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Nonclinical Safety Evaluation of Muraglitazar, a Novel PPARα/γ Agonist

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The toxicity of muraglitazar, an oxybenzylglycine, nonthiazolidinedione peroxisome proliferator–activated receptor (PPAR) α/γ agonist, was evaluated in a comprehensive nonclinical toxicology program that included single-dose oral toxicity studies in mice, rats, and monkeys; repeat-dose toxicity studies in rats, dogs, and monkeys; a battery of *in vitro* and *in vivo* genetic toxicity studies; carcinogenicity studies in mice and rats; reproductive and developmental toxicity studies in rats and rabbits; and studies to investigate species-specific findings. Pharmacologically mediated changes, similar to those observed with other PPARγ agonists, were observed following chronic administration and included subcutaneous edema, hematologic/hematopoietic and serum chemistry alterations, and morphologic findings in the heart and adipose tissue in rats and monkeys. In dogs, a species highly sensitive to PPARγ agonists, muraglitazar caused pronounced species-specific clinical toxicity and degenerative changes in the brain, spinal cord, and testes at high doses and exposures. Muraglitazar was nongenotoxic in the standard battery of genotoxicity studies. Gallbladder adenomas in male mice and adipocyte neoplasms in male and female rats were seen at suprapharmacologic exposures, whereas urinary bladder tumors occurred in male rats at lower exposures. Subsequent investigative studies established that the urinary bladder carcinogenic effect was mediated by urolithiasis rather than a direct pharmacologic effect on urothelium. Muraglitazar had no effects on reproductive function in male and female rats at high systemic exposures, was not teratogenic in rats or rabbits, and demonstrated no selective developmental toxicity. Overall, there were no nonclinical findings that precluded the safe administration of muraglitazar to humans.

Key Words: **dual PPARα/γ; agonist; muraglitazar; toxicology profile; chronic toxicity.**

The incidence of type 2 diabetes, a chronic debilitating disease, is increasing rapidly in industrialized nations, and it is estimated that there will be 221 million diabetic patients worldwide by the year 2010 (King *et al*., 1998). Several agonists of the peroxisome proliferator–activated receptors (PPARs) have been developed for the treatment of type 2 diabetes based on their role in regulating lipid metabolism and insulin sensitization (Berger and Moller, 2002; Lehrke and Lazar, 2005; Yki-Jarvinen, 2004). PPARs are ligand-activated transcription factors that modulate target gene expression by binding to specific peroxisome proliferator response elements (PPREs) in promotor regions of regulated genes (Berger and Moller, 2002; Kersten *et al*., 2000; Nahle, 2004). Three receptor types have been identified in the family including alpha (α), gamma (γ), and delta (δ)—also called beta. PPARα agonists are primarily used to treat dyslipidemia (Cox, 2005), whereas the PPARγ agonists increase glucose utilization, reduce hepatic glucose production, and enhance insulin sensitivity (Saltiel and Horikoshi, 1995; Saltiel and Olefsky, 1996). There has been considerable interest in the development of dual PPARα/γ agonists for the treatment of type 2 diabetes since dyslipidemia often accompanies diabetes.

Muraglitazar, a nonthiazolidinedione/nonfibrate dual PPARα/γ agonist that combined the insulin sensitization and glucose lowering effects of PPARγ agonism with the antidyslipidemic effects of PPARα agonism (Harrity *et al*., 2006), was discontinued from development as a treatment of type 2 diabetes. Prior to its discontinuation, muraglitazar was shown to clinically improve the lipid and glucose profiles in diabetic patients (Cox, 2005). As demonstrated by Buse *et al*. (2005) in a double-blind, placebo-controlled, 24-week monotherapy trial, muraglitazar significantly reduced serum triglycerides, fasting plasma glucose, and glycosylated hemoglobin and increased high density lipoprotein-cholesterol, enabling many patients to achieve the American Diabetes Association goals for glycemic control and effectively improving their lipid profile.

Consistent with anticipated pharmacologic properties, muraglitazar binds with high affinity to both human PPARα and PPARγ ligand-binding domain protein with IC_{50} for binding of 0.25 and 0.19 μmol/l, respectively (Devasthale *et al*., 2005). In addition, muraglitazar potently transactivates full-length human PPARγ or PPARα-mediated reporter gene activity (EC_{50} for transactivations = 0.11 and 0.32 μmol/l, respectively) (Devasthale *et al*., 2005).

Muraglitazar is excreted primarily in the feces, mainly *via* the biliary route; the biotransformation profile in rats, dogs, monkeys, and humans are qualitatively similar. Metabolites of muraglitazar include products of glucuronidation, dealkylation,

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and hydroxylation. The parent drug was the most abundant component in the plasma of mice, rats, dogs, monkeys, and humans; and all human metabolites were produced by all animal species. The recommended clinical dose was 5 mg once daily with an associated mean AUC exposure of 4.884 μg·h/ml.

The toxicity of muraglitazar in animals was well characterized in a comprehensive nonclinical toxicology program to establish a safe starting dose for clinical trials and to identify potential adverse effects during clinical development. The objective of this article is to present the overall nonclinical toxicity profile of muraglitazar.

MATERIALS AND METHODS

The studies were conducted in compliance with U.S. Food and Drug Administration (FDA) Good Laboratory Practice regulations and in accordance with the Guide for the Care and Use of Laboratory Animals.

Test Substance

Muraglitazar is an oxybenzylglycine, nonthiazolidinedione PPAR α and γ agonist and is chemically described as N-[(4-methoxyphenoxy)carbonyl]-N-[[4-[2-(5-methyl-2-phenyl-4-oxazolyl) ethoxy]phenyl]methyl]glycine.

