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COMMENTS

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* *Please use the Section 4 Coding Sheet for Section 4 Documents*

Jean B. Sweeney
Vice President

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8EHQ-0210-00373DH
MR#324971

3M

February 16, 2010

CERTIFIED MAIL

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Document Processing Center
EPA East – Room 6428 Attn: Section 8(e)
Office of Pollution Prevention and Toxics, U.S. EPA
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001

Re: TSCA 8(e) Substantial Risk Notice: Sulfonate-based and Carboxylic-based Fluorochemicals, Docket 8EHQ-0598-373 - Results from a mechanistic investigation of the effect of PFBS, PFHS, and PFOS on lipid and lipoprotein metabolism in transgenic mice

Dear Sir or Madam:

3M is submitting this notice to supplement previous submissions on sulfonyl and carboxylic-based fluorochemicals (FCs), and more specifically our July 28, 2006 and January 8, 2007 submittals concerning data generated by TNO Laboratories in Leiden, Netherlands. These data suggest an effect of perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHS) on body weight, food consumption, and serum cholesterol in 15% dietary fat fed E3 Leiden transgenic mice.

Enclosed is a final report for follow up investigation to the aforementioned study. The current study was conducted at TNO Laboratories in Leiden, Netherlands using APOE*3-Lieden/huCETP transgenic mice. Results help to explain the mechanism of action by which perfluorobutanesulfonate (PFBS), PFHS, and PFOS alter lipid and lipoprotein metabolism in animals treated with these chemicals. An effect noted in this study that has not been clearly demonstrated in other studies on these chemicals was a decrease in high density lipoprotein (HDL) in animals treated with PFOS and PFHS.

If you have any questions, please contact Deanna Luebker at (651) 737-1374 or djluebker@mmm.com.

Sincerely,

Jean B. Sweeney (DL)

Jean B. Sweeney
Vice President
Environmental, Health and Safety Operations

Enclosure

CONTAINS NO CBI

324971

TNO-Report for the study entitled:

Mechanism of the effect of different PFAS's (PFBS vs PFHS vs PFOS) on lipid and lipoprotein metabolism in APOE*3-Leiden/huCETP transgenic mice

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In assignment of: 3M, Medical department

Project number: 031.12685

Study number: 3M#03

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CONFIDENTIAL

Signature and date:

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		Principal Investigator	Team Manager	Study Director

Summary

Introduction and Aim
Perfluorinated alkyl sulphonates are fully fluorinated amphiphilic organic molecules with strong surface-tension reducing properties. They are stable to environmental and metabolic degradation. Perfluorooctanesulfonate (PFOS) is widely dispersed in humans, fish-eating wildlife, and surface waters. Toxicological studies in rats and monkeys have shown a reduction in serum cholesterol after treatment with PFOS; however; such reductions have not been observed among exposed workers. In the present study the focus is put on a further elucidation of the mechanism responsible for the observed changes in plasma triglycerides and cholesterol levels in the atherogenic apoB-containing (VLDL, IDL, LDL) lipoproteins and to investigate the effect of PFBS, PFHS and PFOS on HDL metabolism. The APOE*3-Leiden(E3L)/huCETP transgenic mouse model was used to study these effects. The aim of the study is further to emphasize the differences in biological effects between PFBS, PFHS and PFOS and to publish these data.

Material and methods
The study, which was subdivided in three experiments, had the following design: after a run-in period of 4 weeks on a Western type diet (containing 14 % beef tallow, 1 % corn oil, 0.25 % cholesterol), mice received a Western type diet (control) or a western type diet containing 0.03 % PFBS (31.8 mg/kg body weight/day), 0.006% PFHS (6.0 mg/kg body weight/day), 0.003 % PFOS (3.1 mg/kg body weight/day) or as a positive control 0.03% fenofibrate (31.1 mg/kg body weight /day). In all experiments body weight, food intake, plasma cholesterol, HDL cholesterol and triglycerides were measured after 4 weeks of treatment.

In experiment 1 lipolytic activity (LPL and HL activity) was measured from post-heparin plasma after 5 weeks of treatment and feces were collected for the determination of bile acids, neutral sterols and fatty acids. At the end of the experiment (6 weeks of treatment) VLDL-triglyceride and de novo apoB production was measured and lipid composition of VLDL was determined. In experiment 2 after 4 weeks of treatment at the end of the experiment the gall bladder was cannulated and during 45 minutes bile flow, biliary cholesterol, phospholipids and bile salt output were measured. Directly hereafter the in vivo clearance of VLDL-like triglycerides-rich particles was determined. In experiment 3, next to plasma ApoA1 and CETP activity and mass determinations, the in vivo clearance of autologous labeled HDL was measured after 4 weeks of treatment. From this experiment livers were collected and a part was used for liver histology analysis, a part was used for liver lipid analysis and a part was used for microarray analysis.

Mechanism of action of PFBS
PFBS reduced plasma cholesterol and triglyceride levels by about 25% and 45%, respectively. The data from the physiological experiments indicate that the decreases in lipid levels are caused by increased clearance of VLDL-TG and VLDL-CE and mildly reduced VLDL-particle production. PFBS had no clear effect on HDL-cholesterol and apoA1 levels. PFBS showed no effect on genes involved in HDL metabolism, in line with the unchanged HDL levels. PFBS mildly increased liver weight, but had no effect on ALT and development of hepatosteatosis.

Based on mRNA signals PFBS appears to have mild PPARα-agonistic activity (β-oxidation increased and liver size increased). The present data indicate that PFBS has no increased CVD risk profile.

Mechanism of action of PFHS
PFHS reduced plasma cholesterol and triglyceride levels by about 60% and 75%, respectively. The data from the physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by increased lipolysis and clearance of VLDL-TG and VLDL-CE, and strongly reduced VLDL-TG and VLDL-particle production. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (4.3-fold) was increased.

PFHS strongly decreased HDL-cholesterol (about -75%) and apoA1 (about -75%) levels. Based on mRNA signals we conclude that PFHS reduces HDL levels by down-regulation of apoA1 synthesis and HDL maturation (ABCa1, LCAT). These adverse changes are most likely the result of PXR-agonistic activity. Increased remodeling (PLTP) and decreased uptake (SR-B1) are suggested to be responsible for the formation of larger HDL particles. PFHS increased liver weight, ALT, and resulted in hepatosteatosis, as observed by biochemical and histological measures.

Based on mRNA signals PFHS has strong PPARα-agonistic (lipolysis increased, β-oxidation increased, FA uptake increased, liver size increased) and PXR-agonistic activity (FA uptake increased, FA synthesis increased and HDL synthesis and maturation decreased, liver size increased).

Mechanism of action of PFOS: PFOS reduced plasma cholesterol and triglyceride levels by about 65% and 70%, respectively. A similar mechanism of action is active with PFOS as with PFHS. The data from physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by increased lipolysis and clearance of VLDL-TG, and strongly reduced VLDL-TG and VLDL-particle production. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (2.1-fold) was increased. PFOS increased mRNA levels of cholesterol synthesizing enzymes and cholesterol esterification genes. Moreover, the gene coding for the major rate-limiting enzyme in the bile acid synthetic pathway, and genes involved in biliairy cholesterol excretion were decreased by PFOS. Inhibition of cholesterol metabolism and excretion may form an explanation for increased hepatic cholesterol levels found with PFOS.

PFOS strongly decreased HDL-cholesterol (about -65%) and apoA1 (about -80%) levels. Based on mRNA signals we conclude that PFOS reduces HDL levels by down-regulation of apoA1 synthesis and HDL maturation. These adverse changes are most likely the result of PXR-agonistic activity. PFOS increased liver weight, ALT, and resulted in pronounced hepatosteatosis and liver cholesterol accumulation, as observed by biochemical and histological measures.

Based on mRNA signals PFOS has strong PPARα-agonistic (lipolysis increased, β-oxidation increased, FA uptake increased, liver size increased) and PXR-agonistic activity (FA uptake increased, FA synthesis increased and HDL synthesis and maturation decreased, liver size increased).

Involvement of other nuclear transcription factors in the regulation of lipid and lipoprotein metabolism by PFHS and PFOS
Involvement of CAR and LXR in the changes in lipid and lipoprotein metabolism caused by PFHS and PFOS cannot be fully excluded, but is less likely. Little is know about the role of CAR in lipid metabolism. CAR has been shown to decrease β-oxidation genes as CPT1 and enoyl CoA hydratase. The latter genes were, however, increased in the present experiments. LXR increases fatty acid synthesis by induction of SREBP1c expression, which was 2-fold decreased, however. In addition, LXR induces expression of CETP mRNA, whereas in the present experiments a decrease in CETP activity was found. It cannot be excluded that this is caused by a strongly decreased acceptor pool for CE transfer. Involvement of RXR in the observed effects cannot be excluded, since RXR forms a heterodimer together with a larger number of nuclear transcription factors like PPARα, PXR and LXR. However, direct activation of RXR, for instance with bexarotene leads to opposite effects with increased levels of triglycerides and apoB-containing lipoproteins.

Mechanism of action of fenofibrate
Fenofibrate reduced plasma cholesterol and triglyceride levels by about 40% and 70%, respectively. The data from the physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by strongly increased lipolysis and clearance of VLDL-TG and VLDL-CE, despite the increased VLDL-TG production rate. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (4.6-fold) was increased. LDLR mRNA as marker for increased uptake of VLDL remnant particles was enhanced by 1.5-fold. Fenofibrate paradoxically increases VLDL-TG production despite reducing plasma TG, which may be caused by enhanced hepatic free fatty acid uptake resulting from strongly accelerated peripheral LPL-mediated lipolysis of VLDL or by increased *de novo* hepatic TG synthesis.

Fenofibrate increased HDL-cholesterol (+50%) and formation of large HDL-1 particles, and had no effect on apoA1. Fenofibrate strongly decrease in CETP activity, which was found majorly responsible for the increased HDL levels upon treatment with fenofibrate and PPARα,γ-agonists. Fenofibrate increased liver weight, without effects on ALT and hepatosteatosis, and decreased liver cholesterol content.

Based on mRNA signals fenofibrate has strong PPARα-agonistic activity (lipolysis increased, FA uptake increased, β-oxidation increased; HDL remodeling decreased).

Contents

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1 Introduction & aim

Perfluorinated alkyl sulphonates are fully fluorinated amphiphilic organic molecules with strong surface-tension reducing properties. They are stable to environmental and metabolic degradation. Perfluorooctanesulfonate (PFOS) is widely dispersed in humans, fish-eating wildlife, and surface waters. Toxicological studies in rats and monkeys have shown a reduction in serum cholesterol after treatment with PFOS; however, such reductions have not been observed among exposed workers. In order to obtain more insight into the possible mechanism of action the APOE*3-Leiden transgenic mouse, a well-recognized animal model for hyperlipidemia and atherosclerosis, has been used in a previous study to investigate the in vivo effects of three perfluoro-alkyl-sulphonates with different chain length, PFBS (C4), PFHS (C6) and PFOS (C8), on plasma lipids and lipoproteins and bile acid metabolism. Of the three PFAS, PFHS and PFOS have been withdrawn from the market by 3M in the beginning of 2000 because of environmental issues, whereas PFBS is marketed in various industrial applications.

APOE*3Leiden transgenic mice exhibit elevated plasma cholesterol and triglyceride levels, mainly confined to the VLDL/LDL sized lipoprotein fraction (1). Extensive previous research showed that, in contrast to wild-type mice, APOE*3Leiden transgenic mice are highly responsive to fat and cholesterol feeding as far as the effects on plasma VLDL and chylomicron levels are concerned (2, 3). In addition, we have found that drugs and dietary compounds influencing either the chylomicron and VLDL production and/or the hepatic clearance of lipoproteins exert relatively strong effects on plasma cholesterol and triglyceride levels (4-11, see for review ref. 12). In contrast, in normal wild-type mice the plasma cholesterol and triglyceride levels are very low and (almost) not responsive to diet and hypolipidemic drugs.
This animal model has been proven to be representative for the human situation regarding plasma lipoprotein levels, lipoprotein profiles, its responsiveness to hypolipidemic drugs (like statins, fibrates etc.) (4-8, 10) and nutrition (9, 11). In addition, depending on the level of plasma cholesterol APOE*3Leiden mice develop atherosclerotic lesions in the aorta resembling those found in humans with respect to cellular composition and morphological and immunohistochemical characteristics (3).
TNO in collaboration with the Leiden University Medical Center has recently developed the APOE*3-Leiden(E3L)/huCETP transgenic mouse, which has proven to be very suitable for testing the effects of drugs and nutritional factors on plasma HDL and triglyceride levels, atherosclerosis and metabolic syndrome. In the newly generated mouse, human cholesterol ester transfer protein (huCETP) under control of its natural flanking regions is introduced into the APOE*3-Leiden mouse resulting in a more human-like lipoprotein profile with transfer of cholesterol ester from HDL to the apoB-containing lipoproteins in exchange for triglycerides. As a result of this adverse lipoprotein distribution and the higher amount of atherogenic apoB-containing lipoproteins, the E3L.CETP transgenic mice develop increased atherosclerosis on a Western-type diet as compared to E3L transgenic mice (13).
The E3L.CETP transgenic mice respond to treatment with (registered) drugs as fibrates (14), statins (15), niacin (16) the CETP inhibitor torcetrapib (7) at similar dosages and in a similar way to humans, with decreases in the apoB-containing lipoproteins and an increase in HDL levels.

From the previous study in APOE*3-Leiden transgenic mice, it was concluded that PFHS and PFOS have strong cholesterol and triglycerides lowering effects. Lipoprotein profiles of PFHS and PFOS treated APOE*3-Leiden mice also showed the formation of a "large HDL" particle, presumably an apoE-, cholesteryl ester-rich HDL-1 particle. Moreover, PFHS and PFOS treated animals showed increased plasma ALAT levels and liver size, decreased bile acid synthesis and cholesterol-7α-hydroxylase activity, increased fatty acid oxidation, and decreased body weight, epididymal fat and increased energy expenditure via burning of fat, all suggesting PPARα agonist activity for these two chemicals. PFBS differed markedly from PFHS and PFOS by having no or only minor effects on the measured parameters, whereas PFHS appeared to have intermediate effects taking into account the higher dose applied as compared to PFOS.

In the present study the focus is put on a further elucidation of the mechanism responsible for the observed changes in plasma triglycerides and cholesterol levels in the atherogenic apoB-containing (VLDL, IDL, LDL) lipoproteins and to investigate the effect of PFBS, PFHS and PFOS on HDL metabolism. Therefore, in this study the more appropriate APOE*3-Leiden(E3L)/huCETP transgenic

mouse model was used. The aim of the study is further to emphasize the differences in biological effects between PFBS, PFHS and PFOS and to publish these data.

1.1 Aim of the study

To elucidate the mechanism of action responsible for the observed changes in plasma triglycerides and cholesterol levels in the atherogenic apoB-containing (VLDL, IDL, LDL) lipoproteins by PFHS and PFOS and to investigate the effect of PFBS, PFHS and PFOS on HDL metabolism in E3L.CETP transgenic mice. To emphasize the differences in biological effects between PFBS, PFHS and PFOS and to publish these data.

2 Materials and methods

2.1 Test substances & reference substance

Test substances:	PFBS (perfluorobutanesulfonate): L-7038, lot 2 (1999)
	PFHS (perfluorohexanesulfonate): 127498-80, lot L9051
	PFOS (perfluorooctanesulfonate): FC-95, lot 217
Reference substance:	Fenofibrate: F6020, lot 117K1486

PFBS, PFHS and PFOS were provided by 3M Medical Department (sent to TNO on 10-Jun-04). Fenofibrate was purchased from Sigma (St. Louis, USA).

2.2 Mice

Based on the large difference in half-life between males (17h) and females (3h) in CD-1 mice, we have used males in the proposed experiments. One-hundred-twenty male heterozygous APOE*3Leiden.CETP mice, 7-10 weeks of age, from the SPF breeding stock at TNO-Biosciences (Leiden) have been used, and housed during the experiment in macrolon cages (maximal 4 mice per cage), in clean-conventional animal rooms at TNO-Leiden (relative humidity 50-60%, temperature ~21 °C, light cycle 7 am to 7 pm). Individual animals are marked by ear punch-holes. Mice were supplied with food and acidified tap water *ad libitum*.

2.3 Animal welfare

Experiments were performed conform to the rules and regulations set forward by the Netherlands Law on Animal Experiments. Experiments had been approved by the Animal Experiment Committee of TNO under registration number 2483. The study director was entitled to terminate experiments in case of serious unexpected animal discomfort.

2.4 Diets

Mice received Western type diet (WTD; semi synthetic diet containing 14% beef tallow, 1% corn oil and 0.25% of cholesterol), purchased from ABdiets (Woerden, The Netherlands). This resulted in total cholesterol (TC) plasma levels of about 8 mmol/L and triglyceride levels of about 2 mmol/L. Diets were renewed once per week.

2.5 Compound administration

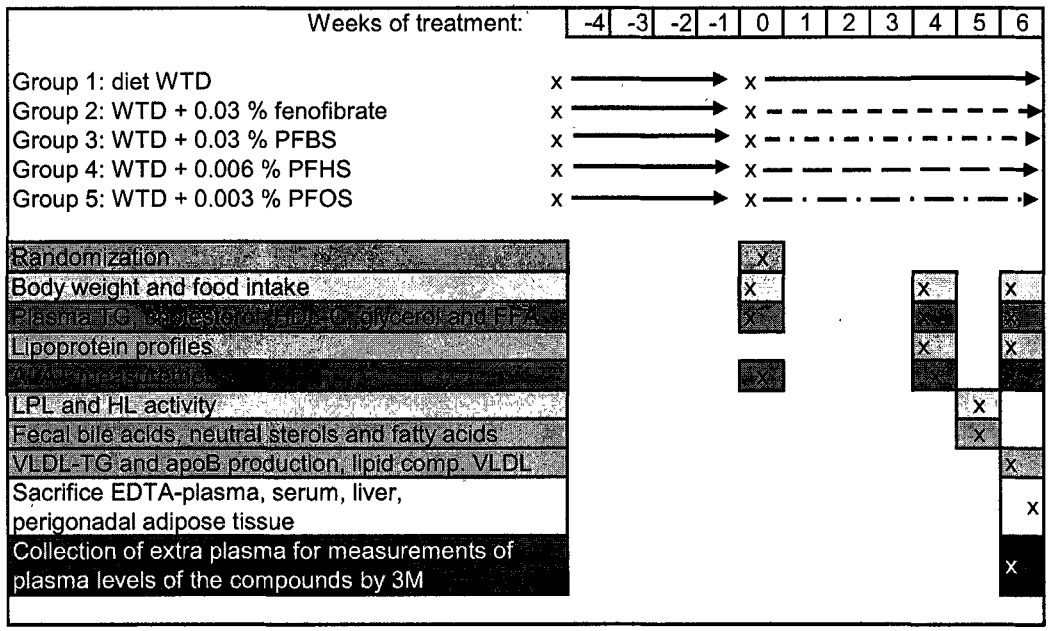
The required quantities of PFBS, PFHS and PFOS were supplied by 3M (sent to TNO on 10-Jun-04). Fenofibrate (Sigma, St. Louis) was used as a positive control. All compounds were administered orally as admix to the WTD. Preparation of all diets (with different compounds) were performed according to: Operation procedure number 14 of "Laboratorium procedures Lipiden" entitled "Preparation of diet chunks from powdered food". The lyophilized diet chunks were stored in vacuum bags in an alarm-secured -20°C room.

2.6 Treatment groups

Group 1: WTD diet
Group 2: WTD + 0.03 % fenofibrate (w/w) or 31.8* mg/kg body weight/day (positive control)
Group 3: WTD + 0.03 % PFBS (w/w) or 31.1* mg/kg body weight/day
Group 4: WTD + 0.006 % PFHS (w/w) or 6.0* mg/kg body weight/day
Group 5: WTD + 0.003 % PFOS (w/w) or 3.1* mg/kg body weight/day
* Average intake of compounds based on average body weight and food intake in the three indicated experiments

2.7 Study design

Design experiment 1



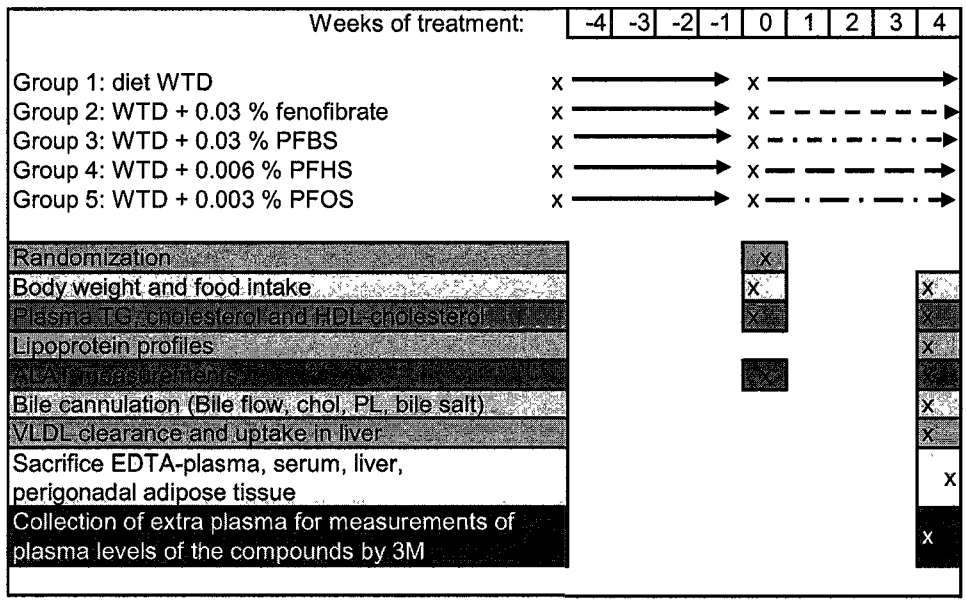
Experiment 1 was performed according to the scheme as outlined above.

In short:
A run-in period of 4 weeks was started with 54 male E3L.CETP mice on a semi synthetic western type diet (containing 14 % beef tallow, 1 % corn oil, 0.25 % cholesterol). In week 0 the animals were randomized on body weight, plasma cholesterol, HDL-cholesterol and triglycerides (after 4h fasting) in 5 groups of 8 animals and the 6-weeks treatment was started. Body weight, food intake, plasma cholesterol, HDL-cholesterol, triglycerides, glycerol and free fatty acids (after 4h fasting) were measured at t=0 weeks and 4 and 6 weeks after start of treatment. Plasma ALAT as a parameter for liver damage and lipoprotein profiles were measured in pooled samples per group at t=0, 4, 6 weeks (ALAT) and t=4, 6 weeks (lipoprotein profiles). Extra plasma (without radioactivity) was collected at 6 weeks for measurements of plasma levels of the compounds by 3M.

After 5 weeks of treatment lipolytic activity (LPL and HL activity) was measured from postheparin plasma from fasted mice (4h fasting) and feces was collected for the determination of bile acids, neutral sterols and fatty acids. At the end of the experiment VLDL-triglyceride and de novo apoB production was measured and lipid composition of VLDL was determined. After sacrifice EDTA-plasma, serum and perigonadal adipose tissue and liver was collected. Both tissues were directly frozen in liquid nitrogen and stored below - 70 °C.

The run-in period of study 1 started on 25 March 2008, mice were sacrificed on 6 June 2008.

Design experiment 2



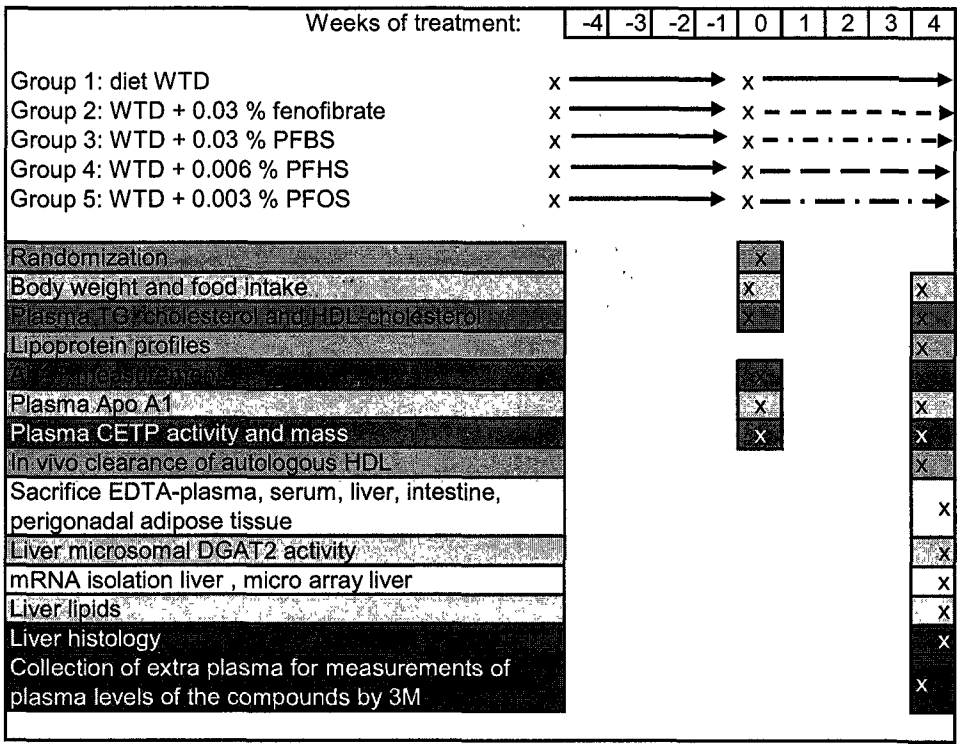
Experiment 2 was performed according to the scheme as outlined above.

In short:
A run-in period of 4 weeks was started with 38 male E3L.CETP mice on a semi synthetic western type diet (containing 14 % beef tallow, 1 % corn oil, 0.25 % cholesterol). In week 0 the animals were randomized on body weight, plasma cholesterol, HDL-cholesterol and triglycerides (after 4h fasting) in 5 groups of 6 animals and the 4-weeks treatment was started. Body weight, food intake, plasma cholesterol, HDL-cholesterol and triglycerides (after 4h fasting) were measured at t=0 and 4 weeks. Plasma ALAT as a parameter for liver damage and lipoprotein profiles was measured in pooled samples per group at t=0 and 4 weeks (ALAT) and t=4 weeks (lipoprotein profiles). Extra plasma (without radioactivity) was collected at 4 weeks for measurements of plasma levels of the compounds by 3M.

After 4 weeks of treatment at the end of the experiment the gall bladder was cannulated and during 45 minutes bile flow, biliary cholesterol, phospholipids and bile salt output were measured. Directly hereafter the in vivo clearance of VLDL-like triglycerides-rich particles was determined. After sacrifice EDTA-plasma, serum and perigonadal adipose tissue and liver was collected. The perigonadal adipose tissue and liver were directly frozen in liquid nitrogen and stored below -70 °C.

The run-in period of study 2 started on 16 June 2008, mice were sacrificed on 15 August 2008. The extra mice from study 3, used for bile cannulation were sacrificed on 2 October 2008.

Design experiment 3



Experiment 3 was performed according to the scheme as outlined above.

In short:
A run-in period of 4 weeks was started with 38 male E3L.CETP mice on a semi synthetic western type diet (containing 14 % beef tallow, 1 % corn oil, 0.25 % cholesterol). In week 0 the animals were randomized on body weight, plasma cholesterol, HDL-cholesterol and triglycerides (after 4h fasting) in 5 groups of 7 animals and the 4-weeks treatment was started. Body weight, food intake, plasma cholesterol, HDL cholesterol and triglycerides and plasma ApoA1, CETP activity and mass (after 4h fasting) was measured at t=0 and 4 weeks. ALAT as a parameter for liver damage and lipoprotein profiles was measured in pooled samples per group at t=0 and 4 weeks (ALAT) and t=4 weeks (lipoprotein profiles). Extra plasma (without radioactivity) was collected at 4 weeks for measurements of plasma levels of the compounds by 3M.

After 3 weeks of treatment one mouse of each group was sacrificed by CO_2 and serum was collected from the retro-orbital vein. A part of the liver was directly frozen in liquid nitrogen. HDL was isolated and radiolabeled with ^{3}H-cholesteryl oleyl ether. The in vivo clearance of autologous HDL was determined in 5 mice per group for 24 hr after an injection of ^{3}H-cholesteryl oleyl ether labeled autologous HDL. After sacrifice EDTA-plasma, serum and small intestine, perigonadal adipose tissue and liver were collected. The small intestine was directly frozen in liquid nitrogen and stored below -70 °C for mRNA isolation. The liver was cut in four parts; two parts of the liver were directly frozen in liquid nitrogen and stored below - 70 °C for mRNA isolation and liver lipid analysis, one part was used fixed in buffered formalin for liver histology and a part was used for the isolation of microsomes. Perigonadal adipose tissue was directly frozen in liquid nitrogen and stored below - 70 °C. One mouse per group was used for an extra bile cannulation at t=5 weeks.

The run-in period of study 3 started on 28 July 2008, mice were sacrificed on 25 September 2008, except the extra mice from study 3. These mice, used as extra mice for the bile cannulation experiment for study 2, were sacrificed on 2 October 2008.

The last measurement (CETP activity) was performed on 7 September 2009. Liver histology analysis was performed in week 38, 2009.

2.8 Measurements experiment 1

1) Body weight and food intake (per cage) at 0, 4 and 6 weeks.
2) Plasma cholesterol, HDL cholesterol, triglycerides, glycerol and free fatty acids (after 4h fasting) at 0, 4 and 6 weeks.
   Plasma cholesterol and triglycerides were determined using kit "Cholesterol CHOD-PAP" and kit "Triglycerides GPO-PAP" both from Roche (Mannheim, Germany). Plasma glycerol was determined using "the free glycerol determination kit" from Sigma (St. Louis, USA). Plasma free fatty acids were determined using kit "NEFA C" from WAKO (Neuss, Germany). Plasma HDL-cholesterol was determined using kit "Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) after the precipitation of apoB containing lipids by $MnCl_2$ (0.2 mol/L) and heparin (500 IU/mL).
3) Lipoprotein profiles at group level at 4 and 6 weeks. Lipoproteins were separated by FPLC analysis using an AKTA apparatus. Analyses were performed in freshly obtained pooled samples per group. Cholesterol and phospholipid profiles were measured in the fractions using kit "Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) and kit "Phospholipids" from Instruchemie (Delfzijl, the Netherlands), respectively.
4) Plasma ALAT at group level at 0, 4 and 6 weeks. Plasma ALAT was measured using the spectrophotometric assay of the Roche Reflotron plus system (Mannheim, Germany).
5) Lipoprotein lipase and hepatic lipase activity at 5 weeks. Lipolytic activity (lipoprotein lipase and hepatic lipase activity) was determined as described previously (18). In short: Postheparin plasma from fasted mice (4h) was collected from the tail vein at 20 minutes after intraperitoneal injection of heparin (0.5 IU/g body weight). Postheparin plasma triacylglycerol hydrolase activity was determined in the presence or absence of 1 mol/L NaCl to estimate both the lipoprotein lipase (LPL) and hepatic lipase (HL) activity. LPL activity was calculated as the portion of total lipase activity inhibited by 1 mol/L NaCl.
6) VLDL-triglyceride and VLDL-apoB production and lipid composition of nascent VLDL at 6 weeks. The rate of hepatic VLDL-triglyceride production, de novo apoB secretion and lipid composition of nascent VLDL was determined in 4 hr (or overnight) fasted mice as described previously (18, 19). In short: Mice were anesthetized with fluanisone-fentanyl-midazolam intraperitoneally and injected intravenously with 0.1 ml phosphate-buffered saline containing 100 µCi Tran^{35}S-label (ICN Biomedicals, Irvine, USA) to measure de novo apoB synthesis. After 30 min, the animals received a Triton WR1339 injection (500 mg/kg body weight), which virtually completely inhibited VLDL clearance by blocking LPL mediated lipolysis. Blood samples were drawn 0, 15, 30, 60 and 90 min after Triton WR1339 injection and plasma triglycerides concentrations were measured. After 90 minutes mice were killed and blood was collected by heart punction for isolation of VLDL and subsequent determination of de novo apoB synthesis and VLDL composition. For that purpose VLDL particles (density < 1.019) were separated from other lipoproteins by density gradient ultracentrifugation. The protein content of the VLDL fraction was determined by a Lowry protein determination, and triglycerides and total cholesterol concentrations were determined using kit "Cholesterol CHOD-PAP" and kit "Triglycerides GPO-PAP" both from Roche (Mannheim, Germany). Phospholipid and free cholesterol concentrations were determined using kit "Phospholipids" and kit "Free cholesterol C" both from Instruchemie (Delfzijl, The Netherlands). The ^{35}S-apoB content of VLDL was measured after selective precipitation of apoB with isopropanol. The VLDL-TG production rate was calculated by curve fitting (trendlines with the equation: plasma TG=a*time + b, in which a is the calculated production rate). Lipid composition of VLDL was calculated per newly synthesized ApoB and expressed as µmol/dpm ApoB.
7) Fecal excretion of bile acids, neutral sterols and fatty acids at 5 weeks (n= 6 per group). Fecal bile acids, neutral sterols and fatty acids were determined in feces collected during a 48-72 h time period in 3 subgroups at 2 consecutive time points during week 5, by gas chromatographic (GC) analysis as described previously (19, 20).
8) After sacrifice at 6 weeks (end of VLDL-triglyceride and VLDL-apoB production experiment) EDTA-plasma (also needed for isolation of VLDL and subsequent determination of de novo apoB synthesis and VLDL composition), serum, perigonadal adipose tissue and liver. EDTA-plasma and serum were collected from heart punction. The liver and perigonadal adipose tissue was weighed and directly frozen in liquid nitrogen and stored below -70 °C.

9) Plasma taken at 6 weeks after 4h fasting was sent to 3M for measurements of plasma levels of compounds. Samples were sent on dry ice by Fedex courier on December 1st 2008 and arrived at the 3M center on December 2nd 2008.

2.9 Measurements experiment 2

1) Body weight and food intake (per cage) at 0 and 4 weeks.
2) Plasma cholesterol, HDL cholesterol and triglycerides (after 4h fasting) at 0 and 4 weeks. Plasma cholesterol and triglycerides were determined using kit "Cholesterol CHOD-PAP" and kit "Triglycerides GPO-PAP" both from Roche (Mannheim, Germany). Plasma HDL-cholesterol was determined using kit "Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) after the precipitation of apoB containing lipids by $MnCl_2$ (0.2 mol/L) and heparin (500 IU/mL).
3) Lipoprotein profiles at group level at 4 weeks. Lipoproteins were separated by FPLC analysis using an AKTA apparatus. Analyses were performed in freshly obtained pooled samples per group. Cholesterol and phospholipid profiles were measured in the fractions using kit "Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) and kit "Phospholipids" from Instruchemie (Delfzijl, the Netherlands) respectively.
4) Plasma ALAT at group level at 0 and 4 weeks. Plasma ALAT was measured using the spectrophotometric assay of the Roche Reflotron plus system (Mannheim, Germany).
5) Bile cannulation, bile flow, biliary cholesterol, phospholipids, bile acids at 4 weeks. Determination of biliary lipid secretion and bile flow was determined as described previously (20). In short: The common bile duct of anesthetized mice was ligated, and the gallbladder was cannulated. Bile was collected in 15 minutes intervals for 45 minutes. Bile flow was measured. Cholesterol and phospholipid concentrations in bile were determined determined using kit "Cholesterol CHOD-PAP" and kit "Phospholipids" from Roche (Mannheim, Germany) and Instruchemie (Delfzijl, The Netherlands), respectively. Total bile acids were determined in bile using kit "total bile acids assay" from Lucron Bioproducts (Milsbeek, the Netherlands)
6) Clearance of VLDL-like particles and uptake in liver at 4 weeks. The in vivo clearance of VLDL-like triglycerides-rich particles was adapted from what was described previously (19). In short: Directly after bile cannulation experiment VLDL-like emulsion particles containing 200 µCi ^{3}H-triolein and 20 µCi ^{14}C-cholesteryl oleate were injected into the tail vein at a dose of 1 mg triglycerides per mouse. At 2, 5, 10, 20 and 30 min blood samples (50 µl) were taken from the tail vein. ^{3}H and ^{14}C activities were counted in 10 µl serum and corrected for total serum volume. At the end of the experiment liver, heart, perigonadal fat, spleen and muscle (femoralis) were collected. All tissues were weighed. Lipids were extracted from the tissues by an overnight incubation at 60 °C in 500 µl Solvable (Perkin-Elmer, Wellesley, USA) and radioactivity was measured. The clearance rate of VLDL-like particles was calculated by curve fitting (trendlines with the equation: % of injected dose=$b*e^{time/a}$, in which a is the calculated half life of the labeled VLDL-like particles).
7) After sacrifice at 4 weeks (end of in vivo VLDL clearance experiment) collection of EDTA-plasma, remainder liver and perigonadal adipose tissue. EDTA-plasma was collected from heart punction. The remainder of the livers and perigonadal fat tissues were directly frozen in liquid nitrogen and stored below -70 °C.
8) 15 µl of (non-radioactive) plasma taken at 4 weeks after 4h fasting was sent to 3M for measurements of plasma levels of compounds. Samples were sent on dry ice by Fedex courier on December 1st 2008 and arrived at the 3M center on December 2nd 2008.

2.10 Measurements experiment 3

1) Body weight and food intake (per cage) at 0 and 4 weeks.
2) Plasma cholesterol, HDL-cholesterol and triglycerides (after 4h fasting) at 0 and 4 weeks. Plasma cholesterol, HDL-cholesterol and triglycerides were determined using kit "Cholesterol CHOD-PAP" and kit "Triglycerides GPO-PAP" both from Roche (Mannheim, Germany). Plasma HDL-cholesterol was determined using kit "Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) after the precipitation of apoB containing lipids by $MnCl_2$ (0.2 mol/L) and heparin (500 IU/mL).
3) Lipoprotein profiles at group level at 4 weeks. Lipoproteins were separated by FPLC analysis using an AKTA apparatus. Analyses were performed in freshly obtained pooled samples per group. Cholesterol and phospholipid profiles were measured in the fractions using kit

"Cholesterol CHOD-PAP" from Roche (Mannheim, Germany) and kit "Phospholipids" from Instruchemie (Delfzijl, the Netherlands), respectively.

4) Plasma ALAT at group level at 0 and 4 weeks. Plasma ALAT was measured using the spectrophotometric assay of the Roche Reflotron plus system (Mannheim, Germany).

5) Plasma CETP activity (17) and mass (16) at 0 and 4 weeks. CETP activity was determined using kit "Roar CETP Activity assay kit" from Roar Biomedical Inc (New York, USA). CETP mass was determined using the CETP ELISA from Daiichi Pure Chemicals Co. (Tokyo, Japan).

6) Plasma ApoA1 concentration at 0 and 4 weeks. Plasma ApoA1 concentrations were determined using a sandwich ELISA (16). Rabbit anti-mouse ApoA1 polyclonal antibody from Abcam Plc. (Cambridge, UK) was coated overnight (3 µg/mL) onto Costar strips (New York, USA) at 4°C and was incubated with diluted mouse plasma (dilution 1:400,000) for 90 min at 37°C. Subsequently, goat anti-mouse ApoA1 antibody from Rockland Immunochemicals Inc. (Gilbertsville, USA; dilution 1:3,000) was added and incubated for 90 min at 37°C. Finally, HRP-conjugated rabbit anti-goat IgG antibody from Rockland Immunochemicals Inc. (Gilbertsville, USA; dilution 1:15,000) was added and incubated for 90 min at 37°C. HRP was detected by incubation with tetramethylbenzidine from Organon Teknika (Boxtel, The Netherlands). Purified mouse ApoA1 from Biodesign International (Saco, USA) was used as a standard.

7) In vivo clearance of autologous HDL at 4 weeks. The radiolabeling of autologous HDL and in vivo clearance of autologous HDL was performed as described previously (14). In short: For the radiolabeling of autologous HDL, one mouse from each experimental group was euthanized by CO_2, and blood was drawn from the retro-orbital vein. (One piece of the liver was collected and directly frozen in liquid nitrogen and stored below -70 °C for mRNA isolation and liver lipids determination). Serum was collected and HDL was isolated after density ultracentrifugation. HDL (0.32 µmol HDL-cholesterol) was radiolabeled by incubation (37°C, 24 h) with ^{3}H-cholesteryl oleyl ether labeled egg yolk phosphatidylcholine vesicles (40 µCi, 0.5 mg of phospholipid) in the presence of lipoprotein-deficient serum (1 mL) from E3Leiden.CETP mice. Subsequently, HDL was reisolated after density ultracentrifugation. For the in vivo clearance of autologous HDL, mice were injected via the tail vein with a trace of autologous ^{3}H-cholesteryl oleyl ether labeled HDL (0.1 µCi in PBS) at 8 am. Blood was collected at 1, 2, 4, 8 and 24 h after injection and ^{3}H-activity was counted in plasma samples to determine the plasma decay of ^{3}H-cholesteryl oleyl ether. HDL clearance was calculated by curve fitting timeframe 0-8 h (trendlines with the equation % of injected dose=$100*e^{a*time}$, in which a is the calculated fractional catabolic rate of HDL).

8) After sacrifice at 4 weeks (end of in vivo HDL clearance experiment) collection of EDTA-plasma, serum, liver, small intestine and perigonadal adipose tissue. EDTA-plasma and serum was collected from heart punction. The liver was weighed and cut in 4 parts, two parts and the small intestine were directly frozen in liquid nitrogen and stored below -70 °C. One part was fixed in phosphate buffered formalin (10%) for liver histology. The remainder of the liver was used to isolate microsomes for DGAT activity measurements (per liver microsomes were isolated) Perigonadal adipose tissue was weighed and directly frozen in liquid nitrogen and stored below -70 °C.

9) 15 µl of (non-radioactive) plasma taken at 4 weeks after 4h fasting was sent to 3M for measurements of plasma levels of compounds. Samples were sent on dry ice by Fedex courier on December 1st 2008 and arrived at the 3M center on December 2nd 2008

10) DGAT2 activity in liver microsomes. The DGAT activity in liver microsomes was determined as described previously (7, 18) in the presence of 5 mmol/L or 100 mmol/L $MgCl_2$. Since DGAT2 activity is inhibited at higher concentrations of $MgCl_2$ (100 mmol/L), DGAT2 activity can be calculated by subtracting the DGAT activity in the presence of 100 mmol/L $MgCl_2$ (only DGAT1 activity) from the DGAT activity in the presence of 5 mmol/L $MgCl_2$ (DGAT1 and DGAT2 activity).

11) Micro array analysis livers: RNA was isolated from collected liver pieces. After quality control of the RNA, microarray analysis was carried out at Service XS B.V. (Leiden, the Netherlands) using the Affymetrix technology platform and Affmetrix GeneChip® mouse genome MOE430-2.0 arrays. Data were sent to TNO Zeist for gene expression data analysis (21).

12) Liver lipids (FC, CE, TG and PL) in 6 samples per group and liver histology in 5 samples per group. Liver lipids were determined by a HPTLC analysis as previously described (22).

2.11 Statistical analysis

Significance of differences between the groups was calculated non-parametrically, using the computer program SPSS. A Kruskall-Wallis test for several independent samples was used, followed by a Mann-Whitney U-test for independent samples. A P-value < 0.05 was considered statistically significant. The resulting p-values were given in separate tables in the report.

3 Deviations from the protocol

Experiment 1

- Lipolytic activity determination: Post-heparin plasma was collected in week 5 instead of week 4 of treatment. Instead of 1 IU heparin/g body weight 0.5 IU heparin/g body weight heparin was injected intraperitoneally, 20 minutes before collection of post-heparin plasma.
- Feces were collected during a 48 h and a consecutive 72 h time period in 3 subgroups instead of 2 consecutive time points of 48 hours.
- We decided not to collect liver pieces for histology analysis, liver lipid analysis and mRNA isolation and subsequent micro array analysis in experiment 1 but in experiment 3, since injection of Triton WR-1339 (used to block VLDL clearance) could have an effect on liver gene expression and liver lipids. Intestine was collected in experiment 3 instead of experiment 1.
- At sacrifice no serum was collected, only EDTA-plasma. All of the plasma was used for VLDL isolation (via ultracentrifugation).
- Lipid composition of VLDL is expressed per dpm ^{35}S-ApoB instead of per mg protein, since the protein concentration was very low in the PFHS and PFOS treatment, and could not be measured properly.

Experiment 2

- 5 extra mice from experiment 3 (one mouse per group) were used to perform extra bile cannulations for determination of bile flow, biliary cholesterol, phospholipids, bile acids. These extra mice were used since some mice from experiment 2 had to be excluded from analysis because of a very low bile production. The excluded mice cooled off too much during the cannule placement operation, and the mice were not warm enough during the bile collection, which affected bile production drastically.
- Clearance of VLDL-like particles and uptake in liver at 4 weeks: We decided to only measure uptake in liver of VLDL-like particles at the end of the VLDL-clearance experiment, instead of taking liver biopts at every blood sampling point, to minimize loss of mice. Next to the liver, uptake of VLDL-like particles was measured in heart, muscle (femoralis), perigonal fat and spleen.
- At sacrifice no serum was collected, only EDTA-plasma. No intestine was collected and stored in liquid nitrogen.

Experiment 3

- Treatment was started with 5 groups of 7 animals instead of 5 groups of 6 animals. The extra mouse per group was used for an extra bile cannulation (see also deviations in experiment 2).
- Instead of 0.4 µM HDL per group, 0.32 µM HDL was used for radiolabeling. The reason for this was that less than 0.4 µM HDL was isolated from the PFHS and PFOS treatment groups.
- We decided not to collect liver pieces for histology analysis, liver lipid analysis and mRNA isolation and subsequent micro array analysis in experiment 1 but in experiment 3, since injection of Triton WR-1339 (used to block VLDL clearance) could have an effect on liver gene expression and liver lipids. Intestine was collected in experiment 3 instead of experiment 1 for that same reason.
- After consultation with the Sponsor (see email correspondence of 28-Jan-2009), we decided to perform micro array analysis on the livers instead of liver and intestinal mRNA analysis of some selected genes.
- Microsomes were isolated per liver instead of combining 2 livers; per group 5 remainders of livers were used for microsomes isolation.

4 Results

4.1 Results study 1

4.1.1 Markers of general well-being

During the study one mouse (mouse 22) was put in a separate cage because of an open wound from fighting. Another mouse (mouse 15) showed some scabs in the belly area. No other specific clinical signs were observed during the study. At sacrifice, besides the livers no macroscopic differences were observed between the groups. Livers were visibly somewhat enlarged in the fenofibrate group and strongly enlarged in the PFHS and PFOS group.

4.1.2 Body weight

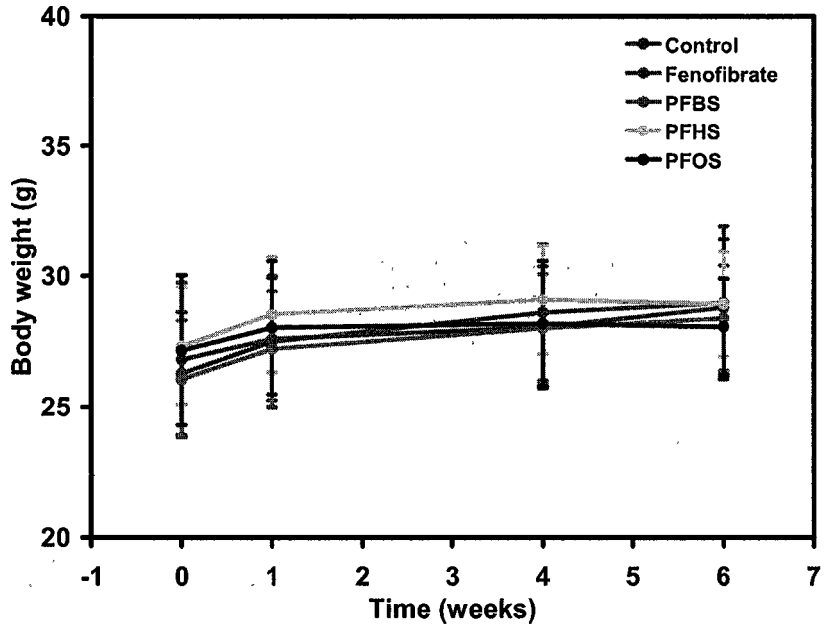
Values are absolute values (grams) and are means ± S.D. from 8 mice per group. Individual body weights are given in appendix I.

Body weight (g)		time (weeks)			
		0	1	4	6
Control	avg	26.3	27.5	28.6	29.0
	sd	2.4	2.5	2.6	2.9
Fenofibrate	avg	26.8	27.6	28.0	28.8
	sd	2.9	2.3	2.3	2.6
PFBS	avg	26.1	27.2	28.0	28.4
	sd	1.7	1.8	1.8	1.8
PFHS	avg	27.3	28.5	29.1	28.9
	sd	2.2	2.2	2.1	2.0
PFOS	avg	27.2	28.0	28.2	28.1
	sd	2.9	2.6	2.4	1.8

Body weight: P-value		time (weeks)			
		0	1	4	6
Between groups		0.635	0.781	0.782	0.888
Control vs:	Fenofibrate	0.574	0.798	1.000	0.959
	PFBS	1.000	0.959	0.645	0.959
	PFHS	0.382	0.328	0.574	0.878
	PFOS	0.382	0.645	0.721	0.721
Fenofibrate vs	PFBS	0.279	0.382	0.382	0.505
	PFHS	0.878	0.721	0.721	0.798
	PFOS	0.959	0.878	0.798	0.382
PFBS vs	PFHS	0.234	0.279	0.161	0.505
	PFOS	0.279	0.505	1.000	0.721
PFHS vs	PFOS	0.878	0.798	0.442	0.382

During the study an increase in body weight was seen. Between groups no significant differences were observed.

Figure 4.1.2 Body weight



* p<0.05 vs. control

4.1.3 Liver and perigonadal fat weight

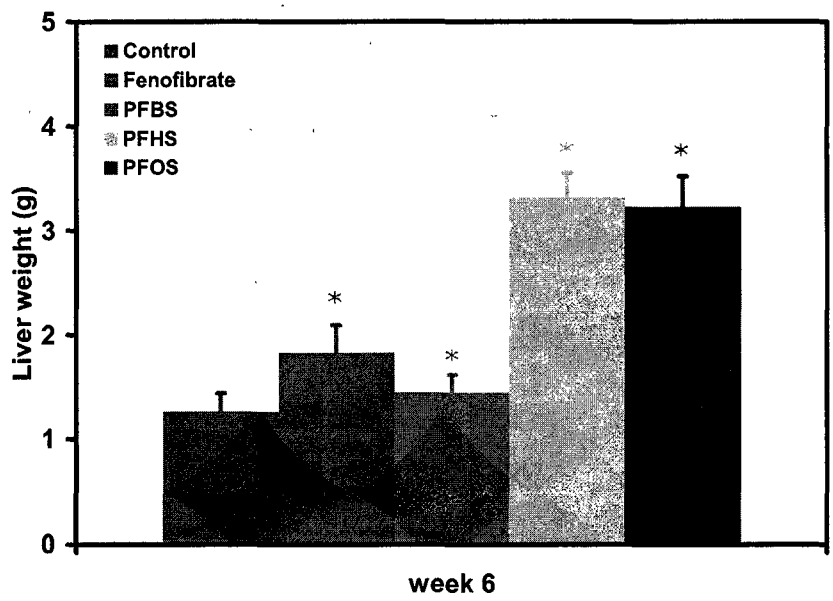
Values are absolute values (grams) and are means ± S.D. from 8 mice per group. Individual tissue weights are given in appendix II.

