CLOFIBRATE

This substance was considered by previous working groups, in February 1980 (IARC, 1980) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

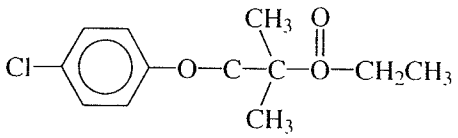
Chem. Abstr. Serv. Reg. No.: 637-07-0

Chem. Abstr. Name: 2-(4-Chlorophenoxy)-2-methylpropanoic acid, ethyl ester

IUPAC Systematic Name: Ethyl 2-(*para*-chlorophenoxy)-2-methylpropionate

Synonyms: *para*-Chlorophenoxyisobutyric acid ethyl ester; 2-(*para*-chlorophenoxy)-2-methylpropionic acid ethyl ester; ethyl *para*-chlorophenoxyisobutyrate; ethyl 2-(*para*-chlorophenoxy)isobutyrate; ethyl 2-(4-chlorophenoxy)isobutyrate; ethyl α-(*para*-chlorophenoxy)isobutyrate; ethyl α-(4-chlorophenoxy)isobutyrate; ethyl α-(*para*-chlorophenoxy)-α-methylpropionate; ethyl α-(4-chlorophenoxy)-α-methylpropionate; ethyl 2-(4-chlorophenoxy)-2-methylpropionate; ethyl clofibrate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}H_{15}ClO_3$ Relative molecular mass: 242.70

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless to pale-yellow liquid (Gennaro, 1995)

(*b*) *Boiling-point*: 158–160 °C (at 25 mm Hg) (Gennaro, 1995)

(*c*) *Density*: 1.138–1.144 at 25 °C (Hassan & Elazzouny, 1982)

(*d*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Hassan & Elazzouny, 1982).

(*e*) *Solubility*: Practically insoluble in water; miscible with acetone, chloroform, diethyl ether and ethanol (Budavari, 1995; Gennaro, 1995)

1.1.4 *Technical products and impurities*

Clofibrate is available as 500-mg capsules which may also contain gelatin, D&C Red 28, D&C Red 30, D&C Yellow 10 (Quinoline Yellow), FD&C Blue 1 (Brilliant Blue FCF), FD&C Red 3 (Erythrosine) or FD&C Yellow 6 (Sunset Yellow FCF) (Thomas, 1991; Medical Economics, 1996).

Trade names and designations of the chemical and its pharmaceutical preparations include: Amotril; Anparton; Apolan; Arterioflexin; Artes; Artevil; Ateculon; Ateriosan; Aterosol; Atheromide; Atheropront; Atrofort; Atrolen; Atromid; Atromid-S; Atromidin; Atrovis; AY 61123; Azionyl; Bioscleran; Cartagyl; Citiflus; Claripex; Claripex CPIB; Cloberat; Clobrat; Clobren SF; Clof; Clofibral; Clofibrat; Clofinit; Clofipront; Clofirem; CPIB; Deliva; ECPIB; EPIB; Estaprol; Geromid; Healthstyle; Hyclorate; ICI 28257; Ipolipid; Klofiran; Levatrom; Lipavil; Lipavlon; Lipilim; Lipomid; Liponorm; Liporan; Liprinal; Lobetrin; Lostat; MG 46; Miscleron; Misclerone; Neo-Atromid; NSC 79389; Normet; Normolipol; Novofibrate; Recolip; Regelan; Sclerovasal; Serotinex; Sklerolip; Skleromexe; Sklero-Tablinen; Ticlobran; Xyduril; Yoclo.

1.1.5 *Analysis*

Methods for the analysis of clofibrate have been reviewed (Hassan & Elazzouny, 1982).

Several international pharmacopoeias specify high-performance liquid chromatography (HPLC) or titration with hydrochloric acid as the assays for purity of clofibrate, and HPLC or gas chromatography with flame ionization detection (GC/FID) for determining impurities and decomposition products. Methods are also specified for determining acid, heavy metal, arsenic and *para*-chlorophenol content. The assays for clofibrate in capsules apply titration with hydrochloric acid, or HPLC or GC/FID methods using standards (Council of Europe, 1984; Society of Japanese Pharmacopoeia, 1992; British Pharmacopoeial Commission, 1993; United States Pharmacopeial Convention, 1994).

1.2 Production and use

1.2.1 *Production*

Clofibric acid was first synthesized in 1947 (Windholz, 1976), but the ethyl ester, clofibrate, was not reported until 1961 (Budavari, 1995). Clofibrate is prepared by condensing phenol with ethyl 2-chloro-2-methylpropionate in the presence of a suitable dehydrochlorinating agent and then chlorinating the aromatic ring (Gennaro, 1995).

1.2.2 *Use*

The efficacy of clofibrate in reducing serum cholesterol levels was first reported in 1962 (Thorp & Waring, 1962). Clofibrate was first marketed in the United States of America in 1967 (Wysowski *et al.*, 1990).

Clofibrate is used as a hypolipidaemic drug. It reduces elevated plasma concentrations of triglycerides by reduction of elevated concentrations of very low-density lipoproteins (VLDLs) within two to five days after initiation of therapy. It is less effective in reducing low-density lipoprotein (LDL) cholesterol and the plasma concentration of total cholesterol. It is mostly effective in the treatment of type III hyperlipoproteinaemia. It may also be helpful in some patients with type IIb, type IV or type V hyperlipoproteinaemia (see Glossary, p. 448) (Goodman Gilman *et al.*, 1990; Reynolds, 1993; Larsen *et al.*, 1994).

The usual daily dose is 2 g (20–30 mg/kg bw per day) taken orally in two or three divided doses (Reynolds, 1993; Vidal, 1995).

The cellular mechanisms responsible for the hypolipidaemic effects of fibrate drugs have not been clarified fully but include: activation of lipoprotein lipase, suppression of free fatty acid release from adipose tissue, inhibition of hepatic triglyceride synthesis and increased secretion of cholesterol (see IARC, 1983) into bile. Therapy with clofibrate does not significantly reduce the rate of synthesis of VLDL triglycerides, but such treatment is associated with an increase in the rate of catabolism of VLDL particles (Larsen *et al.*, 1994). The mobilization of deposits of cholesterol in tissues is accompanied by regression and disappearance of xanthomas (Goodman Gilman *et al.*, 1990).

Clofibrate has been used in the prophylaxis of ischaemic heart diseases but it is no longer recommended for this purpose, because of adverse effects seen during long-term treatment: increased incidences of cholecystitis, gallstones and in some cases of certain cardiovascular disorders and excess deaths found in the WHO Cooperative Trial on the use of clofibrate in the primary prevention of ischaemic heart disease (Reynolds, 1993). Some patients have also shown a paradoxical rise in LDL (Goodman Gilman *et al.*, 1990).

Clofibrate has been used in the treatment of neonatal jaundice (Gabilan *et al.*, 1990; Erkul *et al.*, 1991; Gabilan *et al.*, 1991).

Following the report of a WHO-sponsored cooperative study of the use of clofibrate in the primary prevention of ischaemic heart disease (Committee of Principal Investigators, 1978), it was withdrawn in the Federal Republic of Germany and Norway in early 1979. In a number of other countries, including France, Italy, Sweden, Switzerland, the United Kingdom and the United States, practitioners were advised to reserve its use for patients with high plasma lipid concentrations that are refractory to dietary measures and to consider carefully the risks and benefits of the treatment (United States Food and Drug Administration, 1979; WHO, 1979a; Expert Panel, 1988). It was reintroduced in the Federal Republic of Germany in August 1979 (WHO, 1979b). In the United Kingdom, clofibrate is now rarely prescribed (Dunnigan, 1992).

In the United States, clofibrate represented 80.9% of the cholesterol-lowering medications used in 1978, 41.2% in 1983 and 3.5% in 1988. Gemfibrozil (see monograph, pp. 428–429), lovastatin and cholestyramine are now used more commonly (Wysowski *et al.*, 1990).

1.3 Occurrence

Clofibrate is not known to occur as a natural product.

No quantitative data on occupational exposure levels were available to the Working Group.

The National Occupational Exposure Survey conducted between 1981 and 1983 in the United States by the National Institute for Occupational Safety and Health indicated that about 325 employees were potentially occupationally exposed to clofibrate. The estimate was based on a survey of companies and did not involve measurements of actual exposure (United States National Library of Medicine, 1996).

1.4 Regulations and guidelines

Clofibrate is listed in the following pharmacopoeias: British, Brazilian, Chinese, Czech, Egyptian, European, French, Greek, Hungarian, Indian, Italian, Japanese, Netherlands, Nordic, Portuguese, Romanian, Swiss and United States (Reynolds, 1993).

2. Studies of Cancer in Humans

2.1 Case–control study

A population-based case–control study in Kansas, United States, investigated a large number of possible risk factors for soft-tissue sarcoma (Hoar Zahm *et al.*, 1989). One of the factors examined was medical treatment with cholesterol-lowering drugs (among which was clofibrate). Among white males, aged 21 years or older, a total of 139 newly diagnosed (1976–82) and histologically confirmed cases of soft-tissue sarcomas were identified through the University of Kansas Cancer Data Service (50% deceased). Deceased cases were not excluded from the study. Three controls were matched to each case by age and vital status. For living cases, controls were selected either from the Health Care Financing Administration files or by telephone random digit dialling. For deceased cases, controls were selected from Kansas state mortality files. Exposure information was obtained from interviews with study subjects or with their next-of-kin. The response rate was 93%. The distribution of proxy type was similar among the cases and controls. Among users of cholesterol-lowering drugs (5 cases and 20 controls), a nonsignificant excess of soft-tissue sarcoma was seen (odds ratio, 1.7; 95% confidence interval (CI), 0.5–5.0). The increased risk was found only among deceased subjects (odds ratio, 1.9; 95% CI, 0.5–6.4; 4 cases, 15 controls). [The Working Group noted that no adjustment was made for confounders, that all medical data, such as on use of cholesterol-lowering drugs, were self- or proxy-reported and that the inclusion of deceased controls may have overrepresented the prevalence of their use.]

