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Evidence against Peroxisome Proliferation-Induced Hepatic Oxidative Damage

LEVELS OF ESTERIFIED ISOPROSTANES IN LIVERS OF MICE FED A DIET CONTAINING [4-CHLORO-6-(2,3-XYLIDINO)-2-PYRIMIDINYLTHIO]ACETIC ACID (WY-14,643)

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ABSTRACT. It has been proposed that nongenotoxic peroxisome proliferators may cause hepatocellular cancer by an oxidative damage-mediated mechanism(s). The argument for this hypothesis is based mainly on the noted ability of peroxisome proliferators to induce significantly H_2O_2-producing peroxisomal β-oxidation while causing a minimal induction of H_2O_2-degrading catalase. The recent discovery, accurate determination, and use of isoprostanes as a sensitive indicator of oxidative damage prompted us to investigate whether induction of hepatic peroxisomal β-oxidation in male B6C3F1 mice is accompanied by elevated levels of isoprostanes in those livers. The data show that while 7 days of feeding mice a diet containing 100 ppm [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (WY-14,643) increased peroxisomal β-oxidation by 16-fold and catalase activity by only 2-fold, hepatic levels of esterified F_2-isoprostanes were not altered. These levels were 2.8 ± 0.5 ng/g liver in control mice and 2.4 ± 0.1 ng/g liver in mice fed the experimental diet for 7 days. Consequently, it is concluded that oxidative stress does not appear to occur in response to peroxisome proliferation, as evidenced by the lack of increase in hepatic levels of F_2-isoprostanes in livers of mice treated with the potent peroxisome proliferator WY-14,643. BIOCHEM PHARMACOL 53;9:1369–1374, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. peroxisome proliferation; isoprostanes; oxidative damage; hepatocarcinogenesis; lipid peroxidation

Various hypolipidemic drugs, herbicides, and phthalate ester plasticizers cause peroxisome proliferation [1, 2]. Chronic exposure to these chemicals results eventually in the development of liver tumors in rodents via, as of yet, undefined mechanisms [3, 4]. Since peroxisome proliferators and their metabolites are not directly genotoxic [5, 6], attention has been focused on possible indirect mechanisms to explain the peroxisome proliferator-induced hepatocarcinogenesis.

Treatment of animals with peroxisome proliferators causes large increases in the activity of the H_2O_2-producing peroxisomal β-oxidation enzymes [7–9], while causing only minimal increases in the activity of peroxisomal catalase and decreasing the activity of glutathione peroxidase [7, 9, 10]. Consequently, it was hypothesized that an imbalance between H_2O_2 production and its degradation could lead to H_2O_2-mediated oxidative damage, which eventually causes carcinogenesis in the livers of treated animals [11, 12]. However, this hypothesis is undermined by the findings showing that H_2O_2 levels do not increase in livers of animals treated with peroxisome proliferators [13], and the lack of a quantitative correlation between hepatic peroxisome proliferation and liver cancer [14]. More direct evidence against the oxidative damage hypothesis was presented recently, where chronic treatment with the peroxisome proliferator [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (WY-14,643) failed to increase exhaled ethane or hepatic conjugated dienes [15]. Hence, several investigators have suggested that hepatocarcinogenesis due to peroxisome proliferators may result from the promotion of spontaneously initiated cells and implicate DNA replication as a crucial factor in the carcinogenic activity of these compounds [14, 16–19].

F_2-Isoprostanes are produced *in vivo* by free radical-catalyzed peroxidation of arachidonic acid, via a cyclooxygenase-independent reaction, and have been proposed as a valuable sensitive tool to assess oxidative stress *in vivo* [20–24]. It has been shown that hepatic F_2-isoprostanes were elevated significantly in response to oxidative stress caused

