of this enzyme within 10 days (De Matteis & Gibbs, 1975).

Sprague-Dawley rats given a diet containing 2.5% griseofulvin for 12 days showed a 40% decrease in CYP, a twofold increase in NADPH-cytochrome *c* reductase, a 50% decrease in NADH-cytochrome *c* reductase, a 56% decrease in aryl hydroxylase and a 56% decrease in benzphetamine demethylase activity (NADPH and NADH), whereas the activity of glutathione *S*-transferase was increased twofold. Complexation of metyrapone with CYP was increased by 40% (Williams & Simonet, 1986). Rats also showed decreased activity of microsomal stearyl coenzyme A desaturase (75%) when given a diet containing 2.5% griseofulvin (Williams & Simonet, 1988).

When expression of multi-drug resistance genes was examined in male Swiss albino mice given griseofulvin at 2.5% in diet for up to 12 weeks, increased P-glycoprotein production was observed until 8 weeks. As treatment progressed, the expression began to decrease, and at 12 weeks complete loss of expression of P-glycoprotein was seen in affected cells. Northern blotting revealed increased expression of *mdr*2 (multi-drug resistant gene 2) and, to a lesser extent, increased *mdr1a* mRNA (Preisegger *et al*., 1996).

Male Swiss albino mice ‘intoxicated with griseofulvin’ (given in feed, amount not specified) showed increased Tau (a microtubule-associated protein) mRNA expression in the liver. At 4.5 months, the expression was 30-fold higher than that in controls. The increased Tau mRNA expression was due to preferential splicing to yield isoform 1. Expression of isoforms 2 and 3 eventually became undetectable. The increase in liver Tau protein did not match the increased mRNA expression. Recovery of Tau splicing patterns occurred within 30 days of withdrawal (Kenner *et al*., 1999).

A diet containing 0.5% griseofulvin was given to dd-Y mice for 2, 4, 6, 8 or 16 days, and the mRNA levels of selected liver, skin and peripheral blood cell enzymes were studied. In the liver, mRNA expression of δ-aminolaevulinic acid synthase and haem oxygenase-1 was increased. Similar increases were reported in peripheral blood cells. The changes in expression of these mRNAs in the skin were not significant. In liver, peripheral blood cells and skin ferrochelatase, mRNA expression remained less affected or unchanged, suggesting that inhibition of ferrochelatase by griseofulvin is post-transcriptional. The expression increased rapidly during the first 4 days, and when treament was stopped, expression began to decline to control levels. Erythrocyte protoporphyrin concentrations had increased by fivefold at 4 days and were 25-fold

higher by 16 days of treatment. When treatment was stopped, the concentrations returned to control values (Inafuku *et al*., 1999).

Genes thought to be important in hepatocellular proliferation were studied to determine their expression after exposure to griseofulvin. Male C3H mice given a diet containing 2.5% griseofulvin for 5 or 14 months had increased expression of c-*fos*, AP-1, NFκB, PPARβ, PPARγ, RARα, RARβ and RARγ. Expression of catalase, AOX, CYP4a1, the activated receptor α (PPARα) and the retinoid X receptor-α and γ (RXR) were down-regulated (Nagao *et al*., 1998).

4.5 Genetic and related effects

The genotoxicity of griseofulvin has been reviewed (Knasmüller *et al.*, 1997).

4.5.1 *Humans*

No data were available to the Working Group.

4.5.2 *Experimental systems* (see Table 1 for references)

Griseofulvin did not induce SOS repair in *Escherichia coli* or a response in the *Bacillus subtilis rec* test, nor did it induce reverse mutation in various *Salmonella typhimurium* strains when tested either in the absence or in the presence of an endogenous metabolic system. Griseofulvin did not induce recombination or mutation in *Saccharomyces cerevisiae*, but induced DNA damage, somatic mutation and mitotic recombination in *Drosophila melanogaster*. It did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro* or gene mutation in mouse lymphoma or Chinese hamster V79 cells. Griseofulvin induced micronucleus formation in a number of rodent cell lines and in human lymphocytes *in vitro*. In a study of micronucleus formation in isolated human lymphocytes, 99% of the micronuclei contained whole chromosomes (kinetochore-positive), indicating an aneuploidic event. In addition, griseofulvin altered the cell cycle of lymphocytes, thereby increasing the percentage of triploid cells. It induced aneuploidy in R3-5 cells *in vitro* and in mouse germ cells *in vivo.* Griseofulvin induced transformation of Syrian hamster embryo cells. It also induced sister chromatid exchange in bone-marrow cells and chromosomal aberrations in spermatocytes of mice treated *in vivo*, but it did not induce micronucleus formation in the bone-marrow cells of mice treated *in vivo*. Griseofulvin induced abnormal sperm morphology in one mouse strain but not in another.