Tissue weight (g)		Liver	Perigonadal fat
Control	avg	1.27	0.62
	sd	0.18	0.24
Fenofibrate	avg	1.83	0.50
	sd	0.27	0.11
PFBS	avg	1.45	0.51
	sd	0.17	0.15
PFHS	avg	3.32	0.45
	sd	0.23	0.14
PFOS	avg	3.23	0.38
	sd	0.29	0.10

Tissue weight: P-value		Liver	Perigonadal fat
Between groups		**<0.001**	0.077
Control vs:	Fenofibrate	**0.001**	0.234
	PFBS	**0.028**	0.328
	PFHS	**<0.001**	0.083
	PFOS	**<0.001**	**0.007**
Fenofibrate vs	PFBS	**0.003**	0.798
	PFHS	**<0.001**	0.442
	PFOS	**<0.001**	0.065
PFBS vs	PFHS	**<0.001**	0.645
	PFOS	**<0.001**	0.130
PFHS vs	PFOS	0.645	0.382

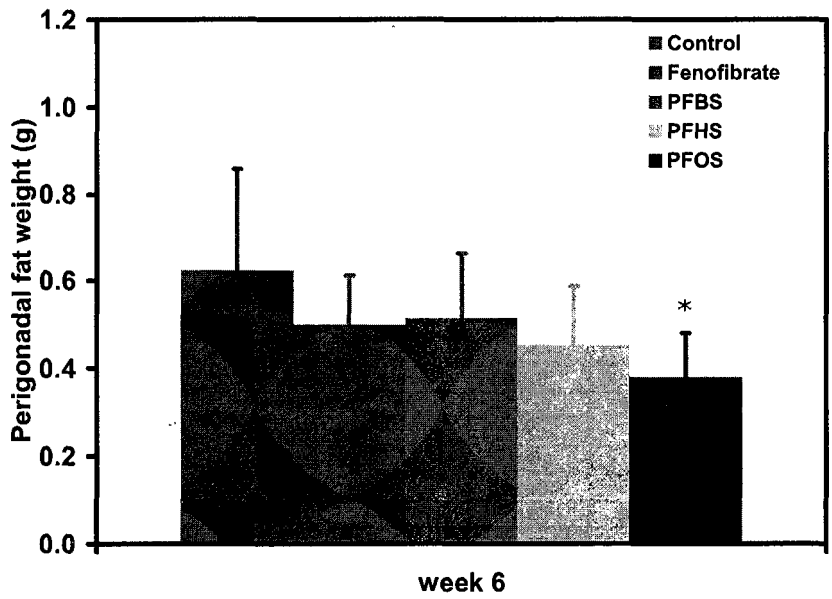
Livers were significantly enlarged in all treatment groups. Fenofibrate and PFBS treatment increased liver weights by respectively +44% and +15%. The PFHS and PFOS groups showed a robust induction in liver weights, respectively by +162% and +155 %, which was also significantly higher as compared to the PFBS group. Although a slight decrease was seen in all treatment groups, only PFOS significantly decreased perigonadal fat weight (by -39%, p=0.007).

Figure 4.1.3a Liver weight



* p<0.05 vs. control

Figure 4.1.3b Perigonadal fat weight



* p<0.05 vs. control

4.1.4 Food intake

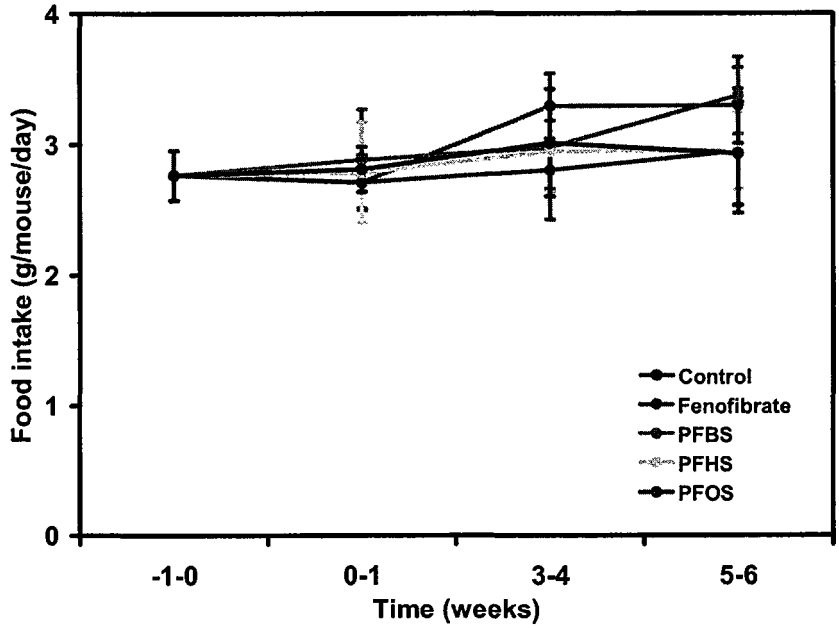
Values are absolute values (gram/mouse/day) and are means ± S.D. from 3-4 cages per group. Food intake values per individual cage are given in appendix III.

Food intake (g/day/mouse)		time (weeks)			
		-1-0	0-1	3-4	5-6
Control	avg	2.8	2.7	2.8	2.9
	sd	0.2	0.2	0.4	0.5
Fenofibrate	avg	2.8	2.7	3.3	3.3
	sd	0.2	0.2	0.2	0.3
PFBS	avg	2.8	2.9	3.0	3.4
	sd	0.2	0.3	0.1	0.6
PFHS	avg	2.8	2.8	2.9	2.9
	sd	0.2	0.4	0.3	0.3
PFOS	avg	2.8	2.8	3.0	2.9
	sd	0.2	0.2	0.4	0.4

Food intake: P-value		time (weeks)		
		0-1	3-4	5-6
Between groups		0.848	0.253	0.384
Control vs:	Fenofibrate	1.000	0.114	0.400
	PFBS	0.400	0.700	0.400
	PFHS	1.000	0.700	1.000
	PFOS	0.700	0.400	1.000
Fenofibrate vs	PFBS	0.400	0.114	1.000
	PFHS	1.000	0.114	0.229
	PFOS	0.629	0.400	0.229
PFBS vs	PFHS	1.000	1.000	0.400
	PFOS	1.000	0.700	0.229
PFHS vs	PFOS	1.000	1.000	1.000

All groups showed a similar food intake.

Figure 4.1.4 Food intake



* p<0.05 vs. control

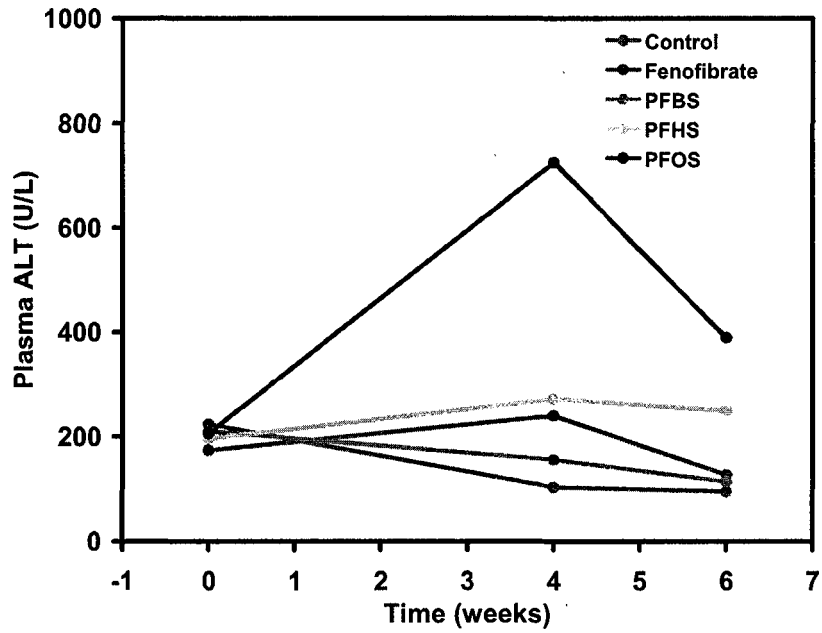
4.1.5 Plasma ALT

Values are absolute values (U/L) from measurements in pooled plasma samples per group (8 mice per group) at t=0, 4 and 6.

Group	Plasma ALAT (U/L)		
	t=0 weeks	t=4 weeks	t=6 weeks
Control	174	240	128
Fenofibrate	224	104	96
PFBS	210	156	115
PFHS	196	272	250
PFOS	206	726	390

Although no statistical analysis could be performed on pooled ALT levels, PFOS and to a lesser extend PFHS seem to increase plasma ALT levels.

Figure 4.1.5 Plasma ALT



4.1.6 Plasma cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 8 mice per group. Individual plasma cholesterol levels are given in appendix IV.

Cholesterol (mmol/L)		time (weeks)		
		0	4	6
Control	avg	7.9	8.1	9.0
	sd	1.5	1.9	1.1
Fenofibrate	avg	7.8	5.0	5.6
	sd	1.5	0.6	1.1
PFBS	avg	7.9	6.2	6.8
	sd	1.5	0.9	1.4
PFHS	avg	8.1	3.2	3.6
	sd	1.5	0.7	0.6
PFOS	avg	8.0	3.0	3.2
	sd	1.6	0.7	1.1

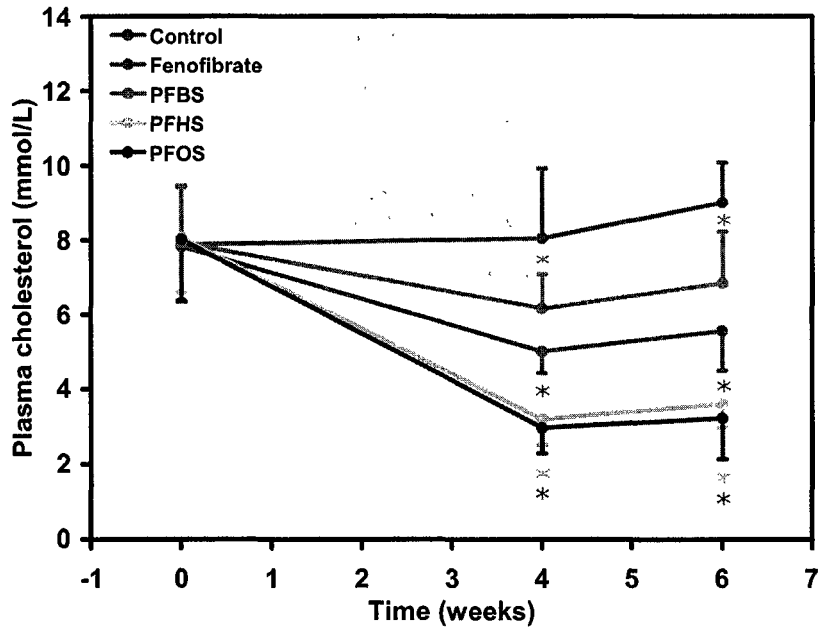
Cholesterol: P-value		time (weeks)		
		0	4	6
Between groups		0.999	**<0.001**	**<0.001**
Control vs:	Fenofibrate	1.000	**0.001**	**<0.001**
	PFBS	1.000	**0.050**	**0.010**
	PFHS	0.959	**<0.001**	**<0.001**
	PFOS	0.959	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.959	**0.028**	0.065
	PFHS	0.878	**<0.001**	**<0.001**
	PFOS	0.798	**<0.001**	**0.002**
PFBS vs	PFHS	0.878	**<0.001**	**<0.001**
	PFOS	0.878	**<0.001**	**<0.001**
PFHS vs	PFOS	1.000	0.574	0.382

All treatment groups showed a significant reduction in plasma cholesterol at t=4 and 6 weeks of treatment. Fenofibrate decreased plasma cholesterol by -38% at both time points.

PFBS, PFHS and PFOS treatment reduced plasma cholesterol levels respectively by -23%, -60% and -63% after 4 weeks of treatment and by -24%, -60% and -64% at 6 weeks of treatment.

PFHS and PFOS decreased cholesterol levels to a larger extent than PFBS ($p<0.001$ at both time points). PFHS and PFOS showed a similar inhibition.

Figure 4.1.6 Plasma cholesterol



* $p<0.05$ vs. control

4.1.7 Plasma HDL-cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 8 mice per group. Individual plasma HDL-cholesterol levels are given in appendix V.

HDL-Cholesterol (mmol/L)		time (weeks)		
		0	4	6
Control	avg	0.94	1.36	1.50
	sd	0.17	0.21	0.41
Fenofibrate	avg	0.96	2.06	2.31
	sd	0.19	0.32	0.54
PFBS	avg	0.93	1.52	1.46
	sd	0.23	0.24	0.50
PFHS	avg	1.05	0.85	0.69
	sd	0.32	0.16	0.08
PFOS	avg	0.94	0.62	0.44
	sd	0.25	0.12	0.13

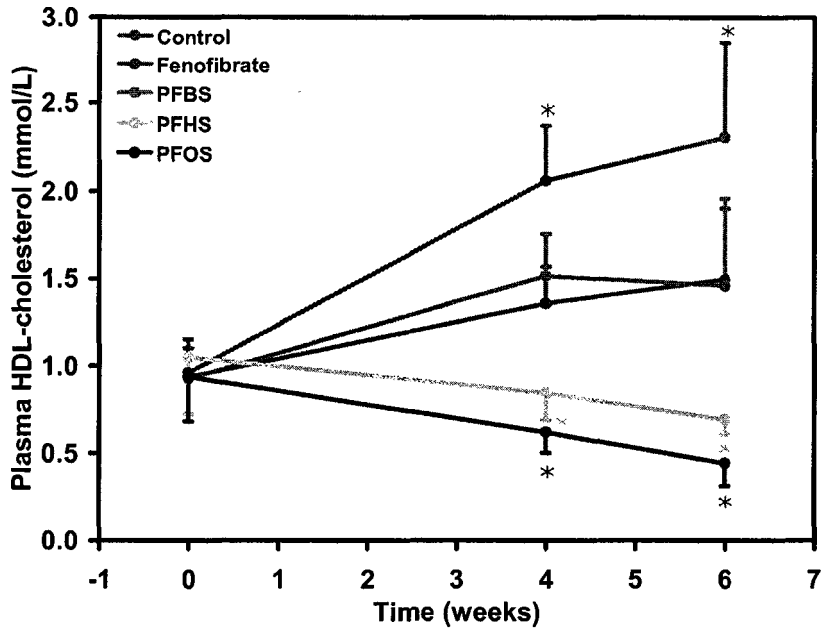
HDL-Cholesterol: P-value		time (weeks)		
		0	4	6
Between groups		0.789	**<0.001**	**<0.001**
Control vs:	Fenofibrate	0.721	**0.001**	**0.007**
	PFBS	0.878	0.328	0.959
	PFHS	0.328	**0.001**	**<0.001**
	PFOS	0.959	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.574	**0.003**	**0.010**
	PFHS	0.442	**<0.001**	**<0.001**
	PFOS	0.574	**<0.001**	**<0.001**
PFBS vs	PFHS	0.328	**<0.001**	**0.010**
	PFOS	0.959	**<0.001**	**<0.001**
PFHS vs	PFOS	0.442	**0.028**	**0.001**

Plasma-HDL levels were increased at t=4 and 6 weeks in the fenofibrate group (respectively by +52% and +54%).

PFBS treatment showed no significantly different plasma HDL-cholesterol levels as compared to the control group, but both PFHS and PFOS significantly decreased HDL-cholesterol after 4 and 6 weeks of treatment (PFHS respectively by -38% and -54%, PFOS by -54% and -70%). Compared to PFBS treatment, PFHS and PFOS also significantly decreased HDL-cholesterol levels.

When comparing PFHS and PFOS treatment, PFOS significantly decreased HDL-cholesterol to a larger extent (p=0.028 and p=0.001 at t=4 and 6 weeks respectively).

Figure 4.1.7 Plasma HDL-cholesterol



* p<0.05 vs. control

4.1.8 Plasma triglycerides

Values are absolute values (mmol/L) and are means ± S.D. from 8 mice per group. Individual plasma triglycerides levels are given in appendix VI.

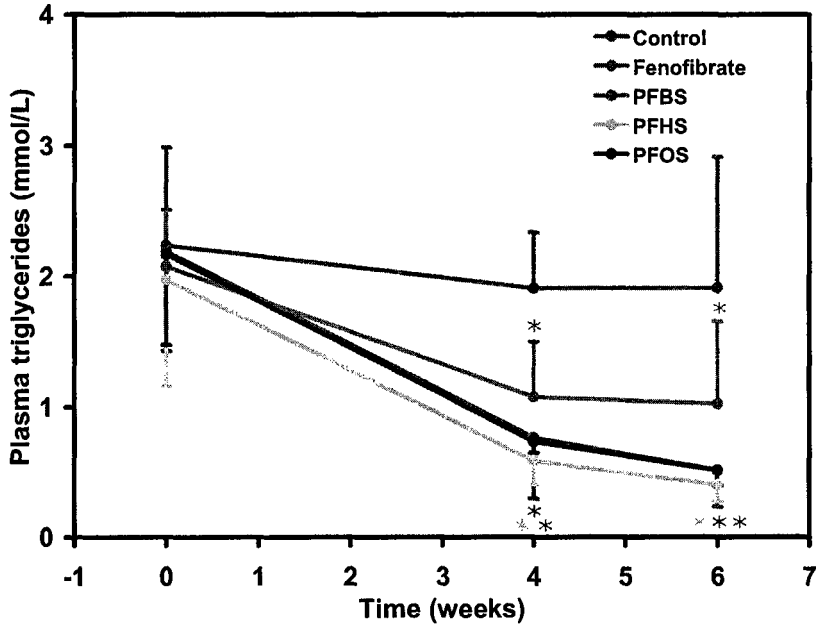
Triglycerides (mmol/L)		time (weeks)		
		0	4	6
Control	avg	2.24	1.91	1.91
	sd	0.75	0.43	1.01
Fenofibrate	avg	2.19	0.77	0.52
	sd	0.76	0.47	0.29
PFBS	avg	2.08	1.08	1.02
	sd	0.43	0.42	0.63
PFHS	avg	1.97	0.59	0.41
	sd	0.81	0.18	0.13
PFOS	avg	2.17	0.74	0.52
	sd	0.70	0.09	0.12

Triglycerides: P-value		time (weeks)		
		0	4	6
Between groups		0.971	**<0.001**	**<0.001**
Control vs:	Fenofibrate	1.000	**0.002**	**0.001**
	PFBS	0.798	**0.003**	**0.028**
	PFHS	0.721	**<0.001**	**<0.001**
	PFOS	0.798	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.959	**0.028**	**0.015**
	PFHS	0.878	0.798	0.234
	PFOS	1.000	0.195	0.234
PFBS vs	PFHS	0.574	**0.003**	**0.001**
	PFOS	0.959	**0.015**	**0.002**
PFHS vs	PFOS	0.382	**0.050**	0.161

All treatment groups showed significant reductions in plasma triglycerides at t=4 and 6 weeks of treatment. Fenofibrate decreased plasma triglycerides by -60% and -73%, respectively.

PFBS, PFHS and PFOS treatment reduces plasma triglycerides levels respectively by -44%, -69% and -61% at t=4 weeks of treatment and by -46%, -79% and -73% at t=6 weeks of treatment. PFHS and PFOS decreased triglycerides levels to a larger extent than PFBS (respectively p=0.003 and p=0.015 at t=4 weeks and p=0.001 and p=0.002 at t= 6 weeks). When comparing the PFHS and PFOS group, at t=4 weeks a borderline significance was seen (p=0.05) towards lower triglycerides levels in the PFHS group, but at t=6 weeks no significant differences were seen between groups.

Figure 4.1.8 Plasma triglycerides



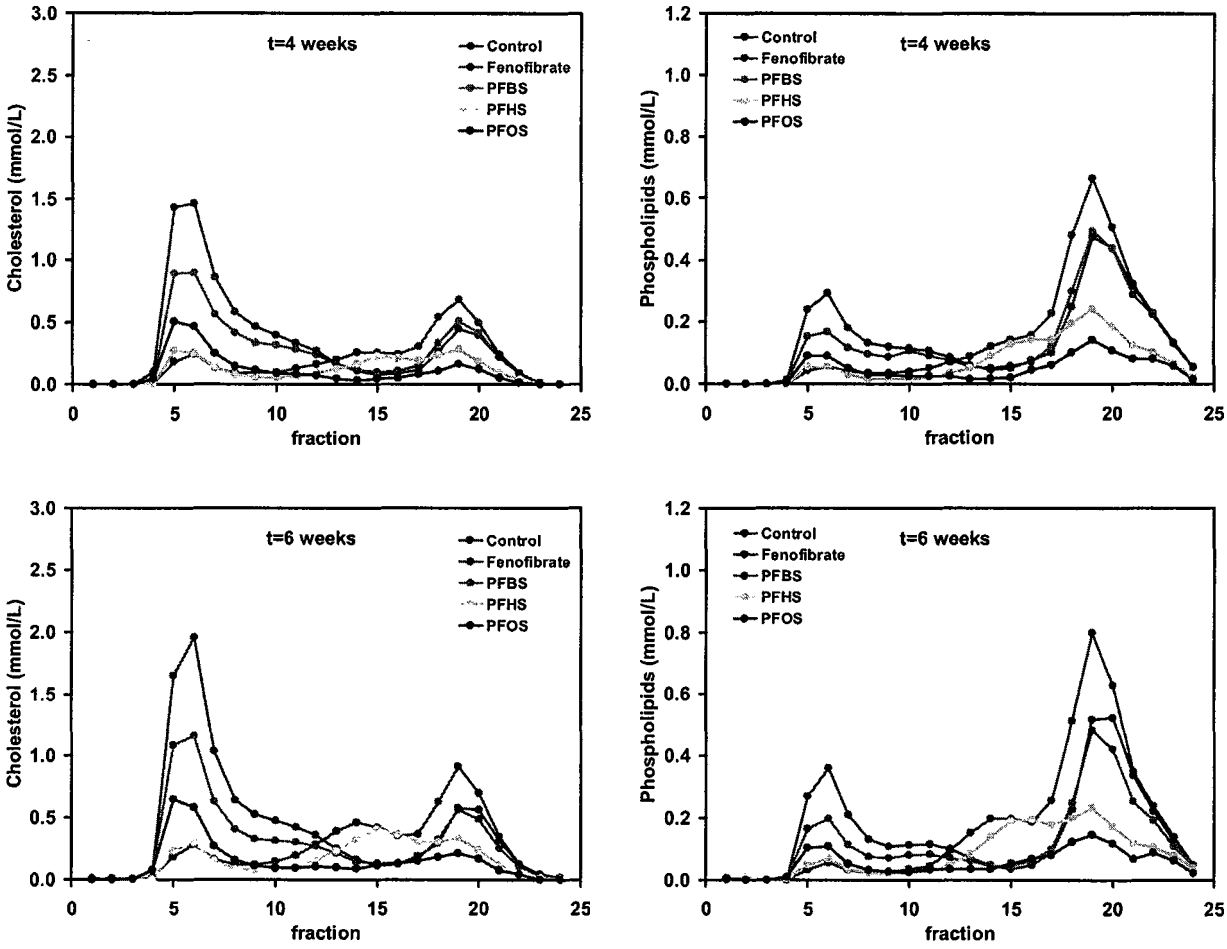
* p<0.05 vs. control

4.1.9 Lipoprotein profiles

Lipoproteins were separated on a superose column. Values are absolute values (mM) from cholesterol (left column) and phospholipids (right column) measurements in pooled plasma per group (with 8 mice per group) at t=4 and 6. We consider fractions 4-8 as VLDL; 9-14 as LDL; 13-16 as large-HDL (HDL1) and 17-24 as HDL.

After 4 and 6 weeks of treatment, plasma cholesterol levels were decreased in the VLDL-LDL peak in all groups as compared to the control group. Fenofibrate and PFHS showed the strongest and a similar reduction, PFOS seemed to decrease VLDL to a lesser extend than PFHS. PFBS decreased VLDL-LDL to a lesser extent than PFHS and PFOS. HDL was increased in the fenofibrate group. Whereas PFBS showed no clear effects on HDL, both PFHS and PFOS strongly decreased the HDL peak. PFHS seemed to decrease HDL to a lesser extent than PFOS and in the profile of the PFHS group HDL1 (large HDL) was increased. The changes in the lipoproteins (apoB-containing lipoproteins (VLDL-LDL) and HDL) are in line with changes in plasma cholesterol and HDL.

Figure 4.1.9 Lipoprotein profiles



4.1.10 Plasma free glycerol

Values are absolute values (mmol/L) and are means ± S.D. from 8 mice per group. Individual plasma free glycerol levels are given in appendix VII.

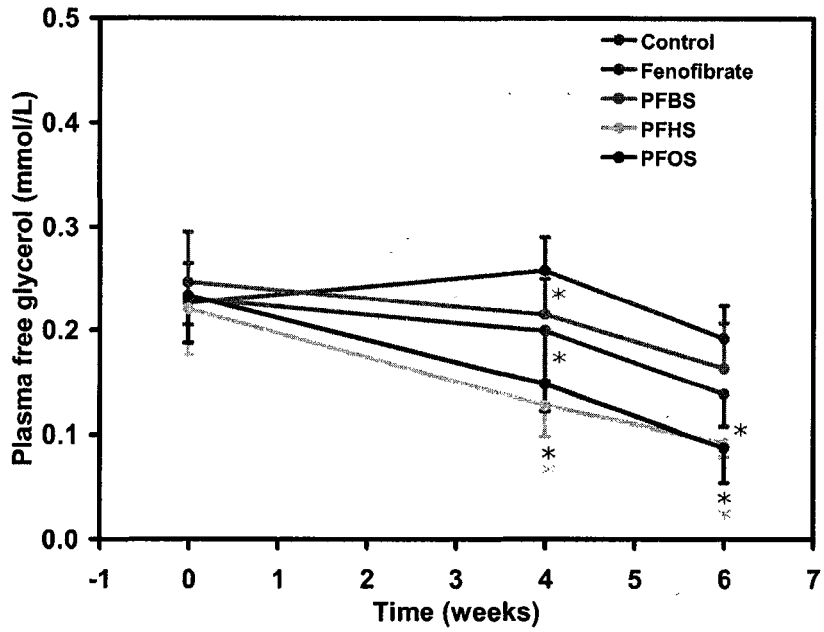
Free glycerol (mmol/L)		time (weeks)		
		0	4	6
Control	avg	0.23	0.26	0.19
	sd	0.04	0.03	0.03
Fenofibrate	avg	0.23	0.20	0.14
	sd	0.03	0.05	0.03
PFBS	avg	0.25	0.21	0.16
	sd	0.05	0.03	0.04
PFHS	avg	0.22	0.13	0.09
	sd	0.04	0.03	0.01
PFOS	avg	0.23	0.15	0.09
	sd	0.05	0.03	0.03

Free glycerol: P-value		time (weeks)		
		0	4	6
Between groups		0.724	**<0.001**	**<0.001**
Control vs:	Fenofibrate	0.878	**0.021**	**0.005**
	PFBS	0.442	**0.028**	0.130
	PFHS	0.721	**<0.001**	**<0.001**
	PFOS	0.959	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.382	0.878	0.195
	PFHS	0.442	**0.007**	0.001
	PFOS	1.000	**0.028**	**0.015**
PFBS vs	PFHS	0.234	**0.001**	**<0.001**
	PFOS	0.505	**0.001**	**0.002**
PFHS vs	PFOS	0.574	0.195	0.574

Fenofibrate, PFHS and PFOS showed significant reductions in free glycerol levels at 4 and 6 weeks of treatment (respectively by -23%, -51% and -43% at t=4 weeks and by -28%, -52% and -54% at t=6 weeks). PFBS showed a transient decrease at t=4 weeks (by -17%), but at t=6 weeks plasma free glycerol levels were not significantly different from the control group.

PFHS and PFOS decreased free glycerol levels to a larger extent than PFBS (respectively $p=0.001$ and $p=0.001$ at t=4 weeks and $p<0.001$ and $p=0.002$ at t=6 weeks). No significant differences were seen between the PFHS and PFOS group.

Figure 4.1.10 Plasma free glycerol



* p<0.05 vs. control

4.1.11 Plasma free fatty acids

Values are absolute values (mmol/L) and are means ± S.D. from 8 mice per group. Individual plasma free fatty acid levels are given in appendix VIII.

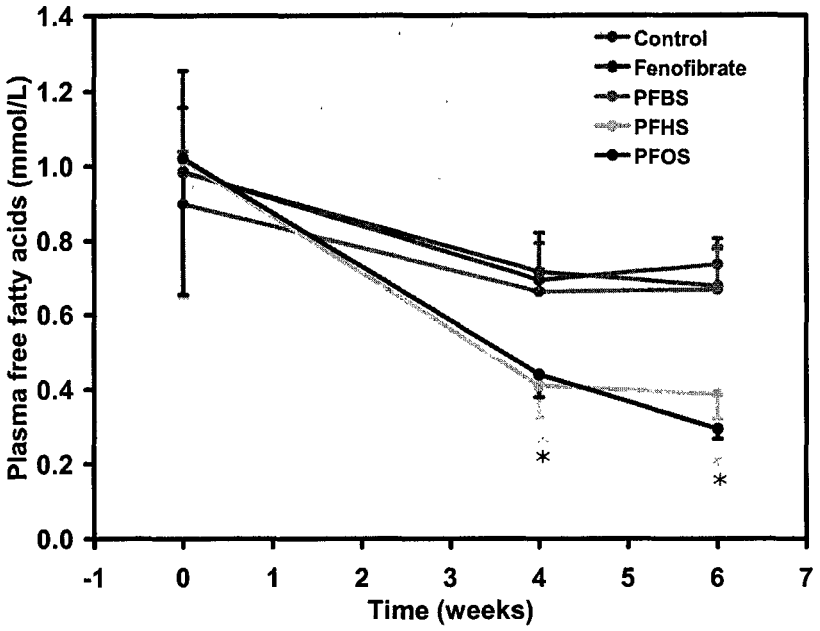
Free fatty acids (mmol/L)		time (weeks)		
		0	4	6
Control	avg	0.99	0.69	0.74
	sd	0.27	0.10	0.07
Fenofibrate	avg	0.98	0.72	0.68
	sd	0.17	0.11	0.10
PFBS	avg	0.90	0.66	0.67
	sd	0.14	0.06	0.12
PFHS	avg	1.02	0.41	0.39
	sd	0.37	0.09	0.06
PFOS	avg	1.02	0.44	0.29
	sd	0.37	0.06	0.03

Free fatty acids: P-value		time (weeks)		
		0	4	6
Between groups		0.941	**<0.001**	**<0.001**
Control vs:	Fenofibrate	0.721	0.721	0.382
	PFBS	0.721	0.574	0.279
	PFHS	0.959	**<0.001**	**<0.001**
	PFOS	0.959	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.382	0.382	0.798
	PFHS	0.798	**<0.001**	**<0.001**
	PFOS	0.798	**<0.001**	**<0.001**
PFBS vs	PFHS	0.721	**<0.001**	**<0.001**
	PFOS	0.505	**<0.001**	**<0.001**
PFHS vs	PFOS	0.798	0.574	**0.003**

Free fatty acid levels were similar in the control, fenofibrate and PFBS groups. Both PFHS and PFOS significantly decreased free fatty acid levels after 4 and 6 weeks of treatment (PFHS respectively by -41% and -48%, PFOS respectively by -37% and -60%).

After 4 weeks of treatment no significant differences were seen between the PFHS or PFOS group but after 6 weeks the PFOS group showed significantly lower FFA levels than the PFHS group (p=0.003).

Figure 4.1.11 Plasma free fatty acids



* p<0.05 vs. control

4.1.12 Post-heparin LPL and HL activity

Values are absolute values (µmol FFA/hr/mL) and are means ± S.D. from 8 mice per group. Individual post-heparin LPL and HL activity values are given in appendix XII.

Lipolytic activity (µmol FFA/hr/mL)		HL activity	LPL activity
Control	avg	7.4	12.0
	sd	0.9	2.5
Fenofibrate	avg	12.3	25.1
	sd	1.4	3.1
PFBS	avg	5.3	14.3
	sd	2.1	2.4
PFHS	avg	8.5	20.8
	sd	3.0	5.1
PFOS	avg	9.0	18.5
	sd	1.4	2.9

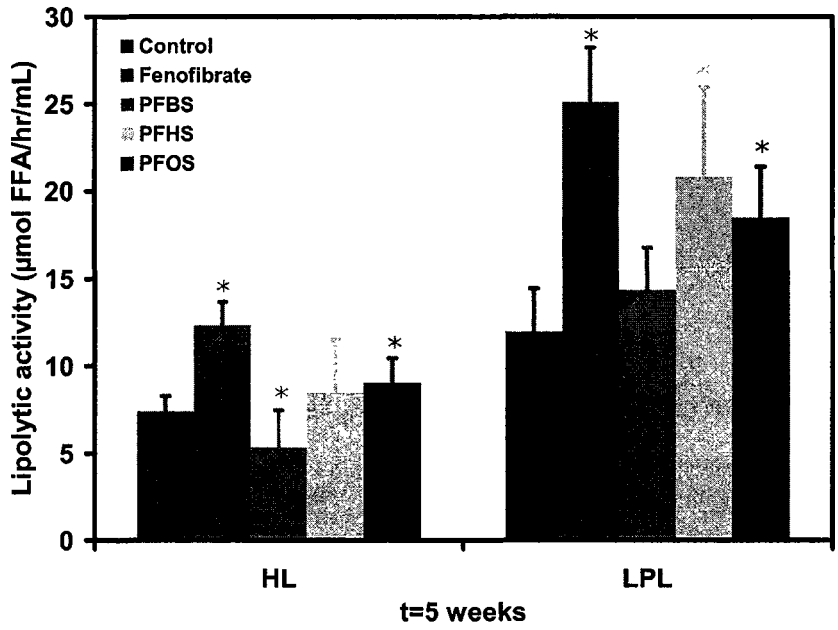
Lipolytic activity: P-values		HL activity	LPL activity
Between groups		**<0.001**	**<0.001**
Control vs:	Fenofibrate	**<0.001**	**<0.001**
	PFBS	**0.050**	0.105
	PFHS	0.798	**0.001**
	PFOS	**0.021**	**0.001**
Fenofibrate vs	PFBS	**<0.001**	**<0.001**
	PFHS	**0.010**	**0.050**
	PFOS	**<0.001**	**0.001**
PFBS vs	PFHS	**0.050**	**0.007**
	PFOS	**0.003**	**0.010**
PFHS vs	PFOS	0.442	0.328

Post-heparin lipoprotein lipase activity was strongly induced after fenofibrate treatment (by +110%). Both PFHS and PFOS increased LPL activity to a lesser extent (respectively by +74% and +54%).

Hepatic lipase activity was significantly increased in the fenofibrate group and the PFOS group (respectively by +67% and +22%). In contrast PFBS significantly decreased hepatic lipase activity by -28% (p=0.05).

PFBS had significantly lower levels of LPL and HL activity as compared to PFHS (0.007 and 0.05, respectively) and PFOS (0.010 and 0.003, respectively).

Figure 4.1.12 Post heparin LPL and HL activity



* p<0.05 vs. control

4.1.13 Fecal lipids

Values are absolute values (µmol/100 gram mouse/day) or relative values (% of total neutral sterols, phytosterols, bile acids and fatty acids, respectively) and are means ± S.D. from 3 (combined) cages per group at 2 consecutive time points. Values per (combined) cage per time point are given in appendix XIII.

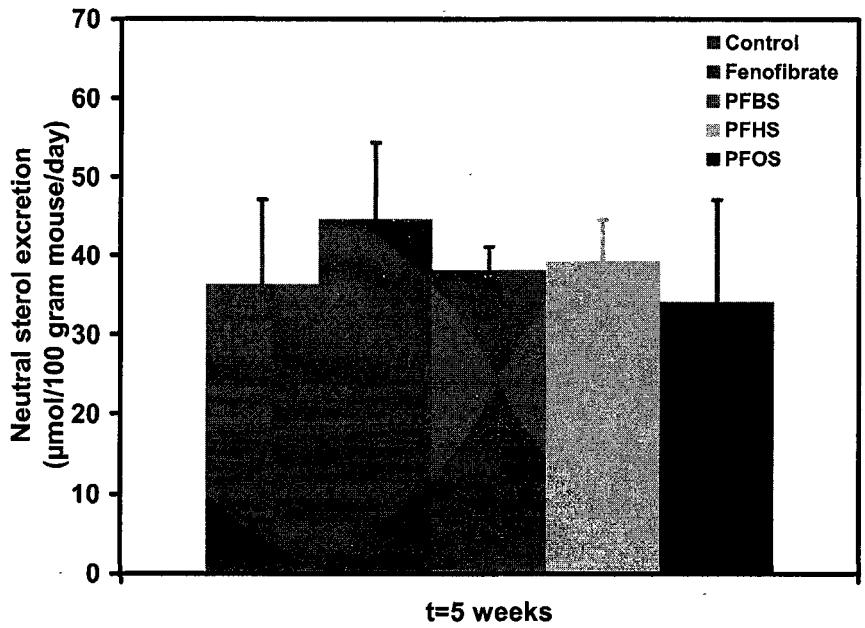
Fecal lipids (µmol/100 gram mouse/day)		Total neutral sterols	Total phytosterols	Total bile acids	Total fatty acids
Control	avg	36.3	3.5	5.5	257
	sd	10.7	0.7	1.6	222
Fenofibrate	avg	44.5	3.4	3.4	410
	sd	9.8	0.6	0.8	121
PFBS	avg	38.1	3.6	5.0	240
	sd	3.0	0.4	1.4	79
PFHS	avg	39.1	2.9	3.3	159
	sd	5.2	0.2	0.9	128
PFOS	avg	34.0	3.4	2.8	188
	sd	12.9	0.6	0.3	86

Fecal lipids: P-value		Total neutral sterols	Total phytosterols	Total bile acids	Total fatty acids
Between groups		0.462	0.175	**0.001**	**0.030**
Control vs:	Fenofibrate	0.180	0.937	**0.015**	0.180
	PFBS	0.310	1.000	0.589	0.485
	PFHS	0.310	0.065	**0.004**	0.394
	PFOS	0.818	0.937	**0.002**	0.937
Fenofibrate vs	PFBS	0.394	0.485	0.093	**0.009**
	PFHS	0.310	0.394	0.699	**0.026**
	PFOS	0.180	0.818	0.240	**0.009**
PFBS vs	PFHS	1.000	**0.002**	**0.015**	0.065
	PFOS	0.937	0.937	**0.002**	0.240
PFHS vs	PFOS	0.589	0.132	0.093	0.310

Nor fecal neutral sterols (figure 4.1.13a), neither fecal phytosterols (figure 4.1.13b) were affected by fenofibrate, PFBS, PFHS or PFOS treatment. Total bile acid excretion was significantly lower in the fenofibrate, PFHS and PFOS groups (figure 4.1.13c). Fecal bile acids were decreased by -38%, -41% and -50%, respectively.

Although the fenofibrate group showed a somewhat higher fatty acid excretion, none of the treatment groups were significantly different from the control group (figure 4.1.13d).

Figure 4.1.13a Total neutral sterol excretion



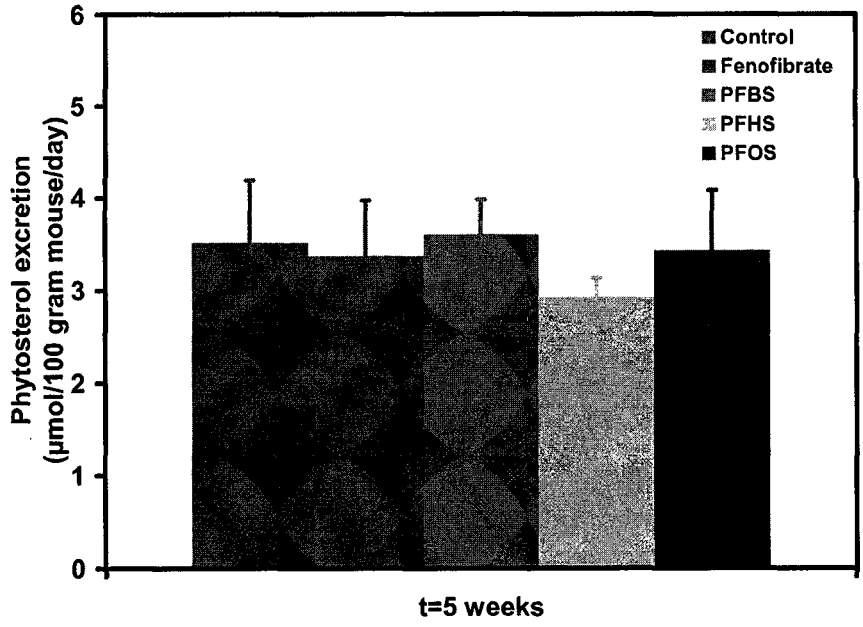
* p<0.05 vs. control

Fecal neutral sterols (%)		coprostanol	cholesterol	cholestanol	lathosterol
Control	avg	0.14	93.7	3.0	3.2
	sd	0.04	0.6	1.2	0.7
Fenofibrate	avg	0.12	94.8	2.0	3.1
	sd	0.04	0.8	0.4	0.7
PFBS	avg	0.12	94.4	2.6	2.9
	sd	0.02	0.4	0.5	0.2
PFHS	avg	0.12	93.1	4.1	2.6
	sd	0.04	0.9	1.2	0.5
PFOS	avg	0.14	92.4	4.5	3.0
	sd	0.04	1.5	1.6	0.5

Fecal neutral sterols: P-value		coprostanol	cholesterol	cholestanol	lathosterol
Between groups		0.673	**0.002**	**0.004**	0.401
Control vs:	Fenofibrate	0.699	**0.026**	0.240	0.485
	PFBS	0.589	0.093	0.310	0.937
	PFHS	0.310	0.310	0.132	0.132
	PFOS	0.699	0.093	0.240	0.818
Fenofibrate vs	PFBS	0.937	0.394	0.065	0.937
	PFHS	0.937	**0.015**	**0.004**	0.240
	PFOS	0.310	**0.002**	**0.002**	0.818
PFBS vs	PFHS	0.818	**0.041**	**0.041**	0.132
	PFOS	0.240	**0.002**	**0.004**	0.818
PFHS vs	PFOS	0.394	0.589	0.818	0.180

The fecal neutral sterol composition of the fenofibrate group was somewhat changed, the percentage excreted cholesterol was slightly but significantly increased as compared to the control group (94.8% vs 93.7%). The other treatment groups showed the same composition as the control group.

Figure 4.1.13b Total phytosterol excretion



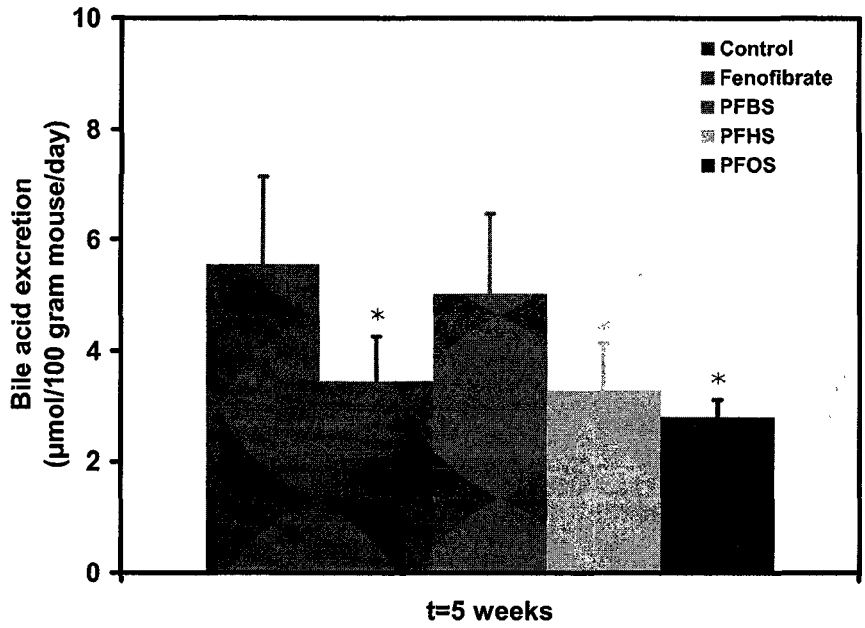
* $p<0.05$ vs. control

Fecal phytosterols (%)		campesterol	stigmasterol	b-sitosterol
Control	avg	26.1	9.2	64.7
	sd	0.5	0.5	0.7
Fenofibrate	avg	26.9	8.4	64.7
	sd	0.9	1.3	1.1
PFBS	avg	26.1	8.7	65.2
	sd	0.2	1.2	1.1
PFHS	avg	26.1	9.5	64.4
	sd	1.4	3.7	2.5
PFOS	avg	24.2	14.8	61.0
	sd	1.1	1.8	1.3

Fecal phytosterols: P-value		campesterol	stigmasterol	b-sitosterol
Between groups		**0.006**	**0.011**	**0.009**
Control vs:	Fenofibrate	0.180	0.310	0.818
	PFBS	0.485	0.485	0.485
	PFHS	0.818	1.000	0.937
	PFOS	**0.002**	**0.002**	**0.002**
Fenofibrate vs	PFBS	0.065	0.818	0.699
	PFHS	0.310	1.000	0.818
	PFOS	**0.002**	**0.002**	**0.002**
PFBS vs	PFHS	1.000	1.000	0.818
	PFOS	**0.002**	**0.002**	**0.002**
PFHS vs	PFOS	**0.041**	**0.026**	**0.009**

Only PFOS treatment affected fecal phytosterol composition as compared to the control group; PFOS showed an increased stigmasterol (14.8 % vs 9.2 %) and a decreased b-sitosterol and campesterol content (61.0% vs 64.7% and 24.2% vs 26.1%, respectively).

Figure 4.1.13c Total bile acid excretion



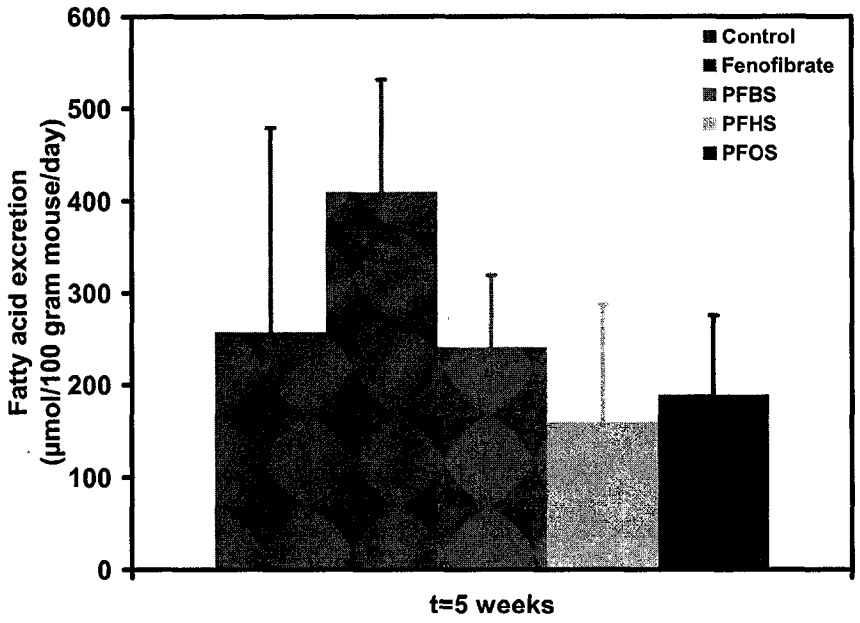
* p<0.05 vs. control

Fecal bile acids (%)		a-muricholate	deoxycholate	cholate	lithocholate	b-muricholate	w-muricholate	hyodeo/urso
Control	avg	7.1	14.2	12.7	10.6	25.5	23.5	6.6
	sd	1.1	5.1	4.2	1.4	2.8	1.7	1.0
Fenofibrate	avg	6.0	10.6	13.4	16.6	18.4	18.5	16.4
	sd	1.2	4.3	4.5	3.7	6.6	2.0	5.3
PFBS	avg	6.5	17.9	11.3	17.3	24.5	29.2	12.5
	sd	2.4	7.8	5.2	7.3	13.4	14.6	5.5
PFHS	avg	6.3	16.7	15.1	11.1	16.6	24.8	8.8
	sd	2.9	10.5	8.3	5.4	8.8	11.9	3.9
PFOS	avg	5.5	13.5	13.5	6.3	14.1	26.3	6.7
	sd	2.4	10.5	6.1	3.0	7.9	12.4	2.1

Fecal bile acids: P-value		a-muricholate	deoxycholate	cholate	lithocholate	b-muricholate	w-muricholate	hyodeo/urso
Between groups		0.642	0.490	0.816	**0.003**	0.149	0.342	**0.003**
Control vs:	Fenofibrate	0.180	0.310	0.818	**0.004**	0.093	**0.002**	**0.002**
	PFBS	0.818	0.394	0.589	0.065	0.589	0.485	**0.015**
	PFHS	0.394	0.937	0.589	0.937	0.065	0.589	0.132
	PFOS	0.310	0.394	0.818	**0.026**	**0.041**	1.000	0.699
Fenofibrate vs	PFBS	0.818	0.180	0.485	0.937	0.699	0.394	0.310
	PFHS	0.699	0.394	1.000	0.093	0.937	0.132	**0.015**
	PFOS	0.485	0.699	0.937	**0.002**	0.310	0.394	**0.002**
PFBS vs	PFHS	0.937	0.818	0.240	0.132	0.485	0.485	0.240
	PFOS	0.589	0.240	0.589	**0.004**	0.132	0.818	**0.041**
PFHS vs	PFOS	0.394	0.589	0.818	0.180	0.699	0.937	0.394

Fecal bile acid composition was significantly changed after treatment with fenofibrate, PFBS and PFOS. Fenofibrate increased the percentage lithochate and hyodeoxycholate/ ursocholate (16.6% vs 10.6 % and 16.4% vs 6.6%, respectively) and decreased w-muricholate content (18.5% vs 23.5%). PFBS only increased hyodeoxycholate/ ursocholate content significantly (12.5% vs 6.6%), while PFOS decreased the percentage of lithocholate and b-muricholate (6.3% vs 10.6% and 14.1% vs 25.5% respectively).