Animals

The species used in the toxicology studies included CD-1 (Harlan Laboratories, Dublin, VA) and Crl:CD-1 (ICR) BR mice (Charles River Laboratories, Raleigh, NC), Hsd:Sprague-Dawley (Harlan Laboratories, Dublin, VA, Indianapolis, IN, and Frederick, MD), and Crl:CD (SD) IGS BR rats (Charles River Laboratories, Raleigh, NC, Kingston, NY, and Portage, MI), New Zealand White Hra:(NZW)SPF rabbits (Covance Research Products, Inc. Denver, PA), Beagle dogs (Harlan-Ridglan Research Farms Inc., Mt. Horeb, WI and Marshall Farms, North Rose, NY) and captive-bred Cynomolgus monkeys (Charles River Biomedical Resources Foundation, Houston, TX, Three Springs Scientific, Perkasie, PA, and Bristol-Myers Squibb, Somerville, NJ). All animals were housed in environmentally controlled rooms maintained on a 12-h light-dark cycle with a targeted humidity range of 30–70% and a targeted temperature range of 64°F–79°F and were provided water and certified feed *ad libitum*.

Muraglitazar was administered by oral gavage (mice, rats, and rabbits), nasogastric intubation (monkeys), or in capsules (dogs). The carrier was alkaline 96% polyethylene glycol (PEG)-400 and 4% 1M NaOH.

Doses Administered

Single-dose toxicity studies of muraglitazar were conducted by the oral route at doses of 500–4000 mg/kg in mice (five per sex per group) and rats (five per sex per group) and 10–1000 mg/kg in male monkeys (three per group).

Chronic oral toxicity studies were conducted over a wide range of doses in 20 rats per sex per group (0.3–300 mg/kg) for 6 months and in monkeys (four per sex per group) at doses of 0.4–5 mg/kg for 1 year.

Chronic Studies

Dose Selection

Rat. Muraglitazar was well tolerated when administered to rats at doses of 3–300 mg/kg for up to 1 month. Minimal to moderate decreases in erythrocyte count and hematocrit were observed at all doses; vacuolation and hyperplasia of adipocytes were observed at ≥ 30 mg/kg; and at 300 mg/kg, drug-related changes included increased liver and heart (> 30%) weights and multifocal fibroplasia/fibrosis of adipose tissue. Based on these results, 300 mg/kg was selected as the high dose in the definitive chronic study; additional doses of 0.3, 3, and 30 mg/kg were selected to investigate dose-response relationships. The doses selected for the 6-month oral toxicity study were associated with muraglitazar plasma concentrations 0.7–312 times and 0.6–376 times human therapeutic exposure in male and female rats, respectively (Table 1).

Monkey. Doses in the 1-year monkey study were based on results of a 9-month oral toxicity study conducted at doses of 0.4, 2, and 10 mg/kg (lowered to 5 mg/kg during week 17 due to edema). After 4 months of dosing in the 9-month study, dose-related edema (transient at 0.4 mg/kg) was observed at all doses. At ≥ 2 mg/kg, decreases in erythrocytic parameters and hypocellularity of the bone marrow were observed, and at 10/5 mg/kg, increases in fat lobule size were noted. The doses selected for the 1-year oral toxicity study, 0.4, 2, and 5 mg/kg, were associated with muraglitazar plasma concentrations 2–29 times and 2–44 times human therapeutic exposure in male and female monkeys, respectively (Table 1).

TABLE 1
Multiples of Human Exposure for Muraglitazar in Pivotal Toxicity Studies

Species	Type of study (sampling time)	Dose (mg/kg/day)	Exposure multiple[a]	
			Male	Female
Mouse	2-year oral carcinogenicity	1	3	5
	(day 177)	5	17	25
		20	62	87
		40	141	154
Rat	6-month oral toxicity	0.3	0.7	0.6
	(day 168)	3	8	5
		30	53	59
		300	312	376
Rat	2-year oral carcinogenicity	1	1	2
	(day 178)	5	8	7
		30	37	45
		50	48	59
Monkey	9-month oral toxicity	0.4	3	4
	(day 275)	2	17	17
		10/5[b]	53	59
Monkey	1-year oral toxicity	0.4	2	2
	(day 362)	2	14	9
		5	29	44
Pregnant rat	Toxicokinetics[c]	1.8		5
	(DG 15)	18		56
		180		406
Lactating rat	Toxicokinetics[d]	1.5		3
	(DL 4)	5		14
		15		38
		45		126
Pregnant rabbit	Toxicokinetics[c]	2.5		0.2
	(DG 19)	12		1
		60		5

Note. DG, gestation day; DL, lactation day.

[a]AUC(TAU) at steady state at 5 mg/day doses in a multiple ascending dose study in type 2 diabetic human subjects (CV168002): 4.884 μg·h/ml. AUC exposure in animals at steady state (animal exposure divided by human exposure).

[b]Dose was reduced from 10 to 5 mg/kg after 16 weeks of dosing.

[c]Supports embryo-fetal development study in rats or rabbits.

[d]Supports pre- and postnatal development studies in rats.

Parameters Evaluated

Rats. In the 6-month oral toxicity study, rats were observed daily for clinical signs of toxicity; detailed physical examinations were performed pretest and weekly; individual body weights and food consumption were recorded weekly; ophthalmic examinations were performed on all animals pretest and after a daily dose during weeks 13 and 26; water intake (fasted) over an 18-h period was determined during weeks 14 and 27; blood analyses were collected from the tail vein (fasted animals) at weeks 12 and 24 for hematology and clinical chemistry and from the heart at study termination for blood coagulation testing; urinalyses were conducted on urine samples collected over an 18-h fasted period during weeks 14 and 27; all animals were necropsied at study termination and subjected to a thorough gross and microscopic evaluation of tissues.

Monkeys. In the 1-year oral toxicity study, monkeys were observed twice daily for clinical signs of toxicity; detailed physical examinations were performed pretest and weekly; food consumption (qualitative) and individual body weights were recorded weekly; blood was collected from fasted monkeys pretest and at 6 and 12 months for hematology and clinical chemistry analyses, at 10 months to assess blood plasma volume using Evans Blue dye, and at study termination for blood coagulation tests; urinalyses were conducted on samples collected pretest and at 6 and 12 months; ophthalmoscopic examinations were conducted pretest and at 6 and 12 months; electrocardiograms (unanesthetized animals) and echocardiographic examinations were recorded pretest and at 6 and 12 months; echocardiographic measurements included left ventricular internal diameter during systole and left ventricular internal diameter during diastole, left ventricular posterior wall thickness during systole and left ventricular posterior wall thickness during diastole, intraventricular septal thickness during systole and intraventricular septal thickness during diastole, and E point to septal separation; all animals were necropsied at study termination and subjected to a thorough gross and microscopic evaluation of tissues.