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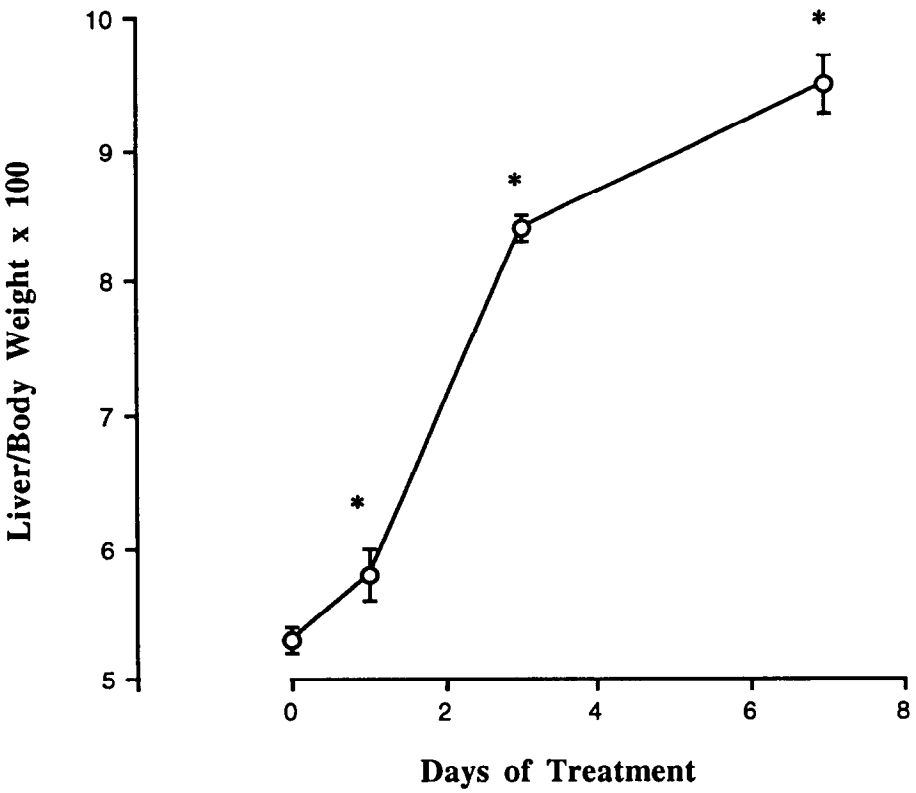

FIG. 1. Hepatomegaly induced by WY-14,643. Mice were fed a diet containing 100 ppm WY-14,643 for the designated durations. Data are means ± SEM from 6–12 mice per group. Key: * $P < 0.05$ compared with control values.

by carbon tetrachloride, vitamin E and selenium deficiencies, as well as in patients with conditions involving oxidative stress [24–26]. Since the measurement of isoprostanes is considered a far superior approach to assess oxidative stress status *in vivo* compared with conventional methods, the present study was designed to investigate whether oxidative damage occurs in livers of mice treated with the potent peroxisome proliferator WY-14,643, using F_2-isoprostanes as an indicator.

MATERIALS AND METHODS

Animal Treatment

Male B6C3F1 mice (Charles River, Portage, MI) weighing 25 ± 2 g were maintained on a daily cycle of alternating 12-hr periods of light and dark. All mice received a standard powdered diet, *ad lib.*, for 7 days prior to receiving diet containing 100 ppm WY-14,643 for 1, 3, and 7 days. WY-14,643 was obtained from ChemSyn Science Laboratories (Lenexa, KS). Control mice continued to receive drug-free diet and were killed after 1, 3, and 7 days. Since no differences were observed in monitored parameters among control mice, these animals were pooled together and identified as the zero day group. Following exsanguination, livers were excised quickly, were either homogenized or freeze-clamped, and then were kept at −80° until analyzed.

Determination of Peroxisomal Enzyme Activities

Peroxisomal β-oxidation was evaluated using the method of Lazarow and DeDuve [27], and catalase was measured by the method of Aebi [28], as previously reported [8, 9]. Mice were anesthetized with pentobarbital (50 mg/kg, i.p.), and livers were excised and homogenized in 0.25 M sucrose (20%, w/v). Cyanide-insensitive palmitoyl-CoA oxidation was used as a measure of peroxisomal enzyme β-oxidation and was assayed by measuring the rate of NAD^+ reduction spectrophotometrically at 340 nm. Catalase was assayed by monitoring the rate of H_2O_2 degradation spectrophotometrically at 240 nm.

Measurements of F_2-Isoprostanes

Lipids were extracted from mice livers and were subsequently subjected to base hydrolysis to liberate free F_2-isoprostanes from the acylated derivatives [22]. Free F_2-isoprostanes were then quantitated, following derivatization, employing gas chromatography/negative-ionization mass spectrometry as described in detail previously [22].

Statistical Analysis

Data are presented as means ± SEM from 5–12 mice per group, and were analyzed by Student's *t*-test for significance at $P < 0.05$.

