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HEPATIC PEROXISOME PROLIFERATION

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HEPATIC PEROXISOME PROLIFERATION

SUMMARY

Peroxisomes are subcellular organelles found in all eukaryote cells. In the liver they are usually round and measure about 0.5 - 1.0μm. In rodents they contain a prominent crystalloid core but this may be absent in newly formed rodent peroxisomes and human peroxisomes. A major role of the peroxisomes is the breakdown of long chain fatty acids thereby complementing mitochondrial fatty acid metabolism.

Many chemicals are known to increase the number of peroxisomes in rat and mouse hepatocytes. This peroxisome proliferation is accompanied by replicative DNA synthesis and liver growth. No clear structure-activity relationships are apparent. Many of these peroxisome proliferators contain acid functions which can modulate fatty acid metabolism.

Two mechanisms have been proposed for the induction of peroxisome proliferation. One is based on the existence of one or several specific cytosolic receptors which bind the peroxisome proliferator facilitating its translocation to the cell nucleus and activation of the expression of specific genes. The second, perhaps more general hypothesis, involves chemically mediated perturbation of lipid metabolism. These two hypotheses are not mutually exclusive.

Many peroxisome proliferators have been shown to induce hepatocellular tumours when administered at high dose levels for long periods to rats and mice despite being uniformly non-genotoxic. Three mechanisms have been proposed to explain the induction of tumours. One is based on increased production of active oxygen species due to imbalanced production of peroxisomal enzymes; it is proposed that these reactive oxygen species cause indirect DNA damage with subsequent tumour formation. An alternative mechanism is the promotion of endogenous lesions by sustained DNA synthesis

and hyperplasia. Thirdly, it is conceivable that sustained growth stimulation may be sufficient for tumour formation.

Marked species differences are apparent in responses to peroxisome proliferators. Rats and mice are extremely sensitive, hamsters show an intermediate response, whilst guinea pigs, monkeys and man appear to be relatively insensitive or non-responsive at dose levels that produce a marked response in rodents. These species differences may be reproduced in vitro using primary culture hepatocytes isolated from a variety of species including man.

The available experimental evidence suggests a strong association and a probable causal link between peroxisome proliferator-elicited liver growth and the subsequent development of liver tumours in rats and mice. Since man is insensitive or unresponsive, at therapeutic dose levels, to peroxisome proliferator-induced hepatic effects it is reasonable to conclude that the levels of exposure encountered to these non-genotoxic agents do not present a hepatocarcinogenic hazard to man. This conclusion is supported by the available, albeit limited epidemiological data.

A: INTRODUCTION

Peroxisomes are subcellular organelles which are found in most eukaryotic cells. During the last decade an increasing number of chemical compounds have been found to induce peroxisome proliferation in the liver cells of rats and mice. This peroxisome proliferation is part of a unique hepatomegaly which is characterised by a massive increase in the number of peroxisomes, proliferation of the smooth endoplasmic reticulum and stimulation of cell division within the liver. In mutagenicity assays peroxisome proliferators yield negative results despite inducing liver tumours in long term rodent bioassays. Thus peroxisome proliferators may be regarded as non-genotoxic carcinogens.

To clarify the significance to man of the carcinogenicity of these compounds ECETOC established a Task Force with the following terms of reference:

- describe peroxisome proliferation and related phenomena,

- review the chemicals causing peroxisome proliferation and if appropriate discuss structure-activity relationships,

- evaluate these chemicals in terms of mutagenicity and carcinogenicity,

- describe the mechanisms leading to peroxisome proliferation and subsequent carcinogenicity,

- evaluate the species differences in peroxisome proliferation,

- evaluate the hazard to man of chemicals which induce peroxisome proliferation.

B: PEROXISOME PROLIFERATORS; DEFINITION OF THE PROBLEM

1. Morphological and Biochemical Characteristics

Peroxisomes are abundant in cells which are involved in lipid metabolism; they are often associated with lipid droplets within the cell (Gorgas, 1987). Peroxisomes are enclosed by a single unit membrane and show a positive staining for peroxidase activity (due to the presence of catalase). Their shape and size varies greatly depending on the cell type investigated. In the rodent liver peroxisomes occupy about 1.5% of the hepatocytic volume (Stäubli, 1969). In electron micrographs they generally appear to be round with a diameter of about 0.5 - 1.0μm and contain an electron-dense crystalloid core, which contains the enzyme urate oxidase. Human hepatic peroxisomes are generally smaller and lack the crystalloid core. Dumb-bell shaped and ring-like peroxisomes have been observed in sebacious glands (Gorgas, 1984). Serial sections and structural reconstruction have shown that in such cells peroxisomes are organised in a reticulum throughout the cell with swollen areas and thin interconnecting strands. A similar peroxisomal reticulum has been demonstrated in mouse hepatocytes (Gorgas, 1985). A reticular structure was also suggested on the basis of studies of peroxisome development (Lazarow and Fujiki, 1985).

Peroxisomes contain a large number of enzymes (Table 1) many of which are intimately involved in lipid metabolism, eg. breakdown of lipid (ie. the β-oxidation system), the synthesis of plasmologens (ether lipids), the synthesis of cholesterol and the synthesis of long chain fatty esters which are found in waxes and pheromones. Most of these functions are also performed by other subcellular organelles, eg. microsomes or mitochondria, and it is often difficult to assess the contribution of the peroxisomal enzymes to the total cellular reactions. In the case of fatty acid β-oxidation it would appear that very long fatty acids which are poorly oxidised by the mitochondrial system are good substrates for the peroxisomal system so in this manner the two systems may complement each other (Boeck et al, 1980; Fahimi and Sies, 1987). In this respect it is of interest that children suffering from Zellweger's syndrome, a hereditary disease

characterised by a lack of peroxisomes, have very high blood levels of long chain fatty acids. Such children also tend to lack plasmologens, which suggests that peroxisomes are essential for ether lipid synthesis (Wanders et al, 1987).

Administration of a variety of substances to laboratory rodents results in a proliferation of the hepatocytic (and to a certain extent renal) peroxisome compartment (Table 2). The degree of peroxisome proliferation varies markedly depending upon the substance inducing the change. The proliferation may be accompanied by changes in the morphology of the peroxisomes; often coreless peroxisomes are induced. Other substances induce a different kind of matrical inclusion body which may even distend the shape of the organelle (Hruban et al, 1966) or treatment may result in the formation of very large peroxisomes which tend to form groups within the cytoplasm. Recently, the proliferation of the peroxisomal membrane without a marked increase in the number of organelles has been reported (Fahimi, 1989). Presumably these morphological changes are reflections of different biochemical situations but their physiological and toxicological significance is unclear. Alterations in peroxisome structure can also be observed in the absence of apparent peroxisome proliferation.

2. The Rodent Peroxisome Proliferation Phenomenon

Peroxisome proliferators elicit marked liver enlargement which is characterised by hyperplasia (increase in cell number and content of DNA) and hypertrophy (increase in cell size). The hyperplasia is due to stimulation of replicative (semi-conservative) DNA synthesis and subsequent cell division. The hypertrophy is characterised by the proliferation of the peroxisomes and the smooth endoplasmic reticulum (SER). The latter is accompanied by the specific induction of cytochrome P450 IVA (P452) which exhibits high specificity for the ω-oxidation of fatty acids (eg lauric acid hydroxylation). The peroxisome proliferation is accompanied by selective increases in the specific activities of certain peroxisomal enzymes, particularly those involved in the β-oxidation of fatty acids (eg acyl CoA oxidase, EC 3.99.3). The measurement of acyl CoA oxidase with palmitoyl CoA as substrate (cyanide insensitive palmitoyl CoA oxidation) allows a rapid

and accurate assessment of peroxisome proliferation which correlates well with peroxisome volume density determinations by electron microscopy and morphometric analysis (Lock et al, 1989).

Liver growth occurs rapidly as shown by studies with fenofibrate and diethyl hexyl phthalate (DEHP); there is an initial growth period lasting 3 - 4 days which is accompanied by increased ^{3}H-thymidine incorporation (a measurement of DNA synthesis) reaching a peak at the third day and which subsequently reverts to normal levels; liver growth continues more slowly for a further week or two. Morphometric studies suggest that this additional increase occurs as a result of hepatocyte enlargement. The enlarged liver returns to normal after a period of 2 - 3 weeks following cessation of the test substance administration. If administration is continued the liver remains enlarged (Mitchell et al, 1985a) and other aspects of the phenomenon become apparent. These include the deposition of lipofuscin (which for many years has been regarded as the "age pigment" but may result from the polymerisation of oxidised lipid within lysosomes) and eventually the formation of liver nodules and ultimately tumours. The interval between the early changes and the eventual appearance of tumours is measured in terms of several months. The rates of appearence of liver nodules and tumours are related and differ from compound to compound.

C: COMPOUNDS CAUSING PEROXISOME PROLIFERATION

When a clearly defined structure-activity relationship exists between a set of chemical compounds and their pharmacological or toxicological effects it may be possible to elucidate their mechanism of action.

A large number of chemicals cause peroxisome proliferation in rats and mice; their potencies as peroxisome proliferators and hepatic carcinogens vary over at least two orders of magnitude. Lists of these chemicals have been presented in various formats, generally based on the table produced by Reddy and Lalwani (1983) who divided them into clofibrate and its analogues, plasticisers, other compounds and dietary and other factors. Other lists have been prepared (Cohen and Grasso, 1981; Hawkins et al, 1987; Mitchell, 1985a; Stott, 1988; Vamecq and Draye, 1989; Lock et al, 1989; and Holloway, 1989) but these are not comprehensive. Compounds, and physiological factors, which have been shown to induce peroxisome proliferation are listed in Table 2 in which they are arranged into a number of chemically related structural groups:

2-Phenoxy-acetic acids (including clofibrate etc), (Table 2a),
2-Phenoxy acetic acid analogues, (Table 2b),
n-Alkyl carboxylic acids (Table 2c) and their precursors (Table 2d),
Long chain alkyl dicarboxylic acids and their precursors (Table 2e),
"Alkyl-aryl" carboxylic acids and their precursors (Table 2f),
O-Substituted Benzoic acids and their precursors (Table 2g),
Non-carboxylic acids and precursors (Table 2h),
Other compounds and physiological factors (Table 2i).

Compounds which are known, or expected, to be metabolised to an active proliferator have been listed as precursors.

1. <u>2-Phenoxy-acetic acids</u> (Table 2a)

This is the largest group of peroxisome proliferators and the most extensively studied although remarkably few variantions in the substitution pattern have been evaluated; about 90% of the reports are on compounds with dialkyl substitutions on the acetic acid (R_1, R_2) and different substituents at the 4 position on the benzene ring.

$$\text{C}_6\text{H}_5\text{-O-C}(R_1)(R_2)\text{-COOR}_3$$

(ring positions 1–5 numbered)

The compounds have been generally administered as esters which are, or are assumed to be, rapidly hydrolysed to the carboxylic acid as the active proliferator. In the case of clofibric acid for which data are available on the free acid and a number of different esters, clofibrate, SaH 42-348, simfibrate and dulofibrate, variations of the ester group make little difference to the activity.

The few reports suggest that there is no specific requirement for substituents at R_1, R_2 or on the 4-position; the structures in Table 2a indicate that the substituents in these positions can range from hydrogen to large complex organic moieties. It is likely that the simple unsubstituted compounds would be active if they were not rapidly excreted. This suggestion is supported in the case of 4-chlorophenoxy acetic acid which is inactive <u>in vivo</u> in the mouse (Lundgren <u>et al</u>, 1987a,b) and active <u>in vitro</u> in rat hepatocytes (Lake <u>et al</u>, 1988). Rapid excretion probably also accounts for the lack of activity of 4-fluoro isobutyric acid in which the R_1 and R_2 are methyl groups (Azarnoff <u>et al</u>, 1976).

The lack of activity of the clofibrate analogues with chlorine in the 2- or 3- position of the benzene ring suggests that the structural requirements for peroxisome proliferators are complex (Azarnoff <u>et al</u>,

1976). No reports are available of compounds with dialkyl groups at R_1, R_2 and other substituents at position 2 or 3 on the aromatic ring. Until such compounds have been studied it is not possible to assess the role of such substituents. The only compounds of this type which have been studied were reported by Harrison (1984) (cf Compounds I-IV Table 2a, page 45) in which the 2-position of the aromatic ring is joined to the acetic acid residue. The lack of activity may be related to the rigidity of the molecules which consequently cannot be considered as close analogues of clofibrate for the purpose of structure-activity considerations.

2. <u>2-Phenoxy-acetic acid analogues</u> (Table 2b)

These compounds are similar to the phenoxyacetic acids but the oxygen atom at the 1 position of the aromatic ring is replaced by sulphur or nitrogen.

$$\text{C}_6\text{H}_5\text{-X-CH}_2\text{-COOH}$$

3. <u>Alkyl carboxylic acids</u> (Table 2c)

A considerable variety of substituted n-alkyl carboxylic acids with chain lengths from C_2 to C_{10} are active peroxisome proliferators. All of the active compounds are resistant to metabolism at C_2 due to the presence of various substituents.

The simplest of the groups of chemicals shown to be potent peroxisome proliferators are the substituted hexanoic acids. The effects of chain length and substituents have been studied by Lundgren <u>et al</u> (1987b), who concluded that the most effective inducer had an ethyl group at C_2. Until compounds with substitutions of four or more carbons at the 2-position or some 2,2 or 3,3 disubstituted short chain (C_4-C_{10}) alkanoic

acids have been studied the above conclusions must be considered as tentative.

4. n-Alkyl carboxylic acid precursors (Table 2d)

Included in the table are a number of compounds which are expected to be metabolised to n-alkyl carboxylic acids, some of which are peroxisome proliferators and some not. Tetrachloroethylene and trichlorethylene are converted to trichloroacetic acid, a known peroxisome proliferator. Similarly halothane is metabolised to trifluoroacetic acid which could account for its reported activity. The low rate of oxidation of 1-H,1-H-pentadecafluorooctanol to the corresponding acid may explain why this compound is not a peroxisome proliferator. Hydrolysis of the plasticisers diethylyhexyl adipate (DEHA) and diethylhexyl phthalate (DEHP) leads to the formation of 2-ethylhexanol which is readily oxidised to 2-ethylhexanoic acid, an active peroxisome proliferator. DEHA metabolism to adipic acid and two equivalents of 2-ethyl hexanoic acid accounts for the total activity. In contrast, DEHP forms one equivalent of 2-ethylhexanoic acid and one of monoethyl hexylphthalic acid, both of which are active proliferators. Monoethyl hexylphthalic acid will be considered later. Overall the activity of this set of compounds supports the view that a poorly metabolisable carboxylic acid function is required to produce peroxisome proliferation.

5. Dicarboxylic acids and precursors (Table 2e)

The active peroxisome proliferators in this group have the β-position relative to the carboxyl blocked by with dimethyl substituents or a sulphur atom. A number of compounds are clearly metabolic precursors of this type of molecule.

6. Alkyl-aryl carboxylic acids and precursors (Table 2f)

This group comprises a set of compounds which have a carboxylic acid function or a mixture of alkyl and aryl groups in more or less linear arrangement which could be comparable to a long chain fatty acid. In all cases, metabolism is blocked by the aryl ring, the alkyl substituents or a hetero atom or a combination of these substituents.

Whether the precursors DG-5685 and DH-6463 act directly or through the carboxylic acid which would be expected from metabolism of the dioxane ring is unknown at present.

7. Ortho-substituted benzoic acids and precursors (Table 2g)

This group is based on the mono alkyl phthalic acid esters, the active metabolites of the dialkyl phthalates. Hydrolysis of the dialklyphthalates produces mono alkylphthalic acid which is a metabolically stable ester and the corresponding alcohol, both of which contribute to peroxisome proliferation. The activity of the mono-alklyphthalates varies with the alcohol component of the ester. In the case of mono ethylhexylphthalate, in vitro studies indicated that the metabolite mono (2-ethyl-5-oxohexyl) phthalate (metabolite VI) was a potent peroxisome proliferator (Mitchell et al, 1985a).

The fact that Aspirin (a mono acetylphthalate) is a peroxisome proliferator suggests that bulky groups are not essential for activity.

Tibric acid and lactofen are complex molecules with a number of functional groups. They are active peroxisome proliferators and it seems likely that they do not act in the same way as the phthalates. This underlines the fact that data on related compounds, which would be necessary for useful structure-activity considerations, are lacking.

8. Non-carboxylic acids and precursors (Table 2h)

This group has diverse structures with a variety of ionisable functional groups, such as sulphonic acids, sulphamic acids and tetrazoles. Some of these compounds eg. perfluoro-octyl sulphonic acid may mimic fatty acids.

