Is Peroxisome Proliferation an Obligatory Precursor Step in the Carcinogenicity of Di(2-ethylhexyl)phthalate (DEHP)?

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Di(2-ethylhexyl)phthalate (DEHP), a peroxisome proliferator, has been listed by the International Agency for Research on Cancer (IARC) and by the National Toxicology Program as a possible or reasonably anticipated human carcinogen because it induces dose-related increases in liver tumors in both sexes of rats and mice. Recently, the suggestion has been advanced that DEHP should be considered unlikely to be a human carcinogen because it is claimed that the carcinogenic effects of this agent in rodents are due to peroxisome proliferation and that humans are nonresponsive to this process. An IARC working group recently downgraded DEHP to "not classifiable as to its carcinogenicity to humans" because they concluded that DEHP produces liver tumors in rats and mice by a mechanism involving peroxisome proliferation, which they considered to be not relevant to humans. The literature review presented in this commentary reveals that, although our knowledge of the mechanism of peroxisome proliferation has advanced greatly over the past 10 years, our understanding of the mechanism(s) of carcinogenicty of peroxisome proliferators remains incomplete. Most important is that published studies have not established peroxisome proliferation per se as an obligatory pathway in the carcinogenicity of DEHP. No epidemiologic studies have been reported on the potential carcinogenicity of DEHP, and cancer epidemiologic studies of hypolipidemic fibrate drugs (peroxisome proliferators) are inconclusive. Most of the pleiotropic effects of peroxisome proliferators are mediated by the peroxisome proliferator activated receptor (PPAR), a ligand-activated transcription factor that is expressed at lower levels in humans than in rats and mice. In spite of this species difference in PPAR expression, hypolipidemic fibrates have been shown to induce hypolipidemia in humans and to modulate gene expression (e.g., genes regulating lipid homeostasis) in human hepatocytes by PPAR activation. Thus, humans are responsive to agents that induce peroxisome proliferation in rats and mice. Because peroxisome proliferators can affect multiple signaling pathways by transcriptional activation of PPAR-regulated genes, it is likely that alterations in specific regulated pathways (e.g., suppression of apoptosis, protooncogene expression) are involved in tumor induction by peroxisome proliferators. In addition, because DEHP also induces biological effects that occur independently of peroxisome proliferation (e.g., morphologic cell transformation and decreased levels of gap junction intercellular communication), it is possible that some of these responses also contribute to the carcinogenicity of this chemical. Last, species differences in tissue expression of PPARs indicate that it may not be appropriate to expect exact site correspondence for potential PPAR-mediated effects induced by peroxisome proliferators in animals and humans. Because peroxisome proliferation has not been established as an obligatory step in the carcinogenicity of DEHP, the contention that DEHP poses no carcinogenic risk to humans because of species differences in peroxisome proliferation should be viewed as an unvalidated hypothesis. *Key words:* apoptosis, cell proliferation, di(2-ethylhexyl)phthalate, hypolipidemic drugs, peroxisome proliferation, peroxisome proliferator activated receptor. *Environ Health Perspect* 109:437–442 (2001). [Online 24 April 2001]
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Di(2-ethylhexyl)phthalate (DEHP) has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B) (*1*) and by the National Toxicology Program as reasonably anticipated to be carcinogenic to humans (*2*). These listings are based on dosed feed studies conducted by the National Toxicology Program which demonstrated that DEHP induced dose-related increases in liver tumors in both sexes of rats and mice (*3*,*4*). No epidemiologic studies on DEHP carcinogenicity have been reported.