RESULTS

Hepatomegaly Due to WY-14,643

As expected, mice fed WY-14,643 showed marked hepatomegaly. This hepatomegaly was evidenced by an increase in

TABLE 1. Changes in catalase activity due to WY-14,643

Days of feeding WY-14,643	Enzyme activity (mU/mg protein)
0	457 ± 16
1	595 ± 25*
3	805 ± 44*
7	1020 ± 15*

Data are means ± SEM from 4–12 mice per group.
* $P < 0.05$ compared with the control (0 days) group.

liver/body weight ratios (Fig. 1). After 24 hr, ratios increased significantly from control values of 5.3 ± 0.1 to 5.8 ± 0.2% (Fig. 1). Ratios continued to climb and reached 1.5-fold after 3 days and 2-fold after 7 days of feeding mice a diet containing this potent peroxisome proliferator (Fig. 1). These increases were due to liver enlargement since animal body weights were not altered significantly by the treatment (data not shown).

Peroxisomal Enzyme Activities in Mice Fed WY-14,643

Basal hepatic peroxisomal β-oxidation activity of 2.6 ± 0.1 mU/mg protein showed a significant increase to 11 ± 0.6 mU/mg protein after 24 hr of consuming diet containing WY-14,643 (Fig. 2). There was a concomitant slight increase in catalase activity (Table 1). Consuming diet that contained WY-14,643 for up to 7 days further increased peroxisomal β-oxidation, which reached 10- and 16-fold after 3 and 7 days, respectively (Fig. 2), while there was only a 2-fold increase in catalase activity after 7 days (Table 1).

Hepatic Levels of Esterified Isoprostanes

The concentration of esterified F_2-isoprostanes in livers of control animals was 2.8 ± 0.5 ng/g (Fig. 3). There were no significant changes in these levels in livers of mice fed the diet containing WY-14,643, compared with the control mice (Fig. 3). F_2-Isoprostane levels were 2.2 ± 0.1 ng/g after day 1 of treatment and 2.4 ± 0.1 ng/g after 7 days (Fig. 3).

DISCUSSION

Delineating the mechanism(s) by which various peroxisome proliferating agents cause liver cancer has been the focus of extensive research efforts for many years. Numerous studies have presented evidence for, as well as against, a proposed oxidative damage theory [11–19]. In support of the oxidative damage hypothesis, it has been observed that peroxisome proliferators cause a significant induction in the activities of enzymes responsible for the production of H_2O_2, while leading to a slight increase, no change, or a decrease in the activity of enzymes involved in the degradation of H_2O_2 [8, 9, 11, 12]. This was hypothesized to result in the accumulation of H_2O_2 which is proposed to subsequently cause oxidative damage [11, 12]. In support of this hypothesis, hepatocarcinogenicity due to peroxisome proliferators was inhibited by exogenously administered anti-oxidants [29]. Furthermore, it has been reported that treatment with peroxisome proliferators elevates hepatic