Dog Toxicity/Investigative Studies

The toxicologic potential of muraglitazar in dogs (three per sex per group) was evaluated in a 1-month oral toxicity study using doses of 0.2, 2, 20, and 200 mg/kg and two subsequent 1-month investigative studies with six/sex/group or six males per group (5, 10, 20, and/or 200 mg/kg). The subsequent investigative studies were designed to determine the reproducibility and reversibility of the treatment-related microscopic changes in the brain, spinal cord, and testes in the 1-month oral toxicity study and to establish no-observed-effect levels (NOELs) for these findings.

Genotoxicity

The potential genotoxicity of muraglitazar was investigated in a standard battery of *in vitro* and *in vivo* genetic toxicity studies including an exploratory bacterial mutagenicity assay, Ames reverse mutation assay in *Salmonella* and *Escherichia coli*, cytogenetics study using primary human lymphocytes, and oral micronucleus study in rats.

Reproductive Toxicity Studies

A battery of reproductive and developmental toxicity studies was conducted in rats and rabbits with muraglitazar. These studies evaluated the effects on fertility of muraglitazar in male and female rats (25 per sex per group), as well as the potential embryo-fetal toxicity and teratogenicity in 22 presumed pregnant rats and rabbits per group, and prenatal/postnatal toxicity in 25 presumed pregnant rats per group.

Carcinogenicity Studies

Traditional lifetime carcinogenicity studies (up to 2 years) were conducted (Covance Laboratories Inc., Vienna, VA) in mice and rats with muraglitazar (Tannehill-Gregg *et al.*, 2007). In mice, two studies were conducted—doses of 1, 5, or 20 mg/kg were evaluated in the first (initial) and 40 mg/kg in the second (Supplementary). The first study contained two vehicle control groups, whereas one vehicle control group was included in the second study; all groups, including controls, contained 60 mice per sex per group. In addition, 19 mice per sex per group were designated for toxicokinetic analysis in each study and received muraglitazar by oral gavage once daily at 1, 5, 20, or 40 mg/kg for 26 weeks. In the carcinogenicity study in rats, 65 rats per sex group were given muraglitazar by oral gavage once daily at 1, 5, 30, or 50 mg/kg for up to 105 weeks. Two control groups (65 rats per sex per group) were given the same vehicle used in mice, alkaline PEG-400 solution. The dose volume for all studies was 5 ml/kg. In the rat carcinogenicity study, toxicokinetic analyses were conducted on blood samples collected from study animals rather than from separate satellite groups.

RESULTS

Safety Multiples of Muraglitazar

The systemic exposures (AUC) achieved in mice, rats (nongravid, gravid, lactating), monkeys, and pregnant rabbits in toxicology studies were equivalent to or substantially higher than mean human AUC exposure at an efficacious clinical dose of 5 mg/day. Table 1 provides the multiples of human exposure achieved in pivotal toxicology studies. Since muraglitazar is highly bound (> 99%) to mouse, rat, monkey, and human serum proteins, multiples to human exposure were calculated using total concentration of the drug. These multiples demonstrate that adequate doses were utilized in the toxicity studies to ensure a comprehensive evaluation of the toxicity of muraglitazar and to assess potential human risk.

Muraglitazar Modulation of PPAR Target Gene Expression in Mice

As previously described, muraglitazar potently stimulates full-length human PPARγ- and PPARα-mediated reporter gene expression (EC_{50} for PPARγ and PPARα transactivation = 0.11 and 0.32 μmol/l, respectively) (Devasthale *et al.*, 2005). In a chimeric Gal4/mouse PPAR-mediated reporter gene assay, muraglitazar shows mouse PPARγ agonist activity at levels comparable with its human PPARγ activity (EC_{50} for mouse PPARγ = 0.09 μmol/l for muraglitazar and 0.08 μmol/l for the PPARγ agonist rosiglitazone; the PPARα agonist fenofibric acid was inactive), and mouse PPARα agonist activity that is weaker than its human PPARα activity (observed EC_{50} for mouse PPARα = 23.8 μmol/l for muraglitazar and 16.3 μmol/l for fenofibric acid; rosiglitazone was inactive). The differential mouse and human PPARα activity is likely due to mouse/rodent-specific interactive disparities of muraglitazar with dissimilar amino acids in the mouse and human PPARα ligand-binding domain (Boettcher *et al.*, 2003).

Acute Toxicity Studies

Muraglitazar demonstrated a low order of acute toxicity; the minimum lethal dose was 2000 mg/kg in mice, greater than

4000 mg/kg in rats, and was greater than 1000 mg/kg in monkeys. Single doses of 2000 mg/kg and greater caused decreased activity and death in mice; a single dose of 4000 mg/kg resulted in unkempt appearance, loose feces, and decreased body weights in rats; and a single dose of 1000 mg/kg in monkeys reduced rectal temperature. There was no macroscopic evidence of target organ toxicity at the highest doses evaluated in rodents; monkeys were returned to stock following the postdose observation period.

Chronic Toxicity Studies

Pharmacologically Mediated Effects

Edema. Swelling (edema) of the limbs, jaw, abdomen, head, and/or whole body (generalized) was observed at the overtly toxic dose of 300 mg/kg in rats administered muraglitazar for up to 6 months (see Supplementary Table 1). In monkeys, slight to severe edema was observed following chronic treatment ($\geq$ 3 months) at 2–10 mg/kg with a few transient observations of edema at the lowest dose evaluated (0.4 mg/kg). Edema in monkeys was first characterized by periocular swelling that was generally noted during the third month in the high-dose group (10 mg/kg: 9-month study; 5 mg/kg: 1-year study). During subsequent weeks, edema was observed at $\geq$ 2 mg/kg and was noted mainly in the axilla, cheeks, and legs in both sexes and the scrotum in males (see Supplementary Table 2). Due to the severity of the edema observed at 10 mg/kg in the 9-month study, the high dose was reduced to 5 mg/kg during week 17. However, the severity of the edema was not appreciably different after the dose was reduced. Over the course of the 9-month and 1-year studies, the frequency of edema, its severity, and the affected area varied for individual animals.