2.2 Clinical trials

A randomized trial of the World Health Organization, started in 1965 to determine whether clofibrate would lower the incidence of ischaemic heart disease in men, raised concern over a nonsignificant excess of cancer deaths in treated subjects (58 versus 42 in placebo-treated controls) (Committee of Principal Investigators, 1978, 1980; IARC, 1980). The greatest excesses were for cancers of the gastrointestinal and respiratory tracts. Results of a further four years of follow-up of this trial to the end of 1982 subsequently became available (Committee of Principal Investigators, 1984). On average, the total follow-up period was 13.2 years, 5.3 of which were during the actual treatment phase (range, four to eight years) and 7.9 thereafter. Three groups of men, divided according to their cholesterol level, were studied, comprising 208 000 man–years of observation. The first two groups included subjects in the upper third of the serum cholesterol distribution, randomly allocated either to treatment with clofibrate (1.6 g daily) or to receive an olive oil-containing placebo. The third group was composed of half of the men in the lowest third of the distribution, who received an olive oil-containing placebo. At the conclusion of follow-up, the age-standardized rates of death from malignant neoplasms per 1000 per annum were 2.4, 2.4 and 2.3, respectively (based on 206, 197 and 173 deaths from malignant neoplasms). However, the age-standardized death rates for malignant neoplasms during the treatment phase had been 2.0 (42 deaths), 1.2 (25 deaths) and 1.7 (30 deaths), respectively.

The Coronary Drug Project, a randomized and double blind trial in the United States and Puerto Rico, started in 1966, investigated the effects of lipid-lowering drugs on 8341 men, aged 30–64 years with a history of myocardial infarction. The first results, with a mean follow-up of 6.2 years study (5–8.5 years), showed no increase in the cancer death rate in the clofibrate (1.8 g/day)-treated group (10 deaths in 1103 patients) compared with that of a placebo-treated group (24 deaths in 2789 patients) (Coronary Drug Project Research Group, 1975). After a mean follow-up of 15 years (including 8.8 years after termination of the trial), with definite information about vital status for 98.9% of subjects, the clofibrate group had somewhat lower cancer mortality (3.4%) than did the placebo group (4.4%). This was also the case for lung cancer (13 deaths in 1103 clofibrate-treated men (12/1000) and 53 deaths in 2789 placebo-treated men (19/1000)) and for cancer of the gastrointestinal tract (4/1000 versus 6/1000) (Canner *et al.*, 1986).

In the Stockholm Ischaemic Heart Disease study (Carlson & Rosenhamer, 1988), 555 patients with ischaemic heart disease, under 70 years of age, were treated with clofibrate and nicotinic acid ($n = 279$) or with a placebo ($n = 276$) (not blind). Because of cancer, 10 subjects among the treated group and 6 among the controls withdrew from the trial. The numbers of cancer deaths during the five years of treatment were four in the treatment group and six in the control group.

Recently, Law *et al.* (1994) conducted a meta-analysis to assess whether low serum cholesterol concentration increases mortality from causes other than ischaemic heart disease. The data were derived from the 10 largest cohort studies, two international studies and 28 randomized trials, supplemented by unpublished data. Only the trials provided information about cholesterol-lowering drugs. Extended observation after the

trial period had ended was available for six of the trials and provided information on the risk for cancer 5–10 years after treatment with cholesterol-lowering drugs (about 15 years after the start of treatment). The overall relative odds estimate of the risk for cancer was 0.9 (95% CI, 0.7–1.1; based on 232 treated patients. The meta-analysis did not provide estimates of relative risk for cancer mortality for the clofibrate trial separately. [The Working Group noted that the numbers of cancer deaths provided cannot be compared directly because of differences in survival between clofibrate-treated subjects and those who did not receive the drug.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

In a study reported as a summary in a monograph, groups of 25 male and 25 female Alderley Park mice [age not specified] were given 0 (control), 1000 (therapeutic level in humans), 2500 or 5000 (maximum tolerated dose, MTD) mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 18 months. Major organs and abnormalities were examined histologically. Mortality was similar in all groups. No difference in the incidence of any tumour type between control and treated groups was reported (Tucker & Orton, 1995).

In another study reported as a summary in a monograph, groups of 51 male and 51 female C57Bl/10J mice, six weeks of age, were given clofibrate [purity not specified] at daily dose levels of 150, 250 and 350 mg/kg bw in the diet for 18 months. The untreated control groups comprised 151 males and 151 females. There was body-weight reduction of about 10% the higher-dose group. Mortality was similar in all groups (70–80% survival at 18 months). There was a significant increase in liver weights in treated males and females in the two higher-dose groups. Full necropsy and histological examinations were carried out on all animals. No difference in tumour incidence between treated and control groups was reported (Tucker & Orton, 1995).

3.1.2 *Rat*

A group of 15 male Fischer 344 rats, weighing 84–100 g [age not specified], was given 0.5% (v/w) clofibrate [purity not specified; equivalent to about 250 mg/kg bw per day] in the diet for up to 28 months. A group of 15 untreated males served as controls. Of the treated animals, one rat was killed at 13 months and three more between 17 and 21 months. The remaining 11 rats were killed between 24 and 28 months. One or more hepatocellular carcinoma developed in 10/11 rats compared with 0/14 controls which survived to 28 months ($p < 0.001$); five of the animals with hepatocellular carcinomas showed pulmonary metastases. In addition, among the treated animals, pancreatic exocrine acinar carcinomas were found in 2/11 rats, whereas none was found in controls (Reddy & Qureshi, 1979). [The Working Group noted the small number of animals.]

A group of 25 male weanling Fischer 344 rats, weighing approximately 100 g, was fed 5000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 72 (when the first tumour appeared)–97 weeks (total intake, 25–33 g/rat). A group of 25 untreated males served as controls. The study was terminated at 129 weeks, when all surviving animals were killed. Among the treated rats, malignant tumours developed at various sites. Hepatocellular carcinomas were found in 4/25 treated rats; among the other tumours observed in treated rats were a pancreatic exocrine acinar carcinoma in one rat and pancreatic exocrine acinar adenomas in three rats. Treated and control rats developed similar numbers of leukaemias and tumours of the testis (Svoboda & Azarnoff, 1979).

Groups of 70 (control) and 74 (continuous treatment) male Sprague-Dawley rats, seven weeks of age, were given 0 or 400 mg/kg bw clofibrate [purity not specified] daily in the diet for up to 113 weeks. A third group of 28 (recovery group) male Sprague-Dawley rats was given 400 mg/kg bw clofibrate in the diet for 42–95 weeks and then held for a further 16–18 weeks before killing. Three to five rats in each group were sacrificed at 4–10-week intervals beginning at week 4 and ending at week 113, when zero to five animals remained per group. Only the liver and abnormal organs were examined histologically. Hyperplastic (neoplastic) nodules did not occur before week 68 of treatment. In rats treated for 68 weeks or longer, the incidence of hyperplastic (neoplastic) nodules was: controls, 0/36; continuous treatment, 19/36; and recovery group, 1/16. The only hepatocellular carcinomas found were in two rats in the continuous treatment group at week 95 (Greaves *et al.*, 1986).

3.1.3 *Marmoset*

In a study reported as a summary in a monograph, groups of 11–16 male and 11–16 female marmosets were given clofibrate [purity not specified] in water containing 0.5% w/w polysorbate 80 by gastric instillation at intended dose levels of 100, 150, 250 and 300 mg/kg bw per day. Effective doses were 94, 157, 213 and 263 mg/kg bw per day. Groups of 20 males and 20 females were untreated or treated with the vehicle only. The study was terminated after 6.5 years due to premature deaths. Necropsy was performed and all major organs were examined histologically. Causes of death varied and were not related to clofibrate treatment. There was no effect on liver weight in any of the clofibrate-treated groups. A statistically significant ($p < 0.01$) increase in kidney weight was seen in the higher-dose groups. There were no histological changes in the liver that could be attributed to clofibrate. No liver tumour or other treatment-related tumour was found in clofibrate-treated marmosets (Tucker & Orton, 1995).

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Groups of 7–20 male C3H/Hen, C57Bl/6N and BALB/cA mice, six weeks of age, were subjected to partial hepatectomy. After 24 h, mice were given 20 mg/kg bw *N*-nitrosodiethylamine (NDEA) by intraperitoneal injection. Six hours later, they were given basal diet or a diet containing 1000 mg/kg diet (ppm) clofibrate [purity not specified] until week 20, at which time the experiment was terminated and all surviving

animals were killed. Livers were analysed for the number and size distribution of glucose-6-phosphatase-deficient enzyme-altered islands by a stereological method (for statistical comparisons, Welch's test was used). NDEA alone induced many more and larger enzyme-altered islands in C3H mice than in the other two strains. In C3H mice, administration of clofibrate in addition to NDEA increased the number and volume of enzyme-altered islands. In the other two strains, clofibrate had no enhancing effect (Lee *et al.*, 1989). [The Working Group noted the absence of a group given clofibrate alone.]

