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FUNCTIONAL ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPARα) BY ENVIRONMENTAL CHEMICALS IN RELATION TO THEIR TOXICITIES

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ABSTRACT

Peroxisome proliferator-activated receptor α (PPARα) may work in the processes of both physiological and toxicological response to various endogenous or exogenous substances. The literature on the study of functional activation of PPARα by environmental chemicals in relation to their toxicities were reviewed. Environmental chemicals that were found to induce peroxisomes (peroxisome proliferators) and to activate the function of PPARα included plasticizers, herbicides, and organic solvents that have carboxyl groups in their parent substances or their metabolites. Several studies have showed species differences in the constitutive expression of PPARα and activation of PPARα, which may result in species differences in the induction of transcription of the genes encoding several peroxisomal enzymes. Although much information has supported the view that PPARα is primarily involved in the hepatic carcinogenicity of peroxisome proliferators, conflicting evidence exists. Most of the peroxisome proliferators have been shown to induce reproductive and developmental disorders, which might, in part, be associated with the functional activation of PPARα. Few epidemiological studies on the effect of peroxisome proliferators on humans have been conducted. The effect of perfluorooctanoic acid on humans was evaluated from the aspect of lipid metabolism in one study, which concluded that there was no effect.

Key Words: herbicides, organic solvents, peroxisome proliferator-activated receptor (PPARα), plasticizers plasticizers, transcriptional activation

INTRODUCTION

A peroxisome is a subcellular organelle in many plant and animal cells, in which hydrogen peroxide-producing enzymes and those involved in β-oxidation of fatty acids are found[1,2]. It is a globular body, approximately 0.5 μm in diameter, consisting of one membrane and a granular matrix. The ratio of mitochondria to peroxisomes in a cell is 4 : 1. Chemicals that induce the peroxisome are called peroxisome proliferators, which include plasticizers, harbicides, and

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antilipidemic drugs. These chemicals increase the size and number of peroxisomes and induce enlargement of the liver and expression of peroxisomal enzymes[3-5].

The peroxisome proliferator-activated receptor (PPAR), a member of the nuclear hormonal receptor family, has been recognized as a receptor that mediates the proliferation of peroxisomes. In the past ten years, research on PPARs has developed dramatically; a PPAR (PPARα, one of its subtypes) was cloned by Issemann and Green[6], and two other subtypes, PPARβ(δ) and PPARγ, were found in succession[7]. As a result, these receptors have been demonstrated to have various important functions, including energy metabolism within the body, homeostasis of intracellular lipids, maintenance of mitochondrial function, cell growth signaling, and control of apoptosis[8,9]. On the other hand, PPARα is also known to be related to the adverse effects of peroxisome proliferators[5].

Ligands of PPARα

There are 200 or more ligands, including long-chain fatty acids, fibrate antilipidemic drugs, thiazolidinediones, non-steroidal anti-inflammatory drugs, plasticizers such as phthalic esters, herbicides, and organic solvents[10]. Of these chemicals, only the possible environmental ligands of PPARα listed in Table 1 were dealt with. It is noted that these chemicals or their metabolites include a carboxyl group.

Organ distribution of PPAR

Braissant *et al*. investigated the organ distribution of PPARα in mature rats, using immunostaining with *in situ* hybridization and polyclonal antibody[11]. It was suggested that different subtypes of PPAR (α, β, ρ) are expressed in many cell types. PPARα was predominantly expressed in the hepatic and myocardial, crypt, and proximal renal tubule cells.

PPARα and the target genes

Figure 1 shows a functional activation of PPARα by environmental chemicals[12]. A complex of PPARα–peroxisome proliferators such as phthalic acid esters forms a heterodimer with RXR (retinoid X receptor), combines with PPRE (peroxisome proliferator response element), and regulates transcriptional activation of the target genes. Thus, ligands of PPARα influence the