In the case of the tetrazoles, which are isoelectric to carboxylic acids, eg LY171883 (Eacho et al, 1986), the requirement for an acid function was

demonstrated by blocking the acid hydrogen of the tetrazol by N-methylation (Eacho et al, 1989). The relative activity of the analogues A to F (cf. Table 2h) increased with increasing methylene chain length to n=8 and declined slightly with n=9. In a smaller series, a similar increase with increasing methylene chain length was observed with LY16443, and compounds I and J. It was concluded that the strong peroxisome proliferators, including compounds E, L and J assume conformations in which the tetrazole is in closer proximity to the acetophenone function than in the relatively inactive analogues such as compound H. This has been recently supported by structural molecular orbital calculations which suggest a preferred separation of 3.9 - 6.1 Å between the acid tetrazole moiety and the acetophenone phenyl groups for the induction of β-oxidation. It is of interest that clofibric acid has a low energy configuration with a phenyl to carbon distance of 3.9 - 6.1 Å (Foxworthy et al, 1990a).

9. Other Compounds and Physiological Factors (Table 2i)

There are few reports on non-acidic substances and changes in the physiological status of animals causing peroxisome proliferation. The role of these compounds or physiological status is further discussed in Section D.

10. Evaluation

Currently this is a field of activity investigated by several laboratories. From the above discussion it is apparent that there is no clear structural-activity relationship and that any relationship would be very complex. Nevertheless, one common factor is the presence of an acid function in either the parent molecule or a metabolite.

D: MECHANISMS OF PEROXISOME PROLIFERATION

Induction of peroxisome proliferation in the liver is marked by dramatic changes in many characteristic features of hepatocytes. These involve a change in the activity of peroxisomal cytosolic and mitochondrial enzymes, proliferation of smooth endoplasmic reticulum and peroxisomes, stimulation of DNA synthesis and other changes. Morphologically, it is not possible to trace the changes back to a single event which can be characteristic of initiation of peroxisome proliferation. In many cases the transient formation of lipid droplets is the earliest observable change, often appearing within 24 hrs (Capuzzi et al, 1983). This is followed by the induction of peroxisomes within a week, dependent on compound and dose. Two mechanisms have been proposed for the induction of peroxisome proliferation:

- the activation of specific genes by the chemical or its metabolite, either directly or through a specific receptor,

- substrate overload, either the result of extra-hepatic lipolysis causing an influx of fatty acids into the liver, or by perturbation of hepatic lipid metabolism by the peroxisome proliferators or their metabolites.

1. Receptor Mediated Mechanism: A Chemical Perspective

Lalwani et al (1983a, 1987) reported the presence of a protein which specifically bound the hypolipidaemic agent nafenopin. Other authors using computer-aided molecular modelling have developed structure-activity relationships which might also suggest a receptor mediated mechanism (Lake et al, 1986, 1987, 1988; Lewis et al, 1987; Foxworthy and Eacho, 1988; Eacho et al, 1989).

2. Receptor Mediated Mechanism: Biological Evidence

The following observations support a ligand-receptor mediated mechanism as proposed by Lalwani et al (1983a, 1987):

- the tissue-specific biological response in transplanted hepatocytes (Reddy et al, 1984a; Rao et al, 1986a);
- the inducibility in cultured hepatocytes of peroxisome proliferation (Gray et al, 1982; Mitchell et al, 1984; Furukawa et al, 1984; Bieri et al, 1984; Foxworthy and Eacho, 1986);
- the induction of similar, specific changes in protein composition in the livers of rats exposed to structurally dissimilar peroxisome proliferators (Usuda et al, 1988: Sharma et al, 1988);
- the rapid and significant increase in the rate of synthesis of mRNAs for peroxisomal β-oxidation and microsomal ω-oxidation enzymes (P450 IVA) in liver (Reddy et al, 1986; Hardwick et al, 1987) and the rapidity of the transcriptional response of these genes;
- the detection of a specific binding protein in liver cytosol for nafenopin, a peroxisome proliferator.

These findings imply that peroxisome proliferators act directly on hepatocytes to induce peroxisome proliferation. Lalwani et al (1983a) described a saturable pool of binding sites for nafenopin in the cytosol of rat liver. The degree of binding correlated with the ability of this agent to induce peroxisome proliferation in various tissues. A 70kD protein that binds ^{3}H-nafenopin has been isolated from rat liver and partially characterised; when clofibric acid and ciprofibrate, two compounds closely related to nafenopin, were used as affinity ligands, the same 70kD protein was isolated (Lalwani et al, 1987). Recently, other workers using high specific activity ^{3}H-nafenopin or ^{3}H-ciprofibrate have failed to detect any specific binding of these agents to hepatic microsomal or cytosolic fractions (Milton et al, 1988) and have thereby cast doubt on the existence of a specific cytosolic receptor for peroxisome proliferators. These authors showed that these peroxisome proliferators reversibly bind to serum albumin and suggested that this accounts for the observed binding. Resolution of these conflicting views on binding proteins requires further research.

The diverse nature of the chemical structures producing peroxisome proliferation suggests that agents do not interact with a single type of binding protein with a single recognition site. Hence it has been suggested (Reddy and Rao, 1986) that a binding protein with multiple recognition sites exists or, alternatively, that several binding proteins with different ligand binding properties are involved. Issemann and Green (1990) have recently reported cloning and characterisation of a member of the nuclear hormone receptor family that can be activated by peroxisome proliferators. This receptor has therefore been termed "PPAR" for peroxisome proliferator activated receptor. These studies showed that a chimeric receptor using the DNA binding domain of the oestrogen receptor and the putative ligand binding domain of PPAR was able to activate an oestrogen-responsive gene in the presence of peroxisome proliferators. When the chimeric receptor was tested using several different peroxisome proliferators (Wy-14,643, nafenopin, MEHP, clofibrate, and trichloroacetic acid) a good correlation was observed between their ability to activate the chimeric receptor and their potency either as peroxisome proliferators or as rat liver carcinogens. These data suggest that the PPAR may mediate some of the biological effects of peroxisome proliferators. Further support for this suggestion comes from a study indicating that the tissue specific expression of the receptor compares well with the tissue specific induction of acyl CoA oxidase by peroxisome proliferators (Nemali et al, 1988; Green, unpublished). At present the significance of these findings is not clear since no ligand binding to the receptor was detected using the potent peroxisome proliferator nafenopin (Isseman and Green, 1990).

3. Perturbation of Lipid Metabolism Mechanism: A Chemical Perspective

Ikeda et al (1988) have suggested that the ultimate structure inducing peroxisome proliferation is an "unmetabolisable lipophilic anion". This permits classification of peroxisome proliferators into sets of compounds which are not metabolisable for different reasons. In particular, the compounds are often resistant to metabolism through the fatty acid β-oxidation cycle either because they cannot form an acyl-CoA ester, or if formed, such esters cannot undergo β-oxidation. The presence of a carboxylic acid functional group in most of the compounds is apparent from Table 2,

although many of the chemicals are administered as esters which are believed to hydrolyse readily to the corresponding carboxylic acid. In a number of cases the administered compound is metabolised into a carboxylic acid which is the proximate peroxisome proliferator. These have been listed in the tables as precursors, for example, perchlorethylene and trichlorethylene are converted to trichloracetic acid (Table 2) and tiadenol and heptamethylnonane converted to the corresponding α, ω-dicarboxylic acids.

To undergo β-oxidation, carboxylic acids must form acyl-CoA esters which are then dehydrogenated by the acyl-CoA oxidase introducing a double bond. The formation of acyl-CoA esters has been demonstrated with a variety of peroxisome proliferators including the substituted n-alkyl carboxylic acids (Becker and Harris, 1983), dicarboxylic acids (Vamecq et al, 1985, 1989), and phenoxy-acetic acids (clofibrate, nafenopin and ciprofibrate) (Bronfmann et al, 1986). The formation of CoA esters by these compounds shows that two methyl groups on the α-carbon atom does not sterically inhibit the formation of the ester. In the absence of evidence to the contrary it can be assumed that all the phenoxy acetic acid analogues and the 'alkyl-aryl' carboxylic acids will form acyl CoA esters. If the α or β- position is blocked the compound cannot undergo β-oxidation. This applies to almost all of the active peroxisome proliferators which have carboxylic acid functional groups, including the ortho-substituted benzoic acids.

4. Perturbation of Lipid Metabolism by Substrate Overload: Biological Evidence

The ability of structurally diverse chemicals to elicit peroxisome proliferation has led to the proposal that perturbation in lipid metabolism is the event which initiates the process (Bremer and Norman, 1982: Elcombe and Mitchell, 1986; Berge et al, 1987). This concept has become widely accepted as the more likely mechanism (Sharma et al, 1988); nevertheless the exact way in which this occurs is still not clear. The observation that peroxisome proliferation is induced by a variety of physiological perturbations (Table 2i) as well as high fat diets (Hori et al, 1981; Ishii et al, 1980a,b; Osmundsen, 1982; Neat et al, 1980, 1981) supports the idea

that substrate overload and alterations in lipid metabolism could initiate the process. Observations that lead to a 'lipid perturbation' hypothesis are:

- hypolipidaemic activity of the chemical agents,
- transient accumulation of lipid within hepatocytes,
- induction of peroxisomal enzymes involved in fatty acid oxidation but NOT other peroxisomal enzymes,
- induction of microsomal P-450 IVA (a fatty acid hydroxylase),
- increased levels of CoA and carnitine,
- increased transport of lipid to liver (eg high fat diets, hyperthyroidism, chemically induced diabetes) results in peroxisome proliferation,
- many peroxisome proliferators form CoA esters and mixed triglycerides.

There is considerable evidence that peroxisome proliferators alter hepatic lipid metabolism. For example, peroxisomal β-oxidation and microsomal ω-oxidation are increased along with the activities of carnitine acetyltransferase and carnitine octanoyl-transferase, while the levels of CoA and carnitine are elevated. In contrast the activities of other peroxisomal enzymes such as D-amino acid oxidase, urate oxidase and catalase are hardly affected. As a result of these findings, it has been proposed (Elcombe and Mitchell, 1986) that increased levels of intrahepatic lipid may be an important factor in the genesis of peroxisome proliferation. The accumulation of hepatic lipid can occur in a number of ways, and the structurally diverse chemicals that produce peroxisome proliferation may act at many different loci to perturb lipid metabolism.

It may be that fat accumulation in the liver itself is not the stimulus for the induction since treatment of rats with methotrexate, which induces fatty liver of the large droplet type, does not trigger peroxisome proliferation (Berge et al, 1987; Price et al, 1985). Nevertheless many peroxisome proliferators cause a transient accumulation of small droplets of neutral lipid within the hepatocytes. This is probably a reflection of the perturbed lipid metabolism and not the initial stimulus for the peroxisome

proliferation phenomenon (Mann et al, 1985; Mitchell, 1985; Elcombe et al, 1985). Studies have shown that the proximate peroxisome proliferator derived from DEHP, namely metabolite VI (mono (2-ethyl-5-oxo-hexyl)phthalate), causes a concentration dependent decrease in the oxidation of palmitic acid by isolated rat hepatocytes and competitively inhibits medium chain octanoyl (but not palmitoyl) carnitine oxidation in isolated mitochondria (Elcombe and Mitchell, 1986; Mitchell, 1985). The precise site of action of metabolite VI within the mitochondrium is not known; it may be direct inhibition of a mitochondrial oxidation enzyme specific for medium chain fatty acids, or of carnitine octanoyl transferase responsible for the transport of medium chain acylcarnitines across the mitochondrial membrane (Mitchell, 1985; Lock et al, 1989). The inhibition by metabolite VI of long chain fatty acid metabolism (such as palmitic acid) in hepatocytes, but not in isolated mitochondria, may be the result of depletion of essential CoA by metabolite VI per se, or through sequestration by medium chain fatty acids. Studies with isolated hepatocyte cultures have shown a rapid depletion of CoA following the addition of mono(2-ethylhexyl) phthalate (Mitchell, 1985; Lock et al, 1989). Inhibition of mitochondrial β-oxidation due to sequestration of CoA would lead to the accumulation of medium (C_6-C_{10}) and long chain (C_{12}-C_{20}) fatty acids or their CoA esters in the cell. In fact, a rapid and marked accumulation of hexanoyl CoA has been reported following the addition of metabolite VI to isolated hepatocytes (Elcombe, unpublished observations).

Furthermore, it is possible that these fatty acids could induce microsomal cytochrome P-450 IVA and peroxisome proliferation in order to maintain lipid homeostasis. In support of this hypothesis, it has been shown that medium chain (C_6-C_8) fatty acids induce cytochrome P-450 IVA in cultured hepatocytes. It is also known that cytochrome P-450 IVA preferentially metabolises long chain fatty acids (Orton and McCormick, 1982). Hence, the accumulated long chain fatty acids could be converted to long chain dicarboxylic acids (Robbins, 1968) by the newly induced cytochrome P-450 IVA. Furthermore long chain dicarboxylic acids (C_{14}-C_{18}) induce peroxisomal β-oxidation but not microsomal ω-oxidation in cultured hepatocytes. These long chain dicarboxylic acids are also able to stimulate ^{3}H-thymidine incorporation into DNA in isolated rat hepatocytes in culture (Lock et al,

1989) thus contributing to the hyperplasia seen following the administration of peroxisome proliferators in vivo.

The proposed mechanism of peroxisome proliferation due to DEHP is illustrated in Figure 1. This requires the induction of cytochrome P-450 IVA prior to the induction of peroxisomal enzymes such as acyl CoA oxidase. Several studies (Bell et al, 1991; Bell and Elcombe, 1991; Bieri et al, 1991) suggest that cytochrome P-450 IVA is indeed induced prior to acyl CoA oxidase, both in vivo and in primary rat hepatocyte cultures. Furthermore, inhibition of protein synthesis by cycloheximide did not prevent the transcription of cytochrome P-450 IVA mRNA but inhibited the transcription of acyl CoA oxidase in mRNA (Milton et al, 1990; Bieri et al, 1991). This suggests the requirement of a protein, possibly cytochrome P-450 IVA, for the subsequent induction of acyl CoA oxidase.

The inhibition of liver enzymes by other peroxisome proliferators has also been demonstrated. 2-Hydroxy-3-Propyl-4-[6-(tetrazol-5-yl) hexyloxy] acetophene (4-THA) causes a concentration-dependent inhibition of oleate, but not octanoate oxidation, in isolated rat hepatocytes (Foxworthy and Eacho, 1988). These authors also showed that 4-THA is a competitive inhibitor of carnitine palmitoyl transferase-I in rat liver mitochondria (Foxworthy and Eacho, 1988). It is plausible that the structure-activity relationship observed with 4-THA and its analogues are merely a reflection of the ability of these compounds to inhibit this enzyme. Other inhibitors of mitochondrial fatty acid oxidation, such as valproic acid and 2-[5-(4-chlorophenyl-pentyl] oxiran 2-carboxylate (Becker and Harris, 1983; Turnbull et al, 1984: Olson et al, 1986) also cause peroxisome proliferation in rodents (Horie and Suga, 1985; Bone et al, 1982; Draye and Vamecq, 1987).

Chlopromazine at 0.5%, but not at 0.1%, in the diet was shown to induce peroxisome proliferation. The observation that chlorpromazine is an in vitro inhibitor of mitochondrial carnitine palmitoyltransferase and cytochrome oxidase and of peroxisomal carnitine octanoyltransferase (Leighton et al, 1984; Vamecq, 1987) suggests interference with fatty acid metabolism. Chlopromazine has indeed been shown to induce fatty livers in rats (Price et al, 1985).

Becker and Harris (1983) reported that valproic acid caused a marked depletion in hepatic CoA and identified the formation of valproyl-CoA plus an apparent CoA ester metabolite of valproyl CoA and proposed that either valproyl CoA itself or the sequestration of CoA caused the inhibition of fatty acid metabolism. Bronfmann _et al_ (1986) also identified compounds that possessed the characteristics of CoA thio esters of clofibrate, nafenopin and ciprofibrate in _in vitro_ rat liver microsomal incubations. The possibility that the acyl CoA esters of the peroxisome proliferators may be the initiators has been considered (Berge _et al_, 1984; Bronfmann _et al_, 1986).

Some of the non-carboxylic acids may also inhibit β-oxidation of fatty acids; Foxworthy and Eacho (1988) have shown that the tetrazoles inhibit carnitine palmitoyl transferase-1 and the oxidation of oleic acid. It is possible that other non-carboxylic acids, eg. sulphonic acid may inhibit β-oxidation of fatty acids.

For compounds to exert their effect via enzyme inhibition it is necessary to attain and maintain sufficiently high concentrations within the cell. Metabolism and excretion could account for the observation that several compounds which should induce peroxisome proliferation are inactive _in vivo_ eg:

- perfluorooctanoate, which is an active proliferator in male but not female rats (Ikeda _et al_, 1985). (The female rat eliminates perfluro-octanoate from its body more rapidly than the male; Hanhijarvi _et al_, 1982).

- 4-chlorophenoxy acetic acid which is active _in vitro_ (Lake _et al_, 1988) but was found not to be a peroxisome proliferator _in vivo_ (Lundgren _et al_, 1987a) probably because the compound can be rapidly cleared _in vivo_ whilst _in vitro_ it is maintained at a high concentration throughout the induction period.