3.2.2 *Rat*

Groups of 30 male Fischer rats weighing 135–150 g [age not specified] were given 100 mg/L (ppm) NDEA in the drinking-water for two weeks. One week later the rats were fed 0 or 5000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 48 weeks, at which time the experiment was terminated. Clofibrate significantly ($p < 0.001$) enhanced the development of liver tumours in rats previously exposed to NDEA: 25/28 rats given NDEA plus clofibrate had liver-cell tumours [type not specified] versus 5/18 (three hepatocellular carcinomas) in the group given NDEA alone (Reddy & Rao, 1978). [The Working Group noted the absence of a group given clofibrate alone.]

A group of 54 female rats [strain and age not specified] was treated twice with 50 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) in citrate buffer (pH 6.0) intravenously at one-week intervals. Twenty-six treated rats were given 20.8 mg/day clofibrate in milk [route and volume not specified] on five days per week for one year. The remaining 28 animals received milk only. One year after the first injection of MNU, all animals were killed. Complete necropsy and histological examination were performed. The authors reported that clofibrate had no effect on the incidence of tumours induced by MNU (Anisimov *et al.*, 1981).

A group of 68 female rats [strain and age not specified] was treated intravenously with 1.5 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) in water/lipid emulsion three times at one-week intervals. Thirty-six rats were then given 20.8 mg/day per rat clofibrate in milk [route and volume not specified] five days per week for one year at which time the experiment was terminated. The remaining 32 DMBA-treated animals were given milk only. One year after the first DMBA injection, animals were killed and complete necropsy and histological examination were performed. There was a decreased incidence (1.8 times lower) and a decreased multiplicity of mammary adenocarcinoma in clofibrate-treated animals compared with the animals treated with DMBA alone (1.21 versus 1.77, respectively). There was no difference in the incidence of other tumour types between the two groups (Anisimov *et al.*, 1981).

Three groups of 13–17 male rats [strain and age not specified], weighing 200–220 g, were given 14 mg/kg bw dimethylhydrazine dihydrochloride (DMH) weekly by subcutaneous injection for 20 weeks. Two of these groups were given 25 mg/animal clofibrate [purity not specified] by gastric instillation on five days per week either beginning 10 days before or concomitantly with DMH treatment, and the third group was given water. All rats were killed 25 weeks after the start of DMH treatment. The incidence of intestinal tumours was 100% in all three groups. There was no difference in the

number of intestinal tumours per animal. The mean volume of intestinal tumours was significantly smaller in groups treated with DMH and clofibrate compared with the group treated with DMH alone ($p < 0.05$, Student's t test). The percentage of tumours without invasion was considerably higher in animals that began clofibrate treatment before DMH treatment than in those only treated concomitantly or given no clofibrate (Berstein *et al.*, 1982). [The Working Group noted that the strong carcinogenic effect of DMH in all groups may have precluded the detection of modulating effects.]

Groups of 15–25 male Fischer 344 rats, weighing 80–90 g [age not specified], were given 40 mg/L (ppm) NDEA in the drinking-water for five weeks (total dose, 32 mg/rat). One week later, the rats were given 0, 1000, 2500, 5000 or 10 000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 19 weeks. At the end of clofibrate treatment, all surviving animals were killed. Body-weight gain was depressed, especially in the two highest-dose groups. Livers were fixed and sliced at 2 mm intervals and tumours larger than 1 mm in diameter were counted visually. At the lower-dose levels, clofibrate significantly increased the multiplicity of liver tumours initiated by NDEA: 12.5 ± 5.7 (0% clofibrate, 20/20 survivors), 22.2 ± 15.1 ($p < 0.025$, Student's t test) (1000 ppm clofibrate, 13/15 survivors) and 19.1 ± 8.3 ($p < 0.005$) (2500 ppm clofibrate, 23/25 survivors); 5000 ppm clofibrate had no effect (12.0 ± 4.6, 11/15 survivors) and 10 000 ppm clofibrate significantly decreased ($p < 0.05$) the multiplicity of liver tumours (7.8 ± 5.3, 17/20 survivors) (Mochizuki *et al.*, 1982). [The Working Group noted the absence of a group given clofibrate alone and that the counting technique used is susceptible to multiple counting of large lesions.]

Three groups of 10 male Fischer 344 rats, weighing 90–100 g [age not specified], were concomitantly given 40 mg/L (ppm) NDEA in the drinking-water and fed 0, 1000 or 2500 mg/kg diet (ppm) clofibrate [purity not specified] for five weeks. The total intake of NDEA was 31, 26.5 and 25.9 mg/rat, respectively, in the three groups. All rats survived and were killed 25 weeks after the start of the experiment. Livers were fixed and sliced at 2 mm intervals and tumours larger than 1 mm in diameter were counted visually. Clofibrate significantly increased the multiplicity of liver tumours initiated by NDEA: 12.4 ± 5.4 (0% clofibrate), 25.3 ± 14.1 ($p < 0.025$, Student's t test) (1000 ppm clofibrate) and 22.6 ± 8.7 ($p < 0.01$) (2500 ppm clofibrate) (Mochizuki *et al.*, 1983). [The Working Group noted the absence of a group given clofibrate alone and that the counting technique used is susceptible to multiple counting of large lesions.]

Two groups of 15 male Fischer 344 rats, eight weeks of age, were given basal diet containing 200 mg/kg diet (ppm) 2-acetylaminofluorene (2-AAF) for eight weeks, then maintained on basal diet for a further two weeks, after which the animals received 730 mg/kg diet (ppm) clofibrate [purity not specified] for 24 weeks or the basal diet. Another two groups of 9 or 12 rats were given basal diet without 2-AAF for 10 weeks and then given 730 ppm clofibrate in the diet for 24 weeks or the basal diet. Some animals from each group were killed at six weeks after the start of clofibrate treatment and the remainder were killed after 24 weeks of clofibrate treatment. The livers were analysed for altered foci and neoplasms. Clofibrate slightly enhanced the incidence of 2-AAF-induced foci at week 34. The incidence of foci/cm^2 was 5.4 ± 1.5 in animals given 2-AAF and clofibrate and 4.3 ± 2.2 in those given 2-AAF only. No significant

increase in the incidence of liver neoplasms (nodules) was observed in the clofibrate-treated group (2/9 versus 0/9 in controls) (Numoto *et al.*, 1984)

Groups of 20 male Fischer 344 rats, five weeks of age, were given 0 or 500 mg/kg diet (ppm) *N*-nitrosoethylhydroxyethylamine in the diet for two weeks, followed by 3500 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 24 weeks. All animals survived to the end of the experiment at week 27 when they were killed. Clofibrate did not increase the incidence or multiplicity of renal tubular-cell adenomas and adenocarcinomas (Kurokawa *et al.*, 1988).

Groups of 60–70 male Fischer 344 rats, four weeks of age, were given 0 or 200 mg/kg bw NDEA as a single intraperitoneal injection in physiological saline. Two weeks later, the animals were fed diets containing 0 or 3000 mg/kg diet (ppm) clofibrate [purity not specified] for up to 64 weeks. All animals were subjected to partial hepatectomy at week 3. At weeks 8, 20, 32, 49 and 64, 7–22 rats were killed from the various groups. Clofibrate alone did not induce hepatocellular carcinomas and only a few, small preneoplastic foci were observed at the end of the study. However, in animals treated with NDEA, clofibrate increased the total number of glutathione *S*-transferase placental form (GST-P)-positive and -negative preneoplastic lesions from week 32 onward ($p < 0.05$, Student's *t* test) and the incidence of hepatocellular carcinomas: 12/26 (NDEA plus 3000 ppm clofibrate) versus 4/17 (NDEA alone) (Hosokawa *et al.*, 1989).

Groups of 15 male Fischer 344 rats, six weeks of age, were given 0 or 500 mg/L (ppm) *N*-nitrosobutyl(4-hydroxybutyl)amine (NBHBA) in the drinking-water for four weeks. Subsequently, rats were given 2500, 5000 or 10 000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for four weeks, followed by a three-week interval during which they were fed 30 000 mg/kg (ppm) uracil in the diet. The clofibrate treatment was then resumed for a further nine weeks. A further group of animals treated with NBHBA and uracil only served as controls. The experiment was terminated at 20 weeks. The incidence of urinary bladder hyperplasias and papillomas in control animals (NBHBA only) and in animals treated with NBHBA and clofibrate was similar. The density of hyperplasias (number of lesions/10 cm basement membrane) was significantly increased ($p < 0.01$, Student's *t* test) in all clofibrate-treated groups (Hagiwara *et al.*, 1990).

Groups of male Fischer 344 rats [exact numbers not specified], seven weeks of age, were given 0 or 3000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 30 weeks, followed by a basal diet or a diet containing 100 ppm 2-AAF for up to 78 weeks. Three weeks after the start of the experiment, partial hepatectomy was performed on all animals. Five rats fed clofibrate were killed at week 30 and three to seven rats from each group were killed at week 48; all surviving animals were killed at 78 weeks. The authors reported that clofibrate inhibited the development of GST-P-positive focal lesions and hepatocellular carcinomas induced by subsequent feeding of 2-AAF (Mutai *et al.*, 1990). [The Working Group noted the unusual design.]