The finding that DEHP was not mutagenic in *Salmonella*, with or without metabolic activation, but that it induced hepatomegaly and hepatic peroxisome proliferation in rats and mice and reduced serum concentrations of cholesterol and triglycerides, similar to clofibrate and other hypolipidemic drugs, has stimulated extensive research efforts aimed at understanding the potential role of peroxisome proliferation in hepatocarcinogenesis. In light of the developing mechanistic information on peroxisome proliferation, some reviewing agencies have been urged to reexamine their cancer classification for DEHP. For example, Doull et al. (*5*) contend that "DEHP should be classified as unlikely to be a human carcinogen under any known conditions of human exposure," because "DEHP exerts its tumorigenic response through the process of peroxisome proliferation," an effect to which "humans are almost completely nonresponsive." However, while acknowledging that the mechanism of rodent hepatocarcinogenesis of DEHP is not fully understood, Doull et al. (*5*) claim that "the hepatocarcinogenic effects of DEHP in rodents result directly from the receptor-mediated, threshold-based mechanism of peroxisome proliferation, a well understood process associated uniquely with rodents." This view is based to a large extent on a "strong concordance between peroxisome proliferation and hepatocarcinogenesis in rats and mice" (*5*) and the claimed "scientific consensus that its [DEHP] hepatocarcinogenic action in rodents is associated with peroxisome proliferation and increased hepatocyte replication."

Likewise, an IARC working group recently downgraded DEHP to "not classifiable as to its carcinogenicity to humans" (group 3) because they concluded that DEHP produces liver tumors in rats and mice by a mechanism involving peroxisome proliferation, which they judged to be not relevant to humans (*6*). The term "responsive" is used in this commentary to indicate the induction of increased volume density of peroxisomes and/or peroxisomal enzyme activities in liver cells resulting from *in vivo* or *in vitro* exposure to agents that have been shown to induce these effects in rats and/or mice. The main issue addressed in this commentary is whether or not current scientific data support the claim that the carcinogenic effects of DEHP observed in rats and mice result from peroxisome proliferation and therefore do not represent a potential cancer risk to humans. I evaluate this issue by reviewing available data relevant to possible mechanism(s) of carcinogenicity of DEHP and other peroxisome proliferators and by examining how that information might impact our understanding of potential cancer risk from DEHP. In addition, a future research direction is suggested for evaluating potential biological effects of peroxisome proliferators in rodent and human tissues.

Our knowledge of the mechanism of peroxisome proliferation has advanced greatly over the past 10 years; however, the actual

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mechanistic events involved in the carcinogenicity of DEHP or other peroxisome proliferators have not been adequately elucidated. Consequently, current mechanistic data do not rule out the possibility that DEHP poses a cancer risk to humans by mechanisms independent of peroxisome proliferation.

What Is the Mechanistic Basis for the Correlation between Peroxisome Proliferation and Liver Cancer Induction in Rats and Mice?

Based on an apparent correlation between peroxisome proliferation and hepatocarcinogenesis, Reddy et al. (*7*) proposed that hypolipidemic peroxisome proliferators may represent a novel class of chemical carcinogens. This diverse group of chemicals, including hypolipidemic fibrate drugs, plasticizers (e.g., DEHP), various pesticides, and solvents, have been found to induce peroxisome proliferation and liver cancer in rats and mice. Several peroxisome proliferators also induce testicular neoplasms (*8*).

An IARC working group that evaluated the role of peroxisome proliferation in chemical carcinogenesis (*9*) acknowledged that the mechanism of tumor induction by peroxisome proliferators is incomplete, but still concluded that peroxisome proliferator-induced liver cancer in rats and mice was likely due to oxidative stress from peroxisome proliferation and/or increased hepatocellular proliferation. Because data on hypolipidemic fibrate drugs indicated little peroxisome proliferation in human liver and no induction in cultured human hepatocytes, the IARC working group recommended that tumor responses in rats or mice occurring secondary to peroxisome proliferation could be discounted in overall evaluations if *a*) information is available to exclude mechanisms of carcinogenesis other than those related to peroxisome proliferation, *b*) peroxisome proliferation and hepatocellular proliferation were demonstrated under the conditions of the bioassay, and *c*) such effects have not been found in adequate human studies. For DEHP, insufficient data are available to establish criteria *a* and *c*, and regarding criterion *b*, hepatocellular proliferation induced by DEHP is not a sustained response with continued exposure to this agent.

If peroxisome proliferation alone is predictive of liver cancer outcome, then similar levels of peroxisome proliferation should lead to similar incidences of liver tumors in experimental animals. However, this is not always the case. For example, at doses of DEHP and Wy-14,643 that produce similar levels of peroxisome proliferation in rat liver, Wy-14,643 produced an earlier and much greater liver tumor response than did DEHP (*10*). Thus, peroxisome proliferation alone does not provide an adequate mechanistic explanation for the different carcinogenic potencies of DEHP and WY-14,643 in the rat liver.