The evidence therefore suggests that there is an association between the perturbation of lipid metabolism and peroxisome proliferation. Nevertheless no single event can be identified as the key to the induction process.

Despite the apparent conflict between the receptor hypothesis and the substrate overload hypothesis of peroxisome proliferation the two mechanisms are not necessarily mutually exclusive and can be combined as shown in Figure 2.

E: SPECIES DIFFERENCES IN PEROXISOME PROLIFERATION

1. In Vivo Studies using Peroxisome Proliferators

Many attempts have been made to reproduce in other species the phenomenon of peroxisome proliferation which is readily seen in rats and mice. A number of compounds readily induce hepatic carcinoma in these species (Table 3). A review of the literature on species differences in respect of palmityl-CoA oxidising systems is presented below and in Table 4 .

1.1. Hamsters

Clofibrate (Holloway, 1989) and clobuzarit (Orton et al, 1984) failed to stimulate peroxisomal β-oxidation in hamsters, despite achieving or exceeding plasma concentrations which led to marked proliferation in rats and mice. Similarly, studies with fenofibrate (Pourbaix et al, 1984; Reddy et al, 1982), LY171883 (Eacho et al, 1986), bezafibrate (Watanabe et al, 1989), methylclofenapate (Reddy et al, 1982a), and nafenopin (Lake et al, 1989) demonstrated little or no effect on peroxisomal β-oxidation when compared to studies in rats and mice. These experiments clearly indicate that hamsters are less responsive to peroxisome proliferators than rats and mice.

1.2. Guinea Pigs

Studies with clofibrate (Oesch et al, 1988), LY171833 (Eacho et al, 1986), DEHP (Osumi and Hashimoto, 1978), monoethyl hexyl phthalate (MEHP) (Mitchell et al, 1985a), methylclofenapate, trichloroacetic acid (Elcombe, unpublished data) and nafenopin (Lake et al, 1989) at doses exceeding the effective dose levels in rats, have consistently failed to elicit peroxisome proliferation in guinea pigs.

Small (3-fold or less) increases in peroxisomal β-oxidation were reported following the administration of tiadenol (Oesch et al, 1988) and bezafibrate (Watanabe et al, 1989) to guinea pigs. In comparison,

experiments in rats with equivalent doses resulted in a stimulation of peroxisomal β-oxidation (13-fold and 19-fold respectively).

These data illustrate the poor response given by guinea pigs to peroxisome proliferators.

1.3. Dogs

Little published information is available on the effect of peroxisome proliferators in dogs. Orton et al (1984) showed the lack of effect of clobuzarit on peroxisomal β-oxidation in dogs, despite achieving similar peak serum concentrations to those observed in experiments with rats and mice. Eacho et al (1986) have shown that dogs did not respond to LY171883, while similar doses led to a 7-fold increase in peroxisomal β-oxidation in rats. Watanabe et al (1989) found no induction of β-oxidation in dogs administered bezafibrate at 30mg/kg/day for 2 weeks. However, no data were shown for rats at this dose level (although 100mg/kg/day for 2 weeks resulted in a 13-fold response).

The limited data suggest that the dog is insensitive to peroxisome proliferators.

1.4. Non-human primates

Administration of clofibrate (350mg/kg/day for 2 weeks) did not elicit peroxisome proliferation in marmosets, despite achieving a plasma concentration of 0.6mM; this concentration led to an 11-fold increase in β-oxidation in rats (Holloway, 1989). Studies by the same author failed to demonstrate peroxisome proliferation in rhesus monkeys administered 300mg/kg/day clofibrate for 2 weeks. However, the interpretation of this study was confounded by variations in the plasma concentrations achieved (ie. 0.1 - 0.47mM, mean of 0.15mM).

Subjective electron microscopic examination of livers from rhesus monkeys administered 200mg/kg/day fenofibrate for 12 months did not reveal proliferation of peroxisomes (Blane and Pinaroli, 1984).

Clobuzarit at a dose of 45mg/kg/day for 2 weeks (peak serum concentration 120μg/ml) did not elicit peroxisome proliferation in marmosets. In contrast, under similar conditions rat and mouse studies revealed an approximate 3-fold increase of peroxisomal β-oxidation at serum concentrations of clobuzarit of 98 and 96μg/ml respectively (Orton et al, 1984). LY171183 (Eacho et al, 1986) and bezafibrate (Watanabe et al, 1989) at doses of 200mg/kg/day for 2 weeks and 125mg/kg/day for 13 weeks respectively did not cause peroxisome proliferation in rhesus monkeys. However, similar dose levels of these compounds induced peroxisomal β-oxidation by 7 - 13 fold in rats and mice.

DEHP did not stimulate peroxisomal β-oxidation in marmosets (Rhodes et al, 1986) or cynomolgus monkeys (Short et al, 1987), however, the latter study only involved two monkeys per group. Furthermore, primates appear not to hydrolyse DEHP extensively to MEHP, hence the delivered liver dose of proximate peroxisome proliferator is very low (Rhodes et al, 1986).

Lake et al (1989) have demonstrated the insensitive nature of marmosets to nafenopin, only a small (2-fold) increase in β-oxidation being seen after 250mg/kg/day for 3 weeks. A clear no effect level of 50mg/kg/day in the marmoset was noted; in contrast, this dose given to rats, resulted in a 10-fold increase in peroxisomal β-oxidation.

More recently, Makowska et al (1991) have underlined the relatively refractive nature of the marmoset in studies using ciprofibrate. A dose of 80 - 100mg/kg/day of ciprofibrate administered to marmosets for 26 weeks resulted in a 3.5-fold increase in β-oxidation, whereas a dose of 20mg/kg/day given to rats for 26 weeks resulted in a 35-fold increase. These data clearly illustrate the marked species differences in sensitivity. This is emphasised by the work of Reddy et al (1984b) where ciprofibrate administered to rhesus monkeys (50mg/kg increasing to 200mg/kg/day for 3 weeks) and cynomolgus monkeys (400mg/kg/day for 7 weeks) resulted in 5-fold and 13-fold increases in peroxisomal β-oxidation. These data suggest that monkeys are 40 - 70 times less sensitive than rats to the effects of ciprofibrate.

In summary, marmosets appear to be essentially non-responsive, while other primate species appear also to be either non-responsive or markedly less sensitive than rodents to the hepatic effects of peroxisome proliferators.

1.5. Man

A number of studies have been conducted on patients who had been treated with hypolipidaemic agents for several months to several years, in an attempt to elucidate the effect of peroxisome proliferators in the human liver (Table 5).

Two of these studies showed a marginal increase in hepatocellular peroxisome content. Hanefeld et al (1983) used a quantitative technique based on that of Weibel (1969) to compare the number of peroxisomes found in biopsy specimens from 16 hypolipidaemic patients before and after receiving treatment with clofibrate for three to four months. He found that there was a 50% increase in the mean number of peroxisomes but that the volume density (i.e. the percentage of the cell volume occupied by these organelles) was increased by only 23%. Statistically, the mean increase in the number of organelles was significant whereas the increase in volume density was not. Hinton et al (1991) studied 7 patients who were biopsied before and after 6 - 24 months treatment with 2mg/kg/day ciprofibrate. No differences were observed histologically on comparing the specimens before and after treatment. Ultrastructurally no differences were observed after a qualitative examination. However, a morphometric analysis using a method similar to those used by Hanefeld et al (1983) revealed a slight (30%) increase in the proportion of the hepatocyte cytoplasm occupied by peroxisomes. No changes were observed in the proportion of hepatocyte occupied by mitochondria or lysosomes. As in previous studies, considerable inter-individual variation in the volume of hepatocytes occupied by peroxisomes was observed. Consequently the biological significance of such small changes is questionable.

Three other studies failed to demonstrate peroxisome proliferation in man. Thus, De la Iglesia (1982) studied liver biopsies from 9 patients

on long term (17 - 25 months) treatment with gemfibrozil. It was found that the content of hepatic peroxisomes in the treated patients varied widely from cell to cell according to the location in the hepatic lobule but peroxisome proliferation as observed in rodents does not occur. The appearance of livers from the treated patients was similar to that described in the literature for normal individuals. No peroxisome proliferation was found in a group of 22 males and 6 females who had received fenofibrate treatment for between 6 and 36 months compared to 14 untreated individuals. All fenofibrate patients were receiving the drug at or shortly before the time of biopsy (Blumcke et al, 1983). Gariot et al (1987) compared liver biopsies from 12 hyperlipidaemic patients receiving a mean daily dose of 317mg fenofibrate and from 15 patients receiving a low fat diet. There was no difference in the volume density or in the number of peroxisomes between test and control groups assessed by the method of Weibel (1969). There was a slight increase in the volume density of mitochondria in the group receiving fenofibrate.

Unfortunately, no data are available on the effects of hypolipidaemic agents on peroxisomal enzyme activities in man.

2. Species Differences in Carcinogenicity

Administration of clofibrate to marmosets at daily doses of 94, 157, 218 and 263mg/kgbw did not elicite hepatomegaly or peroxisome proliferation; furthermore no carcinogenic effects were seen in the liver or any other organ (Tucker et al, 1988, Tucker, personnal communication). Apart from the forgoing data on marmosets published information is not available on the carcinogenicity of peroxisome proliferators in laboratory species other than rats and mice. However, two epidemiology studies have been performed.

The principal study on clofibrate was carried out by WHO co-ordinated study (Oliver et al, 1978) on 15,745 males. Those with high cholesterol levels were assigned at random to receive either 1.6g clofibrate daily (5,331) or an olive oil placebo (5,296); there was a further control group with a lower cholesterol level (5,118) who also received the placebo. The average length of the trial was 5.8 years but records of deaths continued for another year.

There were more deaths from all causes in the clofibrate treated group than in controls (high cholesterol). The difference was statistically significant ($p < 0.05$). Fifty-eight patients in the clofibrate treated group developed neoplasia compared with 42 in the high cholesterol and 41 in the low cholesterol controls respectively (Table 6). This difference was not statistically significant, and there was no evidence of change in the pattern of, or numbers of organs effected.

A study with gemfibrozil also gave the opportunity to evaluate the cancer incidence (Frick et al, 1987). A total of 18,966 Finnish male subjects were screened and 4,081 were included in the trial; 2,051 subjects were assigned to receive gemfibrozil and 2,030 were assigned to receive the placebo. The cancer incidence is listed in Table 7. There was no significant difference in the total number of cancers or specific cancers between the two groups with the exception of 5 basal cell carcinomas of the skin in the gemfibrozil group and none in the placebo group. This difference was of borderline statistical significance according to the Fischer exact test ($p=0.062$); 4.8 tumours would have been expected based on the national cancer register of Finland. No statistically significant differences were found in any of the analyses of other specific cancer type.

This study must be considered of limited value for evaluation of carcinogenic potential because of the short periods of exposure to the drugs.

3. In vitro Studies of Peroxisome Proliferation

Studies made to evaluate species differences in vivo are frequently confounded by variations in administered dose, target organ dose, or differences in routes and rates of biotransformation. Hence several laboratories have attempted to eliminate these confounding factors by using in vitro hepatocyte culture systems. Cultured hepatocytes from a variety of species have been used to assess differences in response to peroxisome proliferators (Table 8). These studies have shown marked species differences in response to the proximate peroxisome proliferators; mouse and rat

hepatocytes responded, while guinea pig, monkey and human hepatocytes did not.

The phenomenon of peroxisome proliferation is also associated with a stimulation of S-phase DNA synthesis and induction of cytochrome P450IVA. At least with one potent peroxisome proliferator, methylclofenapate, species differences in response were also observed in these parameters. Methylclofenapate increased peroxisomal β-oxidation, S-phase and P-450IVA (lauric acid hydroxylase) in rat hepatocytes, but not in guinea pig, marmoset or human hepatocytes (Elcombe and Styles, 1989).

4. Evaluation

It is apparent that marked species differences exist in the response to peroxisome proliferators. Rats and mice are extremely sensitive while other species such as guinea pigs, monkeys and man are essentially non-responsive at dose levels that produce a marked response in rodents. This species specificity cannot be explained by simple variations in the disposition and biotransformation of the compounds but appears to be due to intrinsic biochemical differences between the hepatocytes of the various species.

F: MECHANISMS OF CARCINOGENICITY OF PEROXISOME PROLIFERATORS

A variety of peroxisome proliferators have been shown to induce hepatocellular tumours in rodents (Table 3). The mechanism underlying this carcinogenic effect is discussed in this section.

1. Direct Genotoxicity

The relationship between mutagenicity and carcinogenicity is well documented. In general, peroxisome proliferators give negative results in tests for genotoxic activity and are therefore considered to be non-mutagenic.

A variety of peroxisome proliferators have given negative results in the Ames *Salmonella typhimurium* reversion assay, DNA repair assays and tests for clastogenic activity. Particularly interesting are the negative results obtained with nafenopin, DEHP and chlorinated paraffins in hepatic *in vivo* DNA repair assays and with nafenopin in the rat liver micronucleus assay (Butterworth *et al*, 1984; Bentley *et al*, 1987; Ashby *et al*, 1990; Meyer & Bentley, unpublished observations). Furthermore, Von Däniken *et al* (1981, 1984) could not demonstrate covalent binding of clofibrate, fenofibrate, DEHP or DEHA to rat liver DNA and Goel *et al* (1985) could demonstrate no covalent binding of nafenopin or WY-14,643. It should be noted that the ^{32}P postlabelling assay, possibly the most sensitive indicator for adduct formation, gave negative results with a variety of peroxisome proliferators (Gupta *et al*, 1985). These findings strongly suggest that direct DNA damage is not a prerequisite for the carcinogenicity of peroxisome proliferators.

2. The Induction of Indirect DNA Damage

The observation that many carcinogenic peroxisome proliferators were not mutagenic led Reddy *et al* (1980) to postulate that such compounds formed a unique class of carcinogens and that there was a causal link between peroxisome proliferation and carcinogenicity. This postulate was based on

the finding that the enzyme pattern in induced peroxisomes differed from that of control peroxisomes. Certain oxidases, in particular fatty acyl CoA oxidase (the first step of peroxisomal β-oxidation) are markedly induced whilst catalase activity is generally only slightly increased or even repressed (Reddy & Lalwani, 1983; Cohen & Grasso, 1981; Lazarow & de Duve, 1976; Hawkins et al, 1987; Lake et al, 1989; Nemali et al, 1989). For example nafenopin, Wy-14,643 and BR 931 induced fatty acyl CoA oxidase 20 fold, whilst catalase gene expression was induced less that 2 fold (Nemali et al, 1989). Reddy et al (1980) postulated that these alterations could lead to excess H_2O_2 production within the cell with increased formation of oxygen radicals which could eventually lead to oxidative DNA damage.

Subsequent investigations have shown that treatment of animals with peroxisome proliferators also reduces the efficiency of other defence systems against oxidative damage, since vitamin E levels (Lake et al, 1990) and glutathione peroxidase activity are reduced (Furukawa et al, 1985; Elliott & Elcombe, 1987). Glutathione peroxidase is a cytosolic enzyme which is responsible for inactivating peroxides produced in the cytosol or leaking from the peroxisomes. Jones et al (1981), using isolated hepatocytes, have demonstrated the excretion of oxidised glutathione (GSSG) following incubation with substrates for peroxisomal oxidases and Conway et al (1987) have demonstrated the biliary excretion of oxidised glutathione from isolated perfused livers when oleate was added to the perfusate. The excretion of GSSG was most marked when livers from rats which had been treated with nafenopin were used. These findings indicate that glutathione peroxidase is involved in the inactivation of H_2O_2 produced in the peroxisomes. Consequently decreases in the level of this cytosolic enzyme should enhance the effects of the enzyme imbalance within the peroxisome.

Considerations of enzyme kinetics suggest that intracellular steady state hydrogen peroxide concentrations should be increased following treatment with peroxisome proliferators (Tomaszewski et al, 1986). Increases in the amount of lipid peroxidation (assessed as conjugated dienes) and accumulation of lipofuscin following treatment of animals with peroxisome proliferators are often taken as indications that this is indeed the case (Reddy et al, 1982a; Cattley et al, 1987). Moreover, when several parameters were compared during

chronic feeding studies with Wy-14,643 and DEHP under conditions where both induced liver tumours, the extent of lipofuscin accumulation appeared to correlate well with the potency of the compounds as carcinogens (Conway et al, 1989). On the other hand, Elliott and Elcombe (1987) failed to demonstrate excess lipid peroxidation assessed as malonaldehyde production in livers of animals treated with DEHP, MEHP, clofibrate or methylclofenapate. Lake et al (1990) also failed to demonstrate excess lipid peroxidation in livers of vitamin E deficient rats or iron loaded mice treated with nafenopin. In these experiments nafenopin appeared to prevent lipid peroxidation as a consequence of the vitamin deficiency when peroxidation was assessed via malonaldehyde production. The evidence for lipid peroxidation is equivocal although accumulation of lipofucsin is a common observation.