Table 1. Ligands of PPARα

Commercial category	Environmental chemicals
Plasticizer	Di-(2-ethylhexyl) phthalate (DEHP)
	Di-(2-ethylhexyl) adipate (DEHA)
	Dicyclohexyl phthalate (DCHP)
	Butylbenzyl phthalate(BBP)
	Dibutyl phthalate (DBP)
	Perfluorooctanoic acid (PFOA)
Herbicide	2,4-dichlorophenoxyacetic acid(2,4-D)
	2,4,5-trichlorophenoxyacetic acid (2,4,5-T)
	dicamba (2-methoxy-3,6-dichlorobenzoic acid)
Solvents	Trichloroethylene
	Tetrachloroethylene

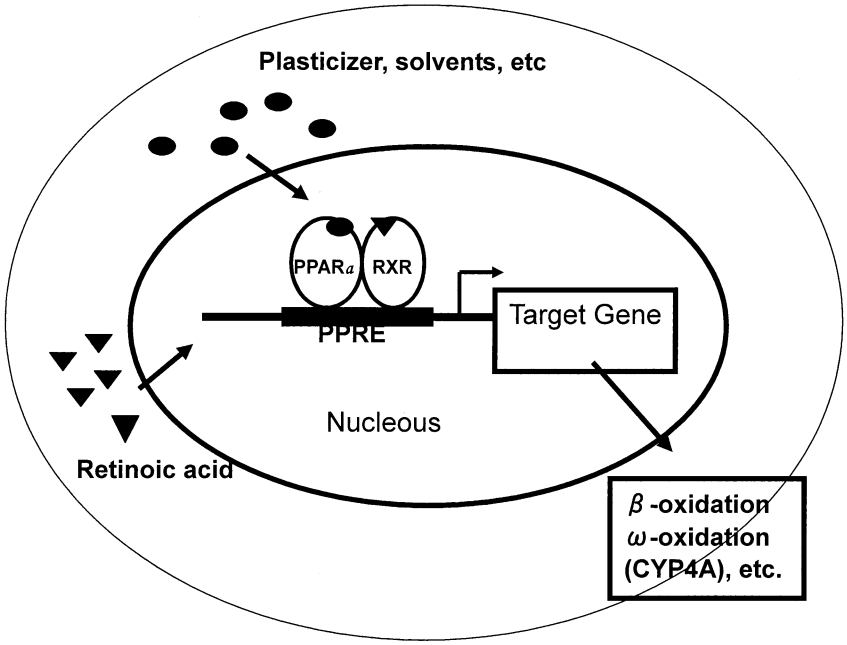

Figure 1: Mechanism of transcriptional activation of PPARα by phthalic esters, reported by Auwerx *et al*[11]. with slight modification.

expression of enzymes involved in β-oxidation of fatty acids, ω-oxidations (CYP4A), and metabolism of lipoprotein.

PPARα and carcinogenicity

Based on the fact that many peroxisome proliferators are carcinogenic to the liver, a relationship between carcinogenicity and PPARα has been noted[2,13]. David *et al*. administered DEHP to rats and mice for 104 weeks at 0~12500 and 0~6000 ppm, respectively[14,15]. Proliferation of peroxisomes was observed at an exposure concentration of 2500 ppm or more for rats, and 500 ppm or more for mice. Moreover, an increase in hepatocellular tumors (adenomas and carcinomas) was observed at 12500 and 1500 ppm in rats and mice, respectively. In contrast, exposure to DEHP did not induce peroxisome proliferation in primates[16]. Based on these data, in 2000 the IARC downgraded DEHP to "not classifiable as to carcinogenicity to humans." Melnick[17] objected to this decision because: 1) although determining whether the activation of PPARα is deeply involved in the development of hepatocellular tumor is a top priority, understanding of the mechanism(s) of carcinogenicity of peroxisome proliferators remains incomplete; 2) cancer epidemiological studies of DEHP or hypolipidemic fibrate drugs (peroxisome proliferators) are inconclusive; 3) although most of the pleiotropic effects of peroxisome proliferators are mediated by the PPARα, hypolipidemic fibrates have been shown to modulate the target gene expression, and thereby induce hypolipidemia even in humans, in whom expression is lower than in rats or mice; and 4) DEHP also induces biological effects that occur independent of peroxisome proliferation (e.g., morphologic cell transformation), and it is possible that some of these responses also contribute to the carcinogenicity of this chemical. Roberts *et*