Although increased replicative DNA synthesis and cell division have been suggested as the mechanism of peroxisome proliferator-mediated carcinogenesis, persistent increases in hepatocyte proliferation do not occur with DEHP or most hypolipidemic fibrate drugs. Except for Wy-14,643 (*10*), cell replication rates return to control levels within 10–30 days of continuous treatment. Thus, the sustained increase in cell replication that has been seen with Wy-14,643 (*10*) does not apply to other peroxisome proliferators (*11*). Although cell proliferation is an integral component of chemical carcinogenesis, scientific data do not substantiate the hypothesis that induction of cancer by nongenotoxic carcinogens occurs solely by a transient increase in cell division (*12*). For peroxisome proliferators, other reported effects such as suppression of apoptosis (*13*), inhibition of gap junction intercellular communication (*9*), activation of Kupffer cells (*14*), induced protooncogene expression (*15,16*), or a combination of these factors (e.g., disruption in the balance between hepatocyte proliferation and apoptosis) (*13*) may be involved in the carcinogenic process.

Are Data from Studies in Humans or Human Tissues on the Induction of Peroxisome Proliferation and Potential Carcinogenicity of Peroxisomal Proliferators, Including DEHP, Reliable for Assessing Human Risk?

Because of the commercial value of many agents that induce peroxisome proliferation, research efforts have focused on the mechanism of toxicity/carcinogenicity of these chemicals with the goal of elucidating a species-specific response. It has been often claimed that humans are refractory to adverse effects of peroxisome proliferators. This is based on reports that chemicals (including DEHP and its metabolites) that induce peroxisome proliferation in rat or mouse hepatocyte cultures do not induce peroxisome proliferation in cultured human hepatocytes (*17,18*), and that no increase in liver cancer has been observed in studies of patients treated with hypolipidemic fibrate drugs. In addition, induction of peroxisomal β-oxidation and DNA synthesis and suppression of apoptosis were observed in rat hepatocytes incubated with nafenopin, mono(2-ethylhexyl)phthalate (MEHP; the primary metabolite of DEHP), or diisononylphthalate, but not in cultured human hepatocytes (*19–22*).

Because of the public health implications of the claims that humans are nonresponsive to adverse effects of peroxisome proliferators, it is necessary to evaluate the data used to make these claims for adequacy and potential confounders. First, the fact that hypolipidemia (one of the pleiotropic effects of peroxisome proliferators in rodents) is also induced by hypolipidemic fibrate drugs in humans demonstrates that humans are responsive to these agents. Second, a moderate increase in peroxisome number or volume density was observed in liver biopsies taken from patients treated with clofibrate or ciprofibrate (*23*). Third, Perrone et al. (*24*) reported that two peroxisome proliferators, clofibrate and ciprofibrate, induced a 2- to 3-fold increase in peroxisomal enzyme activity in cultured human hepatocytes.

With respect to the relationship between cell proliferation and tumor outcome, issues such as whether the response is transient or sustained and the specific population of cells that are responsive need to be determined. For example, cell culture studies cannot establish a sustained response, and the balance between cell replication and cell loss in preneoplastic hepatic foci may be more relevant to the carcinogenic process for peroxisome proliferators than measurements of cell proliferation in normal hepatocytes (*11,12,25*). The finding that 22 weeks of exposure to WY-14,643 induced a greater carcinogenic response in the liver of old rats (starting age of 15 months of age) compared to young rats (starting age of 2 months), but that peroxisome proliferation and sustained hepatocellular proliferation were not different in relation to age, suggests that the greater accumulation of spontaneously initiated foci in old rats may be a critical factor in the carcinogenic mechanism of this compound (*25*). Human cell culture studies have focused mainly on responses to peroxisome proliferators in normal hepatocytes, although some studies have examined responses in the human HepG2 hepatoma cell line.

