Annu. Rev. Pharmacol. Toxicol. 1995. 35:483–507

MECHANISMS OF HEPATOCARCINOGENICITY OF PEROXISOME-PROLIFERATING DRUGS AND CHEMICALS

Brian G. Lake
BIBRA Toxicology International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, England

KEY WORDS: peroxisome proliferation, oxidative stress, cell replication, tumor promotion, species differences

ABSTRACT

A wide variety of chemicals have been shown to produce liver enlargement, peroxisome proliferation, and induction of peroxisomal and microsomal fatty acid–oxidizing enzyme activities in rats and mice. Moreover, certain peroxisome proliferators have been shown to increase the incidence of liver tumors in these two species. This review describes the characteristics of peroxisome proliferation in rodent liver and in vitro in primary hepatocyte cultures and gives examples of the range of different classes of chemicals that produce this effect. Mechanisms of initiation of peroxisome proliferation in rodent hepatocytes, including peroxisome proliferator–activated receptors, are also described. Peroxisome proliferators are not considered to be genotoxic carcinogens, and proposed mechanisms of liver tumor formation include induction of sustained oxidative stress, a role for enhanced cell replication, and the promotion of spontaneous preneoplastic lesions. Data are also presented on species differences in response and key issues concerning the risk assessment to humans of rodent liver peroxisome proliferators.

INTRODUCTION

Peroxisomes (or microbodies) are single membrane–limited cytoplasmic organelles present in the cells of animals, plants, fungi, and protozoa. DeDuve

0362-1642/95/0415-0483$05.00

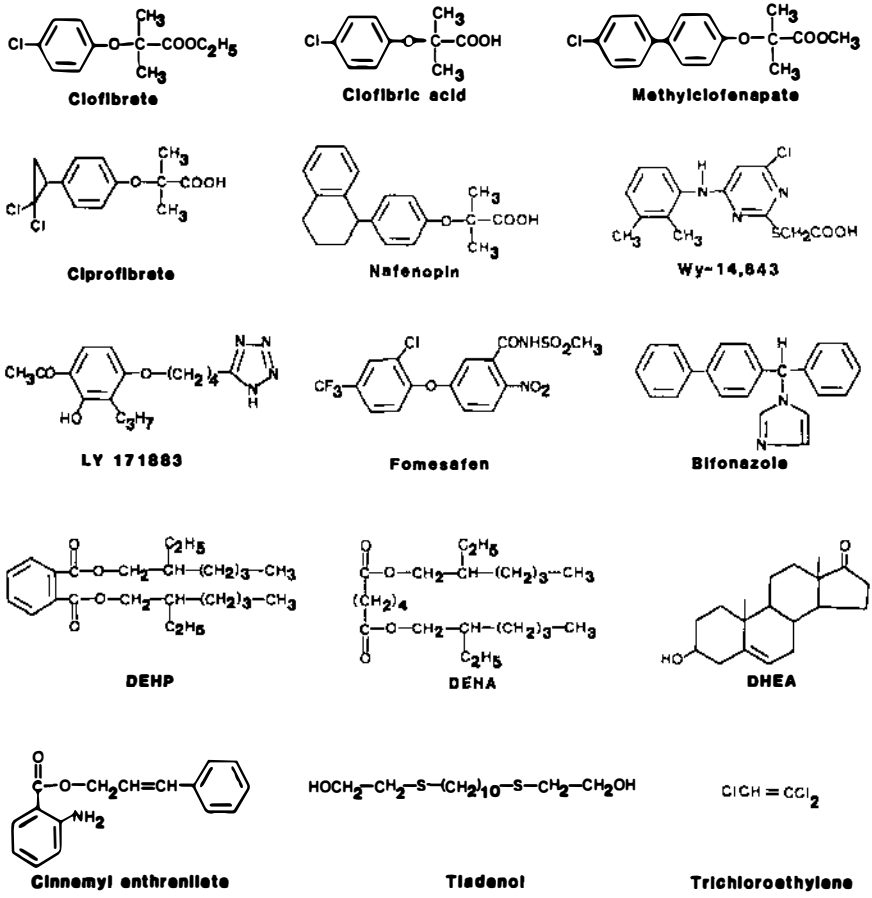

Figure 1 Structures of some compounds known to produce peroxisome proliferation in rodent hepatocytes (3, 4, 6, 14, 18, 20, 21).

and coworkers demonstrated that rat liver peroxisomes contain both catalase, which destroys hydrogen peroxide, and a number of hydrogen peroxide–generating oxidase enzymes (1). Further work by Lazarow & DeDuve (2) revealed that liver peroxisomes, like mitochondria, contain a complete fatty acid β-oxidation cycle. In rat hepatocytes, peroxisomes are normally spherical or oval in shape, with a diameter of approximately 0.5 μm, and they are estimated to account for around 2% of the total cytoplasmic volume and cellular protein (3–7). Peroxisomes have several important functions in intermediary metabolism (7), and peroxisomal disorders can have serious consequences for the organism (8).

Paget (9) and Hess and coworkers (10) demonstrated that clofibrate, a hypolipidemic drug (Figure 1), could produce peroxisome proliferation in rodent liver. Peroxisome proliferation is an important topic of study because many chemicals have been shown to produce this effect in rodent liver, and some of these agents are also hepatocarcinogenic (3, 4, 11–17). Indeed, Reddy and coworkers (11) have suggested that peroxisome proliferators constitute a novel class of chemical carcinogens.

The purpose of this review is to briefly consider the various mechanisms of peroxisome proliferator–induced hepatocarcinogenicity in rodents and the relevance of these tumors to humans. Several other reviews on various aspects of peroxisome proliferation have previously been published (3, 4, 6, 12–21). In addition, rodent liver peroxisome proliferators may also produce tumors in other organs such as the testes and the pancreas (22).

Characteristics of Hepatic Peroxisome Proliferation

The administration of peroxisome proliferators to rats and mice results in a marked increase in liver weight, which is associated with both morphological and biochemical changes (3, 4, 13, 14, 16, 18). Liver enlargement is due to both hepatocyte hyperplasia and hypertrophy. Morphological examination reveals an increase in both the size and number of peroxisomes and increased smooth endoplasmic reticulum. Unlike the livers of untreated animals, many coreless peroxisomes (which lack the characteristic crystalline nucleoid that contains urate oxidase) are observed. The major biochemical alterations consist of the induction of both peroxisomal and microsomal fatty acid–oxidizing enzyme activities. The activity of the peroxisomal fatty acid β-oxidation cycle is normally determined either by measuring overall activity (e.g. as cyanide-insensitive palmitoyl-CoA oxidation) or by assaying the first rate-limiting enzyme of the cycle, namely acyl-CoA oxidase (2, 7). It should be noted that there is a differential induction of peroxisomal enzyme activities: The β-oxidation cycle enzymes can be markedly induced, but much smaller increases are observed in other peroxisomal enzymes such as D-amino acid oxidase and catalase. The stimulation of microsomal fatty acid–oxidizing enzymes (normally measured as lauric acid 12-hydroxylase) is caused by induction of cytochrome P450 isoenzymes in the CYP4A subfamily (18, 23).

Other aspects of the effects of acute and chronic administration of peroxisome proliferators in rodent liver are described elsewhere (3, 4, 13–20, 24). Peroxisome proliferators have also been reported to produce mitochondrial proliferation and changes in mitochondrial enzyme activities (6, 16, 24–26). Other studies have reported the induction of microsomal UDPglucuronosyl transferase towards bilirubin and certain other substrates and of both microsomal and cytosolic epoxide hydrolase activities (27, 28).

Rodent Liver Peroxisome Proliferators

Figure 1 shows the structure of some known rodent liver peroxisome proliferators, such as hypolipidemic and other classes of therapeutic agents; phenoxyacetic acid and other classes of herbicides; phthalate and other classes of plasticizers; steroids; solvents and other industrial chemicals; food flavors; and natural products (6, 14, 20, 21). Although peroxisome proliferators appear to be chemically unrelated, or structurally diverse, investigators have demonstrated that some compounds have similar three-dimensional structures, and other investigators have reported both qualitative and quantitative structure-activity relationships for peroxisome proliferation in rodent hepatocytes (21, 29–31).

A feature of many, but not all, peroxisome proliferators is the presence of an acidic function (18, 21, 25). This acidic function is normally a carboxyl group, either present in the parent structure or generated by metabolism. For example, clofibrate is metabolized to clofibric acid, and tiadenol is oxidized to metabolites containing carboxyl groups (21). Alternatively, the compound may contain a chemical group that is a bioisostere (32) of a carboxyl group, as exemplified by the tetrazole and sulphonamide moieties (Figure 1) of LY 171883 and Fomasefen, respectively. For many compounds the "proximate" peroxisome proliferator may be the parent structure or a metabolite containing a carboxyl group or another acidic function. Glucuronide or other phase II metabolites are not considered active peroxisome proliferators in rodent liver (21). Other compounds may require more extensive phase I metabolism to produce the proximate peroxisome proliferators. For example, di-(2-ethylhexyl)phthalate (DEHP) is initially hydrolyzed to its corresponding monoester and alcohol components; the former is further metabolized by side chain oxidation, and the latter is converted to 2-ethylhexanoic acid (33, 34). Certain peroxisome proliferators have chiral centers that may result in enantiomers with differing potencies (21).

Marked compound potency differences are known to exist (21, 35). Figure 2*A* demonstrates that the hypolipidemic agent ciprofibrate is orders of magnitude more potent than DEHP, which is somewhat more potent than another plasticizer, di-(2-ethylhexyl)adipate (DEHA).

Assessment of Peroxisome Proliferation

Chemicals may be screened for their ability to produce peroxisome proliferation in rodent hepatocytes, after either in vivo or in vitro administration, by both biochemical and morphological markers. The most sensitive marker enzyme activities include palmitoyl-CoA oxidation, acyl-CoA oxidase, lauric acid 12-hydroxylase, and carnitine acetyltransferase; catalase is not recommended, as this enzyme shows only a relatively small increase in activity.