Evidence has been obtained that griseofulvin interacts with the formation of microtubuli (Sloboda *et al*., 1982) and can therefore disturb the correct distribution of chromosomes between daughter cells during cell division (colchicine-like effect) (Sehgal *et al.*, 1990).

Table 1. Genetic and related effects of griseofulvin

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli PQ37, SOS repair test	–	NT	1000 µg/test	Venier *et al.* (1989)
Bacillus subtilis H17/M45, *rec* test	–	NT	100 µg/disc	Ueno & Kubota (1976)
Bacillus subtilis H17/M45, HLL3g/HJ-15, *rec* test	NT	–	NR	Suter & Jaeger (1982)
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–	500 µg/plate	Bruce & Heddle (1979)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	400 µg/plate	Wehner *et al. (*1978)
Salmonella typhimurium TA100, TA1530, TA1532, TA1535, TA1537, TA1538, TA98, TA1950, TA1975, TA1978, G46, reverse mutation	NT	–	500 µg/plate	Léonard *et al. (*1979)
Salmonella typhimurium TA100, TA1535, TA1937, TA98, TA97, reverse mutation	–	–	333 µg/plate	Zeiger *et al.* (1992)
Saccharomyces cerevisiae D61.M, recombination or mutation	–	NT	1600	Albertini *et al*. (1993)
Drosophila melanogaster, DNA damage	+		3000 in feed	Inoue *et al*. (1995)
Drosophila melanogaster, somatic mutation or mitotic recombination	+		3000 in feed	Inoue *et al*. (1995)
Drosophila melanogaster, eye, mitotic recombination	+		35.3 in feed	Rodriguez-Arnaiz & Aranda (1994)
Unscheduled DNA synthesis, primary Fischer 344 rat hepatocytes *in vitro*	–	NT	353	Williams *et al.* (1989)
Gene mutation, mouse lymphoma L5178Y cells, trifluorothymidine resistance *in vitro*	–	NT	150	Stopper *et al. (*1994)
Gene mutation, Chinese hamster V79 cells, 6-thioguanine resistance, forward mutation *in vitro*	–	NT	10	Kinsella (1982)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–	NT	10	Kinsella (1982)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+	+	10	Seelbach *et al.* (1993)
Micronucleus formation, mouse lymphoma L5178 cells *in vitro*	+	NT	12.5	Stopper *et al*. (1994)
Micronucleus formation, Chinese hamster lung V79 cells *in vitro*	+	+	10	Kalweit *et al.* (1999)
Aneuploidy, R3-5 hybrid cell line *in vitro*	+	NT	15	Bourner *et al. (*1998)
Cell transformation, Syrian hamster embryo cells	–	NT	1	Amacher & Zelljadt (1983)
Cell transformation, Syrian hamster embryo cells	+	NT	8	Gibson *et al.* (1995)
Cell transformation, rat 3T3 cells	–[c]	NT	25	Seif (1980)
Micronucleus formation, human lymphocytes *in vitro*[d]	+	NT	5	Kolachana & Smith (1994)
Micronucleus formation, human lymphocytes *in vitro*[d]	+	NT	15.2	Migliore *et al.* (1996)
Gap junction intercellular communication, Chinese hamster V79 cells *in vitro*	–	NT	5.0	Kinsella (1982)
Sister chromatid exchange, Swiss albino mouse bone-marrow cells *in vivo*	+		100 ip × 1	Curry *et al*. (1984)
Micronucleus formation, (C57BL/6×C3H/He)F1 hybrid female mouse bone-marrow cells *in vivo*	–		8000 ip × 5	Bruce & Heddle (1979)
Micronucleus formation, BALB/c mouse bone-marrow cells *in vivo*	–		2000 ip × 1	Léonard *et al.* (1979)
Chromosomal aberrations, BALB/c mouse bone-marrow cells *in vivo*	–		2000 ip × 1	Léonard *et al.* (1979)
Chromosomal aberrations, Swiss mouse spermatocytes *in vivo*	+		500 po × 1	Fahmy & Hassan (1996)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Aneuploidy, ICR mouse oocytes *in vivo*	+		1000 po × 1	Mailhes *et al.* (1993)
Aneuploidy, (102/El×C3H/E1)F_1 hybrid mouse sperm *in vivo*	+		1000 po × 1	Qinghua *et al.* (1999)
Sperm morphology, (C57BL/6×C3H/He)F_1 hybrid mice *in vivo*	+		8000 ip × 5	Bruce & Heddle (1979)
Sperm morphology, BALB/c mice *in vivo*	–		1500 ip × 1	Léonard *et al.* (1979)