With respect to their carcinogenic potential in humans, cancer epidemiologic studies of hypolipidemic fibrate drugs are considered to be "of limited value because of the short time periods involved" (*23*) or "inconclusive because of inconsistent results and insufficient duration of follow-up" (*26*). No epidemiologic studies on the potential carcinogenicity of DEHP have been reported. Thus, current epidemiologic data neither demonstrate an increase in liver cancer in patients treated with hypolipidemic drugs nor do they support the assertion that humans are resistant to the adverse effects of DEHP or other peroxisome proliferators.

Several other important issues impact evaluations of peroxisome proliferation in

human hepatocytes. For one, responsiveness to receptor-mediated gene induction may vary greatly among individuals due to differences in genetic factors, diet, lifestyle, health status, and so on. Few studies use large enough populations to evaluate potential interindividual variability in response. Second, because peroxisome proliferation is a reversible process, evaluations of human liver samples obtained several days after exposure may not provide meaningful results. Greater individual characterization of exposure and other biological factors, including those that might affect the pharmacokinetics of the receptor ligand, are needed for evaluations of potential human responsiveness to peroxisome proliferators. In addition, the quality of human liver samples used for *in vitro* studies may vary substantially compared to livers obtained from laboratory animals. Hepatocytes from rodent livers are harvested immediately after death, whereas several hours may elapse before harvesting human hepatocytes. Cellular degradative changes occurring during this time interval may affect the responsiveness of human hepatocyte cultures. Studies that do not evaluate functional responsiveness of human hepatocyte preparations are of limited value for assessing induction of peroxisome proliferation. Several recent studies with human hepatocyte cultures have used tumor growth factor β1 (TGFβ1) to induce apoptosis and epidermal growth factor as a positive control for induction of DNA synthesis and suppression of apoptosis (*21*). An additional consideration is that tissue culture assays may not be studying the appropriate cell population with respect to cancer risk from peroxisome proliferators. Future studies may need to examine responses in preneoplastic cells rather than responses only in normal hepatocytes. Also, consideration should be given to the possibility that conditions (e.g., timing and duration of exposure) that work best for rodent hepatocytes may not be optimal for human cells.

What Is the Mechanistic Basis for Biological Effects Induced by DEHP or Other Peroxisome Proliferators?

The discovery of the peroxisome proliferator activated receptor (PPAR) (*27*), a ligand-activated transcription factor, provides a mechanistic basis for understanding how peroxisome proliferators modulate gene expression leading to the induction of peroxisomal enzymes. Several PPAR subtypes have been identified, with PPARα implicated in hepatic responses. Binding of a peroxisome proliferator (ligand) to PPARα activates this nuclear hormone receptor. Ligand-bound PPARα regulates gene transcription by forming heterodimers with the retinoid X receptor, RXR (*28*) (i.e., L-PPARα-RXR), which can bind specifically to peroxisome proliferator response elements (PPREs) in the promoter region of peroxisome proliferator regulated genes. This interaction leads to transcriptional activation of these regulated genes, i.e.,

L–PPARα–RXR + regulated gene-PPRE → regulated gene mRNA↑

resulting in increased hepatic DNA synthesis, hepatomegaly, peroxisome proliferation, and the induction of several peroxisomal and microsomal enzymes. Suppression of hepatocyte apoptosis (programmed cell death) by peroxisome proliferators is also mediated by PPARα (*29*). The transcriptional effects of activated PPARα may be further modulated by interactions with other transcriptional factors (coactivators or suppressors).

Human PPARs are responsive to MEHP, the primary metabolite of DEHP. MEHP has been shown to stimulate transcriptional activation of human or mouse PPARα in transfected COS-1 monkey kidney cells, whereas DEHP was inactive (*30*). MEHP also induced transcriptional activation of human and mouse PPARγ.

Transgenic mice lacking PPARα (PPARα knock-out mice) do not elicit hepatic peroxisome proliferation, replicative DNA synthesis, or liver enlargement with exposure to peroxisome proliferators (*31*). These mice also display abnormal lipid metabolism and show no increase in liver tumor incidence after 11 months of feeding with Wy14,643 (*32,33*). Thus, it was suggested (*32,33*) that peroxisome proliferation, hepatocyte replicative DNA synthesis, hypolipidemia, and peroxisome proliferator-induced hepatocarcinogenesis are mediated by PPARα. However, no 2-year carcinogenicity studies have been reported for peroxisome proliferators in PPARα-null mice; such information is necessary to evaluate the possible occurrence of late-developing tumors arising by mechanisms independent of PPARα. Studies in PPARα-null mice compared to wild-type mice reflect outcomes linked to gene expression pathways modulated by this receptor. It is important to note that this model does not represent potential human responses to peroxisome proliferators because, unlike humans, PPARα-null mice lack a functional PPARα.