Figure 4.1.13d Total fatty acid excretion



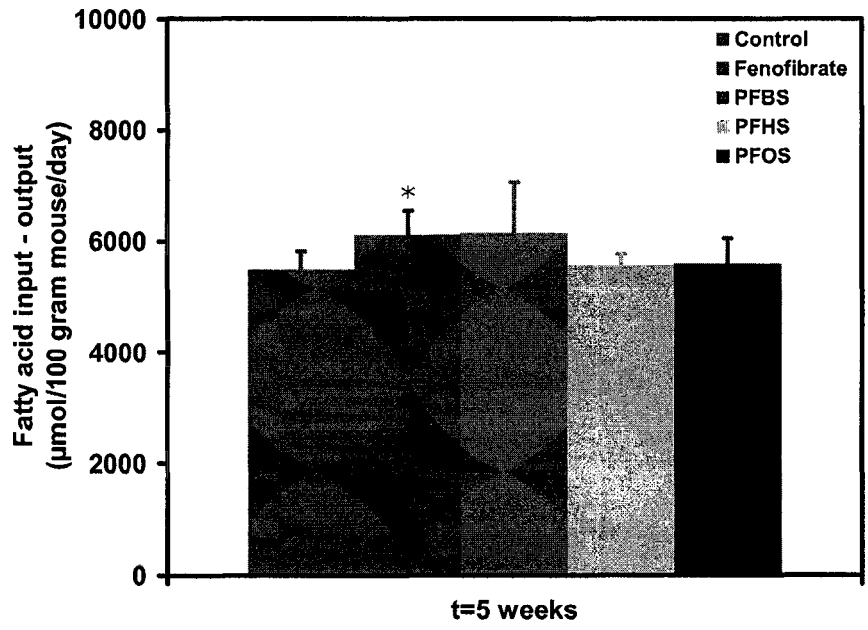
* p<0.05 vs. control

Fecal fatty acids (%)		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Control	avg	2.7	46.5	0.68	42.7	6.7	0.75	0.03
	sd	0.5	1.4	0.07	2.5	1.1	0.08	0.04
Fenofibrate	avg	2.2	46.3	0.62	43.9	6.2	0.66	0.06
	sd	0.3	2.7	0.05	2.5	1.2	0.09	0.03
PFBS	avg	2.6	48.0	0.65	41.8	6.3	0.62	0.03
	sd	0.2	0.7	0.03	1.4	0.7	0.03	0.02
PFHS	avg	2.6	46.8	0.86	41.0	7.9	0.73	0.02
	sd	0.8	4.1	0.24	4.5	0.6	0.10	0.04
PFOS	avg	2.4	46.3	0.69	43.1	6.8	0.74	0.05
	sd	0.4	1.9	0.07	3.0	0.8	0.04	0.03

Fecal fatty acids: P-value		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Between groups		0.121	0.192	0.377	0.159	0.053	**0.034**	0.145
Control vs:	Fenofibrate	0.093	0.937	0.240	0.310	0.485	0.093	0.310
	PFBS	0.589	0.041	0.394	0.699	0.485	**0.002**	0.937
	PFHS	0.818	0.180	0.310	0.065	**0.041**	0.937	0.485
	PFOS	0.240	0.937	0.699	1.000	0.937	0.818	0.589
Fenofibrate vs	PFBS	**0.041**	0.132	0.180	0.093	0.589	0.818	0.065
	PFHS	0.093	0.394	**0.310**	0.180	**0.065**	0.310	**0.041**
	PFOS	0.485	0.818	0.132	0.589	0.180	0.065	0.589
PFBS vs	PFHS	0.180	0.937	0.394	0.065	**0.015**	0.180	0.132
	PFOS	0.240	0.065	0.394	0.589	0.310	**0.002**	0.310
PFHS vs	PFOS	0.180	0.310	0.394	0.093	**0.026**	0.818	0.132

Although changes in fatty acid composition were small, PFBS decreased the percentage of C18:2 (0.62% vs 0.75%) and PFHS increased the percentage of C18:1 (7.9% vs 6.7%) significantly. No effects of fenofibrate or PFOS were seen.

Figure 4.1.13e Fatty acid balance



* p<0.05 vs. control

Fatty acid balance (µmol/100 gram mouse/day)		Fatty acid input-output
Control	avg	5492
	sd	326
Fenofibrate	avg	6114
	sd	436
PFBS	avg	6146
	sd	910
PFHS	avg	5565
	sd	209
PFOS	avg	5593
	sd	458

Fatty acid balance: P-value		Fatty acid input-output
Between groups		0.096
Control vs:	Fenofibrate	**0.015**
	PFBS	0.240
	PFHS	0.589
	PFOS	0.589
Fenofibrate vs	PFBS	0.589
	PFHS	**0.015**
	PFOS	0.240
PFBS vs	PFHS	0.180
	PFOS	0.310
PFHS vs	PFOS	0.937

The fatty acid balance (fatty acid input – output) was significantly increased by fenofibrate treatment (by 11% p=0.015). The perfluor alkyl sulfonate compounds were not significantly changed.

4.1.14 VLDL-triglycerides and de novo ApoB production

Values are absolute values (mmol/L for plasma triglycerides, µmol/h for VLDL-TG production rate, 10^4 dpm/ml/h for de novo ApoB synthesis, µmol/10^4 dpm for TG production per de novo synthesized ApoB and µmol/10^4 dpm for lipid composition per de novo synthesized ApoB) and are means ± S.D. from 7-8 mice per group. Individual values are given in appendix XIV.

Plasma triglycerides (mmol/L)		Time after Triton WR1339 injection (min)				
		0	15	30	60	90
Control	avg	1.4	2.6	4.1	6.4	8.7
	sd	0.4	0.5	0.7	1.0	1.4
Fenofibrate	avg	0.5	2.0	4.0	7.6	11.1
	sd	0.3	0.5	0.5	1.0	1.5
PFBS	avg	0.8	2.1	3.6	6.1	8.9
	sd	0.3	0.6	0.8	1.0	1.5
PFHS	avg	0.3	0.6	1.0	1.6	2.2
	sd	0.1	0.2	0.3	0.5	0.7
PFOS	avg	0.5	0.6	0.9	1.2	1.6
	sd	0.2	0.2	0.3	0.4	0.5

Plasma triglycerides: P-value		Time after Triton WR1339 injection (min)				
		0	15	30	60	90
Between groups		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Control vs:	Fenofibrate	**0.002**	0.072	0.955	**0.029**	**0.006**
	PFBS	**0.021**	0.094	0.336	0.694	0.955
	PFHS	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	PFOS	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fenofibrate vs	PFBS	0.065	0.798	0.195	**0.021**	**0.028**
	PFHS	0.195	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	PFOS	0.878	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PFBS vs	PFHS	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	PFOS	**0.028**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PFHS vs	PFOS	**0.028**	0.959	0.505	0.130	0.105

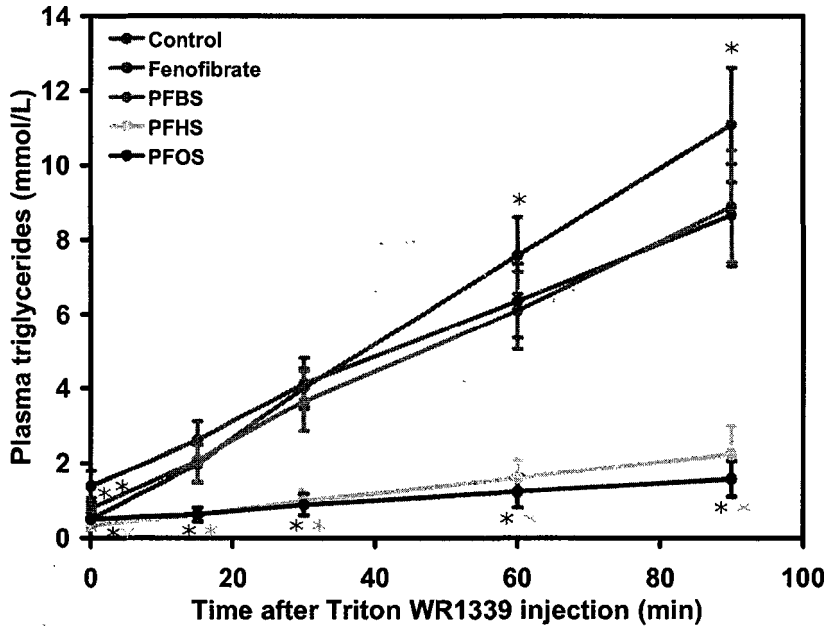
VLDL-TG production rate (µmol/h)		production rate
Control	avg	6.3
	sd	1.4
Fenofibrate	avg	9.3
	sd	1.7
PFBS	avg	6.9
	sd	1.5
PFHS	avg	1.7
	sd	0.7
PFOS	avg	0.9
	sd	0.4

VLDL-TG production rate: P-value		production rate
Between groups		**<0.001**
Control vs:	Fenofibrate	**0.006**
	PFBS	0.536
	PFHS	**<0.001**
	PFOS	**<0.001**
Fenofibrate vs	PFBS	**0.021**
	PFHS	**<0.001**
	PFOS	**<0.001**
PFBS vs	PFHS	**<0.001**
	PFOS	**<0.001**
PFHS vs	PFOS	**0.028**

At t=0 min (before Triton WR1339 injection) fenofibrate, PFHS, PFOS and to a lesser extent PFBS treatment decreased plasma triglycerides levels (in line with the plasma samples, taken at 4 weeks and 6 weeks of treatment). The increase in plasma triglycerides was more or less the same in the control group and the PFBS group. Fenofibrate increased VLDL-triglycerides to a higher extent than the control group. Both PFHS and PFOS showed a significantly reduced VLDL triglycerides production.

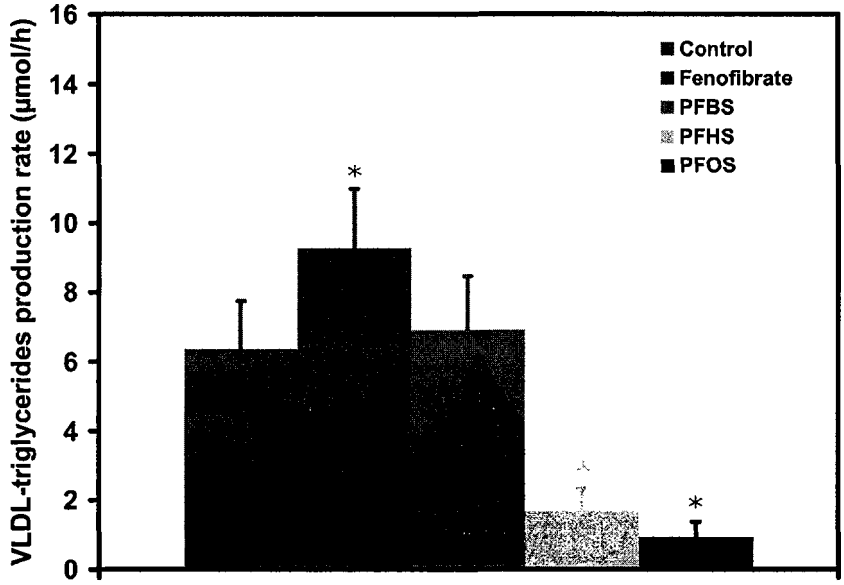
In figure 4.1.14b the VLDL-triglycerides production rate (=slope figure 4.1.14a) is shown. Fenofibrate significantly increased the production rate by +46%. PFHS and PFOS strongly decrease TG production rate, respectively by -74% and -86%, whereas PFBS showed no effects. PFHS and PFOS decreased VLDL-TG production rate as compared to PFBS ($p<0.001$ for both treatments).

Figure 4.1.14a Plasma triglycerides after Triton WR1339 injection



* p<0.05 vs. control

Figure 4.1.14b VLDL-triglycerides production rate



* p<0.05 vs. control

De Novo ApoB synthesis (10^4 dpm/mL/h)		ApoB synthesis
Control	avg	6.1
	sd	1.0
Fenofibrate	avg	6.4
	sd	0.5
PFBS	avg	5.1
	sd	0.9
PFHS	avg	1.5
	sd	0.3
PFOS	avg	0.8
	sd	0.2

De Novo ApoB synthesis: P-value		ApoB synthesis
Between groups		**<0.001**
Control vs:	Fenofibrate	0.463
	PFBS	0.072
	PFHS	**<0.001**
	PFOS	**<0.001**
Fenofibrate vs	PFBS	**0.005**
	PFHS	**<0.001**
	PFOS	**<0.001**
PFBS vs	PFHS	**<0.001**
	PFOS	**<0.001**
PFHS vs	PFOS	**0.001**

TG production per de novo synthesized ApoB (µmol/10^4 dpm)		TG production per ApoB
Control	avg	0.80
	sd	0.09
Fenofibrate	avg	1.12
	sd	0.19
PFBS	avg	1.09
	sd	0.21
PFHS	avg	0.86
	sd	0.24
PFOS	avg	0.86
	sd	0.20

TG production per de novo synthesized ApoB: P-value		TG production per ApoB
Between groups		**0.007**
Control vs:	Fenofibrate	**<0.001**
	PFBS	**0.014**
	PFHS	0.397
	PFOS	0.536
Fenofibrate vs	PFBS	0.798
	PFHS	0.065
	PFOS	**0.010**
PFBS vs	PFHS	0.065
	PFOS	0.065
PFHS vs	PFOS	0.878

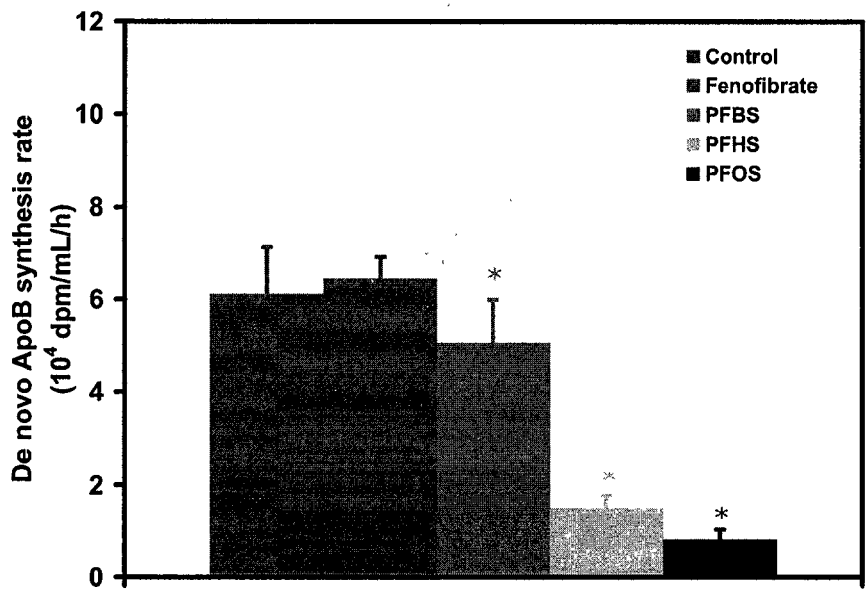
Fenofibrate showed no effects on de novo ApoB synthesis rate (figure 4.1.14c). Since fenofibrate increased VLDL-triglycerides production, the newly synthesized VLDL-triglycerides per ApoB were plotted in figure 4.1.14d to examine TG content of newly synthesized VLDL.

Fenofibrate showed triglycerides-rich newly synthesized ApoB particles (increase in triglycerides of +40% as compared to the control). PFBS showed a decreased ApoB synthesis (by -17%) and an increase in triglycerides-rich newly synthesized particles (increase in triglycerides of +36% as compared to the control).

Both PFHS and PFOS strongly inhibited ApoB synthesis (respectively by -76% and -87%), resulting in newly synthesized VLDL particles with similar triglycerides content as the control group.

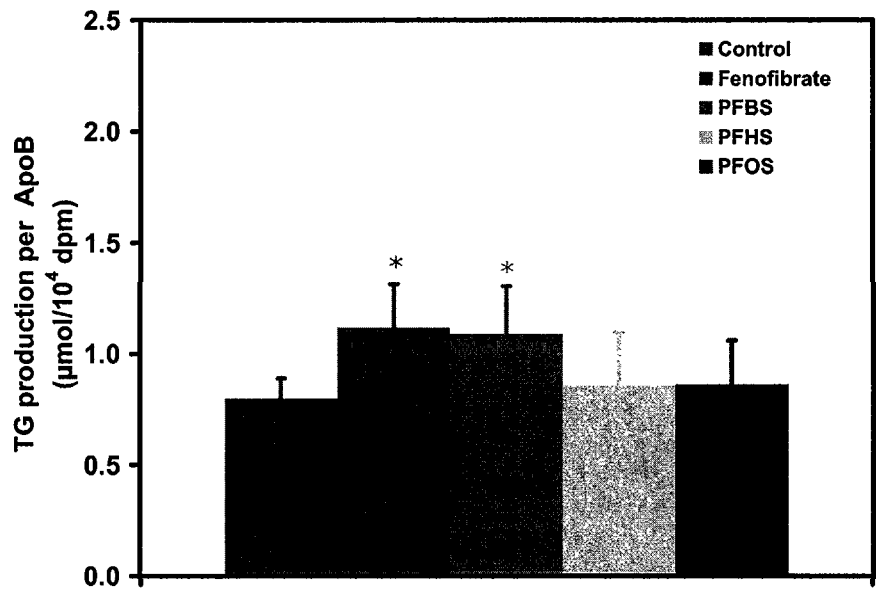
PFHS and PFOS had a significantly reduced de novo ApoB synthesis rate and TG production per ApoB as compared to PFBS.

Figure 4.1.14c De novo ApoB synthesis



* p<0.05 vs. control

Figure 4.1.14d TG production per ApoB



* p<0.05 vs. control

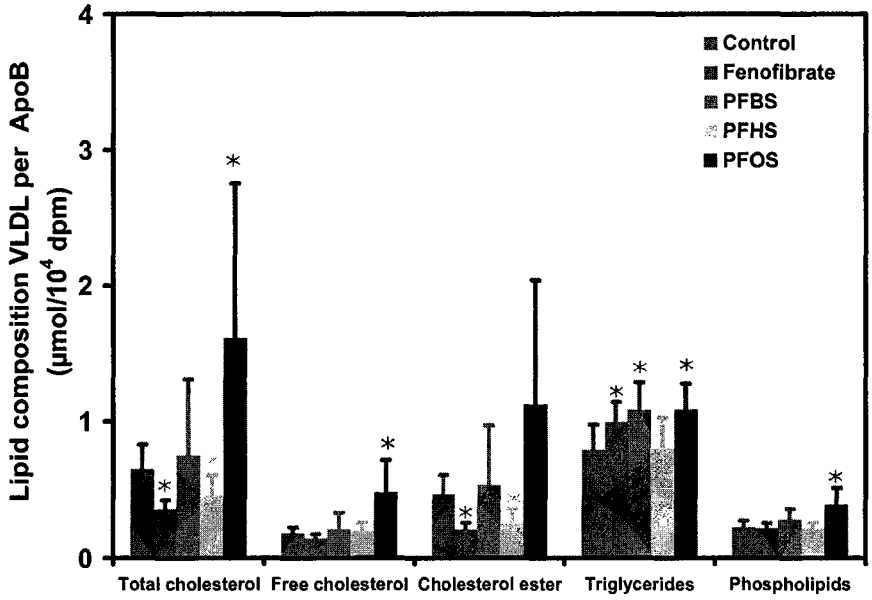
Lipid composition VLDL (μmol/10^4 dpm ApoB)		Total cholesterol	Free cholesterol	Cholesterol ester	Triglycerides	Phospholipids
Control	avg	0.65	0.18	0.47	0.79	0.23
	sd	0.18	0.04	0.14	0.19	0.05
Fenofibrate	avg	0.36	0.15	0.21	0.99	0.22
	sd	0.07	0.03	0.05	0.15	0.04
PFBS	avg	0.75	0.21	0.54	1.08	0.29
	sd	0.55	0.12	0.43	0.20	0.08
PFHS	avg	0.46	0.20	0.25	0.80	0.22
	sd	0.15	0.06	0.11	0.22	0.04
PFOS	avg	1.61	0.49	1.12	1.09	0.39
	sd	1.15	0.23	0.92	0.18	0.12

Lipid composition VLDL: P-value		Total cholesterol	Free cholesterol	Cholesterol ester	Triglycerides	Phospholipids
Between groups		**<0.001**	**<0.001**	**<0.001**	**0.012**	**0.001**
Control vs:	Fenofibrate	**0.001**	0.152	**0.001**	**0.040**	1.000
	PFBS	0.613	0.955	0.536	**0.014**	0.054
	PFHS	**0.029**	0.613	**0.006**	0.779	0.955
	PFOS	**0.014**	**<0.001**	0.094	**0.014**	**0.001**
Fenofibrate vs	PFBS	**0.001**	0.130	**<0.001**	0.382	**0.050**
	PFHS	0.065	**0.050**	0.442	0.083	1.000
	PFOS	**<0.001**	**<0.001**	**<0.001**	0.234	**<0.001**
PFBS vs	PFHS	**0.007**	0.574	**0.010**	**0.028**	0.083
	PFOS	**0.007**	**0.003**	0.065	1.000	**0.028**
PFHS vs	PFOS	**<0.001**	**<0.001**	**0.001**	**0.021**	**<0.001**

Lipid composition of isolated VLDL (isolated VLDL is the sum of newly synthesized VLDL and VLDL present in plasma at t=0 min), calculated as µmol lipid per newly synthesized ApoB, was significantly different from control in all treatment groups. Fenofibrate decreased cholesterolester and increased triglycerides content, respectively by -55% and +26%. PFBS significantly increased triglycerides content by 37%, PFHS showed a decreased cholesterolester composition by -46%. PFOS treatment increased free cholesterol, triglycerides and phospholipids content in VLDL, respectively by +164%, +38% and +74%.

When compared to the PFHS group, the PFOS group showed an isolated VLDL with increased free cholesterol, triglycerides and phospholipids, indicative for larger particles. A compared to PFBS, PFHS induced smaller particles and PFOS larger particles.

Figure 4.1.14e Lipid composition VLDL



* p<0.05 vs. control

4.2 Results study 2

4.2.1 Markers of general well-being

No specific clinical signs were observed during the study. At sacrifice, besides the livers no macroscopic differences were observed between the groups. Livers were visibly somewhat larger in the fenofibrate group and strongly enlarged in the PFHS and PFOS group. One mouse (mouse 21) showed some white spots in the liver and bile stones in the bladder.

4.2.2 Body weight

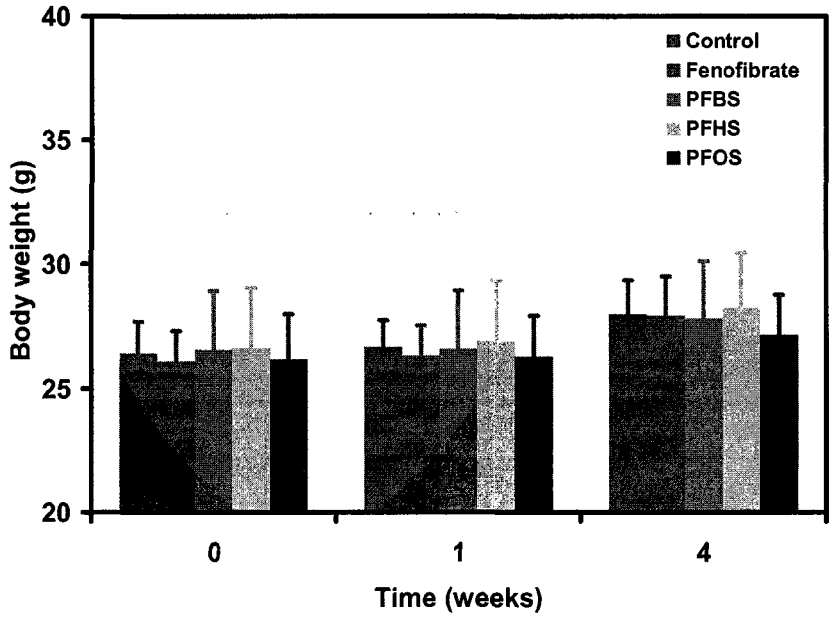
Values are absolute values (g) and are means ± S.D. from 6 mice per group. Individual values are given in appendix I.

Body weight (g)		time (weeks)		
		0	1	4
Control	avg	26.4	26.7	28.0
	sd	1.3	1.1	1.4
Fenofibrate	avg	26.1	26.3	27.9
	sd	1.2	1.2	1.6
PFBS	avg	26.6	26.6	27.8
	sd	2.3	2.3	2.3
PFHS	avg	26.6	26.9	28.2
	sd	2.4	2.4	2.2
PFOS	avg	26.2	26.3	27.2
	sd	1.8	1.6	1.6

Body weight: P-value		time (weeks)		
		0	1	4
Between groups		0.981	0.993	0.913
Control vs:	Fenofibrate	0.394	0.818	0.937
	PFBS	1.000	1.000	0.818
	PFHS	1.000	0.937	0.937
	PFOS	0.937	0.818	0.485
Fenofibrate vs	PFBS	0.818	0.937	0.818
	PFHS	0.818	0.818	0.937
	PFOS	0.937	0.937	0.589
PFBS vs	PFHS	1.000	0.937	0.699
	PFOS	0.818	0.818	0.699
PFHS vs	PFOS	0.699	0.699	0.485

During the study mice gained body weight in all groups. There were no significant differences between groups.

Figure 4.2.2 Body weight



* $p<0.05$ vs. control

4.2.3 Liver and perigonadal fat weight

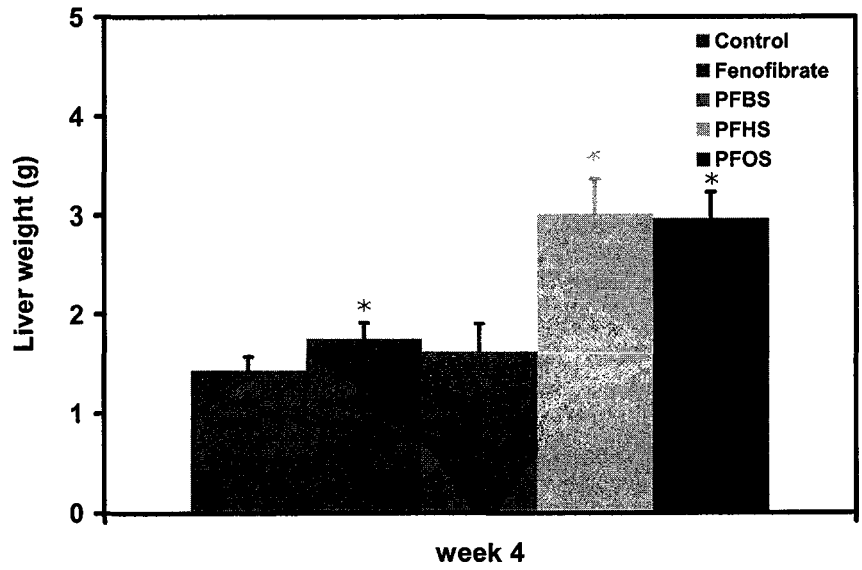
Values are absolute values (g) and are means ± S.D. from 6 mice per group. Individual values are given in appendix II.

Tissue weight (g)		Liver	Perigonadal fat
Control	avg	1.43	0.50
	sd	0.14	0.17
Fenofibrate	avg	1.76	0.58
	sd	0.16	0.12
PFBS	avg	1.63	0.58
	sd	0.28	0.09
PFHS	avg	3.01	0.40
	sd	0.35	0.11
PFOS	avg	2.97	0.41
	sd	0.26	0.12

Tissue weight: P-value		Liver	Perigonadal fat
Between groups		**<0.001**	**0.034**
Control vs:	Fenofibrate	**0.009**	0.240
	PFBS	0.310	0.132
	PFHS	**0.002**	0.310
	PFOS	**0.002**	0.485
Fenofibrate vs	PFBS	0.394	0.818
	PFHS	**0.002**	**0.026**
	PFOS	**0.002**	**0.041**
PFBS vs	PFHS	**0.002**	**0.015**
	PFOS	**0.002**	**0.026**
PFHS vs	PFOS	1.000	0.937

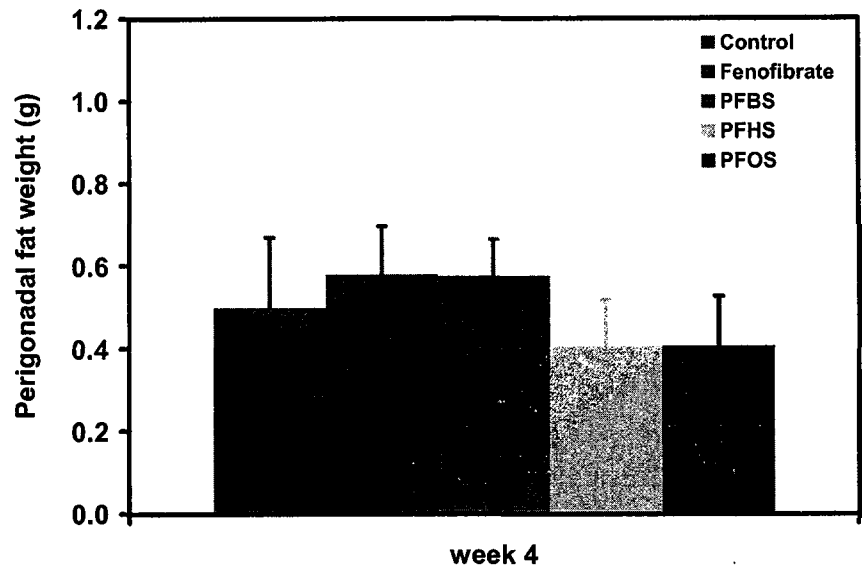
Liver weights were significantly increased in the fenofibrate, PFHS and PFOS group. After 4 weeks of treatment PFBS treatment did not result in significantly increased livers. Fenofibrate increased liver weights by +23%. The PFHS and PFOS groups showed a robust induction in liver weights, respectively by +110% and +107%. Although a slight decrease in perigonadal fat weight was seen after PFOS and PFHS treatment, this was not significantly different from the control group. Liver weights in the PFHS and PFOS groups were significantly higher as compared to the PFBS group, whereas weights of perigonadal fat were significantly decreased in the PFHS and PFOS groups.

Figure 4.2.3a Liver weight



* p<0.05 vs. control

Figure 4.2.3b Perigonadal fat weight



* p<0.05 vs. control

4.2.4 Food intake

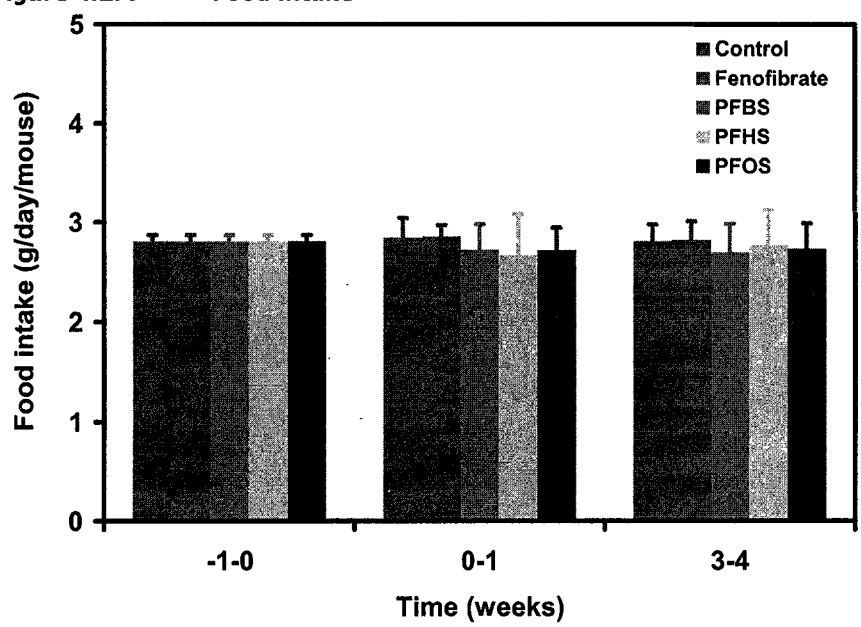
Values are absolute values (g/day/mouse) and are means ± S.D. or range from 2-3 cages per group. Individual values per cage are given in appendix III.

Food intake (g/day/mouse)		time (weeks)		
		-1-0	0-1	3-4
Control	avg	2.8	2.8	2.8
	sd/range	0.1	0.1	0.1
Fenofibrate	avg	2.8	2.9	2.8
	sd/range	0.1	0.1	0.1
PFBS	avg	2.8	2.7	2.7
	sd/range	0.1	0.2	0.2
PFHS	avg	2.8	2.7	2.8
	sd	0.1	0.4	0.4
PFOS	avg	2.8	2.7	2.7
	sd	0.1	0.2	0.2

Food intake: P-value		time (weeks)	
		0-1	3-4
Between groups		0.865	0.920
Control vs:	Fenofibrate	1.000	0.667
	PFBS	0.667	0.667
	PFHS	0.800	0.800
	PFOS	0.400	0.800
Fenofibrate vs	PFBS	0.667	0.667
	PFHS	0.800	1.000
	PFOS	0.800	0.800
PFBS vs	PFHS	1.000	0.800
	PFOS	1.000	0.800
PFHS vs	PFOS	1.000	1.000

All groups showed a similar food intake during the study.

Figure 4.2.4 Food intake



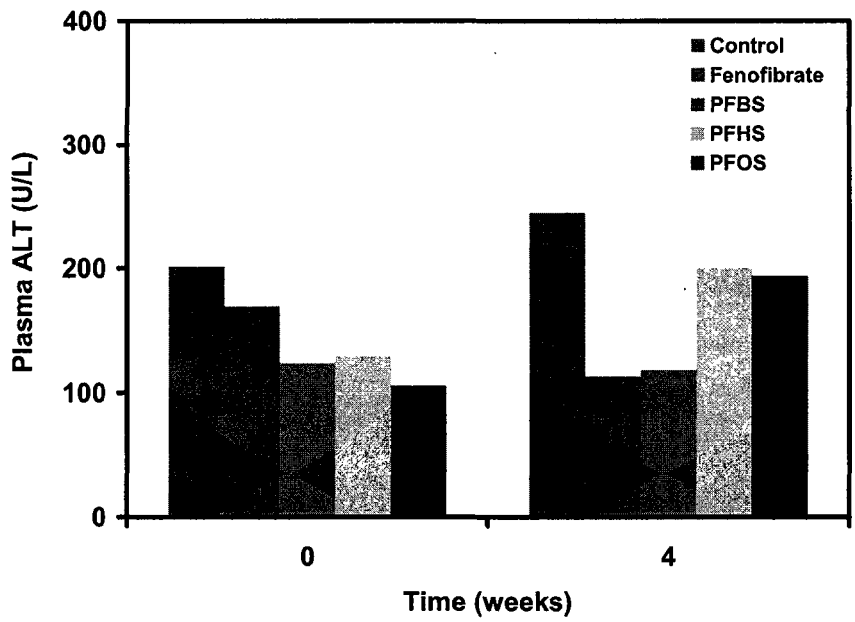
4.2.5 Plasma ALT

Values are absolute values (U/L) from measurements in pooled plasma samples per group (6 mice per group) at t=0 and 4.

Group	Plasma ALAT (U/L)	
	t=0 weeks	t=4 weeks
Control	201	245
Fenofibrate	169	113
PFBS	123	118
PFHS	129	200
PFOS	105	194

At t=0 weeks the control group showed a higher ALT level as compared to the other groups. After 4 weeks of treatment, the control group still had the highest plasma ALT level, but PFHS and PFOS showed, with respect to t=0 levels, a higher increase in plasma ALT.

Figure 4.2.5 Plasma ALT



4.2.6 Plasma cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 6 mice per group. Individual values are given in appendix IV.

Cholesterol (mmol/L)		time (weeks)	
		0	4
Control	avg	8.4	7.6
	sd	1.3	1.2
Fenofibrate	avg	8.4	4.9
	sd	2.9	0.5
PFBS	avg	8.7	6.3
	sd	1.9	1.7
PFHS	avg	8.5	2.5
	sd	2.7	0.7
PFOS	avg	8.6	3.0
	sd	2.3	0.7

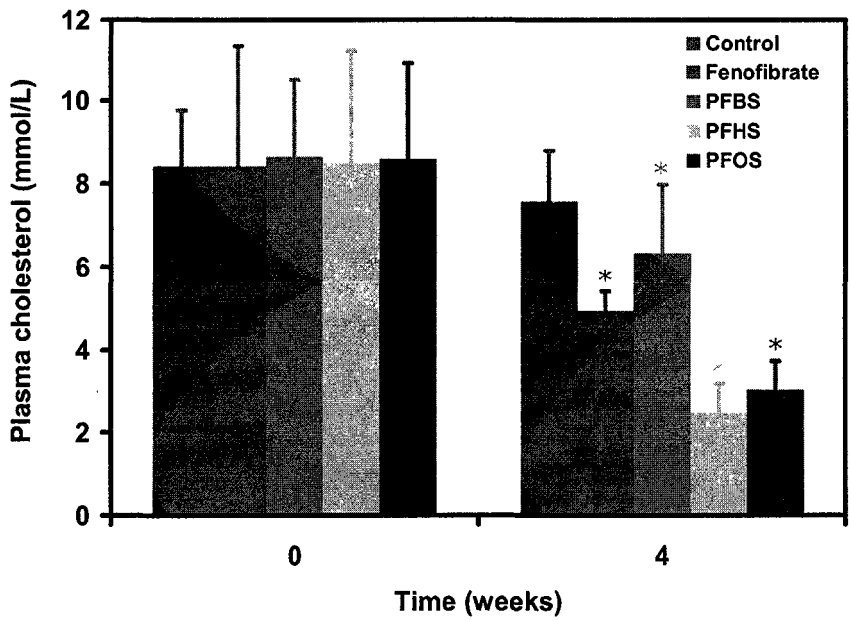
Cholesterol: P-value		time (weeks)	
		0	4
Between groups		0.985	**<0.001**
Control vs:	Fenofibrate	0.818	**0.002**
	PFBS	0.394	**0.041**
	PFHS	0.589	**0.002**
	PFOS	1.000	**0.002**
Fenofibrate vs	PFBS	1.000	0.065
	PFHS	0.937	**0.002**
	PFOS	0.937	**0.002**
PFBS vs	PFHS	0.818	**0.002**
	PFOS	0.699	**0.002**
PFHS vs	PFOS	0.818	0.180

As was seen in study 1, all treatment groups showed a significant reduction in plasma cholesterol at t=4 weeks of treatment. Fenofibrate decreased plasma cholesterol by -35%.

PFBS, PFHS and PFOS treatment reduced plasma cholesterol levels respectively by -16%, -67% and -60% after 4 weeks of treatment.

PFHS and PFOS decreased cholesterol levels to a larger extent than PFBS (p=0.002). PFHS and PFOS showed a similar inhibition.

Figure 4.2.6 Plasma cholesterol



* p<0.05 vs. control

4.2.7 Plasma HDL-cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 6 mice per group. Individual values are given in appendix V.

HDL-Cholesterol (mmol/L)		time (weeks)	
		0	4
Control	avg	0.96	1.22
	sd	0.47	0.29
Fenofibrate	avg	1.12	2.39
	sd	0.68	0.24
PFBS	avg	1.10	1.72
	sd	0.53	0.37
PFHS	avg	1.03	0.80
	sd	0.37	0.12
PFOS	avg	1.07	0.58
	sd	0.43	0.10

HDL-Cholesterol: P-value		time (weeks)	
		0	4
Between groups		1.000	**<0.001**
Control vs:	Fenofibrate	0.937	**0.002**
	PFBS	1.000	**0.041**
	PFHS	0.937	**0.026**
	PFOS	0.937	**0.002**
Fenofibrate vs	PFBS	0.937	**0.015**
	PFHS	1.000	**0.002**
	PFOS	0.937	**0.002**
PFBS vs	PFHS	0.937	**0.002**
	PFOS	1.000	**0.002**
PFHS vs	PFOS	0.937	**0.009**

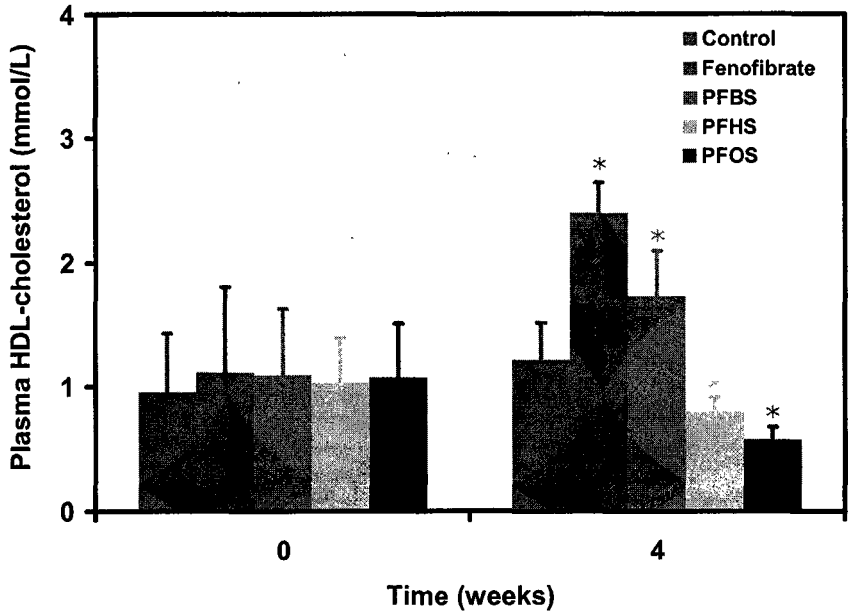
HDL-cholesterol levels were increased after 4 weeks treatment in the fenofibrate group, by +97%. In contrast to experiment 1, PFBS treatment showed a significantly increased plasma HDL-cholesterol, by +42%.

Both PFHS and PFOS significantly decreased HDL-cholesterol after 4 weeks of treatment, by -34% and -54%, respectively.

When comparing PFHS and PFOS treatment, PFOS significantly decreased HDL-cholesterol to a larger extent (p=0.009).

PFHS and PFOS significantly decreased HDL as compared to PFBS.

Figure 4.2.7 Plasma HDL-cholesterol



* $p<0.05$ vs. control

4.2.8 Plasma triglycerides

Values are absolute values (mmol/L) and are means ± S.D. from 6 mice per group. Individual values are given in appendix VI.

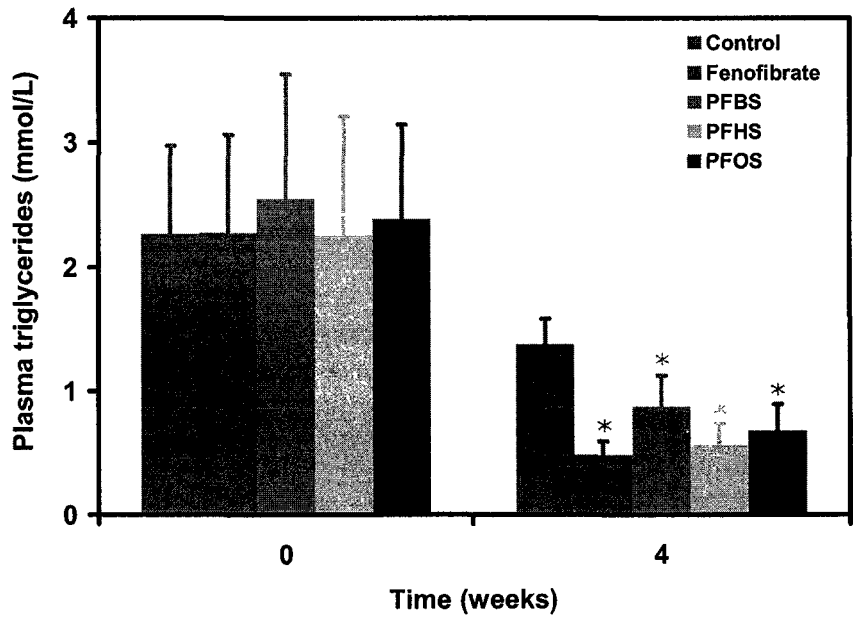
Triglycerides (mmol/L)		time (weeks)	
		0	4
Control	avg	2.26	1.37
	sd	0.71	0.21
Fenofibrate	avg	2.27	0.48
	sd	0.79	0.12
PFBS	avg	2.54	0.87
	sd	1.02	0.25
PFHS	avg	2.25	0.56
	sd	0.96	0.17
PFOS	avg	2.39	0.68
	sd	0.76	0.21

Triglycerides: P-value		time (weeks)	
		0	4
Between groups		0.991	**0.001**
Control vs:	Fenofibrate	1.000	**0.002**
	PFBS	0.818	**0.004**
	PFHS	1.000	**0.002**
	PFOS	0.699	**0.004**
Fenofibrate vs	PFBS	0.818	**0.009**
	PFHS	0.937	0.485
	PFOS	0.818	**0.041**
PFBS vs	PFHS	0.818	**0.026**
	PFOS	0.937	0.180
PFHS vs	PFOS	0.818	0.310

All treatment groups showed significant reductions in plasma triglycerides at t=4 weeks of treatment. Fenofibrate decreased plasma triglycerides by -65%.

PFBS, PFHS and PFOS treatment reduced plasma triglycerides levels respectively by -37%, -59% and -50% after 4 weeks of treatment. PFHS decreased triglycerides levels to a larger extent than PFBS (p=0.026). PFOS showed no significantly different triglycerides levels as compared with PFHS and PFBS.

Figure 4.2.8 Plasma triglycerides



* p<0.05 vs. control

4.2.9 Lipoprotein profiles

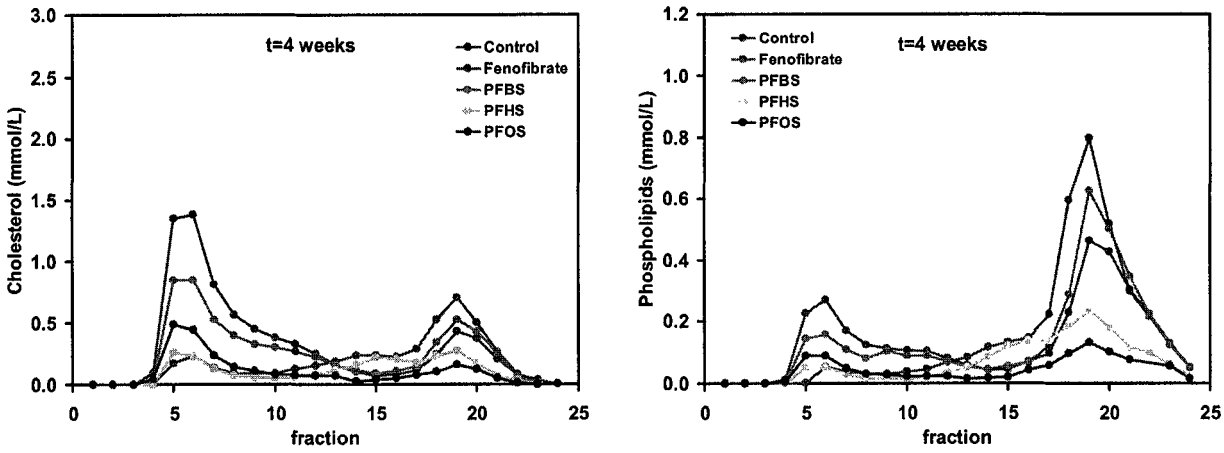
Lipoproteins were separated on a superose column. Values are absolute values (mM) from cholesterol (left column) and phospholipids (right column) measurements in pooled plasma per group (with 6 mice per group) at t=4. We consider fractions 4-8 as VLDL; 9-14 as LDL; 13-16 as large-HDL (HDL1) and 17-24 as HDL.

The profiles are more or less the same as in experiment 1. After 4 weeks of treatment, plasma cholesterol levels were decreased in the VLDL-LDL peak in all groups as compared to the control group. Fenofibrate and PFHS showed the strongest and a similar reduction, PFOS seemed to decrease VLDL to a lesser extent than PFHS. PFBS decreased VLDL to a lesser extend than PFHS and PFOS. HDL was increased in the fenofibrate group, with the appearance of a large HDL1 particle.

Whereas PFBS seemed to increase HDL somewhat, both PFHS and PFOS strongly decreased the HDL peak. PFHS seemed to decrease HDL to a lesser extent than PFOS and in the profile of the PFHS group HDL1 (large HDL) was increased.

The changes in the lipoproteins (ApoB-containing proteins (VLDL-LDL) and HDL) are in line with the changes in plasma cholesterol and HDL.

Figure 4.2.9 Lipoprotein profiles



4.2.10 Biliary bile acids, cholesterol and phospholipids production

Values are absolute values (µL/min/kg mouse for bile flow, nmol/min/kg mouse for biliary bile acids, phospholipids and cholesterol) and are means ± S.D. from 5-7 mice per group. Individual values are given in appendix XV.

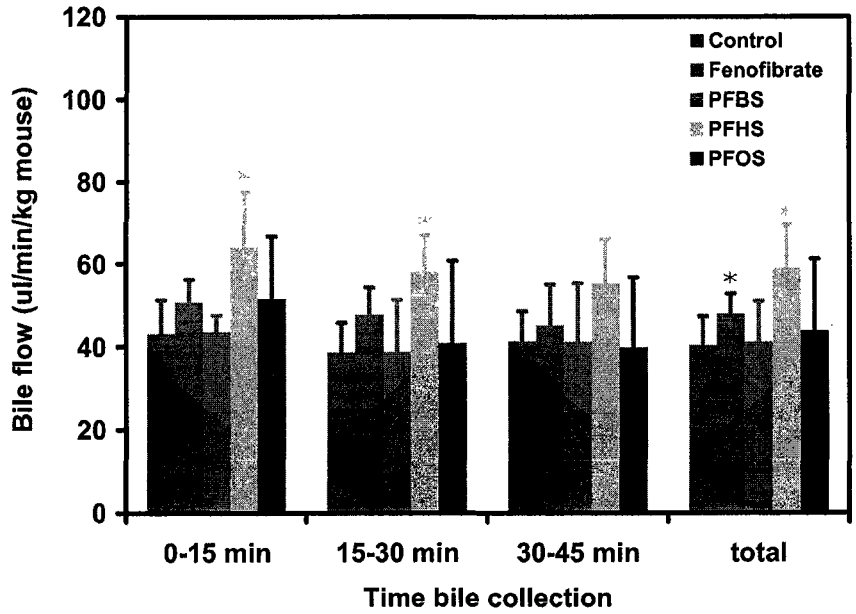
Bile flow (µl/min/kg mouse)		time			
		15 min	30 min	45 min	total
Control	avg	43.2	38.7	41.3	40.3
	sd	7.8	7.1	7.1	6.8
Fenofibrate	avg	50.5	47.4	45.2	47.7
	sd	5.5	6.6	9.5	4.8
PFBS	avg	43.7	38.8	41.2	41.2
	sd	3.6	12.2	13.8	9.4
PFHS	avg	63.9	58.0	54.9	58.9
	sd	13.6	8.9	11.0	10.4
PFOS	avg	51.3	40.8	39.9	44.0
	sd	15.3	19.7	16.6	17.0

Bile flow: P-value		time bile collection			
		0-15 min	15-30 min	30-45 min	total
Between groups		**0.035**	**0.040**	0.245	**0.040**
Control vs:	Fenofibrate	0.329	0.177	0.394	**0.041**
	PFBS	0.690	0.841	0.429	0.931
	PFHS	**0.017**	**0.009**	0.093	**0.004**
	PFOS	0.343	1.000	0.628	1.000
Fenofibrate vs	PFBS	0.126	0.177	0.429	0.126
	PFHS	**0.026**	**0.026**	0.180	**0.041**
	PFOS	1.000	0.445	0.445	0.731
PFBS vs	PFHS	**0.009**	0.052	0.177	**0.030**
	PFOS	0.530	1.000	0.755	1.000
PFHS vs	PFOS	0.101	**0.035**	0.101	0.101

Although bile flow was somewhat increased in the fenofibrate group at all collection points, this was not significantly different from the control group. However, fenofibrate did significantly increase the resulting total bile flow (by 18%, p=0.041).

PFHS treatment increased bile flow, this was significantly different from the control group for bile collected 0-15 min and 15-30 min after the cannula was placed (by +48 and +50%, respectively). The resulting total bile flow was also significantly increased in the PFHS group (by +46%). PFBS and PFOS showed no effects on bile flow.