Groups of 13–14 male Nagase analbuminaemic and 13–14 Sprague-Dawley rats, seven weeks of age, were given 200 mg/kg bw NDEA as a single intraperitoneal injection. Two weeks later, the rats were given 0 or 10 000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for six weeks. Four Nagase analbuminaemic and five

Sprague-Dawley rats were given the diet containing clofibrate without prior treatment with NDEA. Three weeks after the start of the experiment, partial hepatectomy was performed on all animals. The rats were killed at week 8. No GST-P-positive foci were found in the animals fed clofibrate without prior treatment with NDEA. NDEA alone induced significantly more and larger GST-P-positive foci in Nagase analbuminaemic rats than in Sprague-Dawley rats ($p < 0.001$, Student's t test). Clofibrate significantly decreased the number of GST-P-positive foci induced by NDEA in both strains ($p < 0.002$) (de Camargo *et al.*, 1993). [The Working Group noted that some studies suggest that peroxisome proliferators inhibit the histochemical detection of foci.]

Groups of 7–10 male Fischer 344 rats, 12 weeks of age, were given 150 mg/kg bw NDEA as a single intraperitoneal injection or were given 200 mg/kg diet (ppm) 2-AAF in the diet for eight weeks or were untreated. Two weeks later, rats were fed 1000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for up to 37 weeks or received no further treatment. Clofibrate increased the incidence of hepatocellular adenomas following treatment with NDEA (4/8 versus 0/8; $p < 0.05$, Fisher's exact test), but not after treatment with 2-AAF (Cattley *et al.*, 1994).

Groups of four male Sprague-Dawley rats, seven weeks of age, were given 200 mg/kg bw NDEA as a single intraperitoneal injection. After two weeks on basal diet, they were given 200 mg/kg diet (ppm) 2-AAF in the diet for two weeks and were subjected to partial hepatectomy at week 3. Subsequently, two groups of animals were given 3000 mg/kg diet (ppm) clofibrate [purity not specified] for two or four weeks. Another two groups were given basal diet for either two or four weeks. The numbers and areas of GST-P-positive foci of diameter greater than 0.2 mm were measured. Administration of clofibrate for two or four weeks significantly reduced the number and areas of GST-P-positive foci (Yokoyama *et al.*, 1993). [The Working Group noted that some studies have suggested that peroxisome proliferators inhibit the histochemical detection of foci.]

3.2.3 *Hamster*

Groups of 16–22 male Syrian golden hamsters, six weeks of age, were given 500 mg/kg bw *N*-nitrosobis(2-hydroxypropyl)amine (NBHPA) or 0.9% NaCl by subcutaneous injection weekly for five weeks, after which they were given 0, 2500 or 5000 mg/kg diet (ppm) clofibrate [purity not specified] in the diet for 30 weeks, at which time the experiment was terminated. Clofibrate significantly ($p < 0.001$) increased the multiplicity of hepatocellular lesions (including hyperplastic nodules and hepatocellular carcinomas) as measured by the number of lesions/cm^2 in histological sections: 0.5 ± 0.3 (NBHPA alone, 17 hamsters), 1.4 ± 0.5 (NBHPA plus 2500 ppm clofibrate, 16 hamsters) and 1.0 ± 0.3 (NBHPA plus 5000 ppm clofibrate, 17 hamsters). In contrast, 5000 ppm clofibrate significantly ($p < 0.05$) inhibited the development of pancreatic adenocarcinomas and lung neoplasms induced by NBHPA (Mizumoto *et al.*, 1988).

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

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 *Humans*

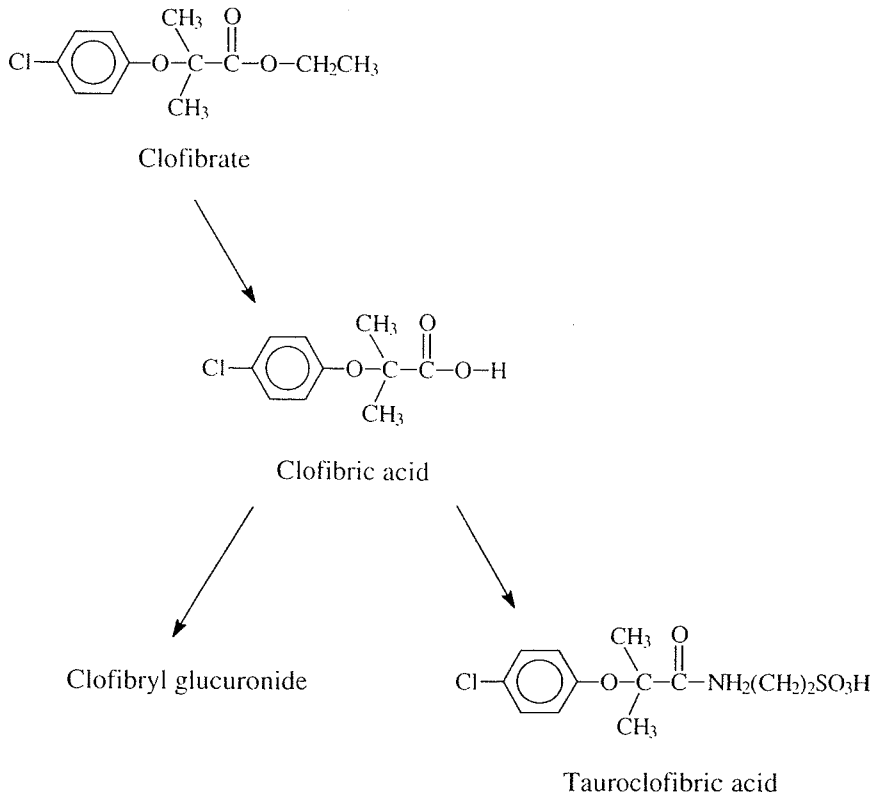
Absorption of clofibrate is typically monitored as circulating clofibric acid (2-(4-chlorophenoxy)isobutyric acid), since the ethyl ester is rapidly hydrolysed by tissue and serum esterases, both *in vivo* and *in vitro*, to the acid (Thorp, 1962). Gugler and Hartlapp (1978) evaluated plasma levels of clofibric acid following single and repeated doses of clofibrate in human volunteers (four men and one woman). Single oral doses of 500–2000 mg [7–28 mg/kg bw est.] resulted in mean peak plasma concentrations of 53–151 μg/mL, that were observed 4–6 h after dosing. At doses of 1000 mg [14 mg/kg bw est.] given twice daily for eight days, peak plasma concentrations of clofibric acid ranged between 200 and 240 μg/mL on the last day. Elimination appeared to be similar for a single dose and for multiple dosing regimens, with mean half-lives of 15–18 h following single doses of 500–2000 mg and 1000 mg twice daily for eight days. Similar plasma levels of clofibric acid were observed in seven male volunteers receiving a single oral administration of 1.3 g [19 mg/kg bw est.] clofibrate (Harvengt & Desager, 1976) and in four men and six women after single oral dosing of 500 mg [7 mg/kg bw est.] clofibrate (Männistö *et al.*, 1975). Cayen *et al.* (1977) reported protein binding levels of 98.5% and 96.8% at serum concentrations of 10 and 100 μg/mL clofibric acid, respectively.

Clofibric acid can undergo conjugation with glucuronic acid in man (Thorp, 1962). The metabolism and elimination of clofibrate and clofibric acid in three and five male subjects, respectively, were described by Emudianughe *et al.* (1983). Elimination was mainly via the urine, with 48-h recoveries of 56% (clofibrate, 565 mg oral dose) [8 mg/kg bw est.] and 80% (clofibric acid, 500 mg oral dose) [7 mg/kg bw est.]. The principal urinary metabolite was clofibryl glucuronide, with only approximately 2% of the dose excreted as clofibric acid. In four male and one female subjects, the total plasma clearance of clofibric acid was greater (6.8 mL/min) following a single oral dose of 2000 mg [29 mg/kg bw est.] than following a single oral dose of 500 mg [7 mg/kg bw est.] (5.6 mL/min) (Gugler & Hartlapp, 1978). The more rapid elimination following the higher dose of clofibrate was attributed to reduced plasma protein binding at higher plasma concentrations (see Figure 1).

4.1.2 *Experimental systems*

The absorption and distribution of orally administered clofibrate in rats appear to be similar to those in humans. Cayen *et al.* (1977) studied the serum levels of radioactivity in male albino Charles River rats given a single oral dose of 0.3 mmol/kg [73 mg/kg] bw [^{14}C]clofibrate or 0.3 mmol/kg [64 mg/kg] bw [^{14}C]clofibric acid. Peak serum concentrations of clofibric acid equivalents between 500 and 1000 nmol/mL (approximately 100–200 μg/mL) were similar to those in humans following single oral doses of

Figure 1. Postulated metabolic pathways of clofibrate



From Emudianughe *et al.* (1983)
Clofibryl glucuronide is the only conjugated form observed in rat, guinea pig, rabbit and man. In the dog and ferret, tauroclofibric acid is also formed, which in cat, was the only conjugate excreted.

clofibrate. Analysis of serum in rats given oral doses of [^{14}C]clofibrate indicated that over 90% of the drug was in the form of unconjugated clofibric acid, consistent with results after oral administration to humans. Groups of five male and five female rats aged three weeks and similar groups aged eight weeks were given 100 or 250 mg/kg bw clofibrate per day by gastric instillation for 16 days (Tucker & Orton, 1995). Blood concentrations of clofibric acid 4 h after the final dose (Table 1) were higher in the older rats than in the weanlings, possibly because of differences in esterase activity. The lack of an intravenous formulation of clofibrate precludes total plasma clearance determinations.