Hematologic/hematopoietic effects. Dose-dependent hematologic and/or hematopoietic changes occurred in rats and monkeys after chronic treatment with muraglitazar. At doses of $\geq$ 30 mg/kg, hematologic changes in rats included generally minimal to mild decreases in red blood cell count, hematocrit, and hemoglobin (3–20%) after 6 months of treatment with no associated effect on bone marrow (Table 2). Additionally, in response to the decreased erythrocytic parameters, there was evidence of an erythrocytic regenerative response characterized by increased polychromasia, anisocytosis, reticulocyte counts, and splenic extramedullary hematopoiesis at the overtly toxic dose of 300 mg/kg in the 6-month study (see Supplementary Table 1).

In monkeys, minimal to mild decreases in erythrocytic parameters occurred in animals administered $\geq$ 2 mg/kg for up to 1 year (Table 2) and were accompanied by hepatic extramedullary hematopoiesis at 10 mg/kg after 4 months. In addition, bone marrow hematopoietic cells were slightly to markedly decreased with no associated change in the myeloid/erythroid ratio at $\geq$ 2 mg/kg after 1 year of dosing. Lastly, increased numbers of white adipocytes were observed in sternal and/or femoral bone marrow of monkeys at $\geq$ 2 mg/kg after 4 months.

Serum chemistry effects. Expected pharmacologically mediated serum chemistry changes occurred across the dose range tested in rats (0.3–300 mg/kg) and monkeys (0.4–5 mg/kg) following chronic treatment ($\geq$ 6 months) with muraglitazar. In rats, these changes generally included decreases in serum glucose, triglycerides, and total cholesterol at doses $\geq$ 3 mg/kg (Table 3). In comparison, decreases in serum triglycerides with no change in serum glucose were noted in monkeys at all doses following 1 year of dosing (data not shown). Other pharmacologically mediated serum chemistry changes included minimal decreases in albumin (4–12%) and protein (4–18%) at 30 and 300 mg/kg in rats (Table 3). There were no significant

TABLE 2
Muraglitazar-Related Erythrocytic Changes in Rats and Monkeys

	Control	3 mg/kg	30 mg/kg	300 mg/kg
Rats				
Males				
Red blood cells ($\times 10^6$/μl) month 6	9.1	9.1	8.5**	7.3**
Hematocrit (%) month 6	48.5	48.2	46.8**	42**
Hemoglobin (g/dl) month 6	15.6	15.5	15.1*	13.2**
Females				
Red blood cells ($\times 10^6$/μl) month 6	7.8	8.0	7.5**	6.3**
Hematocrit (%) month 6	43.0	43.7	42.2	37.5**
Hemoglobin (g/dl) month 6	14.4	14.6	13.9*	12.2**
	Control	0.4 mg/kg	2 mg/kg	5 mg/kg
Monkeys				
Males				
Red blood cells ($\times 10^6$/μl) month 12	6.4	6.2	5.8	5.5*
Hematocrit (%) month 12	40.8	40.9	38.7	38.1*
Hemoglobin (g/dl) month 12	12.9	12.6	12.3	11.9
Females				
Red blood cells ($\times 10^6$/μl) month 12	6.2	6.2	5.7*	5.6*
Hematocrit (%) month 12	39.9	39.1	36.7	37.9
Hemoglobin (g/dl) month 12	12.5	11.9	11.7	11.3*

Note. Mean, $n = 9$–20 (rats) and $n = 3$–4 (monkeys). No drug-related effects at 0.3 mg/kg in rats (data not shown).

Statistically significant, *$p < 0.05$, **$p < 0.01$; statistical comparison to the concurrent control groups (Dunnett test).

TABLE 3
Selected Muraglitazar-Related Changes in Serum Chemistry in Rats at 6 months

	Control	0.3 mg/kg	3 mg/kg	30 mg/kg	300 mg/kg
Males					
Cholesterol (mg/dl)	130.6	133.1	114.5*	96.8**	109.8*
Glucose (mg/dl)	139.2	151.0**	138.4	142.8	130.2
Triglycerides (mg/dl)	86.7	98.9	63.4*	52.6**	48.3**
Total protein (g/dl)	7.03	6.81*	6.92	6.50**	5.73**
Albumin (g/dl)	4.41	4.33	4.33	4.03**	3.68**
Females					
Cholesterol (mg/dl)	139.6	135.8	128.7	124.7*	123.0
Glucose (mg/dl)	150.2	147.2	141.8*	135.6**	129.7**
Triglycerides (mg/dl)	68.3	64.0	70.8	54.1*	44.6**
Total protein (g/dl)	6.82	6.79	6.87	6.45**	6.13**
Albumin (g/dl)	4.62	4.64	4.61	4.25**	4.38**

Note: Mean, $n = 9$–20.

Statistically significant, $*p < 0.05$, $**p < 0.01$; statistical comparison to the concurrent control groups (Dunnett test).

TABLE 4
Muraglitazar-Related Adipose Tissue Findings in Rats at 6 months

Finding	Control M/F	0.3 mg/kg M/F	3 mg/kg M/F	30 mg/kg M/F	300 mg/kg M/F
Hyperplasia, brown adipose tissue[a]					
Mild	—	—	—	—	4/2
Moderate	—	—	—	—	16/18
Fibroplasia/fibrosis, brown adipose tissue[a]					
Minimal to mild	—	—	—	—	18/11
Moderate	—	—	—	—	2/9
Vacuolation, brown adipose cells[a]					
Minimal to mild	—	—	—	7/15	1/2
Moderate	—	—	—	12/5	16/16
Marked	—	—	—	—	3/1
Inflammation, subacute, brown adipose tissue[a]					
Minimal	—	—	—	—	3/2
Hyperplasia, white adipose tissue[b]					
Minimal to mild	—	—	0/16	0/19	20/19

Note. ''—'' Indicates absence of finding/severity in the group.

[a]Located in the interscapular region of the back.

[b]Primarily represents proliferation of brown adipose tissue cells among the white adipose tissue around the mesenteric lymph node and/or mammary gland.

changes in serum chemistry parameters indicative of liver injury in either rats or monkeys (data not shown).