Oxidative stress should induce DNA damage of a type similar to that caused by radiation, this damage should, therefore, be detectable as single strand breaks or alkaline labile sites in the DNA. In agreement with this Fahl et al (1984) have shown that, in the presence of palmitoyl CoA, peroxisomes from ciprofibrate-pretreated animals catalysed the formation of single strand breaks in SV-40 DNA in vitro. Peroxisomes from control animals had no such activity. The authors suggested that H_2O_2 formed by the induced peroxisomes was inducing the DNA damage. In support of this Elliott et al (1986) showed that induced peroxisomes generated higher levels of hydroxyl radical than control peroxisomes in the presence of palmitoyl CoA. However, the in vitro incubations in both these studies contained KCN at concentrations which were sufficient to inhibit the catalase activity (Elliott et al, 1986). Consequently the increased hydroxyl radical production and the induction of single strand breaks reflect the increased fatty acyl CoA oxidase activity of the induced peroxisomes rather than demonstrating an alteration in the balance between H_2O_2 production and inactivation.

The control of cellular peroxide concentrations is complex and requires intact cellular functions (Jones et al, 1981; Chance et al, 1979). Consequently true indications of the induction of indirect DNA damage may only be obtained from experiments in the whole animal. Elliott and Elcombe (1987) failed to demonstrate increased rates of alkaline elution of hepatic nuclear DNA following treatment of rats for up to 28 days using carcinogenic

doses of either DEHP, MEHP, clofibrate or methylclofenapate. Bentley et al (1987) did observe a slight increase in the rate of alkaline elution of hepatic nuclear DNA following treatment of rats with nafenopin for 7 days but not with clofibrate. The effect of nafenopin was not increased by inhibition of catalase activity or treatment of animals with glycolate as a substrate for peroxisomal glycolate oxidase. These findings therefore suggest that the slight increase in alkaline elution rates observed following nafenopin treatment was not a result of oxidative stress and that neither of the peroxisome proliferators induce damage to hepatic DNA.

Oxidative damage may also be assessed by the presence of oxidised DNA bases, particularly 8-hydroxydesoxyguanosine and thymidine glycol (Frenkel et al, 1986; Kasai et al, 1986; Leadon, 1987). Kasai et al (1989) have investigated the formation of 8-hydroxydesoxyguanosine in liver DNA during treatment of rats with ciprofibrate for up to 40 weeks. The authors reported that 8-hydroxydesoxyguanosine levels were increased after 16, 28 and 40 weeks but at 36 weeks levels were not significantly different from the control values. Moreover, control values reported in the paper vary 2 fold in two different experiments. If the variation between the control values is considered to be the normal range then the only truly elevated values were those obtained at 40 weeks. At this time point the rats already carried liver tumours. Thus it would seem unlikely that oxidative DNA damage at this point could have initiated tumour formation although in another study Takagi et al (1990) showed a small (less than two fold but statistically significant) increase in the level of 8-hydroxydeoxyguanosine in rats treated with 1.2% DEHP and 2.5% DEHA. The significance of the findings to the mechanism of carcinogenicity is questionable since at these doses DEHP induced liver tumours whilst DEHA did not (NTP, 1982a,b). Moreover, Hegi et al (1990) failed to detect increased levels of either 8-hydroxydesoxyguanosine or thymidine glycol following treatment of rats with nafenopin for up to 8 weeks. At present, too little is known about variations in endogenous 8-hydroxydesoxyguanosine levels to decide whether a 2-fold increase above the control level is biologically significant.

3. Tumour Initiation and Promotion

3.1. Tumour Initiation

Several peroxisome proliferators have been tested in assays for liver tumour initiation (Garvey et al, 1987; Cattley et al, 1989); all were shown to lack initiating activity - whether assessed in terms of enzyme-altered foci or neoplastic liver nodules (Williams et al, 1987) - consistent with their lack of mutagenicity. Consequently more attention has been paid to their possible role as tumour promoters in two stage carcinogenicity models.

3.2. Tumour Promotion

Peroxisome proliferators have been tested as tumour promoters with confusing results (Table 9), much of this confusion has resulted from the end points used in the studies. In many cases, promotion assays have assessed the ability of a compound to stimulate the growth of enzyme-altered foci in the livers of rats which have previously been treated with an initiating carcinogen such as diethylnitrosamine. The enzyme-altered foci are generally visualised by histochemical or immuno-histochemical methods. In recent years one of the most commonly used histochemical markers has been for γ-glutamyl transpeptidase (GGT) activity. This foetal liver enzyme is not found in hepatocytes of the adult rat liver but is induced in many liver tumours and preneoplastic hepatic lesions. Consequently the stimulation of the formation of GGT staining foci has been widely used in tumour promotion assays. However, peroxisome proliferators appear to inhibit the formation of such foci (De Angelo and Garrett, 1983; Stäubli et al, 1984) and when assessed with this marker the number of GGT staining foci appears to be decreased. This finding is consistent with the observation that tumours induced after chronic treatment with peroxisome proliferators lack γ-glutamyl transpeptidase activity (Rao et al, 1982; 1986b; 1987; 1988; Milano et al, 1987; Yeldandi et al, 1989). The tumours also lack other foetal properties such as expression of glutathione S-transferase P (Rao et al, 1986c; 1988) and α-foetoprotein (Rao et al, 1988). The GGT negative

phenotype of liver lesions induced by ciprofibrate was not altered by subsequent treatment of the rats with 2-acetylaminofluorene (Yeldandi et al, 1989). Interestingly nafenopin also delayed the appearance of GGT activity in cultured rat hepatocytes (Bieri et al, 1984). In view of these findings, tumour promotion assays involving enzyme-altered foci stained for either GGT activity or glutathione S-transferase content cannot be used to assess the tumour promotion potential of peroxisome proliferators. As shown in Table 9 many such assays have been performed. The situation is still confused when assays using other end points such as ATP-ase and glucose-6-phosphatase are considered, since both positive and negative results have also been obtained with these markers. In general, positive results have been found when the formation of tumours was taken as an end point using aflatoxin or diethylnitrosamine as the initiating carcinogen. This suggests that peroxisome proliferators will stimulate the growth of "preneoplastic lesions" induced by other carcinogens.

In keeping with this, Schulte-Hermann et al (1981, 1983) have shown that nafenopin will stimulate DNA synthesis preferentially in spontaneous and nitrosamine induced enzyme-altered foci. Moreover, the same authors have shown that nafenopin will stimulate tumour formation in old rats much more rapidly than in young rats (Kraupp-Grasl, 1990), indicating that the compound may promote the development of spontaneous lesions. The increase in size of preneoplastic lesions is a reflection of the difference between the rates of cell division and cell death. Tumour promoters, eg. phenobarbitone, inhibit programmed cell death (apoptosis) in a manner which is at present poorly understood. Cessation of promoter treatment results in a rapid increase in the number of apoptotic bodies within the liver and reapplication of the promoter during this regression inhibits this process. Studies in which phenobarbitone was used as a tumour promoter and then replaced by nafenopin indicate that peroxisome proliferators will also inhibit apoptosis (Bursch et al, 1984). Consequently, peroxisome proliferators may both stimulate the growth of preneoplastic lesions and inhibit cell turnover (apoptosis) in these lesions. Recent studies have shown that tumour development during treatment with peroxisome proliferators is preceded by the appearance of

rapidly growing foci within the liver. These foci appear diffusely basophilic in H & E staining and form a subclass of the ATP-ase negative foci. Furthermore, in these studies with Wy-14,643 and DEHP, sustained stimulation of DNA synthesis appeared to correlate well with the potency of the two compounds as carcinogens (Marsman et al, 1988).

4. Growth induction

As discussed (cf. Section F.3.), most peroxisome proliferators will also induce DNA synthesis and mitotic activity within the liver with subsequent liver enlargement (Moody and Reddy, 1978b). The induction of DNA synthesis can be reproduced in vitro in primary cultures of adult rat hepatocytes and is, therefore, a direct property of the compounds (Bieri et al, 1984; 1990; Butterworth et al, 1987). Styles et al (1987) have shown that DEHP and methylclofenapate induce DNA synthesis primarily in binucleate cells which upon mitosis produce two tetraploid daughter cells. This suggests that a particular cell population within the liver may be especially susceptible to the growth induction by peroxisome proliferators.

The actual mechanism by which peroxisome proliferators induce cell growth is unclear. Thompson et al (1986) have shown that liver regeneration is controlled by the ordered sequential expression of hepatic cellular protooncogenes. Nafenopin treatment of rats appeared to stimulate the expression of some of these genes (Bentley et al, 1988). This increase in protooncogene expression, although not very marked, was in some cases maintained throughout treatment (up to 28 days). These findings suggest that the mechanisms which control cell growth induced by peroxisome proliferators and normal regenerative cell division are the same. Preliminary observations (Muakkassah-Kelly, 1990) indicate that one of the earliest events after addition of nafenopin to hepatocyte suspensions is an increase in the cytoplasmic calcium concentration. Transient elevation of cytosolic calcium levels has also been reported in response to growth factors (Berridge, 1987). Elevation of cytosolic calcium could contribute to several of the effects of peroxisome proliferators, including activation of serine proteases (Enomoto et al, 1987), effects on cell-cell communication and stimulation of cell growth. It should be borne in mind that many peroxisome proliferators,

particularly the hypolipidaemic drugs, have profound pharmacological effects upon the liver and, when considering the early events described above, it is difficult to distinguish between those which are pharmacologically mediated and those which are related to subsequent pathology. Intracellular processes controlling short term physiological responses commonly control cell division, the difference appearing to be in the strength and duration of the signal. Prolonged hyperstimulation is generally required to stimulate cell division (Berridge, 1987), as would be the case in toxicological studies.

5. Evaluation

Many peroxisome proliferators are potent complete carcinogens in the rodent. From the above discussion it is apparent that there are several mechanisms by which they could induce tumour formation. These include:

- oxidative stress and the induction of indirect DNA damage,
- promotion of spontaneous preneoplastic lesions,
- sustained growth stimulation.

These mechanisms are not mutually exclusive but could cooperate with each other during tumour development as depicted in Figure 3. Thus oxidative stress could contribute to tumour promotion either through the induction of cytotoxicity followed by replacement cell growth or through direct growth stimulation. Cerutti (1985) has suggested that oxidative stress may be an important part of tumour promotion. Another important aspect not discussed above is the reversibility of the effects. In "stop" experiments with clofibrate Greaves et al (1986) showed that nodule formation in the liver was totally reversible up to 95 weeks of treatment if animals were subsequently transfered to a control diet for a further 16 - 18 weeks. These findings suggest that transformation of autonomous growth (a characteristic of the malignant state) occurs at a relatively late stage in the induction of tumours by clofibrate. It is unclear to what extent these findings can be extended to other peroxisome proliferators.

The data are consistent with the following hypothesis. Peroxisome proliferators initially induce a general hyperplastic response in the liver.

If this growth stimulus is maintained the majority of the cells cease to respond (down regulation). A few cells, however, continue to divide (these may be spontaneous lesions) and develop into foci of cellular change (diffusely basophilic) which expand to form a nodular hyperplasia. The cells within these nodules are still dependent upon the external growth stimulus. As the nodule grows the blood supply to the cells alters such that a greater proportion is fed via the hepatic artery (Solt et al, 1977). As the proportion of portal blood decreases the concentration of mitogen reaching the cells also decreases. Thus an environment is created within the nodules in which cells that require less peroxisome proliferator to maintain their growing state are selected. The increase in the proportion of arterial blood reaching the nodular cells also means that they receive more oxygen, a condition favouring oxidative stress. The type of DNA damage reported by Kasai et al (1989) could therefore be important for transition to an autonomous growth state at this stage of tumour development.

It is apparent from this discussion that the mechanism of carcinogenicity arising from peroxisome proliferators is complex and that several different processes are involved. Sustained administration of the peroxisome proliferator is necessary and the consequential liver enlargement is required for tumour development.

G: ASSESSMENT OF HUMAN HEPATO-CARCINOGENIC HAZARD

A procedure for evaluating potential risk to man caused by suspected hepatocarcinogens is provided in schematic form (Figure 4).

Numerous chemicals have been identified as being capable of inducing peroxisome proliferation in the rodent liver (cf. Section C). Those that have been tested for carcinogenicity cause liver tumours in rats and/or mice. This consistent association supports the hypothesis that peroxisome proliferation and the related phenomena may be causally linked to the subsequent appearance of tumours. Current knowledge concerning the mechanism of carcinogenicity of peroxisome proliferators suggests that the risk resulting from human exposure is very low. This conclusion is based on the experimental evidence outlined below.

1. Non-genotoxicity of peroxisome proliferators

There is no evidence that peroxisome proliferators induce direct DNA damage (cf. Section F).

2. Experimental evidence for no-effect levels of liver hepatomegaly and peroxisome proliferation in rodents

Liver enlargement is a characteristic response of laboratory animals to treatment with peroxisome proliferators. This hepatomegaly results from a combination of hypertrophy (ie. increase in cell size) and hyperplasia (ie. increase in cell number).

The current opinion is that peroxisome proliferation and liver growth are closely associated with the development of liver cancer in rats and mice and in their absence liver tumours will not develop even in these sensitive rodent species. When considering dose response relationships two types of threshold may be expected (Figure 5). At very low doses, administration of peroxisome proliferators will not lead to any changes in the liver of rodents (Figure 5, no effect zone 1). This has been shown in feeding studies with

dibutyl, diethylhexyl and diisodecyl phthalates (DBP, DEHP, DIDP) which demonstrated no effect levels for peroxisome proliferation of 104, 52 and 57mg/kg/day respectively (Lake, 1990, unpublished data). With increasing dose the first threshold will be exceeded resulting in the stimulation of peroxisome proliferation and DNA synthesis (Figure 5, effect zone 2). Several studies suggest that a limited amount of liver growth does not automatically lead to tumour development. Thus with DEHA a moderate degree of liver growth and peroxisome proliferation has been demonstrated (Keith *et al*, 1990) at doses equivalent to those used in the NTP bioassay although no tumours were observed (NTP, 1982b). Furthermore fenofibrate and DEHP have induced moderate growth of the liver and induction of peroxisomes at dose levels which do not induce tumours in carcinogenicity assays (Mitchell *et al*, 1985b, Price *et al*, 1986). Similarly asprin (acetylsalicylic acid), a weak peroxisome proliferator, does not produce tumours (Price *et al*, 1986). Thus a second threshold has to be exceeded at which the magnitude of effects is sufficient to induce tumour development in rodents (Figure 5, tumourigenic zone 3). It is most probable that prolonged administration of the compound is required to exceed this tumourigenic threshold since peroxisome proliferator-mediated liver growth can be rapidly and totally reversible on cessation of treatment.

3. Species Differences in Response to Peroxisomal Proliferators

A variety of independent studies have shown that there are marked species differences in the sensitivity to chemicals that cause peroxisome proliferation (Section E). Rats and mice are extremely sensitive, hamsters show a less marked response whilst guinea pigs, primates and man are insensitive or non-responsive.

The available evidence therefore strongly supports the conclusion that exposure to peroxisome proliferators at low levels (eg. at therapeutic doses or at environmental exposure levels) present no risk of tumour development to man. This conclusion is in agreement with the available epidemiological data.

Table 1. Some Enzymes present in mammalian peroxisomes.