Table 1. Blood clofibric acid (μg/mL) concentrations in rats after clofibrate administration

Dose (mg/kg)	Three-week-old rats		Eight-week-old rats	
	Male	Female	Male	Female
100	158 ± 41	98 ± 56	328 ± 42	279 ± 81
250	301 ± 55	170 ± 96	509 ± 110	336 ± 29

From Tucker & Orton, 1995

Following a single intraperitoneal injection to rats of 113 mg/kg bw [^{14}C]clofibrate, 85% of the ^{14}C dose was recovered in the urine within 24 h of administration (70% as clofibryl glucuronide and 15% as clofibric acid) (Emudianughe *et al.*, 1983). In comparison with the same authors' study in humans, a higher dose rate on a mg/kg basis was administered to rats and a different route of administration used. A higher percentage of the dose appeared as unconjugated clofibric acid in the urine of rats than that seen with humans. Odum and Orton (1983) measured hepatic microsomal glucuronyl transferase activity towards clofibric acid in male Alderley Park strain rats, noting an increase in enzyme activity associated with postnatal maturation. Feeding rats with 4000 mg/kg diet (ppm) clofibrate for 14 days did not appear to increase the activity of glucuronyl transferase towards clofibric acid. Bronfman *et al.* (1986) characterized the activity of microsomal fractions from male Sprague-Dawley rats with respect to formation of coenzyme A (CoA) thioesters of clofibric acid *in vitro*. It has been speculated that formation of CoA thioesters of clofibric acid *in vivo* may mediate the pharmacological or toxic effects of clofibrate (Tomaszewski & Melnick, 1994).

Baldwin *et al.* (1980) measured the distribution of ^{14}C following administration of 0.4 mmol/kg bw [^{14}C]clofibrate [97 mg/kg] given either as a single intragastric or intraperitoneal dose (acute) or twice daily for 14 days (chronic) in rats. The units reported were 10^{-5} mmol clofibric acid equivalents per gram of tissue (CFE). Twelve hours after the last dose, levels of radioactivity were similar in liver (6 CFE) and a variety of other tissues for both dosing regimens. Notable exceptions were blood (14 CFE acute versus 7 CFE chronic) and epididymal fat (3 CFE acute versus 11 CFE chronic).

Differences in rates of elimination between humans and rats for clofibric acid have been observed. Baldwin *et al.* (1980) calculated a half-life of 4.1 h in rats, based on elimination of ^{14}C-labelled clofibrate in male Harlan Sprague-Dawley rats given a single dose of 97 mg/kg bw by the intraperitoneal or oral route. The authors attributed the higher elimination rate in rats to the differences in serum protein binding reported by Cayen *et al.* (1977) who found that at concentrations of 10 and 100 µg/mL clofibric acid, the proportions of protein binding in rat serum were 87.2% and 75.4%, respectively, lower than that reported for human serum (see Section 4.1.1).

4.2 Toxic effects

Because clofibrate is readily metabolized to clofibric acid in humans and animals, the toxic effects of clofibric acid are summarized together with those of clofibrate in this section.

4.2.1 *Humans*

Several studies have documented the pharmacological reduction in plasma levels of serum triglycerides and cholesterol in humans treated with clofibrate. Larsen *et al.* (1994) reported the effects of oral clofibrate in 12 human patients with hyperlipoproteinaemia type III treated with clofibrate for eight weeks [1 mg/kg bw est.]. Reductions in circulating total cholesterol (approx. 40%), in VLDL cholesterol (approx. 60%) and triglycerides (approx. 50%) were observed, as was an increase in circulating high-density

lipoprotein cholesterol (approx. 9%). The mechanism of this pharmacological effect is unclear.

Various adverse effects have been attributed to the administration of clofibrate. The most common is cholelithiasis. Bateson *et al.* (1978) found a strong association between clofibrate therapy and gallstones in patients with hyperlipidaemia (the prevalence of gall-bladder disease was about four times that expected, $p < 0.001$). This effect was associated with the elevated cholesterol concentration of the bile in the clofibrate-treated patients. [The route of exposure was probably oral, but the dose levels and duration of administration were not specified.]

An association of clofibrate therapy with skeletal myopathy has been described in several case reports, summarized in reviews by Rush *et al.* (1986) and London *et al.* (1991). Exposures ranged from 750 to 4000 mg [11–57 mg/kg bw est.] per day and duration of treatment from 3 to 730 days (mean, 56 days). Clofibrate-associated myopathy was characterized by muscle pain, elevated levels of leakage enzymes such as creatine phosphokinase, aspartate aminotransferase and lactate dehydrogenase, and muscle weakness. In some cases, cardiac myopathy accompanied the effect on skeletal muscle (McGarvey, 1973; Smals *et al.*, 1977; Scionti *et al.*, 1984). Single cases of association between clofibrate treatment and eosinophilic pneumonia (Hendrickson & Simpson, 1982), erythema multiforme (Murata *et al.*, 1988) and interstitial nephritis (Cumming, 1980) have been reported.

Because of effects noted in rodents, the effects of clofibrate and clofibric acid on human hepatocytes have been studied following in-vivo and in-vitro exposures. Schwandt *et al.* (1978) studied liver biopsies from 40 patients before and after administration of clofibrate (1.5 g/day in 27 patients and 0.5 g/day in 13 patients). A tendency to decreased fatty infiltration was the only effect noted by light microscopy. Hanefeld *et al.* (1980) also described a regression of fatty infiltration after 3–5 months of clofibrate treatment (2 g/day) [29 mg/kg bw est.]. Ultrastructural evaluation revealed an increase in smooth endoplasmic reticulum as well as increased inner membranes of mitochondria. Selective alterations in microbodies (peroxisomes) were not observed. The effect of longer-term treatment (six months to seven years) with clofibrate elicited similar but more marked ultrastructural alterations, with an increase in microbodies (peroxisomes), although peroxisomal ultrastructural effects were not quantified. A second study (Hanefeld *et al.*, 1983) compared biopsies before and during clofibrate therapy (2 g/day for 3–94 months) [29 mg/kg bw est.]. Volume density and numbers of mitochondria both increased by approximately 30%. The numerical density of peroxisomes (a stereological estimation) was increased by approximately 50%, but there was no statistically significant increase in the volume density of peroxisomes (a direct measurement).

4.2.2 *Experimental systems*

Many of the pharmacological effects of clofibrate observed in experimental animals are qualitatively similar to those observed in humans. Reductions in serum cholesterol and/or triglyceride levels in rats treated with clofibrate have been reported from several studies (Anthony *et al.*, 1978; Barnard *et al.*, 1980; Watanabe *et al.*, 1987). In the study

described in Section 4.1.1 (Tucker & Orton, 1995), in which weanling and mature rats were dosed by gastric instillation with clofibrate, this effect was not statistically significant and measurements other than those of alkaline phosphatase (increase) were not reported. A dose-dependent increase in liver weights and, at the high dose, a loss of glycogen from hepatocytes and hypertrophy and eosinophilia of centrilobular cells were observed.

Hepatic effects of clofibrate treatment in experimental animals include peroxisome proliferation, which is characterized by increases in numbers of hepatocellular peroxisomes and levels of peroxisomal enzymes, and hepatocellular hyperplasia. In one study, male and female Sprague-Dawley rats were given 1500–9000 mg/kg diet (ppm) clofibrate in the diet [90–540 mg/kg bw est.] for up to 13 weeks. Increases in peroxisomal fatty acyl CoA oxidase activity were observed after one week (at all doses in males and at the highest dose in females) and after 13 weeks (at 4500 or 9000 ppm doses in males and females). Increases in relative liver weights and peroxisomal volume densities (measured only after 13 weeks) were observed at similar levels of exposure. In the male rats ingesting 9000 ppm clofibrate for 13 weeks, both peroxisomal fatty acyl CoA oxidase activities and peroxisomal volume densities were increased approximately seven-fold. Hepatocyte nuclear bromodeoxyuridine-labelling indices, an indirect measure of cell replication, were increased at one week of exposure, but decreased at 13 weeks of exposure. These effects of clofibrate did not produce any increase in circulating alanine aminotransferase or aspartate aminotransferase activities, which are markers of hepatocellular injury (Tanaka *et al.*, 1992). Similar effects on liver weights, peroxisomal enzymes and/or cell replication have been reported for male Wistar rats (Price *et al.*, 1986) given clofibrate, as well as male Fischer 344 rats (Eacho *et al.*, 1991; Marsman *et al.*, 1992) and male Sprague-Dawley rats (Barrass *et al.*, 1993) given clofibric acid. Statistically significant increases in peroxisomal β-oxidation and/or lauroyl CoA oxidase activities were observed in male C57Bl/6, ATL/OLA, C3H/He, BALB/c and A/J strain mice but not in male C57Bl/10 or CBA/Ca mice given 5000 ppm clofibrate [625 mg/kg bw est.] in the diet for 10 days. However, peroxisomal catalase activity and relative liver weights were increased in all of the strains examined (Lundgren & DePierre, 1989). In NMRI mice treated similarly for up to 25 days, increased relative liver weights and increased cytoplasmic volume density of peroxisomes were measured (Meijer *et al.*, 1991).