Adipose tissue effects. Dose- and time-dependent adipose tissue changes were observed in rats and monkeys following chronic treatment with muraglitazar. In rats after 6 months of dosing at 30 and 300 mg/kg, muraglitazar-related gross pathologic changes consisted of subcutaneous fat masses beneath the skin of the flank, axillary, and interscapular regions (Supplementary Table 1). Microscopically at $\geq$ 3 mg/kg, there was generally dose-related hyperplasia of brown and white adipose cells in adipose tissue throughout the body. The hyperplasia was most apparent in samples collected from the macroscopically apparent masses in the skin (Table 4). Additionally, there was increased vacuolation, fibroplasia/fibrosis, and subacute inflammation of subcutaneous brown adipose tissue masses from the interscapular regions at 30 and/or 300 mg/kg (Table 4). In monkeys, only microscopic adipose tissue changes were observed following 1 year of treatment (Table 5). These included dose-related increases in white adipocytes and decreases in brown adipocytes in representative fat depots (subcutaneous and perirenal) at all doses; increased white adipocytes in the bone marrow, pancreas, adrenal cortex, and thyroid gland (females) at 2 and 5 mg/kg; and increases in white adipocytes in the parathyroid glands in females at 5 mg/kg.

Heart effects. Dose- and time-dependent heart weight increases and correlative morphologic changes occurred in both rats and monkeys following administration of high doses of muraglitazar. In rats, absolute heart weights were increased 16 and 51% in males and 17 and 35% in females at 30 and 300 mg/kg, respectively, after 6 months of treatment. Microscopically, increased heart weights were accompanied by minimal to mild myocardial (cardiac muscle cell) hypertrophy at the overtly toxic dose of 300 mg/kg (data not shown). In monkeys, heart weights were increased 35% in females at 5 mg/kg after 1 year and correlated with echocardiographic evidence of increased thickness of the left ventricular wall during diastole and systole and an increase in the calculated shortening fraction due to a decrease in the systolic left ventricular chamber diameter (Table 6). Notably, there were no correlative microscopic, electrocardiographic, or ultrastructural changes (data not shown) associated with the heart weight increases or echocardiographic findings after 1 year of treatment. No heart weight increases were observed in monkeys following up to 1 year of dosing at 2 mg/kg.

Liver effects. Following 6 months of treatment, liver weights in rats were minimally to moderately increased in males at 300 mg/kg (27% absolute weight) and in females at 30 and 300 mg/kg (15–64% absolute weight; Supplementary Table 1). These liver weight increases were not accompanied by increased serum transaminase activities (ALT, AST) or correlative histopathologic or ultrastructural findings. Muraglitazar-related hepatic findings in male and female monkeys were limited to minimally increased (23% absolute weight) liver weights with no light microscopic or ultrastructural correlate in males or females (Supplementary Table 2) at 5 mg/kg following 1 year of treatment. The liver weight increases in rats and monkeys may be attributed in part to PPARα activity given muraglitazar stimulates full-length PPARα-mediated reporter gene activity in transiently transfected CV-1 cells; and similar to PPARα agonists, muraglitazar induces acyl CoA oxidase gene expression in rodent livers. The EC_{50} value at rat PPARα with muraglitazar is 31.6 μmol/l (43% activation relative to standard). By comparison, the EC_{50} for the PPARα agonist

TABLE 5
Muraglitazar-Related Adipose Tissue Findings in Monkeys at 1 year[a]

Finding	Control M/F	0.4 mg/kg M/F	2 mg/kg M/F	5 mg/kg M/F
White adipose tissue (increased)				
Adrenal gland, cortex, zona reticularis				
Minimal to slight	—	—	1/0	2/2
Femoral marrow				
Moderate	0/1	1/0	3/3	3/1
Marked	—	—	—	0/3
Pancreas, interstitium				
Minimal to slight	0/1	—	1/3	1/2
Parathyroid				
Minimal to slight	2/2	—	0/3	2/4
Perirenal				
Marked	—	1/1	4/1	3/2
Subcutaneous				
Moderate	1/0	2/1	2/2	1/2
Marked	—	1/1	2/2	2/2
Thyroid gland, interstitium				
Slight to moderate	—	2/0	3/3	3/4
Brown adipose tissue (decreased)				
Perirenal				
Minimal to slight	0/1	1/2	3/2	—
Moderate	4/3	0/1	—	—
Subcutaneous				
Minimal to slight	2/2	3/2	—	—
Moderate	2/0	—	—	—

Note. ''—'' Indicates absence of finding/severity in the group.
[a]The relative amount of adipose tissue (white and/or brown) in the perirenal or subcutaneous fat depots and other tissues in control and muraglitazar-treated groups were subjectively graded.

TABLE 6
Selected Muraglitazar-Related Echocardiographic Changes in Female Monkeys

	Control	0.4 mg/kg	2 mg/kg	5 mg/kg
Heart weight (g)				
Absolute	11.586 ± 2.081	13.099 ± 3.548	12.781 ± 1.692	15.632 ± 2.408
Relative[a]	0.352 ± 0.036	0.335 ± 0.038	0.376 ± 0.035	0.455* ± 0.047
LVIDd				
Pretest	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.7 ± 0.2
Month 6	1.7 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Month 12	1.6 ± 0.2	1.8 ± 0.3	1.6 ± 0.0	1.6 ± 0.2
LVIDs				
Pretest	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	1.0 ± 0.1
Month 6	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.2
Month 12	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	0.7 ± 0.1**
FS%				
Pretest	43 ± 5	44 ± 6	41 ± 1	42 ± 6
Month 6	34 ± 3	41 ± 6	41 ± 2	48 ± 9**
Month 12	36 ± 6	45 ± 8	42 ± 8	58 ± 3**
LVPWd				
Pretest	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
Month 6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
Month 12	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1**
LVPWs				
Pretest	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
Month 6	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.2
Month 12	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1**
IVSd				
Pretest	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Month 6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Month 12	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.1*
IVSs				
Pretest	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
Month 6	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Month 12	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.7 ± 0.2*

Note. LVIDs, left ventricular internal diameter during systole; LVIDd, left ventricular internal diameter during diastole; FS, fractional shortening; LVPWd, left ventricular posterior wall thickness diastole; LVPWs, left ventricular posterior wall thickness systole; IVSd, intraventricular septal thickness during diastole; IVSs, intraventricular septal thickness during systole. Mean ± SD, $n = 3$–4.
[a]Percent heart to body weight ratio.
Statistically significant, *$p < 0.05$; **$p < 0.01$.

fenofibric acid at the rat PPARα is 12.0 μmol/l (118% activation relative to standard); the PPARγ agonist rosiglitazone was inactive.