NAD(P)-linked dehydrogenases:	Glycerol-3-phosphate dehydrogenase (NAD), NADH-glycolate reductase, Isocitrate dehydrogenase (NADP), Acyl-dihydroxyacetone phosphate-NADPH oxidoreductase, Xanthine dehydrogenase.
Enzymes of Hydrogen Peroxide Metabolism:	Glycolate oxidase, D-amino acid oxidase, Urate oxidase (not in primates), Catalase.
Transferases:	Carnitine acetyl transferase, Carnitine octanoyl transferase, Dihydroxyacetone phosphate acyl tranferase, Glycolate aminotransferase.
Others:	NADH-cytochrome c reductase (antimycin insenstive), Acyl CoA oxidase, Acyl CoA synthetase.

le 2a. Phenoxy-acetic acids and related compounds causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Structure: phenyl ring (positions 1–5) –O–C(R_1)(R_2)–$COOR_3$

Common Name	R1	R2	2	3	4	R3	PCoA	EM	Dose	Reference
CPA					Cl	-	0		1,250	Lundgren et al, 1987a
							0		1,250	Lundgren et al, 1987b
2,4-D			Cl		Cl	-	++		2,500	Kawashima et al, 1984
							++		1,250	Lundgren et al, 1987a
							++		1,250	Lundgren et al, 1987b
2,4,5-T			Cl	(5-Cl)	Cl	-	++		2,500	Kawashima et al, 1984
							+		1,250	Lundgren et al, 1987a
							+		1,250	Lundgren et al, 1987b
MCPA			CH_3		Cl	-	+	**	200mg/kg	Hietanen et al, 1985
								**	200mg/kg	Vainio et al, 1983
	CH_3	CH_3				C_2H_5		**	2,500	Azarnoff et al, 1976
	CH_3	CH_3			Br	C_2H_5		**	2,500	Azarnoff et al, 1976
	CH_3	CH_3			I	C_2H_5		**	2,500	Azarnoff et al, 1976
	CH_3	CH_3	Cl			C_2H_5		NC	2,500	Azarnoff et al, 1976
	CH_3	CH_3		Cl		C_2H_5		NC	2,500	Azarnoff et al, 1976
Clofibric acid	CH_3	CH_3			Cl	-	+++		2,500	Lundgren et al, 1987b
Clofibrate	CH_3	CH_3			Cl	C_2H_5		**	2,500	Azarnoff et al, 1976
							+++		2,500	Lundgren et al, 1987a
								**	500mg/kg	Hess et al, 1965
							+++	**	5,000	Lazarow and de Duve, 1976

Table 2a. Phenoxy-acetic acids and related compounds causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
(cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure R1	R2	2	3	4	R3	PCoA	EM	Dose	Reference
Clofenic acid (methyl clofenopate)	CH_3	CH_3			Cl	CH_3		**	2,500	Azarnoff et al, 1976
							+++	**	2,000	Lalwani et al, 1983b
							+++		1,000	Lalwani et al, 1981b
Nafenopin (SU-13437)		CH_3	CH_3				+++	**	2,500	Reddy et al, 1973
								**	2,500	Azarnoff et al, 1976
							+			Lungren et al, 1987b
							++		1,000	Lalwani et al, 1983a
Ciprofibrate	CH_3	CH_3			Cl Cl			**	2,500	Lalwani et al, 1983b
							+++	+++	2,500	Dwivedi et al, 1989
								**	2,500	Azarnoff et al, 1976
Fenofibrate	CH_3	CH_3			CO, Cl	i-C_3H_7	++	**	2,500	Lalwani et al, 1983b
							+		5,000	Pourbaix et al, 1984
							+++		2,000	Lalwani et al, 1981b
Bezafibrate	CH_3	CH_3			Cl, $-CH_2-CH_2-NH-CO-$, Cl	-	+	**	2,500	Lalwani et al, 1983b
							++	++	200mg/kg	Sharma et al, 1988

Table 2a. Phenoxy-acetic acids and related compounds causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
(cont. 2) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure R1	R2	2	3	4	R3	PCoA	EM	Dose	Reference
Halofenate (MK185)					Cl	$-CH_2-CH_2-NH-CO-CH_3$		**	2,500	Azarnoff et al, 1976
S-8527	CH_3	C_2H_5			O, COOH	-	+		1,250	Lundgren et al, 1987b
								**	2,500	Azarnoff et al, 1976
SaH 42-348	O, Cl				Cl	N, CH_3		++	1,000	Moody and Reddy, 1976
								**	1,500	Reddy et al, 1975
								**	2,500	Azarnoff et al, 1976
Simfibrate	CH_3	CH_3			Cl	bis $-CH_2-CH_2-CH_2$		+	1,000 mg/kg	Hirai and Ogawa, 1973

Table 2a. Phenoxy-acetic acids and related compounds causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
(cont. 3) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure R1	R2	2	3	4	R3	PCoA	EM	Dose	Reference
AT-308	CH_3	CH_3			(oxadiazolyl-pyridyl structure)	C_2H_5	Liver/BW ratio increased		1,000	Imai and Shimamato, 1973
Beclobric Acid	CH_3	C_2H_5			CH_2-C6H4-Cl		+++		In vitro (0.3 mmol)	Kocarek and Feller, 1987
LF 2151 (reduced fenofibrate)	CH_3	CH_3			H-C-OH-C6H4-Cl		(+)	NC	1,500	Pourbaix et al, 1984

Table 2a. Phenoxy-acetic acids and related compounds causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
(cont. 4) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Structures of clofibrate and analogs (I - V)

Common Name	Structure	PCoA	EM	Dose	Reference
	I	0		160 mg/kg	Harrison, 1984
	II	0		160 mg/kg	Harrison, 1984
	III	0		140 mg/kg	Harrison, 1984
	IV	0		160 mg/kg	Harrison, 1984
	V	0		60 mg/kg	Harrison, 1984
	A		**	300 mg/kg	Magnusson & Magnusson, 1976

able 2b. Phenoxy-acetic acid analogues causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
	General Structure: $C_6H_5-X-CH_2-COOH$ (X is variable, see below)				
	$Cl-C_6H_4-S-C(CH_3)_2-CO_2C_2H_5$		NC	2,500	Azarnoff et al, 1976
	$Cl-C_6H_4-NH-C(CH_3)_2-CO_2C_2H_5$		NC	2,500	Azarnoff et al, 1976
WY-14,643	2,3-dimethylphenyl$-NH-$(6-Cl-pyrimidinyl)$-S-CH_2CO_2H$		**	2,500	Azarnoff et al, 1976
		+++		1,250	Lundgren et al, 1987b
		+++		2,000	Lalwani et al, 1981a,b
		+++		1,000	Lazarow, 1977
			**	1,250	Reddy et al, 1978
BR-931	2,3-dimethylphenyl$-NH-$(pyrimidinyl)$-S-CH_2-CO-NH-CH_2-CH_2-OH$	+++	++	2,000	Lalwani et al, 1987b
			**	1,250	Reddy et al, 1978
		+++		2,000	Lalwani et al, 1981b

able 2c. n-Alkyl Carboxylic Acids causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
TCA	CCl_3-COOH	++	++	200 mg/kg	Elcombe, 1985
		+		500 mg/kg	Goldsworthy and Popp, 1987
PFBA	perfluro-n-butyric acid $CF_3-CF_2-CF_2-COOH$		**	200	Ikeda et al, 1985
Valproic acid	2-Propyl pentanoic acid $CH_3-CH_2-CH_2-CH(CH_2-CH_2-CH_3)-COOH$	++		1,000	Horie and Suga, 1985
Dimethrin	$(CH_3)_2C=CH-C-C(CH_3)_2-CO_2-CH_2-C_6H_3(CH_3)_2$		**	20,000	Hruban et al, 1974
Hexanoic acid	hexanoic acid $CH_3-CH_2-CH_2-CH_2-CH_2-COOH$	0		10,000	Lundgren et al, 1987b
			**	20,000	Moody and Reddy, 1978
	2-Methyl hexanoic acid $CH_3-CH_2-CH_2-CH_2-CH(CH_3)-COOH$	0		10,000	Lundgren et al, 1987b
	2-Ethyl hexanoic acid $CH_3-CH_2-CH_2-CH_2-CH(CH_2-CH_3)-COOH$	+		10,000	Lundgren et al, 1987b
		++		2,000 mg/kg	Keith et al, 1988
			**	20,000	Moody and Reddy, 1978

able 2c. n-Alkyl Carboxylic Acids causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure		PCoA	EM	Dose	Reference
	2-Propyl hexanoic acid	$CH_3-CH_2-CH_2-CH_2-CH(CH_2-CH_2-CH_3)-COOH$	+		10,000	Lundgren et al, 1987b
	trans-2-Hexanoic acid	$CH_3-CH_2-CH_2-CH=CH-COOH$	0		10,000	Lundgren et al, 1987b
	3-Ethyl hexanoic acid	$CH_3-CH_2-CH(CH_2-CH_3)-CH_2-CH_2-COOH$	0		10,000	Lundgren et al, 1987b
	4-Ethyl hexanoic acid	$CH_3-CH_2-CH(CH_2-CH_3)-CH_2-CH_2-COOH$	0		10,000	Lundgren et al, 1987b
PFOA	perfluro-n-octanoic acid	$CF_3-(CF_2)_6-COOH$		**	200	Ikeda et al, 1985
				**	50 mg/kg	Pastoor et al, 1987
PFDA	perfluro-n-decanoic acid	$CF_3-(CF_2)_8-COOH$	+++	++	100 mg/kg	Ikeda et al, 1985
CMTTD	1-Mono(carboxy methyl thio) tetra decane	$CH_3-(CH_2)_{13}-S-CH_2-COOH$	++		750 mg/kg	Berge et al, 1989
			++	++	500 mg/kg	Berge et al, 1989
CETTD	1-Mono(carboxy ethyl thio) tetra decane	$CH_3-(CH_2)_{13}-S-CH_2-CH_2-COOH$	+	+	800 mg/kg	Berge et al, 1989
PMA	Palmitic acid	$CH_3-(CH_2)_{14}-COOH$	0	NC	1,000 mg/kg	Berge et al, 1989

able 2d. n-Alkyl Carboxylic Acid precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure		PCoA	EM	Dose	Reference
PCE	Tetrachloroethylene	$Cl_2C=CCl_2$	++		1,000 mg/kg (mouse)	Goldsworthy and Popp, 1987
			0		(rat)	
TCE	Trichloroethylene	$Cl_2C=CHCl$	++		1,000 mg/kg (mouse)	Goldsworthy and Popp, 1987
			0		(rat)	
			++	+++	1,500 mg/kg (mouse)	Elcombe, 1985
			0	0	(rat)	
PENT	Pentachloroethane	$H-CCl_2-CCl_3$	0		150 mg/kg (mouse)	Goldsworthy and Popp, 1987
			0		(rat)	
	Halothane	$F_3C-CHBrCl$		**	0.25% air 7h/d	Ross and Cardell, 1972
Isooctane	2,2,4-Trimethylpentane	$CH_3-C(CH_3)_2-CH_2-CH(CH_3)-CH_3$	++	0	1,350 mg/kg	Lock et al, 1987
	3-Methyl heptane (2-Ethyl hexane)	$CH_3-CH_2-CH_2-CH_2-CH(CH_3)-CH_2-CH_3$	0		10,000	Lundgren et al, 1987b
	2-Ethyl hexanol	$CH_3-CH_2-CH_2-CH_2-CH(CH_2-CH_3)-CH_2OH$	0		10,000	Lundgren et al, 1987b
			++		1,150 mg/kg	Keith et al, 1988
				**	20,000	Moody and Reddy, 1978

Table 2d. (cont. 1) n-Alkyl Carboxylic Acid precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
PFOL	1-H,1-H-Pentadecafluro octanol $CF_3-(CF_2)_6-CH_2OH$		NC	200	Ikeda et al, 1985
PFO	perflurooctane $CF_3-(CF_2)_6-CF_3$		NC	200	Ikeda et al, 1985
PFD	perflurodecane $CF_3-(CF_2)_8-CF_3$		NC	200	Ikeda et al, 1985
DEHA	Di-(2-ethylhexyl) adipate $COO\cdot CH_2-CH(CH_2-CH_3)-CH_2-CH_2-CH_2-CH_3$ / $(CH_2)_4$ / $COO\cdot CH_2-CH(CH_2-CH_3)-CH_2-CH_2-CH_2-CH_3$	++		1,600 mg/kg	Keith et al, 1988
		++	+	25,000	Barber et al, 1987
	Di-(2-ethylhexyl) phosphate $[CH_3-CH_2-CH_2-CH(CH_2-CH_3)-CH_2-]_2-PO_4$	++		20,000	Lundgren et al, 1987b
	Di-(2-ethylhexyl) sebacate $COO\cdot CH_2-CH(CH_2-CH_3)-CH_2-CH_2-CH_2-CH_3$ / $(CH_2)_8$ / $COO\cdot CH_2-CH(CH_2-CH_3)-CH_2-CH_2-CH_2-CH_3$		**	20,000	Moody & Reddy, 1978

Table 2d. (cont. 2) n-Alkyl Carboxylic Acid precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
	Hexyl alcohol $CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-OH$		NC	20,000	Moody and Reddy, 1978
	2-Ethyl hexyl aldehyde $CH_3-CH_2-CH_2-CH(CH_2-CH_3)-CH_2-CHO$		**	20,000	Moody and Reddy, 1978
	Hexyl aldehyde $CH_3-CH_2-CH_2-CH_2-CH_2-CHO$		NC	20,000	Moody and Reddy, 1978
Citral	$CH_3-C(CH_3)=CH-CH_2-CH_2-C(CH_3)H=CH-CHO$	+	**	2,400 mg/kg	Jackson et al, 1987
NTPC 12 (Chlorowax 500C)	C_{10-13} , 58% Cl	++	++	1,000 mg/kg	Elcombe et al, 1989
NTPC 23 (Chlorowax 40)	C_{23-26} , 43% Cl	0	0	1,000 mg/kg	Elcombe et al, 1989
Cereclor 56L	C_{10-12} , 56% Cl	+++	++	1,000 mg/kg	Elcombe et al, 1989
Chloroparaffin 40G	C_{14-17} , 40% Cl	+	+	1,000 mg/kg	Elcombe et al, 1989

Table 2e. Dicarboxylic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
Medica 16	$HOOC-CH_2-C(CH_3)_2-(CH_2)_{10}-C(CH_3)_2-CH_2-COOH$	++	++	2,500	Hertz et al, 1988
HDDA	$HOOC-(CH_2)_{14}-COOH$ Hexadecanedioic acid	0		750 mg/kg	Berge et al, 1989
Adipic acid	$HOOC-(CH_2)_4-COOH$		NC	20,000	Moody and Reddy, 1978
Niadenate		+++		5,000	Berge and Aarsland, 1985
		+++		5,000	Bakke and Berge, 1982
Nicotinic acid		0		2,500	Berge and Aarsland, 1985
	$CH_3-C(CH_3)_2-CH_2-C(CH_3)_2-CH_2-CH(CH_3)-CH_2-C(CH_3)_2-CH_3$ 2,2,4,4,6,8,8-heptylmethyl nonane	+++	**	10,000	Ikeda et al, 1988

able 2e. Dicarboxylic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
Tiadenol	$HO-CH_2-CH_2-S-(CH_2)_{10}-S-CH_2-CH_2-OH$	+++	**	550 mg/kg	Oesch & Schladt, 1987
			**	5,000	Martin and Feldman, 1974
		+++		5,000	Berge et al, 1984
		+++		3,000	Berge and Aarsland, 1985
		+++		300 mg/kg	Facino et al, 1988
		++	**	2,000	Lalwani et al, 1983b
BCMTD (Tiadenol metabolite)	Bis-(carboxymethythio)-1,10-decane $HOOC-CH_2-S-(CH_2)_{10}-S-CH_2-COOH$	+++	+++	500 mg/kg	Berge et al, 1989
			NC	20,000	Moody & Reddy, 1978
Gemcadiol	2,2,9,9-tetramethy-1,10-decanediol $HOH_2C-C(CH_3)_2-(CH_2)_6-C(CH_3)_2-CH_2OH$		**	300 mg/kg	Fitzgerald et al, 1986

able 2f. Alkyl-aryl Carboxylic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
ICI 55897 (Clobuzart)	Cl–C6H4–C6H4–CH_2–O–C(CH_3)(CH_3)–COOH	+		3,750	Lundgren et al, 1987b
		++	+	10mg/kg	Orton et al, 1984
Gemfibrozil	(CH_3)(CH_3)C6H3–O–$(CH_2)_3$–C(CH_3)(CF_3)–COOH		**	2,000	Fukuda et al, 1978
		+		2,000	Lalwani et al, 1983b
LK-903	CH_3–$(CH_2)_{13}$–C6H4–CH=C(CH_3)–CO_2–CH_2–CH(OH)–CH_2OH	++		500	Hayashi et al, 1981
Cetaben	Sodium p-hexadecylamino benzoate CH_3–$(CH_2)_{15}$–NH–C6H4–CO_2Na		+	200 mg/kg	Fort et al, 1983
RMI-14,514	CH_3–$(CH_2)_{13}$–(furan)–COOH		**	2,000	Lalwani et al, 1983b

able 2f. Alkyl-aryl Carboxylic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
DL-040	(indandione)–CH_2–NH–C6H4–COOH	++	++	400 mg/kg (rhesus monkey)	Lalwani et al, 1985
	Cl–C6H4–CH=CH–CH_2–N(piperazine)N–CH_2–CH_2–C6H4–COOH		**	2,500	Azarnoff et al, 1976
POCA	Ethyl 4-(4-chlorophenoxy) butanoate Cl–C6H4–$(CH_2)_5$–C(CH_2–O)–COOH	+++	+	1,000	Vamecq et al, 1987
		+		2,000	Bone et al, 1982
OKY-046	(imidazolyl)N–CH_2–C6H4–CH=CH–COOH	0		500 mg/kg	Watanake et al, 1986
OKY-1581	(pyridyl)–CH_2–C6H4–CH=C(CH_3)–COOH	++		500 mg/kg	Watanake et al, 1986
DG5685	C6H5–O–C6H4–CH_2–(1,3-dioxane)–(pyridyl)	++	**	1,000	Lalwani et al, 1983b
DH6463	C6H5–O–C6H4–CH_2–(1,3-dioxane)–(pyrimidinyl)	+	**	1,000	Lalwani et al, 1983b

able 2g. Ortho-substituted Benzoic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insnsitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
Asprin	Acetyl salicylic acid	+		10,000	Lundgren et al, 1987b
		+		10,000	Ishii and Suga, 1979
			**	5,000	Hruban et al, 1966, 1974
		++		630 mg/kg	Oesch and Schladt, 1987
o-Phthalic acid		0		10,000	Lundgren et al, 1987b
			NC	850mg/kg	Lake et al, 1975
Tibric acid (CP-18,524)			**	2,500	Azarnoff et al, 1976
			**	2,500	Reddy & Krishnakantha, 1975
Lactofen		+++	**	250	Butler et al, 1988
MNOP	Mono-n-octyl phthalate	+	NC	715 mg/kg	Lake et al, 1984
MEHP	Mono-(2-ethyl hexyl) phthalate	++		10,000	Lundgren et al, 1987b
	Diethyl phthalate		NC	20,000	Moody and Reddy, 1978

able 2g. cont. 1) Ortho-substituted Benzoic Acids and precursors causing peroxisome proliferation as measured by induction of cyanide insnsitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure	PCoA	EM	Dose	Reference
DBP	Di(n-butyl) phthalate	+	**	25,000	Barber et al, 1987
DnHP	Di(n-hexyl) phthalate	0	NC	20,000	Mann et al, 1985
DNOP	Di-n-ocytl phthalate	0	NC	1000mg/kg	Lake et al, 1984
		0	NC	20,000	Mann et al, 1980
DEHP	Di-(2-ethylhexyl) phthalate	+		10,000	Lundgren et al, 1987b
		++	**	25,000	Barber et al, 1987
		+	**	2,000	Moody and Reddy, 1978
		++	+	20,000	Ganning et al, 1983
		+++		20,000	Osumi and Hashimoto, 1978
			**	2,000mg/kg	Lake et al, 1975
		+++	**	2,000	Mann et al, 1985
DINP	Di(isononyl) phthalate	++	**	25,000	Barber et al, 1987
DIDP	Di(isodecyl) phthalate	+++	**	25,000	Barber et al, 1987
DUP	Di(undecyl) phthalate	+	**	25,000	Barber et al, 1987
BBP	Butyl, benzyl phthalate	+	**	25,000	Barber et al, 1987