Figure 4.2.10a Bile flow



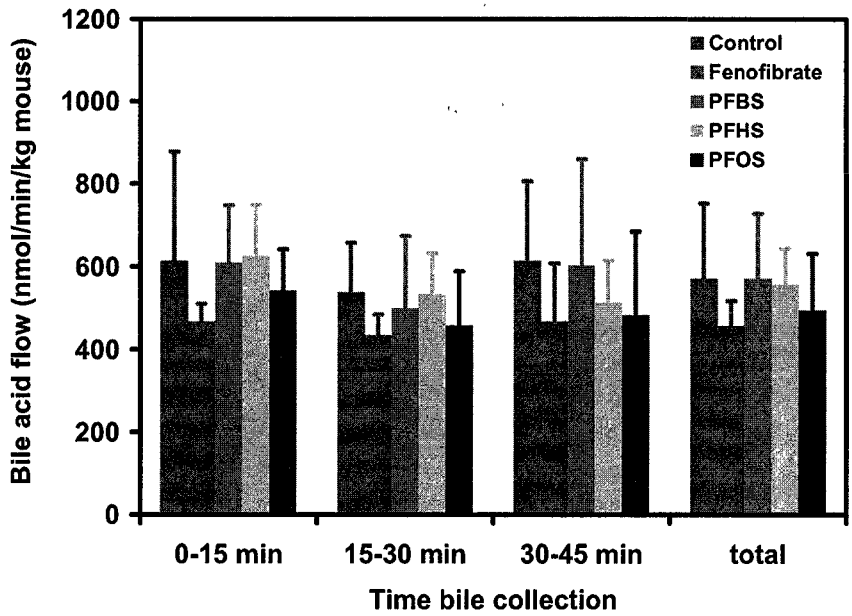
* p<0.05 vs. control

Bile acid flow (nmol/min/kg mouse)		time			
		15 min	30 min	45 min	total
Control	avg	614	539	615	573
	sd	262	119	189	179
Fenofibrate	avg	468	436	469	457
	sd	43	48	140	60
PFBS	avg	610	500	603	571
	sd	137	173	255	156
PFHS	avg	625	535	514	558
	sd	123	96	101	85
PFOS	avg	543	459	483	495
	sd	100	130	201	136

Bile acid flow: P-value		time bile collection			
		0-15 min	15-30 min	30-45 min	total
Between groups		0.284	0.489	0.498	0.379
Control vs:	Fenofibrate	0.329	0.126	0.180	0.240
	PFBS	1.000	0.690	0.931	0.792
	PFHS	0.931	0.931	0.394	0.937
	PFOS	0.876	0.432	0.181	0.445
Fenofibrate vs	PFBS	0.126	0.537	0.429	0.126
	PFHS	0.065	0.180	0.485	0.093
	PFOS	0.181	0.945	0.945	0.945
PFBS vs	PFHS	**0.031**	0.931	0.931	0.429
	PFOS	0.343	0.639	0.343	0.530
PFHS vs	PFOS	0.234	0.234	0.534	0.295

Although fenofibrate and to a lesser extent PFHS and PFOS seemed to decrease bile acid flow, this was not significantly different from the control group. PFBS showed the same bile acid flow as the control group.

Figure 4.2.10b Bile acid flow



* p<0.05 vs. control

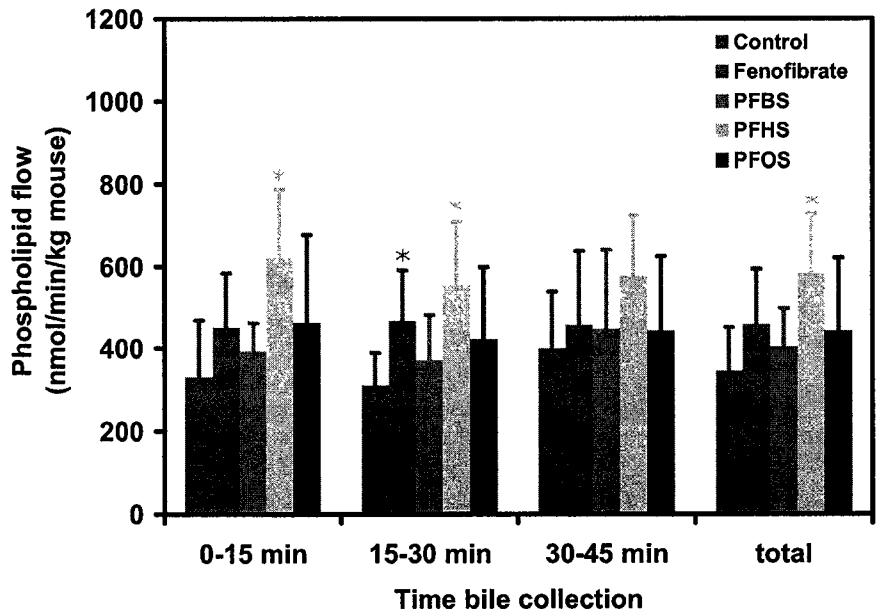
Phospholipid flow (nmol/min/kg mouse)		time			
		15 min	30 min	45 min	total
Control	avg	330	310	400	346
	sd	139	79	139	105
Fenofibrate	avg	451	467	457	459
	sd	132	123	180	136
PFBS	avg	393	372	448	405
	sd	69	109	192	93
PFHS	avg	620	553	576	583
	sd	166	153	148	144
PFOS	avg	463	422	443	443
	sd	214	176	181	178

Phospholipid flow: P-value		time bile collection			
		0-15 min	15-30 min	30-45 min	total
Between groups		0.058	**0.048**	0.370	0.076
Control vs:	Fenofibrate	0.247	**0.030**	0.394	0.180
	PFBS	0.310	0.548	0.931	0.429
	PFHS	**0.017**	**0.004**	0.132	**0.009**
	PFOS	0.149	0.268	0.836	0.295
Fenofibrate vs	PFBS	0.247	0.429	0.792	0.792
	PFHS	0.132	0.310	0.310	0.132
	PFOS	0.628	0.366	0.731	0.534
PFBS vs	PFHS	**0.030**	0.126	0.126	0.052
	PFOS	0.876	0.639	0.876	0.876
PFHS vs	PFOS	0.101	0.101	0.138	0.138

Although biliary phospholipid flow was somewhat increased in the fenofibrate group at all collection points, this was only different from the control group at t=15-30 min (by +51% , p=0.030). The calculated total phospholipid flow, however, was not significantly changed.

PFHS treatment increased phospholipid flow, this was significantly different from the control group for bile collected 0-15 min and 15-30 min after the cannula was placed (by +88 and +78% respectively. The resulting total phospholipid flow was also significantly increased in the PFHS group (by +68%). PFBS and PFOS showed no effects on biliary phospholipid flow.

Figure 4.2.10c Phospholipid flow



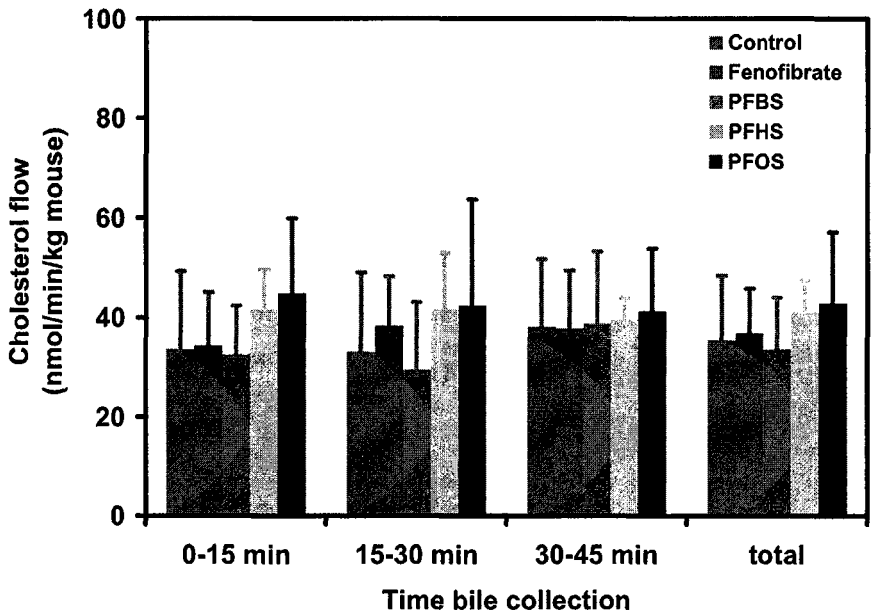
* p<0.05 vs. control

Cholesterol (nmol/min/kg mouse)		time			
		15 min	30 min	45 min	total
Control	avg	33.6	33.0	38.1	35.4
	sd	15.7	16.1	13.7	13.0
Fenofibrate	avg	34.3	38.3	37.8	36.8
	sd	10.8	9.9	11.6	9.0
PFBS	avg	32.5	29.4	38.8	33.6
	sd	9.8	13.7	14.5	10.4
PFHS	avg	41.8	41.6	39.6	41.0
	sd	7.9	11.5	4.4	6.6
PFOS	avg	44.9	42.4	41.2	42.8
	sd	15.1	21.3	12.7	14.4

Cholesterol flow: P-value		time bile collection			
		0-15 min	15-30 min	30-45 min	total
Between groups		0.284	0.386	0.984	0.398
Control vs:	Fenofibrate	0.792	0.429	1.000	0.589
	PFBS	0.841	0.841	1.000	0.662
	PFHS	0.247	0.177	0.818	0.240
	PFOS	0.268	0.432	0.731	0.295
Fenofibrate vs	PFBS	0.931	0.126	1.000	0.429
	PFHS	0.093	0.699	0.937	0.310
	PFOS	0.234	0.945	0.628	0.295
PFBS vs	PFHS	0.177	0.126	0.792	0.247
	PFOS	0.202	0.343	0.755	0.268
PFHS vs	PFOS	0.628	0.836	0.945	0.945

For biliary cholesterol flow, no significant changes were seen between groups.

Figure 4.2.10d Cholesterol flow



* p<0.05 vs. control

4.2.11 In vivo clearance of VLDL-like TG-rich particles and uptake in tissues

Values are relative values (% of injected dose for plasma decay and uptake in different tissues) and absolute values (min for half life) and are means ± S.D. from 4-6 mice per group. Individual values are given in appendix XVI.

[^{3}H]-triolein decay in plasma (% of injected dose)		Time after injection of [^{3}H]-triolein labeled VLDL-like particles (min)					
		0	2	5	10	20	30
Control	avg	100.0	98.2	90.5	76.8	54.7	37.4
	sd	0.0	6.6	8.8	6.0	6.8	9.9
Fenofibrate	avg	100.0	78.1	63.8	35.0	11.5	5.1
	sd	0.0	13.6	13.9	14.1	7.4	2.5
PFBS	avg	100.0	93.0	81.1	55.4	28.0	13.8
	sd	0.0	7.6	6.9	10.4	11.4	7.2
PFHS	avg	100.0	80.4	62.2	37.6	16.5	7.6
	sd	0.0	8.5	9.0	12.4	6.1	3.2
PFOS	avg	100.0	78.6	61.6	42.4	21.7	12.0
	sd	0.0	8.5	4.0	6.2	5.5	3.8

[^{3}H]-triolein decay in plasma: P-value		Time after injection of [^{3}H]-triolein labeled VLDL-like particles (min)				
		2	5	10	20	30
Between groups		**0.019**	**0.004**	**0.004**	**0.002**	**0.001**
Control vs:	Fenofibrate	0.056	**0.016**	**0.008**	**0.008**	**0.008**
	PFBS	0.286	0.190	**0.016**	**0.016**	**0.032**
	PFHS	**0.004**	**0.004**	**0.004**	**0.004**	**0.004**
	PFOS	**0.017**	**0.004**	**0.004**	**0.004**	**0.004**
Fenofibrate vs	PFBS	0.190	0.111	0.111	0.063	**0.032**
	PFHS	1.000	0.931	0.931	0.177	0.177
	PFOS	0.931	0.931	0.247	0.052	**0.017**
PFBS vs	PFHS	0.067	**0.019**	0.067	0.171	0.114
	PFOS	**0.038**	**0.010**	0.114	0.476	0.762
PFHS vs	PFOS	0.818	0.937	0.589	0.180	**0.041**

[^{3}H]-triolein half-life (min)		Half-life
Control	avg	21.0
	sd	6.2
Fenofibrate	avg	6.8
	sd	0.9
PFBS	avg	10.2
	sd	3.3
PFHS	avg	8.1
	sd	1.3
PFOS	avg	10.4
	sd	1.9

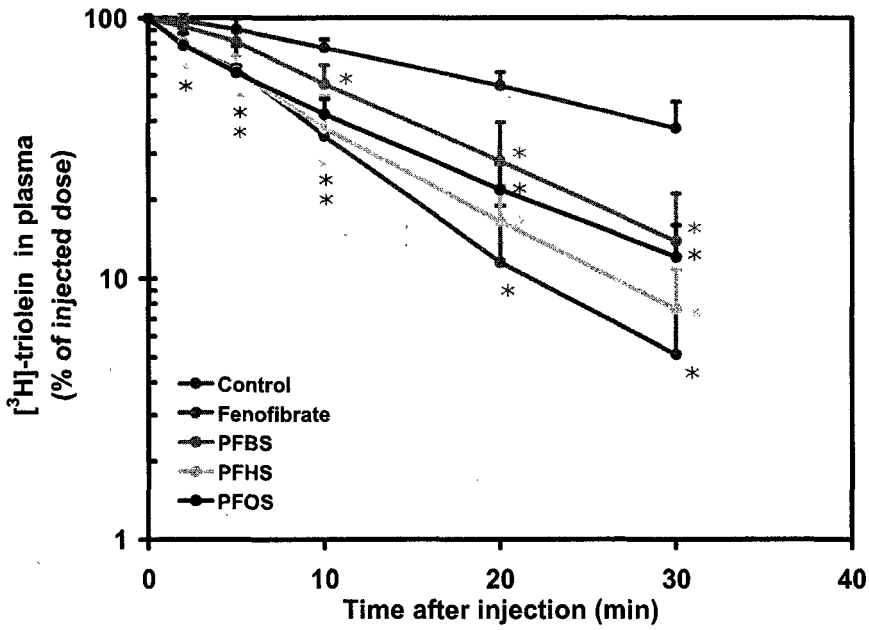
[^{3}H]-triolein half-life: P-value		Half-life
Between groups		**0.001**
Control vs:	Fenofibrate	**0.008**
	PFBS	**0.032**
	PFHS	**0.004**
	PFOS	**0.004**
Fenofibrate vs	PFBS	0.063
	PFHS	0.082
	PFOS	**0.009**
PFBS vs	PFHS	0.352
	PFOS	0.914
PFHS vs	PFOS	**0.041**

The clearance of VLDL-like [^{3}H]-triolein labeled particles in plasma (as a measure for VLDL-TG clearance) was significantly increased in all treatment groups (figure 4.2.11a). Fenofibrate showed the strongest clearance, followed by PFHS, whereas PFOS and PFBS showed a comparable clearance.

In figure 4.2.11b the [^{3}H]-triolein half-life (=1/slope figure 4.2.11a) is shown. Fenofibrate significantly decreased TG half life by -68%. PFBS and PFOS both reduced [^{3}H]-triolein half-life by -51% and PFHS showed a decreased of -61%, which was a significantly stronger decrease as the PFOS group.

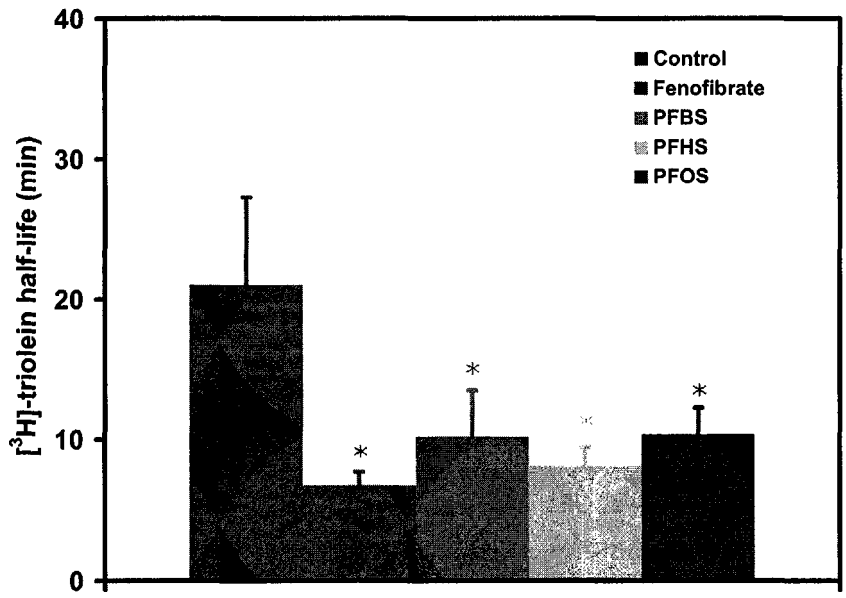
The half-life of VLDL-TG did not significantly differ between PFBS on the one hand and PFHS and PFOS on the other hand.

Figure 4.2.11a [^{3}H]-triolein decay in plasma



* p<0.05 vs. control

Figure 4.2.11b [^{3}H]-triolein half-life



* p<0.05 vs. control

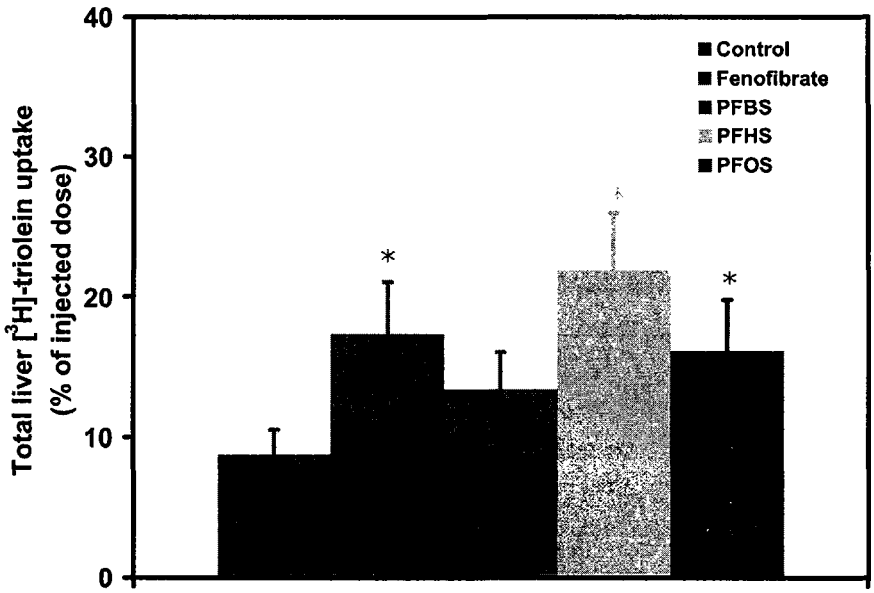
Total liver [^{3}H]-triolein uptake was increased in all treatment groups. Fenofibrate, PFHS and PFOS showed a significant induction of respectively +98%, +149% and +84%. PFBS showed a trend towards a higher uptake (+53%, p=0.063).

PFHS showed a significantly decreased [^{3}H]-triolein uptake in the heart and the spleen (by -52% and -53% respectively), PFOS increased uptake in gonadal white adipose tissue (gWAT, by 164%) and fenofibrate showed a higher uptake in muscle and gonadal fat (by 65% and 162% respectively).

[^{3}H]-triolein tissue uptake (% of injected dose)		liver	heart	spleen	muscle	gWAT
Control	avg	8.7	1.99	0.77	4.82	0.78
	sd	1.8	0.61	0.18	1.66	0.21
Fenofibrate	avg	17.3	1.26	0.53	7.96	2.04
	sd	3.7	0.45	0.17	1.79	0.87
PFBS	avg	13.4	1.81	0.57	6.38	1.45
	sd	2.6	0.98	0.14	2.16	1.79
PFHS	avg	21.8	0.96	0.36	6.97	1.82
	sd	4.2	0.37	0.13	1.22	1.41
PFOS	avg	16.1	1.46	0.47	6.37	2.05
	sd	3.6	0.75	0.21	1.43	1.77

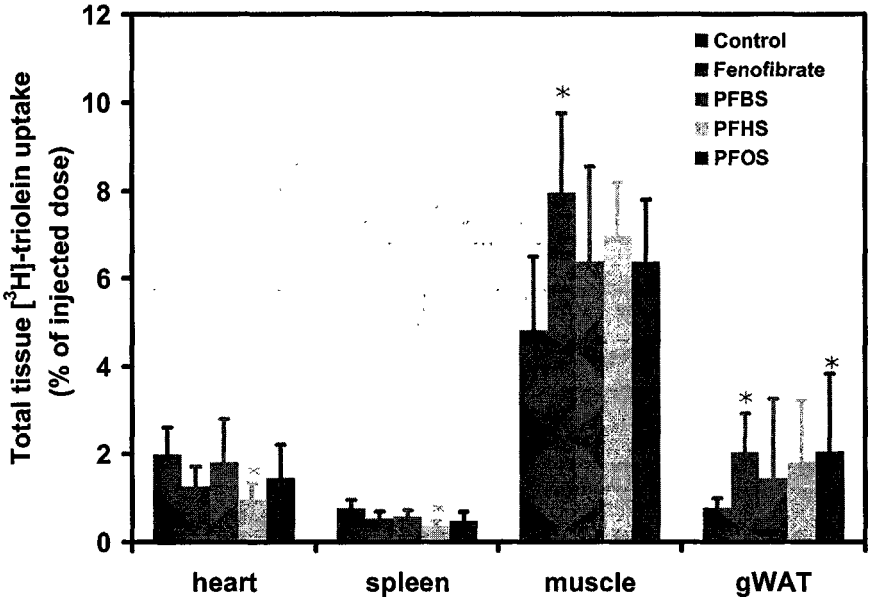
[^{3}H]-triolein tissue uptake: P-value		liver	heart	spleen	muscle	gWAT
Between groups		**0.002**	0.143	**0.049**	0.129	**0.043**
Control vs:	Fenofibrate	**0.008**	0.151	0.095	**0.032**	**0.008**
	PFBS	0.063	0.905	0.190	0.190	0.730
	PFHS	**0.004**	**0.017**	**0.009**	0.052	0.082
	PFOS	**0.004**	0.329	0.082	0.247	**0.004**
Fenofibrate vs	PFBS	0.190	0.413	0.730	0.413	0.286
	PFHS	0.126	0.329	0.126	0.247	0.429
	PFOS	0.792	0.662	**0.009**	**0.004**	**0.004**
PFBS vs	PFHS	**0.010**	0.114	0.114	0.762	0.476
	PFOS	0.257	0.762	0.610	0.914	0.257
PFHS vs	PFOS	0.132	0.310	0.310	0.699	0.485

Figure 4.2.11c Total liver [^{3}H]-triolein uptake



* p<0.05 vs. control

Figure 4.2.11d Total tissue [^{3}H]-triolein uptake



* p<0.05 vs. control

The clearance of VLDL-like [^{14}C]-cholesteryl oleate particles (as a measure for VLDL-cholesterolester clearance) was significantly increased in the fenofibrate group and the PFBS and PFHS groups (figure 4.2.11e). PFOS showed a similar decay as the control group. Fenofibrate showed the strongest increase, whereas PFBS and PFHS showed a comparable decay.

In figure 4.2.11f the [^{14}C]-cholesteryl oleate half-life (=1/slope figure 4.2.11e) is shown. Fenofibrate significantly decreased CE half life by -80%. PFBS and PFHS reduced half-life by -60% and -54% respectively. PFOS was not significantly different from the control group and the PFBS group.

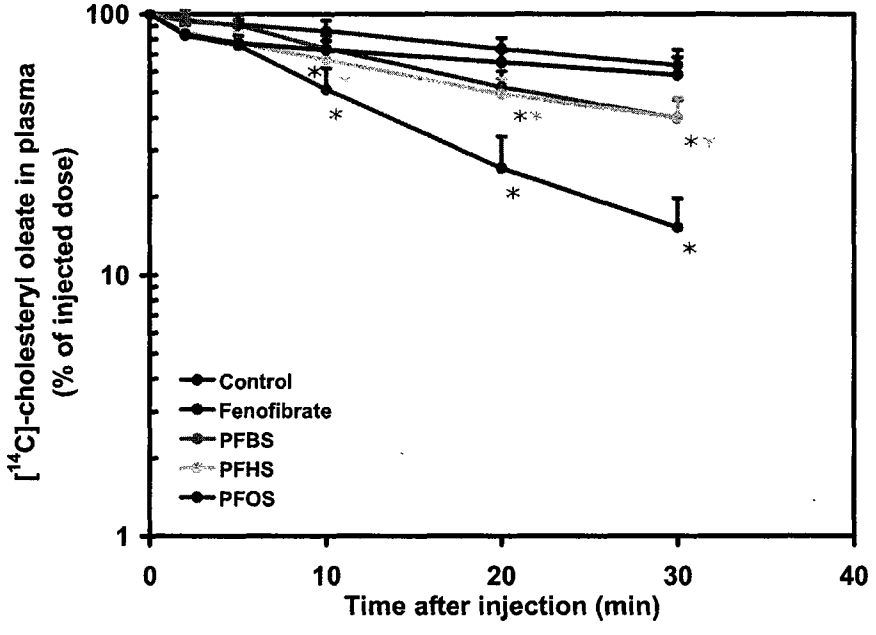
[^{14}C]-cholesteryl oleate decay in plasma (% of injected dose)		Time after injection of [^{14}C]-cholesteryl oleate labeled VLDL-like particles (min)					
		0	2	5	10	20	30
Control	avg	100.0	93.9	90.9	86.0	73.4	63.5
	sd	0.0	11.6	9.3	8.6	7.6	9.1
Fenofibrate	avg	100.0	82.6	75.8	51.3	25.6	15.2
	sd	0.0	13.4	12.9	10.5	8.2	4.4
PFBS	avg	100.0	95.0	90.5	73.7	52.4	39.7
	sd	0.0	7.5	5.1	8.7	7.8	7.0
PFHS	avg	100.0	85.0	77.7	66.6	49.2	40.0
	sd	0.0	7.6	7.3	6.9	7.2	7.4
PFOS	avg	100.0	83.5	77.4	72.9	65.1	58.2
	sd	0.0	7.6	5.0	5.7	7.0	9.8

[^{14}C]-cholesteryl oleate decay in plasma: P-value		Time after injection of [^{14}C]-CO labeled VLDL-like particles (min)				
		2	5	10	20	30
Between groups		0.185	**0.036**	**0.002**	**<0.001**	**<0.001**
Control vs:	Fenofibrate	0.151	0.056	**0.008**	**0.008**	**0.008**
	PFBS	1.000	0.730	0.063	**0.016**	**0.016**
	PFHS	0.247	0.052	**0.009**	**0.004**	**0.004**
	PFOS	0.177	0.052	**0.030**	0.126	0.429
Fenofibrate vs	PFBS	0.286	0.190	**0.032**	**0.016**	**0.016**
	PFHS	0.792	0.931	**0.030**	**0.009**	**0.004**
	PFOS	0.537	0.662	**0.009**	**0.004**	**0.004**
PFBS vs	PFHS	0.067	**0.019**	0.257	0.914	1.000
	PFOS	0.067	**0.019**	0.914	**0.038**	**0.010**
PFHS vs	PFOS	0.937	0.818	0.180	**0.009**	**0.002**

[^{14}C]-cholesteryl oleate half-life (min)		Half-life
Control	avg	56.6
	sd	26.2
Fenofibrate	avg	11.0
	sd	1.7
PFBS	avg	22.5
	sd	6.1
PFHS	avg	26.3
	sd	6.7
PFOS	avg	92.4
	sd	84.2

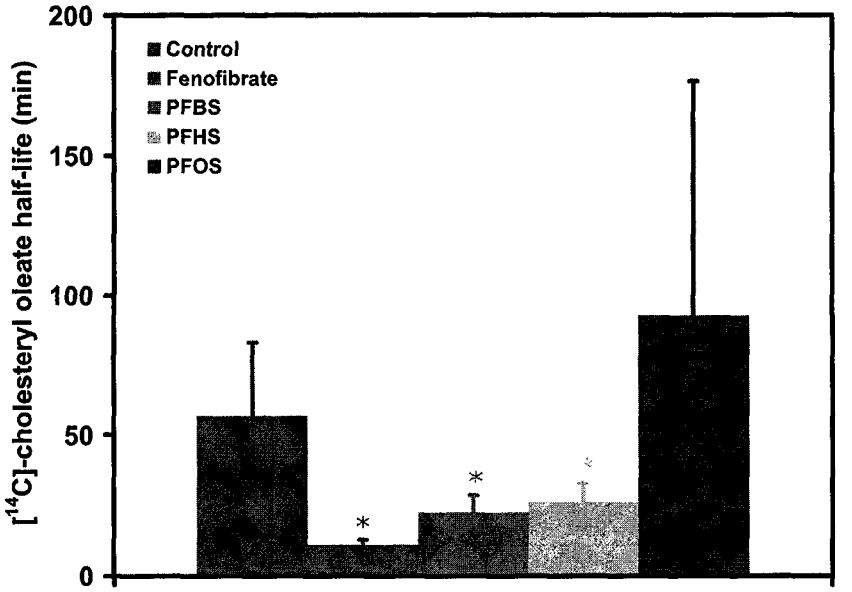
[^{14}C]-cholesteryl oleate half-life: P-value		Half-life
Between groups		**<0.001**
Control vs:	Fenofibrate	**0.008**
	PFBS	**0.016**
	PFHS	**0.009**
	PFOS	0.931
Fenofibrate vs	PFBS	**0.016**
	PFHS	**0.004**
	PFOS	**0.004**
PFBS vs	PFHS	0.610
	PFOS	**0.010**
PFHS vs	PFOS	**0.004**

Figure 4.2.11e [^{14}C]-cholesteryl oleate decay



* p<0.05 vs. control

Figure 4.2.11f [^{14}C]-cholesteryl oleate half-life



* p<0.05 vs. control

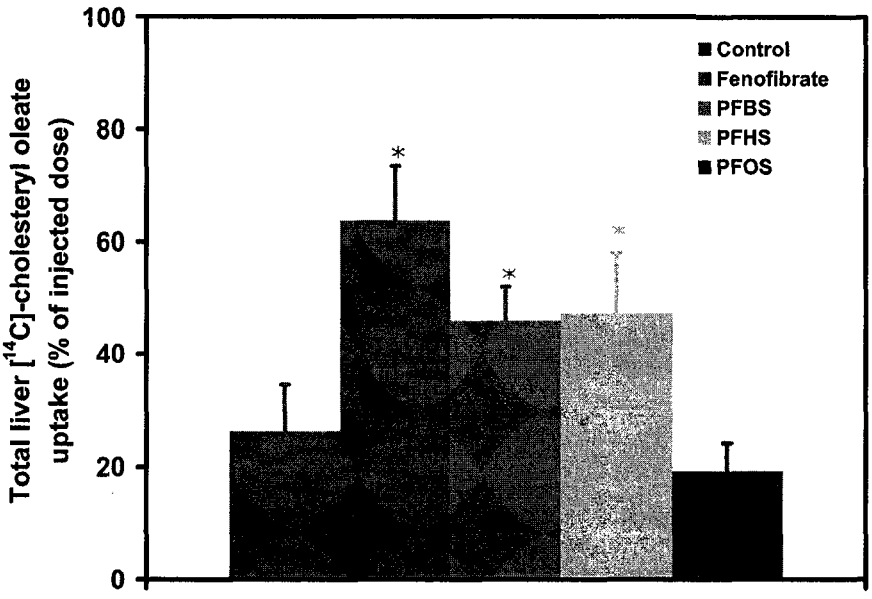
Total liver [^{14}C]-cholesteryl oleate was increased in all treatment groups, except PFOS. Fenofibrate, PFBS and PFHS showed a significant induction of respectively +142%, +74% and +79%.

PFHS showed a significantly decreased [^{14}C]-cholesteryl oleate uptake in the spleen (by -52%), other tissues were not significantly affected by any treatment.

[^{14}C]-cholesteryl oleate tissue uptake (% of injected dose)		liver	heart	spleen	muscle	gWAT
Control	avg	26.3	1.40	1.04	1.02	0.02
	sd	8.1	0.32	0.23	1.42	0.03
Fenofibrate	avg	63.8	1.45	0.79	1.95	0.39
	sd	9.7	0.64	0.20	0.59	0.17
PFBS	avg	45.9	1.50	0.88	1.70	0.18
	sd	3.5	1.04	0.27	1.56	0.30
PFHS	avg	47.1	1.05	0.50	1.91	0.33
	sd	10.9	0.45	0.19	1.20	0.32
PFOS	avg	19.1	0.96	0.63	0.55	0.41
	sd	5.0	0.37	0.30	0.70	0.64

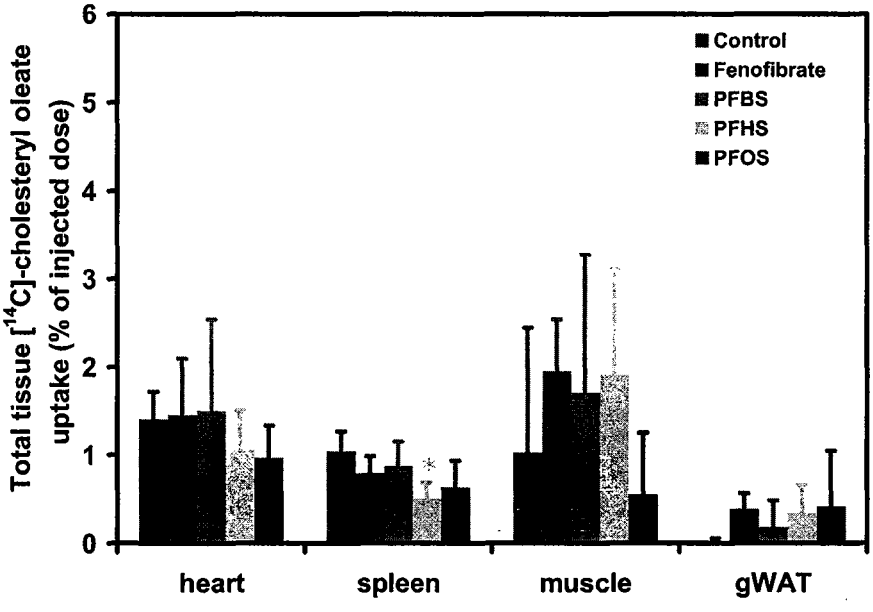
[^{14}C]-cholesteryl oleate tissue uptake: P-value		liver	heart	spleen	muscle	gWAT
Between groups		**<0.001**	0.381	**0.045**	0.124	0.122
Control vs:	Fenofibrate	**0.008**	1.000	0.310	0.421	0.008
	PFBS	**0.016**	0.556	0.730	0.413	0.556
	PFHS	**0.017**	0.329	**0.009**	0.429	0.082
	PFOS	0.126	0.082	0.126	0.792	0.177
Fenofibrate vs	PFBS	**0.016**	0.905	0.556	0.413	0.286
	PFHS	**0.030**	0.329	**0.030**	1.000	0.792
	PFOS	**0.004**	0.177	0.537	**0.017**	0.329
PFBS vs	PFHS	0.352	0.476	0.067	0.610	0.352
	PFOS	**0.010**	0.476	0.257	0.114	0.610
PFHS vs	PFOS	**0.004**	0.818	0.394	**0.026**	0.699

Figure 4.2.11g Total liver [^{14}C]-cholesteryl oleate uptake



* p<0.05 vs. control

Figure 4.2.11h Total tissue [^{14}C]-cholesteryl oleate uptake



* p<0.05 vs. control

4.3 Results study 3

4.3.1 Markers of general well-being

No specific clinical signs were observed during the study. At sacrifice, besides the livers no macroscopic differences were observed between the groups. Livers were visibly somewhat increased in the fenofibrate group and largely increased in the PFHS and PFOS group. One mouse (mouse 23) showed a granular liver.

4.3.2 Body weight

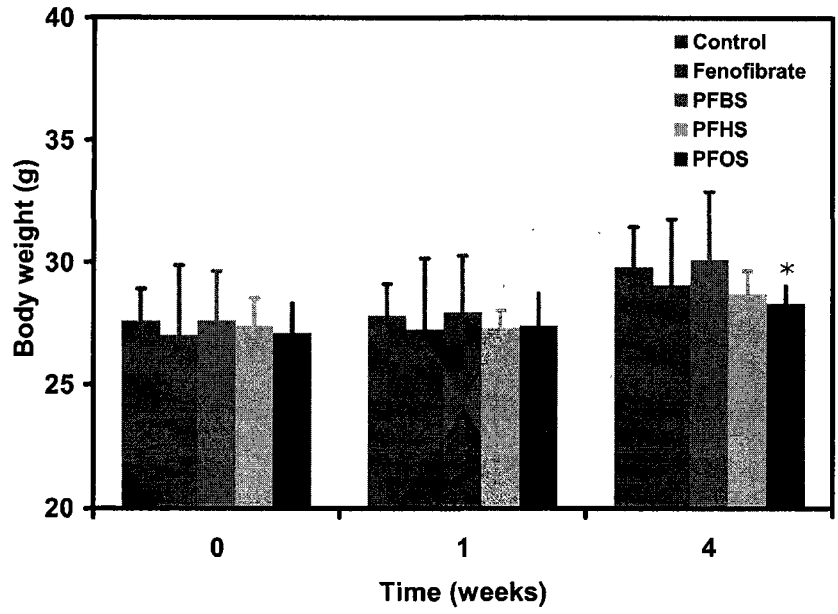
Values are absolute values (g) and are means ± S.D. from 6-7 mice per group. Individual values are given in appendix I.

Body weight (g)		time (weeks)		
		0	1	4
Control	avg	27.6	27.8	29.8
	sd	1.3	1.3	1.6
Fenofibrate	avg	27.0	27.2	29.0
	sd	2.9	2.9	2.7
PFBS	avg	27.6	27.9	30.1
	sd	2.0	2.3	2.7
PFHS	avg	27.4	27.3	28.7
	sd	1.1	0.8	0.9
PFOS	avg	27.1	27.4	28.3
	sd	1.2	1.3	0.7

Body weight: P-value		time (weeks)		
		0	1	4
Between groups		0.741	0.737	0.262
Control vs:	Fenofibrate	0.318	0.383	0.310
	PFBS	0.902	0.620	0.937
	PFHS	0.710	0.456	0.132
	PFOS	0.456	0.383	**0.015**
Fenofibrate vs	PFBS	0.620	0.456	0.485
	PFHS	0.318	0.318	0.818
	PFOS	0.456	0.456	0.937
PFBS vs	PFHS	1.000	0.902	0.394
	PFOS	0.456	0.902	0.132
PFHS vs	PFOS	0.535	0.902	0.589

During the study mice gained body weight in all groups. In contrast to study 1 and 2, the PFOS group showed a small but significantly decreased body weight after 4 weeks of treatment (-5%).

Figure 4.3.2 Body weight



* p<0.05 vs. control

4.3.3 Liver and perigonadal fat weight

Values are absolute values (g) and are means ± S.D. from 6 mice per group. Individual values are given in appendix II.

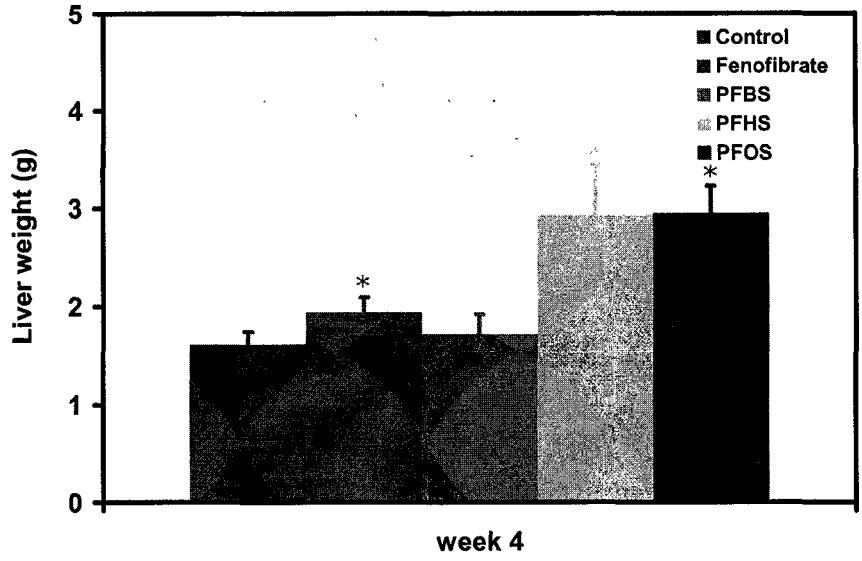
Tissue weight (g)		Liver	Perigonadal fat
Control	avg	1.61	0.56
	sd	0.13	0.16
Fenofibrate	avg	1.94	0.50
	sd	0.16	0.14
PFBS	avg	1.72	0.52
	sd	0.20	0.18
PFHS	avg	2.93	0.36
	sd	0.52	0.05
PFOS	avg	2.95	0.39
	sd	0.27	0.13

Tissue weight: P-value		Liver	Perigonadal fat
Between groups		**<0.001**	0.083
Control vs:	Fenofibrate	**0.004**	0.485
	PFBS	0.310	0.699
	PFHS	**0.002**	**0.009**
	PFOS	**0.002**	0.132
Fenofibrate vs	PFBS	0.093	0.937
	PFHS	**0.026**	0.093
	PFOS	**0.002**	0.132
PFBS vs	PFHS	**0.004**	0.132
	PFOS	**0.002**	0.180
PFHS vs	PFOS	0.699	0.818

Livers were significantly increased in the fenofibrate, PFHS and PFOS group. Fenofibrate increased liver weights by +20%. The PFHS and PFOS groups showed a robust induction in liver weights, respectively by +82% and +83%. Liver weight in the PFHS and PFOS groups was significantly higher as compared to the PFBS group.

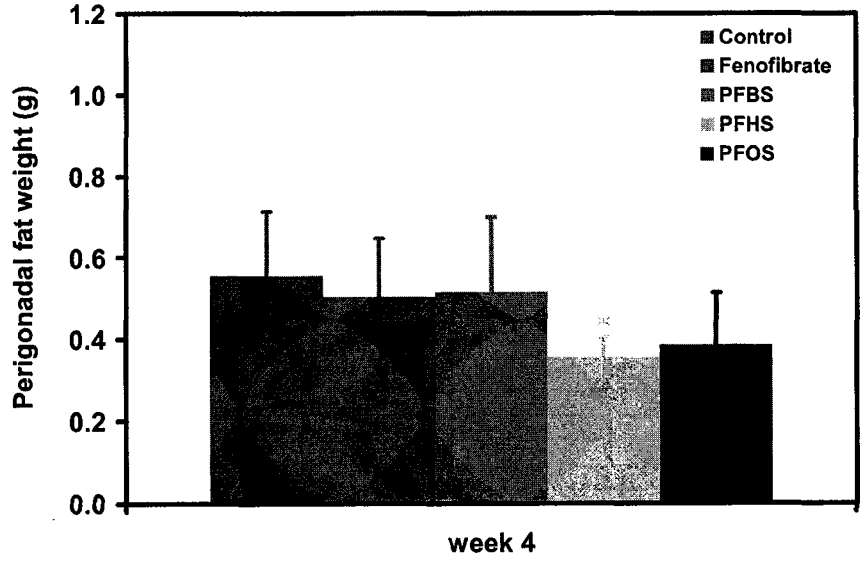
Although a decrease in perigonadal fat weight was seen after PFOS and PFHS treatment, this was only significantly different in the PFHS group (by -36%).

Figure 4.3.3a Liver weight



* p<0.05 vs. control

Figure 4.3.3b Perigonadal fat weight



* p<0.05 vs. control

4.3.4 Food intake

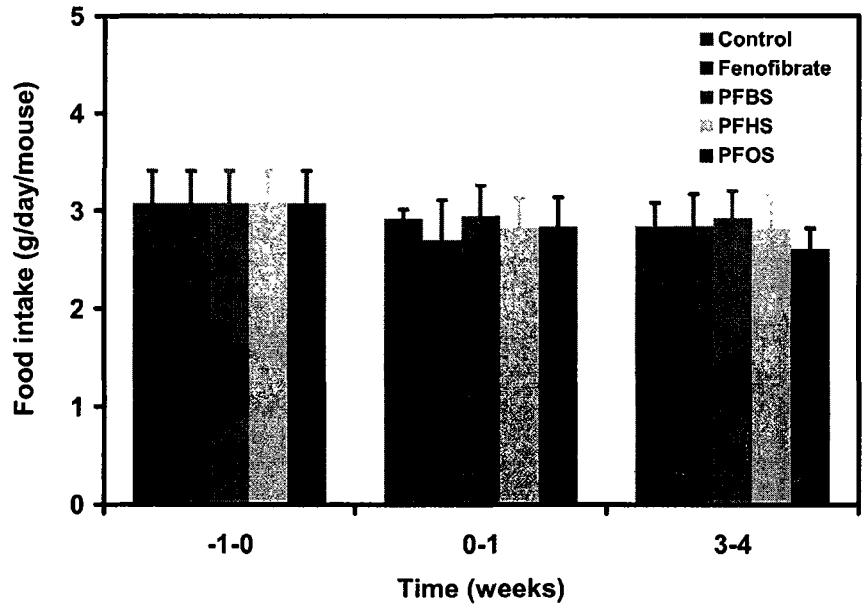
Values are absolute values (g/day/mouse) and are means ± S.D. from 3 cages per group. Individual values per cage are given in appendix III.

Food intake (g/day/mouse)		time (weeks)		
		-1-0	0-1	3-4
Control	avg	3.1	2.9	2.8
	sd	0.3	0.1	0.2
Fenofibrate	avg	3.1	2.7	2.8
	sd	0.3	0.4	0.3
PFBS	avg	3.1	2.9	2.9
	sd	0.3	0.3	0.3
PFHS	avg	3.1	2.8	2.8
	sd	0.3	0.3	0.3
PFOS	avg	3.1	2.8	2.6
	sd	0.3	0.3	0.2

Food intake: P-value		time (weeks)	
		0-1	3-4
Between groups		0.925	0.498
Control vs:	Fenofibrate	1.000	1.000
	PFBS	1.000	1.000
	PFHS	0.700	1.000
	PFOS	0.700	0.200
Fenofibrate vs	PFBS	0.700	1.000
	PFHS	1.000	1.000
	PFOS	1.000	0.400
PFBS vs	PFHS	0.700	0.400
	PFOS	0.700	0.200
PFHS vs	PFOS	1.000	0.400

All groups showed a similar food intake during the study.

Figure 4.3.4 Food intake



* p<0.05 vs. control

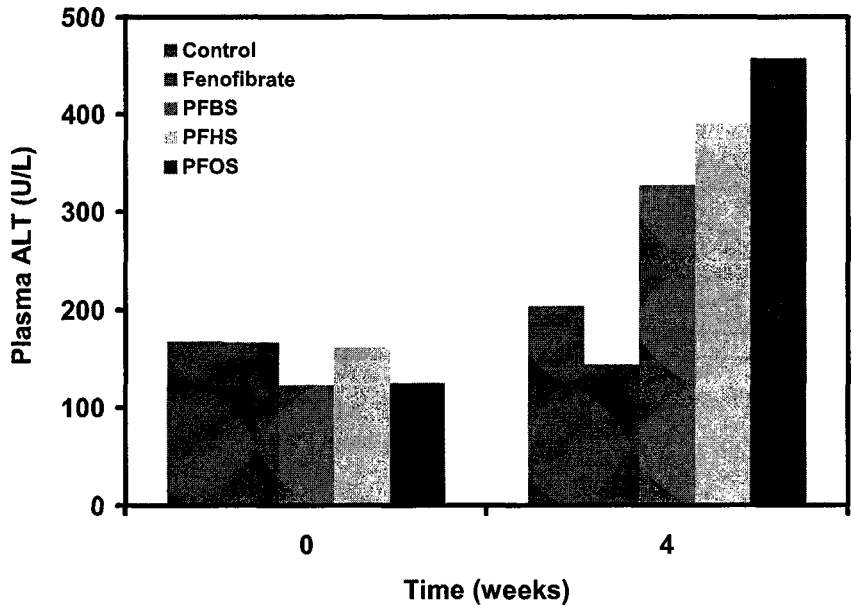
4.3.5 Plasma ALT

Values are absolute values (U/L) from measurements in pooled plasma samples per group (6-7 mice per group) at t=0 and 4.

Group	Plasma ALAT (U/L)	
	t=0 weeks	t=4 weeks
Control	168	204
Fenofibrate	167	145
PFBS	123	328
PFHS	162	391
PFOS	125	458

After 4 weeks from treatment PFHS and PFOS showed higher ALT levels than the control group. In contrast to studies 1 and 2 PFBS treatment also seem to increase plasma ALT.

Figure 4.3.5 Plasma ALT



4.3.6 Plasma cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 6-7 mice per group. Individual values are given in appendix IV.

Cholesterol (mmol/L)		time (weeks)	
		0	4
Control	avg	7.3	7.9
	sd	1.0	0.8
Fenofibrate	avg	7.7	5.1
	sd	1.2	0.1
PFBS	avg	7.6	6.1
	sd	1.9	1.1
PFHS	avg	7.5	2.4
	sd	1.7	0.5
PFOS	avg	7.7	2.6
	sd	1.4	0.6

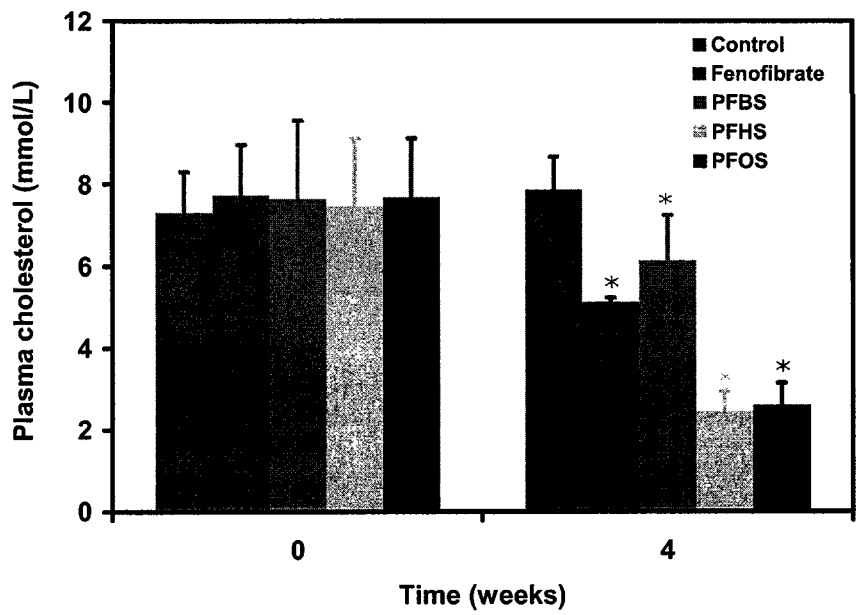
Cholesterol: P-value		time (weeks)	
		0	4
Between groups		0.941	**<0.001**
Control vs:	Fenofibrate	0.535	**0.002**
	PFBS	0.620	**0.026**
	PFHS	0.535	**0.002**
	PFOS	0.620	**0.002**
Fenofibrate vs	PFBS	1.000	**0.015**
	PFHS	0.805	**0.002**
	PFOS	1.000	**0.002**
PFBS vs	PFHS	1.000	**0.002**
	PFOS	0.710	**0.002**
PFHS vs	PFOS	0.805	0.699

As was seen in experiments 1 and 2, all treatment groups showed a significant reduction in plasma cholesterol at t=4 weeks of treatment. Fenofibrate decreased plasma cholesterol by -35%.

PFBS, PFHS and PFOS treatment reduced plasma cholesterol levels respectively by -22%, -69% and -67% after 4 weeks of treatment.

PFHS and PFOS decreased cholesterol levels to a larger extent than PFBS (p=0.002). PFHS and PFOS showed a similar inhibition.

Figure 4.3.6 Plasma cholesterol



* p<0.05 vs. control

4.3.7 Plasma HDL-cholesterol

Values are absolute values (mmol/L) and are means ± S.D. from 6-7 mice per group. Individual values are given in appendix V.