Nonpharmacologically Mediated Effects

Thyroid gland effects. Mild to marked distention of thyroid follicles with colloid was observed in male monkeys at ≥ 0.4 mg/kg/day after 4 months (interim evaluation for the 9-month study), but this finding was not present after 9 months or 1 year of treatment at any dose. Mild to moderate elevations in serum total thyroxine, without correlative microscopic evidence of increased thyroid follicular activity, were noted in male (21–61%) and female (18–24%) monkeys administered 2 and 5 mg/kg for 6 months (interim evaluation for the 12-month study) and/or 1 year (See Supplementary Table 2). Serum levels of thyroid-stimulating hormone (TSH) were unchanged, and there was no clinical indication of hyperthyroidism in any monkeys.

Dog-Specific Toxicities

In dogs, muraglitazar was poorly tolerated at doses ≥ 2 mg/kg. Pharmacologically mediated changes in hematology, serum chemistry parameters, adipose tissue, and bone marrow were similar to those described previously for rats and monkeys; additionally, the brain, spinal cord, testes, and liver were identified as target organs (see Supplementary Table 3). Severe clinical toxicity and death occurred at 200 mg/kg, a dose resulting in nonrelevant systemic exposures (AUC approximately 90–130 times the mean human AUC at 5 mg/day).

Central Nervous System Effects

At the poorly tolerated dose of 200 mg/kg, there was minimal to moderate vacuolation (principally in cerebellar nuclei and thalamus) and axonal swelling and degeneration in the brain and spinal cord. The moderate vacuolation in the

brain at 200 mg/kg was considered an exacerbation of a background lesion given minimal vacuolation in cerebellar/vestibular nuclei and thalamus occurs as a background (present in control animals from three muraglitazar dog studies) or spontaneous histopathologic change in immersion-fixed brains of laboratory Beagle dogs (Wells and Wells, 1989). A dose of 20 mg/kg, which resulted in systemic exposure (AUC) that was 3.5 times the mean human efficacious exposure at 5 mg/day, was considered a NOEL for vacuolation and axonal swelling/degeneration of the brain and spinal cord in dogs. There were no microscopic alterations in the brain/spinal cord following treatment with muraglitazar in mice or rats for up to 2 years and monkeys for up to 1 year with systemic exposures (AUC) up to 154, 376, and 44 times, respectively, the mean human efficacious exposure at 5 mg/day.

Testicular Effects

Minimal to mild testicular toxicity was seen at doses of 10 and 20 mg/kg in dogs with more pronounced testicular injury at the overtly toxic dose of 200 mg/kg. Specifically, there were syncytial cells in seminiferous tubules at $\geq$ 10 mg/kg, increased sloughed germinal epithelial cells in the epididymides at 10 and 200 mg/kg, sperm stasis and an increased incidence and severity of seminiferous tubular atrophy/degeneration at 20 and 200 mg/kg. The NOEL for testicular toxicity was established as 5 mg/kg, a dose resulting in systemic exposure (AUC) 0.4 times that seen at a clinical dose of 5 mg/day. There was no evidence of similar testicular lesions in any of the other animal species evaluated for significantly greater duration at high exposure multiples.

Genotoxic Assessment

Muraglitazar demonstrated no mutagenic activity in the bacterial reverse mutation assay and no clastogenic activity in primary human lymphocytes. In addition, muraglitazar was not clastogenic in the *in vivo* micronucleus test up to a maximum tolerated dose (1200 mg/kg) in female rats or the limit dose (2000 mg/kg) in male rats (data not shown).

Carcinogenic Assessment

In male mice, administered muraglitazar at 20 and 40 mg/kg with systemic exposures $\geq$ 62 times that seen at a clinical dose of 5 mg/day, a low incidence of benign gallbladder adenoma (incidences of 1/60 and 2/60 mice, respectively) occurred and was considered drug-related (Tannehill-Gregg *et al*., 2007). In rats, muraglitazar-related tumors were observed in the urinary bladder and adipose tissue. An increased incidence of transitional cell papillomas and carcinomas of the urinary bladder were noted in males at 5, 30, and 50 mg/kg (8, 37, and 48 times, respectively, the mean human exposure at 5 mg/day). In addition to the urinary bladder tumorigenic response, there was an increased incidence of subcutaneous liposarcoma (malignant tumor of adipose cells) in male rats (48 times human exposure at 5 mg/day) and lipoma (benign tumor of adipose cells) in female rats (59 times human exposure at 5 mg/day) at 50 mg/kg (Tannehill-Gregg *et al*., 2007).

Reproductive Effects

In the study on fertility and early embryonic development in rats, muraglitazar had no effect on reproductive function in males at 600 mg/kg (maximum dose tested) or in females at 60 mg/kg with AUC exposures > 250 and 29 times, respectively, the mean human AUC at 5 mg/day (See Supplementary Table 4). In female rats, altered estrous cycling as well as reductions in ovulation, fertility, implantation, and litter size were observed only at 600 mg/kg, a dose that caused overt toxicity (excess salivation, soft feces, perioral substance, rales, reduced body weight gain/body weight loss, and reduced food consumption during gestation).