al[18]. objected to this review because: 1) antilipidemic drugs cause peroxisome proliferation and cell proliferation in rodent, but not in human hepatocytes; 2) hypolipidemic effects of antilipidemic drugs in humans are mediated by activation of PPARα leading to regulation of gene expression of apolipoprotein (Apo A1, etc.), but not to the induction of the gene battery associated with rodent peroxisome proliferation and cancer; 3) species differences in molecular sequences of PPARα–response elements (PPREs) were observed; and 4) there was no evidence that antilipidemic drugs epidemically increased the incidence of carcinoma. For this controversy to be resolved, carcinogenic mechanisms mediated by PPARα and species differences among these mechanisms should be clarified.

Organic solvents

Exposure to trichloroethylene or tetrachloroethylene was found to induce peroxisome proliferation due to the metabolites in these substances[19-21]. A study on whether these chemicals acted as a ligand of PPARα or PPARγ was conducted by Maloney and Waxman[22]. COS-1 cells transfected with human or mouse PPARα and PPARγ expression plasmids and a PPRE-luciferase reporter were stimulated with trichloroethylene, tetrachloroethylene, or their metabolites for 24 hours. A determination of luciferase activity indicated that mouse and human PPARα were activated by both trichloroacetic and dichloroacetic acid. No species difference was observed in terms of this activation. The activation, however, was not observed with the parent chemicals or the metabolites, including chloral hydrate or trichloroethanol. In contrast, PPARγ was not activated by trichloroacetic acid or dichloroacetic acid. These results suggested that peroxisome proliferation due to trichloroethylene or tetrachloroethylene might be associated with the ligation of their metabolites, trichloroacetic acid or dichloroacetic acid, respectively, to PPARα.

Nakajima *et al*. administered trichloroethylene to wild-type SV/129 mice and PPARα-null mice, and demonstrated that a metabolite of trichloroethylene was a ligand for PPARα[23]. In wild-type mice with PPARα, the number of peroxisomes in the liver was increased, and the expression of target gene products such as enzymes involved in the β-oxidation of fatty acids, or CYP4A, was enhanced after exposure to trichloroethylene. These phenomena were not observed in PPARα-null mice, and, therefore, peroxisome proliferation after exposure to trichloroethylene was judged to be caused by ligation of metabolites to PPARα. Interestingly, although no sex difference was observed for induction of peroxisomes associated with exposure to trichloroethylene, a stronger induction of the target gene products was observed in males than in females. This difference might be caused by a sex difference in PPARα expression, because no sex difference was observed for the biotransformation of trichloroethylene. Nakajima's findings also suggest that the relationship between transcriptional activation of PPARα and peroxisome proliferation is not unequivocal.

Plasticizers

Di (2-ethylhexyl) phthalate (DEHP), a typical plasticizer, is hydrolyzed into mono (2-ethylhexyl) phthalate (MEHP) and 2-ethylhexyl alcohol (2-EH) by lipase. MEHP is further metabolized to a conjugate with UDP-glucuronyltransferase, or to dicarboxylic acids with CYP4A, alcohol dehydrogenases (ADH), and aldehyde dehydrogenases (ALDH). 2-EH is oxidized into 2-ethylhexanoic acid (2EHA) under the catalytic effect of ADH and ALDH. Maloney and Waxman[21] studied the activation of human and mouse PPARα and PPARγ associated with DEHP and its metabolites, using the same technique as that used for trichloroethylene and tetrachloroethylene. DEHP did not activate human or mouse PPARα or PPARγ. MEHP, however, activated both human and mouse PPARα. It was noted that MEHP also activated human and

mouse PPARγ, which effect was not observed on other peroxisome proliferators such as trichloroacetic acid, dichloroacetic acid, or wy-14,643. 2-EH did not activate human or mouse PPARα or PPARγ. On the other hand, 2EHA, a 2-EH metabolite, activated human and mouse PPARα at higher concentrations than those activated by MEHP. In contrast to MEHP, 2EHA did not activate PPARγ. These results suggest that DEHP metabolites with carboxyl groups activated both human and mouse PPARα. It should be noted that MEHP also activated the PPARγ of both species.