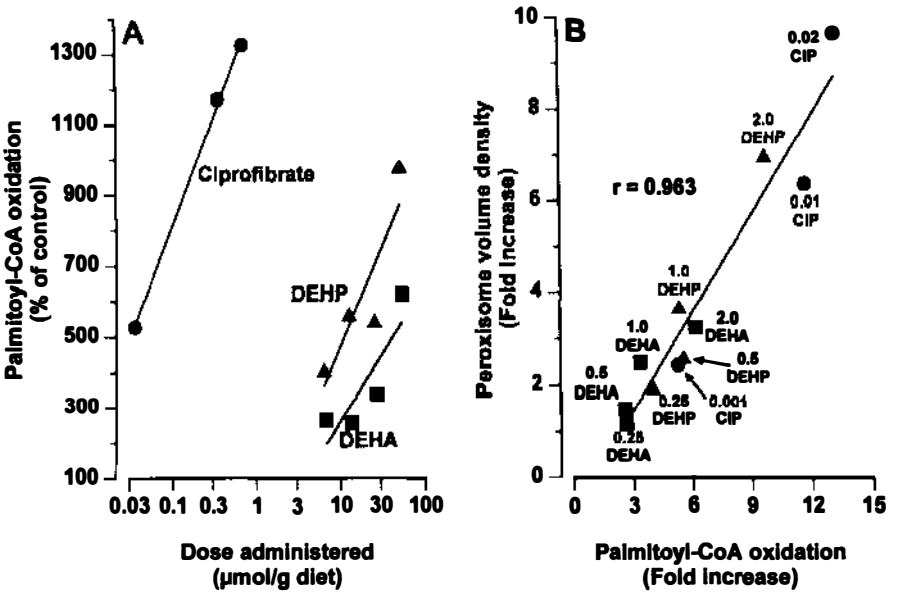

Figure 2 (*A*) Comparison of the potency of ciprofibrate (CIP; ●), DEHP (▲), and DEHA (■) to produce peroxisome proliferation (assessed as induction of palmitoyl-CoA oxidation) in the livers of male F344 rats after 30 days treatment. (*B*) There is an excellent correlation between induction of peroxisomal fatty acid β-oxidation and peroxisome morphometry. (Data from Reference 35.)

Carnitine acetyltransferase is localized in peroxisomal, mitochondrial, and microsomal fractions (36, 37), and although peroxisome proliferators can markedly induce this activity, it may be due to induction both in peroxisomes and in other subcellular compartments. Peroxisome proliferation may also be assessed by ultrastructural examination of liver sections, and in some studies staining with 3,3′-diaminobenzidine has been employed to help identify peroxisomes from other intracellular organelles. For small increases in peroxisome numbers a quantitative morphometric, rather than a qualitative subjective, procedure should be employed. In addition, care should be taken to compare effects in the same area of the liver lobule. For example, in the rat, hepatic peroxisome proliferation, as opposed to cell replication, is more marked in centrilobular than in periportal hepatocytes (6, 38–42).

Good correlations have been reported for rat liver between the induction of peroxisomal fatty acid β-oxidation and organelle proliferation (19, 35, 43, 44). For example, Figure 2*B* demonstrates an excellent correlation between induction of palmitoyl-CoA oxidation and changes in peroxisome morphometry in male F344 rats fed various dietary levels of ciprofibrate, DEHP, and DEHA (35). Good correlations have also been observed in rat hepatocytes between

the induction of peroxisomal and microsomal fatty acid–oxidizing enzyme activities (19, 44, 45). A reduction in plasma or serum cholesterol and particularly triglyceride levels may indicate that the test compound is a peroxisome proliferator, but several rodent studies have demonstrated that a hypolipidemic effect is not necessarily associated with hepatic peroxisome proliferation (21, 30).

Peroxisome Proliferation in Vitro

Studies conducted in this laboratory (21, 45–47) and by several other groups (6, 18, 20, 30, 34, 48–51) have demonstrated that peroxisome proliferation may be readily demonstrated in vitro in primary rat and mouse hepatocyte cultures employing standard hepatocyte media and culture conditions. In general, the characteristics of peroxisome proliferation in vivo—including the stimulation of DNA synthesis, increased peroxisome numbers, and changes in morphology—and a differential induction of enzyme activities have also been observed in cultured hepatocytes. These studies demonstrate that the factors responsible for the induction of peroxisome proliferation are intrahepatic in nature and are retained in cell culture. Peroxisome proliferation may also be observed in vitro in certain cell lines (52), cultured rat hepatocyte spheroids (53), and cultured precision-cut rat liver slices (54).

MECHANISMS OF INDUCTION OF HEPATIC PEROXISOME PROLIFERATION

Proposed mechanisms for the initiation of peroxisome proliferation in rodent hepatocytes include a receptor hypothesis, a substrate overload hypothesis, and a hypothesis that peroxisome proliferators serve as substrates for peroxisomal enzymes (4, 16, 18, 20, 31, 55). Most attention has focused on the identification of receptors and the effect of peroxisome proliferators on lipid metabolism, and it should be noted that these two hypotheses are not mutually exclusive (18, 20, 55). In the substrate overload hypothesis it is proposed that peroxisome proliferation is an adaptive response to a perturbation of lipid metabolism and also involves induction of CYP4A isoenzymes (18, 20, 34, 44, 55). Certainly peroxisome proliferators inhibit fatty acid oxidation, form coenzyme A esters, increase levels of fatty acids, and displace fatty acids from the cytosolic fatty acid–binding protein (6, 18, 20, 30, 31, 34, 55, 56).

Although the presence of cytosolic peroxisome proliferator–binding proteins has been reported in some studies (4, 12, 57, 58), more recently Green and coworkers have cloned a peroxisome proliferator–activated receptor (PPAR) from mouse liver (59, 60). This protein is a member of the steroid hormone receptor superfamily, has a molecular weight of around 52 kDa, and possesses both putative ligand- and DNA-binding domains. When a chimeric receptor

expression vector containing regions that encode the putative ligand-binding domain of this mouse PPAR and the DNA-binding domain of the human estrogen receptor was transfected into COS1 cells, it could be activated by peroxisome proliferators (59, 60). Other studies have demonstrated that PPAR is involved in the activation of genes for both peroxisomal and microsomal (i.e. CYP4A subfamily) fatty acid–oxidizing enzymes (61, 62, 63). Several different PPARs have now been described in the mouse (59, 64, 65, 65a), rat (66, 67), *Xenopus laevis* (61, 68), and human (69, 70). The administration of peroxisome proliferators may increase the levels of certain PPARs in rodent liver (65, 67).

Figure 3 summarizes some possible mechanisms of induction of peroxisome proliferation in rodent hepatocytes. Clearly there is much scope for further studies to fully elucidate the precise mechanism(s) of peroxisome proliferation in rodent hepatocytes and to determine differences between rodent and human hepatocytes. Peroxisome proliferation in rodents can also be produced by various physiological factors—such as feeding high-fat diets, vitamin E deficiency, and starvation—which may be related to xenobiotic-induced organelle proliferation (4, 18, 20). Although some evidence has been obtained for the formation of coenzyme A esters of peroxisome proliferators, this could not be a universal mechanism because while most peroxisome proliferators contain an acidic function, this is not always a free carboxyl group (18, 20, 21, 29–31, 56). While molecular modeling studies have identified a putative peroxisome proliferator–binding site in one PPAR, the binding of nafenopin has not been observed (31, 59, 71). In contrast, certain fatty acids, such as oleic and arachidonic acids, may act as endogenous ligands because they have been shown both to bind to and to activate PPARs in in vitro expression systems (66, 68, 69, 71, 72). Assuming that peroxisome proliferators do interact directly with PPARs, the binding may be only of a transient nature. Steroid hormone receptors are associated with heat shock proteins, and PPAR activation could conceivably involve displacement of the peroxisome proliferator and a heat shock protein such as HSP72 (73, 74). PPARs have been shown to bind to DNA as a heterodimer with retinoid X receptor (RXR), and another member of the steroid hormone receptor superfamily, namely the chicken ovalbumin upstream promoter transcription factor (COUP-TF), has been shown to act as a repressor (75–78). In addition, studies with three mouse liver PPARs (designated α, δ, γ) have demonstrated differences in activation by some peroxisome proliferators and fatty acids (65a). Moreover, the PPAR δ and γ forms interfered with the activation of the PPAR α form by Wy-14,643, suggesting that some PPARs may be dominant repressors of other forms (65a).

Multiple PPARs, together with activating receptors (e.g. RXR) and repressing receptors (e.g. COUP-TF and possibly truncated receptors), may account for known tissue and species differences in response to peroxisome prolif-

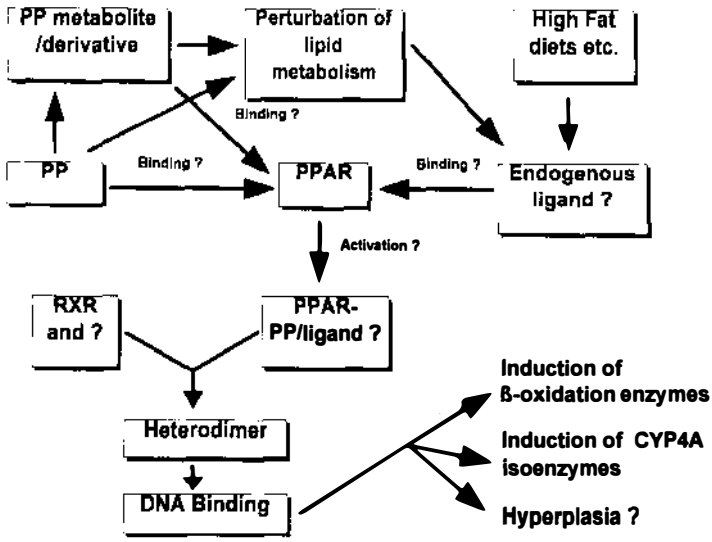

Figure 3 Some possible mechanisms of induction of peroxisome proliferation in rodent hepatocytes. The peroxisome proliferator (PP) may either bind directly to or otherwise activate one or more peroxisome proliferator–activated receptors (PPARs) that bind to DNA as a heterodimer with retinoid X receptor (RXR) and possibly other receptors. Alternatively, the PP may require metabolism (e.g. formation of an acidic function and/or a coenzyme A ester) that could interact with a PPAR either directly or via an endogenous ligand (a fatty acid?) formed as a consequence of a perturbation of lipid metabolism.

erators (3, 18, 20, 59). One human PPAR has been shown to be activated by rodent peroxisome proliferators in an in vitro expression system (70). As peroxisome proliferation does not appear to occur in humans to any significant extent (see below), further work is required to assess the significance of this study. Modulating factors may include levels of expression of PPARs, activators and repressors in human hepatocytes, and differences between the regulatory elements of human and rodent genes for enzymes such as acyl-CoA oxidase (62, 70, 79).