In studies of embryo-fetal development, muraglitazar was not teratogenic at doses up to 180 mg/kg in rats (406 times mean human AUC at 5 mg/day) or 60 mg/kg in rabbits (five times mean human AUC at 5 mg/day). In rats and rabbits, embryo-fetal toxicity (increased resorptions, abortions, decreased fetal body weights, and/or minor reductions in skeletal ossification) was limited to the highest doses tested (rat: 180 mg/kg; rabbit: 60 mg/kg). Common classical signs of maternal toxicity, reduced food consumption, and/or clinical signs were observed in pregnant rabbits at 12 and 60 mg/kg/day, indicating that the embryo-fetal effects observed at 60 mg/kg did not represent selective development toxicity (see Supplementary Table 5). In rats, no overt signs of maternal toxicity were apparent. However, the embryo-fetal toxicity in rats was considered likely the consequence of pharmacologically mediated metabolic effects in the dam (see Supplementary Table 6). The NOELs (18 mg/kg in rats and 12 mg/kg in rabbits) resulted in systemic exposure (AUC) 56 times (rats) and equivalent to (rabbits) that observed in humans at a clinical dose of 5 mg/day.

In the studies of pre- and postnatal development in rats, decreased pup viability and observations suggestive of subcutaneous hemorrhage and poor physical condition of pups at birth and/or during lactation occurred at doses of 5–135 mg/kg (see Supplementary Table 7). These doses resulted in systemic exposures (AUC) 14 to greater than 126 times the mean human AUC exposure at 5 mg/day; the NOEL for these findings was established at 1.5 mg/kg (three times the mean human AUC at 5 mg/day). In contrast to findings in the embryo-fetal development study, dose-dependent maternal toxicity was seen in pregnant/lactating dams generally at $\geq$ 5 mg/kg, possibly due to the longer duration of dosing in this study design (pre- and postnatal development study). Last, no effects on post-weaning behavior or reproductive function were seen in the offspring derived from dams dosed up to 15 mg/kg (38 times the mean human AUC at 5 mg/day).

DISCUSSION

The administration of muraglitazar resulted in pharmacologically mediated changes (Table 7) similar to those observed with the PPARγ agonists rosiglitazone and pioglitazone (Actos Summary basis of approval, 1999; Avandia Summary basis of approval, 1999). The potential toxicity of muraglitazar was investigated at high-exposure multiples (animal:human) and as such provided a comprehensive toxicity evaluation in animals.

The edema observed in rats and monkeys treated chronically with muraglitazar is a common finding in animals administered other PPAR dual and/or gamma agonists (El Hage, 2005). Although the cause of the edema associated with PPARγ treatment is not fully understood, expanded plasma volume is generally considered a major factor in its development (Martens *et al*., 2002; Nesto *et al*., 2003; Pickavance *et al*., 1999). Previous studies demonstrated increases in plasma volume in rats treated with the PPARγ agonist, troglitazone (Horikoshi *et al*., 1994), and in dogs administered rosiglitazone (Avandia Summary basis of approval, 1999). The persistent minimal to mild decreases in the erythrocytic parameters (RBC counts, hematocrit, and hemoglobin) in muraglitazar-treated rats and monkeys, along with reductions in serum total protein and albumin (rats) were consistent with hemodilution as a consequence of increased plasma volume (Nesto *et al*., 2003; Pickavance *et al*., 1999; Rothwell *et al*., 2002). Although there was no safety margin established for edema in monkeys with muraglitazar, edema is a common side effect with treatment of PPARγ agonists and clinically monitorable.

Whereas the reductions in serum lipids in rats and monkeys were attributed to PPARα/γ agonist activities of muraglitazar, the reduction in serum glucose levels in muraglitazar-treated rats was considered a pharmacologic effect of PPARγ agonism (Saltiel and Horikoshi, 1995; Saltiel and Olefsky, 1996). Similar lipid changes have been described in animals treated with the PPARα agonists clofibrate and fenofibrate (Hertz *et al*., 1995; Schoonjans *et al*., 1996; Staels *et al*., 1992), whereas both hypoglycemic and hypolipidemic effects have been observed with the PPARγ agonists, troglitazone, pioglitazone, and ciglitazone (Ikeda *et al*., 1990; Kazumi *et al*., 1996; Lee *et al*., 1994; Sohda *et al*., 1992). The lipid-lowering effects of PPARγ agonists may result from a direct action on the liver through modification of fatty acid synthesis. Alternatively, the hypolipidemic effect may occur indirectly from reduction in plasma insulin or changes in adiposity and adipose tissue composition (Horton *et al*., 1998; Smith, 2002).

Alterations in brown and white adipocytes similar to those observed with muraglitazar have been described in rats, dogs, and monkeys treated with PPARγ agonists (Actos Summary basis of approval, 1999; Avandia Summary basis of approval, 1999; Rothwell *et al*., 2002; Williams *et al*., 1993). The adipose changes have been attributed to PPARγ-mediated recruitment and differentiation of preadipocytes (adipocyte precursor cells) into adipocytes in response to dietary lipids by activation of genes involved in lipid synthesis and storage pathways (Smith, 2002; Speigelman, 1998; Tai *et al*., 1996; Watkins *et al*., 2002).

Heart weight increases similar to those seen with muraglitazar have been observed in rodents and nonrodents treated with other dual and gamma PPAR agonists (de la Iglesia *et al*., 1998; El Hage, 2005; Herman *et al*., 1997; Horikoshi *et al*., 1994; McGuire *et al*., 1997; Rothwell *et al*., 1997). The increases in heart weights associated with these drugs have been attributed to cardiac hypertrophy secondary to plasma

TABLE 7
Selected Drug-Related Findings and Multiples of Human Exposure in Rat and Monkey Chronic Toxicity Studies with Muraglitazar

		Exposure multiple[a]	
Species	Selected findings	LOEL	NOEL
Rat	Mortality	312	59
	Edema	312	59
	Decreased erythroid parameters	53	8
	Adipose tissue		
	Hyperplasia	5	0.7
	Fibroplasia/fibrosis subacute inflammation	312	53
	Heart		
	Increased weights	53	8
	Myocardial cell hypertrophy	312	53
	Various tissues/organs		
	Increased adipocytes[b]	1	0.7
	Urinary bladder		
	Urothelial hyperplasia[b]	8	2
Monkey	Edema	2	ND
	Decreased erythroid parameters	9	4
	Adipose tissue		
	Decreased brown adipocytes	2	ND
	Increased white adipocytes	2	ND
	Heart		
	Increased weights	44	9
	Left ventricular wall thickening	44	9
	Bone marrow		
	Hypocellularity	9	4
	Increased adipocytes	9	4
	Various tissues/organs		
	Increased adipocytes	2	ND

Note. ND, not determined; findings present at lowest dose tested. NOEL, no-observed-effect level; LOEL, lowest observed-effect level.