The transcriptional activation of PPARα in response to phthalic esters and adipic ester was evaluated by analyzing the expression of PPARα-mRNA and the target gene products. An increase in PPARα-mRNA was found in the groups administered butylbenzyl phthalate (BBP), dicyclohexyl phthalate (DCHP), DEHP, or di(2-ethylhexyl) adipate (DEHA). This increase was proportional to the molecular weight and lipid solubility (log Pow) of the parent chemicals (Fig. 2). Since these parent substances do not activate PPARα, the lipid solubility of their metabolites may increase with an increase of parent substances, resulting in easier transfer into the nucleus or in stronger ligations.

The reproductive toxicity of DEHP has been investigated in relation to PPARα in several studies (Table 2). Ward *et al*. administered DEHP at 12000 ppm to Sv/129 wild-type mice and PPARα-null mice for 24 weeks[24]. Activation of PPARα at this exposure concentration was confirmed at the mRNA-level. In the DEHP-exposed group, all wild-type mice died from cystic alteration of the renal tubules by the 16th week, although no weight loss or death was observed in PPARα-null mice. Although hepatomegaly and liver disorders (hepatocytomegaly and cytoplasmic granular hepatocyte eosinophilia) were observed in wild-type mice exposed to DEHP, no such abnormality was found in PPARα-null mice similarly exposed. Kidney and testes disorders were observed in both wild-type and PPARα-null mice. Renal toxicity appeared earlier in wild-type mice, and severe testicular toxicity was observed in wild-type mice. These results suggest that toxicity of DEHP mediated by PPARα was found in the liver, kidney, and

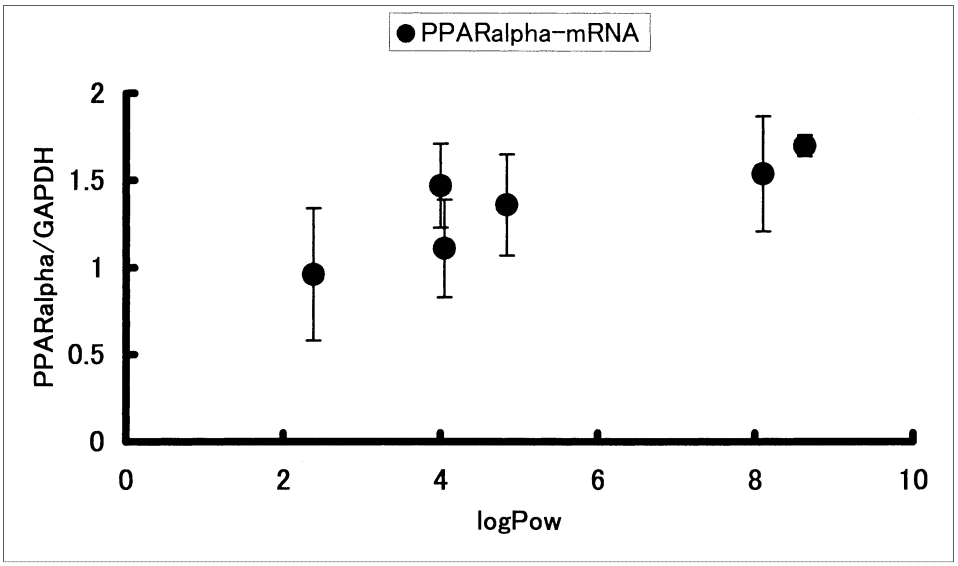

Figure 2: Relationship between lipid solubility (octanol/water partition coefficients) of phthalic esters and PPARα-mRNA level in the liver. Each point and bar represents the mean ± SD.