Table 2h. Non-carboxylic acid groups and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure		PCoA	EM	Dose	Reference
	perfluoro octyl sulphonic acid	$CF_3-(CF_2)_7-SO_3H$	++	**	200	Ikeda et al, 1987
LS2265	$Cl-C_6H_4-C(=O)-C_6H_4-O-C(CH_3)_2-CONHCH_2CH_2SO_3H$		+++	**	2,500	Lanwani et al, 1983b
Fomesafen	$CF_3-C_6H_3(Cl)-O-C_6H_3(NO_2)-CONHSO_2CH_3$		++		50mg/kg	Elcombe, 1986 and unpublished data
Substitued Tetrazoles	$CH_3-C(=O)-C_6H(OH)(C_3H_7)-O-(CH_2)_n-C$ (tetrazole, N-R)					
A	n = 1	R = H	0		2,500	Eacho et al, 1989
B	n = 3	R = H	0		2,500	Eacho et al, 1989
LY171883 (C)	n = 4	R = H	+++		2,500	Eacho et al, 1989
4THA (D)	n = 6	R = H	+++		2,500	Eacho et al, 1989
E	n = 8	R = H	+++		2,500	Eacho et al, 1989
F	n = 9	R = H	++		2,500	Eacho et al, 1989
G	n = 4	R = CH_3	0		2,500	Eacho et al, 1989

Table 2h. Non-carboxylic acid groups and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name	Structure		PCoA	EM	Dose	Reference
Substitued Tetrazoles	$CH_3-C(=O)-C_6H(OH)(C_3H_7)-CH_2-O-C_6H_4-(CH_2)_n-C$ (tetrazole, N-R)					
LY16443 (H)	n = 1	R = H	0		2,500	Eacho et al, 1989
I	n = 2	R = H	+		2,500	Eacho et al, 1989
J	n = 3	R = H	+++		2,500	Eacho et al, 1989
K	$CH_3-C_6H(OH)(C_3H_7)-CH_2-O-C_6H_4-CH_2-C(CH_3)_2-CH_2-C$ (tetrazole, N-R)		0		2,500	Eacho et al, 1989
L	$CH_3-C_6H(OH)(C_3H_7)-CH_2-O-C_6H_4-CH_2-C$ (tetrazole, N-H)		+++		2,500	Eacho et al, 1989

able 2h. Non-carboxylic acid groups and precursors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 2) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name		PCoA	EM	Dose	Reference
Isoprenaline	sympathomimetric-adrenergenic amine HO-C6H3(HO)-CH(OH)-CH2-NH-CH(CH3)CH3		**	5mg/100g (2x daily)	Barni et al, 1981
Thyroid Hormone			+	20ug/100g	Fringes and Reith, 1982

able 2i. Other Compounds and Physiological factors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a subjective increase in peroxisome number and size.

Common Name		PCoA	EM	Dose	Reference
	Ethionine		**	5,000 in water	Wood, 1965
	Phenobarbitone		**	1,000	Burger and Herdson, 1966
	Carbon Tetrachloride		**	0.75ml/ 100kg	Bassi, 1960
	1-Benzylimidazole C6H5-CH2-N(imidazole)	+ 0		64mg/kg 1,800	Oesch and Schladt, 1987 Schladt et al, 1987
	Chlorpromazine phenothiazine-N-CH2-CH2-CH2-N(CH3)2	++	(+)	5,000	Vamecq et al, 1987
	Tridiphane Cl2C6H3-C(CH2-O)-CH2-CCl3	+	**	500mg/kg	Moody and Hammock, 1987

able 2i. Other Compounds and Physiological factors causing peroxisome proliferation as measured by induction of cyanide insensitive Palmityl CoA and electron microscopy.
cont. 1) Dose in ppm unless otherwise stated. Gradings used are:
for PCoA: 0 = less than 2 fold increase above control, + = 2 - 4 fold increase above control, ++ = 5 - 8 fold increase above control, +++ = >9 fold increase above control.
for electron microscopy (EM) where peroxisome proliferation has been measured by morphometric analysis, 0 = less than 2 fold increase from control, + = 2 - 4 fold increase, ++ = 4 fold increase and +++ = 5 fold increase or greater; when there has been no morphometric examination NC = no change and ** = a increase in peroxisome number and size.

Common Name		PCoA	EM	Dose	Reference
DHEA	dihydro-epiandrosterone acetate	+++		6,000	Leighton et al, 1987
Vitamin E Defienciency			**		Dabholkar, 1981
		++			Reddy et al, 1981
Riboflavin Deficiency			**		Tandler et al, 1968
High Fat Diet		++	**	30%)in	Ishii et al, 1980
		+		15%)diet	Neat et al, 1980a,b
		+		25%)	Thomassen et al, 1985
Genetic Obesity		+			Murphy et al, 1979
Diabetic rats (alloxan treated)		+		150mg/kg	Horie et al, 1981
Cold adaptation		+			Pollera et al, 1983
Cholestyramine		ND		100mg/kg	Bakke & Berge, 1984
Endotoxaemia			+	300ug/rat	Levy et al, 1968
Benzbromarone	(3,5-dibromo-4-hydroxyphenyl)-1(2-ethyl-3-benzofuranyl) methanone	+++	+++	2,000	Butler et al, 1990
Mivinolin			+	4.2mg/kg	Rebuffat et al, 1988

Table 3; Incidence of Liver Carcinomas in Mice and Rats fed various Hypolididaemic Agents
(Effective numbers of animals are number alive at first tumour or number of animals on which the tumour incidence was based).
(Dietary levels are given as mg/kg body weight unless indicated in percentage terms)

Compound	Species	Dietary Level	Expt. Duration	Number of Animals			Number with Tumours		Reference
				Sex	Initial	Effective	No	%	
Methly-clofenapate	Rat (F344)	0.1%	19 m	M	14	14	14	100	Reddy et al, 1982a
Gemfibrozil	Rat CD-Charles River	0.00	24 m	M	50	29	1	4	Fitzgerald et al, 1981
		0.006%			50	30	6	20	
		0.06%			50	31	23	24	
		0.00	24 m	F	50	24	9	37	
		0.006%			50	31	5	16	
		0.06%			50	27	3	11	
	Rat CD-Charles River	0.00	18 m	M	72	62	16	26	Fitzgerald et al, 1981
		0.006%			72	59	27	76	
		0.06%			72	61	20	33	
		0.00	18 m	F	72	58	4	7	
		0.006%			72	57	1	2	
		0.06%			72	54	4	7	

Table 3 (cont.1); Incidence of Liver Carcinomas in Mice and Rats fed various Hypolididaemic Agents
(Effective numbers of animals are number alive at first tumour or number of animals on which the tumour incidence was based).
(Dietary levels are given as mg/kg body weight unless indicated in percentage terms)

Compound	Species	Dietary Level	Expt. Duration	Number of Animals			Number with Tumours		Reference
				Sex	Initial	Effective	No	%	
Chlorinated Paraffins (C12, 60% Cl)	Rat F344N	0	24 m	M	50	50	0	0	NTP, 1986a
		312			50	50	13	26	
		625			50	50	18	37	
		0	24 m	F	50	50	0	0	
		312			50	50	5	10	
		625			50	50	8	16	
	Mice B6C3F1	0	24 m	M	50	50	22	46	NTP, 1986a
		125			50	50	36	72	
		250			50	50	47	94	
		0	24 m	F	50	50	3	6	
		125			50	50	22	44	
		250			50	50	31	64	
Chlorinated Paraffins (C23, 43% Cl)	Rat F344N	0	24 m	M	50	50	0	0	NTP, 1986b
		1875			50	50	3	6	
		3750			50	50	3	6	
		0	24 m	F	50	50	1	2	
		100			50	50	2	4	
		300			50	50	1	2	
		900			50	50	2	4	
	Mice B6C3F1	0	24 m	M	50	50	19	38	NTP, 1986b
		2500			50	49	26	52	
		5000			50	49	26	52	
		0	24 m	F	50	50	4	8	
		2500			50	49	3	6	
		5000			50	50	10	20	

Table 3 (cont. 2); Incidence of Liver Carcinomas in Mice and Rats fed various Hypolididaemic Agents
(Effective numbers of animals are number alive at first tumour or number of animals on which the tumour incidence was based).
(Dietary levels are given as mg/kg body weight unless indicated in percentage terms)

Compound	Species	Dietary Level	Expt. Duration	Number of Animals			Number with Tumours		Reference
				Sex	Initial	Effective	No	%	
Diethylhexyl phthalate	Rat F344N	0.00	24 m	M	50	50	4	8	NTP, 1982
		0.6%			49	49	7	14	
		1.2%			49	49	17	34	
		0.00	24 m	F	50	50	0	0	
		0.6%			49	49	8	16	
		1.2%			50	50	21	42	
	Mice B6C3F1	0.00	24 m	M	50	50	23	46	NTP, 1982
		0.3%			48	48	39	81	
		0.6%			50	50	35	70	
		0.00	24 m	F	50	50	1	2	
		0.3%			50	50	19	38	
		0.6%			50	50	35	70	
Diethylhexyl phthalate	Mice B6C3F1	0.00	24 m	M	50	50	13	26	NTP, 1981
		1.2%			50	50	20	40	
		2.5%			50	49	28	57	
		0.00	24 m	F	50	50	3	6	
		1.2%			50	50	19	38	
		2.5%			50	49	18	36	
Trichlor-ethylene	Mice B6C3F1	0	24 m	M	20	20	1	5	NCI, 1976
		1200			50	50	26	52	
		2400			50	48	31	65	
		0	24 m	F	20	20	0	0	
		900			50	50	4	8	
		1800			48	47	11	23	

Table 3 (cont 3); Incidence of Liver Carcinomas in Mice and Rats fed various Hypolididaemic Agents
(Effective numbers of animals are number alive at first tumour or number of animals on which the tumour incidence was based).
(Dietary levels are given as mg/kg body weight unless indicated in percentage terms)

Compound	Species	Dietary Level	Expt. Duration	Number of Animals: Sex	Initial	Effective	Number with Tumours: No	%	Reference
LY 171883	Mice B6C3F1	0.00	24 m	M	60	60	11	18	Bendle et al, 1990
		0.0075%			60	60	9	15	
		0.0225%			60	60	8	13	
		0.075%			60	60	18	30	
		0.00	24 m	F	60	60	0	0	
		0.0075%			60	60	3	5	
		0.0225%			60	60	14	23	
		0.075%			60	60	19	30	
Clofibrate	Rat	0.25%	18 m	M	25	25?	4	16	Svoboda and Azarnoff, 1979
		0.25%	26 m		5	5?	2	40	Reddy and Rao, 1977a
		0.5%	28 m		15	11	10	91	Reddy and Qureshi, 1979
Wy-14,643	Rat F344	0.1%	16 m	M	15	15	15	100	Reddy et al, 1979
	Csb mouse	0.1% for 6 m then 0.05% for 8.5 m		M	20	18	18	100	
Nafenopin	Rat F344	0.1%	25 m	M	15	15?	11	73	Reddy and Rao, 1977a,b
	Csb mouse	0.1% for 12 m then 0.05% for 8 m		M	20	9	9	100	Reddy et al, 1976
		0.1% for 12 m then 0.05% for 8 m		F	20	12	12	100	

Table 3 (cont.4); Incidence of Liver Carcinomas in Mice and Rats fed various Hypolididaemic Agents
(Effective numbers of animals are number alive at first tumour or number of animals on which the tumour incidence was based).
(Dietary levels are given as mg/kg body weight unless indicated in percentage terms)

Compound	Species	Dietary Level	Expt. Duration	Number of Animals: Sex	Initial	Effective	Number with Tumours: No	%	Reference
BR-931	Rat F344	0.05%	19 m	M	10	10	7	70	Reddy et al, 1980
		0.2%	16 m	M	20	20	20	100	
	Csb mouse	0.2%	19 m	F	15	12	11	92	
Tibric Acid	Rat F344	0.2% for 7.5 m then 0.1% for 4 m then 0.05% for 5 m		M	35	31	30	97	Reddy et al, 1980
Lactofen	Rat	0.2%	24 m	M + F	Statistically significant increase in hepatic neoplastic nodules in both sexes.				EPA, 1987
	Mouse	0.025%	18 m	M + F	Statistically significant increase in the incidence of combined hepatic adenomas and carcinomas in both sexes.				

Table 4. Species Differences in Peroxisome Proliferation, in vivo, measured by Palmitoyl-CoA oxidising systems. Nominal scores for PCo are (+ = positive response, > 3 fold; +/- = intermediate responses, > 2-3 fold; - = negative response, < 2 fold; changes in the magnitude of PCoA response are indicated where appropriate).

Chemical	Species	Dose * Duration	PCoA	Comments	Reference
Clofibrate	Mouse	0.6% * 2w	+ (x 12)	Plasma Clofibric acid conc = 0.43mM	Holloway, 1989
	Rat	0.2% * 2w	+ (x 11)	Plasma Clofibric acid conc = 0.62mM	Holloway, 1989
		0.4% * 2w	+ (x 28)	Plasma Clofibric acid conc = 1.24mM	Holloway, 1989
		0.6% * 2w	+ (x 29)	Plasma Clofibric acid conc = 0.98mM	Holloway, 1989
		0.25% * 4w	+ (x 13)		Oesch et al, 1988
	Hamster	0.2% * 2w	- (x 1.2)		Holloway, 1989
		0.4% * 2w	- (x 1.6)		Holloway, 1989
		0.6% * 2w	- (x 0.7)	Plasma Clofibric acid conc = 0.92mM	Holloway, 1989
		0.5% * 6w	+/-(x 2.1)		Reddy et al, 1982
	Marmoset	350mg/kg * 2w	- (x 0.7)	Plasma Clofibric acid conc = 0.60mM	Holloway, 1989
	Rhesus	300mg/kg * 2w	-	Plasma Clofibric acid conc = 0.1 - 0.47 (mean =0.15mM declining with time)	Holloway, 1989
	Guinea Pig	0.25% * 4w	- (x 0.8)		Oesch et al, 1988
Fenofibrate	Rat	0.05% * 1w	+ (x 9)		Oesch et al, 1988
		0.25% * 1w	+ (x 12)		Oesch et al, 1988
	Hamster	0.2% * 6w	+ (x 5)		Reddy et al, 1982
				Plasma fenofibric acid LF2151(metabolite)	
		0.05% * 3w	- (x 0.8)	9.3ug/ml 5.3ug/ml	Pourbaix et al, 1984
		0.5% * 3w	- (x 1.7)	175ug/ml 8.6ug/ml	Pourbaix et al, 1984
	Rhesus Monkey	200mg/kg * 12m	-	Subjective electron microscopy only	Blane and Pinaroli, 1984
	Guinea Pig	0.05% * 4w	- (x 1.2)		Oesch et al, 1988
		0.5% * 3w	- (x 1.1)		Oesch et al, 1988

Table 4 (cont. 1), Species Differences in Peroxisome Proliferation, in vivo, measured by Palmitoyl-CoA oxidising systems. Nominal scores for PCo are (+ = positive response, > 3 fold; +/- = intermediate responses, > 2-3 fold; - = negative response, < 2 fold; changes in the magnitude of PCoA response are indicated where appropriate).