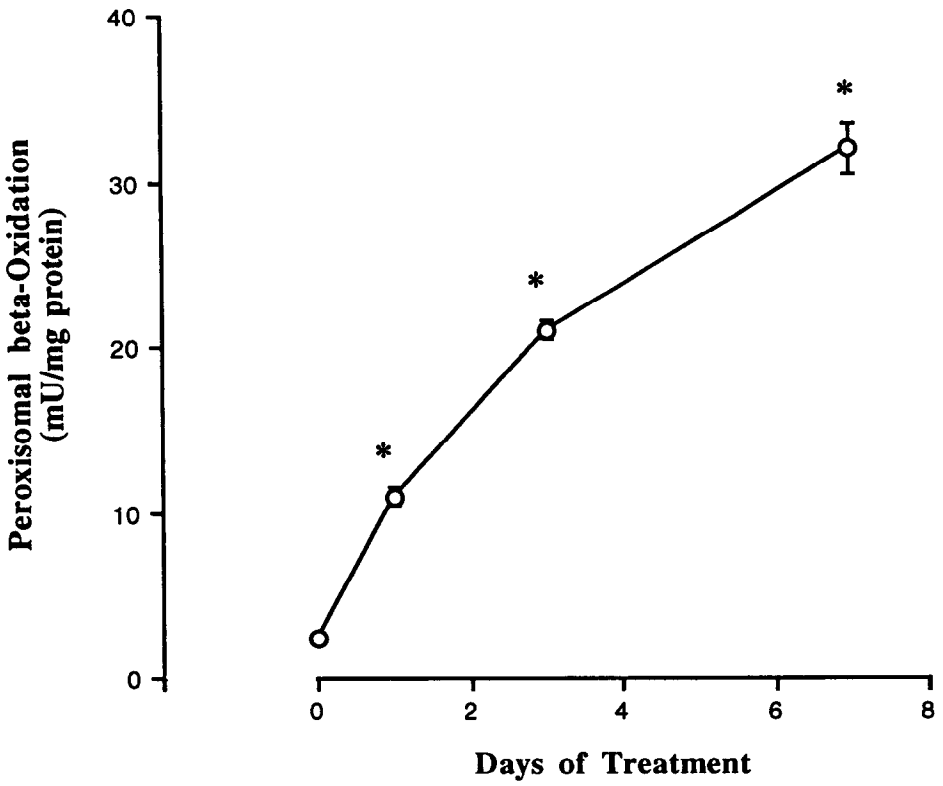

FIG. 2. Peroxisomal β-oxidation in livers of mice fed WY-14,643. Enzyme activities were determined as described under Materials and Methods, in livers of the mice presented in Fig. 1. Data are means ± SEM from 6–12 mice per group. Key: * $P < 0.05$ compared with control values.

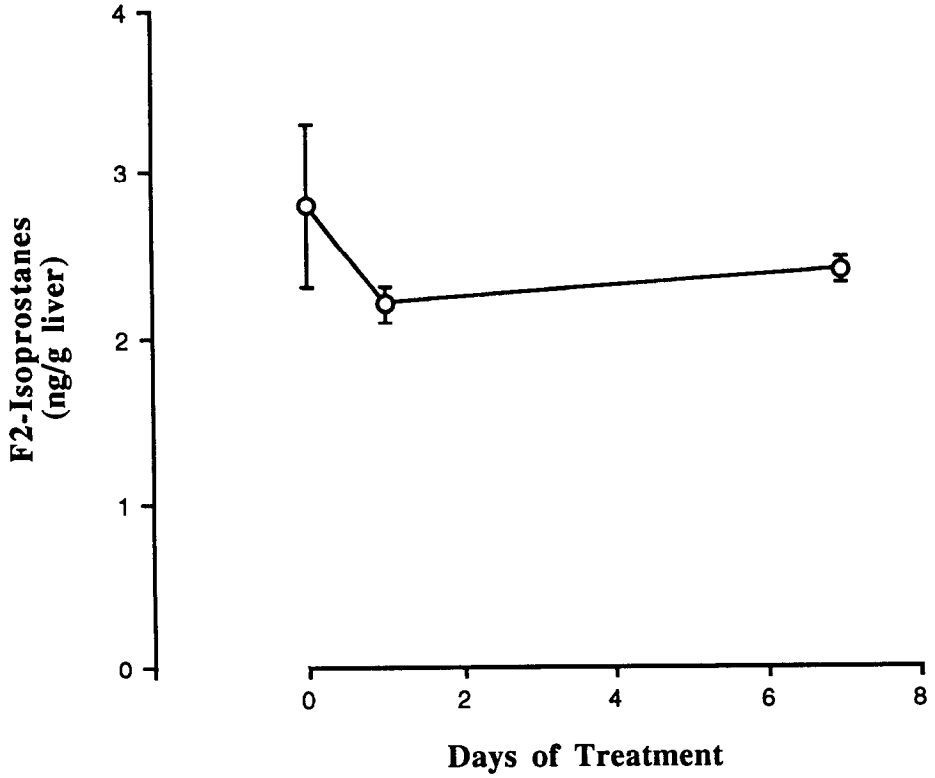

FIG. 3. Hepatic levels of esterified F_2-isoprostanes in treated mice. Lipids extracted from livers of mice treated as depicted in Fig. 1 were subjected to base hydrolysis, and free F_2-isoprostanes were quantitated as described under Materials and Methods. Data are means ± SEM from 5 mice per group.

levels of lipofuscin and 8-hydroxydeoxyguanosine, metabolites that are presumed to reflect oxidative damage [14, 30–32].