NR, not reported

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneal injection; po, oral gavage

[c] A 40-fold stimulation was reported of polyoma-virus A_2-induced cell transformation at a concentration of 2 ng/mL.

[d] Micronuclei were 94–99% kinetochore-positive in these assays.

4.6 Mechanistic considerations

Griseofulvin is reported to alter thyroid hormone homeostasis in rats. The underlying mechanism for this effect is unknown, but it could be related to enzyme induction or to its anti-mitotic activity through tubulin binding. Chronic liver damage associated with porphyria, Mallory body formation, enhanced cell proliferation, liver enlargement and enzyme induction may all contribute to the hepatocarcinogenic effect of griseofulvin in mice.

Griseofulvin can be considered genotoxic by virtue of its ability to induce micronuclei and aneuploidy in rodent cells *in vitro* and *in vivo* and in human cells *in vitro*. It did not induce gene mutation in bacteria or cultured mammalian cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Griseofulvin is an antifungal drug given orally for the treatment of dermatophyte and ringworm infections of the scalp, hair, nails and skin. It is also used as an antifungal agent in veterinary medicine.

5.2 Human carcinogenicity data

Griseofulvin was mentioned in the report of a cohort study designed to screen 215 drugs for carcinogenicity. Although an excess of thyroid cancer was reported among users of griseofulvin in a 9-year follow-up, no results for this drug were reported in a 15-year follow-up, implying that no significant association was observed for cancer at any site.

5.3 Animal carcinogenicity data

Griseofulvin was tested by oral administration in two studies in mice and in one study each in rats and hamsters. It produced hepatocellular adenomas and carcinomas in mice and thyroid follicular-cell adenomas and carcinomas in rats. The incidence of tumours was not increased in hamsters.

5.4 Other relevant data

Griseofulvin induces hepatic enlargement and accumulation of protoporphyrin in mice by inhibiting ferrochelatase. Hepatic porphyria is accompanied by cell damage, necrosis and inflammation. Administration of griseofulvin to mice increased P-glycoprotein in hepatic membranes and resulted in the formation of Mallory bodies. Griseo-

fulvin induced the cytochrome P450 (CYP) 2A5 enzyme concentration in mouse liver. These effects may be related to its hepatocarcinogenic effects. Short-term treatment of rats by gavage caused thyroid gland enlargement, decreased serum thyroxine concentrations and increased serum concentrations of thyroid-stimulating hormone. Griseofulvin binds to tubulin, thereby interfering with the normal polymerization of microtubule protein.

Griseofulvin was teratogenic in rats and cats.

No data were available on the genetic and related effects of griseofulvin in humans. Griseofulvin induced sister chromatid exchange in bone-marrow cells and chromosomal aberration in spermatocytes, but it did not cause micronucleus formation or chromosomal aberrations in bone-marrow cells of mice. It induced aneuploidy *in vivo* and *in vitro* and micronucleus formation in cells *in vitro*. Griseofulvin did not induce recombination or mutation in fungi, but it induced DNA damage and somatic mutation or mitotic recombination in insects. Griseofulvin was not mutagenic and did not induce DNA damage in bacteria.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of griseofulvin.

There is *sufficient evidence* in experimental animals for the carcinogenicity of griseofulvin.

Overall evaluation

Griseofulvin is *possibly carcinogenic to humans (Group 2B)*.

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