Table 2 Genital organ disorders associated with phthalic esters

Phthalic esters (author(s))	Dose and duration	Animal (sex)	Male genital organ	Female genital organ or reproductive effects	Other organs
DFEP (Ward *et al*.)	12000 ppm × 24 weeks	SV/129 mice (M)	Decreased spermatogenesis and giant cells in epididymis		Weight loss, liver and kidney disorders
DEHP (Kurata *et al*.)	100, 500, 2500 mg/kg × 13 weeks	Marmosets (M, F)	No change	No change	
DEHP (Peters *et al*.)	Single dose of 1 g/kg (on the 8 and 9 gestational days)	SV/129 mice (F)		Decreased survival rate of fetus	Retarded maternal weight gain, elevated metallothioneine and zinc concentration, decreased zinc concentration in fetus
DEHP (Davis *et al*.)	2 g/kg x 7-12 days/	SD rats (F)		Prolonged sexual cycle, suppressed ovulation, contracted preovulatory follicles, decreased estradiol, elevated FSH	
DEHP, DBP, DEP, DHP (Lamb *et al*.)	0.01-0.3% × 105 days	CD-1 mice (M, F)	Decreased sperm, increased abnormal sperm, reduced sperm motility	Decreased survival rate of newborn pups	

testes; toxicity without relation to this receptor was also found in the kidney and testes.

Kurata *et al*. investigated the genital organ toxicity of this chemical using 100~2500 mg/kg of DEHP administered to marmosets for 13 weeks[16]. In this study, hepatic peroxisome proliferation was not observed, suggesting a lack of the activation of PPARα by DEHP. The authors also reported that DEHP did not induce testicular damage, decrease of blood testosterone, or estradiol concentration. Considering all of the above findings, the relationships among PPARα, male genital organ disorders associated with DEHP, and the effect of DEHP on sex hormones seems inarguable. The toxicity of DEHP is attributable to that of MEHP or its metabolites, which have been shown to inhibit mitochondrial respiratory function in Sertoli cells[25]. Considering the deep involvement of PPARα in fatty acid oxidation in mitochondria[26] and the findings that PPARα constitutively expresses in Sertoli cells in the testes[11], further study on the testicular damage mediated by PPARα is expected.

Phthalic esters also have female genital organ toxicity. Davis *et al*. administered 2 g/kg of DEHP to female Sprague-Dawley rats to examine the effect of the chemical on female genital organs[27]. DEHP induced a prolongation of the female sexual cycle, a suppression of ovulation, a decrease in the size of ovarian follicles before ovulation (not a decrease in the number of granulosa cells), a decrease in estradiol, and an increase in FSH. Histopathologically, polycystic ovary was observed in DEHP-treated rats. These results indicate that estradiol formation was decreased due to the effect of DEHP on preovulatory granulosa cells. It is possible that MEHP, not DEHP, inhibited aromatase activity, resulting in inhibition of estradiol formation from test-

osterone, and this might be a possible pathway of female genital organ damage associated with DEHP, based on the fact that MEHP decreased the content of aromatase independent of FSH-cAMP[28]. The results above are interesting in that PPARα expresses in the ovarian follicular cells, where disorders associated with DEHP occur. However, the exposure concentration used was too high, as was the case in Ward *et al*[24].

Peters *et al*. reported on PPARα-independent teratogenicity and reproductive disorders of DEHP[29]. Female Sv/129 wild-type and PPARα-null mice were mated with a male with the same genotype, and 1 g/kg of DEHP was administered orally on the 8th and 9th gestational days. Autopsies were performed on the 10th and 18th days. An increase in liver weight in dams and a decrease in the number of living fetuses were observed in both wild-type and PPARα-null mice, suggesting that these adverse effects were independent of PPARα. Zinc is known to be essential for embryonic and fetal development, suggesting that alterations in zinc level may contribute to the mechanisms underlying reproductive toxicity (maternal toxicity and fetal toxicity) and teratogenicity by DEHP. In the study by Peters *et al*., the influence on the fetus was observed to occur simultaneously with a decrease in zinc concentration in the maternal liver, and thus, the involvement of zinc might not be negligible.

Lamb *et al*. studied the effect of four phthalic esters including DEHP on reproductivity and toxicity of male genital organs in mice[30]. Diethyl phthalate (DEP) had a slight effect on body and liver weights, and no effect was observed on fertility. Dibutyl phthalate (DBP) caused a decrease in litter size and the survival rates of pups. Dihexyl phthalate (DHP) and DEHP caused a dose-response decrease in litter size and the survival rate of pups, leading to a reduction in fertility. The effect appeared at exposure concentrations of 0.01% DEHP, and was stronger in mice exposed to DEHP than in those exposed to DHP. DHP and DEHP also decreased sperm concentration and reduced sperm motility, and increased the rate of abnormal spermatozoa. Testicular atrophy was also induced by both chemicals. Interestingly, although no pups were born after mating a female exposed to 0.3% DEHP with a male in the control group, 20% of females in the control group gave birth after mating with a male exposed to 0.3% DEHP. DEHP did have toxicity for the male genital organ, but the effect of the chemical might be stronger in females; i.e., in this case, the effect of DEHP on fertility might reflect a greater influence on females than on males.