In contrast, results of several studies undermine the oxidative stress hypothesis. For example, it has been observed that *in vivo* levels of H_2O_2 were comparable in livers of animals treated with the peroxisome proliferator perfluorooctanoic acid and control animals [13]. Furthermore, a quantitative correlation between peroxisome proliferation, lipofuscin levels, and hepatocarcinogenicity does not exist [14]. Also, it was found recently that compounds not arising from lipid peroxidation diminish the accuracy of lipofuscin measurements [33]. While peroxisome proliferation was accompanied by increases in levels of 8-hydroxydeoxyguanosine in the liver, no increases were observed in renal levels of this adduct [31, 32]. Indeed, 8-hydroxyguanosine levels appeared to decrease in kidneys of rats treated with peroxisome proliferators [32], despite the fact that peroxisomes are known to proliferate significantly in the kidneys, albeit to a lesser extent than in the liver, in response to these chemicals [2, 31, 32]. Furthermore, treatment of animals with any of several peroxisome proliferators for extended periods of time did not alter hepatic levels of 8-hydroxydeoxyguanosine [34, 35]. Eventually, 8-hydroxydeoxyguanosine levels increased, yet not in parallel to the reported hepatocarcinogenic potencies of the proliferators used [35]. A recent study shows a lack of coordination between the time course and extent of peroxisome proliferation and changes in levels of hepatic 8-hydroxydeoxyguanosine produced by two peroxisome proliferators [36]. Therefore, the question of whether or not peroxisome proliferators cause oxidative damage remained unanswered, awaiting the discovery of a sensitive reliable indicator(s) of oxidative injury.

F_2-Isoprostanes are peroxidation products of arachidonic acid formed by a non-cyclooxygenase free radical-catalyzed mechanism. A substantial body of evidence suggests that isoprostanes provide a far more reliable measure of oxidative injury than other previously available markers [23, 37–40]. Furthermore, measuring isoprostanes esterified to tissue lipids is particularly valuable when oxidative injury is localized to a particular organ as is the case with peroxisome proliferators and the liver [23]. The results of this study show that while the potent peroxisome proliferator WY-14,643 caused a 16-fold increase in hepatic peroxisomal β-oxidation and only a 2-fold increase in catalase activity within 7 days (Fig. 2 and Table 1), hepatic concentrations of esterified F_2-isoprostanes were unaltered in those animals (Fig. 3). This remarkable imbalance between H_2O_2 production (peroxisomal β-oxidation) and its destruction (catalase) would be expected to lead to the accumulation of H_2O_2. Subsequently, H_2O_2 would diffuse to other cellular organelles and structures and elicit oxidative stress [11, 12]. H_2O_2 has been shown to cause rapid and substantial increase in levels of F_2-isoprostanes [37]. It may be argued that the absence of an increase in hepatic levels of F_2-isoprostanes in livers of mice treated with WY-14,643 is due to a decrease in the availability of arachidonic acid, the precursor of F_2-isoprostanes. However, previous studies [41, 42] have shown that peroxisome proliferators do not alter serum or hepatic arachidonate concentrations. These find-

ings suggest very strongly that esterified F_2-isoprostanes do not increase despite a significant proliferation of peroxisome, due to the inability of these livers to create and/or maintain the environment necessary to peroxidize arachdonic acid. In support of this conclusion, Conway and Popp [15] recently reported that a dose of WY-14,643 ten times higher than ours given to rats for a full year also failed to increase exhaled ethane or hepatic conjugated dienes. Therefore, our results strongly suggest that peroxisome proliferation is not accompanied by oxidative damage to the liver and confirm the need to continue the search for alternative potential mechanisms for the hepatocarcinogenic effect of peroxisome proliferators.