HEPATOCARCINOGENICITY IN RATS AND MICE

Many peroxisome proliferators have been reported to produce liver tumors, including hepatocellular carcinoma, in rats and/or in mice (3, 4, 11, 13, 17, 19). Potent compounds (e.g. ciprofibrate, Wy-14,643) may produce a 100% incidence of tumors in rat liver within a relatively short period (e.g. 40–60 weeks), whereas weaker agents (e.g. clofibrate, DEHP) may require two years of administration to produce a lower tumor incidence (3, 4, 17, 35, 80–82).

Although peroxisome proliferators may be hepatocarcinogenic in rats and mice, they are not considered to be genotoxic agents. Several studies have shown that peroxisome proliferators do not bind covalently to DNA after in vivo administration to rats and mice (13, 17, 19, 83, 84), and short-term administration does not result in DNA adducts identified by the sensitive ^{32}P-postlabeling technique (85). However, chronic administration for 18 months did produce DNA adducts, the nature of which awaits elucidation (84, 86). The chronic administration of peroxisome proliferators and other non-genotoxic carcinogens may lead to genetic alterations that can be detected by DNA adduct analysis. Peroxisome proliferators have been shown to be essentially negative in a range of short-term tests for genotoxicity, including the Ames test and unscheduled DNA synthesis (3, 13, 17, 19, 83, 84). Although a few positive results (e.g. chromosomal aberrations) have been reported (87–89), the significance of these findings is unclear; the positive results may be due to the perturbation of cellular processes rather than direct DNA damage (90).

Several mechanisms have been proposed to account for peroxisome proliferator–induced hepatocarcinogenesis in rodents. These hypotheses include (*a*) induction of sustained oxidative stress, (*b*) a role for enhanced cell replication, and (*c*) the promotion of spontaneous preneoplastic lesions. Some of the experimental data in support of these hypotheses are briefly reviewed below, together with other effects of peroxisome proliferators.

Oxidative Stress

The oxidative stress hypothesis (Figure 4) is based on the observation that chronic administration of peroxisome proliferators produces a sustained oxidative stress in rodent hepatocytes due to an imbalance in the production and degradation of hydrogen peroxide (4, 12, 15, 83). Peroxisome proliferators markedly induce the enzymes of the peroxisomal fatty acid β-oxidation cycle but produce only a small increase in catalase activity. The first enzyme of the β-oxidation cycle, acyl-CoA oxidase, unlike its mitochondrial counterpart, produces hydrogen peroxide; hence, the cyclic oxidation of a single fatty acid molecule can result in the production of several molecules of hydrogen peroxide (2, 19, 83). Any excess hydrogen peroxide not destroyed by peroxisomal catalase can easily diffuse through the peroxisomal membrane where it will be a substrate for cytosolic selenium-dependent glutathione peroxidase (Figure 4). However, this enzyme activity and that of other enzymes such as superoxide dismutase and glutathione *S*-transferases are often reduced by the administration of peroxisome proliferators (19, 25, 83, 91).

These enzyme changes are postulated to result in increased intracellular levels of hydrogen peroxide that can attack membranes and DNA either directly or via other reactive oxygen species (e.g. hydroxyl radical). Another

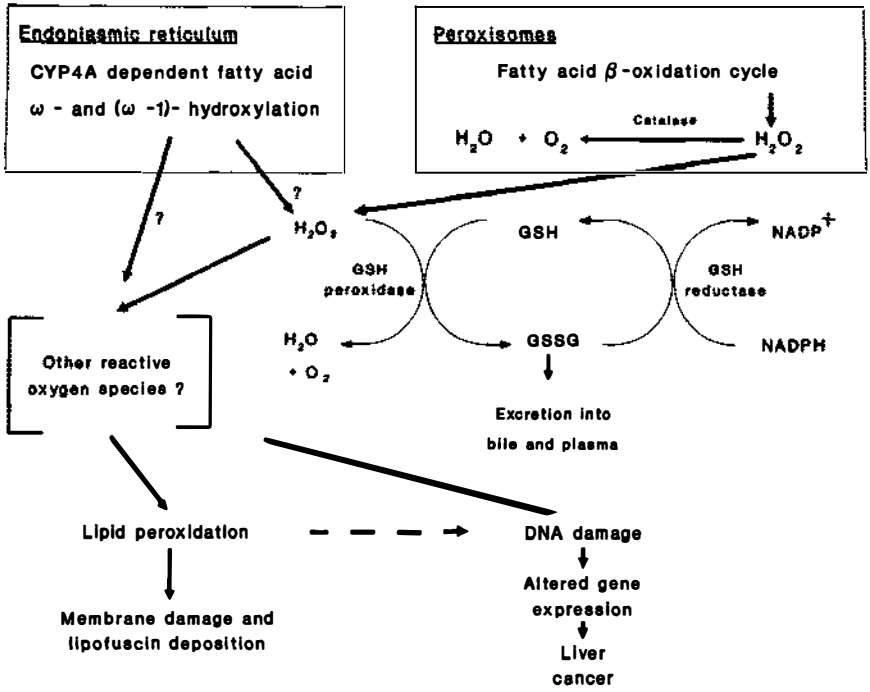

Figure 4 Postulated mechanism for the hepatocarcinogenicity of peroxisome proliferators caused by sustained oxidative stress (4, 12, 83). The imbalance in production and degradation of peroxisomal hydrogen peroxide results in increased cytosolic levels of this and other reactive oxygen species, which are believed to result in cell injury and ultimately tumor formation.

possible source of reactive oxygen species is CYP4A-dependent metabolism of fatty acids and other endogenous substrates (Figure 4). This source does not appear to have been extensively studied, although in one in vitro study with liver microsomes from ciprofibrate-treated rats, little hydrogen peroxide was formed in either the presence or absence of a fatty acid substrate (92). The reduction of glutathione peroxidase, superoxide dismutase, and glutathione *S*-transferase activities may result in a reduced ability of rodent hepatocytes to detoxify active forms of oxygen and organic hydroperoxides (e.g. products of lipid peroxidation). Conversely, peroxisome proliferators induce hepatic aldehyde dehydrogenase and cytosolic epoxide hydrolase activities (19, 27, 28). The induction of these enzymes could be construed as increasing the capacity of hepatocytes to detoxify certain aldehydes and fatty acid epoxides produced as a consequence of lipid peroxidation.

Several experimental observations support a role for enhanced oxidative

stress in the mechanism of toxicity of peroxisome proliferators in rodent liver. These observations include (*a*) increased peroxisomal hydrogen peroxide formation, (*b*) evidence from in vitro studies that fatty acid metabolism in peroxisomal fractions can result in hydroxyl radical formation and DNA damage, (*c*) increased lipid peroxidation, (*d*) increased lipofuscin deposition, (*e*) effects on levels of hepatic antioxidants, (*f*) inhibition of tumor formation by antioxidants, and (*g*) effects on hepatic DNA. Some of these data are briefly discussed below, but not all the available experimental data support the findings listed above. Investigations with liver homogenates, isolated hepatocytes, and isolated perfused liver systems have suggested that peroxisome proliferators can increase the capacity for hydrogen peroxide production in rodent hepatocytes (19). Two in vitro studies have demonstrated that the hydrogen peroxide formed from the metabolism of fatty acids by peroxisomal fractions from rats pretreated with peroxisome proliferators can result in both hydroxyl radical formation (93) and damage to added DNA (94). Although these studies appear to provide support for the oxidative stress hypothesis, it should be noted that cyanide was added to inhibit catalase activity. Hence, care needs to be taken in extrapolating these in vitro results to potential in vivo effects of peroxisome proliferators.

In some studies the chronic administration of peroxisome proliferators has been reported to increase lipid peroxidation and lipofuscin deposition in rodent liver (17, 19, 25, 83, 91, 95, 96). Lipofuscin deposition is a feature of the chronic administration of a number of peroxisome proliferators; the pigment is only observed in nonnodular and nontumorous portions of the liver (25, 96). The material is stored within hepatocytes in lysosomal residual bodies, and lipofuscin deposition is associated with the induction of lysosomal enzyme activities (24, 95). Because many peroxisome proliferators contain a free carboxyl group (Figure 1), they can form coenzyme A esters and, hence, can enter intermediary metabolism and be incorporated into triglycerides (56). Thus, lipofuscin deposition observed after treatment with some agents may not represent lipid peroxidation but, rather, may be due to the storage in lysosomes of modified triglycerides that are resistant to catabolism by intracellular lipases.

In some studies peroxisome proliferators have been reported to produce effects on levels of hepatic antioxidants, such as vitamin E and reduced glutathione (17, 19). Reddy and coworkers (81, 97) have compared the hepatocarcinogenicity of ciprofibrate in rats fed either a control diet or a diet containing one of two antioxidants, namely ethoxyquin and 2(3)-*tert*-butyl-4-hydroxyanisole (BHA). Treatment with ciprofibrate alone for 60 weeks produced a 100% incidence of liver tumors (81, 97). However, although all rats coadministered ciprofibrate with either ethoxyquin or BHA also had liver tumors, the total number and size of these tumors were reduced. Although this

study suggests that antioxidants may ameliorate against the magnitude of peroxisome proliferator–induced tumor formation, these results are at variance with those of other investigations. For example, the hepatocarcinogenicity of both ciprofibrate and nafenopin was greater in rats fed diets containing high levels of vitamin E than in those fed low levels of vitamin E (98, 99).