The relationship between PPARα and DEHP reproductive toxicity reported by Lamb *et al*. was investigated. A diet containing 0.05% DEHP was given to male and female Sv/129 wild-type and PPARα-null mice *ad libitum* (F0). After one month of administration, wild-type and PPARα-null mice were mated, respectively. Neonates (F1) were reared in the same way as their parents, and were mated after maturation. Litter size and the survival rates of their neonates in F1 and F2 generations were observed. The neonatal survival rates of F1 and F2 were calculated to be about 60% in the DEHP-exposed group of wild-type mice, indicating that the rates were clearly lower than that (97%) of the control group. Litter size was also smaller in DEHP-exposed wild-type mice than in control mice. On the other hand, in PPARα-null mice, no difference in survival rate of neonates or in litter size of F1 and F2 was observed between the DEHP-exposed mice and the control mice. Exposure to DEHP had no significant influence on male genital organs at this exposure concentration. Therefore, PPARα might be involved in reproduetive toxicity of DEHP, to which females might have the key; this mechanism is now under research.

Herbicides

Maloney and Waxman studied the activation of PPAR associated with 2,4-dichlorophenoxy acetic acid (2,4-D) and 2- methyl-4-chlorophenoxyacetic acid (MCPA), in addition to that of the

above-mentioned peroxisome proliferators[22]. In contrast to other peroxisome proliferators, neither 2,4-D nor MCPA directly activated human or mouse PPARα or PPARγ. Therefore, the authors considered that metabolites of 2,4-D and MCPA might be responsible for the proliferation of peroxisomes. It was confirmed in the recent study that 2,4-D did induce peroxisome proliferation, and an increase in PPARα protein and PPARα-mRNA as well as an increase in the expression of the target gene products, although whether 2,4-D itself or its metabolites is the active ligand is still unclear (data not shown).

Dicamba (2-methoxy-3,6-dichlorobenzoic acid), widely used as an herbicide, is also a peroxisome proliferator. Espandiari *et al*. administered 0~1% dicamba to female and male Sprague-Dawley rats for 3 weeks[31,32]. Although dicamba had no effect on relative liver weight or food consumption, enzyme activities involved in β-oxidation of fatty acids or in CYP4A (hydroxylation activity of lauryl acid) in liver peroxisomes increased in the 1% dicamba group. These results suggest that dicamba might cause activation of PPARα.

Species differences in activation of PPARα

Species differences were observed in the induction of peroxisome proliferations and the transcription of genes encoding several peroxisomal enzymes by environmental chemicals[33]. According to Maloney and Waxman[22], activation of PPARα by Wy-14,643 and perfluorooctanoic acid (PFOA), a plasticizer, was stronger in mice than in humans. Another study showed that the activation by Wy-14,643 was stronger in rats than in humans[34]. These results suggest that species differences in induction of peroxisome proliferations and peroxisomal enzymes are due, in part, to difference in PPARα activation. Species differences were also observed in constitutive expression of PPARα, suggesting that these species differences might also contribute to differences in activation.

Effect of peroxisome proliferators on humans

Few studies have investigated the effect on humans of the above-mentioned peroxisome proliferators through the mediation of PPARα. Gilliland and Mandel examined the effect of PFOA on humans, based on the fact that this chemical acted as a ligand for animal PPARα and influenced lipid metabolism, leading to hepatocellular necrosis and hypolipidemia[35]. A cross-sectional epidemiological study was conducted in 115 workers with occupational exposure to PFOA in order to examine the relationship between exposure to PFOA and levels of liver enzymes, lipoproteins, and cholesterol. No association was confirmed, and it was concluded that no effect on humans was found under this exposure condition.