Accumulation of lipofuscin pigment was observed to increase markedly over the duration of exposure in male Fischer 344 rats given 5000 mg/kg diet (ppm) clofibric acid in the diet [300 mg/kg bw est.] for up to 22 weeks (Marsman *et al.*, 1992). In male Sprague-Dawley rats given approximately 500 mg/kg bw clofibrate in the diet for 22 days, increases in hepatic lipofuscin (three to four times control levels) were completely prevented by simultaneous feeding with vitamin E, although no effect on peroxisomal β-oxidation activity was observed (Stanko *et al.*, 1995). However, in male Wistar and Fischer 344 rats given 2500 mg/kg diet (ppm) clofibrate [150 mg/kg bw est.] for 78–79 weeks, only minimal changes in hepatic hydrogen peroxide concentration were observed (Tamura *et al.*, 1990a,b). A slight (two- to three-fold) increase was observed in levels of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in hepatic DNA of male

Fischer 344 rats fed 5000 ppm clofibric acid for 22 weeks in the diet (Cattley & Glover, 1993). Similar feeding with clofibrate gave rise to a two-fold increase in levels of this adduct, compared with controls, after one month. Levels were also slightly elevated at 2, 3, 6, 9 and 12 months, but the increases were statistically significant ($p < 0.05$) only at 2 and 12 months (Takagi *et al.*, 1990). Peroxisomal hydrogen peroxide may injure cellular constituents via the production of hydroxyl radical, as demonstrated in liver fractions prepared from male Alpk/Ap (Wistar-derived) rats given 200 mg/kg bw clofibrate daily by gastric instillation for nine days (Elliott *et al.*, 1986).

The induction of peroxisome proliferation appears to be a direct result of the action of clofibric acid on hepatocytes. Incubation of primary cultures of rat hepatocytes with clofibric acid (50–250 μM) for up to 72 h resulted in an up to six-fold increase in the induction of peroxisomal β-oxidation (Foxworthy & Eacho, 1986). This response is probably mediated by the peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear steroid hormone receptor superfamily. In the presence of clofibric acid, PPARα and retinoic acid-X-receptor α form a heterodimer which binds to the response elements located in the promoter region of several peroxisomal genes, such as that of the rat acyl CoA oxidase gene, and facilitate transcriptional activity (Issemann *et al.*, 1993). The critical role of PPARα in mediating responses to clofibrate has been demonstrated with knockout mice (derived from Sv/129 strain embryonic stem cells) that do not express the receptor (Lee *et al.*, 1995). These mPPARα$^{-/-}$ mice (F2 homozygotes; hybrids of Sv/129×C57Bl/6N), when given 5000 mg/kg diet (ppm) clofibrate in the diet for two weeks [625 mg/kg bw est.], failed to show the increases in liver weight, in peroxisome and in mRNA levels for peroxisomal enzymes, including acyl CoA oxidase, that are seen with wild-type mice.

Because of the difficulty in conducting repeat biopsy studies of hepatic responses in human patients, investigators have studied the response to clofibrate in primary human hepatocytes and in human hepatoma cell lines. Treatment of primary cultures of human hepatocytes with clofibric acid at concentrations of 1–1000 μM for up to 72 h did not induce peroxisomal β-oxidation activity or increase numbers of peroxisomes (Blaauboer *et al.*, 1990). Another marker enzyme of peroxisome proliferation, carnitine acetyltransferase, was not induced in human primary hepatocyte cultures exposed to 500 μM clofibric acid for 48 h (Butterworth *et al.*, 1989). The lack of response in these primary human hepatocyte cultures contrasts with results obtained with human hepatoma cell lines. Treatment of human hepatoma (Hep) EBNA2 cells with 100–1000 μM clofibrate for up to five days resulted in increased peroxisomal acyl CoA oxidase activity and acyl CoA oxidase mRNA content (Scotto *et al.*, 1995). Treatment of human HepG2 cells with 250–1000 μM clofibric acid for two days increased the activities of peroxisomal palmitoyl CoA oxidase and catalase (Chance *et al.*, 1995). The significance of these results is unclear because of the low magnitude of the response (≤ 3-fold) seen and uncertainty about how well these cells model potential responses in human tissues.

The central role of PPARα in mediating the hepatic effects of fibrate drugs in rodents indicated that characterization of human PPARα could be important for the extrapolation of effects in rodents to humans. Tugwood *et al.* (1996) found generally low (but variable) expression of PPARα mRNA in 10 human liver samples compared with rodent liver

samples. They characterized the function of human PPARα cDNA clones isolated from two livers. One had a deleted segment leading to a C-terminal truncation of the receptor; the other had non-conservative codon substitutions at amino acid positions 71 and 123. Both clones failed to activate transcription under conditions in which the mouse wild-type PPARα clone is active, indicating a non-functional human receptor. Thus, the insensitivity of human liver to the adaptive effects of peroxisome proliferators may be attributable to low expression of PPARα and/or genetic variations in the PPARα gene that result in lack of response to peroxisome proliferators.

Comparison of laboratory animal species suggests that sensitivity to induction of peroxisome proliferation is species-dependent. The hepatic effects of 300–350 mg/kg bw clofibrate administered orally for two weeks to male marmosets (n = 12), C57Bl/10J mice (n = 3) and AP rats (n = 3) were reported briefly (Tucker & Orton, 1995). For the mice, the replicate was the pooled livers from five mice per cage. Serum concentrations of clofibric acid were: marmoset, 117 ± 34 μg/mL at 4 h after the final dose; mice, undetectable at autopsy (between 10 h and 12 h); rats, 268 ± 35 μg/mL at autopsy (between 10 h and 12 h). The limit of detection of clofibric acid was 30 μg/mL. The failure to detect clofibric acid in mice may be a function of the time of last consumption of medicated diet and the short half-life (< 4 h) of the compound in this species. The results of the liver analyses are presented in Table 2. No effect upon any of the parameters was observed in marmosets. Liver weight was increased in comparison with concurrent controls by 18% in mice and 54% in rats. The parameters indicative of microsomal oxidase activity and, especially, peroxisomal activity were clearly more strongly affected in rats than in mice. Palmitoyl CoA reduction activity was increased 27.5-fold in rats and 3.9-fold in mice.

A long-term study in marmosets also suggests the relative insensitivity to the hepatic effects of clofibrate of this primate species compared with rodents. Groups of male and female marmosets (*Callithrix jacchus*) were given clofibrate by gastric instillation for up to 343 weeks at doses of 0 (undosed), 0 (vehicle), 94, 157, 213 and 263 mg/kg bw per day (Tucker & Orton, 1995). Initially, there were 20 marmosets of each sex in each control group and 10 of each sex in each dose group. A substantial number of premature deaths occurred. The numbers of survivors at week 343 (sexes combined and controls combined) in each of the groups were 61, 14, 14, 9 and 8. Causes of death were unrelated to clofibrate treatment. Because of the premature deaths, the numbers were supplemented at weeks 30 and 143. An increase in kidney weight was observed in the higher-dose groups, but no corresponding change in renal histopathology to account for this observation. No change in relative liver weight was observed and no histological change in any tissue was attributable to treatment. In particular, there was no evidence of changes in the levels of hepatic peroxisomes (by transmission electron microscopy) on three animals per sex in control and highest-dose groups. [Methods used for the evaluation of this end-point were not described.] Hepatic iron deposits of unknown etiology were observed in all marmosets.

In rodents, modulation of enzyme activities by clofibrate is not limited to peroxisomes, but may also extend to mitochondrial, cytosolic and endoplasmic reticulum enzymes within the cell. For example, clofibrate increases the hepatic expression of an

Table 2. Interspecies comparison of the effect of clofibrate upon parameters of hepatic microsomal and peroxisomal activities

	Marmoset		C57Bl/10J mouse		AP rat	
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Liver weight relative to body weight (%)	4.68 ± 0.41	4.63 ± 0.48	5.91 ± 0.36	6.98 ± 0.54*	4.80 ± 0.50	7.39 ± 0.29***
Cytochrome P450 content (nmol/mg protein)	0.36 ± 0.09	0.41 ± 0.08	0.74 ± 0.14	0.87 ± 0.09	0.37 ± 0.04	0.73 ± 0.02***
Catalase (k.sec/g)	29.5 ± 3.1	36.3 ± 3.1	66.0 ± 16.7	84.3 ± 13.6	76.6 ± 1.3	120.3 ± 2.7**
Palmitoyl coenzyme A oxidase (μmol NAD^+ reduced/min/mg protein)	0.15 ± 0.03	0.11 ± 0.03	0.34 ± 0.13	1.33 ± 0.23**	0.2 ± 0.0	5.5 ± 0.7***
Crotonyl coenzyme A oxidase (μmol NAD^+ reduced/min/mg protein)	5.3 ± 0.2	6.0 ± 0.3	15.1 ± 3.4	17.7 ± 3.5	12.1 ± 2.0	28.6 ± 1.2**

From Tucker & Orton (1995)
Results are means ± SD
*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$ Student's t test

enzyme of the CYP4A family associated with fatty acid ω-hydroxylase activity. Male Long-Evans rats given 80 mg/kg bw of the enantiomers or racemix mixture of the clofibrate analogue, 2-(4-*para*-chlorophenyloxy)2-phenyl ethanoic acid, by gastric instillation for three consecutive days, had elevated hepatic CYP4A1 and lauric acid 12-hydroxylase activity (Chinje & Gibson, 1990). Clofibrate (730 mg/kg diet (ppm) for 24 weeks) inhibited the expression of γ-glutamyl transpeptidase in liver homogenates of male Fischer 344 rats in which this enzyme activity had been induced by prior feeding of 2-AAF (Numoto *et al.*, 1984).