Oxygen radical attack on DNA is known to result in a variety of modified DNA bases including 8-hydroxydeoxyguanosine (8-OH-dG). Treatment with a number of peroxisome proliferators has been reported to increase levels of 8-OH-dG in rat hepatic DNA (100, 101). While elevated levels of 8-OH-dG appear to imply oxidative stress, such increases are small and do not correlate with potency. For example, there is little difference between the levels of 8-OH-dG produced in male F344 rats fed diets containing 0.025% ciprofibrate, 1.2% DEHP, or 2.5% DEHA (100, 101). However, although ciprofibrate is a potent hepatocarcinogen, DEHP produced only a small incidence of tumors after two years, and DEHA was not hepatocarcinogenic in the rat (80, 81, 102). Most studies of 8-OH-dG have utilized whole liver homogenates (i.e. nuclear and mitochondrial DNA), and normal levels of 8-OH-dG in mitochondrial DNA are 16-fold greater than in nuclear DNA (103). Indeed, recent studies suggest that peroxisome proliferators may increase 8-OH-dG levels primarily in mitochondrial DNA (104). A few studies have examined the effects of peroxisome proliferators on other modified DNA bases, and generally, treatment with peroxisome proliferators does not result in DNA strand breaks, as detected by the alkaline elution assay (18–20).

In summary, although evidence of oxidative damage to hepatocytes has been obtained in some studies, the magnitude of such effects does not correlate with the ability of peroxisome proliferators to produce tumors. Moreover, the literature contains many anomalous findings that question the probability of sustained oxidative stress being solely responsible for peroxisome proliferator–induced liver tumor formation.

Cell Replication

Many studies have demonstrated that cell proliferation is an important factor in the development of tumors by both genotoxic and nongenotoxic agents (105–107). For example, an enhanced rate of cell replication can increase the frequency of spontaneous mutations and the probability of converting DNA adducts from both endogenous and exogenous sources into mutations before they can be repaired. Moreover, cell replication is clearly important for the promotion and progression of initiated cells into tumors. Peroxisome proliferators are known to produce a burst of cell replication in rodent hepatocytes during the first few days of administration (4, 14, 19, 39–41, 108). In some, but not all, studies peroxisome proliferators have been shown to produce a sustained stimulation of replicative DNA synthesis (19, 39, 41, 109–115). The

majority of these studies have employed the more sensitive continuous infusion (e.g. osmotic pump), rather than the pulse-labeling technique, to administer the DNA precursor. For example, when rat hepatocyte labeling index values were determined over 7-day periods, Wy-14,643 produced a 3.8- to 11.6-fold sustained increase in replicative DNA synthesis between the 8th and 365th day of treatment (39). Apart from intrinsic compound potency, dose is an important factor in determining whether a particular peroxisome proliferator can produce either a transient or a sustained stimulation of cell replication in rodent hepatocytes. Thus low doses of Wy-14,643, methylclofenapate, and nafenopin do not produce a sustained stimulation of replicative DNA synthesis, whereas higher doses do produce this effect (113–116). Peroxisome proliferators may exert lobular differences in effects on replicative DNA synthesis. For example, while short-term administration of Wy-14,643 produces a panlobular stimulation of cell replication in the mouse (40), the acute and chronic administration of this and other peroxisome proliferators primarily produces a periportal stimulation of DNA synthesis in the rat (39–42).

Promotion of Liver Lesions

Peroxisome proliferators have been examined as initiators and promoters of rodent hepatocarcinogenesis (117). In keeping with their nongenotoxic properties, peroxisome proliferators do not produce positive results in initiation studies. However, when appropriate morphological markers are employed, peroxisome proliferators are efficient promoters of certain genotoxic carcinogen-induced lesions, and differences between peroxisome proliferators and other promoters such as sodium phenobarbitone have been reported (117, 118).

Several studies have demonstrated the presence of numerous foci of putative preneoplastic cells in the livers of untreated old rats and mice (119, 120). These lesions are considered to represent spontaneously initiated cells because they have similar biological characteristics to those of cells initiated by genotoxic carcinogens. The ability of peroxisome proliferators to produce tumors in young compared to in old rats has been investigated in studies with nafenopin (121) and Wy-14,643 (122). In both studies more liver adenomas and carcinomas were produced in old than in young rats, which is consistent with the promotion of spontaneously initiated hepatocytes by peroxisome proliferators. A study of Wy-14,643 in the rat has demonstrated a significant morphological continuity between peroxisome proliferator–induced basophilic foci and the development of tumors (123). Moreover, the basophilic foci were highly proliferative; they had cell replication rates higher than nonfocal hepatocytes in treated animals and many-fold higher than in hepatocytes in untreated rats. However, such foci appear to require the continued presence of the peroxisome proliferator (123), and in other studies some evidence for the reversibility of peroxisome proliferator–induced liver lesions has been reported (124, 125).

Other Factors

Several studies have shown that nongenotoxic carcinogens can affect apoptosis (i.e. gene-directed cell death) in rodent liver (126). Nafenopin has been reported to inhibit the apoptosis that occurs in rat liver after withdrawal of a mitogenic agent (127) and to inhibit apoptosis in hepatocytes and a rat hepatocyte cell line in vitro (128). In contrast, treatment with Wy-14,643 for 22 weeks was found to increase the incidence of apoptotic bodies in rat liver (112). Transforming growth factor–β_1 (TGF-β_1) is produced by the nonparenchymal cells of the liver, is a potent inhibitor of DNA synthesis in hepatocytes, and can also initiate apoptosis (129, 130). The insulin-like growth factor II–mannose-6-phosphate (IGFII-Man6P) receptor catalyzes the proteolytic activation of latent TGF-β_1 (131), and the administration of peroxisome proliferators has been shown to increase both TGF-β_1 and IGFII-Man6P receptor gene expression in rat liver (116, 132). The time course and lobular distribution of peroxisome proliferator effects on various growth factors and apoptosis in rodent liver may be important in the mechanism of tumor formation. Liver tumors formed by treatment with Wy-14,643 have been reported to contain only low levels of TGF-β_1 and the IGFII-Man6P receptor (133).

Peroxisome proliferators, like some other nongenotoxic carcinogens, have been reported to inhibit intercellular communication in rodent liver, which may be important in tumor promotion (134). Other studies have demonstrated that peroxisome proliferators can modulate intracellular calcium concentrations that may be associated with effects on various cellular signaling pathways, DNA synthesis, and possibly tumor promotion (135, 136). Other work has suggested that peroxisome proliferators inhibit acyl-CoA synthetase and stimulate protein kinase C, which may promote tumor formation (137).

SPECIES DIFFERENCES

Many studies have investigated species differences in hepatic peroxisome proliferation (3, 13, 14, 16, 18, 20, 138). Such studies have been conducted either in vivo or in vitro in primary hepatocyte cultures and have focused mainly on the measurement of organelle proliferation and induction of peroxisomal and microsomal enzyme activities. Clearly the rat and mouse may be considered responsive species to peroxisome proliferators. Studies with a variety of compounds—including ciprofibrate, clobuzarit, DEHP, LY 171883, and nafenopin—have demonstrated that the Syrian hamster exhibits an intermediate response, whereas in most studies the guinea pig is either nonresponsive or refractory (138–143).

When assessing species differences in response, several factors should be considered, including the disposition, metabolism, and dose of the test com-

pound; sex differences; and intrahepatic differences in response. For example, trichloroethylene produces peroxisome proliferation in the mouse but has little effect in the rat (48). Metabolic studies have demonstrated that trichloroethylene is extensively metabolized to trichloroacetic acid in mouse but not in rat hepatocytes and that trichloroacetic acid can produce peroxisome proliferation in rat and mouse hepatocytes both in vivo and in vitro (48). An example of compound disposition is provided by DEHP, which is known to be more extensively absorbed after oral administration in the rat than in the marmoset (144). However, the observed species difference is supported by the observation that DEHP metabolites produce peroxisome proliferation in rat hepatocytes in vitro but not in marmoset hepatocytes (33, 34). In general, in vitro studies with rat, mouse, Syrian hamster, guinea pig, and primate hepatocytes have paralleled the results of in vivo studies in these species (18, 20, 34, 47–51).

Several in vivo studies in primates have failed to provide any evidence of significant peroxisome proliferation in both New and Old World monkeys (18, 20, 138). However, studies with ciprofibrate and DL-040 have reported peroxisome proliferation in primates, albeit at high doses (145, 146). Several studies have been conducted on humans receiving hypolipidemic agents (e.g. ciprofibrate, clofibrate, fenofibrate, and gemfibrozil) that are known to produce peroxisome proliferation in rodent liver (20). Most studies have failed to detect any significant changes, although clofibrate was reported to produce a small increase in the number of peroxisomes, and ciprofibrate reportedly produced a small increase in the proportion of the hepatocyte cytoplasm occupied by peroxisomes (20, 147). However, owing to the observed large interindividual variation in peroxisome morphometrics, together with cell-to-cell and lobular variations, it is difficult to attach any clear significance to these findings.

Few studies have evaluated species differences in cell replication and hepatocarcinogenesis. Although nafenopin and Wy-14,643 are potent mitogens in rat liver, they do not appear to stimulate replicative DNA synthesis in Syrian hamster hepatocytes after either acute or chronic treatment (114, 115). Peroxisome proliferators can stimulate DNA synthesis in cultured rat hepatocytes, but methylclofenapate has been shown not to increase DNA synthesis in guinea pig, marmoset, and human hepatocytes, and nafenopin was also ineffective in human hepatocytes (51, 148, 149). In one report nafenopin did increase DNA synthesis in marmoset hepatocytes, but this finding was dependent on the particular culture conditions that were used (150). With respect to tumor formation, both nafenopin and Wy-14,643 produced liver nodules and hepatocellular carcinoma after 60 weeks in rats; they did not produce such lesions in Syrian hamsters (115). Two studies have suggested that peroxisome proliferators may not produce liver lesions in marmosets. In an ongoing study ciprofibrate has been shown not to produce liver lesions after three years'

treatment (151), and in a 6.5-year study clofibrate was shown not to increase liver weight nor to produce tumors (152).