Chemical	Species	Dose * Duration	PCoA	Comments	Reference
Clobuzarit	Mouse	20mg/kg * 2w	+ (x 3.8)	peak serum conc. = 96ug/ml	Orton et al, 1984
		60mg/kg * 2w	+ (x 9.6)	peak serum conc. = 208ug/ml	Orton et al, 1984
	Rat	3mg/kg * 2w	+/-(x 2.7)	peak serum conc. = 98ug/ml	Orton et al, 1984
		10mg/kg * 2w	+ (x 4.7)	peak serum conc. = 208ug/ml	Orton et al, 1984
	Hamster	10mg/kg * 2w	- (x 1.6)	peak serum conc. = 155ug/ml	Orton et al, 1984
		25mg/kg * 2w	- (x 1.3)	peak serum conc. = 261ug/ml	Orton et al, 1984
	Marmoset	15mg/kg * 2w	- (x 1.2)	peak serum conc. = 41ug/ml	Orton et al, 1984
		45mg/kg * 2w	- (x 1.0)	peak serum conc. = 120ug/ml	Orton et al, 1984
	Dog	15mg/kg * 2w	- (x 1.1)	peak serum conc. = 93ug/ml	Orton et al, 1984
		45mg/kg * 2w	- (x 0.9)	peak serum conc. = 208ug/ml	Orton et al, 1984
LY 171883	Mouse	0.05-0.5% * 2w	+ (x 16)	0.5% was equivalent to 625mg/kg, at 125mg/kg PCo was 7 fold increased.	Eacho et al, 1986
	Rat	0.05-0.5% * 2w	+ (x 20)	0.5% was equivalent to 365mg/kg, at 215mg/kg PCo was 7 fold increased.	Eacho et al, 1986
	Hamster	0.1-0.5% * 2w	+ (x 3)	0.5% was equivalent to 418mg/kg.	Eacho et al, 1986
	Guinea Pig	200mg/kg * 2w	- (x 1.2)		Eacho et al, 1986
	Dog	200mg/kg * 2w	- (x 1.2)		Eacho et al, 1986
	Rhesus Monkey	200mg/kg * 2w	- (x 1.1)		Eacho et al, 1986

Table 4 (cont. 2), Species Differences in Peroxisome Proliferation, in vivo, measured by Palmitoyl-CoA oxidising systems. Nominal scores for PCo are (+ = positive response, > 3 fold; +/- = intermediate responses, > 2-3 fold; - = negative response, < 2 fold; changes in the magnitude of PCoA response are indicated where appropriate).

Chemical	Species	Dose * Duration	PCoA	Comments	Reference
Bezafibrate	Mouse	100mg/kg * 2w	+ (x 13)		Watanabe et al, 1989
	Rat	100mg/kg * 2w	+ (x 13)		Watanabe et al, 1989
	Guinea Pig	100mg/kg * 2w	+/-(x 3)		Watanabe et al, 1989
	Rabbit	100mg/kg * 2w	- (x 1.2)		Watanabe et al, 1989
	Hamster	100mg/kg * 2w	+/-(x 2.2)		Watanabe et al, 1989
	Dog	30mg/kg * 2w	- (x 1.2)		Watanabe et al, 1989
	Rhesus Monkey	125mg/kg * 13w	- (x 1.3)		Watanabe et al, 1989
Ciprofibrate	Cat	50mg/kg * 1w then 100mg/kg * 2w	+ (x 5)	4.4 fold increase in volume density of peroxisomes.	Reddy et al, 1984
	Rhesus Monkey	50mg/kg * 1w then 100mg/kg * 3w then 200mg/kg * 3w	+ (x 5)	2.9 fold increase in volume density of peroxisomes.	Reddy et al, 1984
	Cynomologus	400mg/kg * 7w	+ (x 13)		
DL-040	Rhesus Monkey	300mg/kg * 1w then 400mg/kg * 10w	+ (x 7)	Controls not killed at same time (Controls at 7w and test at 11 w). 5.4 fold increase in peroxisome volume density	Reddy, 1985

Table 4 (cont. 3), Species Differences in Peroxisome Proliferation, in vivo, measured by Palmitoyl-CoA oxidising systems. Nominal scores for PCo are (+ = positive response, > 3 fold; +/- = intermediate responses, > 2-3 fold; - = negative response, < 2 fold; changes in the magnitude of PCoA response are indicated where appropriate).

Chemical	Species	Dose * Duration	PCoA	Comments	Reference
DEHP	Mouse		+ (x 7)		Osumi and Hashimoto, 1978
	Rat		+ (x 21)		Osumi and Hashimoto, 1978
		2,000mg/kg * 2w	+ (x 4.2)	7 fold increase in peroxisome vol density.	Rhodes et al, 1986
		6,000ppm * 3w	+ (x 3)		Short et al, 1987
		12,000ppm * 3w	+ (x 4.2)		Short et al, 1987
		25,000ppm * 3w	+ (x 7.7)		Short et al, 1987
	Guinea Pig		- (x 1.1)		Osumi and Hashimoto, 1978
	Marmoset	2,000mg/kg * 2w	- (x 1)	No significant change in peroxisome volume density.	Rhodes et al, 1986
	Cynomologus	100mg/kg * 25d	- (x 1.2)	Only 2 animals/group	Short et al, 1987
		500mg/kg * 25d	- (x 0.9)	Only 2 animals/group	Short et al, 1987
MEHP	Rat	250mg/kg * 3d	+ (x 3.3)		Mitchell et al, 1985
	Guinea Pig	250mg/kg * 3d	- (x 1.8)		Mitchell et al, 1985
		150mg/kg * 10d	- (x 1.1)		Mitchell et al, 1985
Methyl-clofenapate	Rat	5-25mg/kg * 10d	+		
	Guinea Pig	25-50mg/kg * 3w	-		Elcombe, 1989, unpublished
					Elcombe, 1989, unpublished
	Hamster	0.2% * 6w	+ (x 7)		Reddy et al, 1982

Table 4 (cont. 4), Species Differences in Peroxisome Proliferation, in vivo, measured by Palmitoyl-CoA oxidising systems. Nominal scores for PCo are (+ = positive response, > 3 fold; +/- = intermediate responses, > 2-3 fold; - = negative response, < 2 fold; changes in the magnitude of PCoA response are indicated where appropriate).

Chemical	Species	Dose * Duration	PCoA	Comments	Reference
Trichloro-acetic acid	Mouse	50-200mg/kg * 10d	+ (x 5)	9 fold increase in peroxisome vol density.	Elcombe, 1985
	Rat	50-200mg/kg * 10d	+ (x 6)	4 fold increase in peroxisome vol density.	Elcombe, 1985
	Guinea Pig	400mg/kg * 10d	- (x 1.1)	No change in peroxisome volume density.	Elcombe, 1985
Nafenopin	Rat	1.5-50mg/kg * 3w	+ (x 10)	at 50mg/kg	Lake et al, 1989
	Hamster	50-250mg/kg * 3w	+ (x 3.5)	+/- (*2.5 PCo) at 50mg/kg	Lake et al, 1989
	Guinea Pig	50-250mg/kg * 3w	- (x 1.3)	no change at 50mg/kg	Lake et al, 1989
	Marmoset	50-250mg/kg * 3w	+/-(x 2)	no change at 50mg/kg	Lake et al, 1989
Tiadenol	Rat	0.5% * 1w	+ (x 19)		Oesch et al, 1988
	Guinea Pig	0.5% * 4w 1.0% * 4w	+/-(x 2.2) +/-(x 2.6)		Oesch et al, 1988 Oesch et al, 1988

Table 5. Studies designed to investigate Peroxisomal Response to Hypolipidaemic Agents Administered to Human Volunteers.

Treatment	Number of Subjects	Type of Hyperlipidaemia	Peroxisome Increase	Author
Gemfibrozil	9	IIa,b, IV	-ve (qualitative)	de la Iglesia et al, 1982
Fenofibrate	38	Not Known	-ve (qualitative)	Blumcke et al, 1983
Ciprofibrate	7	II, III, IV	30% (Vol) (quantitative)	Hinton et al, 1991
Fenofibrate	12	IIa,b, IV	-ve (quantitative)	Gariot et al, 1987
Clofibrate	16	II, III, IV, V	30% (Nos) (quantitative)	Hanefeld et al, 1983

Table 6. Cancer Incidence observed at post-mortem in Man treated with Clofibrate, a hypolipidaemic agent (after Oliver et al, 1978). Rate expressed as incidence /1,000 persons.

Nature of Neoplasm	Clofibrate		Control (High Cholesterol)		Control (Low Cholesterol)	
	Number	Rate	Number	Rate	Number	Rate
Malignant	58	1.7	42	1.3	41	1.3
Benign	3	0	0	0	1	0

Table 7. Cancer Incidence in man observed at post-mortem following treatment with Gemfibrozil (after Frick et al, 1987). Rate expressed as incidence /1,000 persons. (+) indicates a single cancer not represented amongst the other organs listed.

Type of Cancer	Gemfibrozil		Placebo	
	Number	Rate	Number	Rate
Lung	5	2.4	5	2.5
Colon/Rectum	3	1.5	4	2.0
Stomach	1	0.5	4	2.0
Leukaemia	2	1.0	1	0.5
Basal Cell Carcinoma	5	2.4	0	0
Others (+)	15	-	12	-

Table 8. Species Differences in Hepatocyte Peroxisome Proliferation in vitro (+ = positive response; - = negative response; ND = not determined)

Chemical	Mouse	Rat	Guinea Pig	Dog	Marmoset	Monkey	Human	Reference
Clofibric acid	+	+	-	ND	-	-	-	Elcombe, 1986 Elcombe, (unpublished) Blauber et al, 1990 Bichet et al, 1990
Fomesafen	+	+	-	ND	-	ND	-	Elcombe, 1986
MEHP and Metabolites VI and XI	ND	+	-	ND	-	ND	-	Mithcell et al, 1985 Elcombe & Mitchell, 1986 Bichet et al, 1990
Ethylhexanoic acid	+	+	-	ND	-	ND	ND	Elcombe, unpublished
Methylclofenapate	ND	+	-	ND	-	ND	-	Elcombe & Styles, 1989
Trichloroacetic acid	+	+	-	ND	-	ND	-	Elcombe, (unpublished) Tyson et al, 1987
Ciprofibrate	ND	+	ND	ND	ND	ND	-	Foxworthy et al, 1990 Allen et al, 1987
MINP	ND	+	ND	ND	-	ND	ND	Benford et al, 1986
MIDP	ND	+	ND	ND	-	ND	ND	Benford et al, 1986
LY 171883	ND	+	ND	-	ND	-	ND	Foxworthy et al, 1990
Benzfibrate	ND	+	ND	-	ND	-	ND	Foxworthy et al, 1990
Nafenopin	ND	+	ND	ND	-	ND	ND	Bentley, (unpublished)
Beclobric acid	ND	+	ND	ND	ND	-	-	Blauber et al, 1990
Benzbromarone	ND	+	ND	ND	ND	ND	-	Bichet et al, 1990

able 9, Promotion Studies with Peroxisome Proliferators using Rats

Compound	Strain	Sex	Dose	Initiator	Endpoint	Result	Comment	Reference
Clofibrate	F344	M	0.073% in diet for 6 or 24 w	AAF at 0.02% in diet for 8 w	GGT and Fe excluding foci	Increased GCT at 24 w, no effect on Fe foci.		Numoto et al, 1984
	F344	M	0.3% in diet for 8, 20, 32, 49 or 64 w	DEN, 200mg/kg	GST-P foci and tumours	Decreased GST-P +ve foci, increased GST-P -ve foci, tumours increased		Hosokawa, 1989
	F344	M	0.1, 0.25, 0.5, 1.0% in diet for 19 w	DEN, 40 ppm in water for 5 w	Tumours > 1 mm diam	Increased at 0.1 and 0.25%, no effect at 0.5%, decreased at 1.0%.	Decrease in BW gain at 0.25% and >.	Mockizuchi et al, 1982
	F344	M	0.5% in diet for 48 w	DEN, 100 ppm in water for 2 w	Tumours	Increased.		Reddy and Rao, 1978
WY-14,643	F344	F	0.05 or 0.1% in diet for 28 or 60 w	DEN, 10mg/kg, 20 h after PH, IG	Multiple histo-chemical foci	Increases ATP and G6P foci (0.05 > 0.1%).		Glauertet al, 1986
	F344	M	0.1% in diet for 48 w	DEN, 100 ppm in water for 2 w	Tumours	Increased.		Reddy and Rao, 1978
	F344	M	0.1% in diet for 11, 22 and 54 w	DEN, 160 mg/kg ip	ATP, G6P, GGT foci and tumours	Increased ATP foci and tumours, no effect on G6P and GGT foci.		Cattley et al, 1989
BR931	S.D.	M	0.2% in diet for 2 or 6 w	DEN, 160 mg/kg ip	GGT foci	Decreased GGT foci		Shinozuka et al, 1983

Table 9 (cont.1), Promotion Studies with Peroxisome Proliferators using Rats

Compound	Strain	Sex	Dose	Initiator	Endpoint	Result	Comment	Reference
Nafenopin	TIF:RAIF	M & F	0.05% in diet for 2, 4 or 8 w	DEN, 15 mg/kg ip, (neonates)	GGT foci	Decreased		Staubli et al, 1984
	F344	M	0.93% in diet for 6 or 24 w	AAF at 0.02% in diet for 8 w	GGT and Fe excluding foci	Decreased GGT (6 & 24 w), no effect on Fe foci.	BW gain depression	Numoto et al, 1984
	F344	M	0.1% in diet for 22 or 56 w	DEN, 200mg/kg ip + 0.03% AAF in diet 2 w and single CCl4 gavage	GGT foci and tumours	Decreased GGT foci, increased tumours.		Preat et al, 1986a, b
	F344	M	0.01 & 0.02% in diet for 23 w, 0.05 & 0.1% in diet for 24 w	AAF, 0.02% in diet for 10 or 8 w	Tumours	No effects.	BW gain depression	Numoto et al, 1986
	Wistar	M & F	100 mg/kg BW via diet	Aflatoxin B1, 5 mg/kg for F and 2 mg/kg for M	H&E, GGT foci and tumours	Increased basophilic foci and tumours.	BW gain depression	Kraupp-Graal, YEAR??
DEHP	F344	F	1.2% in diet for 3 or 6 m	DEN, 150 mg/kg ip	H&E, GGT, ATP and G6P, toluidene blue and Fe excluding foci	No effect.		Popp et al, 1985
	S.D.	M	2% in diet for 5 or 10 w	DEN, 30 mg/kg ip after partial hepatectomy	GGT foci	No effect.		DeAngelo & Garrett, 1983
	F344	M	1.2% in diet for 12 or 24 w	AAF, 0.02% in diet for 7 w	H&E and Fe excluding foci, and tumours	No effect.	BW gain depression	Williamset al, 1987
	S.D.	F	10, 100, 200 and 500 mg/kg 3*/w for 11 w	DEN, 8 mg/kg at 3 w of age	ATP and GGT foci	Increased ATP foci, no effect on GGT foci.		Oesterle & Demi, 1988

Figure 1. Proposed Mechanisms of Di(-2-ethylhxyl) Phthalate (DEHP) Elicited Liver Growth