Some extrahepatic effects of clofibrate in experimental animals are analogous to those observed in the liver. For example, in male but not female Fischer 344 rats given 400 mg/kg bw clofibrate daily by intraperitoneal injection for three consecutive days, increased renal content of CYP4A2 mRNA was observed (Sundseth & Waxman, 1992). Clofibric acid caused an increase in renal peroxisomal palmitoyl CoA oxidase activity in male Wistar rats given 200 mg/kg bw clofibric acid daily by gastric instillation for 10 consecutive days (Chandoga *et al.*, 1994). Administration of 400 mg/kg bw clofibrate per day in the diet to male Wistar rats for 3 months or longer resulted in diminished size of thyroid follicles, with calcium deposition in the colloid and hypertrophy of the Golgi apparatus (Price *et al.*, 1988).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Schneider and Kaffarnik (1975) reported three cases of male impotence in patients with type IV hyperlipoproteinaemia who were treated with a controlled diet and clofibrate. The complaints of impotence were made within one year of beginning treatment with the drug. Two of the patients reported improvement of the symptoms three and four weeks after interruption of clofibrate therapy; one patient again complained of impotence when clofibrate therapy was resumed.

4.3.2 *Experimental systems*

In a study reported only in abstract, no change in number of resorption sites, litter size, fetal weight or no teratogenic effect was found in rats when dams were given 0.6 mg/kg bw clofibrate per day in feed or 1 or 140 mg/kg bw clofibrate per day by gastric instillation from day 6 to day 20 of gestation (Diener & Hsu, 1966). When doses of 200 mg/kg bw per day were given to both male and female rats by gastric instillation, both before and during gestation, a significant decrease in litter size was observed, and with a dose of 500 mg/kg bw, the number of pregnancies decreased from 7/8 in controls to 0/8 in treated animals. No such effect was found when female rabbits were treated similarly (Pantaleoni & Valeri, 1974). In female albino rats given 50 mg clofibrate per day orally during the entire period of mating, gestation and lactation, the liver weight at birth of the offspring was significantly higher than that of control pups, while there was no difference in birth weight between the groups (Chhabra & Kurup, 1978). The offspring of Wistar rats given 8000 mg/kg diet (ppm) clofibrate in the diet for one week

on gestational days 13, 15, 17, 19 or 21 weighed significantly less than the offspring of control rats. Maternal weight gain was reduced in treated animals compared with controls (Cibelli *et al.*, 1988). An abnormal postnatal fetal thrombosis syndrome in rats has been described, consisting of an extension of the normal thrombosis in the umbilical arteries and causing necrosis of the tail or parts of the hindlimbs (Dange *et al.*, 1975). In pregnant Dutch rabbits given 0 or 5000 ppm clofibrate in the diet throughout pregnancy, no effect on fertility or litter size and no skeletal abnormality were detected (Tucker & Orton, 1995). Nishimura and Tanimura (1976) found that the rabbit fetus serum accumulates a higher concentration of clofibrate than maternal serum.

In albino rats, the serum of newborn pups of dams that had received clofibrate (50 mg/day) orally during mating, gestation and lactation contained 93 nmol/mL clofibric acid. This decreased to 48 nmol/mL on day 12 and 31 nmol/mL at the time of weaning. Placenta collected before birth from clofibrate-fed dams contained about 80 nmol/g clofibric acid. This indicates that the drug crosses the placenta. The activity of mitochondrial glycerol phosphate dehydrogenase in hepatic mitochondria isolated from newborn rats of dams that were fed the drug was almost three times the level observed for control offspring. The activity increased and remained at a higher level during lactation but, when the young animals were weaned, it rapidly decreased to about the same level as that seen in control animals. This suggests that the drug may also pass to the offspring via the mother's milk (Chhabra & Kurup, 1978).

Clofibrate (150 mg/kg bw per day) given continuously to female Wistar/H-Riop rats from gestational day 16 to the end of lactation (22nd day post-partum) produced a decrease in birth weight, an increase in perinatal mortality and an increase in liver weight at the age of 22 days. Investigations in which the dam received doses of 150 mg/kg bw per day during four time intervals between gestational day 16 and postnatal day 22 showed that the increase in liver weight was associated with exposure between delivery and postnatal day 15. When the drug was administered in the last week of pregnancy and the young were dissected on postnatal days 1, 8, 15 or 22, increased liver weight was observed in neonates but not subsequently. The authors suggested that this transient increase in liver weight might be related to enzyme induction rather than to hepatotoxicity (Nyitray *et al.*, 1980).

Pregnant Swiss ICR mice were given clofibrate by subcutaneous injections at various dosages (480 and 960 mg/kg bw) and time intervals, and embryos were removed on days 17 or 18 of gestation. In embryos removed on day 17, the level of intestinal catalase activity of the proximal and distal halves did not differ between treated groups and controls. In embryos removed on day 18, a dose-dependent rise in catalase activities in the proximal half of the small intestine in treated groups was observed, but a plateau was attained with repeated injections (Calvert *et al.*, 1979).

Clofibrate treatment of pregnant female rats has been found to increase the number of liver peroxisomes and the levels of fatty acid oxidation enzymes in fetuses, suggesting that the treatment induces fetal peroxisome proliferation (Cibelli *et al.*, 1988; Stefanini *et al.*, 1989). In mice, 400 mg/kg bw oral clofibrate treatment initiated at day 6 of gestation produced a 4–5-fold increase in levels of peroxisomal membrane protein 70, a 1.5-

to 2-fold increase in dihydroxyacetone phosphate acyltransferase specific activity and a 1.2–1.8-fold increase in catalase specific activity in fetal liver of 19 days gestation. Electron microscopy showed amplification of endoplasmic reticulum and peroxisomes in the fetal liver. There was a general increase in peroxisomal proteins between gestational days 13 and 19 in all fetal tissues except the placenta, and the effect of clofibrate in the lung and the placenta was evident by gestational day 13 (Wilson *et al.* 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 Experimental systems (see also Table 3 for references and Appendices 1 and 2)

Clofibrate is not mutagenic in *Salmonella typhimurium* in the presence or absence of microsomal preparations. In the yeast *Saccharomyces cerevisiae*, clofibrate induced neither gene conversion nor mitotic recombination.

In single studies, clofibrate did not induce unscheduled DNA synthesis in cultured hepatocytes or DNA strand breaks in L1210 cells. However, the ability of *N*-ethyl-*N*-nitrosourea to produce single-strand DNA breaks and of *N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea to produce both single-strand DNA breaks and interstrand cross-links in L1210 cells was enhanced by prior treatment of the cells with clofibrate (Lawson & Gwilt, 1993).

Clofibrate was not mutagenic in Chinese hamster lung V79 cells, in the presence of a rat hepatocyte metabolic activation system. As reported in an abstract, clofibrate did not induce resistance to 6-thioguanine in the granuloma pouch assay in rats.

Clofibrate did not induce chromosomal aberrations in three studies with cultured mammalian cell lines nor micronucleus formation in a study with cultured rat hepatocytes.

In morphological transformation studies with Syrian hamster embryo (SHE) cells, clofibrate had no effect in one study, but was reported in another study to have increased the frequency of transformation. The administration of clofibrate alone was also without effect in the C3H/10T1/2 C18 cell transformation system, whereas it did enhance the frequency of transformation produced by prior treatment with 3-methylcholanthrene. Weak inhibition of gap-junctional intercellular communication in Chinese hamster V79 cells was reported to occur with high concentrations of clofibrate.

No evidence was seen of DNA adduct formation by clofibrate in the livers of male Fischer 344 rats given three doses of 250 mg/kg at 24-h intervals and killed 2 h after the final dose. DNA was analysed by ^{32}P-postlabelling with an estimated limit of detection of 1 adduct in 10^{10} nucleotides and no adduct was detected in hepatocytes treated *in vitro* with 10^{-3} M clofibrate for 4 h (Gupta *et al.*, 1985).

In vivo, clofibrate did not induce unscheduled DNA synthesis in rat hepatocytes. Neither did it induce sister chromatid exchange in rat peripheral blood lymphocytes or bone marrow cells of Chinese hamsters.