Are all biological effects of DEHP or its metabolites mediated by PPARα? Definitely not. For example, oral administration of DEHP to rats induced liver mitochondrial swelling and reduced succinate dehydrogenase (SDH) activity (*34*), and studies with isolated rat liver mitochondria showed that MEHP caused uncoupling of oxidative phosphorylation and inhibition of SDH activity (*35*). Whether these effects are related to a cancer outcome is unknown; however, they demonstrate that DEHP or its metabolites can elicit biological effects in the liver that are independent of PPARα. Further discussion on extraperoxisomal effects of peroxisome proliferators in laboratory animals and humans can be found in a comprehensive review by Youssef and Badr (*36*).

The induction of reproductive toxicity, teratogenicity, and altered zinc metabolism by DEHP in wild-type mice and in PPARα-null mice indicates that these effects are not mediated through PPARα-dependent mechanisms (*37*). Ward et al. (*38*) found that toxic liver lesions observed in wild-type mice fed DEHP were not present in treated PPARα-null mice. However, the PPARα-null mice did develop delayed toxic lesions in the kidney (severe nephropathy) and testis (tubular degeneration and reduced spermatogenesis), indicating that DEHP can induce renal and testicular toxicity independent of PPARα. Whether these effects are mediated by a different PPAR isoform is unknown.

In a study of morphologic transformation in Syrian hamster embryo (SHE) cells, Tsutsui et al. (*39*) found that DEHP but not clofibrate induced morphologic transformation. Cell transformation by DEHP was enhanced by metabolic activation, which also resulted in chromosome aberrations. Wy-14,643, a more potent carcinogen than DEHP, was more active in inducing chromosome aberrations. These results suggest that chromosomal damage may contribute to the carcinogenic activities of some peroxisome proliferators. DEHP increased the frequency of morphologic transformation and decreased the levels of gap junction intercellular communication in SHE cells (*40*). The induction of morphologic cell transformation, inhibition of gap-junction intercellular communication, and activation of Kupffer cells appear to be independent of peroxisome proliferation (*9,14*) and may represent additional effects that contribute to the carcinogenicity of certain peroxisome proliferators.

What Is the Basis for Species Differences in Responses Mediated by PPARα?

Humans and guinea pigs have been labeled as nonresponsive species even though they express a functionally active PPARα (*30,41–44*). The lack of response of human hepatocyte cultures has been attributed to low levels of PPARα expression in human liver (*45*). Human liver samples were reported to have 10-fold lower levels of PPARα mRNA than mouse liver (*43*). However, comparisons of mRNA levels across species may not accurately represent equivalent differences in PPARα protein levels because of possible differences in rates of translation of message

to protein and differences in stability of the specific mRNA and protein products. In addition, because human RNA was generally obtained from liver samples that were frozen several hours after death, the reported low levels of PPARα mRNA and protein in human liver may in part reflect losses occurring before the samples were frozen. PPARα mRNA and the corresponding functional protein (i.e., the protein that mediates transcriptional activation through PPRE) are also expressed in guinea pig liver, but at much lower levels than in mice (*46,47*). The level of liver PPARα mRNA in humans was reported to be approximately 10% of that in mice, 20% of that in rats, and two times higher than that in guinea pigs (*46*). PPARαs from all of these species had similar binding affinities for rat acyl CoA oxidase (ACO) PPRE and comparable activities in reporter gene assays, indicating their functional similarity.

A human peroxisomal ACO promoter gene was cloned and found to be responsive to peroxisome proliferators in reporter gene assays (*48*); in contrast, a follow-up study found the human ACO gene promoter to be inactive in a sample human population (*49*). The lack of induction of peroxisomal ACO activity in this group was suggested to be due to an inactive PPRE sequence in the ACO gene promoter rather than to lower cellular concentrations of PPARα (*49*). In line with this suggestion, it is important to note that there are no data on whether, or to what extent (i.e., population distribution), inactive PPRE sequences exist in the promoter region of genes that directly impact the potential carcinogenicity of peroxisome proliferators in humans.