CONCLUSIONS

Key issues concerning the risk assessment to humans of rodent liver peroxisome proliferators include (*a*) genotoxicity; (*b*) compound potency, no effect levels, and likely human exposure; (*c*) precise mechanism(s) of hepatocarcinogenesis; and (*d*) species differences in response.

Peroxisome proliferators are generally considered nongenotoxic agents (20, 84) that should be assessed differently than genotoxic carcinogens (153). Human exposure is dependent on the usage of the particular chemical: While hypolipidemic agents are administered only to a restricted population of humans, exposure to plasticizers (e.g. DEHP, DEHA) is more widespread. Compound potency to produce both peroxisome proliferation and liver tumors varies greatly, and it should be noted that rodent peroxisome proliferators exhibit clear no-effect levels for peroxisome proliferation and for tumor formation (Figure 5). For example, in the rat no-effect levels for tumor formation have been observed in studies with bezafibrate, clofibrate, DEHA, or DEHP (80, 102, 154). Moreover, the threshold for tumor formation in rodents (Figure 5) is appreciably higher than the threshold for peroxisome proliferation and other effects, as illustrated in Figure 2 and in other studies (20, 35, 154).

Several mechanisms have been proposed to account for why peroxisome

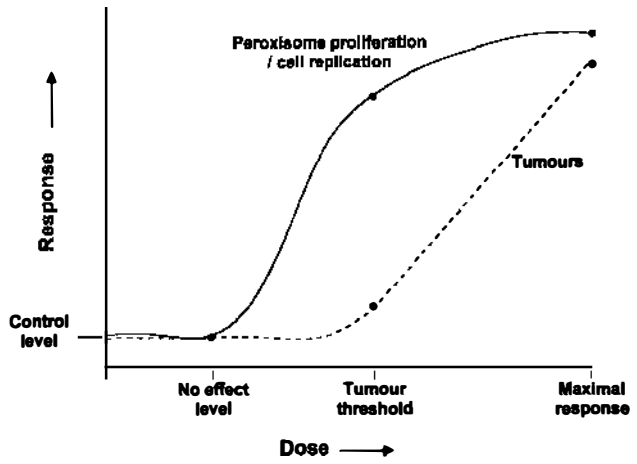

Figure 5 Dose-response relationships for peroxisome proliferation, cell replication, and tumor formation in rodent liver. Many studies have demonstrated that there are clear no-effect levels for organelle proliferation, induction of enzyme activities, etc, but that the threshold for liver tumor formation occurs at higher doses (i.e. where there is sustained stimulation of peroxisome proliferation and possibly cell replication, etc).

proliferators produce tumors in rodent liver. Although some studies have demonstrated that peroxisome proliferation may produce oxidative damage in hepatocytes, sustained oxidative stress does not appear to be solely responsible for peroxisome proliferator–induced hepatocarcinogenesis. In general, there is a poor quantitative correlation between markers of oxidative stress (e.g. levels of lipid peroxidation, 8-OH-dG in DNA, etc) and compound potency to produce tumors. In addition, the oxidative stress hypothesis suggests that there should be some correlation between the magnitude of peroxisome proliferation (in particular the balance between hydrogen peroxide–producing and –degrading enzymes) and subsequent tumor formation. For example, in Figure 2*B* liver tumors are produced in male F344 rats by 0.01 and 0.02% ciprofibrate and 2.0% DEHP but not by ≤2.0% DEHA (35, 80–82, 102). Although potent peroxisome proliferators are more likely to produce liver tumors than weaker agents, the magnitude of peroxisome proliferation does not always correlate with tumor formation. Indeed peroxisome proliferators (e.g. Wy-14,643, methylclofenapate) at dose levels that produce a sustained stimulation of cell replication produce liver tumors more rapidly than other agents (e.g. DEHP, clofibric acid) that produce a similar magnitude of peroxisome proliferation but not a sustained stimulation of cell replication (4, 19, 39, 112, 115).

If the various mechanisms for peroxisome proliferator–induced hepatocarcinogenesis are combined, then a role for increased cell replication in tumor formation is readily identified (Figure 6). Thus if hepatocytes are transformed by either oxidative stress or alternative mechanisms, such initiated cells may be promoted and progress to tumors by enhanced cell replication. Peroxisome

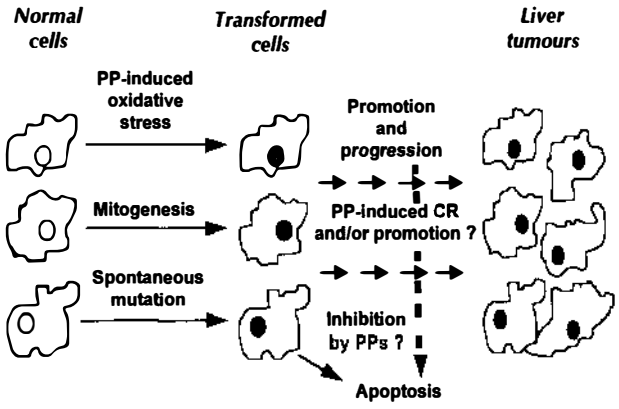

Figure 6 Some possible mechanisms of peroxisome proliferator (PP)–induced hepatocarcinogenicity. Hepatocytes may become initiated by sustained oxidative stress–induced damage, by enhanced cell replication (CR), or spontaneously. Growth selection of initiated cells and their promotion and progression to hepatocellular carcinoma may involve enhanced CR, effects on growth factors, and inhibition of apoptosis.

proliferators are effective promoters of particular populations of initiated cells, and peroxisome proliferators can influence the rates of cell replication and cell death in particular populations of hepatocytes (112, 117, 120, 123). Thus the basophilic nodules that appear to be precursors of peroxisome proliferator–induced tumors have high cell replication rates and peroxisome proliferators can also affect rates of apoptosis and growth factor expression (e.g. TGF-β_1) in rodent liver (123, 126, 128, 131, 132). Further studies are required to elucidate the precise mechanism(s) of peroxisome proliferator–induced tumor formation in rodent liver, although it is clear that both peroxisome proliferation and cell replication are important biomarkers.

With respect to species differences in response, most studies suggest that the rat and mouse are responsive, but primates and humans are either much less responsive or essentially refractory. Apart from the observed lack of effect on organelle proliferation and induction of peroxisomal and microsomal fatty acid–oxidizing enzymes, the limited data available suggest that species such as the Syrian hamster and primates are not susceptible to peroxisome proliferator–induced liver tumor formation.

Current literature suggests that rodent peroxisome proliferators are nongenotoxic agents that as a class of chemicals do not appear to pose any serious hazard for humans. However, further elucidation of the mechanism(s) of liver tumor formation in susceptible species (i.e. rat and mouse) is desirable. From such studies the most appropriate biomarkers of tumor formation could be identified and examined in studies of species differences, possibly including in vitro studies with human hepatocytes. Finally, further carcinogenicity studies in partially (e.g. Syrian hamster) and essentially nonresponsive (e.g. guinea pig) species would strengthen the conclusion that peroxisome proliferators do not constitute any significant hazard to humans.

ACKNOWLEDGMENTS

I am most grateful to the UK Ministry of Agriculture, Fisheries, and Food for their generous financial support of BIBRA studies on hepatic peroxisome proliferation.

Literature Cited

1. DeDuve C, Baudhuin P. 1966. Peroxisomes (microbodies and related particles). *Physiol. Rev.* 46:323–57
2. Lazarow PB, DeDuve C. 1976. A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. USA* 73:2043–46
3. Cohen AJ, Grasso P. 1981. Review of the hepatic response to hypolipidaemic