CYP4A

The CYP4 family consists of 18 subfamilies, one of which is CYP4A[36]. It has been confirmed that CYP4A includes 12 isozymes found in nine species of mammals. CYP4A catalyzes mainly ω-oxidation of medium- and long-chain fatty acids, although chemical substrates other than fatty acids are also catalyzed. We studied the relationship between PPARα-mRNA induction and CYP4A inducibility for phthalic esters (Fig 2). A positive relationship was found, suggesting that CYP4A could be a promising indicator for PPARα induction.

REFERENCES

1) Lock, E.A., Mitchell, A.M. and Elcombe, C.R.: Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Ann. Rev. Pharmacol. Toxicol*.. 29,145–63 (1989).
2) Reddy, J.K., Azarnoff, D.L. and Hignite, C.E.: Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature*, 283, 397–298 (1980).
3) Berge, R.K., Hosoy, L.H., Aarsland, A., Bakke, O.M. and Farstad, M.: Enzymatic changes in rat liver associated with low and high doses of a peroxisome proliferator. *Toxicol. Appl. Pharmacol*., 73, 35–41 (1984).
4) Budroe, J.D., Umemura, T., Angeloff, K. and Williams, G.M.: Dose-response relationships of hepatic acyl-CoA oxidase and catalase activity and liver mitogenesis induced by the peroxisome proliferator ciprofibrate in C57BL/6N and BALB/c mice. *Toxicol. Appl. Pharmacol*., 113, 192–198 (1992).
5) Motojima, K.: Peroxisome proliferator-activated receptor (PPAR): structure, mechanisms of activation and diverse functions. *Cell Struct. Funct*., 18, 267–277 (1993).
6) Issemann, I. and Green, S.: Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, 347(6294), 645–650 (1990).
7) Gonzalez, F.J.: Recent update on the PPAR alpha-null mouse. *Biochimie*., 79,139–144. (1997)
8) Fruchart, J.C.: Peroxisome proliferator-activated receptor-alpha activation and high-density lipoprotein metabolism. *Am. J. Cardiol*., 88, 24N–29N (2001).
9) Tanaka, N. and Aoyama, T.: Peroxisome proliferator-activated receptor (PPAR). *Nippon Naika Gakkai Zasshi*, 90, 2033–2039 (2001). (in Japanese)
10) Kersten, S. and Wahli, W.: Peroxisome proliferator activated receptor agonists. *EXS*, 89, 141–51 (2000).
11) Braissant, O., Foufelle, F., Scotto, C., Dauca, M. and Wahli, W.: Differential expression of peroxisome proliferators-activated receptors (PPARs): Tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology*, 137, 354–366 (1996).
12) Auwerx, J., Schoonjans, K., Fruchart, J.C. and Staels, B.: Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects. *J. Atheroscler. Thromb*., 3, 81–89 (1996).
13) Reddy, J.K., Scarpelli, D.G., Subbarao, V. and Lalwani, N.D.: Chemical carcinogens without mutagenic activity: peroxisome proliferators as a prototype. *Toxicol. Pathol*., 11, 172–180 (1983)
14) David, R.M., Moore, M.R., Cifone, M.A., Finney, D.C. and Guest, D.: Chronic peroxisome proliferation and hepatomegaly associated with the hepatocellular tumorigenesis of di(2-ethylhexyl) phthalate and the effects of recovery. *Toxicol. Sci*. 50, 195–205 (1999).
15) David, R.M., Moore, M.R., Finney, D.C. and Guest, D.: Chronic toxicity of di(2-ethylhexyl) phthalate in rats. *Toxicol. Sci*., 55, 433–443 (2000).
16) Kurata, Y., Kidachi, F., Yokoyama, M., Toyota, N., Tsuchitani, M. and Katoh, M.: Subchronic toxicity of di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation,testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol. Sci*., 42, 49–56 (1998).
17) Melnick, R.L.: Is peroxisome proliferation an abligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? *Environ. Health Perspect*., 109, 437–442 (2001).
18) Roberts, R., Moffat, G. and Ashby, J.: Exposure to peroxisome proliferators: reassessment of the potential carcinogenic hazard. *Environ. Health Perspect*., 109, A462–464 (2001).
19) Elcombe, C.R.: Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: a biochemical human hazard assessment. *Arch. Toxicol. Suppl*. 8, 6–17 (1985).
20) Elcombe, C.R., Rose, M.S. and Pratt, I.S. : Biochemical, histological, and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: possible relevance to species differences in hepatocarcinogenicity. *Toxicol. Appl. Pharmacol*., 79, 365–376 (1985).
21) Goldsworthy, T.L. and Popp, J.A.: Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. *Toxicol. Appl. Pharmacol*., 88, 225–233 (1987).
22) Maloney, E.K. and Waxman, D.J.: trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol. Appl. Pharmacol*., 161, 209–218 (1999).
23) Nakajima, T., Kamijo, Y., Usuda, N., Liang, Y., Fukushima, Y., Kametani, K., Gonzalez, F.J. and Aoyama, T.: Sex-dependent regulation of hepatic peroxisome proliferation in mice by trichloroethylene via peroxisome proliferators-activated receptor a (PPARa). *Carcinogenesis*, 21, 677–682 (2000).
24) Ward, J.M., Peters, J.M., Perella, C.M. and Gonzalez, F.J.: Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phtalate (DEHP) in peroxisome proliferator-activated receptora-null mice. *Toxicol. Pathol*., 26, 240–246 (1998).
25) Oishi, S.: Effects of phthalic acid esters on testicular mitochondrial functions in the rat. *Arch. Toxicol*., 64, 143–147 (1990).