Is Peroxisome Proliferation an Obligatory Step for Tumor Induction by DEHP and Other Peroxisome Proliferators?

Several investigators of peroxisome proliferator induced responses acknowledge uncertainty on whether or how PPARα might influence the possible carcinogenicity of peroxisome proliferators in humans; this is because the mechanism(s) of carcinogenicity of these agents are not understood (*31,38,41,43*). Although it is well established that peroxisome proliferators alter gene expression by a receptor-mediated process, the molecular mechanisms of modulation of cell proliferation and suppression of apoptosis by peroxisome proliferators are not known (*13*). Furthermore, the sustainability of growth perturbation responses (mitogenesis and apoptosis) have not been fully described for any species, and the extent to which such effects occur in human preneoplastic cells has not been determined.

Current data are insufficient to answer the question of whether tumorigenesis by DEHP or other peroxisome proliferators is restricted to rats and mice. There are no carcinogenicity data on DEHP or other peroxisome proliferators in any other animal species, including those that express low levels of PPARα (e.g., guinea pig). In addition, there are no cancer epidemiology studies on DEHP, and human studies on hypolipidemic fibrate drugs are inconclusive. Furthermore, the potential role of nonreceptor-mediated effects of DEHP and other peroxisome proliferators in human populations have not been evaluated. Because peroxisome proliferation is one of several changes produced by DEHP, it is not possible to conclude that peroxisome proliferation alone is the cause of liver cancers induced in rats and mice.

As in humans, fibrate drugs induce hypolipidemia in guinea pigs in spite of the low expression of PPARα. In addition, peroxisome proliferators did not induce replicative DNA synthesis or peroxisome proliferation in primary cultures of guinea pig hepatocytes, although apoptosis was markedly suppressed by a PPARα mediated mechanism (*19,46*). Thus, suppression of apoptosis appears to be regulated independently from induction of DNA synthesis. Suppression of apoptosis by peroxisome proliferators could prevent the death and removal of preneoplastic cells with damaged DNA and thereby contribute to the carcinogenic process. Because peroxisome proliferators induce suppression of apoptosis in guinea pig hepatocytes without inducing peroxisomal enzymes, it is apparent that certain PPARα regulated pathways are responsive even in species with low levels of PPARα expression. Measurements of apoptosis in human hepatocyte cultures have given mixed results. Hasmall et al. (*20,21*) reported no effect of nafenopin or MEHP on apoptosis in human hepatocytes, whereas Perrone et al. (*24*) observed an increase in spontaneous apoptosis in human hepatocytes incubated with clofibric acid or ciprofibrate. Results from the later study also showed a significant trend for suppression of apoptosis by ciprofibrate in human hepatocytes incubated for 24 hr in the presence of TGFβ, with a marginal response at the higher dose.

The pleiotropic effects of peroxisome proliferators are dependent on the existence of functional PPARs and PPREs in the promoter regions of genes regulated by these agents. Activation of PPARα modulates lipid homeostasis in humans (*50*), by the same receptor-mediated process as that which induces peroxisome proliferation in the rodent liver. Vu-Dac et al. (*51*) showed that fenofibrate induced a 2.5- to 4.5-fold increase in apolipoprotein A-II mRNA in human hepatocytes consequent to PPAR binding to the AII-PPRE. These results demonstrate that fibrates can modulate gene expression in humans by PPAR activation, whether or not peroxisomal enzymes are induced.

The critical steps in peroxisome proliferator-induced carcinogenesis need to be elucidated before it is possible to attribute a particular gene expression pathway (i.e., peroxisomal enzymes) as the cause of tumor induction. The contention that peroxisome proliferation and PPARα expression levels reflect species-specific susceptibility to the carcinogenic effects of peroxisome proliferators is an oversimplification of complex signaling mechanisms; nonresponsive species clearly elicit responses to peroxisome proliferators that are mediated by PPARα (e.g., suppression of apoptosis, hypolipidemic effects). A correlation between peroxisome induction and tumor development in rats and mice may reflect a coincidental observation rather than a causal relationship. Because peroxisome proliferators can affect multiple signaling pathways by transcriptional activation of PPAR-regulated genes, it is possible that chemicals such as DEHP contribute to the cancer process by mechanisms independent of peroxisomal proliferation or mitogenesis (e.g., suppression of the apoptotic removal of initiated cells).