Table 3. Genetic and related effects of clofibrate

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BSD, *Bacillus subtilis* rec strains, differential toxicity	–	–	NR	Kawachi *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NR	Kawachi *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Warren *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	100	Dayan *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Warren *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Dayan *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Warren *et al.* (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Dayan *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Warren *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NR	Kawachi *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Warren *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Dayan *et al.* (1985)
SAS, *Salmonella typhimurium* TA100Fr1, reverse mutation	–	–	500	Dayan *et al.* (1985)
SCG, *Saccharomyces cerevisiae*, gene conversion	–	–	20 000	Schiestel & Reddy (1990)
SCH, *Saccharomyces cerevisiae*, mitotic recombination	–	–	20 000	Schiestel & Reddy (1990)
DIA, DNA single-strand breaks, L1210 cells *in vitro*	–	–	3	Lawson & Gwilt (1993)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus	–	–	243	Dayan *et al.* (1985)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain	–	–	243	Dayan *et al.* (1985)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	–	NT	2400	Williams *et al.* (1989)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	243	Linnainmaa (1984)
CIC, Chromosomal aberrations, Chinese hamster CHL cells *in vitro*	–	NT	250	Ishidate *et al.* (1978)
CIS, Chromosomal aberrations, Syrian hamster lung fibroblasts *in vitro*	–	NT	NR	Kawachi *et al.* (1980)
CIS, Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	72	Tsutsui *et al.* (1993)
TCM, Cell transformation, C3H 10T½ mouse cells	–	NT	1.2	Lillehaug *et al.* (1986)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIA, Micronucleus test, rat hepatocytes *in vitro*	–	NT	243	Müller *et al.* (1993)
TCS, Cell transformation, Syrian hamster embryo cells	+	NT	12	Mikalsen *et al.* (1990)
TCS, Cell transformation, Syrian hamster embryo cells	–	–	72	Tsutsui *et al.* (1993)
DVA, DNA strand breaks, rat hepatocytes *in vivo*	–		200 po × 6	Elliott & Elcombe (1987)
DVA, DNA strand breaks, rat hepatocytes *in vivo*	–		750 diet × 14 d	Nilsson *et al.* (1991)
DVA, DNA strand breaks, Fischer rat liver *in vivo*	–		100 diet × 78 wk	Tamura *et al.* (1991)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		750 diet × 14 d	Nilsson *et al.* (1991)
GVA, Gene mutation, rat granuloma pouch 6-TG resistance *in vivo*	?		4 sc × 1	Maier (1984) (abstract)
SVA, Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		200 po × 14	Linnainmaa (1984)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	–		200 po × 14	Linnainmaa (1984)
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	–		NG	Kawachi *et al.* (1980)
BID, Binding (covalent) to DNA, male F344 rat hepatocytes *in vitro*	–	NT	243	Gupta *et al.* (1985)
BVD, Binding (covalent) to DNA, male F344 rat liver *in vivo* (^{32}P-postlabelling)	–		250 po × 3	Gupta *et al.* (1985)
ICR, Inhibition of intercellular communication, V79 cells	(+)	NT	24	Awogi *et al.* (1984) (abstract)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw; NG, dose not given

Slightly elevated levels of 8-hydroxydeoxyguanosine have been detected in liver DNA of rats fed diets containing clofibrate (see Section 4.2.2).

Oral treatment of Sprague-Dawley-derived SIV 50 rats with ^{14}C-labelled clofibric acid (225 mg/kg) did not lead to detectable radioactivity associated with liver DNA, although protein binding was clearly demonstrated (von Däniken *et al.*, 1981).

4.5 Mechanistic considerations

The role of data on peroxisome proliferation in evaluating carcinogenicity in humans has been discussed. When the data support the conclusion that a tumour response in mice or rats is secondary only to peroxisome proliferation, this should be considered in addressing the potential carcinogenicity of an agent in humans. The report of the Working Group on Peroxisome Proliferation and its Role in Carcinogenesis (IARC, 1995) indicates that the following issues should be considered:

"(*a*) Information is available to exclude mechanisms of carcinogenesis other than those related to peroxisome proliferation.

(*b*) Peroxisome proliferation (increases in peroxisome volume density or fatty acid β-oxidation activity) and hepatocellular proliferation have been demonstrated under the conditions of the bioassay.

(*c*) Such effects have not been found in adequately designed and conducted investigations of human groups and systems."

The weight of evidence indicates that clofibrate, and peroxisome proliferators in general, do not act as direct DNA-damaging agents and that their mechanism of tumour initiation is indirect. Two responses have been proposed to account for liver carcinogenesis by peroxisome proliferators in rodents. These include (i) induction of peroxisome proliferation and (ii) increased hepatocellular proliferation. These responses are not mutually exclusive with respect to tumour formation.

Chronic administration of peroxisome proliferators produces a sustained oxidative stress in rodent hepatocytes due to overproduction of hydrogen peroxide. This can theoretically generate reactive oxygen species which can attack DNA or may affect cells in other ways. There is also evidence from in-vitro experiments that fatty acid metabolism in peroxisomal fractions can result in hydroxyl radical formation and DNA damage. In-vivo observations in support of this hypothesis include increased lipid peroxidation, increased lipofuscin deposition, the effects on levels of hepatic antioxidants and inhibition of tumour formation by antioxidants (Lake 1995). However, some of the evidence suggests that the level of oxidative damage *in vivo* may be too low to account entirely for the carcinogenicity of peroxisome proliferators.

During the first few days of administration, peroxisome proliferators induce cell division in rodent hepatocytes; in some, but not all, studies sustained stimulation of replicative DNA synthesis has also been observed (Lake, 1995). An enhanced rate of cell proliferation can be a critical effect both in tumour initiation, by increasing the frequency of spontaneous mutations and the rate of conversion of DNA adducts into mutations before they are repaired, and in tumour promotion by facilitating clonal expansion of

initiated cells. The promoting activity of clofibrate has been demonstrated in rodent models of multistage hepatocarcinogenesis.

There are clear species differences in the responses of mammalian cells to peroxisome proliferators (Lake, 1995). Biopsy studies have clearly indicated that the responsiveness of human livers to the peroxisome proliferation produced by fibrate drugs is lacking or is much less than that seen in the livers of treated rodents, although similar levels of drug are achieved in the circulation. The striking hepatomegalic effect of peroxisome proliferation is similarly not observed in patients receiving fibrate drugs. In cultures of hepatocytes, peroxisome proliferation and cell proliferation occur with rodent but not human hepatocytes. In rodent liver, hepatomegaly and peroxisome proliferation require expression of functional PPARα, a member of the steroid hormone receptor superfamily. Clofibrate activates rodent PPARα *in vitro*. The insensitivity of human liver to the effects of peroxisome proliferators is consistent with the low level of PPARα in human livers, as well as observations of genetic variations that render the human PPARα receptor inactive as compared to PPARα expressed in rodent liver (Tugwood *et al.*, 1996). In non-human primates, administration of clofibrate and other peroxisome proliferators has also failed to elicit the hepatomegaly and peroxisome proliferation induced in rodent liver.

Clofibrate-induced peroxisome proliferation and cell proliferation have been demonstrated in feeding studies in rats conducted under bioassay conditions. Peroxisome proliferation has not been found in studies of human groups and systems using clofibrate. Taken together, these findings indicate that the increased incidence of liver tumours in rodents treated with clofibrate results from a mechanism that would not be operative in humans.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Clofibrate was introduced in the 1960s to reduce plasma concentrations of triglycerides and cholesterol in patients at high risk of coronary heart disease. Since the late 1970s, its use has decreased considerably.

5.2 Human carcinogenicity data

In 1978, a randomized trial of the World Health Organization, conducted to determine whether clofibrate treatment would lower the incidence of ischaemic heart disease in men, raised concern over a nonsignificant excess of deaths from cancer in treated subjects.

Subsequently the association between clofibrate and cancer risk was examined in three randomized trials and a small case–control study. A further four-year follow-up of the WHO trial showed no difference in the age-standardized death rates from malignant neoplasms. In two other trials, there was also no difference in cancer deaths between

clofibrate-treated patients and a placebo-treated group. A meta-analysis of results from six trials also found no excess cancer mortality due to use of clofibrate as a cholesterol-lowering drug. The case–control study, that had several methodological limitations, showed a nonsignificant excess of soft-tissue sarcoma.

5.3 Animal carcinogenicity data

Clofibrate was tested for carcinogenicity by oral administration in the diet in two experiments in mice and in three experiments in rats, and in one experiment in marmosets by gastric instillation. No increase in incidence of tumours was reported in mice or marmosets. In rats, clofibrate produced hepatocellular carcinomas.

Clofibrate was tested in several experiments by combined administration with other chemicals. It enhanced the hepatocarcinogenicity of *N*-nitrosamines in rats and hamsters. It did not enhance the carcinogenicity of 2-acetylaminofluorene in rat liver.

5.4 Other relevant data

Clofibrate exerts similar pharmacological responses in humans and rodents. Absorption and metabolism of clofibrate are similar in humans and rats. Elimination of clofibric acid, the free acid form of the drug as it appears in the circulation, is more rapid in rats, possibly due to lower binding to plasma proteins.

Clofibrate-induced peroxisome proliferation and cell proliferation have been demonstrated in feeding studies in rats. Peroxisome proliferation has not been found in studies of clofibrate in human livers or hepatocytes.

There are a number of case reports of reversible impotence in men treated with clofibrate. No noteworthy effect on the fetus has been observed in studies in rats or rabbits.

Clofibrate is inactive in most tests for genetic activity, although it induced cell transformation in one study.

Mechanistic considerations

The weight of evidence indicates that clofibrate does not act as a direct DNA-damaging agent and that its mechanism of tumour induction is indirect. Two biological responses have been proposed to account for liver carcinogenesis by peroxisome proliferators in rodents. These are (i) induction of peroxisome proliferation and (ii) increased hepatocellular proliferation. Upon exposure to clofibrate, proliferation of both peroxisomes and cells occurs in rat liver and of peroxisomes in cultured rat hepatocytes, whereas peroxisome proliferation does not occur in human liver or cultured hepatocytes. These observations suggest that the mechanism of liver carcinogenesis in clofibrate-treated rats would not be operative in humans.

5.5 Evaluation[1]

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

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

Overall evaluation

Clofibrate is *not classifiable as to its carcinogenicity in humans (Group 3).*

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[1]For definition of the italicized terms, see Preamble, pp. 22–25.

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