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References

1. Kawashima Y, Katoh H and Kozuka H, Differential effects of altered hormonal state on the induction of acyl-CoA hydrolases and peroxisomal β-oxidation by clofibric acid. *Biochim Biophys Acta* **750:** 365–372, 1983.
2. Reddy JK and Lalwai ND, Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *Crit Rev Toxicol* **12:** 1–58, 1983.
3. Chen H, Huang C-y, Wilson MW, Lay LT, Robertson LW, Chow CK and Glauert HP, Effect of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cell proliferation and toxicity in Sprague–Dawley rats. *Carcinogenesis* **15:** 2847–2850, 1994.
4. James NH and Roberts RA, The peroxisome proliferator class of non-genotoxic hepatocarcinogens synergize with epidermal growth factor to promote clonal expansion of initiated rat hepatocytes. *Carcinogenesis* **15:** 2687–2694, 1994.
5. Butterworth BE, Bermudez E, Smith-Oliver T, Earle L, Cattley R, Martin J, Popp JA, Strom S, Jirtle R and Michalopoulos G, Lack of genotoxic activity of di(2-ethylhexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis* **5:** 1329–1335, 1984.
6. Rao MS, Ide H, Yeldandi AV, Kumar S and Reddy JK, Expression of peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase enzyme and its mRNA in peroxisome proliferator-induced liver tumors. *Carcinogenesis* **15:** 2619–2622, 1994.
7. Lazarow PB, Three hypolipidemic drugs increase hepatic palmitoyl-CoA oxidation in the rat. *Science* **197:** 580–581, 1977.
8. Badr MZ, Induction of peroxisomal enzyme activities by di-(2-ethylhexyl)phthalate in thyroidectomized rats with parathyroid replants. *J Pharmacol Exp Ther* **263:** 1105–1110, 1992.
9. Thottassery J, Winberg L, Youssef J, Cunningham M and Badr M, Regulation of perfluorooctanoic acid-induced peroxisomal enzyme activities and hepatocellular growth by adrenal hormones. *Hepatology* **15:** 316–322, 1992.
10. Furukawa K, Numoto S, Furuya K, Furukawa NT and Williams GM, Effect of the hepatocarcinogen nafenopin, a peroxisome proliferator, on the activities of rat liver glutathione-requiring enzymes and catalase in comparison to the action of phenobarbital. *Cancer Res* **45:** 5011–5019, 1985.
11. Reddy JK, Azarnoff DL and Hignite CE, Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* **283:** 397–398, 1980.
12. Reddy JK, Carcinogenicity of peroxisome proliferators: Evaluation and mechanisms. *Biochem Soc Trans* **18:** 92–94, 1990.
13. Handler JA, Seed CB, Bradford BU and Thurman RG, Induction of peroxisomes by treatment with perfluorooctanoate does not increase rates of H_2O_2 production in intact liver. *Toxicol Lett* **60:** 61–68, 1992.
14. Marsman DS, Goldworthy TL and Popp JA, Contrasting hepatocytic peroxisome proliferation, lipofuscin accumulation and cell turnover for the hepatocarcinogens Wy-14,643 and clofibric acid. *Carcinogenesis* **13:** 1011–1017, 1992.
15. Conway JG and Popp JA, Effect of the hepatocarcinogenic peroxisome proliferator Wy-14,643 *in vivo*: No increase in ethane exhalation or hepatic conjugated dienes. *Toxicol Appl Pharmacol* **135:** 229–236, 1995.
16. Marsman DS, Cattley RC, Conway JG and Popp JA, Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (WY-14,643) in rats. *Cancer Res* **48:** 6739–6744, 1988.
17. Cattley RC, Marsman DS and Popp JA, Age-related susceptibility to the carcinogenic effect of the peroxisome proliferator WY-14,643 in rat liver. *Carcinogenesis* **12:** 469–473, 1991.
18. Eacho PI, Lanier TL and Brodhecker CA, Hepatocellular DNA synthesis in rats given peroxisome proliferating agents: Comparison of WY-14,643 to clofibric acid, nafenopin and LY171883. *Carcinogenesis* **12:** 1557–1561, 1991.
19. Kraupp-Grasl B, Huber W, Taper H and Schulte-Hermann R, Increased susceptibility of aged rats to hepatocarcinogenesis by the peroxisome proliferator nafenopin and the possible involvement of altered liver foci occurring spontaneously. *Cancer Res* **51:** 666–671, 1991.
20. Awad JA, Morrow JD, Takahashi K and Roberts LJ II, Identification of non-cyclooxygenase-derived prostanoid (F_2-isoprostane) metabolites in human urine and plasma. *J Biol Chem* **268:** 4161–4169, 1993.
21. Morrow JD, Minton TA, Mukundan CR, Campbell MD, Zackert WE, Daniel VC, Badr KF, Blair IA and Roberts LJ II, Free-radical-induced generation of isoprostanes *in vivo*. Evidence for the formation of D-ring and E-ring isoprostanes. *J Biol Chem* **269:** 4317–4326, 1994.
22. Morrow JD and Roberts LJ II, Mass spectrometry of prostanoids: F_2-Isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods Enzymol* **233:** 163–174, 1994.
23. Morrow JD and Roberts LJ II, The isoprostanes. Current knowledge and directions for future research. *Biochem Pharmacol* **51:** 1–9, 1996.
24. Morrow JD, Awad JA, Kato T, Takahashi K, Badr KF, Roberts LJ II and Burk RF, Formation of novel non-cyclooxygenase-derived prostanoids (F_2-isoprostanes) in carbon tetrachloride hepatotoxicity: An animal model of lipid peroxidation. *J Clin Invest* **90:** 2502–2507, 1992.
25. Awad JA, Morrow JD, Hill KE, Roberts LJ II and Burk RF, Detection and localization of lipid peroxidation in selenium- and vitamin E-deficient rats using F_2-isoprostanes. *J Nutr* **124:** 810–816, 1994.
26. Morrow JD, Frei B, Longmire AW, Gaziano M, Lynch SM, Shyr Y, Strauss WE, Oates JA and Roberts LJ II, Increase in circulating products of lipid peroxidation (F_2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med* **332:** 1198–1203, 1995.
27. Lazarow PB and DeDuve C, A fatty acyl-CoA oxidizing system in rat liver peroxisomes: Enhancement by clofibrate, a hepolipidemic drug. *Proc Natl Acad Sci USA* **73:** 2043–2046, 1976.
28. Aebi H, Catalase. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer H), pp. 673–684. Academic Press, New York, 1974.
29. Rao MS, Lalwani ND, Watanabe TK and Reddy JK, Inhibitory effect of antioxidants ethoxyquin and 2(3)-*tert*-butyl-4-