26) Aoyama, T., Peters, J.M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T. and Gonzalez, F.J.: Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPARα) J. Biol. Chem., 273, 5678–5684 (1998).
27) Davis, B.J., Marronpot, R.R. and Heindel, J.J.: Di(2-ethylhexyl) phtalate suppresses estradiol and ovulation in cycling rats. *Toxicol. Appl. Pharmacol*., 128, 216–223 (1994).
28) Davis, B.J., Weaver, R., Gaines, L.J. and Heindel, J.J.: Mono-(2-ethylhexyl) phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells. *Toxicol. Appl. Pharmacol*., 128, 224–228 (1994).
29) Peters, J.M., Taubeneck, M.W., Keen, C.L., and Gonzalez, F.J.: Di(2-ethylhexyl)phtalate induces a functional zinc deficiency during pregnancy and teratogenesis that is independent of peroxisome proliferator-activated receptor α. *Teratology*, 56, 311–316. (1997).
30) Lamb, J.C. IV, Chapin, R.C., Teague, J., Lawton, A.D. and Reel, J.R.: Reproductive effects of four phthalic acid esters in the mouse. *Toxicol.Appl. Pharmacol*., 88, 255–269 (1987).
31) Espandiari, P., Ludewig, G., Glauert, H.P. and Robertson, L.W.: Activation of hepatic NF-kappaB by the herbicide Dicamba (2-methoxy-3,6-dichlorobenzoic acid) in female and male rats. *J. Biochem. Mol. Toxicol*., 12, 339–344 (1993).
32) Espandiari, P., Thomas, V.A., Glauert, H.P., O'Brien, M., Noonan, D. and Robertson, L.W.: The herbicide dicamba (2-methoxy-3,6-dichlorobenzoic acid) is a peroxisome proliferator in rats. *Fundam. Appl. Toxicol*., 26, 85–90 (1995).
33) Choudhury, A.I., Chahal, S., Bell, A.R., Tomlinson, S.R., Roberts, R.A., Salter, A.M. and Bell, D.R.: Species differences in peroxisome proliferation; mechanisms and relevance. *Mutat. Res*., 448, 201–212 (2000).
34) Mukherjee, R., Jow, L., Noonan, D. and McDonnell, DP.: Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J. Steroid Biochem. Mol. Biol*., 51, 157–166 (1994).
35) Gilliland, F.D. and Mandel, J.S.: Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: a study of occupationally exposed men. *Am. J. Ind. Med*. 29, 560–8 (1996).
36) Okita, R.T. and Okita, J.R.: Cytochrome P450 4A fatty acid omega hydroxylases. *Curr. Drug Metab*., 2, 265–281 (2001).