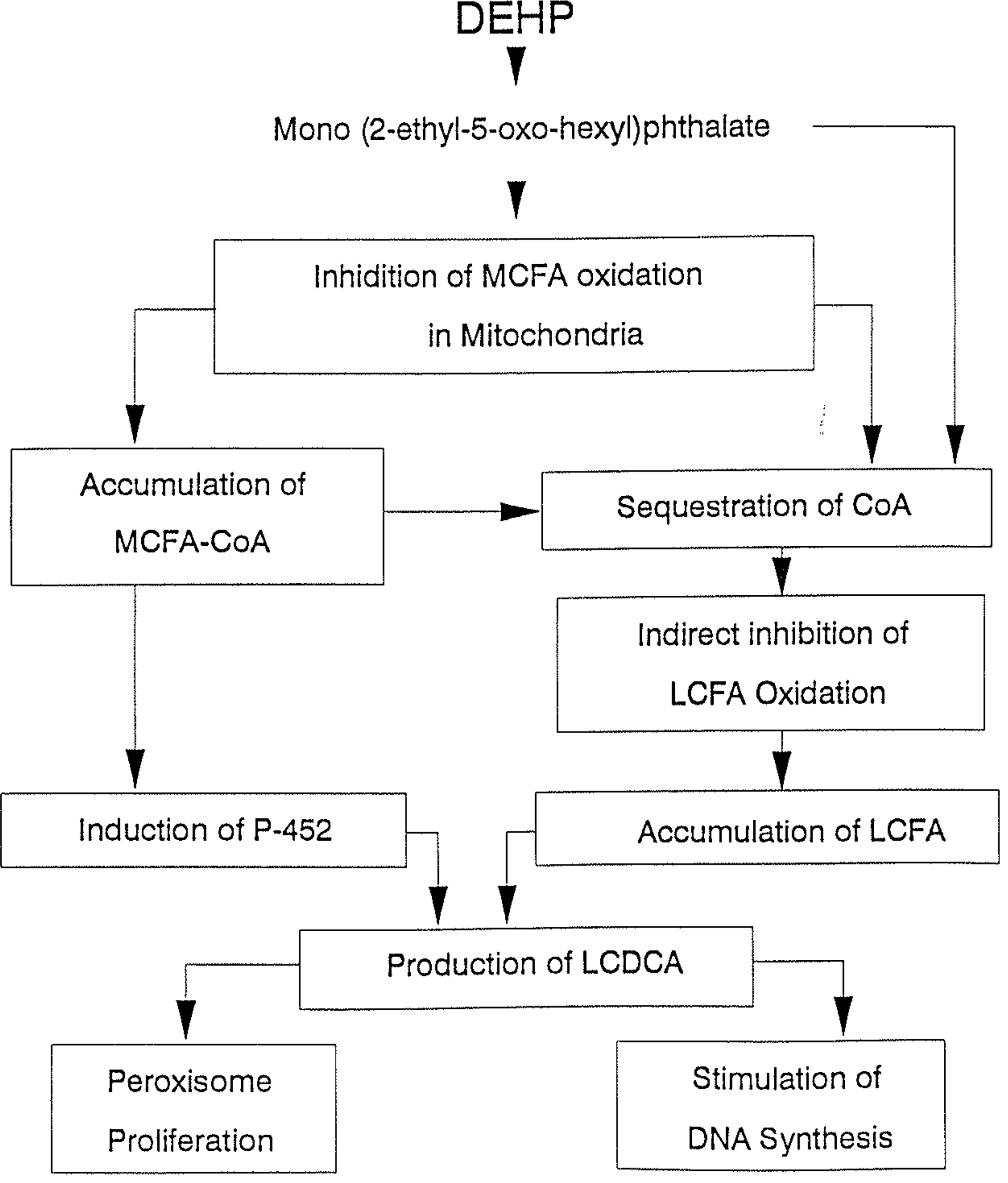


Figure 2. Possible Unifying Receptor-mediated Mechanism for Peroxisome Proliferation

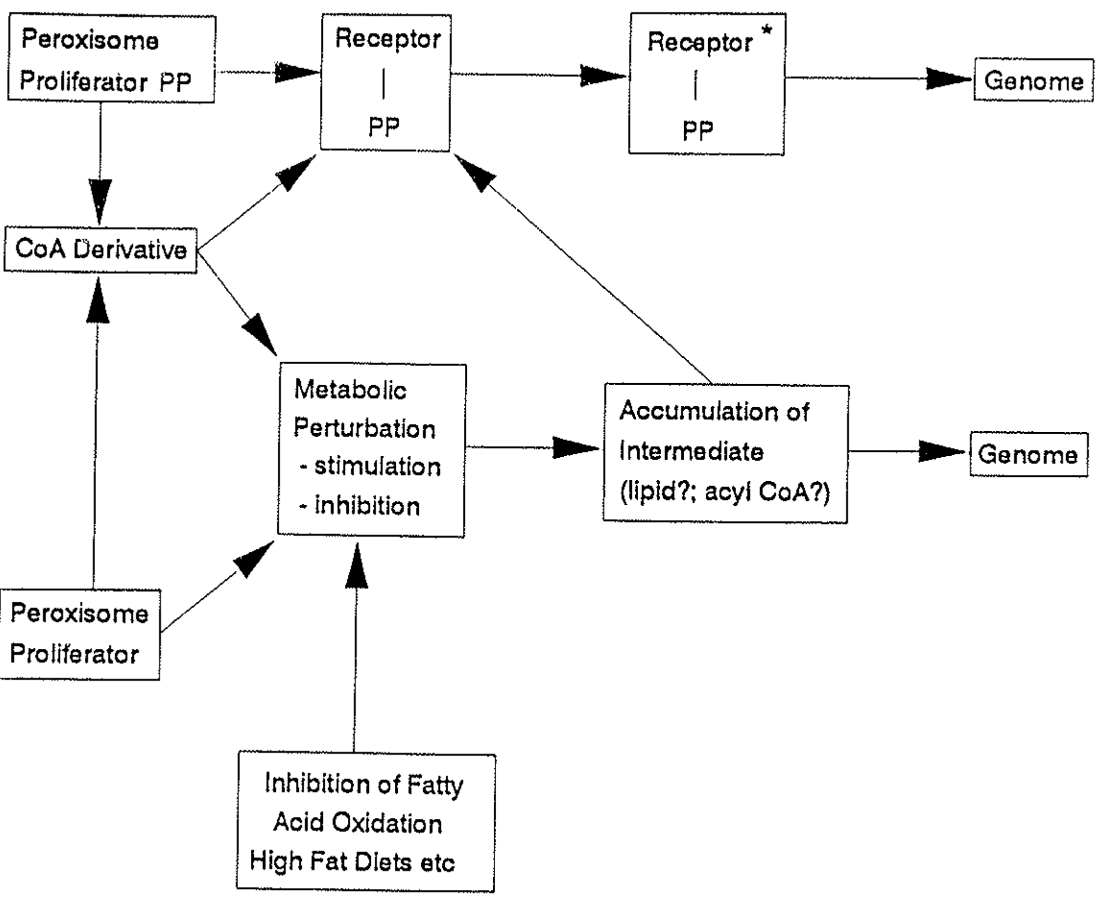


Figure 3. Mechanisms of Tumour Induction by Peroxisome Proliferators

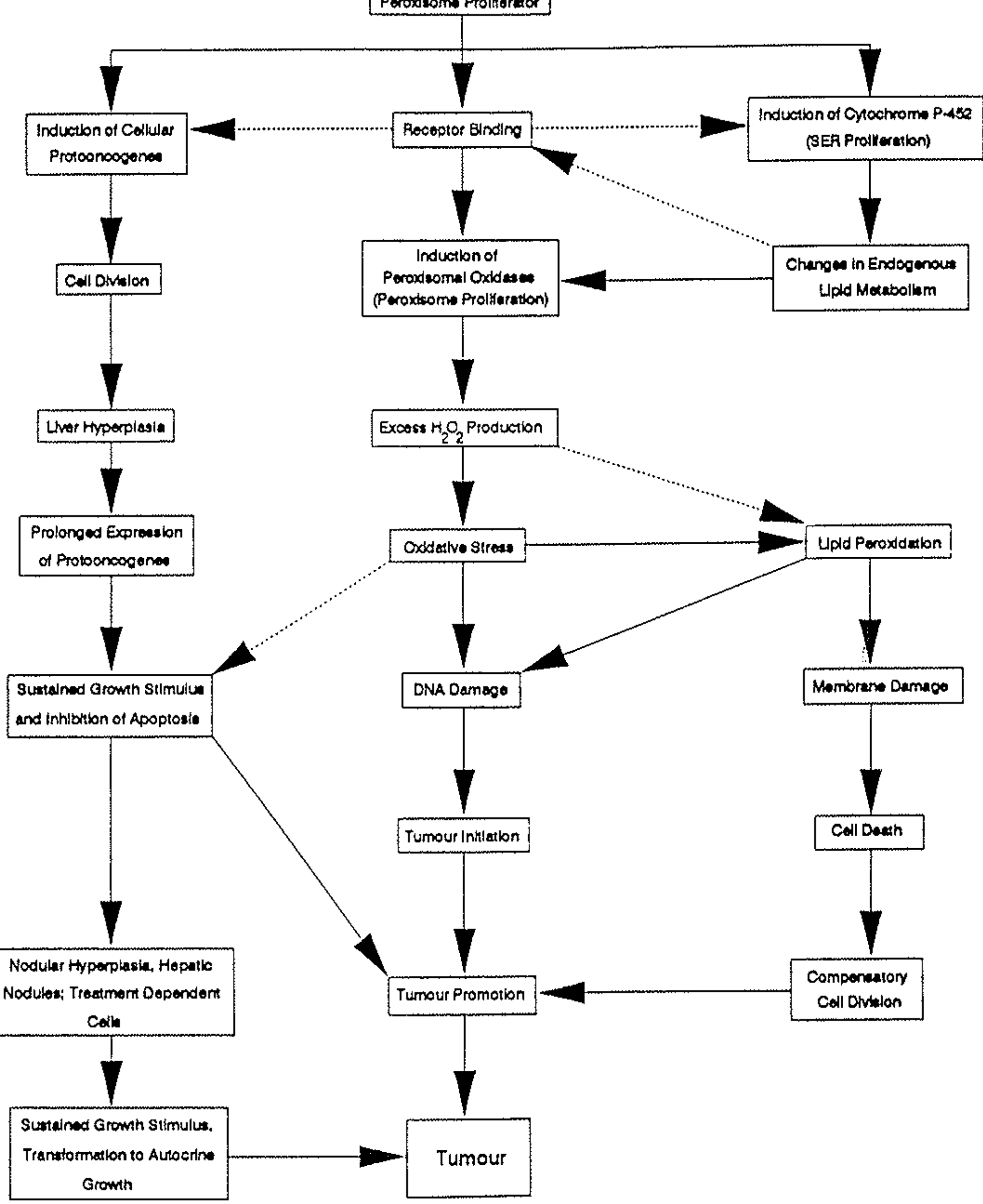


Figure 4. Schematic Diagram for Evaluating the Potential Hazard to Man of Suspected Hepatocarcinogens

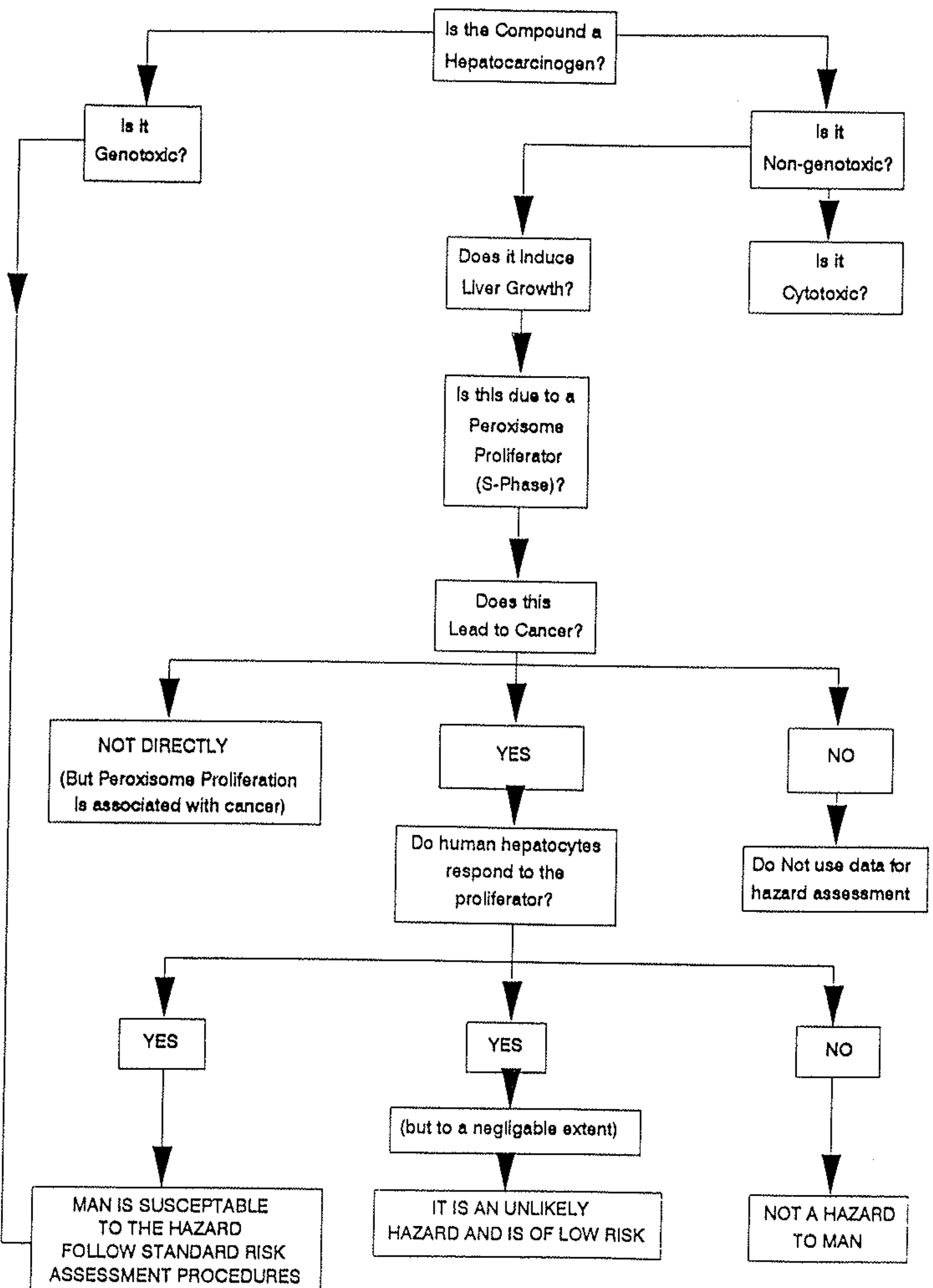
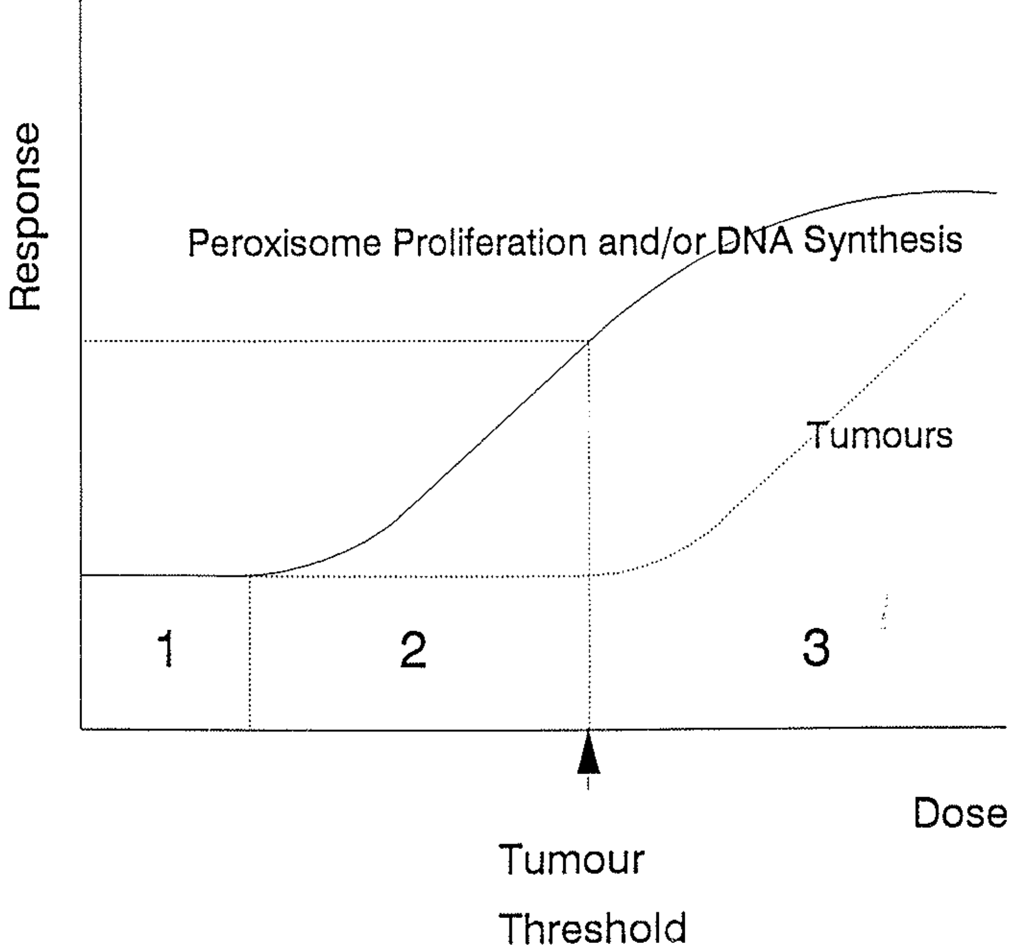


Figure 5. Hypothetical Dose-response curve for effects of Peroxisome Proliferators on the Liver



Zone 1: No observable effects

Zone 2: Peroxisome Proliferation and DNA Synyhesis seen.

Zone 3: Tumour induction

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