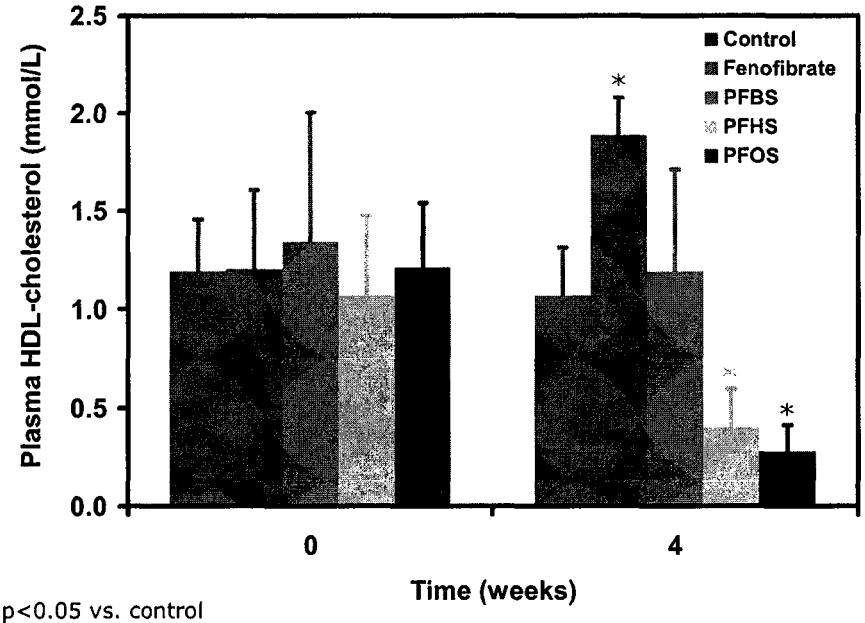
HDL-Cholesterol (mmol/L)		time (weeks)	
		0	4
Control	avg	1.19	1.07
	sd	0.26	0.24
Fenofibrate	avg	1.20	1.89
	sd	0.40	0.19
PFBS	avg	1.34	1.19
	sd	0.66	0.53
PFHS	avg	1.07	0.40
	sd	0.41	0.20
PFOS	avg	1.21	0.28
	sd	0.33	0.13

HDL-Cholesterol: P-value		time (weeks)	
		0	4
Between groups		0.991	**<0.001**
Control vs:	Fenofibrate	0.902	**0.002**
	PFBS	0.902	0.818
	PFHS	0.620	**0.002**
	PFOS	1.000	**0.002**
Fenofibrate vs	PFBS	1.000	0.065
	PFHS	0.710	**0.002**
	PFOS	1.000	**0.002**
PFBS vs	PFHS	0.902	**0.002**
	PFOS	1.000	**0.002**
PFHS vs	PFOS	0.805	0.240

HDL-cholesterol levels were increased after 4 weeks treatment in the fenofibrate group, by +77%. Both PFHS and PFOS significantly decreased HDL-cholesterol after 4 weeks of treatment, by -62% and -74%, respectively. PFBS treatment did not result in significantly altered HDL-cholesterol levels.

When compared to PFBS, PFHS and PFOS treatment showed a decreased plasma HDL-cholesterol, when comparing PFHS and PFOS treatment, no significant changes were seen.

Figure 4.3.7 Plasma HDL-cholesterol



* p<0.05 vs. control

4.3.8 Plasma triglycerides

Values are absolute values (mmol/L) and are means ± S.D. from 6-7 mice per group. Individual values are given in appendix VI.

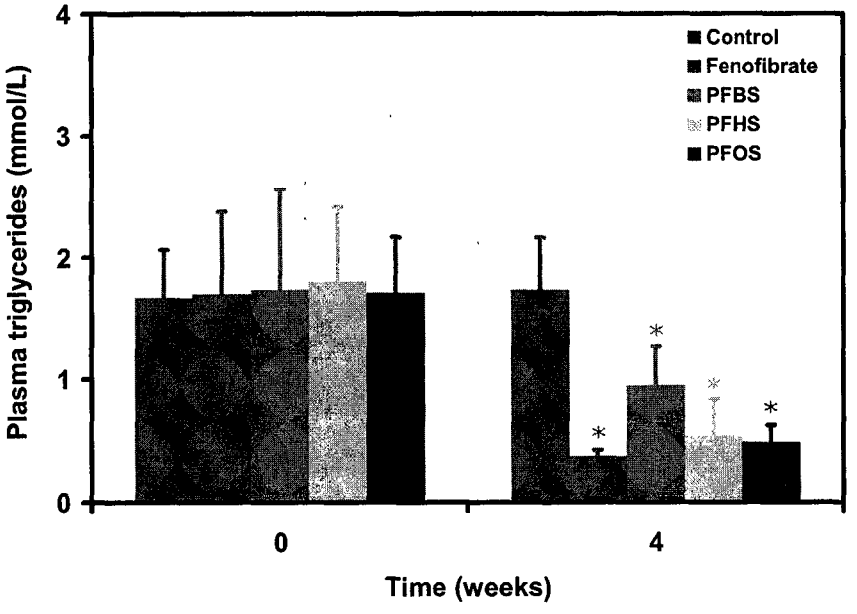
Triglycerides (mmol/L)		time (weeks)	
		0	4
Control	avg	1.67	1.74
	sd	0.39	0.42
Fenofibrate	avg	1.70	0.38
	sd	0.67	0.05
PFBS	avg	1.74	0.95
	sd	0.82	0.32
PFHS	avg	1.81	0.54
	sd	0.61	0.29
PFOS	avg	1.71	0.49
	sd	0.46	0.14

Triglycerides: P-value		time (weeks)	
		0	4
Between groups		0.988	**<0.001**
Control vs:	Fenofibrate	0.710	**0.002**
	PFBS	0.902	**0.004**
	PFHS	0.620	**0.002**
	PFOS	1.000	**0.002**
Fenofibrate vs	PFBS	0.710	**0.002**
	PFHS	0.902	0.180
	PFOS	0.710	0.132
PFBS vs	PFHS	1.000	**0.026**
	PFOS	0.902	0.009
PFHS vs	PFOS	0.805	1.000

All treatment groups showed significant reductions in plasma triglycerides at t=4 weeks of treatment. Fenofibrate decreased plasma triglycerides by -78%.

PFBS, PFHS and PFOS treatment reduced plasma triglycerides levels respectively by -45%, -69% and -72% after 4 weeks of treatment. Both PFHS and PFOS decreased triglycerides levels to a larger extent than PFBS (p=0.002). PFOS showed no significantly different triglycerides levels as compared to PFHS.

Figure 4.3.8 Plasma triglycerides



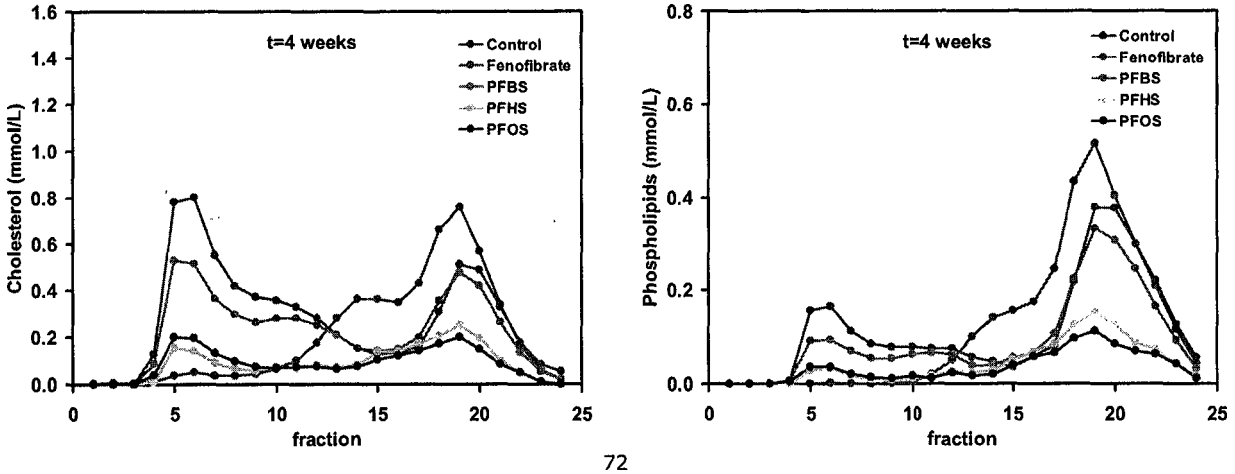
* p<0.05 vs. control

4.3.9 Lipoprotein profiles

Lipoproteins were separated on a superose column. Values are absolute values (mM) from cholesterol (left column) and phospholipids (right column) measurements in pooled plasma per group (with 6-7 mice per group) at t=4 We consider fractions 4-8 as VLDL; 9-14 as LDL; 13-16 as large-HDL (HDL1) and 17-24 as HDL.

The profiles are more or less the same as in experiment 1 and 2. After 4 weeks of treatment, plasma cholesterol levels were decreased in the VLDL-LDL peak in all groups as compared to the control group. Fenofibrate showed the strongest reduction, PFOS seemed to decrease VLDL to a lesser extent than PFHS. PFBS decreased VLDL-LDL to a lesser extend than PFHS and PFOS. HDL was increased in the fenofibrate group, with formation of a HDL1 particle. Whereas PFBS showed no clear effects on HDL, both PFHS and PFOS strongly decreased the HDL peak. PFHS seemed to decrease HDL to a lesser extend than PFOS. In contrary to the profiles in experiment 1 and 2 HDL1 (large HDL) was not increased in the PFHS group. The changes in the lipoprotein profiles are in line with the changes in plasma lipids.

Figure 4.3.9 Lipoprotein profiles



4.3.10 Plasma ApoA1

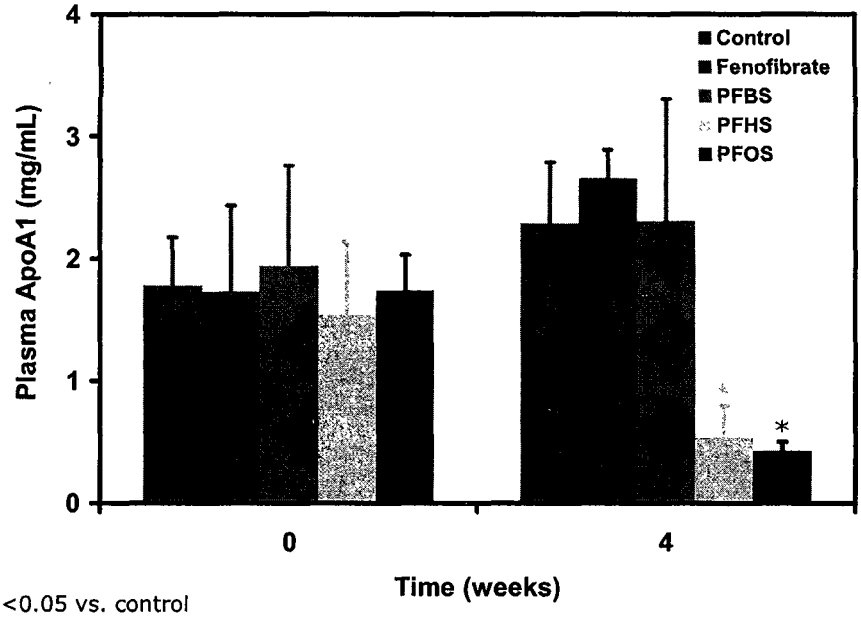
Values are absolute values (mg/mL) and are means ± S.D. from 6-7 mice per group. Individual values are given in appendix IX.

ApoA1 (mg/mL)		time (weeks)	
		0	4
Control	avg	1.78	2.28
	sd	0.40	0.50
Fenofibrate	avg	1.73	2.65
	sd	0.70	0.23
PFBS	avg	1.94	2.30
	sd	0.82	1.00
PFHS	avg	1.54	0.54
	sd	0.60	0.26
PFOS	avg	1.73	0.43
	sd	0.30	0.08

ApoA1: P-value		time (weeks)	
		0	4
Between groups		0.974	**<0.001**
Control vs:	Fenofibrate	0.710	0.180
	PFBS	1.000	0.818
	PFHS	0.620	**0.002**
	PFOS	0.805	**0.002**
Fenofibrate vs	PFBS	0.902	0.180
	PFHS	0.902	**0.002**
	PFOS	0.710	**0.002**
PFBS vs	PFHS	0.805	**0.002**
	PFOS	1.000	**0.002**
PFHS vs	PFOS	0.535	0.394

Whereas PFHS and PFOS stongly inhibited plasma ApoA1 (by -76% and -81% respectively, PFBS and fenofibrate showed not significant effects on ApoA1 levels.

Figure 4.3.10 Plasma ApoA1



* p<0.05 vs. control

4.3.11 Plasma CETP mass

Values are absolute values (µg/mL) and are means ± S.D. from 5-7 mice per group. Individual values are given in appendix X.

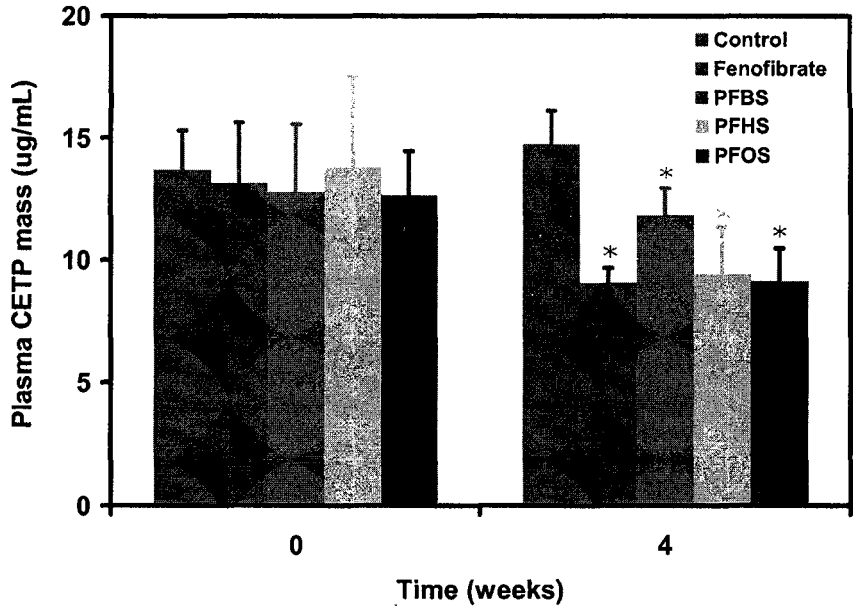
CETP mass (µg/mL)		time (weeks)	
		0	4
Control	avg	13.7	14.7
	sd	1.6	1.4
Fenofibrate	avg	13.1	9.1
	sd	2.5	0.6
PFBS	avg	12.8	11.8
	sd	2.7	1.1
PFHS	avg	13.8	9.4
	sd	3.7	1.9
PFOS	avg	12.6	9.1
	sd	1.8	1.3

CETP mass: P-value		time (weeks)	
		0	4
Between groups		0.950	**0.001**
Control vs:	Fenofibrate	0.710	**0.004**
	PFBS	0.535	**0.009**
	PFHS	1.000	**0.004**
	PFOS	0.383	**0.002**
Fenofibrate vs	PFBS	0.902	**0.004**
	PFHS	0.902	1.000
	PFOS	0.805	0.662
PFBS vs	PFHS	0.802	0.052
	PFOS	1.000	**0.015**
PFHS vs	PFOS	0.805	0.931

Plasma CETP levels were significantly decreased in all treatment groups. Fenofibrate decreased plasma CETP concentration by -38%.

PFBS, PFHS and PFOS treatment reduced plasma CETP levels respectively by -20%, -36% and -38% after 4 weeks of treatment. PFHS and PFOS decreased levels to a larger extent than PFBS (p=0.052 and 0.015 respectively). PFOS and PFHS showed similar CETP levels.

Figure 4.3.11 Plasma CETP mass



* p<0.05 vs. control

4.3.12 Plasma CETP activity

Values are absolute values (pmol/h) and are means ± S.D. from 4-7 mice per group. Individual values are given in appendix XI.

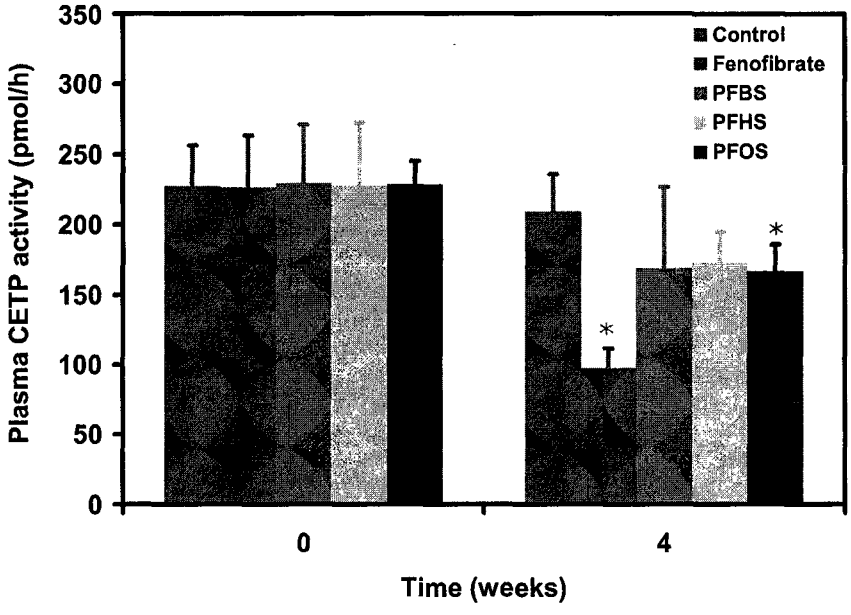
CETP activity (pmol/h)		time (weeks)	
		0	4
Control	avg	227	209
	sd	29	26
Fenofibrate	avg	227	97
	sd	37	14
PFBS	avg	229	169
	sd	41	58
PFHS	avg	228	172
	sd	44	22
PFOS	avg	229	166
	sd	17	19

CETP activity: P-value		time (weeks)	
		0	4
Between groups		0.996	**0.008**
Control vs:	Fenofibrate	0.945	**0.010**
	PFBS	0.945	0.132
	PFHS	0.731	0.052
	PFOS	0.876	**0.009**
Fenofibrate vs	PFBS	1.000	0.114
	PFHS	0.937	**0.016**
	PFOS	0.792	**0.010**
PFBS vs	PFHS	0.818	0.931
	PFOS	0.931	0.818
PFHS vs	PFOS	0.931	0.329

CETP activity levels were significantly decreased after fenofibrate and PFOS treatment. Fenofibrate decreased plasma CETP activity by -53%.

Although PFBS, PFHS and PFOS treatment reduced plasma CETP activity, this was not significantly different in the PFBS group (p=0.132). A trend toward lowered CETP activity was seen in the PFHS group (by -18%, p=0.052). PFOS decreased CETP activity by -20%.

Figure 4.3.12 Plasma CETP activity



* p<0.05 vs. control

4.3.13 In vivo clearance of autologous HDL

Values are relative values (% of injected dose for plasma decay of labeled autologous HDL) and absolute values (pools HDL/h for fractional catabolic rate of HDL and mM HDL/h for catabolic rate of HDL) and are means ± S.D. from 5 mice per group. Individual values are given in appendix XVII.

[^{3}H]-cholesteryl oleyl ether decay in plasma (% of injected dose)		Time after injection of [^{3}H]-cholesteryl oleyl ether labeled autologous HDL (h)					
		0	1	2	4	8	24
Control	avg	100.0	69.7	56.2	38.6	20.3	4.9
	sd	0.0	6.6	4.3	2.5	2.5	1.8
Fenofibrate	avg	100.0	67.7	60.0	46.7	31.7	9.4
	sd	0.0	14.9	5.4	2.1	1.5	1.0
PFBS	avg	100.0	64.3	52.2	32.8	17.0	2.6
	sd	0.0	5.9	8.0	3.4	4.0	0.8
PFHS	avg	100.0	54.6	38.8	23.6	9.7	1.4
	sd	0.0	5.2	4.8	4.7	2.8	0.7
PFOS	avg	100.0	55.4	40.0	23.5	8.7	1.3
	sd	0.0	5.3	4.2	2.7	1.9	0.4

[^{3}H]-cholesteryl oleyl ether decay in plasma: P-value		Time after injection of [3H]-CO-ether labeled autologous HDL (h)				
		1	2	4	8	24
Between groups		**0.018**	**0.004**	**<0.001**	**<0.001**	**<0.001**
Control vs:	Fenofibrate	0.841	0.548	**0.008**	**0.008**	**0.008**
	PFBS	0.222	0.421	**0.016**	0.222	**0.032**
	PFHS	**0.016**	**0.008**	**0.008**	**0.008**	**0.008**
	PFOS	**0.008**	**0.008**	**0.008**	**0.008**	**0.008**
Fenofibrate vs	PFBS	1.000	0.310	**0.008**	**0.008**	**0.008**
	PFHS	0.095	**0.008**	**0.008**	**0.008**	**0.008**
	PFOS	0.222	**0.008**	**0.008**	**0.008**	**0.008**
PFBS vs	PFHS	**0.032**	0.056	**0.008**	**0.016**	0.056
	PFOS	**0.032**	0.095	**0.008**	**0.008**	**0.008**
PFHS vs	PFOS	0.841	0.841	0.841	0.841	0.841

Fractional Catabolic Rate HDL (pools HDL-C/h)		FCR
Control	avg	0.21
	sd	0.02
Fenofibrate	avg	0.16
	sd	0.01
PFBS	avg	0.24
	sd	0.03
PFHS	avg	0.32
	sd	0.04
PFOS	avg	0.33
	sd	0.03

Fractional Catabolic Rate HDL: P-value		FCR
Between groups		**<0.001**
Control vs:	Fenofibrate	**0.008**
	PFBS	0.151
	PFHS	**0.008**
	PFOS	**0.008**
Fenofibrate vs	PFBS	**0.008**
	PFHS	**0.008**
	PFOS	**0.008**
PFBS vs	PFHS	**0.008**
	PFOS	**0.008**
PFHS vs	PFOS	1.000

Catabolic rate HDL (mM HDL-C/h)		CR
Control	avg	0.23
	sd	0.04
Fenofibrate	avg	0.31
	sd	0.05
PFBS	avg	0.25
	sd	0.09
PFHS	avg	0.12
	sd	0.06
PFOS	avg	0.08
	sd	0.04

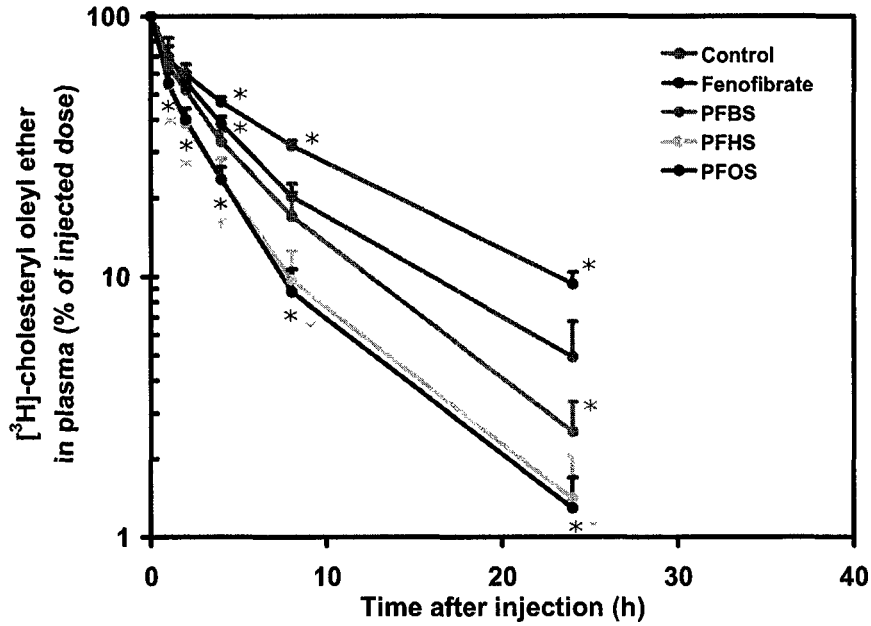
Catabolic rate HDL: P-value		CR
Between groups		**0.001**
Control vs:	Fenofibrate	0.056
	PFBS	1.000
	PFHS	**0.008**
	PFOS	**0.008**
Fenofibrate vs	PFBS	0.222
	PFHS	**0.008**
	PFOS	**0.008**
PFBS vs	PFHS	**0.032**
	PFOS	**0.008**
PFHS vs	PFOS	0.421

The clearance of HDL (measured with a trace of autologous labeled [^{3}H]-cholesteryl oleyl ether) in plasma was significantly increased in the PFHS and the PFOS groups. Fenofibrate decreased HDL clearance. PFBS showed an increased HDL clearance at two time points (4 and 24 h, figure 4.3.13a).

In figure 4.3.13b the fractional catabolic rate of HDL (=slope 0-8 h figure 4.3.13a) is shown. Fenofibrate significantly decreased the fractional catabolic rate by -25%; PFHS and PFOS showed a similarly increased fractional catabolic rate (by 50% and 54%, respectively).

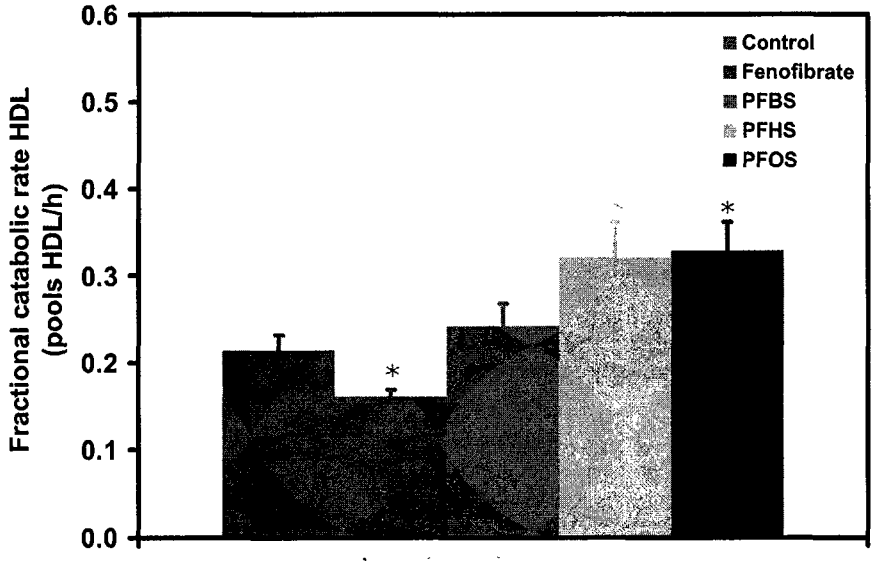
However, calculation of the catabolic rate (figure 4.3.13c), which takes into account the different pool sizes of HDL-cholesterol after the various treatments, showed that the clearance of HDL was actually decreased by PFHS and PFOS treatment (by -48% and -65% respectively). The catabolic rate was somewhat increased by fenofibrate, although this was not significantly different from the control group (+32%, p=0.056). PFBS showed no effects.

Figure 4.3.13a [^{3}H]-cholesteryl oleyl ether decay in plasma



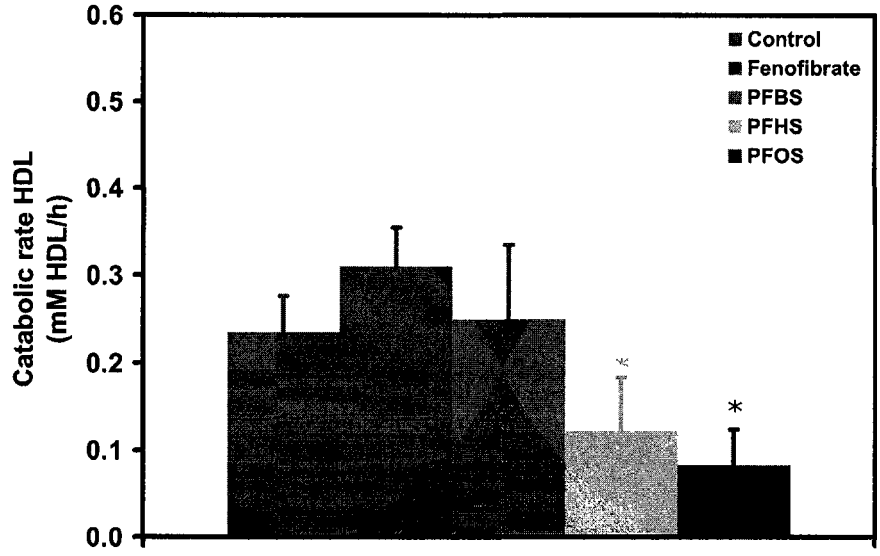
* p<0.05 vs. control

Figure 4.3.13b Fractional catabolic rate of HDL



* p<0.05 vs. control

Figure 4.3.13C Catabolic rate of HDL



* p<0.05 vs. control

4.3.14 Liver microsomal DGAT activity

Values are absolute values (nmol/min/mg protein) and are means ± S.D. from 5 mice per group. Individual values are given in appendix XVIII.

DGAT activity (nmol/min/mg protein)		DGAT-1	DGAT-2
Control	avg	2.2	1.6
	sd	0.8	0.4
Fenofibrate	avg	2.1	2.8
	sd	0.5	1.0
PFBS	avg	2.6	1.7
	sd	0.6	0.7
PFHS	avg	2.6	2.4
	sd	0.9	0.8
PFOS	avg	3.4	2.8
	sd	0.5	0.9

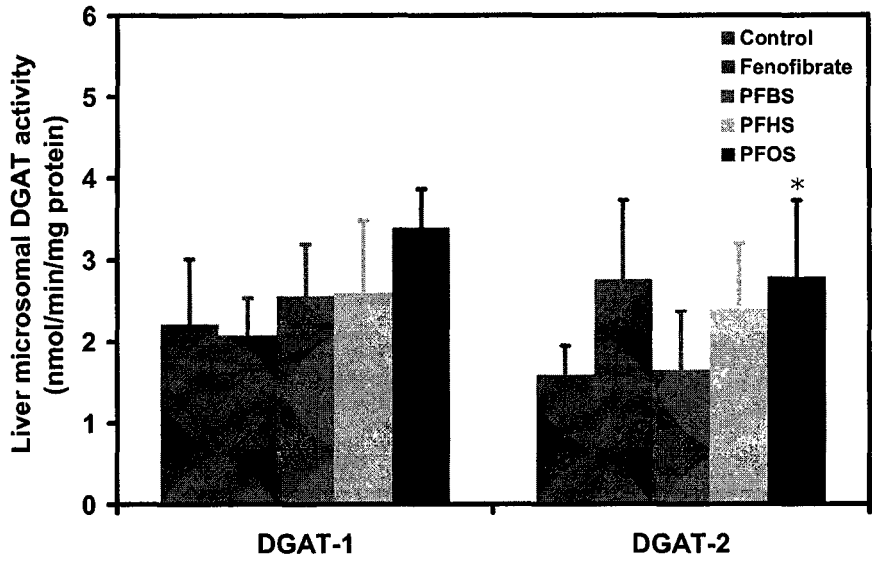
DGAT activity: P-value		DGAT-1	DGAT-2
Between groups		0.068	0.070
Control vs:	Fenofibrate	0.841	0.056
	PFBS	0.222	1.000
	PFHS	0.421	0.151
	PFOS	0.056	**0.016**
Fenofibrate vs	PFBS	0.222	0.151
	PFHS	0.310	0.841
	PFOS	**0.016**	1.000
PFBS vs	PFHS	0.690	0.222
	PFOS	0.095	0.056
PFHS vs	PFOS	0.151	0.841

Hepatic microsomal DGAT-2 activity (which is primarily involved in VLDL assembly in the liver) was significantly increased in the PFOS treatment group (by +75%). Fenofibrate showed a trend towards significance in a higher DGAT-2 activity (by +73%, p= 0.056). PFBS and PFHS showed no significant effects.

None of the treatment groups affected DGAT-1 activity (primarily involved in TG storage in the liver) significantly, although PFOS showed a trend towards significance (+53%, p=0.056).

DGAT-1 and DGAT-2 activities tended to be higher in the PFOS group as compared to the PFBS treated mice.

Figure 4.3.14 Liver microsomal DGAT activity



* p<0.05 vs. control

4.3.15 Liver lipid analysis

Values are absolute values (µg/mg protein) and are means ± S.D. from 6 mice per group. Individual values are given in appendix XIX.

Liver lipids (µg/mg protein)		FC	CE	TC	TG
Control	avg	14.3	24.6	38.9	74.5
	sd	1.4	3.5	3.9	17.5
Fenofibrate	avg	11.7	11.9	23.6	59.9
	sd	0.8	1.5	2.3	15.9
PFBS	avg	11.6	15.9	27.5	61.8
	sd	2.0	6.4	8.3	30.2
PFHS	avg	13.1	24.2	37.3	113.5
	sd	1.6	5.7	7.0	24.8
PFOS	avg	16.6	47.6	64.1	217.2
	sd	1.5	10.3	11.5	47.5

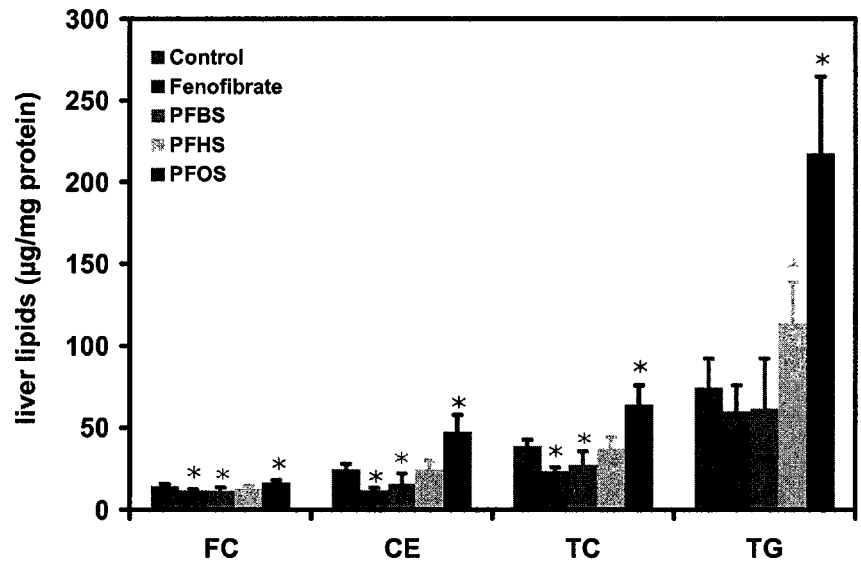
Liver lipids: P-value		FC	CE	TC	TG
Between groups		**0.001**	**<0.001**	**<0.001**	**<0.001**
Control vs:	Fenofibrate	**0.004**	**0.002**	**0.002**	0.240
	PFBS	**0.041**	**0.041**	**0.026**	0.240
	PFHS	0.240	1.000	0.937	**0.015**
	PFOS	**0.026**	**0.002**	**0.002**	**0.002**
Fenofibrate vs	PFBS	0.589	0.394	0.589	0.699
	PFHS	0.065	**0.002**	**0.002**	**0.009**
	PFOS	**0.002**	**0.002**	**0.002**	**0.002**
PFBS vs	PFHS	0.240	0.065	0.065	**0.026**
	PFOS	**0.002**	**0.002**	**0.002**	**0.002**
PFHS vs	PFOS	**0.002**	**0.002**	**0.002**	**0.002**

Hepatic triglycerides were significantly increased after PFHS and PFOS treatment (by +52% and +192%, respectively). PFOS showed a significantly higher increase than PFHS (p=0.002). Both fenofibrate and PFBS seem to decrease hepatic triglycerides somewhat, but this was not significantly different from the control.

Hepatic free cholesterol and cholesterolester levels were affected by both fenofibrate and PFBS, free cholesterol was reduced by -18% and -19% and cholesterolester was decreased by -52% and -36%, respectively. PFHS did not have an effect on hepatic cholesterol levels, but PFOS showed increases in both cholesterol and cholesterol ester (by +16% and +93%, respectively).

PFOS increased hepatic triglycerides and cholesterol levels to a significantly higher extent as compared to PFBS. The same holds true for triglycerides with PFHS; there was a tendency towards increased cholesterolester levels.

Figure 4.3.15 Liver lipids



* p<0.05 vs. control

4.3.16 Liver histology

HPS (haematoxyline-phloxine-saffrane) stained liver slides were examined histopathologically (based on necrosis with inflammatory cells, proteinaceous droplets, hepatocellular hypertrophy and hepatocellular microvacuolation). Per group 5 livers were analyzed.

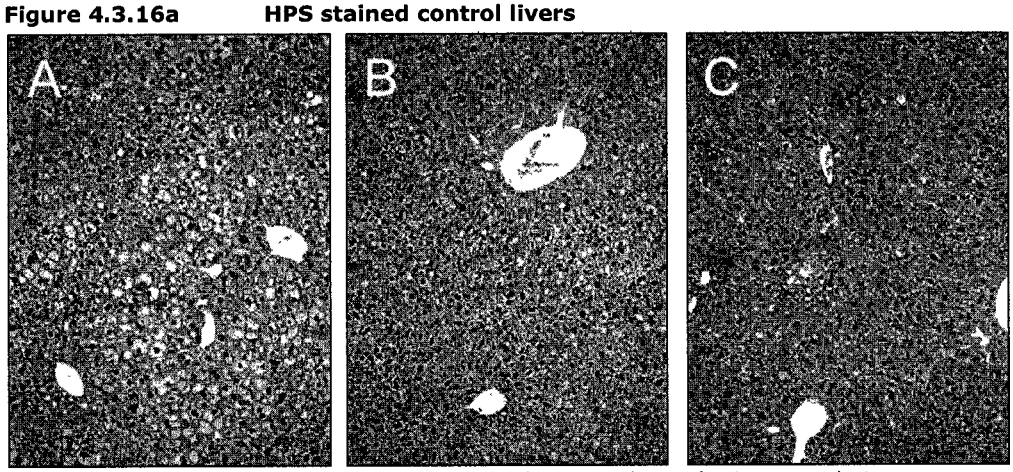
Table 4.3.16 Histopathologically examination of livers

Group	Mouse	Necrosis with inflammatory cells	Proteinaceous droplets	Hepatocellular hypertrophy	Hepatocellular microvacuolation
control	1		S		
control	2		S	Multifocal S	S
control	3		S		
control	4		S		
control	6		S		
fenofibrate	8		VS	S	
fenofibrate	9	VS F	VS	S	
fenofibrate	10	VS MF	VS	S	MD
fenofibrate	13		VS	S	
fenofibrate	14		VS	S	VS F
PFBS	16		VS		M D
PFBS	17	VS F	VS/S		M D
PFBS	18		VS/S		
PFBS	19		VS/S		
PFBS	20		VS		M F
PFHS	22	S F	VS	M	VS F
PFHS	23		VS	S	M D
PFHS	25		VS	M	VS F
PFHS	26	S F	VS	M	VS F
PFHS	27	S F	VS	M	VS F
PFOS	29		S	M	M D
PFOS	31		S	M	M D
PFOS	32	S F	S	M	M D
PFOS	33		S	M	M D
PFOS	35		VS	M	S/VS

VS = very slight
S = slight
M = moderate
F = focal
MF = multifocal
D = diffuse

Control:
Only 1 out of the 5 control ApoE3L. CETP mice, mouse 2, showed hepatocellular hypertrophy and hepatocellular microvacuolation. The liver histology of the other 4 mice appeared to be quite normal (figure 4.3.16a, table 4.3.16). Mouse 1 & 3 appeared to have more glycogen in the liver than mouse 4 & 6. In all control mice, proteinaceous droplets could be detected in the cytoplasm of the hepatocytes (deposition of ApoE3Leiden proteins, ref).

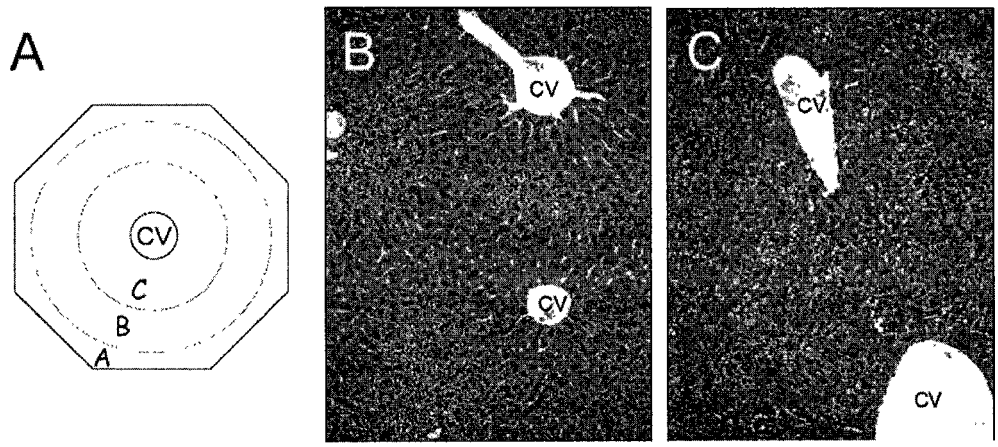
Figure 4.3.16a HPS stained control livers



A: Control (mouse 2, 100x) with hepatocellular hypertrophy and microvacuolation
B: Control (mouse 1, 100x) with more glycogen (white cytoplasm)
C: Control (mouse 4, 100x)

Fenofibrate:
The addition of fenofibrate to the diet resulted in fewer proteinaceous droplets. The hepatocytes were slightly hypertrophic and had a fine granular eosinophilic cytoplasm, especially in zone B & C around the central vein (figure 4.3.16b, table 4.3.16). Mouse 10 showed moderate hepatocellular vacuolation.

Figure 4.3.16b HPS stained livers, treated with fenofibrate

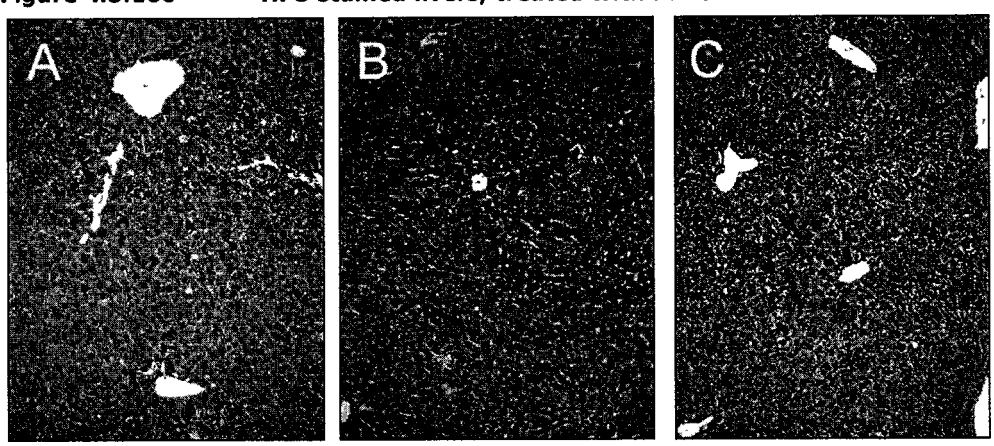


A: Drawing of liver lobule, showing zone A, B and C (100x)
B: Fenofibrate treatment (mouse 8, 100x)
C: Fenofibrate treatment (mouse 10, 100x)

CV= central vein

PFBS:
The addition of PFBS to the diet resulted in fewer proteinaceous droplets. Three out of five mice showed moderate hepatocellular microvacuolation (figure 4.3.16c, table 4.3.16).

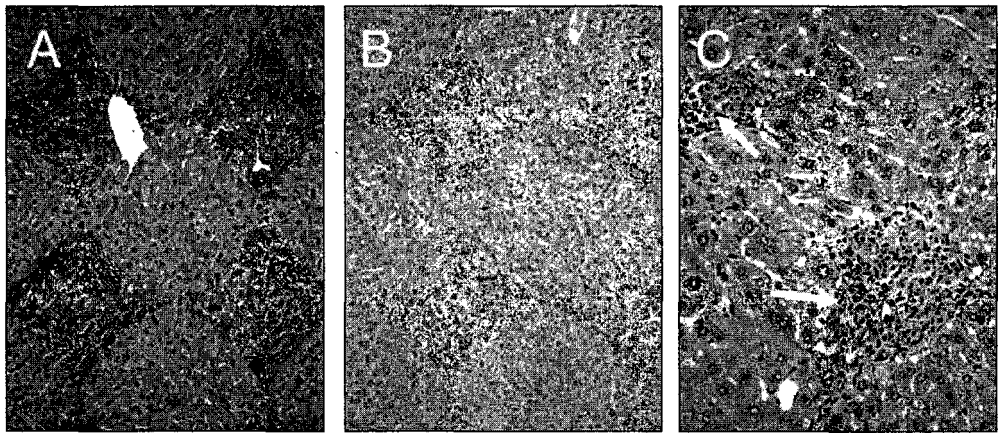
Figure 4.3.16c HPS stained livers, treated with PFBS



A: PFBS treatment (mouse 16, 100x)
B: PFBS treatment (mouse 17, 100x)
C: PFBS treatment (mouse 18, 100x)

PFHS:
The addition of PFHS to the diet resulted in fewer proteinaceous droplets. The hepatocytes were hypertrophic and had a fine granular eosinophilic cytoplasm, especially in zone B & C around the central vein (figure 4.3.16d, table 4.3.16). One mouse (M23) showed moderate diffuse hepatocellular microvacuolation. The other four mice only showed focally, very slight microvacuolation. Focal aggregates of inflammatory cells/necrotic hepatocytes could be detected in the livers of 3 out of 5 mice.

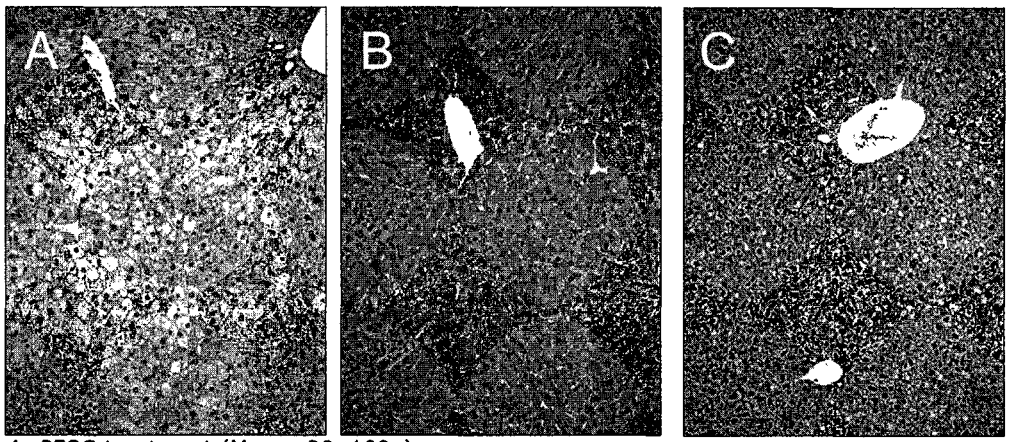
Figure 4.3.16d HPS stained livers, treated with PFHS



A: PFHS treatment (mouse 22, 100x)
B: PFHS treatment (mouse 23, 100x)
C: PFHS treatment (mouse 27, 200x). Aggregates of inflammatory cells (arrows)

PFOS:
The addition of PFOS to the diet resulted in hypertrophic hepatocytes with fine microvacuolation of the cytoplasm (table 4.3.16). Figure 4.3.16e, in which stainings of PFOS, PFHS and control livers were put next to each other, clearly showed the increased lipid filled vacuoles in the hepatocytes after PFOS treatment.

Figure 4.3.16e PFOS treatment vs PFHS treatment vs control



A: PFOS treatment (Mouse 29, 100x)
B: PFHS treatment (Mouse 22, 100x)
C: Control (Mouse 4, 100x)

4.3.17 Liver microarray analysis

Liver microarray analysis and subsequent gene expression data analysis was performed on 6 mice per group. The total transcriptome analysis report is included in appendix XX. In this paragraph tables of gene expression data of selected pathways with transcription factors and genes involved (fold change vs control, with q-values) are shown. Q-values are p-values corrected for multiple tested.

Triglyceride metabolism

Lipolysis
In line with the higher lipolytic activity in plasma, hepatic mRNA signals for LPL (fenofibrate 4.6-fold, PFHS 4.3-fold and PFOS 2.1 fold) were increased.

Fatty acid/triglyceride synthesis
Although a major regulator of fatty acid synthesis, SREBP1c was decreased with PFBS, PFHS and PFOS several genes in the fatty acid synthesis pathway were increased, suggesting that regulation does not take place via activation of LXR but probably via PXR. Several ACS genes and DGAT1 were increased with fenofibrate, PFHS and PFOS. SCD2 was increased by PFHS and PFOS.