drugs in rodents and assessment of its toxicological significance to man. *Food Chem. Toxicol.* 19:585–605
4. Reddy JK, Lalwani ND. 1983. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* 12:1–58
5. Weibel ER, Stäubli W, Gnägi HR, Hess RA. 1969. Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereological methods and normal morphometric data for rat liver. *J. Cell Biol.* 42:68–112
6. Eacho PI, Feller DR. 1991. Hepatic peroxisome proliferation induced by hypolipidemic drugs and other chemicals. In *Antilipidemic Drugs, Medicinal, Chemical and Biochemical Aspects*, ed. DT Witiak, HAI Newman, DR Feller, pp. 375–426. Amsterdam: Elsevier Sci.
7. Mannaerts GP, Van Veldhoven PP. 1993. Metabolic role of mammalian peroxisomes. See Ref. 155, pp. 19–62
8. Wanders RJA, Barth PG, Schutgens RBH, Tager JM. 1993. Peroxisomal disorders. See Ref. 155, pp. 63–98
9. Paget GE. 1963. Experimental studies of the toxicity of Atromid with particular reference to the fine structural changes in the livers of rodents. *J. Atheroscler. Res.* 3:729–37
10. Hess R, Stäubli W, Reiss W. 1965. Nature of the hepatomegalic effect produced by ethyl chlorophenoxyisobutyrate in the rat. *Nature* 208:856–58
11. Reddy JK, Azarnoff DL, Hignite CE. 1980. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283:397–98
12. Rao MS, Reddy JK. 1987. Peroxisome proliferation and hepatocarcinogenesis. *Carcinogenesis* 8:631–36
13. Stott WT. 1988. Chemically induced proliferation of peroxisomes: implications for risk assessment. *Regul. Toxicol. Pharmacol.* 8:125–59
14. Moody DE, Reddy JK, Lake BG, Popp JA, Reese DH. 1991. Peroxisome proliferation and nongenotoxic carcinogenesis: commentary on a symposium. *Fund. Appl. Toxicol.* 16:233–48
15. Rao MS, Reddy JK. 1991. An overview of peroxisome proliferator–induced hepatocarcinogenesis. *Environ. Health Perspect.* 93:205–9
16. Hawkins JM, Jones WE, Bonner FW, Gibson GG. 1987. The effect of peroxisome proliferators on microsomal, peroxisomal, and mitochondrial enzyme activities in the liver and kidney. *Drug Metab. Rev.* 18:441–515
17. Conway JG, Cattley RC, Popp JA, Butterworth BE. 1989. Possible mechanisms in hepatocarcinogenesis by the peroxisome proliferator di(2-ethylhexyl)phthalate. *Drug Metab. Rev.* 21:65–102
18. Lock EA, Mitchell AM, Elcombe CR. 1989. Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu. Rev. Pharmacol. Toxicol.* 29:145–63
19. Lake BG. 1993. Role of oxidative stress and enhanced cell replication in the hepatocarcinogenicity of peroxisome proliferators. See Ref. 155, pp. 595–618
20. Bentley P, Calder I, Elcombe C, Grasso P, Stringer D, Wiegand H-J. 1993. Hepatic peroxisome proliferation in rodents and its significance for humans. *Food Chem. Toxicol.* 31:857–907
21. Lake BG, Lewis DFV. 1993. Structure-activity relationships for chemically induced peroxisome proliferation in mammalian liver. See Ref. 155, pp. 313–42
22. Hinton RH, Price SC. 1993. Extrahepatic peroxisome proliferation and the extrahepatic effects of peroxisome proliferators. See Ref. 155, pp. 487–511
23. Gibson GG. 1989. Comparative aspects of the mammalian cytochrome P450 IV gene family. *Xenobiotica* 19:1123–48
24. Grasso P. 1993. Hepatic changes associated with peroxisome proliferation. See Ref. 155, pp. 639–52
25. Lake BG, Kozlen SL, Evans JG, Gray TJB, Young PJ, Gangolli SD. 1987. Effect of prolonged administration of clofibric acid and di-(2-ethylhexyl)-phthalate on hepatic enzyme activities and lipid peroxidation in the rat. *Toxicology* 44:213–28
26. Berge RK, Kryvi H, Aarsaether N, Aarsland A, Lillehaug JR, Skorve J. 1993. Proliferation of peroxisomes and mitochondria and modulation of lipid metabolizing enzymes by hypolipidaemic non-β-oxidizable fatty acids (3-thia fatty acids). See Ref. 155, pp. 277–97
27. Fournel S, Magdalou J, Pinon P, Siest G. 1987. Differential induction profile of drug-metabolizing enzymes after treatment with hypolipidaemic agents. *Xenobiotica* 17:445–57
28. Moody DE, Gibson GG, Grant DF, Magdalou J, Rao MS. 1992. Peroxisome proliferators, a unique set of drug-metabolizing enzyme inducers: commentary on a symposium. *Drug Metab. Dispos.* 20:779–91
29. Lake BG, Lewis DFV, Gray TJB. 1988. Structure-activity relationships for he-

patic peroxisome proliferation. *Arch. Toxicol. Suppl.* 12:217–24
30. Eacho PI, Foxworthy PS, Herron DK. 1993. Tetrazole substituted acetophenone peroxisome proliferators: structure-activity relationships and effects on lipid hepatic metabolism. See Ref. 155, pp. 343–72
31. Lewis DFV, Lake BG. 1993. Interaction of some peroxisome proliferators with the mouse liver peroxisome proliferator-activated receptor (PPAR): a molecular modelling and quantitative structure-activity relationship study. *Xenobiotica* 23: 79–96
32. Thornber CW. 1979. Isosterism and molecular modification in drug design. *Chem. Soc. Rev.* 8:563–80
33. Mitchell AM, Lhuguenot J-C, Bridges JW, Elcombe CR. 1985. Identification of the proximate peroxisome proliferator(s) derived from di(2-ethylhexyl)phthalate. *Toxicol. Appl. Pharmacol.* 80:23–32
34. Elcombe CR, Mitchell AM. 1986. Peroxisome proliferation due to di(2-ethylhexyl)phthalate (DEHP): species differences and possible mechanisms. *Environ. Health Perspect.* 70:211–19
35. Reddy JK, Reddy MK, Usman MI, Lalwani ND, Rao MS. 1986. Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl)phthalate, and di(2-ethylhexyl)adipate with a hypolipidemic drug. *Environ. Health Perspect.* 65:317–27
36. Bieber LL, Krahling JB, Clarke PRH, Valkner KJ, Tolbert NE. 1981. Carnitine acyltransferases in rat liver peroxisomes. *Arch. Biochem. Biophys.* 211:599–604
37. Ishii H, Fukumori N, Horie S, Suga T. 1980. Effects of fat content in the diet on hepatic peroxisomes of the rat. *Biochim. Biophys. Acta* 617:1–11
38. Bell DR, Bars RG, Gibson GG, Elcombe CR. 1991. Localization and differential induction of cytochrome P450 IVA and acyl-CoA oxidase in rat liver. *Biochem. J.* 275:247–52
39. Marsman DS, Cattley RC, Conway JG, Popp JA. 1988. Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (Wy-14,643) in rats. *Cancer Res.* 48:6739–44
40. Eldridge SR, Tilbury LF, Goldsworthy TL, Butterworth BE. 1990. Measurement of chemically induced cell proliferation in rodent liver and kidney: a comparison of 5-bromo-2′-deoxyuridine and [^{3}H]thymidine administered by injection or osmotic pump. *Carcinogenesis* 11:2245–51
41. Eacho PI, Lanier TL, Brodhecker CA. 1991. Hepatocellular DNA synthesis in rats given peroxisome proliferating agents: comparison of WY-14,643 to clofibric acid, nafenopin and LY 171883. *Carcinogenesis* 12:1557–61
42. Barrass NC, Price RJ, Lake BG, Orton TC. 1993. Comparison of the acute and chronic mitogenic effects of the peroxisome proliferators methylclofenapate and clofibric acid in rat liver. *Carcinogenesis* 14:1451–56
43. Lin LI-K. 1987. The use of multivariate analysis to compare peroxisome induction data on phthalate esters in rats. *Toxicol. Ind. Health* 3:25–47
44. Sharma R, Lake BG, Foster R, Gibson GG. 1988. Microsomal cytochrome P-452 induction and peroxisome proliferation by hypolipidaemic agents in liver. A mechanistic inter-relationship. *Biochem. Pharmacol.* 37:1193–201
45. Lake BG, Gray TJB, Pels Rijcken WR, Beamand JA, Gangolli SD. 1984. The effect of hypolipidaemic agents on peroxisomal β-oxidation and mixed-function oxidase enzyme activities in primary cultures of rat hepatocytes. Relationship between induction of palmitoyl-CoA oxidation and lauric acid hydroxylation. *Xenobiotica* 14:269–76
46. Gray TJB, Beamand JA, Lake BG, Foster JR, Gangolli SD. 1982. Peroxisome proliferation in cultured rat hepatocytes produced by clofibrate and phthalate ester metabolites. *Toxicol. Lett.* 10:273–79
47. Lake BG, Gray TJB, Gangolli SD. 1986. Hepatic effects of phthalate esters and related compounds—*in vivo* and *in vitro* correlations. *Environ. Health Perspect.* 67:283–90
48. Elcombe CR. 1985. Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: a biochemical human hazard assessment. *Arch. Toxicol. Suppl.* 8:6–17
49. Butler EG, England PJ, Williams GM. 1988. Genetic differences in enzymes associated with peroxisome proliferation and hydrogen peroxide metabolism in inbred mouse strains. *Carcinogenesis* 9: 1459–63
50. Bieri F. 1993. Cultured hepatocytes: a useful *in vitro* system to investigate effects induced by peroxisome proliferators and their species specificity. See Ref. 155, pp. 299–311

51. Foxworthy PS, Eacho PI. 1994. Cultured hepatocytes for studies of peroxisome proliferation: methods and applications. *J. Pharmacol. Toxicol. Methods* 31:21–30
52. Osumi T, Yokota S, Hashimoto T. 1990. Proliferation of peroxisomes and induction of peroxisomal β-oxidation enzymes in rat hepatoma H4IIEC3 by ciprofibrate. *J. Biochem.* 108:614–21
53. Roberts RA, Soames AR. 1993. Hepatocyte spheroids: prolonged hepatocyte viability for *in vitro* modeling of non-genotoxic carcinogenesis. *Fund. Appl. Toxicol.* 21:149–58
54. Beamand JA, Price RJ, Cunninghame ME, Lake BG. 1993. Culture of precision-cut liver slices: effect of some peroxisome proliferators. *Food Chem. Toxicol.* 31:137–47
55. Bell DR, Elcombe CR. 1993. Peroxisome proliferation—lipid metabolism and receptors. See Ref. 155, pp. 137–47
56. Bronfman M. 1993. Possible role of CoASH esters on xenobiotic-induced peroxisomal proliferation and non-genotoxic carcinogenesis. See Ref. 155, pp. 217–45
57. Milton MN, Elcombe CR, Kass GEN, Gibson GG. 1988. Lack of evidence for a hepatic peroxisome proliferator receptor and an explanation for the binding of hypolipidaemic drugs to liver homogenates. *Biochem. Pharmacol.* 37:793–98
58. Alvares K, Carrillo A, Yuan PM, Kawano H, Morimoto RI, Reddy JK. 1990. Identification of cytosolic peroxisome proliferator binding protein as a member of the heat shock protein HSP70 family. *Proc. Natl. Acad. Sci. USA* 87: 5293–97
59. Issemann I, Green S. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 347:645–50
60. Green S, Issemann I, Tugwood JD. 1993. The molecular mechanism of peroxisome proliferator action. See Ref. 155, pp. 99–118
61. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli, W. 1992. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 68:879–87
62. TugwoodJD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S. 1992. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl CoA oxidase gene. *EMBO J.* 11:433–39
63. Muerhoff AS, Griffin KJ, Johnson EF. 1992. The peroxisome proliferator-activated receptor mediates the induction of *CYP4A6*, a cytochrome P450 fatty acid ω-hydroxylase, by clofibric acid. *J. Biol. Chem.* 267:19,051–53
64. Chen F, Law SW, O'Malley BW. 1993. Identification of two mPPAR related receptors and evidence for the existence of five subfamily members. *Biochem. Biophys. Res. Commun.* 196:671–77
65. Zhu Y, Alvares K, Huang Q, Rao MS, Reddy JK. 1993. Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver. *J. Biol. Chem.* 268:26,817–20
65a. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, et al. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* 91:7355–59
66. Göttlicher M, Widmark E, Li Q, Gustafsson J-Å. 1992. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA* 89:4653–57
67. Gebel T, Arand M, Oesch F. 1992. Induction of the peroxisome proliferator activated receptor by fenofibrate in rat liver. *FEBS Lett.* 309:37–40
68. Krey G, Keller H, Mahfoudi A, Medin J, Ozato K, et al. 1993. *Xenopus* peroxisome proliferator activated receptors: genomic organization, response element recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *J. Steroid Biochem. Mol. Biol.* 47:1–6
69. Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA. 1992. Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol. Endocrinol.* 6: 1634–41
70. Sher T, Yi H-F, McBride OW, Gonzalez FJ. 1993. cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* 32: 5598–604
71. Morrison V, Elcombe CR. 1995. Peroxisome proliferator-activated receptor: ligand binding studies. *Xenobiotica* 25: In press
72. Göttlicher M, Demoz A, Svensson D, Tollet P, Berge RK, Gustafsson J-Å. 1993. Structural and metabolic requirements for activators of the peroxisome proliferator-activated receptor. *Biochem. Pharmacol.* 46:2177–84
73. Green S, Chambon P. 1988. Nuclear