hydroxyanisole on hepatic tumorigenesis in rats fed ciprofibrate, a peroxisome proliferator. *Cancer Res* **44:** 1072–1076, 1984.
30. Kasai H, Okada Y, Nishimura S, Rao MS and Reddy JK, Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator. *Cancer Res* **49:** 2603–2605, 1989.
31. Takagi A, Sai K, Umemura T, Hasegawa R and Kurokawa Y, Significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats following short-term exposure to the peroxisome proliferators di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate. *Jpn J Cancer Res* **81:** 213–215, 1990.
32. Takagi A, Sai K, Umemura T, Hasegawa R and Kurokawa Y, Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. *Cancer Lett* **57:** 55–60, 1991.
33. Szweda LI, Age-related increase in liver retinyl palmitate: Relationship to lipofuscin. *J Biol Chem* **269:** 8712–8715, 1994.
34. Hegi ME, Ulrich D, Sagelsdorff P, Richter C and Lutz WK, No measurable increase in thymidine glycol or 8-hydroxydeoxyguanosine in liver DNA of rats treated with nafenopin or choline-devoid low-methionine diet. *Mutat Res* **238:** 325–329, 1990.
35. Cattley RC and Glover SE, Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: Relationship to carcinogenesis and nuclear localization. *Carcinogenesis* **14:** 2495–2499, 1993.
36. Huang C-y, Wilson MW, Lay LT, Chow CK, Robertson LW and Glauert HP, Increased 8-hydroxydeoxyguanosine in hepatic DNA of rats treated with the peroxisome proliferators ciprofibrate and perfluorodecanoic acid. *Cancer Lett* **87:** 223–228, 1994.
37. Salahudeen A, Badr K, Morrow J and Roberts J II, Hydrogen peroxide induces 21-aminosteroid-inhibitable F_2-isoprostane production and cytolysis in renal tubular epithelial cells. *J Am Soc Nephrol* **6:** 1300–1303, 1995.
38. Burk RF, Hill KE, Awad JA, Morrow JD, Kato T, Cockell KA and Lyons PR, Pathogenesis of diquat-induced liver necrosis in selenium-deficient rats: Assessment of the roles of lipid peroxidation and selenoprotein P. *Hepatology* **21:** 561–569, 1995.
39. Longmire AW, Swift LL, Roberts LJ II, Awad JA, Burk RF and Morrow JD, Effect of oxygen tension on the generation of F_2-isoprostanes and malondialdehyde in peroxidizing rat liver microsomes. *Biochem Pharmacol* **47:** 1173–1177, 1994.
40. Morrow JD and Roberts LJ II, Quantification of noncyclooxygenase derived prostanoids as a marker of oxidation stress. *Free Radic Biol Med* **10:** 195–200, 1991.
41. Olson CT and Andersen ME, The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. *Toxicol Appl Pharmacol* **70:** 362–372, 1983.
42. Kawashima Y, Nishizawa H and Kozuka H, Reduction by clofibric acid of serum arachidonic acid in rats. Effect on the acyl composition of renal phospholipids. *Biochem Pharmacol* **43:** 2321–2326, 1992.