[a]AUC(TAU) at steady state at 5 mg/day doses in a multiple ascending dose study in type 2 diabetic human subjects (CV168002): 4.884 μg·h/ml. AUC exposure in animals at steady state (animal exposure divided by human exposure).

[b]Additional nonneoplastic target organ changes in oral carcinogenicity study.

volume expansion and associated hemodilution (Arakawa *et al*., 2004; El Hage, 2005; Pickavance *et al*., 1999; Rothwell *et al*., 2002). In addition, the left ventricular wall thickening observed in monkeys treated with muraglitazar was also seen in dogs administered rosiglitazone and has been attributed to increased cardiac workload secondary to plasma volume expansion (PDR Electronic Library, 2004a).

The liver weight increases in nonclinical toxicology studies in mice, rats, and monkeys treated with troglitazone (de la Iglesia *et al*., 1998; Herman *et al*., 1997; Horikoshi *et al*., 1994; McGuire *et al*., 1997; Rothwell *et al*., 1997); in rodents and/or dogs given pioglitazone (Actos Summary basis of approval, 1999) or rosiglitazone (Avandia Summary basis of approval, 1999); in rodents and monkeys treated with PPARα agonists (Gray and de la Iglesia, 1984; Klaunig *et al*., 2003); and in rats and monkeys administered muraglitazar have been attributed to induction of hepatic drug-metabolizing enzyme activity. Following treatment with the PPARα agonists or the structurally distinct PPARγ agonist, troglitazone, morphologic, and biochemical evidence of minimal hepatic peroxisomal enzyme induction in rodents (Herman *et al*., 1997; Klaunig *et al*., 2003; McGuire *et al*., 1997) and bile duct hyperplasia in monkeys have also been described (Rothwell *et al*., 2002). That muraglitazar failed to induce hepatocellular peroxisome proliferation was potentially due to its less robust PPARα activation relative to other PPARα agonists.

The relevance of the thyroid findings in monkeys administered muraglitazar was unclear since the findings have not been described for other PPARα or PPARγ agonists and were not accompanied by changes in serum levels of TSH, microscopic evidence of increased thyroid follicular activity, or any indication of hyperthyroidism in monkeys. The absence of clinically meaningful changes in TSH levels in phase III clinical trials with muraglitazar supports that the findings in the monkey had no established clinical relevance.

While mice, rats, and monkeys tolerated muraglitazar at high systemic exposures, dogs appeared to be uniquely sensitive to muraglitazar as they were the only species in which testicular seminiferous tubular and central nervous system toxicities were observed. In comparison, there was no microscopic evidence of brain or spinal cord alterations in dogs administered pioglitazone (Actos Summary basis of approval, 1999) and rosiglitazone (Avandia Summary basis of approval, 1999), and bilateral testicular degeneration was observed in only one dog treated for 3 months with pioglitazone (Actos Summary basis of approval, 1999).

At exposures $\geq$ 48 times the mean human exposure, muraglitazar increased the incidence of benign gallbladder adenomas in male mice and adipocyte tumors in male and female rats (Tannehill-Gregg et al., 2007). In comparison, increased incidences of benign gallbladder adenomas have not been described in mouse carcinogenicity studies with PPARα agonists (Klaunig *et al*., 2003) and have been seen with only one of the seven PPARγ agonists reviewed by the FDA (El Hage, 2005), suggesting that the gallbladder adenomas in muraglitazar-treated mice were not likely mediated by a direct pharmacologic effect on the gallbladder epithelium. Importantly, there was no evidence of increased cholelithiasis or hepatobiliary disease in phase III clinical trials with muraglitazar (Buse *et al*., 2005). The subcutaneous adipocyte tumors in rats treated with muraglitazar likely were a consequence of chronic pharmacologic stimulation of preadipocytes at high systemic exposures. In comparison, increased incidences of adipocyte tumors (lipomas) were reported in rats given rosiglitazone (Avandia Summary basis of approval, 1999) at comparatively low-exposure multiples.

In male rats given muraglitazar at exposures $\geq$ 8 times the human exposure, the incidences of transitional cell papilloma and carcinoma of the urinary bladder were increased (Tannehill-Gregg et al., 2007). Similarly, an increased incidence of urinary bladder tumors was reported for pioglitazone-treated male rats (Actos Summary basis of approval, 1999). In a subsequent 21-month investigative study of muraglitazar in male rats, chronic mucosal injury proliferation secondary to pharmacologically mediated increases in urinary solids (calcium- and magnesium-containing microcrystalline precipitates, crystals, aggregates, and/or microcalculi) was shown to be the nongenotoxic mechanism for the male rat–specific urinary bladder tumorigenic response (Dominick *et al*., 2006).

The reproductive and developmental changes that occurred with muraglitazar were similar to those observed with PPARγ agonists (PDR Electronic Library, 2004a,b). Like muraglitazar, rosiglitazone altered estrous cyclicity and reduced fertility in female rats (PDR Electronic Library, 2004a). Additionally, embryo-fetal changes similar to those noted in pregnant rats and rabbits administered muraglitazar were observed in pregnant rats treated with pioglitazone and rosiglitazone (PDR Electronic Library, 2004a,b), suggesting an etiology involving pharmacologically mediated effects on maternal lipid metabolism and/or glucose homeostasis during gestation (Catalano et al., 2002; Ghatnekar et al., 2004; Soria et al., 2002; Sugden et al., 2003).

In conclusion, muraglitazar demonstrated good oral tolerability and a spectrum of clinical, clinical pathology, and anatomic changes in rats and monkeys that were pharmacologically mediated and, with the exception of edema, generally occurred at high doses and exposures. Muraglitazar was neither genotoxic nor teratogenic. In carcinogenicity studies, it induced urinary bladder and adipocyte tumors in rats, and a low incidence of gallbladder adenoma in mice. Overall, the toxicity profile of muraglitazar is comparable to that described for PPARγ and other dual agonists.

SUPPLEMENTARY DATA

Supplementary Tables 1–7 are available online at http://toxsci.oxfordjournals.org/.

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