receptors enhance our understanding of transcription regulation. *Trends Genet.* 4:309–14

74. Huang Q, Alvares K, Chu R, Bradfield CA, Reddy JK. 1994. Association of peroxisome proliferator-activated receptor and Hsp 72. *J. Biol. Chem.* 269: 8493–97
75. Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. 1992. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* 358:771–74
76. Green S. 1993. Nuclear hormone receptors. Promiscuous liaisons. *Nature* 361: 590–91
77. Bardot O, Aldridge TC, Latruffe N, Green S. 1993. PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. *Biochem. Biophys. Res. Commun.* 192:37–45
78. Miyata KS, Zhang B, Marcus SL, Capone JP, Rachubinski RA. 1993. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds to a peroxisome proliferator-responsive element and antagonizes peroxisome proliferator-mediated signaling. *J. Biol. Chem.* 268:19,169–72
79. Varanasi U, Chu R, Chu S, Espinosa R, LeBeau MM, Reddy JK. 1994. Isolation of the human peroxisomal acyl-CoA oxidase gene: organization, promoter analysis, and chromosomal localization. *Proc. Natl. Acad. Sci. USA* 91:3107–11
80. National Toxicology Program. 1982. Carcinogenesis bioassay of di(2-ethylhexyl)phthalate (CAS No. 117–81–7) in F344 rats and B6C3F1 mice (feed study). *Tech. Rep. Ser.* No. 217
81. Rao MS, Lalwani ND, Watanabe TK, Reddy JK. 1984. Inhibitory effect of antioxidants ethoxyquin and 2(3)-*tert*-butyl-4-hydroxyanisole on hepatic tumorigenesis in rats fed ciprofibrate, a peroxisome proliferator. *Cancer Res.* 44: 1072–76
82. Milano M, Reddy JK, Prasad JD, Rao MS. 1987. Absence of γ-glutamyltranspeptidase activity in lung metastasis in rats with hepatocellular carcinomas induced by ciprofibrate, a peroxisome proliferator. *Cancer Lett.* 38:65–71
83. Reddy JK, Rao MS. 1989. Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogenesis. *Mutat. Res.* 214:63–68
84. Budroe JD, Williams GM. 1993. Genotoxicity studies of peroxisome proliferators. See Ref. 155, pp. 525–68
85. Gupta RC, Goel SK, Earley K, Singh B, Reddy JK. 1985. ^{32}P-Postlabeling analysis of peroxisome proliferator-DNA adduct formation in rat liver *in vivo* and hepatocytes *in vitro*. *Carcinogenesis* 6:933–36
86. Randerath E, Randerath K, Reddy R, Danna TF, Rao MS, Reddy JK. 1991. Induction of rat liver DNA alterations by chronic administration of peroxisome proliferators as detected by ^{32}P-postlabeling. *Mutat. Res.* 247:65–76
87. Hwang J-J, Hsia MTS, Jirtle RL. 1993. Induction of sister chromatid exchange and micronuclei in primary cultures of rat and human hepatocytes by the peroxisome proliferator, Wy-14,643. *Mutat. Res.* 286:123–33
88. Reisenbichler H, Eckl PM. 1993. Genotoxic effects of selected peroxisome proliferators. *Mutat. Res.* 286:135–44
89. Tsutsui T, Watanabe E, Barrett JC. 1993. Ability of peroxisome proliferators to induce cell transformation, chromosome aberrations and peroxisome proliferation in cultured Syrian hamster embryo cells. *Carcinogenesis* 14:611–18
90. Williams GM. 1989. Methods for evaluating chemical genotoxicity. *Annu. Rev. Pharmacol. Toxicol.* 29:189–211
91. Goel SK, Lalwani ND, Reddy JK. 1986. Peroxisome proliferation and lipid peroxidation in rat liver. *Cancer Res.* 46: 1324–30
92. Capdevila J, Kim YR, Martin-Wixtrom C, Falck JR, Manna S, Estabrook RW. 1985. Influence of a fibric acid type of hypolipidemic agent on the oxidative metabolism of arachidonic acid by liver microsomal cytochrome P-450. *Arch. Biochem. Biophys.* 243:8–19
93. Elliott BM, Dodd NJF, Elcombe CR. 1986. Increased hydroxyl radical production in liver peroxisomal fractions from rats treated with peroxisome proliferators. *Carcinogenesis* 7:795–99
94. Fahl WE, Lalwani ND, Watanabe T, Goel SK, Reddy JK. 1984. DNA damage related to increased hydrogen peroxide generation by hypolipidemic drug-induced liver peroxisomes. *Proc. Natl. Acad. Sci. USA* 81:7827–30
95. Conway JG, Tomaszewski KE, Olson MJ, Cattley RC, et al. 1989. Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and Wy-14,643. *Carcinogenesis* 10: 513–19
96. Reddy JK, Lalwani ND, Reddy MK, Qureshi SA. 1982. Excessive accumulation of autofluorescent lipofuscin in the liver during hepatocarcinogenesis by methyl clofenapate and other hypo-

lipidemic peroxisome proliferators. *Cancer Res.* 42:259–66

97. Lalwani ND, Reddy MK, Qureshi SA, Moehle CM, Hayashi H, Reddy JK. 1983. Noninhibitory effect of antioxidants ethoxyquin, 2(3)-*tert*-butyl-4-hydroxyanisole and 3,5-di-*tert*-butyl-4-hydroxytoluene on hepatic peroxisome proliferation and peroxisomal fatty acid β-oxidation induced by a hypolipidemic agent in rats. *Cancer Res.* 43:1680–87
98. Glauert HP, Beaty MM, Clark TD, Greenwell WS, Tatum V, et al. 1990. Effect of dietary vitamin E on the development of altered hepatic foci and hepatic tumors induced by the peroxisome proliferator ciprofibrate. *J. Cancer Res. Clin. Oncol.* 116:351–56
99. Lake BG, Evans JG, Walters DG, Price RJ. 1991. Comparison of the hepatic effect of nafenopin, a peroxisome proliferator, in rats fed adequate or vitamin E- and selenium-deficient diets. *Hum. Exp. Toxicol.* 10:87–88
100. Kasai H, Okada Y, Nishimura S, Rao MS, Reddy JK. 1989. Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator. *Cancer Res.* 49:2603–5
101. Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y. 1993. Production of 8-hydroxydeoxyguanosine in rodent liver by the administration of peroxisome proliferators. See Ref. 155, pp. 569–94
102. National Toxicology Program. 1982. Carcinogenesis bioassay of di(2-ethylhexyl)adipate (CAS No. 103–23–1) in F344 rats and B6C3F1 mice (feed study). *Tech. Rep. Ser.* No. 212
103. Richter C, Park J-W, Ames BN. 1988. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. USA* 85: 6465–67
104. Cattley RC, Glover SE. 1993. Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: relationship to carcinogenesis and nuclear localization. *Carcinogenesis* 14:2495–99
105. Ames BN, Gold LS. 1991. Endogenous mutagens and the causes of aging and cancer. *Mutat. Res.* 250:3–16
106. Cohen SM, Ellwein LB. 1990. Cell proliferation in carcinogenesis. *Science* 249:1007–11
107. Cohen SM, Ellwein LB. 1991. Genetic errors, cell proliferation and carcinogenesis. *Cancer Res.* 51:6493–505
108. Reddy JK, Rao MS, Azarnoff DL, Sell S. 1979. Mitogenic and carcinogenic effects of a hypolipidemic peroxisome proliferator, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14,643), in rat and mouse liver. *Cancer Res.* 39:152–61
109. Ward JM, Hagiwara A, Anderson LM, Lindsey K, Diwan BA. 1988. The chronic hepatic or renal toxicity of di(2-ethylhexyl)phthalate, acetaminophen, sodium barbital, and phenobarbital in male B6C3F1 mice: autoradiographic, immunohistochemical, and biochemical evidence for levels of DNA synthesis not associated with carcinogenesis or tumor promotion. *Toxicol. Appl. Pharmacol.* 96:494–506
110. Yeldandi AV, Milano M, Subbarao V, Reddy JK, Rao MS. 1989. Evaluation of liver cell proliferation during ciprofibrate-induced hepatocarcinogenesis. *Cancer Lett.* 47:21–27
111. Rao MS, Subbarao V, Yeldandi AV, Reddy JK. 1992. Hepatocarcinogenicity of dehydroepiandrosterone in the rat. *Cancer Res.* 52:2977–79
112. Marsman DS, Goldsworthy TL, Popp JA. 1992. Contrasting hepatocytic peroxisome proliferation, lipofuscin accumulation and cell turnover for the hepatocarcinogens Wy-14,643 and clofibric acid. *Carcinogenesis* 13:1101–17
113. Wada N, Marsman DS, Popp JA. 1992. Dose-related effects of the hepatocarcinogen, Wy-14,643, on peroxisomes and cell replication. *Fund. Appl. Toxicol.* 18:149–54
114. Price RJ, Evans JG, Lake BG. 1992. Comparison of the effects of nafenopin on hepatic peroxisome proliferation and replicative DNA synthesis in the rat and Syrian hamster. *Food Chem. Toxicol.* 30:937–44
115. Lake BG, Evans JG, Cunninghame ME, Price RJ. 1993. Comparison of the hepatic effects of nafenopin and WY-14,643 on peroxisome proliferation and cell replication in the rat and Syrian hamster. *Environ. Health Perspect.* 101: 241–48
116. Lake BG, Rumsby PC, Cunninghame ME, Davies MJ, Price RJ. 1994. Effect of methylclofenapate (MCP) on peroxisome proliferation, cell replication and transforming gr wth factor-β_1 (TGF-β_1) gene expression in rat liver. *Toxicologist* 14:301
117. Popp JA, Cattley RC. 1993. Peroxisome proliferators as initiators and promoters of rodent hepatocarcinogenesis. See Ref. 155, pp. 653–65
118. Cattley RC, Popp JA. 1989. Differences between the promoting activities of the peroxisome proliferator WY-14,643 and