If tumor induction by peroxisome proliferators is mediated by PPARα, then it may not be appropriate to expect exact site correspondence in animals and humans. The tissue distribution of PPARα is different in humans than it is in rats or mice (*43*). In humans, the highest levels of PPARα mRNA are in skeletal muscle and kidney, and low levels were found in the liver. In rats and mice the highest levels of PPARα expression are in the liver and kidney, yet peroxisome proliferators do not appear to cause tumors of the kidney.

The finding of PPARα mRNA and protein in rat and adult human testes suggests that this receptor may be involved in regulating the growth of tubular and interstitial cells in the testis of rats and humans (*52*). The demonstration of a functional PPAR in human breast cancer cell lines that can bind specifically to a PPRE indicates that peroxisome proliferators may transcriptionally activate peroxisome proliferator-regulated genes in human breast epithelial cells (*53*). Furthermore, stimulation of cell proliferation by DEHP in a human breast cancer cell line has been observed (*54*); however, the mechanism of this response has not been elucidated. The potential for peroxisome proliferators to affect human breast cancer risk has not been determined.

It is not known if there is a differential human sensitivity to the effects of peroxisome proliferators or whether there are highly

susceptible individuals in human subpopulations. In a study of 10 individual liver samples, PPARα mRNA levels differed by approximately 10-fold (*43*). Interindividual variability in PPARα base sequences along with possible variable levels of induced PPARα expression by glucocorticoids or nutritional factors may render certain individuals more responsive to peroxisome proliferators than the general population (*55*).

Conclusions and Future Research Needs

Although our understanding of the mechanism(s) of peroxisome proliferation and induction of peroxisomal ACO activity has advanced immensely over the past 10 years, there is still insufficient data to conclude that DEHP poses no cancer risk to humans. The fact that peroxisome proliferators have been shown to modulate gene expression in human hepatocytes (expression of genes regulating lipid homeostasis) and in guinea pig hepatocytes (suppression of apoptosis) through the activation of PPARα without inducing peroxisomal enzymes raises the distinct possibility that transcriptional activation of PPAR-responsive genes associated with the carcinogenic process can occur independently of peroxisome proliferation. Even in rodent liver cells, differences in dose–response relationships among the pleotropic effects of peroxisome proliferators (*22*) suggest that although these processes may be dependent on PPAR activation, they likely occur by independent mechanisms. Further, due to differences in tissue distribution of PPARs in rodents and humans, it may not be appropriate to expect responses to peroxisome proliferators to exhibit site correspondence across species. Issues raised in this commentary cannot be resolved until current hypotheses related to potential associations between oxidative damage, perturbation of growth control (enhancement of cell proliferation and suppression of apoptosis), and the carcinogenicity of DEHP and other peroxisome proliferators are rigorously tested and the mechanistic links between these events are elucidated.

DNA microarray technology could provide a powerful approach to resolve some of the issues raised here. This technique uses cDNA microarrays to simultaneously monitor the expression of thousands of specified genes. Specifically, a study is needed to quantify time- and dose-dependent changes in gene expression and consequent protein profiles resulting from exposure of freshly prepared human hepatocytes (and preneoplastic cells) to MEHP or other peroxisome proliferators. To determine whether and/or to what extent these agents alter the expression of protooncogenes, tumor-suppressor genes, or other genes that regulate mitogenesis or apoptosis, tissue samples from a diverse population of individuals need to be analyzed. Critical to these evaluations would be the demonstration of apolipoprotein A-II induction, confirming the functional responsiveness of human cell cultures. Without this critical positive control, it would not be possible to decipher the absence of an effect as being due to the lack of transcriptional function in human cell cultures rather than lack of responsiveness to these peroxisome proliferators.

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