Table 4.3.16a Gene expression data of triglyceride metabolism pathway with transcription factors and genes involved

Pathway	common name	Average expression (2log)					Fenofibrate vs control		PFBS vs control		PFHS vs Control		PFOS vs Control	
		Control	Fenofibrate	PFBS	PFHS	PFOS	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value
TG metabolism														
	lipolysis													
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	FXR	9.78	9.82	9.77	10.04	10.06	1.03	0.436	-1.01	0.676	-1.19	**0.035**	-1.21	**0.028**
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	PPARg	5.58	5.28	5.48	5.90	6.18	-1.23	0.256	-1.07	0.619	1.25	0.179	-1.52	**0.042**
	LPL	8.92	11.13	8.94	11.01	10.01	4.63	**0.000**	1.02	0.666	4.27	**0.000**	2.13	**0.000**
	HL	10.82	10.42	10.60	10.05	10.33	-1.32	**0.000**	-1.17	0.081	-1.70	**0.000**	-1.40	**0.000**
	ApoC3	14.10	13.98	14.10	14.05	14.08	-1.08	**0.011**	-1.00	0.683	-1.04	0.139	-1.01	0.370
	ApoC1	13.81	13.77	13.75	13.77	13.71	-1.03	0.235	-1.04	0.285	-1.03	0.209	-1.07	**0.020**
	ApoC2	12.49	12.54	12.50	12.27	12.11	1.04	0.402	1 01	0.672	-1.16	0.056	-1.30	**0.003**
	ApoA5	13.14	12.94	12.75	12.14	12.45	-1.15	0.064	-1.32	**0.013**	-2.01	**0.000**	-1.61	**0.000**
	GPIHBP1	7.41	7.52	7.09	7.66	7.85	1.08	0.344	-1.25	0.189	1.19	0.103	1.36	**0.012**
	FA/TG synthesis													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	FXR	9.78	9.82	9.77	10.04	10.06	1.03	0.436	-1.01	0.676	-1.19	**0.035**	-1.21	**0.028**
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	PPARg	5.58	5.28	5.48	5.90	6.18	-1.23	0.256	-1.07	0.619	1.25	0.179	1.52	**0.042**
	SREBP1a/c	11.05	10.90	10.28	10.10	10.03	-1.11	0.350	-1.70	**0.025**	-1.94	**0.000**	-2.03	**0.000**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	FAS	10.06	10.99	9.56	10.68	9.89	1.90	0.068	-1.42	0.403	1.53	0.135	-1.13	0.374
	DGAT1	5.99	6.56	5.92	6.47	6.44	1.48	**0.002**	-1.05	0.581	1.39	**0.006**	1.37	**0.008**
	DGAT2	9.25	9.02	9.13	8.93	9.04	-1.17	**0.046**	-1.09	0.360	-1.25	**0.005**	-1.16	**0.042**
	SCD1	13.15	13.47	12.52	13.39	13.10	1.24	0.207	-1.54	0.109	1.18	0.226	-1.03	0.420
	SCD2	1.98	2.10	2.16	2.70	2.30	1.09	0.247	1.14	0.282	1.65	**0.000**	1.25	**0.013**
	ACLY	11.10	11.02	10.52	11.24	9.79	-1.05	0.476	-1.49	0.292	1.11	0.366	-2.47	**0.003**
	s14	12.36	12.75	12.00	12.59	12.53	1.31	0.326	-1.28	0.516	1.17	0.349	1.12	0.389
	ACS, Acsl1	11.68	12.79	12.07	12.57	12.48	2.15	**0.000**	-1.31	**0.008**	1.85	**0.000**	1.73	**0.000**
	ACS, Acsl3	6.89	8.51	7.14	8.47	7.67	3.07	**0.000**	1.19	0.482	2.98	**0.000**	1.71	**0.016**
	ACS, Acsl4	8.29	8.95	8.54	9.35	8.78	1.58	**0.000**	1.19	0.170	2.08	**0.000**	1.40	**0.001**
	ACS, Acsl5	11.73	12.31	11.58	12.23	11.76	1.49	**0.001**	-1.10	0.437	1.42	**0.002**	1.03	0.407
	ACS, Acss1	2.04	2.03	2 03	2.34	2.28	-1.01	0.487	-1.01	0.671	1.23	**0.021**	1.18	0.057
	ACS, Acss2	10.77	11.58	10.45	11.73	10.43	1.76	0.068	-1.24	0.498	1.95	**0.023**	-1.26	0.257
	ACS, Acsm1	11.23	10.89	11.27	10.98	11.18	-1.27	**0.000**	1.03	0.568	-1.19	**0.004**	-1.04	0.305
	ACS, Acsm2	2.09	1.95	2.34	2.12	2.05	-1.10	0.130	1.19	0.057	1.02	0.360	-1.02	0.371
	ACS, Acsm3	9.74	9.92	9.40	9.34	9.25	1.13	0.168	-1.27	0.080	-1.32	**0.005**	-1.40	**0.001**
	ACS, Acsm5	9.85	9.77	9.53	9.77	9.94	-1.06	0.373	-1.24	0.116	-1.05	0.316	1.06	0.300
	ACS, Acsl6	measured, but not expressed												
	SCD3	measured, but not expressed												
	SCD4	not measured (not on array)												
	ACC, Acaca	not measured (not on array)												
	ACC, Acacb	not measured (not on array)												
	ACS, Acsm4	not measured (not on array)												
	ACS, Acss3	not measured (not on array)												
	S14-THRSP-SPOT14	12.36	12.75	12.00	12.59	12.53	1.31	0.326	-1.28	0.516	1.17	0.349	1.12	0.389

Pathway	common name	Average expression (2log)					Fenofibrate vs control		PFBS vs control		PFHS vs Control		PFOS vs Control	
		Control	Fenofibrate	PFBS	PFHS	PFOS	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value
TG metabolism														
	beta oxidation													
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	PGC1alpha	6.40	6.50	6.21	5.01	5.12	0.11	0.436	-0.18	0.547	-1.39	0.000	-1.27	0.000
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	CAR	7.46	7.86	7.63	7.44	8.32	1.32	0.172	1.13	0.546	-1.01	0.443	1.82	**0.007**
	CPT1a	10.89	11.25	10.90	10.68	10.74	1.28	**0.010**	1.01	0.675	-1.16	0.067	-1.12	0.143
	CPT1b	2.60	5.20	2.65	4.13	3.84	6.08	**0.000**	1.04	0.645	2.91	**0.000**	2.37	**0.000**
	ACO, Acox1	13.55	14.14	13.74	14.09	14.03	1.51	**0.000**	1.14	0.071	1.46	**0.000**	1.39	**0.000**
	ACO, Acox2	10.85	10.99	10.88	11.11	11.26	1.10	0.108	1.02	0.629	1.20	**0.003**	1.33	**0.000**
	ACO, Acox3	2.18	2.05	2.27	1.97	2.07	-1.10	0.243	1.07	0.506	-1.16	0.081	-1.08	0.240
	bifunctional enzym	12.71	14.33	13.17	14.38	14.24	3.08	**0.000**	1.37	**0.008**	3.19	**0.000**	2.89	**0.000**
	thiolase, Acaa1a	10.26	11.17	10.54	11.13	11.12	1.89	**0.000**	1.21	0.154	1.83	**0.000**	1.82	**0.000**
	thiolase, Acaa1b	14.31	14.68	14.45	14.68	14.62	1.29	**0.000**	1.10	0.069	1.29	**0.000**	1.24	**0.000**
	thiolase, Acaa2	12.74	13.02	12.70	12.85	12.82	1.21	**0.000**	-1.03	0.479	1.08	**0.029**	1.06	0.107
	ACS, Acsl1	11.68	12.79	12.07	12.57	12.48	2.15	**0.000**	1.31	**0.008**	1.85	**0.000**	1.73	**0.000**
	ACS, Acsl3	6.89	8.51	7.14	8.47	7.67	3.07	**0.000**	1.19	0.482	2.98	**0.000**	1.71	**0.016**
	ACS, Acsl4	8.29	8.95	8.54	9.35	8.78	1.58	**0.000**	1.19	0.170	2.08	**0.000**	1.40	**0.001**
	ACS, Acsl5	11.73	12.31	11.58	12.23	11.76	1.49	**0.001**	-1.10	0.437	1.42	**0.002**	1.03	0.407
	ACS, Acss1	2.04	2.03	2.03	2.34	2.28	-1.01	0.487	-1.01	0.671	1.23	**0.021**	1.18	0.057
	ACS, Acss2	10.77	11.58	10.45	11.73	10.43	1.76	0.068	-1.24	0.498	1.95	**0.023**	-1.26	0.257
	ACS, Acsm1	11.23	10.89	11.27	10.98	11.18	-1.27	**0.000**	1.03	0.568	-1.19	**0.004**	-1.04	0.305
	ACS, Acsm2	2.09	1.95	2.34	2.12	2.05	-1.10	0.130	1.19	0.057	1.02	0.360	-1.02	0.371
	ACS, Acsm3	9.74	9.92	9.40	9.34	9.25	1.13	0.168	-1.27	0.080	-1.32	**0.005**	-1.40	**0.001**
	ACS, Acsm5	9.85	9.77	9.53	9.77	9.94	-1.06	0.373	-1.24	0.116	-1.05	0.316	1.06	0.300
	ACS, Acsl6	measured, but not expressed												
	CPT1c	measured, but not expressed												
	HNF3b-FoxA-2	8.58	8.37	8.28	8.24	7.68	-1.16	0.322	-1.23	0.401	-1.26	0.156	-1.87	0.003
	HNF4a	9.02	8.77	8.95	8.59	8.77	-1.19	**0.025**	-1.06	0.495	-1.35	**0.000**	-1.19	0.019
	TR	3.53	3.33	3.27	3.17	3.39	-1.15	0.099	-1.20	0.131	-1.28	**0.005**	-1.10	0.161
	FA uptake, transport, binding													
	SREBP2	3.32	3.50	3.24	3.35	3.09	1.13	0.165	-1.05	0.552	1.02	0.400	-1.17	0.077
	SREBP1a/c	11.05	10.90	10.28	10.10	10.03	-1.11	0.350	-1.70	**0.025**	-1.94	**0.000**	-2.03	**0.000**
	PPARg	5.58	5.28	5.48	5.90	6.18	-1.23	0.256	-1.07	0.619	1.25	0.179	1.52	**0.042**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	FABP, Fabp1	14.47	14.61	14.45	14.57	14.55	1.10	**0.012**	-1.02	0.573	1.07	**0.048**	1.06	0.087
	FABP, Fabp2	11.78	12.22	11.40	10.75	10.64	1.36	**0.006**	-1.30	0.069	-2.04	**0.000**	-2.20	**0.000**
	FABP, Fabp4	9.16	10.29	9.76	10.86	10.31	2.19	**0.000**	1.51	0.077	3.25	**0.000**	2.21	**0.000**
	FABP, Fabp6	2.32	2.40	2.36	2.16	2.22	1.05	0.412	1.03	0.643	-1.12	0.199	-1.08	0.303
	FABP, Fabp7	7.51	5.71	7.29	6.37	7.07	-3.48	**0.000**	-1.16	0.546	-2.20	**0.004**	-1.35	0.169
	FATP, Slc27a1	2.82	5.01	3.07	4.35	4.04	4.59	**0.000**	1.20	0.414	2.90	**0.000**	2.33	**0.000**
	FATP, Slc27a2	13.86	14.06	14.00	13.98	13.96	1.15	**0.001**	1.10	0.060	1.08	**0.022**	1.07	**0.043**
	FATP, Slc27a3	measured, but not expressed												
	FATP, Slc27a4	4.48	6.20	4.47	5.99	5.53	3.30	**0.000**	-1.01	0.684	2.84	**0.000**	2.08	**0.000**
	FATP, Slc27a5	13.16	13.06	13.16	13.01	13.06	-1.07	0.181	-1.00	0.683	-1.11	**0.047**	-1.08	0.127
	CD36	9.45	11.23	10.15	11.25	11.22	3.42	**0.000**	1.62	**0.001**	3.48	**0.000**	3.40	**0.000**
	LDLR	6.51	7.11	6.53	6.70	6.21	1.52	**0.016**	1.02	0.672	1.14	0.234	-1.23	0.137
	PCSK9	5.42	6.14	5.31	5.91	4.86	1.64	0.110	-1.08	0.636	1.40	0.172	-1.48	0.142
	Fabp9	measured, but not expressed												
	VLDL assemblage/formation													
	ApoB	14.19	14.15	14.18	14.09	14.04	-1.02	0.374	-1.00	0.672	-1.07	0.073	-1.11	**0.012**
	ApoBEC	6.06	6.06	6.64	6.27	6.10	1.00	0.515	1.50	**0.045**	1.16	0.174	1.03	0.413
	MTTP	8.67	9.18	8.91	9.02	8.96	1.42	**0.000**	1.18	0.171	1.27	**0.007**	1.22	**0.020**
	Apobec2	measured, but not expressed												
	Apobec3	measured, but not expressed												
	Apobec4	measured, but not expressed												
	PL excretion													
	MDR2	10.85	11.20	11.12	11.05	11.05	1.27	**0.003**	1.20	0.079	1.14	0.051	1.14	0.055

B-oxidation
Genes involved in β-oxidation of fatty acids (CPT1a and 1b, ACO, enoyl CoA hydratase, thiolase (acetyl-Coenzyme A acyltransferase) and ACS were increased with fenofibrate, PFHS and PFOS.

Fatty acid uptake, binding
Genes involved in fatty acid uptake and binding, most prominently CD36 and FATP, and to a lesser extent FABP genes were increased with fenofibrate, PFHS and PFOS.

Triglyceride uptake
The major receptor involved in uptake of VLDL remnants and LDL, LDLR, was increased with fenofibrate.

PFBS showed no effect on genes involved in triglyceride metabolism except for mild increases in β-oxidation genes enoyl CoA hydratase and ACS and fatty acid transporter CD36.

Cholesterol metabolism

Uptake
The major receptor involved in uptake of VLDL remnants and LDL, LDLR, was increased with fenofibrate, in line with decreased liver cholesterol levels.

Synthesis and storage
In line with decreased liver cholesterol levels also cholesterol synthetic genes HMGCoA synthase and reductase and squalene synthase were increased with fenofibrate. HMGCoA synthase and squalene synthase were also increased with PFHS.
Cholesterol esterification genes ACAT1 and ACAT2 were increased with fenofibrate, PFHS and PFOS.

Bile acid metabolism and biliairy cholesterol excretion
The gene coding for the major rate-limiting enzyme in the bile acid synthetic pathway, CYP7a1, was strongly decreased with PFHS and PFOS. Also genes involved in bile acid uptake, NTCP, and biliairy excretion, BSEP, were decreased. Genes involved in biliairy cholesterol excretion, ABCg5/g8 were decreased by PFHS and PFHS. Inhibition of cholesterol metabolism and excretion may form an explanation for increased hepatic cholesterol levels found with PFOS.

PFBS showed no effect on genes involved in cholesterol metabolism except for a decrease in SREBP1a, which is not understood well. However, regulation by SREBP1a/c takes place on the protein level, which was not measured.

Table 4.3.16b Gene expression data of cholesterol metabolism pathway with transcription factors and genes involved

Pathway	common name	Average expression (2log)					Fenofibrate vs control		PFBS vs control		PFHS vs Control		PFOS vs Control	
		Control	Fenofibrate	PFBS	PFHS	PFOS	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value
Cholesterol														
	cholesterol synthesis, storage, uptake and metabolism													
	SREBP2	3.32	3.50	3.24	3.35	3.09	1.13	0.165	-1.05	0.552	1.02	0.400	-1.17	0.077
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	SREBP1a/c	11.05	10.90	10.28	10.10	10.03	-1.11	0.350	-1.70	**0.025**	-1.94	**0.000**	-2.03	**0.000**
	synthesis													
	ACLY	11.10	11.02	10.52	11.24	9.79	-1.05	0.476	-1.49	0.292	1.11	0.366	-2.47	**0.003**
	HMG CoA reductase	7.69	8.48	7.59	8.39	7.33	1.73	**0.039**	-1.07	0.629	1.62	**0.045**	-1.28	0.204
	HMG CoA synthase	12.45	12.83	12.50	12.41	12.53	1.29	**0.000**	1.03	0.573	-1.03	0.337	1.05	0.244
	squaleen synthase	8.64	9.50	8.31	9.47	8.53	1.83	**0.003**	-1.25	0.342	1.78	**0.003**	-1.08	0.360
	HMGCS	not measured (not on array)												
	storage													
	ACAT1	11.40	11.88	11.32	11.63	11.54	1.39	**0.000**	-1.06	0.370	1.17	**0.002**	1.10	**0.034**
	ACAT2	9.41	10.33	9.27	10.23	9.37	1.89	**0.004**	-1.10	0.569	1.77	**0.006**	-1.03	0.429
	uptake													
	SREBP2	3.32	3.50	3.24	3.35	3.09	1.13	0.165	-1.05	0.552	1.02	0.400	-1.17	0.077
	SREBP1a/c	11.05	10.90	10.28	10.10	10.03	-1.11	0.350	-1.70	**0.025**	-1.94	**0.000**	-2.03	**0.000**
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	LDLR	6.51	7.11	6.53	6.70	6.21	1.52	**0.016**	1.02	0.672	1.14	0.234	-1.23	0.137
	PCSK9	5.42	6.14	5.31	5.91	4.86	1.64	0.110	-1.08	0.636	1.40	0.172	-1.48	0.142
	NPC1-like1	not measured (not on array)												
	metabolism													
	FXR	9.78	9.82	9.77	10.04	10.06	1.03	0.436	-1.01	0.676	1.19	**0.035**	1.21	**0.028**
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	CAR	7.46	7.86	7.63	7.44	8.32	1.32	0.172	1.13	0.546	-1.01	0.443	1.82	**0.007**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	CYP7A	7.82	7.77	7.57	6.14	5.90	-1.04	0.506	-1.19	0.624	-3.20	**0.037**	-3.78	**0.021**
	IBAT	5.01	4.15	5.61	3.72	3.52	-1.81	0.062	1.52	0.300	-2.45	**0.005**	-2.80	**0.002**
	bsep	11.12	10.67	10.87	10.40	10.32	-1.37	**0.004**	-1.19	0.198	-1.65	**0.000**	-1.74	**0.000**
	ntcp	12.19	12.02	11.88	11.80	11.77	-1.13	0.242	-1.24	0.191	-1.82	**0.020**	-1.34	**0.014**
	excretion													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	ABCG5	11.00	10.65	10.54	9.96	10.13	-1.28	**0.049**	-1.38	0.061	-2.06	**0.000**	-1.83	**0.000**
	ABCG8	7.20	7.05	6.52	5.90	5.95	-1.11	0.369	-1.61	0.061	-2.46	**0.000**	-2.38	**0.000**

HDL metabolism

Synthesis, maturation and remodeling
Genes involved in HDL synthesis, apoA1 the major protein of HDL, and HDL maturation, ABCa1 and LCAT, were decreased by PFHS and PFOS.
PLTP a gene involved in remodeling of HDL, leading to larger particles was increased by PFHS and fenofibrate.

Uptake
The major gene involved in uptake of HDL-cholesterol esters, SR-B1, was decreased by fenofibrate, PFHS and PFOS.
The latter mechanism together with the strong decrease in CETP activity is responsible for the increased HDL levels with fenofibrate. However, this mechanism cannot explain the strong decrease in HDL levels observed with PFHS and PFOS. The decreased HDL levels most likely result from decreased HDL synthesis and maturation (decreased gene expression of apoA1, ABCa1 and LCAT).

PFBS showed no effect on genes involved in HDL metabolism, in line with unchanged HDL levels

Table 4.3.16c Gene expression data of HDL metabolism pathway with transcription factors and genes involved

Pathway	common name	Average expression (2log)					Fenofibrate vs control		PFBS vs control		PFHS vs Control		PFOS vs Control	
		Control	Fenofibrate	PFBS	PFHS	PFOS	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value
HDL metabolism														
	HDL formation													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	ApoA1	14.38	14.34	14.25	13.96	13.85	-1.03	0.365	-1.10	0.148	-1.35	**0.000**	-1.45	**0.000**
	APOA2	14.52	14.55	14.45	14.48	14.50	1.03	0.310	-1.05	0.289	-1.02	0.270	-1.01	0.373
	HDL maturation													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	ABCA1	5.14	5.06	5.16	4.95	5.01	-1.06	0.244	1.01	0.635	-1.14	**0.024**	-1.09	0.092
	LCAT	10.98	10.82	10.99	10.46	10.55	-1.12	0.080	1.01	0.669	-1.43	**0.000**	-1.35	**0.000**
	HDL modelling/destabilisation													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	PLTP	7.65	9.38	7.56	8.85	8.05	3.32	**0.000**	-1.07	0.629	2.28	**0.002**	1.31	0.171
	endothelial lipase	2.90	3.41	2.70	2.85	2.65	1.42	**0.045**	-1.15	0.457	-1.04	0.398	-1.19	0.185
	HL	10.82	10.42	10.60	10.05	10.33	-1.32	**0.000**	-1.17	0.081	-1.70	**0.000**	-1.40	**0.000**
	GPIHBP1	7.41	7.52	7.09	7.66	7.85	1.08	0.344	-1.25	0.189	1.19	0.103	1.36	**0.012**
	CETP	not measured (not on array)												
	HDL uptake													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	FXR	9.78	9.82	9.77	10.04	10.06	1.03	0.436	-1.01	0.676	1.19	**0.035**	1.21	**0.028**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	SRB1	9.50	8.99	9.23	8.29	8.68	-1.42	**0.001**	-1.20	0.168	-2.31	**0.000**	-1.76	**0.000**
	ATPsynth	13.07	13.20	12.96	13.13	13.05	1.10	0.064	-1.07	0.272	1.05	0.203	-1.01	0.424
Pathway	**common name**	**Average expression (2log)**					**Fenofibrate vs control**		**PFBS vs control**		**PFHS vs Control**		**PFOS vs Control**	
		Control	Fenofibrate	PFBS	PFHS	PFOS	Fold change	q-value	Fold change	q-value	Fold change	q-value	Fold change	q-value

Table 4.3.16d Gene expression data of transcription factors and safety parameters

Safety and transcription factors														
	safety													
	ALAT	9.58	9.64	9.25	9.57	9.80	1.04	0.368	-1.26	**0.040**	-1.01	0.441	-1.17	**0.039**
	transcription factors													
	LXRa	6.77	6.76	6.77	6.52	6.42	-1.01	0.505	-1.00	0.692	-1.19	**0.039**	-1.27	**0.008**
	LXRb	4.48	4.33	4.26	4.17	4.37	-1.11	0.271	-1.16	0.304	-1.24	**0.046**	-1.08	0.277
	PPARa	11.03	11.33	10.90	10.54	10.55	1.23	0.055	-1.10	0.453	-1.40	**0.002**	-1.40	**0.003**
	PPARg	5.58	5.28	5.48	5.90	6.18	-1.23	0.256	-1.07	0.619	1.25	0.179	1.52	**0.042**
	CAR	7.46	7.86	7.63	7.44	8.32	1.32	0.172	1.13	0.546	-1.01	0.443	1.82	**0.007**
	FXR	9.78	9.82	9.77	10.04	10.06	1.03	0.436	-1.01	0.676	1.19	**0.035**	1.21	**0.028**
	PXR	6.74	7.10	6.78	7.10	7.41	1.28	**0.010**	1.03	0.622	1.28	**0.006**	1.59	**0.000**
	PGC1alpha	6.40	6.50	6.21	5.01	5.12	0.11	0.436	-0.18	0.547	-1.39	0.000	-1.27	0.000
	PGC1beta	7.00	6.84	6.70	6.53	6.55	-0.16	0.405	-0.30	0.458	-0.47	0.119	-0.44	0.139
	PPARd	measured, but not expressed												
	CYP3A11	13.53	13.39	13.51	13.75	13.73	-1.10	0.100	-1.01	0.651	1.17	**0.007**	1.15	**0.013**

5 Conclusions and comments

In appendix XXI a table is included, in which the effects PFBS, PFHS, PFOS an fenofibrate on all measured parameters (except for the micro-array data) are summarized.

Mechanism of action of PFBS
PFBS reduced plasma cholesterol and triglyceride levels by about 25% and 45%, respectively. The data from physiological experiments indicate that the decreases in lipid levels are caused by increased clearance of VLDL-TG and VLDL-CE and mildly reduced VLDL-particle production.
PFBS showed no effect on genes involved in triglyceride metabolism except for mild increases in β-oxidation genes enoyl CoA hydratase and ACS and fatty acid transporter CD36. PFBS also showed no effect on genes involved in cholesterol metabolism except for a decrease in SREBP1a, which is not understood well. However, regulation by SREBP1a/c takes place on the protein level, which was not measured.

PFBS had no effect on HDL-cholesterol and apoA1 levels. PFBS showed no effect on genes involved in HDL metabolism, in line with the unchanged HDL levels.

PFBS mildly increased liver weight, but had no effect on ALT and development of hepatosteatosis.

Based on mRNA signals PFBS appears to have mild PPARα-agonistic activity (β-oxidation increased and liver size increased)

The present data indicate that PFBS has no increased CVD risk profile.

Mechanism of action of PFHS
PFHS reduced plasma cholesterol and triglyceride levels by about 60% and 75%, respectively.
The data from physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by increased lipolysis and clearance of VLDL-TG and VLDL-CE, and strongly reduced VLDL-TG and VLDL-particle production. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (4.3-fold) was increased.

Although a major regulator of fatty acid synthesis, SREBP1c, was decreased with PFBS, PFHS and PFOS, several genes in the fatty acid synthesis pathway were increased, suggesting that regulation does not take place via activation of LXR but probably via PXR. Several ACS genes, SCD2 and DGAT1 were increased with PFHS. Increased hepatic fatty acid synthesis together with reduced VLDL-TG secretion may form an explanation for the accumulation of triglycerides in the liver. In addition, increased uptake and binding of fatty acids (increased mRNA levels of CD36 and FATP, and to a lesser extent FABP genes) which result from enhanced lipolysis may contribute to development of hepatosteatosis. This occurred, despite the observation that genes involved in β-oxidation of fatty acids (CPT1b, ACO, enoyl CoA hydratase, thiolase (acetyl-Coenzyme A acyltransferase) and ACS were increased with PFHS.

PFHS strongly decreased HDL-cholesterol (about -75%) and apoA1 (about -75%) levels. Although HDL catabolic rate and the major gene involved in uptake of HDL-cholesterol esters, SR-B1 were strongly decreased, we conclude that based on mRNA signals PFHS reduces HDL levels by down-regulation of apoA1 synthesis and HDL maturation (ABCa1, LCAT). The latter adverse changes are most likely the result of PXR-agonistic activity (24). Increased remodeling (PLTP) and decreased uptake (SR-B1) are suggested to be responsible for the formation of larger HDL particles.

PFHS increased liver weight, ALT, and resulted in hepatosteatosis, as observed by biochemical and histological measures.

Based on mRNA signals PFHS has strong PPARα-agonistic (lipolysis increased, β-oxidation increased, FA uptake increased, liver size increased) and PXR-agonistic activity (FA uptake increased, FA synthesis increased and HDL synthesis and maturation decreased, liver size increased).

Mechanism of action of PFOS

PFOS reduced plasma cholesterol and triglyceride levels by about 65% and 70%, respectively.
A similar mechanism of action is active with PFOS as with PFHS.
The data from physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by increased lipolysis and clearance of VLDL-TG, and strongly reduced VLDL-TG and VLDL-particle production. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (2.1-fold) was increased.

Although a major regulator of fatty acid synthesis, SREBP1c, was decreased with PFBS, PFHS and PFOS, several genes in the fatty acid synthesis pathway were increased, suggesting that regulation does not take place via activation of LXR but probably via PXR. Several ACS genes, SCD2 and DGAT1 were increased with PFOS. Increased hepatic fatty acid synthesis together with reduced VLDL-TG secretion may form an explanation for the accumulation of triglycerides in the liver. Also increased uptake and binding of fatty acids (increased mRNA levels of CD36 and FATP, and to a lesser extent FABP genes) which result from enhanced lipolysis may contribute to development of hepatosteatosis. This occurred, despite the observation that genes involved in β-oxidation of fatty acids (CPT1b, ACO, enoyl CoA hydratase, thiolase (acetyl-Coenzyme A acyltransferase) and ACS were increased with PFHS.

PFOS increased mRNA levels of the cholesterol synthesizing enzymes HMGCoA synthase and squalene synthase and cholesterol esterification genes ACAT1 and ACAT2. Moreover, the gene coding for the major rate-limiting enzyme in the bile acid synthetic pathway, CYP7a1, and genes involved in biliairy cholesterol excretion, ABCg5/g8 were decreased by PFOS. Inhibition of cholesterol metabolism and excretion may form an explanation for increased hepatic cholesterol levels found with PFOS.

PFOS strongly decreased HDL-cholesterol (about -65%) and apoA1 (about -80%) levels. Although HDL catabolic rate and the major gene involved in uptake of HDL-cholesterol esters, SR-B1 were decreased, we conclude that based on mRNA signals PFOS reduces HDL levels by down-regulation of apoA1 synthesis and HDL maturation (LCAT). The latter adverse changes are most likely the result of PXR-agonistic activity (24).

PFOS increased liver weight, ALT, and resulted in pronounced hepatosteatosis and liver cholesterol accumulation, as observed by biochemical and histological measures.

Based on mRNA signals PFOS has strong PPARα-agonistic (lipolysis increased, β-oxidation increased, FA uptake increased, liver size increased) and PXR-agonistic activity (FA uptake increased, FA synthesis increased and HDL synthesis and maturation decreased, liver size increased).

Involvement of other nuclear transcription factors in the regulation of lipid and lipoprotein metabolism by PFHS and PFOS.

Involvement of CAR and LXR in the changes in lipid and lipoprotein metabolism caused by PFHS and PFOS cannot be fully excluded, but is less likely. Little is know about the role of CAR in lipid metabolism. CAR has been shown to decrease β-oxidation genes as CPT1 and enoyl CoA hydratase. The latter genes were, however, increased in the present experiments.
LXR increases fatty acid synthesis by induction of SREBP1c expression, which was 2-fold decreased, however. In addition, LXR induces expression of CETP mRNA, whereas in the present experiments a decrease in CETP activity was found. It cannot be excluded that this is caused by a strongly decreased acceptor pool for CE transfer. Involvement of RXR in the observed effects cannot be excluded, since RXR forms a heterodimer together with a larger number of nuclear transcription factors like PPARα, PXR and LXR. However, direct activation of RXR, for instance with bexarotene (25) leads to opposite effects with increased levels of triglycerides and apoB-containing lipoproteins (25).
Using the miceroarray database, we suggest to study the involvement of the above and other transcription factors (ArH) involved in the metabolism of xenobiotics in more detail with respect to changes in other metabolic processes and pathways like glucose metabolism, inflammation and immuneresponse.

Mechanism of action of fenofibrate
Fenofibrate reduced plasma cholesterol and triglyceride levels by about 40% and 70%, respectively. The data from physiological experiments supported by hepatic mRNA levels indicate that the decreases in lipid levels are caused by strongly increased lipolysis and clearance of VLDL-TG and VLDL-CE, despite the increased VLDL-TG production rate. In line with the higher lipolytic activity in plasma, the hepatic mRNA signal for LPL (4.6-fold) was increased. LDLR mRNA as marker for increased uptake of VLDL remnant particles was enhanced by 1.5-fold.
Several ACS genes and DGAT1 mRNA, involved in fatty acid and triglyceride synthesis, were increased with fenofibrate, whereas DGAT2 activity primarily responsible for VLDL secretion tended to be increased by 73% ($p=0.056$). In addition, genes involved in fatty acid uptake and binding, most prominently CD36 and FATP, and to a lesser extent FABP genes were increased. Several genes involved in β-oxidation of fatty acids (CPT1a and 1b, ACO, enoyl CoA hydratase, thiolase (acetyl-Coenzyme A acyltransferase) and ACS were strongly increased with fenofibrate. Taken together the net effect of these changes in metabolic pathways in fatty metabolism is no effect of fenofibrate on liver triglyceride levels.

In conclusion, fenofibrate paradoxically increases VLDL-TG production despite reducing plasma TG, which may be caused by enhanced hepatic free fatty acid uptake resulting from strongly accelerated peripheral LPL-mediated lipolysis of VLDL or by increased *de novo* hepatic TG synthesis.

Fenofibrate reduced liver cholesterol content resulting in enhanced LDLR mRNA levels and mRNA levels of cholesterol synthetic enzymes (HMGCoA synthase and reductase and squalene synthase) and decreased mRNA levels of cholesterol metabolizing enzymes (CYP7a1), the latter leading to reduced fecal bile acid excretion.

Fenofibrate increased HDL-cholesterol (+50%) and formation of large HDL-1 particles, and had no effect on apoA1.
Although the major gene involved in uptake of HDL-cholesterol esters, SR-B1, was decreased, the catabolic rate was not significantly decreased. Fenofibrate strongly decrease in CETP activity, which was found majorly responsible for the increased HDL levels upon treatment with fenofibrate and PPARα,γ-agonists (14,26).

Fenofibrate increased liver weight, without effects on ALT and hepatosteatosis, and decreased liver cholesterol content.

Based on mRNA signals fenofibrate has strong PPARα-agonistic activity (lipolysis increased, FA uptake increased, β-oxidation increased; HDL remodeling decreased).

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7 Appendices

Appendix I Body weight

Study 1

Group	Mouse#	Cage#	Eartag		Body weight (g)			
			L	R	week 0	week 1	week 4	week 6
CONTROL	1	1	2	1	29.7	31.0	32.4	32.2
CONTROL	2	1	0	1	25.5	25.9	26.8	25.9
CONTROL	3	2	1	1	29.7	31.2	32.2	33.2
CONTROL	4	2	2	1	26.9	28.3	29.1	30.6
CONTROL	5	2	1	2	25.3	27.0	28.1	29.9
CONTROL	6	3	1	1	25.5	26.7	28.5	27.7
CONTROL	7	3	2	1	23.9	25.4	26.4	26.6
CONTROL	8	3	1	2	23.6	24.4	25.2	25.6
AVERAGE					26.3	27.5	28.6	29.0
SD					2.4	2.5	2.6	2.9
fenofibrate	9	4	1	0	28.5	28.0	28.9	29.4
fenofibrate	10	4	1	1	29.6	30.2	30.1	31.0
fenofibrate	11	5	1	1	26.5	26.3	26.7	26.6
fenofibrate	12	5	2	1	26.8	27.9	27.9	28.8
fenofibrate	13	6	0	2	30.0	30.0	30.0	32.2
fenofibrate	14	6	1	0	21.0	22.8	23.0	23.7
fenofibrate	15	7	1	0	24.6	27.3	28.2	28.9
fenofibrate	16	7	1	2	27.4	28.2	29.4	29.6
AVERAGE					26.8	27.6	28.0	28.8
SD					2.9	2.3	2.3	2.6
PFBS	17	8	1	0	26.1	26.5	26.7	27.5
PFBS	18	8	0	1	29.6	31.0	31.9	32.0
PFBS	19	8	2	0	26.2	27.7	27.5	28.4
PFBS	20	9	1	0	25.0	25.8	26.4	26.7
PFBS	21	9	1	1	24.8	26.0	27.1	26.7
PFBS	22	10(A)	0	0	24.3	26.1	27.3	27.4
PFBS	23	10	0	1	26.8	28.6	29.3	30.2
PFBS	24	10	2	1	25.6	26.0	27.7	28.1
AVERAGE					26.1	27.2	28.0	28.4
SD					1.7	1.8	1.8	1.8
PFHS	25	11	0	0	27.0	27.7	27.5	27.1
PFHS	26	11	1	1	27.6	28.5	29.8	29.1
PFHS	27	11	1	2	24.9	26.2	27.4	27.0
PFHS	28	12	0	2	28.5	29.8	29.4	28.8
PFHS	29	12	1	2	24.4	25.6	26.8	27.4
PFHS	30	13	1	2	30.7	31.0	31.4	31.1
PFHS	31	13	0	0	25.8	27.5	27.8	28.2
PFHS	32	13	1	0	29.6	31.8	32.5	32.6
AVERAGE					27.3	28.5	29.1	28.9
SD					2.2	2.2	2.1	2.0
PFOS	33	14	0	1	30.8	31.8	31.8	31.6
PFOS	34	14	1	2	26.3	27.7	28.2	28.0
PFOS	35	15	1	1	25.6	25.8	26.5	26.7
PFOS	36	15	1	2	27.4	27.9	27.6	27.5
PFOS	37	15	0	0	25.4	26.5	25.7	26.8
PFOS	38	16	2	2	31.7	31.7	31.2	29.6
PFOS	39	16	0	1	23.0	24.8	25.4	25.9
PFOS	40	16	1	1	27.0	27.9	29.0	28.3
AVERAGE					27.2	28.0	28.2	28.1
SD					2.9	2.6	2.4	1.8

* Week 5: mouse 22 put in separate cage (10A), because of fighting wounds

Study 2

Group	Mouse#	Cage#	Eartag		Body weight (g)		
			L	R	week 0	week 1	week 4
control	1	1	1	0	24.4	25.1	26.3
control	2	1	0	2	25.7	25.6	26.7
control	3	1	1	2	26.1	26.8	27.5
control	4	2	1	0	27.6	27.5	29.3
control	5	2	2	0	27.4	27.6	28.6
control	6	2	1	2	27.3	27.4	29.5
AVERAGE					26.42	26.67	27.98
SD					1.25	1.07	1.35
fenofibrate	7	3	0	1	24.3	24.6	26.3
fenofibrate	8	3	1	1	27.2	27.7	29.9
fenofibrate	9	3	2	0	26.8	26.9	29.1
fenofibrate	10	4	0	1	27.4	27.4	28.6
fenofibrate	11	4	0	2	25.4	25.6	26.0
fenofibrate	12	4	2	0	25.4	25.7	27.6
AVERAGE					26.08	26.32	27.92
SD					1.23	1.21	1.56
PFBS	13	5	0	0	27.0	26.9	28.4
PFBS	14	5	1	2	29.8	29.9	31.4
PFBS	15	5	0	1	25.3	25.2	27.1
PFBS	16	6	0	1	25.8	25.7	27.0
PFBS	17	6	0	2	23.2	23.4	24.4
PFBS	18	6	2	1	28.3	28.4	28.5
AVERAGE					26.57	26.58	27.80
SD					2.33	2.33	2.30
PFHS	19	7	0	0	29.5	29.6	29.3
PFHS	20	7	1	1	26.8	26.7	27.7
PFHS	21	8	2	1	23.3	23.6	25.1
PFHS	22	8	0	2	26.0	27.0	28.4
PFHS	23	9	1	1	24.9	24.8	27.1
PFHS	24	9	0	2	29.2	29.6	31.7
AVERAGE					26.62	26.88	28.22
SD					2.42	2.45	2.22
PFOS	25	10	0	0	24.8	25.1	28.7
PFOS	26	10	0	1	27.8	27.7	28.5
PFOS	27	11	1	0	25.7	26.1	26.8
PFOS	28	11	2	1	28.5	28.3	28.0
PFOS	29	12	1	1	26.5	26.6	26.4
PFOS	30	12	0	2	23.7	23.9	24.5
AVERAGE					26.17	26.28	27.15
SD					1.81	1.63	1.59

Study 3

Group	Mouse#	Cage#	Eartag		Body weight (g)		
			L	R	week 0	week 1	week 4
[redacted]	1	1	0	1	26.9	26.9	29.2
[redacted]	2	1	1	2	29.4	28.4	29.3
[redacted]	3	2	0	1	26.2	26.8	28.1
[redacted]	4	2	2	0	29.3	30.1	32.9
[redacted]	5	2	0	2	26.2	26.3	sacrificed for HDL isolation
[redacted]	6	3	0	0	27.3	28.0	29.3
[redacted]	7	3	2	0	27.7	28.1	29.9
[redacted] AGE					27.6	27.8	29.8
[redacted]					1.3	1.3	1.6
fenofibrate	8	4	2	1	26.7	27.1	28.1
fenofibrate	9	4	1	2	25.4	25.4	27.1
fenofibrate	10	5	2	0	30.6	30.3	32.6
fenofibrate	11	5	1	0	25.2	26.0	sacrificed for HDL isolation
fenofibrate	12	5	1	1	31.4	32.1	32.1
fenofibrate	13	6	0	1	23.9	24.1	26.2
fenofibrate	14	6	2	1	25.7	25.6	28.1
AVERAGE					27.0	27.2	29.0
SD					2.9	2.9	2.7
PFBS	15	1	1	7	25.1	26.2	sacrificed for HDL isolation
PFBS	16	2	1	7	29.0	30.0	31.7
PFBS	17	1	0	7	31.0	31.7	34.5
PFBS	18	1	0	8	28.6	29.0	29.9
PFBS	19	2	1	8	26.6	26.8	29.1
PFBS	20	0	1	9	26.3	26.0	26.5
PFBS	21	1	2	9	26.5	25.8	28.8
AVERAGE					27.6	27.9	30.1
SD					2.0	2.3	2.7
PFHS	22	0	2	10	26.7	27.2	28.9
PFHS	23	2	1	10	29.0	27.8	30.0
PFHS	24	1	0	11	28.6	28.3	29.2
PFHS	25	0	2	11	26.2	26.2	27.3
PFHS	26	1	1	12	26.1	26.4	28.0
PFHS	27	2	0	12	27.4	27.2	28.7
PFHS	28	0	2	12	27.6	27.7	sacrificed for HDL isolation
AVERAGE					27.4	27.3	28.7
SD					1.1	0.8	0.9
PFOS	29	0	0	13	27.6	27.7	28.1
PFOS	30	1	0	13	26.0	27.6	sacrificed for HDL isolation
PFOS	31	0	2	13	26.4	26.3	27.6
PFOS	32	0	1	14	28.4	28.3	29.1
PFOS	33	1	2	14	26.3	25.9	27.4
PFOS	34	0	1	15	28.9	29.6	29.0
PFOS	35	0	2	15	25.9	26.2	28.5
AVERAGE					27.1	27.4	28.3
SD					1.2	1.3	0.7

Appendix II Tissue weight

Study 1

Body weight, liver weight and perigonadal fat weight at sacrifice.

Group	Mouse#	Cage#	Eartag		Body weight	Liver weight	Perigonadal fat weight
			L	R	(g)	(g)	(g)
control	1	1	2	1	32.3	1.62	1.08
control	2	1	0	1	25.7	1.10	0.59
control	3	2	1	1	32.4	1.36	0.42
control	4	2	2	1	30.3	1.35	0.44
control	5	2	1	2	29.0	1.24	0.36
control	6	3	1	1	26.6	1.16	0.70
control	7	3	2	1	27.2	1.23	0.79
control	8	3	1	2	25.5	1.06	0.61
AVERAGE					28.6	1.27	0.62
SD					2.8	0.18	0.24
fenofibrate	9	4	1	0	29.2	1.92	0.61
fenofibrate	10	4	1	1	30.3	2.22	0.60
fenofibrate	11	5	1	1	26.9	1.55	0.52
fenofibrate	12	5	2	1	28.0	1.61	0.59
fenofibrate	13	6	0	2	31.9	1.82	0.56
fenofibrate	14	6	1	0	23.4	1.47	0.41
fenofibrate	15	7	1	0	30.0	2.09	0.31
fenofibrate	16	7	1	2	29.7	1.93	0.39
AVERAGE					28.7	1.83	0.50
SD					2.6	0.27	0.11
PFBS	17	8	1	0	27.3	1.37	0.54
PFBS	18	8	0	1	31.9	1.77	0.80
PFBS	19	8	2	0	28.3	1.53	0.49
PFBS	20	9	1	0	27.2	1.31	0.52
PFBS	21	9	1	1	27.8	1.40	0.58
PFBS	22	10(A)	0	0	26.5	1.55	0.33
PFBS	23	10	0	1	30.1	1.44	0.33
PFBS	24	10	2	1	26.6	1.24	0.52
AVERAGE					28.2	1.45	0.51
SD					1.9	0.17	0.15
PFHS	25	11	0	0	26.9	3.10	0.53
PFHS	26	11	1	1	28.3	3.31	0.66
PFHS	27	11	1	2	27.3	3.45	0.41
PFHS	28	12	0	2	28.9	3.14	0.35
PFHS	29	12	1	2	27.2	3.49	0.34
PFHS	30	13	1	2	31.2	3.30	0.49
PFHS	31	13	0	0	28.0	3.02	0.26
PFHS	32	13	1	0	32.9	3.73	0.58
AVERAGE					28.8	3.32	0.45
SD					2.1	0.23	0.14
PFOS	33	14	0	1	31.3	3.37	0.41
PFOS	34	14	1	2	28.7	3.65	0.20
PFOS	35	15	1	1	26.3	3.08	0.41
PFOS	36	15	1	2	27.7	3.32	0.39
PFOS	37	15	0	0	25.7	3.03	0.33
PFOS	38	16	2	2	29.4	3.43	0.56
PFOS	39	16	0	1	25.6	2.70	0.36
PFOS	40	16	1	1	27.6	3.25	0.37
AVERAGE					27.8	3.23	0.38
SD					2.0	0.29	0.10

Mouse 15: scabs on belly (from fighting?)
Mouse 22: open wound at back from fighting
Mouse 32: white spot on liver
Mouse 38,39,40: pale liver

Study 2

Body weight, liver weight and perigonadal fat weight at sacrifice.

Group	Mouse#	Cage#	Eartag		Body weight	Liver weight	Perigonadal fat weight
			L	R	(g)	(g)	(g)
[illegible]	1	1	1	0	25.7	1.35	0.45
[illegible]	2	1	0	2	26.2	1.37	0.34
[illegible]	3	1	1	2	26.8	1.23	0.43
[illegible]	4	2	1	0	29.2	1.54	0.49
[illegible]	5	2	2	0	28.2	1.49	0.45
[illegible]	6	2	1	2	29.9	1.61	0.83
[illegible]					27.7	1.43	0.50
[illegible]					1.7	0.14	0.17
fenofibrate	7	3	0	1	26.1	1.72	0.55
fenofibrate	8	3	1	1	29.3	1.95	0.58
fenofibrate	9	3	2	0	28.7	1.83	0.37
fenofibrate	10	4	0	1	29.3	1.85	0.63
fenofibrate	11	4	0	2	26.6	1.69	0.62
fenofibrate	12	4	2	0	27.7	1.49	0.72
AVERAGE					28.0	1.76	0.58
SD					1.4	0.16	0.12
[illegible]	13	5	0	0	28.1	1.52	0.45
[illegible]	14	5	1	2	31.3	1.79	0.63
[illegible]	15	5	0	1	27.4	1.52	0.70
[illegible]	16	6	0	1	26.9	1.32	0.60
[illegible]	17	6	0	2	26.3	1.50	0.52
[illegible]	18	6	2	1	28.2	2.10	0.55
[illegible]					28.0	1.63	0.58
[illegible]					1.8	0.28	0.09
PFHS	19	7	0	0	29.3	2.89	0.24
PFHS	20	7	1	1	27.2	2.72	0.43
PFHS	21	8	2	1	25.2	2.78	0.47
PFHS	22	8	0	2	28.2	2.83	0.55
PFHS	23	9	1	1	27.0	3.17	0.42
PFHS	24	9	0	2	32.2	3.64	0.31
AVERAGE					28.2	3.01	0.40
SD					2.4	0.35	0.11
PFOS	25	10	0	0	28.5	3.17	0.21
PFOS	26	10	0	1	28.4	2.93	0.34
PFOS	27	11	1	0	26.8	2.72	0.40
PFOS	28	11	2	1	28.3	2.88	0.56
PFOS	29	12	1	1	27.2	3.37	0.47
PFOS	30	12	0	2	26.0	2.73	0.45
AVERAGE					27.5	2.97	0.41
SD					1.0	0.26	0.12

Mouse 21: White spots in liver, bile stones in bladder

Study 3

Body weight, liver weight and perigonadal fat weight at sacrifice.

Group	Mouse#	Cage#	Eartag		Body weight (g)	Liver weight (g)	Perigonadal fat weight (g)
			L	R			
control	1	1	0	1	28.9	1.61	0.74
control	2	1	1	2	28.9	1.83	0.46
control	3	2	0	1	26.7	1.53	0.58
control	4	2	2	0	30.7	1.69	0.74
control	5	2	0	2	sacrificed for HDL isolation		
control	6	3	0	0	28.0	1.56	0.38
control	7	3	2	0	29.1	1.46	0.43
AVERAGE					28.7	1.61	0.56
SD					1.3	0.13	0.16
fenofibrate	8	4	2	1	27.1	1.98	0.37
fenofibrate	9	4	1	2	26.1	1.93	0.33
fenofibrate	10	5	2	0	31.4	2.07	0.71
fenofibrate	11	5	1	0	sacrificed for HDL isolation		
fenofibrate	12	5	1	1	32.8	2.12	0.56
fenofibrate	13	6	0	1	25.0	1.69	0.46
fenofibrate	14	6	2	1	26.6	1.85	0.59
AVERAGE					28.2	1.94	0.50
SD					3.2	0.16	0.14
PFBS	15	1	1	7	sacrificed for HDL isolation		
PFBS	16	2	1	7	29.8	2.09	0.54
PFBS	17	1	0	7	32.5	1.73	0.72
PFBS	18	1	0	8	28.2	1.62	0.39
PFBS	19	2	1	8	28.1	1.71	0.23
PFBS	20	0	1	9	25.9	1.50	0.52
PFBS	21	1	2	9	28.3	1.67	0.69
AVERAGE					28.8	1.72	0.52
SD					2.2	0.20	0.18
PFHS	22	0	2	10	27.9	3.18	0.28
PFHS	23	2	1	10	29.0	1.88	0.38
PFHS	24	1	0	11	27.9	3.16	0.40
PFHS	25	0	2	11	26.3	3.21	0.33
PFHS	26	1	1	12	26.9	2.98	0.33
PFHS	27	2	0	12	28.3	3.17	0.41
PFHS	28	0	2	12	sacrificed for HDL isolation		
AVERAGE					27.7	2.93	0.36
SD					1.0	0.52	0.05
PFOS	29	0	0	13	26.7	2.89	0.30
PFOS	30	1	0	13	sacrificed for HDL isolation		
PFOS	31	0	2	13	26.4	2.85	0.44
PFOS	32	0	1	14	27.6	3.20	0.52
PFOS	33	1	2	14	26.0	2.78	0.52
PFOS	34	0	1	15	28.2	3.35	0.32
PFOS	35	0	2	15	26.0	2.62	0.21
AVERAGE					26.8	2.95	0.39
SD					0.9	0.27	0.13

Mouse 23: granular liver

Appendix III Food intake

Study 1

Cage#	week-1-0 Food intake (g/mouse/day)
24	2.65
26	2.91
27	3.09
28	2.52
29	2.70
30	2.69
31	2.60
34	3.09
36	2.63
37	2.78
38	3.12
39	2.88
40	2.71
41a	2.78
41b	2.54
42	2.62
43	2.68
AVERAGE	2.8
SD	0.2

Group#	Cage#	Number of mice	Food intake (g/mouse/day)		
			week 0-1	week 3-4	week 5-6
control	1	2	2.74	2.73	2.87
control	2	3	2.90	3.21	3.46
control	3	3	2.49	2.47	2.52
AVERAGE			2.7	2.8	2.9
SD			0.2	0.4	0.5
fenofibrate	4	2	2.94	3.28	3.39
fenofibrate	5	2	2.46	2.98	2.94
fenofibrate	6	2	2.67	3.33	3.22
fenofibrate	7	2	2.76	3.58	3.63
AVERAGE			2.7	3.3	3.3
SD			0.2	0.2	0.3
PFOSA	8	3	3.15	2.98	3.00
PFBS	9	2	2.55	2.85	2.80
PFBS	10	3 (2)*	2.95	3.09	4.08
PFBS	10A	1			3.60
AVERAGE			2.9	3.0	3.4
SD			0.3	0.1	0.6
PFHS	11	3	2.42	2.62	2.70
PFHS	12	2	2.75	2.96	2.88
PFHS	13	3	3.18	3.25	3.27
AVERAGE			2.8	2.9	2.9
SD			0.4	0.3	0.3
PFOS	14	2	2.96	3.48	3.38
PFOS	15	3	2.85	2.76	2.67
PFOS	16	3	2.62	2.79	2.73
AVERAGE			2.8	3.0	2.9
SD			0.2	0.4	0.4

* Week 5: mouse 22 put in separate cage (10A), because of fight wounds

Study 2

Cage#	week-1-0 Food intake (g/mouse/day)
105	2.73
107	2.83
108	2.74
109	2.83
110	2.83
111	2.88
112	2.75
113	2.71
114	2.89
129	2.84
130	2.84
AVERAGE	2.8
SD	0.1

Group#	Cage#	Number of mice	Food intake (g/mouse/day)	
			week 0-1	week 3-4
[illegible]	1	3	2.70	2.69
[illegible]	2	3	2.98	2.92
[illegible]			2.8	2.8
[illegible]			0.1	0.1
fenofibrate	3	3	2.93	2.95
fenofibrate	4	3	2.77	2.69
AVERAGE			2.9	2.8
Range			0.1	0.1
[illegible]	5	3	2.90	2.90
[illegible]	6	3	2.54	2.50
[illegible]			2.7	2.7
[illegible]			0.2	0.2
[illegible]	7	2	2.93	3.00
[illegible]	8	2	2.19	2.35
[illegible]	9	2	2.88	2.94
[illegible]			2.7	2.8
[illegible]			0.4	0.4
PFOS	10	2	2.96	3.01
PFOS	11	2	2.67	2.63
PFOS	12	2	2.51	2.55
AVERAGE			2.7	2.7
SD			0.2	0.2

Study 3

Cage#	week-1-0 Food intake (g/mouse/day)
149	2.94
150	3.65
151	3.34
152	2.87
153	3.17
154	3.31
155	3.35
156	2.89
157	2.87
158	3.21
159	2.86
161	2.39
AVERAGE	3.1
SD	0.3

Group#	Cage#	Number of mice	Food intake (g/mouse/day)	
			week 0-1	week 3-4
[illegible]	1	2	2.80	2.56
[illegible]	2	3	2.97	2.94
[illegible]	3	2	2.96	2.99
[illegible]			2.9	2.8
[illegible]			0.1	0.2
fenofibrate	4	2	2.99	2.96
fenofibrate	5	3	2.87	3.08
fenofibrate	6	2	2.23	2.46
AVERAGE			2.7	2.8
SD			0.4	0.3
[illegible]	7	3	2.94	2.88
[illegible]	8	2	3.25	3.21
[illegible]	9	2	2.63	2.66
[illegible]			2.9	2.9
[illegible]			0.3	0.3
[illegible]	10	2	3.17	3.20
PFHS	11	2	2.65	2.60
PFHS	12	3	2.62	2.61
AVERAGE			2.8	2.8
SD			0.3	0.3
PFOS	13	3	2.79	2.55
PFOS	14	2	2.55	2.43
PFOS	15	2	3.14	2.84
AVERAGE			2.8	2.6
SD			0.3	0.2

Appendix IV Plasma cholesterol

Study 1:

Group	Mouse#	Cage#	Eartag		Plasma cholesterol (mmol/L)		
			L	R	t=0 weeks	t=4 weeks	t=6 weeks
control	1	1	2	1	8.41	8.17	8.49
control	2	1	0	1	10.42	10.29	10.51
control	3	2	1	1	7.78	6.70	8.99
control	4	2	2	1	6.75	5.32	8.02
control	5	2	1	2	5.93	6.32	9.15
control	6	3	1	1	9.64	10.51	10.68
control	7	3	2	1	7.52	8.07	7.97
control	8	3	1	2	6.59	9.02	8.31
AVERAGE					7.88	8.05	9.02
SD					1.55	1.86	1.06
fenofibrate	9	4	1	0	10.58	5.47	7.19
fenofibrate	10	4	1	1	9.12	4.78	6.30
fenofibrate	11	5	1	1	7.35	4.89	4.77
fenofibrate	12	5	2	1	7.68	4.84	4.79
fenofibrate	13	6	0	2	6.56	4.10	4.85
fenofibrate	14	6	1	0	6.80	4.84	5.35
fenofibrate	15	7	1	0	8.46	6.10	6.86
fenofibrate	16	7	1	2	6.16	5.08	4.44
AVERAGE					7.84	5.01	5.57
SD					1.48	0.58	1.06
PFBS	17	8	1	0	7.54	6.54	7.11
PFBS	18	8	0	1	8.06	5.59	5.80
PFBS	19	8	2	0	6.34	4.76	5.40
PFBS	20	9	1	0	6.21	7.02	6.63
PFBS	21	9	1	1	6.62	6.01	5.32
PFBS	22	10(A)	0	0	9.41	7.60	9.30
PFBS	23	10	0	1	8.98	6.42	8.07
PFBS	24	10	2	1	10.34	5.41	7.14
AVERAGE					7.94	6.17	6.85
SD					1.53	0.92	1.38
PFHS	25	11	0	0	9.03	3.97	4.37
PFHS	26	11	1	1	9.97	3.15	4.21
PFHS	27	11	1	2	6.46	2.90	3.89
PFHS	28	12	0	2	7.21	3.09	3.06
PFHS	29	12	1	2	7.46	4.11	4.06
PFHS	30	13	1	2	6.23	2.55	3.26
PFHS	31	13	0	0	8.31	2.14	2.48
PFHS	32	13	1	0	9.83	3.69	3.57
AVERAGE					8.06	3.20	3.61
SD					1.45	0.69	0.65
PFOS	33	14	0	1	7.78	2.49	2.20
PFOS	34	14	1	2	6.27	2.94	2.08
PFOS	35	15	1	1	6.48	2.26	2.45
PFOS	36	15	1	2	6.80	2.30	2.55
PFOS	37	15	0	0	9.12	3.24	3.41
PFOS	38	16	2	2	10.88	4.33	5.10
PFOS	39	16	0	1	7.40	2.89	3.72
PFOS	40	16	1	1	9.42	3.29	4.27
AVERAGE					8.02	2.97	3.22
SD					1.63	0.68	1.09

Study 2:

Group	Mouse#	Cage#	Eartag		Plasma cholesterol (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
[redacted]	1	1	1	0	7.44	7.17
[redacted]	2	1	0	2	11.01	6.62
[redacted]	3	1	1	2	8.36	7.79
[redacted]	4	2	1	0	8.51	7.09
[redacted]	5	2	2	0	7.78	6.83
[redacted]	6	2	1	2	7.44	9.92
[redacted]					8.42	7.57
[redacted]					1.34	1.22
fenofibrate	7	3	0	1	6.42	4.85
fenofibrate	8	3	1	1	7.12	4.92
fenofibrate	9	3	2	0	10.10	4.40
fenofibrate	10	4	0	1	10.39	5.57
fenofibrate	11	4	0	2	12.13	5.40
fenofibrate	12	4	2	0	4.36	4.45
AVERAGE					8.42	4.93
SD					2.92	0.48
[redacted]	13	5	0	0	9.37	6.36
[redacted]	14	5	1	2	11.59	4.71
[redacted]	15	5	0	1	8.60	9.46
[redacted]	16	6	0	1	5.90	6.17
[redacted]	17	6	0	2	7.93	5.26
[redacted]	18	6	2	1	8.51	5.99
[redacted]					8.65	6.33
[redacted]					1.86	1.65
[redacted]	19	7	0	0	12.95	2.39
[redacted]	20	7	1	1	6.09	1.46
[redacted]	21	8	2	1	6.82	3.53
[redacted]	22	8	0	2	6.47	2.89
[redacted]	23	9	1	1	10.67	2.39
[redacted]	24	9	0	2	7.99	2.25
[redacted]					8.50	2.49
[redacted]					2.74	0.69
PFOS	25	10	0	0	6.27	2.82
PFOS	26	10	0	1	12.95	3.18
PFOS	27	11	1	0	7.58	2.44
PFOS	28	11	2	1	7.61	2.61
PFOS	29	12	1	1	9.13	2.80
PFOS	30	12	0	2	7.98	4.33
AVERAGE					8.59	3.03
SD					2.33	0.68

Study 3:

Group	Mouse#	Cage#	Eartag		Plasma cholesterol (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
control	1	1	0	1	5.90	7.33
control	2	1	1	2	6.70	6.90
control	3	2	0	1	8.50	9.16
control	4	2	2	0	7.53	7.94
control	5	2	0	2	6.80	sacrificed for HDL isolation
control	6	3	0	0	8.64	8.31
control	7	3	2	0	7.02	7.49
AVERAGE					7.30	7.85
SD					1.00	0.80
fenofibrate	8	4	2	1	5.57	5.24
fenofibrate	9	4	1	2	7.31	5.05
fenofibrate	10	5	2	0	9.03	5.06
fenofibrate	11	5	1	0	6.76	sacrificed for HDL isolation
fenofibrate	12	5	1	1	8.59	5.03
fenofibrate	13	6	0	1	8.60	5.27
fenofibrate	14	6	2	1	8.21	5.11
AVERAGE					7.73	5.13
SD					1.24	0.10
PFBS	15	1	1	7	4.87	sacrificed for HDL isolation
PFBS	16	2	1	7	9.12	5.78
PFBS	17	1	0	7	8.04	6.37
PFBS	18	1	0	8	10.51	8.20
PFBS	19	2	1	8	5.74	5.83
PFBS	20	0	1	9	7.57	5.08
PFBS	21	1	2	9	7.62	5.52
AVERAGE					7.64	6.13
SD					1.91	1.10
PFHS	22	0	2	10	6.49	1.65
PFHS	23	2	1	10	4.17	2.12
PFHS	24	1	0	11	8.08	2.65
PFHS	25	0	2	11	8.16	2.34
PFHS	26	1	1	12	8.78	2.89
PFHS	27	2	0	12	8.93	2.98
PFHS	28	0	2	12	7.55	sacrificed for HDL isolation
AVERAGE					7.45	2.44
SD					1.66	0.50
PFOS	29	0	0	13	7.70	2.15
PFOS	30	1	0	13	5.78	sacrificed for HDL isolation
PFOS	31	0	2	13	8.71	2.79
PFOS	32	0	1	14	9.29	3.01
PFOS	33	1	2	14	8.49	3.38
PFOS	34	0	1	15	8.16	2.04
PFOS	35	0	2	15	5.62	2.17
AVERAGE					7.68	2.59
SD					1.44	0.55

Appendix V Plasma HDL-cholesterol

Study 1

Group	Mouse#	Cage#	Eartag		Plasma HDL-cholesterol (mmol/L)		
			L	R	t=0 weeks	t=4 weeks	t=6 weeks
[illegible]	1	1	2	1	0.67	1.34	1.37
[illegible]	2	1	0	1	1.19	1.64	1.78
[illegible]	3	2	1	1	0.75	0.92	0.89
[illegible]	4	2	2	1	0.96	1.46	1.26
[illegible]	5	2	1	2	1.07	1.34	1.07
[illegible]	6	3	1	1	0.90	1.46	1.95
[illegible]	7	3	2	1	1.00	1.44	1.70
[illegible]	8	3	1	2	0.94	1.26	1.96
[illegible]					0.94	1.36	1.50
[illegible]					0.17	0.21	0.41
fenofibrate	9	4	1	0	0.91	2.10	3.01
fenofibrate	10	4	1	1	0.94	2.63	2.99
fenofibrate	11	5	1	1	1.12	2.12	2.19
fenofibrate	12	5	2	1	1.20	2.05	2.33
fenofibrate	13	6	0	2	0.94	2.09	2.36
fenofibrate	14	6	1	0	1.03	1.95	2.43
fenofibrate	15	7	1	0	0.56	1.45	1.46
fenofibrate	16	7	1	2	0.99	2.10	1.70
AVERAGE					0.96	2.06	2.31
SD					0.19	0.32	0.54
[illegible]	17	8	1	0	1.10	1.29	1.74
[illegible]	18	8	0	1	0.94	1.53	1.42
[illegible]	19	8	2	0	0.88	1.50	1.58
[illegible]	20	9	1	0	1.17	1.70	1.64
[illegible]	21	9	1	1	1.16	1.81	1.99
[illegible]	22	10(A)	0	0	0.88	1.25	0.61
[illegible]	23	10	0	1	0.47	1.25	0.81
[illegible]	24	10	2	1	0.83	1.81	1.88
[illegible]					0.93	1.52	1.46
[illegible]					0.23	0.24	0.50
[illegible]	25	11	0	0	1.33	0.92	0.83
[illegible]	26	11	1	1	1.39	0.99	0.79
[illegible]	27	11	1	2	1.07	1.02	0.63
[illegible]	28	12	0	2	0.91	0.95	0.66
[illegible]	29	12	1	2	1.35	0.58	0.72
[illegible]	30	13	1	2	1.16	0.87	0.63
[illegible]	31	13	0	0	0.68	0.72	0.60
[illegible]	32	13	1	0	0.51	0.71	0.67
[illegible]					1.05	0.85	0.69
[illegible]					0.32	0.16	0.08
PFOS	33	14	0	1	0.84	0.60	0.31
PFOS	34	14	1	2	0.82	0.53	0.33
PFOS	35	15	1	1	1.19	0.77	0.49
PFOS	36	15	1	2	1.27	0.73	0.54
PFOS	37	15	0	0	0.85	0.75	0.65
PFOS	38	16	2	2	1.20	0.61	0.55
PFOS	39	16	0	1	0.54	0.49	0.31
PFOS	40	16	1	1	0.80	0.49	0.36
AVERAGE					0.94	0.62	0.44
SD					0.25	0.12	0.13

Study 2:

Group	Mouse#	Cage#	Eartag		Plasma HDL- cholesterol (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
control	1	1	1	0	1.46	1.63
control	2	1	0	2	0.22	1.18
control	3	1	1	2	1.20	1.22
control	4	2	1	0	0.77	0.90
control	5	2	2	0	0.73	0.90
control	6	2	1	2	1.37	1.46
AVERAGE					0.96	1.22
SD					0.47	0.29
fenofibrate	7	3	0	1	1.18	2.25
fenofibrate	8	3	1	1	0.32	2.44
fenofibrate	9	3	2	0	0.75	2.25
fenofibrate	10	4	0	1	0.79	2.60
fenofibrate	11	4	0	2	1.35	2.09
fenofibrate	12	4	2	0	2.30	2.74
AVERAGE					1.12	2.39
SD					0.68	0.24
PFBS	13	5	0	0	0.51	1.48
PFBS	14	5	1	2	0.66	1.34
PFBS	15	5	0	1	2.01	1.57
PFBS	16	6	0	1	1.25	1.60
PFBS	17	6	0	2	1.08	2.10
PFBS	18	6	2	1	1.06	2.25
AVERAGE					1.10	1.72
SD					0.53	0.37
PFHS	19	7	0	0	1.08	0.93
PFHS	20	7	1	1	0.75	0.64
PFHS	21	8	2	1	1.35	0.70
PFHS	22	8	0	2	1.51	0.93
PFHS	23	9	1	1	0.96	0.78
PFHS	24	9	0	2	0.53	0.80
AVERAGE					1.03	0.80
SD					0.37	0.12
PFOS	25	10	0	0	1.10	0.45
PFOS	26	10	0	1	0.96	0.49
PFOS	27	11	1	0	0.60	0.64
PFOS	28	11	2	1	1.20	0.61
PFOS	29	12	1	1	1.84	0.72
PFOS	30	12	0	2	0.75	0.54
AVERAGE					1.07	0.58
SD					0.43	0.10

Study 3:

Group	Mouse#	Cage#	Eartag		Plasma HDL-cholesterol (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
CONTROL	1	1	0	1	1.52	1.39
CONTROL	2	1	1	2	1.44	1.02
CONTROL	3	2	0	1	1.24	0.89
CONTROL	4	2	2	0	0.78	1.36
CONTROL	5	2	0	2	1.29	sacrificed for HDL isolation
CONTROL	6	3	0	0	0.93	0.89
CONTROL	7	3	2	0	1.16	0.87
AVERAGE					1.19	1.07
SD					0.26	0.24
fenofibrate	8	4	2	1	1.27	1.70
fenofibrate	9	4	1	2	1.06	1.94
fenofibrate	10	5	2	0	1.34	1.78
fenofibrate	11	5	1	0	1.21	sacrificed for HDL isolation
fenofibrate	12	5	1	1	0.53	1.73
fenofibrate	13	6	0	1	1.12	1.96
fenofibrate	14	6	2	1	1.89	2.22
AVERAGE					1.20	1.89
SD					0.40	0.19
PFBS	15	1	1	7	2.74	sacrificed for HDL isolation
PFBS	16	2	1	7	1.17	1.26
PFBS	17	1	0	7	1.11	0.74
PFBS	18	1	0	8	0.67	0.84
PFBS	19	2	1	8	1.26	0.66
PFBS	20	0	1	9	1.01	1.80
PFBS	21	1	2	9	1.44	1.83
AVERAGE					1.34	1.19
SD					0.66	0.53
PFHS	22	0	2	10	1.19	0.13
PFHS	23	2	1	10	0.24	0.21
PFHS	24	1	0	11	0.88	0.40
PFHS	25	0	2	11	1.52	0.48
PFHS	26	1	1	12	1.29	0.61
PFHS	27	2	0	12	1.26	0.57
PFHS	28	0	2	12	1.11	sacrificed for HDL isolation
AVERAGE					1.07	0.40
SD					0.41	0.20
PFOS	29	0	0	13	1.29	0.31
PFOS	30	1	0	13	0.86	sacrificed for HDL isolation
PFOS	31	0	2	13	1.21	0.37
PFOS	32	0	1	14	1.45	0.05
PFOS	33	1	2	14	1.76	0.34
PFOS	34	0	1	15	0.83	0.40
PFOS	35	0	2	15	1.08	0.21
AVERAGE					1.21	0.28
SD					0.33	0.13

Appendix VI Plasma triglycerides

Study 1

Group	Mouse#	Cage#	Eartag		Plasma triglycerides (mmol/L)		
			L	R	t=0 weeks	t=4 weeks	t=6 weeks
control	1	1	2	1	2.90	2.61	1.61
control	2	1	0	1	1.99	2.09	0.91
control	3	2	1	1	3.59	2.10	3.58
control	4	2	2	1	1.99	1.38	2.28
control	5	2	1	2	1.67	1.81	3.19
control	6	3	1	1	2.69	2.23	1.43
control	7	3	2	1	1.68	1.42	1.04
control	8	3	1	2	1.38	1.60	1.23
AVERAGE					2.24	1.91	1.91
SD					0.75	0.43	1.01
fenofibrate	9	4	1	0	3.43	0.70	0.40
fenofibrate	10	4	1	1	2.77	0.57	0.44
fenofibrate	11	5	1	1	2.00	0.46	0.30
fenofibrate	12	5	2	1	1.60	0.47	0.34
fenofibrate	13	6	0	2	1.72	0.54	0.43
fenofibrate	14	6	1	0	1.27	0.57	0.43
fenofibrate	15	7	1	0	2.96	1.87	1.18
fenofibrate	16	7	1	2	1.78	0.96	0.64
AVERAGE					2.19	0.77	0.52
SD					0.76	0.47	0.29
PFBS	17	8	1	0	1.62	0.75	0.63
PFBS	18	8	0	1	2.37	1.01	0.95
PFBS	19	8	2	0	2.30	1.07	0.78
PFBS	20	9	1	0	1.60	0.71	0.61
PFBS	21	9	1	1	1.74	0.81	0.57
PFBS	22	10(A)	0	0	1.94	1.96	2.47
PFBS	23	10	0	1	2.20	1.39	1.26
PFBS	24	10	2	1	2.85	0.91	0.92
AVERAGE					2.08	1.08	1.02
SD					0.43	0.42	0.63
PFHS	25	11	0	0	1.40	0.35	0.42
PFHS	26	11	1	1	1.76	0.40	0.33
PFHS	27	11	1	2	2.04	0.49	0.33
PFHS	28	12	0	2	1.96	0.59	0.36
PFHS	29	12	1	2	0.90	0.60	0.29
PFHS	30	13	1	2	1.91	0.73	0.59
PFHS	31	13	0	0	2.11	0.65	0.32
PFHS	32	13	1	0	3.72	0.92	0.63
AVERAGE					1.97	0.59	0.41
SD					0.81	0.18	0.13
PFOS	33	14	0	1	3.48	0.76	0.54
PFOS	34	14	1	2	2.33	0.80	0.31
PFOS	35	15	1	1	1.54	0.67	0.52
PFOS	36	15	1	2	1.21	0.67	0.44
PFOS	37	15	0	0	2.66	0.71	0.56
PFOS	38	16	2	2	1.97	0.78	0.59
PFOS	39	16	0	1	2.19	0.89	0.71
PFOS	40	16	1	1	1.96	0.61	0.47
AVERAGE					2.17	0.74	0.52
SD					0.70	0.09	0.12

Study 2

Group	Mouse#	Cage#	Eartag		Plasma TG (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
[redacted]	1	1	1	0	1.81	1.58
[redacted]	2	1	0	2	3.48	1.48
[redacted]	3	1	1	2	1.91	1.57
[redacted]	4	2	1	0	2.74	1.25
[redacted]	5	2	2	0	2.01	1.06
[redacted]	6	2	1	2	1.60	1.29
[redacted]					2.26	1.37
[redacted]					0.71	0.21
fenofibrate	7	3	0	1	1.49	0.40
fenofibrate	8	3	1	1	2.66	0.54
fenofibrate	9	3	2	0	3.14	0.61
fenofibrate	10	4	0	1	2.24	0.59
fenofibrate	11	4	0	2	2.92	0.41
fenofibrate	12	4	2	0	1.18	0.32
AVERAGE					2.27	0.48
SD					0.79	0.12
[redacted]	13	5	0	0	3.05	1.04
[redacted]	14	5	1	2	4.21	1.22
[redacted]	15	5	0	1	1.64	0.99
[redacted]	16	6	0	1	1.41	0.71
[redacted]	17	6	0	2	2.59	0.70
[redacted]	18	6	2	1	2.31	0.57
[redacted]					2.54	0.87
[redacted]					1.02	0.25
[redacted]	19	7	0	0	3.53	0.85
[redacted]	20	7	1	1	1.95	0.51
[redacted]	21	8	2	1	1.44	0.38
[redacted]	22	8	0	2	0.99	0.57
[redacted]	23	9	1	1	2.65	0.42
[redacted]	24	9	0	2	2.94	0.64
[redacted]					2.25	0.56
[redacted]					0.96	0.17
PFOS	25	10	0	0	1.24	0.63
PFOS	26	10	0	1	3.32	0.63
PFOS	27	11	1	0	2.31	0.60
PFOS	28	11	2	1	2.40	0.59
PFOS	29	12	1	1	1.95	0.53
PFOS	30	12	0	2	3.10	1.10
AVERAGE					2.39	0.68
SD					0.76	0.21

Study 3

Group	Mouse#	Cage#	Eartag		Plasma TG (mmol/L)	
			L	R	t=0 weeks	t=4 weeks
control	1	1	0	1	1.64	1.47
control	2	1	1	2	1.38	1.21
control	3	2	0	1	1.50	1.49
control	4	2	2	0	2.14	1.80
control	5	2	0	2	1.16	sacrificed for HDL isolation
control	6	3	0	0	2.24	2.27
control	7	3	2	0	1.64	2.18
AVERAGE					1.67	1.74
SD					0.39	0.42
fenofibrate	8	4	2	1	1.49	0.41
fenofibrate	9	4	1	2	1.36	0.32
fenofibrate	10	5	2	0	1.62	0.43
fenofibrate	11	5	1	0	1.68	sacrificed for HDL isolation
fenofibrate	12	5	1	1	3.15	0.39
fenofibrate	13	6	0	1	1.58	0.33
fenofibrate	14	6	2	1	1.03	0.37
AVERAGE					1.70	0.38
SD					0.67	0.05
PFBS	15	1	1	7	0.57	sacrificed for HDL isolation
PFBS	16	2	1	7	1.46	0.87
PFBS	17	1	0	7	1.63	0.87
PFBS	18	1	0	8	3.30	1.21
PFBS	19	2	1	8	1.55	1.45
PFBS	20	0	1	9	2.04	0.63
PFBS	21	1	2	9	1.63	0.66
AVERAGE					1.74	0.95
SD					0.82	0.32
PFHS	22	0	2	10	1.46	0.41
PFHS	23	2	1	10	0.89	0.44
PFHS	24	1	0	11	2.48	0.40
PFHS	25	0	2	11	1.55	0.31
PFHS	26	1	1	12	1.52	0.57
PFHS	27	2	0	12	2.36	1.11
PFHS	28	0	2	12	2.40	sacrificed for HDL isolation
AVERAGE					1.81	0.54
SD					0.61	0.29
PFOS	29	0	0	13	1.36	0.35
PFOS	30	1	0	13	1.99	sacrificed for HDL isolation
PFOS	31	0	2	13	1.63	0.53
PFOS	32	0	1	14	1.78	0.42
PFOS	33	1	2	14	1.27	0.73
PFOS	34	0	1	15	2.56	0.38
PFOS	35	0	2	15	1.38	0.51
AVERAGE					1.71	0.49
SD					0.46	0.14

Appendix VII Plasma free glycerol

Study 1

Group	Mouse#	Cage#	Eartag		Plasma free glycerol (mmol/L)		
			L	R	t=0 weeks	t=4 weeks	t=6 weeks
control	1	1	2	1	0.25	0.24	0.14
control	2	1	0	1	0.18	0.23	0.17
control	3	2	1	1	0.28	0.29	0.24
control	4	2	2	1	0.24	0.23	0.20
control	5	2	1	2	0.25	0.28	0.20
control	6	3	1	1	0.24	0.31	0.17
control	7	3	2	1	0.17	0.22	0.19
control	8	3	1	2	0.20	0.25	0.22
AVERAGE					0.23	0.26	0.19
SD					0.04	0.03	0.03
fenofibrate	9	4	1	0	0.28	0.11	0.13
fenofibrate	10	4	1	1	0.21	0.23	0.10
fenofibrate	11	5	1	1	0.22	0.22	0.14
fenofibrate	12	5	2	1	0.25	0.23	0.19
fenofibrate	13	6	0	2	0.21	0.17	0.14
fenofibrate	14	6	1	0	0.24	0.22	0.12
fenofibrate	15	7	1	0	0.24	0.17	0.17
fenofibrate	16	7	1	2	0.21	0.26	0.11
AVERAGE					0.23	0.20	0.14
SD					0.03	0.05	0.03
PFBS	17	8	1	0	0.21	0.21	0.16
PFBS	18	8	0	1	0.32	0.25	0.12
PFBS	19	8	2	0	0.27	0.21	0.13
PFBS	20	9	1	0	0.16	0.26	0.15
PFBS	21	9	1	1	0.24	0.21	0.14
PFBS	22	10(A)	0	0	0.29	0.20	0.25
PFBS	23	10	0	1	0.25	0.23	0.21
PFBS	24	10	2	1	0.24	0.15	0.16
AVERAGE					0.25	0.21	0.16
SD					0.05	0.03	0.04
PFHS	25	11	0	0	0.18	0.11	0.08
PFHS	26	11	1	1	0.22	0.11	0.10
PFHS	27	11	1	2	0.16	0.11	0.09
PFHS	28	12	0	2	0.26	0.18	0.08
PFHS	29	12	1	2	0.21	0.11	0.09
PFHS	30	13	1	2	0.30	0.15	0.12
PFHS	31	13	0	0	0.23	0.10	0.08
PFHS	32	13	1	0	0.20	0.15	0.08
AVERAGE					0.22	0.13	0.09
SD					0.04	0.03	0.01
PFOS	33	14	0	1	0.33	0.11	0.08
PFOS	34	14	1	2	0.22	0.14	0.08
PFOS	35	15	1	1	0.18	0.14	0.06
PFOS	36	15	1	2	0.22	0.13	0.05
PFOS	37	15	0	0	0.23	0.19	0.06
PFOS	38	16	2	2	0.19	0.14	0.10
PFOS	39	16	0	1	0.25	0.18	0.14
PFOS	40	16	1	1	0.25	0.15	0.13
AVERAGE					0.23	0.15	0.09
SD					0.05	0.03	0.03

Appendix VIII Plasma free fatty acids

Study 1

Group	Mouse#	Cage#	Eartag		Plasma free fatty acids (mmol/L)		
			L	R	t=0 weeks	t=4 weeks	t=6 weeks
control	1	1	2	1	1.40	0.78	0.60
control	2	1	0	1	1.34	0.67	0.71
control	3	2	1	1	0.78	0.63	0.75
control	4	2	2	1	0.92	0.80	0.82
control	5	2	1	2	1.11	0.84	0.80
control	6	3	1	1	0.70	0.66	0.70
control	7	3	2	1	0.88	0.56	0.74
control	8	3	1	2	0.75	0.61	0.76
AVERAGE					0.99	0.69	0.74
SD					0.27	0.10	0.07
fenofibrate	9	4	1	0	1.27	0.59	0.64
fenofibrate	10	4	1	1	1.17	0.82	0.80
fenofibrate	11	5	1	1	0.93	0.63	0.55
fenofibrate	12	5	2	1	0.96	0.59	0.72
fenofibrate	13	6	0	2	0.74	0.72	0.61
fenofibrate	14	6	1	0	0.97	0.80	0.57
fenofibrate	15	7	1	0	1.02	0.72	0.78
fenofibrate	16	7	1	2	0.81	0.86	0.76
AVERAGE					0.98	0.72	0.68
SD					0.17	0.11	0.10
PFBS	17	8	1	0	0.96	0.59	0.75
PFBS	18	8	0	1	0.98	0.66	0.55
PFBS	19	8	2	0	1.08	0.74	0.77
PFBS	20	9	1	0	0.89	0.65	0.69
PFBS	21	9	1	1	1.00	0.74	0.64
PFBS	22	10(A)	0	0	0.91	0.58	0.45
PFBS	23	10	0	1	0.68	0.69	0.74
PFBS	24	10	2	1	0.70	0.65	0.76
AVERAGE					0.90	0.66	0.67
SD					0.14	0.06	0.12
PFHS	25	11	0	0	1.00	0.39	0.39
PFHS	26	11	1	1	1.81	0.25	0.29
PFHS	27	11	1	2	0.62	0.39	0.32
PFHS	28	12	0	2	0.98	0.47	0.38
PFHS	29	12	1	2	0.90	0.34	0.40
PFHS	30	13	1	2	1.22	0.50	0.50
PFHS	31	13	0	0	0.90	0.44	0.36
PFHS	32	13	1	0	0.70	0.50	0.44
AVERAGE					1.02	0.41	0.39
SD					0.37	0.09	0.06
PFOS	33	14	0	1	1.82	0.37	0.29
PFOS	34	14	1	2	1.10	0.39	0.31
PFOS	35	15	1	1	0.92	0.52	0.28
PFOS	36	15	1	2	1.06	0.52	0.27
PFOS	37	15	0	0	0.66	0.48	0.26
PFOS	38	16	2	2	0.80	0.42	0.35
PFOS	39	16	0	1	0.70	0.41	0.31
PFOS	40	16	1	1	1.10	0.39	0.29
AVERAGE					1.02	0.44	0.29
SD					0.37	0.06	0.03

Appendix IX Plasma ApoA1

Group	Mouse#	Cage#	Eartag		Plasma ApoA1 (mg/mL)	
			L	R	t=0 weeks	t=4 weeks
[redacted]	1	1	0	1	2.40	2.70
[redacted]	2	1	1	2	1.43	2.66
[redacted]	3	2	0	1	1.96	2.69
[redacted]	4	2	2	0	1.26	2.32
[redacted]	5	2	0	2	2.05	sacrificed for HDL isolation
[redacted]	6	3	0	0	1.55	1.70
[redacted]	7	3	2	0	1.79	1.62
[redacted]					1.78	2.28
[redacted]					0.40	0.50
fenofibrate	8	4	2	1	1.84	2.86
fenofibrate	9	4	1	2	1.42	2.43
fenofibrate	10	5	2	0	1.81	2.49
fenofibrate	11	5	1	0	1.63	sacrificed for HDL isolation
fenofibrate	12	5	1	1	0.77	2.83
fenofibrate	13	6	0	1	1.53	2.41
fenofibrate	14	6	2	1	3.09	2.90
AVERAGE					1.73	2.65
SD					0.70	0.23
[redacted]	15	1	1	7	3.53	sacrificed for HDL isolation
[redacted]	16	2	1	7	1.67	1.53
[redacted]	17	1	0	7	1.55	1.35
[redacted]	18	1	0	8	1.13	1.85
[redacted]	19	2	1	8	1.80	4.07
[redacted]	20	0	1	9	1.37	2.30
[redacted]	21	1	2	9	2.50	2.72
[redacted]					1.94	2.30
[redacted]					0.82	1.00
PFHS	22	0	2	10	1.81	0.41
PFHS	23	2	1	10	0.31	0.16
PFHS	24	1	0	11	1.79	0.71
PFHS	25	0	2	11	2.08	0.45
PFHS	26	1	1	12	1.47	0.60
PFHS	27	2	0	12	1.93	0.90
PFHS	28	0	2	12	1.36	sacrificed for HDL isolation
AVERAGE					1.54	0.54
SD					0.60	0.26
PFOS	29	0	0	13	1.38	0.47
PFOS	30	1	0	13	1.49	sacrificed for HDL isolation
PFOS	31	0	2	13	1.93	0.53
PFOS	32	0	1	14	2.19	0.32
PFOS	33	1	2	14	1.95	0.48
PFOS	34	0	1	15	1.67	0.39
PFOS	35	0	2	15	1.54	0.37
AVERAGE					1.73	0.43
SD					0.30	0.08

Appendix X Plasma CETP mass

Group	Mouse#	Cage#	Eartag		Plasma CETP (µg/mL)	
			L	R	t=0 weeks	t=4 weeks
[redacted]	1	1	0	1	11.08	15.53
[redacted]	2	1	1	2	14.19	14.26
[redacted]	3	2	0	1	12.19	16.51
[redacted]	4	2	2	0	14.26	15.47
[redacted]	5	2	0	2	15.29	sacrificed for HDL isolation
[redacted]	6	3	0	0	15.47	13.68
[redacted]	7	3	2	0	13.29	12.82
[redacted]					13.68	14.71
[redacted]					1.61	1.37
fenofibrate	8	4	2	1	9.53	8.32
fenofibrate	9	4	1	2	11.24	not enough plasma left
fenofibrate	10	5	2	0	16.79	9.70
fenofibrate	11	5	1	0	12.19	sacrificed for HDL isolation
fenofibrate	12	5	1	1	12.61	8.99
fenofibrate	13	6	0	1	14.32	9.61
fenofibrate	14	6	2	1	15.23	8.71
AVERAGE					13.13	9.06
SD					2.48	0.59
[redacted] PFBS	15	1	1	7	9.26	sacrificed for HDL isolation
[redacted] PFBS	16	2	1	7	14.93	11.00
[redacted] PFBS	17	1	0	7	16.45	13.29
[redacted] PFBS	18	1	0	8	13.68	12.95
[redacted] PFBS	19	2	1	8	9.08	11.00
[redacted] PFBS	20	0	1	9	13.09	10.61
[redacted] PFBS	21	1	2	9	12.95	11.98
[redacted] AVERAGE					12.78	11.81
[redacted] SD					2.74	1.12
PFHS	22	0	2	10	12.05	7.49
PFHS	23	2	1	10	9.26	not enough plasma left
PFHS	24	1	0	11	16.11	8.80
PFHS	25	0	2	11	12.89	12.40
PFHS	26	1	1	12	19.63	8.22
PFHS	27	2	0	12	16.28	10.12
PFHS	28	0	2	12	10.04	sacrificed for HDL isolation
AVERAGE					13.75	9.41
SD					3.75	1.93
PFOS	29	0	0	13	14.32	8.61
PFOS	30	1	0	13	10.04	sacrificed for HDL isolation
PFOS	31	0	2	13	13.75	9.61
PFOS	32	0	1	14	13.29	9.44
PFOS	33	1	2	14	14.38	11.39
PFOS	34	0	1	15	12.19	7.81
PFOS	35	0	2	15	10.37	7.91
AVERAGE					12.62	9.13
SD					1.81	1.34

Appendix XI Plasma CETP activity

CETP activity

Group	Mouse#	Cage#	Eartag		CETP activity (pmol/h)	
			L	R	t=0 weeks	t=4 weeks
control	1	1	0	1	245	175
control	2	1	1	2	227	211
control	3	2	0	1	202	218
control	4	2	2	0	265	207
control	5	2	0	2	208	sacrificed for HDL isolation
control	6	3	0	0	255	253
control	7	3	2	0	189	192
AVERAGE					227	209
SD					29	26
fenofibrate	8	4	2	1	not enough plasma left	not enough plasma left
fenofibrate	9	4	1	2	214	not enough plasma left
fenofibrate	10	5	2	0	243	107
fenofibrate	11	5	1	0	190	sacrificed for HDL isolation
fenofibrate	12	5	1	1	288	84
fenofibrate	13	6	0	1	231	112
fenofibrate	14	6	2	1	193	87
AVERAGE					227	97
SD					37	14
PFBS	15	1	1	7	not enough plasma left	sacrificed for HDL isolation
PFBS	16	2	1	7	218	169
PFBS	17	1	0	7	250	250
PFBS	18	1	0	8	299	185
PFBS	19	2	1	8	192	202
PFBS	20	0	1	9	230	104
PFBS	21	1	2	9	188	103
AVERAGE					229	169
SD					41	58
PFHS	22	0	2	10	185	142
PFHS	23	2	1	10	188	not enough plasma left
PFHS	24	1	0	11	242	166
PFHS	25	0	2	11	196	194
PFHS	26	1	1	12	265	165
PFHS	27	2	0	12	290	194
PFHS	28	0	2	12	not enough plasma left	sacrificed for HDL isolation
AVERAGE					228	172
SD					44	22
PFOS	29	0	0	13	228	163
PFOS	30	1	0	13	232	sacrificed for HDL isolation
PFOS	31	0	2	13	216	157
PFOS	32	0	1	14	212	190
PFOS	33	1	2	14	not enough plasma left	187
PFOS	34	0	1	15	254	139
PFOS	35	0	2	15	not enough plasma left	162
AVERAGE					229	166
SD					17	19

Appendix XII Post heparin LPL and HL activity

Group	Mouse	LPL + HL activity (µmol FFA/h/mL)	HL activity (µmol FFA/h/mL)	LPL activity (µmol FFA/h/mL)
[illegible]	1	16.62	5.99	10.63
[illegible]	2	24.15	8.63	15.51
[illegible]	3	19.70	7.66	12.04
[illegible]	4	21.64	7.71	13.93
[illegible]	5	16.03	6.73	9.31
[illegible]	6	14.77	6.56	8.21
[illegible]	7	21.01	7.41	13.61
[illegible]	8	20.79	8.32	12.48
[illegible]			7.37	11.97
[illegible]			0.90	2.46
fenofibrate	9	38.29	11.89	26.40
fenofibrate	10	36.46	11.85	24.60
fenofibrate	11	35.48	14.24	21.24
fenofibrate	12	34.58	12.44	22.14
fenofibrate	13	41.82	13.88	27.94
fenofibrate	14	41.60	11.36	30.25
fenofibrate	15	38.49	12.87	25.63
fenofibrate	16	32.51	10.05	22.46
AVERAGE			12.32	25.08
SD			1.36	3.10
PFBS	17	20.17	6.65	13.52
PFBS	18	19.91	7.23	12.69
PFBS	19	20.45	5.78	14.66
PFBS	20	21.07	4.81	16.26
PFBS	21	14.50	3.29	11.22
PFBS	22	21.24	8.67	12.57
PFBS	23	18.29	3.40	14.89
PFBS	24	21.57	2.66	18.91
AVERAGE			5.31	14.34
SD			2.14	2.43
PFHS	25	25.43	9.73	15.70
PFHS	26	26.37	6.09	20.28
PFHS	27	31.74	5.80	25.94
PFHS	28	34.69	14.09	20.60
PFHS	29	24.99	5.51	19.48
PFHS	30	24.67	11.20	13.47
PFHS	31	28.34	6.82	21.52
PFHS	32	37.83	8.40	29.44
AVERAGE			8.45	20.81
SD			3.04	5.12
PFOS	33	31.98	10.04	21.94
PFOS	34	30.83	8.52	22.31
PFOS	35	28.65	9.94	18.71
PFOS	36	20.98	5.79	15.19
PFOS	37	27.94	8.58	19.36
PFOS	38	24.04	9.95	14.09
PFOS	39	26.84	9.74	17.10
PFOS	40	28.61	9.53	19.08
AVERAGE			9.01	18.47
SD			1.43	2.92

Appendix XIII Fecal lipids

Fecal neutral sterols

Group	Cage#	total neural sterols excretion (µmol/100 gr mouse/day)	% of neutral sterols			
			coprostanol	cholesterol	cholestanol	lathosterol
control	1	33.54	0.18	93.21	3.93	2.68
control	2	54.59	0.18	93.87	1.73	4.21
control	3	32.79	0.13	93.20	3.64	3.03
control	1	31.08	0.11	94.06	3.21	2.62
control	2	42.23	0.10	94.69	1.30	3.91
control	3	23.63	0.11	93.08	3.92	2.89
AVERAGE		36.31	0.14	93.68	2.96	3.22
SD		10.75	0.04	0.63	1.15	0.67
fenofibrate	4+5	32.00	0.19	94.08	2.59	3.13
fenofibrate	6	55.13	0.15	95.75	1.64	2.46
fenofibrate	7	43.36	0.12	93.96	2.00	3.92
fenofibrate	4+5	33.96	0.11	94.86	2.45	2.58
fenofibrate	6	49.60	0.08	95.64	1.89	2.40
fenofibrate	7	53.05	0.09	94.52	1.57	3.81
AVERAGE		44.52	0.12	94.80	2.02	3.05
SD		9.81	0.04	0.76	0.42	0.69
PFBS	8	38.02	0.11	94.02	2.80	3.07
PFBS	9	34.74	0.13	94.29	2.81	2.77
PFBS	10+10A	43.30	0.16	95.16	1.69	2.99
PFBS	8	38.40	0.11	93.99	3.02	2.88
PFBS	9	38.16	0.09	94.32	2.86	2.72
PFBS	10+10A	35.69	0.12	94.40	2.22	3.26
AVERAGE		38.05	0.12	94.36	2.57	2.95
SD		2.98	0.02	0.42	0.51	0.20
PFHS	11	33.47	0.17	92.34	5.19	2.31
PFHS	12	33.56	0.16	92.03	5.12	2.69
PFHS	13	43.17	0.12	93.50	2.90	3.47
PFHS	11	37.90	0.10	92.84	4.78	2.28
PFHS	12	40.25	0.09	93.41	4.20	2.30
PFHS	13	46.53	0.08	94.60	2.51	2.81
AVERAGE		39.15	0.12	93.12	4.12	2.64
SD		5.23	0.04	0.93	1.15	0.46
PFOS	14	43.81	0.13	92.50	3.47	3.91
PFOS	15	26.10	0.16	92.60	4.44	2.80
PFOS	16	15.50	0.21	89.74	7.26	2.78
PFOS	14	50.66	0.09	93.93	2.88	3.10
PFOS	15	39.01	0.13	93.71	3.45	2.71
PFOS	16	29.21	0.15	91.93	5.20	2.73
AVERAGE		34.05	0.14	92.40	4.45	3.00
SD		12.86	0.04	1.51	1.61	0.47

Fecal phytosterols

Group	Cage#	total phytosterols excretion (µmol/100 gr mouse/day)	% of phytosterols		
			campesterol	stigmasterol	b-sitosterol
control	1	3.56	27.00	9.10	63.91
control	2	4.53	25.93	9.55	64.52
control	3	3.39	25.97	9.43	64.60
control	1	3.44	25.68	9.52	64.80
control	2	3.73	25.63	8.34	66.02
control	3	2.43	26.42	9.34	64.25
AVERAGE		3.51	26.10	9.21	64.68
SD		0.67	0.52	0.46	0.73
fenofibrate	4+5	2.80	26.83	9.20	63.97
fenofibrate	6	3.99	27.14	7.03	65.83
fenofibrate	7	3.00	28.24	6.47	65.29
fenofibrate	4+5	2.73	26.91	9.68	63.40
fenofibrate	6	3.66	25.40	8.89	65.71
fenofibrate	7	4.04	26.92	9.22	63.86
AVERAGE		3.37	26.91	8.41	64.68
SD		0.60	0.90	1.33	1.05
PFBS	8	3.27	26.43	6.50	67.07
PFBS	9	3.20	26.16	9.44	64.40
PFBS	10+10A	4.24	25.75	8.95	65.30
PFBS	8	3.52	26.10	8.97	64.92
PFBS	9	3.66	26.04	10.13	63.83
PFBS	10+10A	3.71	26.15	8.43	65.41
AVERAGE		3.60	26.11	8.74	65.16
SD		0.38	0.22	1.23	1.11
PFHS	11	2.86	26.93	5.80	67.27
PFHS	12	2.95	26.85	6.28	66.88
PFHS	13	2.56	28.21	6.16	65.62
PFHS	11	3.16	24.70	12.99	62.31
PFHS	12	2.99	25.11	13.36	61.53
PFHS	13	3.04	25.10	12.24	62.66
AVERAGE		2.93	26.15	9.47	64.38
SD		0.21	1.39	3.74	2.51
PFOS	14	4.14	24.36	15.20	60.44
PFOS	15	3.01	25.40	12.36	62.24
PFOS	16	2.35	22.54	16.10	61.36
PFOS	14	3.71	24.79	16.54	58.68
PFOS	15	3.73	24.94	12.79	62.27
PFOS	16	3.65	23.16	15.94	60.90
AVERAGE		3.43	24.20	14.82	60.98
SD		0.64	1.11	1.80	1.34

Fecal bile acids

Group	Cage#	total bile acid excretion (µmol/100 gr mouse/day)	% of bile acids						
			a-muricholate	deoxycholate	cholate	lithocholate	b-muricholate	w-muricholate	hyodeo/urso
[redacted]	1	8.33	7.93	19.10	12.16	10.58	21.94	21.91	6.38
[redacted]	2	5.80	5.78	7.67	19.16	8.52	26.92	26.31	5.64
[redacted]	3	4.22	7.29	15.90	8.58	12.54	24.50	22.82	8.38
[redacted]	1	5.95	8.38	17.35	11.45	11.15	23.01	22.19	6.47
[redacted]	2	4.98	5.80	7.92	15.99	9.60	29.02	24.82	6.86
[redacted]	3	4.01	7.24	17.33	8.60	10.92	27.53	22.68	5.70
[redacted]		5.5	7.1	14.2	12.7	10.6	25.5	23.5	6.6
[redacted]		1.6	1.1	5.1	4.2	1.4	2.8	1.7	1.0
fenofibrate	4+5	3.67	6.56	10.23	12.02	18.35	16.64	17.63	18.57
fenofibrate	6	2.37	5.83	9.99	13.10	19.39	10.93	21.23	19.53
fenofibrate	7	4.37	5.10	7.58	19.89	12.89	25.21	18.78	10.56
fenofibrate	4+5	3.52	8.32	18.88	7.28	15.93	18.92	16.50	14.16
fenofibrate	6	2.54	5.43	9.82	11.03	21.14	11.94	16.52	24.13
fenofibrate	7	4.13	5.06	7.11	17.15	12.00	26.95	20.46	11.27
AVERAGE		3.4	6.0	10.6	13.4	16.6	18.4	18.5	16.4
SD		0.8	1.2	4.3	4.5	3.7	6.6	2.0	5.3
[redacted]	8	4.08	6.38	18.03	8.92	16.49	16.09	40.86	11.53
[redacted]	9	4.21	9.58	27.82	18.10	29.65	47.57	43.85	21.37
[redacted]	10+10A	6.83	4.98	12.73	17.49	11.63	32.23	14.60	6.84
[redacted]	8	6.74	6.50	25.12	5.88	20.57	11.32	40.27	16.51
[redacted]	9	3.46	8.54	17.42	8.27	16.75	23.43	25.28	10.95
[redacted]	10+10A	4.86	2.81	6.50	9.32	8.99	16.59	10.17	7.63
[redacted]		5.0	6.5	17.9	11.3	17.3	24.5	29.2	12.5
[redacted]		1.4	2.4	7.8	5.2	7.3	13.4	14.6	5.5
PFHS	11	3.74	9.81	25.45	19.87	14.44	25.96	35.27	9.03
PFHS	12	3.57	9.31	31.19	26.55	19.39	25.45	35.36	15.22
PFHS	13	3.46	5.72	8.61	19.43	8.03	19.87	19.61	7.47
PFHS	11	4.07	5.67	15.48	10.32	8.35	11.36	30.81	6.91
PFHS	12	3.13	5.76	16.94	10.67	12.09	13.12	23.66	10.42
PFHS	13	1.60	1.73	2.75	3.67	4.04	3.61	4.30	3.52
AVERAGE		3.3	6.3	16.7	15.1	11.1	16.6	24.8	8.8
SD		0.9	2.9	10.5	8.3	5.4	8.8	11.9	3.9
PFOS	14	2.87	5.40	10.53	21.12	10.62	15.61	30.96	9.21
PFOS	15	2.90	7.94	14.67	15.40	8.58	16.71	36.72	7.19
PFOS	16	3.29	8.86	33.87	19.26	1.99	28.04	42.81	8.53
PFOS	14	2.31	3.42	5.60	10.72	5.32	8.37	10.93	6.65
PFOS	15	2.66	4.12	5.99	8.69	4.91	7.21	18.62	4.38
PFOS	16	2.66	3.18	10.28	5.67	6.27	8.66	17.88	4.03
AVERAGE		2.8	5.5	13.5	13.5	6.3	14.1	26.3	6.7
SD		0.3	2.4	10.5	6.1	3.0	7.9	12.4	2.1

Fecal fatty acids

Group	Cage#	total fatty acid excretion (µmol/100 gr mouse/day)	% of fatty acids						
			C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
[redacted]	1	106.3	3.29	48.11	0.68	39.69	7.44	0.79	0.00
[redacted]	2	563.7	1.99	44.01	0.59	46.97	5.57	0.77	0.09
[redacted]	3	146.4	2.66	47.28	0.78	42.34	6.23	0.66	0.05
[redacted]	1	94.4	2.74	45.79	0.73	41.96	7.92	0.87	0.00
[redacted]	2	520.7	2.42	47.12	0.61	43.67	5.46	0.68	0.05
[redacted]	3	110.1	3.05	46.50	0.69	41.36	7.69	0.70	0.00
[redacted]		256.9	2.69	46.47	0.68	42.66	6.72	0.75	0.03
[redacted]		222.1	0.46	1.43	0.07	2.48	1.10	0.08	0.04
fenofibrate	4+5	251.4	2.74	49.74	0.70	39.35	6.82	0.61	0.05
fenofibrate	6	564.9	2.21	47.47	0.60	43.73	5.37	0.58	0.04
fenofibrate	7	386.9	1.83	41.60	0.61	46.64	8.38	0.82	0.11
fenofibrate	4+5	319.7	2.21	46.82	0.64	44.06	5.59	0.64	0.05
fenofibrate	6	535.8	2.06	45.87	0.55	45.43	5.44	0.61	0.04
fenofibrate	7	399.7	2.20	46.56	0.63	44.17	5.67	0.69	0.07
AVERAGE		409.7	2.21	46.34	0.62	43.90	6.21	0.66	0.06
SD		121.5	0.30	2.68	0.05	2.48	1.19	0.09	0.03
PFBS	8	242.8	2.56	48.30	0.67	42.54	5.30	0.58	0.05
PFBS	9	190.7	2.68	48.04	0.68	41.06	6.88	0.62	0.04
PFBS	10+10A	378.4	2.33	46.86	0.63	43.88	5.60	0.66	0.04
PFBS	8	239.0	2.52	48.38	0.66	40.90	6.90	0.59	0.04
PFBS	9	144.1	2.81	47.43	0.62	42.27	6.23	0.65	0.00
PFBS	10+10A	246.0	2.66	48.92	0.66	40.08	7.00	0.65	0.03
AVERAGE		240.2	2.59	47.99	0.65	41.79	6.32	0.62	0.03
SD		78.5	0.16	0.74	0.03	1.37	0.73	0.03	0.02
PFHS	11	85.4	3.18	49.02	1.02	38.52	7.65	0.62	0.00
PFHS	12	101.8	3.12	47.31	1.05	39.47	8.26	0.78	0.00
PFHS	13	122.3	2.91	50.57	0.51	38.64	6.79	0.58	0.00
PFHS	11	101.1	2.89	47.45	0.98	39.69	8.26	0.74	0.00
PFHS	12	127.2	2.61	47.96	0.98	39.50	8.14	0.82	0.00
PFHS	13	418.7	1.16	38.79	0.59	50.06	8.48	0.82	0.10
AVERAGE		159.4	2.64	46.85	0.86	40.98	7.93	0.73	0.02
SD		127.9	0.75	4.13	0.24	4.47	0.62	0.10	0.04
PFOS	14	208.7	2.38	47.10	0.70	41.76	7.23	0.76	0.07
PFOS	15	162.3	2.57	47.50	0.73	40.94	7.51	0.71	0.04
PFOS	16	78.8	3.07	48.02	0.80	39.52	7.81	0.78	0.00
PFOS	14	330.1	1.74	42.76	0.61	47.92	6.10	0.79	0.08
PFOS	15	132.4	2.28	46.94	0.69	43.08	6.26	0.72	0.05
PFOS	16	218.7	2.19	45.49	0.61	45.10	5.87	0.70	0.04
AVERAGE		188.5	2.37	46.30	0.69	43.05	6.79	0.74	0.05
SD		86.3	0.44	1.93	0.07	3.05	0.82	0.04	0.03

Fatty acid balance

Group	Cage#	total fatty acid excretion (µmol/100 gr mouse/day)	total fatty acid input (µmol/100 gr mouse/day)	Fatty acid balance (µmol/100 gr mouse/day)
[illegible]	1	106	5800	5694
[illegible]	2	564	6138	5574
[illegible]	3	146	5178	5032
[illegible]	1	94	5461	5367
[illegible]	2	521	6488	5967
[illegible]	3	110	5427	5317
[illegible]		256.92	5749	5492
[illegible]		222.07	492	326
fenofibrate	4+5	251	6347	6095
fenofibrate	6	565	6476	5911
fenofibrate	7	387	6393	6006
fenofibrate	4+5	320	5998	5678
fenofibrate	6	536	6577	6041
fenofibrate	7	400	7352	6952
AVERAGE		409.73	6524	6114
SD		121.45	451	436
[illegible]	8	243	5407	5164
[illegible]	9	191	5739	5548
[illegible]	10+10A	378	7801	7422
[illegible]	8	239	6048	5809
[illegible]	9	144	5949	5804
[illegible]	10+10A	246	7373	7127
[illegible]		240.17	6386	6146
[illegible]		78.53	965	910
PFHS	11	85	5323	5237
PFHS	12	102	5604	5503
PFHS	13	122	5823	5701
PFHS	11	101	5541	5439
PFHS	12	127	5896	5769
PFHS	13	419	6159	5740
AVERAGE		159.41	5724	5565
SD		127.93	296	209
PFOS	14	209	6276	6067
PFOS	15	162	5239	5077
PFOS	16	79	5148	5069
PFOS	14	330	6430	6099
PFOS	15	132	5865	5732
PFOS	16	219	5732	5514
AVERAGE		188.49	5782	5593
SD		86.31	523	458