phenobarbital in rat liver. *Cancer Res.* 49:3246–51

119. Schulte-Hermann R, Timmermann-Trosiener I, Schuppler J. 1983. Promotion of spontaneous preneoplastic cells in rat liver as a possible explanation of tumor production by nonmutagenic compounds. *Cancer Res.* 43:839–44
120. Grasl-Kraupp B, Huber W, Schulte-Hermann R. 1993. Are peroxisome proliferators tumour promoters in rat liver? See Ref. 155, pp. 667–93
121. Kraupp-Grasl B, Huber W, Taper H, Schulte-Hermann R. 1991. Increased susceptibility of aged rats to hepatocarcinogenesis by the peroxisome proliferator nafenopin and the possible involvement of altered liver foci occurring spontaneously. *Cancer Res.* 51: 666–71
122. Cattley RC, Marsman DS, Popp JA. 1991. Age-related susceptibility to the carcinogenic effect of the peroxisome proliferator WY-14,643 in rat liver. *Carcinogenesis* 12:469–73
123. Marsman DS, Popp JA. 1994. Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14,643. *Carcinogenesis* 15:111–17
124. Svoboda DJ, Azarnoff DL. 1979. Tumors in male rats fed ethyl chlorophenoxyisobutyrate, a hypolipidemic drug. *Cancer Res.* 39:3419–28
125. Greaves P, Irisarri E, Monro AM. 1986. Hepatic foci of cellular and enzymatic alteration and nodules in rats treated with clofibrate or diethylnitrosamine followed by phenobarbital: their rate of onset and their reversibility. *J. Natl. Cancer Inst.* 76:475–84
126. Bursch W, Oberhammer F, Schulte-Hermann R. 1992. Cell death by apoptosis and its protective role against disease. *Trends Pharmacol. Sci.* 13: 245–51
127. Bursch W, Düsterberg B, Schulte-Hermann R. 1986. Growth, regression and cell death in rat liver as related to tissue levels of the hepatomitogen cyproterone acetate. *Arch. Toxicol.* 59:221–27
128. Bayly AC, Roberts RA, Dive C. 1994. Suppression of liver cell apoptosis *in vitro* by the non-genotoxic hepatocarcinogen and peroxisome proliferator nafenopin. *J. Cell Biol.* 125:197–203
129. Massagué J. 1990. The transforming growth factor-β family. *Annu. Rev. Cell Biol.* 6:597–641
130. Oberhammer FA, Pavelka M, Sharma S, Tiefenbacher R, Purchio AF, et al. 1992. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor β_1. *Proc. Natl. Acad. Sci. USA* 89:5408–12
131. Jirtle RL, Carr BI, Scott CD. 1991. Modulation of insulin-like growth factor-II/mannose 6-phosphate receptors and transforming growth factor-β_1 during liver regeneration. *J. Biol. Chem.* 266:22,444–50
132. Rumsby PC, Davies MJ, Price RJ, Lake BG. 1994. Effect of some peroxisome proliferators on transforming growth factor-β_1 gene expression and insulin-like growth factor II/mannose-6-phosphate receptor gene expression in rat liver. *Carcinogenesis* 15:419–21
133. Jirtle RL. 1994. Liver tumor promotion and breast cancer chemoprevention: common mechanisms. In *Non-Genotoxic Carcinogenesis. Schering Foundation Workshop, 10*, ed. A Cockburn, LL Smith, Berlin: Springer-Verlag. pp. 157–71.
134. Klaunig JE, Ruch RJ. 1990. Role of inhibition of intercellular communication in carcinogenesis. *Lab. Invest.* 62: 135–46
135. Bieri F. 1993. Peroxisome proliferators and cellular signaling pathways. A review. *Biol. Cell* 77:43–46
136. Bennett AM, Williams GM. 1993. Calcium as a permissive factor but not an initiation factor in DNA synthesis induction in cultured rat hepatocytes by the peroxisome proliferator ciprofibrate. *Biochem. Pharmacol.* 46:2219–27
137. Bojes HK, Thurman RG. 1994. Peroxisome proliferators inhibit acyl CoA synthetase and stimulate protein kinase C *in vivo*. *Toxicol. Appl. Pharmacol.* 126: 233–39
138. Rodricks JV, Turnbull D. 1987. Interspecies differences in peroxisomes and peroxisome proliferation. *Toxicol. Ind. Health* 3:197–212
139. Makowska JM, Gibson GG, Bonner FW. 1992. Species differences in ciprofibrate induction of hepatic cytochrome P450 4A1 and peroxisome proliferation. *J. Biochem. Toxicol.* 7:183–91
140. Orton TC, Adam HK, Bentley M, Holloway B, Tucker MJ. 1984. Clobuzarit: species differences in the morphological and biochemical response of the liver following chronic administration. *Toxicol. Appl. Pharmacol.* 73:138–51
141. Lake BG, Gray TJB, Foster JR, Stubberfield CR, Gangolli SD. 1984. Comparative studies on di-(2-ethylhexyl)phthalate-induced hepatic peroxisome proliferation in the rat and hamster. *Toxicol. Appl. Pharmacol.* 72: 46–60

142. Eacho PI, Foxworthy PS, Johnson WD, Hoover DM, White SL. 1986. Hepatic peroxisomal changes induced by a tetrazole-substituted alkoxyacetophenone in rats and comparison with other species. *Toxicol. Appl. Pharmacol.* 83: 430–37
143. Lake BG, Evans JG, Gray TJB, Körösi SA, North CJ. 1989. Comparative studies on nafenopin-induced hepatic peroxisome proliferation in the rat, Syrian hamster, guinea pig and marmoset. *Toxicol. Appl. Pharmacol.* 99:148–60
144. Rhodes C, Orton TC, Pratt IS, Batten PL, Bratt H, et al. 1986. Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl)phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. *Environ. Health Perspect.* 65:299–308
145. Reddy JK, Lalwani ND, Qureshi SA, Reddy MK, Moehle CM. 1984. Induction of hepatic peroxisome proliferation in non-rodent species, including primates. *Am. J. Pathol.* 114:171–83
146. Lalwani ND, Reddy MK, Ghosh S, Barnard SD, Molello JA, Reddy JK. 1985. Induction of fatty acid β-oxidation and peroxisome proliferation in the liver of Rhesus monkeys by DL-040, a new hypolipidemic agent. *Biochem. Pharmacol.* 34:3473–82
147. Hanefeld M, Kemmer C, Kadner E. 1983. Relationship between morphological changes and lipid-lowering action of *p*-chlorophenoxyisobutyric acid (CPIB) on hepatic mitochondria and peroxisomes in man. *Atherosclerosis* 46: 239–46
148. Elcombe CR, Styles JA. 1989. Species differences in peroxisome proliferation. *Toxicologist* 9:63
149. Parzefall W, Erber E, Sedivy R, Schulte-Hermann R. 1991. Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat tumor promoters. *Cancer Res.* 51:1143–47
150. Bieri F, Stäubli W, Waechter F, Muakkassah-Kelly S, Bentley P. 1988. Stimulation of DNA synthesis but not of peroxisomal β-oxidation by nafenopin in primary cultures of marmoset hepatocytes. *Cell Biol. Int. Rep.* 12: 1077–87
151. Graham MJ, Wilson SA, Winham MA, Spencer AJ, Rees JA, et al. 1994. Lack of peroxisome proliferation in marmoset liver following treatment with ciprofibrate for 3 years. *Fund. Appl. Toxicol.* 22:58–64
152. Tucker MJ, Orton TC. 1993. Toxicological studies in primates with three fibrates. See Ref. 155, pp. 425–47
153. Weisburger JH. 1994. Does the Delaney clause of the U.S. food and drug laws prevent human cancers? *Fund. Appl. Toxicol.* 22:483–93
154. Hartig F, Stegmeier K, Hebold G, Özel M, Fahimi HD. 1982. Study of liver enzymes: peroxisome proliferation and tumor rates in rats at the end of carcinogenicity studies with bezafibrate and clofibrate. *Ann. NY Acad. Sci.* 386:464–67
155. Gibson G, Lake B, eds. 1993. *Peroxisomes: Biology and Importance in Toxicology and Medicine.* London: Taylor & Francis