Appendix XIV VLDL-triglycerides and de novo ApoB production

VLDL-triglycerides production

Group	Mouse#	Body weight (g)	Plasma triglycerides (mmol/L)					Slope	VLDL-TG production (µmol/h)
			t=0 min	t=15 min	t=30 min	t=60 min	t=90 min		
[redacted]	1	32.3	1.90	3.22	4.755	6.681	8.478	0.073	6.33
[redacted]	3	32.4	1.76	2.84	4.515	6.468	7.965	0.070	6.11
[redacted]	4	30.3	1.30	3.20	5.157	8.373	11.532	0.113	9.25
[redacted]	5	29.0	1.76	2.33	3.975	5.589	8.715	0.077	6.03
[redacted]	6	26.6	0.96	2.03	3.498	5.433	7.26	0.070	5.04
[redacted]	7	27.2	1.00	2.30	3.591	6.159	8.817	0.087	6.37
[redacted]	8	25.5	1.00	2.15	3.324	5.748	7.875	0.077	5.30
[redacted]		29.0	1.38	2.58	4.12	6.35	8.66	0.081	6.35
[redacted]		2.8	0.41	0.50	0.70	1.00	1.38	0.015	1.38
fenofibrate	9	29.2	0.50	1.84	3.618	7.002	9.474	0.102	8.06
fenofibrate	10	30.3	0.70	2.42	4.323	8.223	10.419	0.111	9.09
fenofibrate	11	26.9	0.23	1.40	3.564	7.332	10.065	0.114	8.25
fenofibrate	12	28.0	0.46	1.69	3.876	7.494	12.102	0.131	9.87
fenofibrate	13	31.9	0.28	1.79	3.792	6.843	12.036	0.128	11.05
fenofibrate	14	23.4	0.23	1.44	3.531	6.321	9.837	0.107	6.77
fenofibrate	15	30.0	1.04	2.52	4.302	7.779	10.722	0.109	8.85
fenofibrate	16	29.7	0.60	2.60	5.025	9.654	14.028	0.151	12.09
AVERAGE		28.7	0.50	1.96	4.00	7.58	11.09	0.119	9.25
SD		2.6	0.28	0.48	0.52	1.02	1.53	0.016	1.71
PFBS	17	27.3	0.57	1.97	3.456	6.087	8.895	0.092	6.79
PFBS	18	31.9	1.11	2.66	4.629	7.017	10.965	0.107	9.20
PFBS	19	28.3	1.00	2.88	4.932	7.854	10.806	0.108	8.25
PFBS	20	27.2	0.71	1.30	2.529	5.019	7.803	0.081	5.94
PFBS	21	27.8	0.57	1.64	3.612	6.192	8.388	0.089	6.67
PFBS	22	26.5	1.19	2.30	3.45	5.214	6.993	0.064	4.57
PFBS	23	30.1	0.74	2.18	3.702	6.537	9.795	0.100	8.13
PFBS	24	26.6	0.43	1.52	2.79	4.866	7.497	0.078	5.59
AVERAGE		28.2	0.79	2.05	3.64	6.10	8.89	0.090	6.89
SD		1.9	0.28	0.56	0.82	1.04	1.50	0.015	1.55
PFHS	25	26.9	0.28	0.44	0.696	0.969	1.467	0.013	0.94
PFHS	26	28.3	0.41	0.65	0.921	1.338	1.755	0.015	1.14
PFHS	27	27.3	0.41	0.67	1.002	1.626	2.253	0.021	1.53
PFHS	28	28.9	0.31	0.80	1.362	2.202	3.195	0.032	2.48
PFHS	29	27.2	0.23	0.47	0.852	1.518	2.235	0.023	1.66
PFHS	30	31.2	0.37	0.82	1.404	2.286	3.261	0.032	2.71
PFHS	31	28.0	0.18	0.30	0.555	1.089	1.311	0.014	1.02
PFHS	32	32.9	0.48	0.84	1.089	1.782	2.349	0.021	1.85
AVERAGE		28.8	0.33	0.62	0.99	1.60	2.23	0.021	1.66
SD		2.1	0.10	0.20	0.30	0.48	0.72	0.008	0.66
PFOS	33	31.3	0.54	0.70	0.99	1.26	1.80	0.014	1.16
PFOS	34	28.7	0.49	0.86	1.48	2.13	2.46	0.022	1.74
PFOS	35	26.3	0.34	0.45	0.63	0.80	1.12	0.009	0.60
PFOS	36	27.7	0.46	0.56	0.79	1.19	1.49	0.012	0.90
PFOS	37	25.7	0.39	0.56	0.89	1.55	1.94	0.018	1.26
PFOS	38	29.4	0.59	0.62	0.74	0.98	1.23	0.007	0.59
PFOS	39	25.6	0.90	0.92	0.98	1.10	1.57	0.007	0.49
PFOS	40	27.6	0.40	0.41	0.62	0.93	1.04	0.008	0.60
AVERAGE		27.8	0.51	0.63	0.89	1.24	1.58	0.012	0.92
SD		2.0	0.18	0.18	0.28	0.42	0.48	0.006	0.44
[redacted]	2	25.7	0.76	1.61	2.478	3.714	4.356	0.040	2.78

De novo ApoB synthesis and TG production per ApoB

Group	Mouse#	Body weight (g)	µl serum used for VLDL isolation	ml VLDL after UC	35S dpm in ApoB per 400 µl VLDL	de novo ApoB synthesis (* 10^4 dpm/ml/hr)	TG production per ApoB (µmol/10^6 dpm)
	1	32.3	200	1.197	4756.4	4.74	0.917
	3	32.4	245	1.210	6062.0	4.99	0.841
	4	30.3	280	1.216	10320.5	7.47	0.908
	5	29.0	220	1.202	6896.9	6.28	0.735
	6	26.6	280	1.162	8837.6	6.11	0.689
	7	27.2	200	1.183	7309.8	7.20	0.722
	8	25.5	300	1.202	8982.9	6.00	0.770
		29.0				6.11	0.80
		2.8				1.02	0.09
fenofibrate	9	29.2	240	1.178	8178.3	6.69	0.917
fenofibrate	10	30.3	185	1.219	6455.8	7.09	0.940
fenofibrate	11	26.9	250	1.208	8164.6	6.58	1.036
fenofibrate	12	28.0	270	1.201	8402.8	6.23	1.257
fenofibrate	13	31.9	280	1.208	7718.3	5.55	1.387
fenofibrate	14	23.4	160	1.199	5371.3	6.71	0.958
fenofibrate	15	30.0	300	1.234	9005.4	6.17	1.062
fenofibrate	16	29.7	300	1.216	9726.5	6.57	1.377
AVERAGE		28.7				6.45	1.12
SD		2.6				0.46	0.19
PFOS	17	27.3	185	1.218	5776.8	6.34	0.872
PFOS	18	31.9	190	1.214	4434.8	4.72	1.358
PFOS	19	28.3	185	1.215	4785.3	5.24	1.236
PFOS	20	27.2	200	1.187	4695.1	4.64	1.045
PFOS	21	27.8	140	1.186	3184.5	4.50	1.185
PFOS	22	26.5	280	1.212	5217.1	3.76	1.019
PFOS	23	30.1	280	1.205	6610.5	4.74	1.266
PFOS	24	26.6	290	1.212	9286.6	6.47	0.722
AVERAGE		28.2				5.05	1.09
SD		1.9				0.93	0.21
PFHS	25	26.9	190	1.217	1514.1	1.62	0.479
PFHS	26	28.3	195	1.212	1545.3	1.60	0.559
PFHS	27	27.3	175	1.203	1306.0	1.50	0.831
PFHS	28	28.9	250	1.204	2057.2	1.65	1.152
PFHS	29	27.2	220	1.206	1523.0	1.39	0.975
PFHS	30	31.2	300	1.190	2655.5	1.76	1.098
PFHS	31	28.0	320	1.207	1387.7	0.87	0.929
PFHS	32	32.9	320	1.191	2419.8	1.50	0.833
AVERAGE		28.8				1.49	0.86
SD		2.1				0.27	0.24
PFOS	33	31.3	190	1.197	874.7	0.92	0.899
PFOS	34	28.7	200	1.216	1059.7	1.07	1.257
PFOS	35	26.3	200	1.206	651.1	0.65	0.780
PFOS	36	27.7	220	1.200	846.6	0.77	0.934
PFOS	37	25.7	210	1.197	1216.1	1.15	0.945
PFOS	38	29.4	330	1.203	987.9	0.60	0.745
PFOS	39	25.6	340	1.196	1188.7	0.70	0.613
PFOS	40	27.6	300	1.167	1039.2	0.67	0.712
AVERAGE		27.8				0.82	0.86
SD		2.0				0.21	0.20
	2	25.7	190	1.199	6243.1	6.57	0.367

Lipid composition per ApoB

Group	Mouse#	Lipid in isolated VLDL per ApoB (µmol/10^4 ApoB)				
		Total cholesterol	Free cholesterol	Cholesterol ester	Triglycerides	Phospholipids
[illegible]	1	0.98	0.26	0.71	1.14	0.32
[illegible]	3	0.71	0.22	0.49	0.90	0.25
[illegible]	4	0.43	0.15	0.28	0.83	0.19
[illegible]	5	0.74	0.18	0.57	0.68	0.24
[illegible]	6	0.64	0.17	0.47	0.61	0.20
[illegible]	7	0.51	0.14	0.36	0.64	0.18
[illegible]	8	0.57	0.16	0.41	0.74	0.21
[illegible]		0.65	0.18	0.47	0.79	0.23
[illegible]		0.18	0.04	0.14	0.19	0.05
fenofibrate	9	0.38	0.15	0.23	0.87	0.23
fenofibrate	10	0.37	0.12	0.25	0.88	0.20
fenofibrate	11	0.27	0.12	0.15	0.93	0.19
fenofibrate	12	0.35	0.13	0.22	1.00	0.20
fenofibrate	13	0.34	0.17	0.17	1.17	0.25
fenofibrate	14	0.29	0.12	0.18	0.82	0.17
fenofibrate	15	0.50	0.20	0.30	1.06	0.26
fenofibrate	16	0.38	0.19	0.19	1.23	0.28
AVERAGE		0.36	0.15	0.21	0.99	0.22
SD		0.07	0.03	0.05	0.15	0.04
[illegible]	17	0.51	0.13	0.37	0.86	0.21
[illegible]	18	0.62	0.19	0.43	1.36	0.32
[illegible]	19	0.49	0.17	0.32	1.17	0.27
[illegible]	20	0.57	0.17	0.40	1.02	0.24
[illegible]	21	0.48	0.16	0.33	1.09	0.27
[illegible]	22	2.10	0.51	1.58	1.16	0.44
[illegible]	23	0.77	0.22	0.55	1.27	0.33
[illegible]	24	0.49	0.15	0.34	0.75	0.22
[illegible]		0.75	0.21	0.54	1.08	0.29
[illegible]		0.55	0.12	0.43	0.20	0.08
[illegible]	25	0.25	0.12	0.13	0.41	0.14
[illegible]	26	0.48	0.14	0.34	0.60	0.19
[illegible]	27	0.42	0.17	0.25	0.84	0.23
[illegible]	28	0.41	0.21	0.20	0.94	0.24
[illegible]	29	0.45	0.20	0.25	0.77	0.22
[illegible]	30	0.45	0.24	0.21	1.11	0.24
[illegible]	31	0.43	0.25	0.18	0.76	0.23
[illegible]	32	0.79	0.31	0.48	0.99	0.28
[illegible]		0.46	0.20	0.25	0.80	0.22
[illegible]		0.15	0.06	0.11	0.22	0.04
PFOS	33	0.62	0.32	0.30	1.23	0.34
PFOS	34	0.88	0.33	0.55	1.35	0.34
PFOS	35	0.81	0.37	0.44	1.09	0.34
PFOS	36	1.22	0.40	0.82	1.02	0.32
PFOS	37	0.90	0.28	0.62	0.76	0.26
PFOS	38	3.96	0.97	3.00	1.21	0.65
PFOS	39	2.42	0.67	1.75	1.10	0.47
PFOS	40	2.06	0.55	1.52	0.95	0.43
AVERAGE		1.61	0.49	1.12	1.09	0.39
SD		1.15	0.23	0.92	0.18	0.12
[illegible]	2	0.55	0.14	0.41	0.53	0.21

Appendix XV Biliary bile acids, cholesterol and phospholipids

Bile flow

Group	Mouse	volume (µL)				Bile flow (µL/min/kg mouse)			
		15 min	30 min	45 min	total	15 min	30 min	45 min	total
[redacted]	1	11.70	8.10	8.00	27.80	30.35	21.01	20.75	24.04
[redacted]	2	14.80	0.50	13.40	28.70	37.66		34.10	34.10
[redacted]	3	19.10	13.10	16.50	48.70	47.51	32.59	41.04	40.38
[redacted]	4	16.60	17.30	18.40	52.30	37.90	39.50	42.01	39.80
[redacted]	5	16.70	14.60	16.30	47.60	39.48	34.52	38.53	37.51
[redacted]	6	16.30	16.30	16.80	49.40	36.34	36.34	37.46	36.71
[redacted]	7	23.90	22.10	23.80	69.80	54.75	50.63	54.52	53.30
[redacted]						43.20	38.71	41.28	40.30
[redacted]						7.76	7.13	7.07	6.76
Fenofibrate	7	9.20	2.80	3.90	15.90	23.50	7.15	9.96	13.54
Fenofibrate	8	20.50	22.30	13.20	56.00	46.64	50.74	30.03	42.47
Fenofibrate	9	24.10	19.20	18.70	62.00	55.98	44.60	43.44	48.01
Fenofibrate	10	22.10	24.10	26.70	72.90	50.28	54.84	60.75	55.29
Fenofibrate	11	18.30	19.20	20.60	58.10	45.86	48.12	51.63	48.54
Fenofibrate	12	22.30	16.10	16.70	55.10	53.67	38.75	40.19	44.20
extra fenofibrate	12	19.40	17.70	23.20	60.30	39.43	35.98	47.15	40.85
AVERAGE						50.49	47.41	45.21	47.70
SD						5.50	6.60	9.53	4.80
[redacted]PFBS	13	14.30	15.30	4.90	34.50	38.93	36.30	11.63	27.28
[redacted]PFBS	14	19.40	13.70	15.40	48.50	41.32	29.18	32.80	34.43
[redacted]PFBS	15	18.50	14.40	15.40	48.30	45.01	35.04	37.47	39.17
[redacted]PFBS	16	15.70	13.30	15.20	44.20	38.91	32.96	37.67	36.51
[redacted]PFBS	17	18.00	14.40	12.90	45.30	45.63	36.50	32.70	38.28
[redacted]PFBS	18								
extra PFBS	21	20.30	25.50	27.80	73.60	47.82	60.07	65.49	57.79
AVERAGE						43.74	38.75	41.23	41.24
SD						3.57	12.23	13.78	9.43
PFHS	19	9.60	0.50	0.40	10.50	21.84	1.14	0.91	7.96
PFHS	20	19.00	21.00	14.80	54.80	46.57	51.47	36.27	44.77
PFHS	21	22.70	22.10	22.80	67.60	60.05	58.47	60.32	59.61
PFHS	22	23.90	21.30	22.70	67.90	56.50	50.35	53.66	53.51
PFHS	23	33.90	26.20	24.70	84.80	83.70	64.69	60.99	69.79
PFHS	24	36.70	34.80	32.70	104.20	75.98	72.05	67.70	71.91
extra PFHS	24	25.40	21.30	21.00	67.70	60.69	50.90	50.18	53.92
AVERAGE						63.92	57.99	54.85	58.92
SD						13.55	8.88	10.96	10.41
PFOS	25	16.50	10.60	9.40	36.50	38.60	24.80	21.99	28.46
PFOS	26	18.00	10.00	13.30	41.30	42.25	23.47	31.22	32.32
PFOS	27	22.10	18.30	14.80	55.20	54.98	45.52	36.82	45.77
PFOS	28	21.20	19.50	20.50	61.20	49.94	45.94	48.29	48.06
PFOS	29	23.50	18.70	18.40	60.60	57.60	45.83	45.10	49.51
PFOS	30	13.70	8.80	9.90	32.40	35.13	22.56	25.38	27.69
extra PFOS	34	34.00	32.90	29.90	96.80	80.38	77.78	70.69	76.28
AVERAGE						51.27	40.84	39.93	44.01
SD						15.30	19.71	16.64	17.01

mouse 1 mouse not warm enough
mouse 2 canula wrong, hardly any flow, after 30 min adjusted
mouse 7 mouse not warm enough
mouse 13 mouse not warm enough
mouse 18 mouse died during anaesthesia
mouse 19 mouse not warm enough

Bile acid flow

Group	Mouse	Bile acid conc in bile (mM)			Bile acid flow (nmol/min/kg mouse)			
		15 min	30 min	45 min	15 min	30 min	45 min	Total
Control	1	14.09	8.18	14.71	428	172	305	302
Control	2	11.25		12.47	424		425	425
Control	3	16.88	16.23	18.17	802	529	746	692
Control	4	8.71	10.52	10.15	330	415	427	391
Control	5	13.09	13.46	14.34	517	465	552	511
Control	6	12.60	15.34	16.88	458	558	632	549
extra control	7	17.64	14.35	16.67	966	726	909	867
AVERAGE		13.78	13.98	14.78	614	539	615	573
SD		3.61	2.20	3.05	262	119	189	179
Fenofibrate	7		12.47	19.36		89	193	141
Fenofibrate	8	9.07	8.95	9.43	423	454	283	387
Fenofibrate	9	9.19	9.43	9.67	514	421	420	452
Fenofibrate	10	8.95	9.07	9.31	450	497	565	504
Fenofibrate	11	9.19	9.31	9.43	421	448	487	452
Fenofibrate	12	8.95	9.07	9.43	480	351	379	404
extra fenofibrate	12	13.12	12.38	14.35	517	445	677	546
AVERAGE		9.74	9.70	10.27	468	436	469	457
SD		1.66	1.32	2.00	43	48	140	60
PFBS	13	8.95	9.07	9.43	304	329	110	247
PFBS	14	9.55	8.83	9.19	395	258	301	318
PFBS	15	15.72	14.34	13.21	708	502	495	568
PFBS	16	16.75	19.75	22.32	652	651	841	715
PFBS	17	16.11	11.37	27.42	735	415	897	682
PFBS	18							
extra PFBS	21	11.76	11.26	7.34	562	676	481	573
AVERAGE		13.98	13.11	15.90	610	500	603	571
SD		3.15	4.20	8.65	137	173	255	156
PFHS	19							
PFHS	20	8.95	11.25	8.83	417	579	320	439
PFHS	21	10.64	7.51	8.83	639	439	532	537
PFHS	22	10.15	8.71	10.10	574	438	542	518
PFHS	23	9.19	9.67	9.43	769	626	575	657
PFHS	24	9.43	9.07	8.95	717	653	606	659
extra PFHS	24	10.50	9.34	10.12	637	475	508	540
AVERAGE		9.81	9.26	9.38	625	535	514	558
SD		0.71	1.23	0.61	123	96	101	85
PFOS	25	11.98	11.37	12.84	462	282	282	342
PFOS	26	15.09	18.30	17.00	638	430	531	533
PFOS	27	8.82	7.88	8.15	485	359	300	381
PFOS	28	11.26	12.75	13.49	562	586	651	600
PFOS	29	8.82	11.76	8.82	508	539	398	481
PFOS	30	12.38	16.91	15.33	435	382	389	402
extra PFOS	34	8.82	8.15	11.76	709	634	831	725
AVERAGE		11.02	12.45	12.48	543	459	483	495
SD		2.38	3.98	3.22	100	130	201	136

mouse 1	mouse not warm enough
mouse 2	canula wrong, hardly any flow, after 30 min adjusted
mouse 7	mouse not warm enough
mouse 13	mouse not warm enough
mouse 18	mouse died during anaesthesia
mouse 19	mouse not warm enough

Phospholipid flow

Group	Mouse	PL conc in bile (mg/dL)			PL flow (nmol/min/kg mouse)			
		15 min	30 min	45 min	15 min	30 min	45 min	Total
Control	1	417	646	696	163	175	186	175
Control	2	633		722	307		317	317
Control	3	544	582	836	333	245	443	340
Control	4	379	443	430	185	225	233	215
Control	5	620	684	747	316	304	371	331
Control	6	557	773	811	261	362	392	338
Control	7	787	635	913	556	415	642	538
AVERAGE		577	623	743	330	310	400	346
SD		147	123	168	139	79	139	105
Fenofibrate	7		671	976		62	125	94
Fenofibrate	8	366	443	430	220	290	167	226
Fenofibrate	9	709	760	747	512	437	419	456
Fenofibrate	10	925	938	874	600	663	685	649
Fenofibrate	11	900	760	696	532	472	464	489
Fenofibrate	12	620	1039	785	429	519	407	452
extra fenofibrate	12	812	913	989	413	424	601	479
AVERAGE		722	809	754	451	467	457	459
SD		209	209	189	132	123	180	136
PFBS	13	544	823	722	238	386	108	244
PFBS	14	709	773	823	378	291	348	339
PFBS	15	493	646	595	286	292	288	289
PFBS	16	938	1217	1065	471	517	517	502
PFBS	17	722	633	1788	425	298	754	492
PFBS	18							
extra PFBS	21	660	597	395	407	463	334	401
AVERAGE		704	773	933	393	372	448	405
SD		159	257	539	69	109	192	93
PFHS	19							
PFHS	20	696	684	747	418	454	350	407
PFHS	21	595	608	709	461	458	552	490
PFHS	22	811	747	874	591	485	605	560
PFHS	23	760	709	773	821	592	608	673
PFHS	24	811	912	925	795	848	808	817
extra PFHS	24	812	736	825	636	483	534	551
AVERAGE		747	733	809	620	553	576	583
SD		87	101	81	166	153	148	144
PFOS	25	696	836	1001	347	267	284	299
PFOS	26	722	1065	1471	393	322	592	436
PFOS	27	572	597	559	406	351	266	341
PFOS	28	534	660	648	344	391	404	380
PFOS	29	307	585	509	228	346	296	290
PFOS	30	1557	1683	1595	705	490	522	573
extra PFOS	34	787	787	812	816	789	740	782
AVERAGE		739	888	942	463	422	443	443
SD		394	388	437	214	176	181	178

mouse 1	mouse not warm enough
mouse 2	canula wrong, hardly any flow, after 30 min adjusted
mouse 7	mouse not warm enough
mouse 13	mouse not warm enough
mouse 18	mouse died during anaesthesia
mouse 19	mouse not warm enough

Cholesterol flow

Group	Mouse	Cholesterol in bile (mmol/L)			Cholesterol flow (nmol/min/kg mouse)			
		15 min	30 min	45 min	15 min	30 min	45 min	Total
Control	1	o sample le	0.63	0.45		13.2	9.3	11.3
Control	2	0.51		1.11	19.1		37.8	37.8
Control	3	0.75	0.81	1.08	35.5	26.3	44.3	35.4
Control	4	0.54	0.93	1.02	20.5	36.7	42.8	33.3
Control	5	0.79	0.54	0.55	31.3	18.6	21.3	23.8
Control	6	0.58	0.66	0.64	21.2	24.0	24.1	23.1
extra control	7	1.08	1.17	1.07	59.2	59.3	58.1	58.9
AVG		0.75	0.82	0.91	33.6	33.0	38.1	35.4
SD		0.21	0.24	0.25	15.7	16.1	13.7	13.0
Fenofibrate	7	no sample le	o sample left					
Fenofibrate	8	0.60	0.64	0.70	28.0	32.7	21.1	27.3
Fenofibrate	9	0.63	0.57	0.64	35.3	25.4	28.0	29.6
Fenofibrate	10	0.60	0.66	0.63	30.2	36.2	38.3	34.9
Fenofibrate	11	0.48	0.88	0.96	22.0	42.5	49.6	38.0
Fenofibrate	12	0.69	0.99	0.99	37.0	38.4	39.8	38.4
extra fenofibrate	12	1.35	1.52	1.07	53.4	54.7	50.3	52.8
AVERAGE		0.73	0.88	0.83	34.3	38.3	37.8	36.8
SD		0.32	0.35	0.19	10.8	9.9	11.6	9.0
PFBS	13	0.54	0.72	0.21	18.2	26.1	2.4	15.6
PFBS	14	0.58	0.67	0.66	24.1	19.7	21.6	21.8
PFBS	15	1.04	0.69	0.85	46.6	24.2	32.0	34.3
PFBS	16	0.63	0.85	0.88	24.4	28.1	33.3	28.6
PFBS	17	0.63	0.60	1.50	28.7	21.9	49.0	33.2
PFBS	18							
extra PFBS	21	0.81	0.89	0.89	38.7	53.2	58.0	50.0
AVERAGE		0.74	0.74	0.96	32.5	29.4	38.8	33.6
SD		0.19	0.12	0.32	9.8	13.7	14.5	10.4
PFHS	19							
PFHS	20	0.81	1.16	1.19	37.6	59.5	43.0	46.7
PFHS	21	0.51	0.46	0.54	30.6	27.1	32.6	30.1
PFHS	22	0.69	0.64	0.70	39.0	32.4	37.8	36.4
PFHS	23	0.64	0.75	0.63	53.9	48.5	38.4	46.9
PFHS	24	0.58	0.55	0.66	44.4	39.9	44.7	43.0
extra PFHS	24	0.74	0.82	0.82	45.2	41.9	41.3	42.8
AVERAGE		0.66	0.73	0.76	41.8	41.6	39.6	41.0
SD		0.11	0.24	0.23	7.9	11.5	4.4	6.6
PFOS	25	1.32	1.23	1.64	50.9	30.5	36.0	39.1
PFOS	26	1.17	1.05	0.99	49.4	24.6	30.9	35.0
PFOS	27	1.02	1.07	1.10	56.2	48.5	40.4	48.4
PFOS	28	0.82	0.98	0.98	41.2	44.8	47.1	44.4
PFOS	29	0.49	1.02	1.19	28.3	46.8	53.6	42.9
PFOS	30	0.65	0.82	0.89	22.9	18.6	22.5	21.3
extra PFOS	34	0.81	1.07	0.82	65.1	82.9	58.2	68.8
AVERAGE		0.90	1.03	1.09	44.9	42.4	41.2	42.8
SD		0.29	0.12	0.27	15.1	21.3	12.7	14.4

mouse 1	mouse not warm enough
mouse 2	canula wrong, hardly any flow, after 30 min adjusted
mouse 7	mouse not warm enough
mouse 13	mouse not warm enough
mouse 18	mouse died during anaesthesia
mouse 19	mouse not warm enough

Appendix XVI In vivo clearance of VLDL-like TG-rich particles and uptake in liver

Plasma DECAY 3H

[redacted]

	1	2	3	4	5	6	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
2	110.88	94.29	105.45	100.00	88.86	102.16	98.15	6.59	2.95	5
5	104.79	85.62	100.35	95.12	77.79	93.48	90.47	8.84	3.95	5
10	94.01	73.24	77.04	78.21	69.79	85.65	76.79	5.96	2.67	5
20	66.22	42.66	58.42	58.48	55.72	58.37	54.73	6.85	3.06	5
30	46.14	21.46	34.12	42.35	45.12	43.94	37.40	9.90	4.43	5

mouse excluded, not warm enough

SERUM DECAY 3H (t2 = 100%)

control

	1	2	3	4	5	6	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
5	94.50	90.80	95.17	95.13	87.54	91.50	92.03	3.21	1.44	5
10	84.78	77.67	73.06	78.21	78.54	83.84	78.27	3.83	1.71	5
20	59.72	45.24	55.40	58.48	62.70	57.14	55.79	6.48	2.90	5
30	41.61	22.76	32.36	42.35	50.78	43.01	38.25	10.85	4.85	5

SERUM HALF-LIFE 3H

	1	2	3	4	5	6				
k el	0.032	0.053	0.040	0.031	0.023	0.031	0.04	0.01	0.01	5
t 1/2	21.73	13.08	17.42	22.29	29.75	22.50	21.01	6.25	2.79	5

Mouse/ORGAN MASS (g)

control

	1	2	3	4	5	6	average	std	SEM	n
mouse	25.7	26.2	26.8	29.2	28.2	29.9	28.06	1.56	0.7	5
liver	1.350	1.370	1.230	1.540	1.490	1.610	1.45	0.15	0.067	5
heart	0.117	0.136	0.130	0.142	0.144	0.120	0.13	0.01	0.004	5
spleen	0.099	0.099	0.099	0.123	0.110	0.103	0.11	0.01	0.004	5

ORGAN DISTRIBUTION 3H (% DOSIS)

control

	1	2	3	4	5	6	average	std	SEM	n
liver	12.24	10.51	10.27	9.13	6.76	7.07	8.75	1.76	0.79	5
heart	3.82	2.91	1.85	2.26	1.48	1.43	1.99	0.61	0.27	5
spleen	0.65	0.66	0.96	0.72	0.96	0.55	0.77	0.18	0.08	5
muscle	5.99	7.10	5.72	4.78	3.54	2.97	4.82	1.66	0.74	5
gWAT	0.47	1.01	0.50	0.92	0.84	0.62	0.78	0.21	0.10	5

Plasma DECAY 3H

Group 2 **fenofibrate**

	7	8	9	10	11	12	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
2	101.22	75.49	94.93	89.01	62.91	67.92	78.05	13.64	6.10	5
5	99.33	61.03	85.15	68.26	55.58	48.93	63.79	13.89	6.21	5
10	74.89	41.59	56.25	28.38	28.43	20.55	35.04	14.06	6.29	5
20	40.12	11.13	24.18	6.60	9.67	5.88	11.49	7.41	3.32	5
30	18.42	5.17	9.36	3.84	4.00	3.24	5.12	2.47	1.10	5

mouse excluded, not warm enough

SERUM DECAY 3H (t2 = 100%)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
5	98.14	80.84	89.69	76.68	88.35	72.04	81.52	7.54	3.37	5
10	73.99	55.10	59.25	31.88	45.20	30.25	44.33	13.16	5.88	5
20	39.64	14.75	25.47	7.41	15.37	8.66	14.33	7.17	3.20	5
30	18.20	6.85	9.86	4.32	6.36	4.77	6.43	2.19	0.98	5

SERUM HALF-LIFE 3H

	7	8	9	10	11	12				
k el	0.063	0.100	0.084	0.119	0.102	0.113	0.10	0.01	0.01	5
t 1/2	11.09	6.90	8.22	5.82	6.78	6.16	6.78	0.92	0.41	5

Mouse/ORGAN MASS (g)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
mouse	26.1	29.3	28.7	29.3	26.6	27.7	28.32	1.16	0.52	5
liver	1.720	1.950	1.830	1.850	1.690	1.490	1.76	0.18	0.08	5
heart	0.125	0.146	0.147	0.131	0.124	0.124	0.13	0.01	0.01	5
spleen	0.085	0.076	0.108	0.082	0.077	0.079	0.08	0.01	0.01	5

ORGAN DISTRIBUTION 3H (% DOSIS)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
liver	14.08	16.35	23.07	18.61	14.14	14.35	17.31	3.69	1.65	5
heart	3.66	1.50	1.87	1.21	0.69	1.04	1.26	0.45	0.20	5
spleen	0.65	0.65	0.73	0.43	0.51	0.32	0.53	0.17	0.07	5
muscle	7.97	9.79	7.64	7.27	5.48	9.61	7.96	1.79	0.80	5
gWAT	2.04	2.13	1.17	2.98	2.78	1.12	2.04	0.87	0.39	5

Plasma DECAY 3H

	13	14	15	16	17	18	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00		100.00	0.00	0.00	4
2	84.17	96.20	88.73	101.91	84.97		92.96	7.58	3.79	4
5	82.48	84.54	72.32	88.14	79.42		81.10	6.86	3.43	4
10	72.43	53.93	41.34	62.68	63.71		55.41	10.36	5.18	4
20	52.17	36.23	14.41	22.77	38.65		28.01	11.45	5.72	4
30	28.22	15.52	6.40	10.26	23.11		13.82	7.23	3.62	4
	mouse excluded, cooled off				mouse died					

SERUM DECAY 3H (t2 = 100%)

PFBS

	13	14	15	16	17	18	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00		100.00	0.00	0.00	4
5	97.99	87.87	81.50	86.49	93.46		87.33	4.92	2.46	4
10	86.05	56.06	46.58	61.50	74.97		59.78	11.86	5.93	4
20	61.98	37.66	16.23	22.35	45.49		30.43	13.49	6.74	4
30	33.53	16.13	7.21	10.07	27.20		15.15	8.85	4.43	4

SERUM HALF-LIFE 3H

	13	14	15	16	17	18				
k el	0.039	0.063	0.097	0.085	0.048		0.07	0.02	0.01	4
t 1/2	17.86	10.93	7.18	8.15	14.56		10.21	3.31	1.66	4

Mouse/ORGAN MASS (g)

PFBS

	13	14	15	16	17	18	average	std	SEM	n
mouse	29.3	31.3	27.4	26.9	26.3		27.98	2.26	1.13	4
liver	1.520	1.790	1.520	1.320	1.500		1.53	0.19	0.10	4
heart	0.120	0.142	0.123	0.122	0.117		0.13	0.01	0.01	4
spleen	0.056	0.127	0.088	0.092	0.103		0.10	0.02	0.01	4

ORGAN DISTRIBUTION 3H (% DOSIS)

PFBS

	13	14	15	16	17	18	average	std	SEM	n
liver	12.57	15.18	15.74	12.53	10.06		13.38	2.62	1.31	4
heart	2.15	2.91	1.54	2.21	0.60		1.81	0.98	0.49	4
spleen	0.91	0.66	0.57	0.37	0.69		0.57	0.14	0.07	4
muscle	7.19	5.94	8.52	7.50	3.57		6.38	2.16	1.08	4
gWAT	0.62	0.65	1.08	0.00	4.05		1.45	1.79	0.90	4

Plasma DECAY 3H

Group 4 **PFHS**

	19	20	21	22	23	24	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
2	75.24	69.83	73.55	88.17	88.57	87.20	80.43	8.47	3.46	6
5	68.94	52.84	55.16	74.06	54.76	67.72	62.25	9.05	3.69	6
10	48.71	38.49	26.12	55.35	24.35	32.34	37.56	12.45	5.08	6
20	21.18	16.26	8.12	25.07	11.84	16.26	16.45	6.12	2.50	6
30	8.92	8.14	4.80	13.06	4.49	6.28	7.62	3.20	1.30	6

SERUM DECAY 3H (t2 = 100%)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
5	91.63	75.67	75.00	83.99	61.83	77.67	77.63	9.97	4.07	6
10	64.74	55.11	35.51	62.78	27.49	37.09	47.12	15.75	6.43	6
20	28.15	23.28	11.04	28.43	13.37	18.65	20.49	7.39	3.02	6
30	11.85	11.66	6.53	14.81	5.07	7.20	9.52	3.80	1.55	6

SERUM HALF-LIFE 3H

	19	20	21	22	23	24				
k el	0.078	0.077	0.102	0.070	0.102	0.093	0.09	0.01	0.01	6
t 1/2	8.86	8.99	6.83	9.96	6.78	7.48	8.15	1.31	0.53	6

Mouse/ORGAN MASS (g)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
mouse	29.3	27.2	25.2	28.2	27.0	32.2	28.18	2.39	0.98	6
liver	2.890	2.720	2.780	2.830	3.170	3.640	3.01	0.35	0.14	6
heart	0.142	0.132	0.101	0.116	0.128	0.157	0.13	0.02	0.01	6
spleen	0.094	0.155	0.073	0.097	0.102	0.101	0.10	0.03	0.01	6

ORGAN DISTRIBUTION 3H (% DOSIS)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
liver	25.93	22.04	25.47	23.83	17.18	16.31	21.79	4.15	1.69	6
heart	1.09	1.49	0.77	1.23	0.59	0.59	0.96	0.37	0.15	6
spleen	0.38	0.59	0.26	0.38	0.33	0.23	0.36	0.13	0.05	6
muscle	7.14	7.54	6.84	8.61	4.88	6.81	6.97	1.22	0.50	6
gWAT	1.03	0.91	1.26	0.83	2.45	4.43	1.82	1.41	0.58	6

Plasma DECAY 3H

Group 5 **PFOS**

	25	26	27	28	29	30	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
2	73.11	70.90	79.23	94.43	79.87	73.93	78.58	8.53	3.48	6
5	63.63	57.01	67.75	62.47	61.35	57.64	61.64	3.98	1.63	6
10	47.09	34.40	48.18	46.09	43.84	34.75	42.39	6.23	2.54	6
20	27.71	20.65	23.32	26.80	19.28	12.71	21.74	5.52	2.26	6
30	14.99	13.27	13.83	14.60	10.74	4.83	12.04	3.84	1.57	6

SERUM DECAY 3H (t2 = 100%)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
5	87.03	80.42	85.51	66.15	76.81	77.97	78.98	7.48	3.05	6
10	64.42	48.52	60.81	48.81	54.89	47.00	54.07	7.24	2.95	6
20	37.90	29.13	29.43	28.38	24.14	17.19	27.70	6.82	2.79	6
30	20.51	18.72	17.46	15.46	13.45	6.54	15.36	4.97	2.03	6

SERUM HALF-LIFE 3H

	25	26	27	28	29	30				
k el	0.057	0.060	0.064	0.063	0.072	0.098	0.07	0.02	0.01	6
t 1/2	12.25	11.63	10.81	11.02	9.59	7.05	10.39	1.86	0.76	6

Mouse/ORGAN MASS (g)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
mouse	28.5	28.4	26.8	28.3	27.2	26.0	27.53	1.03	0.42	6
liver	3.170	2.930	2.720	2.880	3.370	2.730	2.97	0.26	0.10	6
heart	0.133	0.131	0.121	0.120	0.118	0.117	0.12	0.01	0.00	6
spleen	0.101	0.098	0.094	0.074	0.080	0.062	0.08	0.02	0.01	6

ORGAN DISTRIBUTION 3H (% DOSIS)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
liver	17.74	19.80	18.52	11.49	17.43	11.53	16.09	3.64	1.48	6
heart	2.63	1.09	1.90	1.68	0.71	0.75	1.46	0.75	0.31	6
spleen	0.43	0.67	0.43	0.76	0.36	0.18	0.47	0.21	0.09	6
muscle	8.17	7.66	5.07	6.98	4.73	5.63	6.37	1.43	0.58	6
gWAT	1.03	1.36	1.50	1.17	1.60	5.65	2.05	1.77	0.72	6

Plasma DECAY 14C

	1	2	3	4	5	6	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
2	110.13	92.26	103.51	99.46	74.50	99.73	93.89	11.58	5.18	5
5	107.98	90.27	96.01	96.21	74.98	97.16	90.93	9.32	4.17	5
10	100.64	87.47	85.94	89.28	71.93	95.25	85.97	8.61	3.85	5
20	87.83	65.39	82.80	78.41	66.09	74.20	73.38	7.61	3.40	5
30	86.54	49.93	63.97	75.25	61.97	66.15	63.45	9.11	4.07	5

mouse excluded, cooled off

SERUM DECAY 14C (t2 = 100%)

control

	1	2	3	4	5	6	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
5	98.05	97.84	92.75	96.73	100.65	97.43	97.08	2.84	1.27	5
10	91.39	94.81	83.02	89.76	96.55	95.50	91.93	5.62	2.51	5
20	79.75	70.87	79.99	78.83	88.72	74.40	78.56	6.74	3.01	5
30	78.58	54.12	61.80	75.66	83.19	66.32	68.22	11.44	5.11	5

SERUM HALF-LIFE 14C

	1	2	3	4	5	6				
k el	0.010	0.023	0.015	0.011	0.007	0.016	0.01	0.01	0.00	5
t 1/2	72.96	30.40	45.01	66.01	97.63	43.87	56.58	26.24	11.74	5

MOUSE/ORGAN MASS (g)

control

	1	2	3	4	5	6	average	std	SEM	n
mouse	25.7	26.2	26.8	29.2	28.2	29.9	28.06	1.56	0.7	5
liver	1.350	1.370	1.230	1.540	1.490	1.610	1.45	0.15	0.067	5
heart	0.117	0.136	0.130	0.142	0.144	0.120	0.13	0.01	0.004	5
spleen	0.099	0.099	0.099	0.123	0.110	0.103	0.11	0.01	0.004	5

ORGAN DISTRIBUTION 14C (% DOSIS)

control

	1	2	3	4	5	6	average	std	SEM	n
liver	27.48	38.23	28.74	26.78	17.66	20.24	26.33	8.06	3.61	5
heart	1.82	1.59	1.68	1.41	1.45	0.87	1.40	0.32	0.14	5
spleen	0.77	0.94	1.31	0.88	1.26	0.81	1.04	0.23	0.10	5
muscle	0.00	2.87	2.23	0.00	0.00	0.00	1.02	1.42	0.63	5
gWAT	0.00	0.03	0.00	0.00	0.07	0.00	0.02	0.03	0.01	5

Plasma DECAY 14C

Group 2 **fenofibrate**

	7	8	9	10	11	12	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
2	101.04	77.43	96.22	97.36	67.90	74.13	82.61	13.40	5.99	5
5	97.73	69.87	92.26	86.66	67.08	63.00	75.77	12.88	5.76	5
10	91.97	54.53	66.87	50.64	45.74	38.87	51.33	10.47	4.68	5
20	71.30	23.96	39.52	20.03	25.03	19.40	25.59	8.16	3.65	5
30	52.59	13.27	22.27	13.15	16.15	11.02	15.17	4.37	1.95	5

mouse excluded, cooled off

SERUM DECAY 14C (t2 = 100%)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	5
5	96.72	90.23	95.88	89.01	98.79	84.98	91.78	5.53	2.47	5
10	91.02	70.42	69.49	52.01	67.36	52.44	62.35	9.31	4.16	5
20	70.56	30.94	41.07	20.57	36.86	26.17	31.12	8.18	3.66	5
30	52.05	17.14	23.15	13.51	23.78	14.86	18.49	4.73	2.12	5

SERUM HALF-LIFE 14C

	7	8	9	10	11	12				
k el	0.024	0.066	0.054	0.076	0.055	0.070	0.06	0.01	0.00	5
t 1/2	29.37	10.53	12.88	9.10	12.70	9.97	11.04	1.68	0.75	5

MOUSE/ORGAN MASS (g)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
mouse	26.1	29.3	28.7	29.3	26.6	27.7	28.32	1.16	0.52	5
liver	1.720	1.950	1.830	1.850	1.690	1.490	1.76	0.18	0.08	5
heart	0.125	0.146	0.147	0.131	0.124	0.124	0.13	0.01	0.01	5
spleen	0.085	0.076	0.108	0.082	0.077	0.079	0.08	0.01	0.01	5

ORGAN DISTRIBUTION 14C (% DOSIS)

fenofibrate

	7	8	9	10	11	12	average	std	SEM	n
liver	48.82	57.57	69.98	77.98	56.41	56.90	63.77	9.75	4.36	5
heart	1.18	2.29	1.98	1.10	0.84	1.05	1.45	0.64	0.29	5
spleen	0.98	0.97	0.97	0.62	0.83	0.55	0.79	0.20	0.09	5
muscle	1.50	2.68	1.91	2.38	1.27	1.49	1.95	0.59	0.26	5
gWAT	0.53	0.58	0.27	0.48	0.45	0.15	0.39	0.17	0.08	5

Plasma DECAY 14C

GROUP	PFBS									
	13	14	15	16	17	18	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00		100.00	0.00	0.00	4
2	86.24	96.32	93.93	104.07	85.81		95.03	7.52	3.76	4
5	86.06	91.68	88.29	97.01	85.14		90.53	5.08	2.54	4
10	80.91	63.21	71.51	84.12	76.13		73.74	8.75	4.37	4
20	66.91	61.00	44.53	47.34	56.77		52.41	7.76	3.88	4
30	58.58	47.30	31.77	36.44	43.42		39.73	6.95	3.48	4
	mouse excluded, cooled off					mouse died				

SERUM DECAY 14C (t2 = 100%)

	PFBS									
	13	14	15	16	17	18	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00		100.00	0.00	0.00	4
5	99.79	95.18	94.00	93.22	99.22		95.40	2.67	1.34	4
10	93.81	65.62	76.13	80.84	88.72		77.83	9.65	4.83	4
20	77.58	63.33	47.41	45.49	66.16		55.60	10.66	5.33	4
30	67.93	49.11	33.83	35.02	50.60		42.14	8.94	4.47	4

SERUM HALF-LIFE 14C

	13	14	15	16	17	18				
k el	0.015	0.024	0.040	0.040	0.026		0.03	0.01	0.00	4
t 1/2	46.83	28.41	17.16	17.29	27.18		22.51	6.13	3.06	4

Mouse/ORGAN MASS (g)

	PFBS									
	13	14	15	16	17	18	average	std	SEM	n
mouse	28.1	31.3	27.4	26.9	26.3		27.98	2.26	1.13	4
liver	1.520	1.790	1.520	1.320	1.500		1.53	0.19	0.10	4
heart	0.120	0.142	0.123	0.122	0.117		0.13	0.01	0.01	4
spleen	0.056	0.127	0.088	0.092	0.103		0.10	0.02	0.01	4

ORGAN DISTRIBUTION 14C (% DOSIS)

	PFBS									
	13	14	15	16	17	18	average	std	SEM	n
liver	33.82	47.53	46.84	48.33	40.76		45.87	3.46	1.73	4
heart	2.05	2.90	1.33	1.38	0.39		1.50	1.04	0.52	4
spleen	1.26	1.03	0.92	0.48	1.07		0.88	0.27	0.14	4
muscle	6.10	3.98	1.46	0.75	0.63		1.70	1.56	0.78	4
gWAT	0.00	0.00	0.09	0.00	0.62		0.18	0.30	0.15	4

Plasma DECAY 14C

Group 4 **PFHS**

	19	20	21	22	23	24	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
2	87.15	76.23	77.80	93.89	81.88	93.04	85.00	7.57	3.09	6
5	80.98	65.54	74.76	86.27	75.83	82.77	77.69	7.35	3.00	6
10	64.90	63.52	55.88	76.12	67.91	71.05	66.56	6.92	2.82	6
20	50.02	48.81	36.95	53.51	47.41	58.38	49.18	7.17	2.93	6
30	35.06	45.05	27.84	46.59	39.65	45.55	39.96	7.38	3.01	6

SERUM DECAY 14C (t2 = 100%)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
5	92.92	85.98	96.09	91.89	92.61	88.97	91.41	3.50	1.43	6
10	74.47	83.33	71.83	81.07	82.93	76.36	78.33	4.79	1.95	6
20	57.40	64.04	47.50	57.00	57.90	62.75	57.76	5.84	2.38	6
30	40.23	59.10	35.78	49.62	48.43	48.96	47.02	8.13	3.32	6

SERUM HALF-LIFE 14C

	19	20	21	22	23	24				
k el	0.032	0.018	0.039	0.026	0.027	0.025	0.03	0.01	0.00	6
t 1/2	21.46	37.67	17.96	26.46	25.67	28.29	26.25	6.73	2.75	6

Mouse/ORGAN MASS (g)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
mouse	29.3	27.2	25.2	28.2	27.0	32.2	28.18	2.39	0.98	6
liver	2.890	2.720	2.780	2.830	3.170	3.640	3.01	0.35	0.14	6
heart	0.142	0.132	0.101	0.116	0.128	0.157	0.13	0.02	0.01	6
spleen	0.094	0.155	0.073	0.097	0.102	0.101	0.10	0.03	0.01	6

ORGAN DISTRIBUTION 14C (% DOSIS)

PFHS

	19	20	21	22	23	24	average	std	SEM	n
liver	49.59	27.99	61.71	49.33	48.4	45.75	47.13	10.89	4.45	6
heart	0.97	1.85	1.08	1.06	0.890	0.44	1.05	0.45	0.19	6
spleen	0.53	0.84	0.40	0.49	0.439	0.29	0.50	0.19	0.08	6
muscle	2.33	3.73	1.67	1.96	1,778	0.00	1.91	1.20	0.49	6
gWAT	0.27	0.02	0.28	0.00	0.76	0.66	0.33	0.32	0.13	6

Plasma DECAY 14C

Group 5 **PFOS**

	25	26	27	28	29	30	average	std	SEM	n
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
2	79.76	77.83	84.08	97.66	84.26	77.32	83.49	7.56	3.09	6
5	78.42	71.44	79.37	85.58	76.10	73.48	77.40	4.99	2.04	6
10	78.30	67.32	74.51	80.32	70.70	66.49	72.94	5.72	2.34	6
20	73.82	68.67	62.95	70.75	57.81	56.80	65.13	7.03	2.87	6
30	73.97	65.65	53.61	57.03	47.97	51.16	58.23	9.81	4.00	6

SERUM DECAY 14C (t2 = 100%)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
2	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00	6
5	98.32	91.79	94.39	87.62	90.31	95.03	92.91	3.79	1.55	6
10	98.16	86.50	88.61	82.24	83.90	85.99	87.57	5.64	2.30	6
20	92.55	88.22	74.86	72.44	68.61	73.46	78.36	9.64	3.94	6
30	92.74	84.35	63.76	58.39	56.93	66.17	70.39	14.71	6.00	6

SERUM HALF-LIFE 14C

	25	26	27	28	29	30				
k el	0.003	0.005	0.016	0.018	0.020	0.015	0.01	0.01	0.00	6
t 1/2	239.02	150.68	43.32	39.61	35.36	46.21	92.37	84.23	34.39	6

Mouse/ORGAN MASS (g)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
mouse	28.5	28.4	26.8	28.3	27.2	26.0	27.53	1.03	0.42	6
liver	3.170	2.930	2.720	2.880	3.370	2.730	2.97	0.26	0.10	6
heart	0.133	0.131	0.121	0.120	0.118	0.117	0.12	0.01	0.00	6
spleen	0.101	0.098	0.094	0.074	0.080	0.062	0.08	0.02	0.01	6

ORGAN DISTRIBUTION 14C (% DOSIS)

PFOS

	25	26	27	28	29	30	average	std	SEM	n
liver	14.95	17.87	28.00	15.14	21.60	17.31	19.15	4.96	2.03	6
heart	0.84	0.78	1.54	1.15	0.45	1.03	0.96	0.37	0.15	6
spleen	0.51	1.01	0.57	0.98	0.48	0.24	0.63	0.30	0.12	6
muscle	1.39	1.45	0.00	0.46	0.00	0.00	0.55	0.70	0.29	6
gWAT	0.43	0.00	0.18	0.00	0.17	1.66	0.41	0.64	0.26	6

Appendix XVII In vivo clearance of autologous HDL

HDL isolation

Group#	Mouse#	Isolated HDL (mmol/L)	Quantity (ml)	µmol HDL	Needed for 0.32 µmol (ml)
control	5	0.256	2	0.51	1.25
fenofibrate	11	0.402	2	0.80	0.80
PFBS	15	0.360	2	0.72	0.89
PFHS	28	0.179	2	0.36	1.79
PFOS	30	0.164	2.4	0.39	1.95

NB: Groups PFHS and PFOS not enough for 0.4 µmol. Therefore all groups 0.32 µmol HDL labeled, in stead of 0.4 µmol.

Group#	Mouse#	3H dpm after dialysis (10 µl) vial 1	vial 2	3H dpm per 200 µl	needed 200000 dpm/200 µl stock	ml+	ml PBS	
control	5	18493.9	18398.2	368921	1.5	ml+	1.27	ml PBS
fenofibrate	11	18179.9	16939.5	351194	1.5	ml+	1.13	ml PBS
PFBS	15	19041.4	20370.3	394117	1.5	ml+	1.46	ml PBS
PFHS	28	17957	17930.9	358879	1.5	ml+	1.19	ml PBS
PFOS	30	19147.3	19078.8	382261	1.5	ml+	1.37	ml PBS

Group	Mouse#	% of injected dose in time (h) 0	1	2	4	8	24	$t_{1/2}$ (h)	FCR (pools HDL-C/h)	HDL-C (mM)	CR (mM HDL-C/h)
control	1	100.0	74.9	57.5	40.7	21.3	4.3	3.39	0.204	1.39	0.284
control	2	100.0	63.6	56.8	39.9	20.5	3.6	3.28	0.211	1.02	0.215
control	3	100.0	71.4	58.2	39.2	20.7	3.7	3.32	0.209	0.89	0.185
control	4	100.0	76.7	60.0	38.9	22.6	8.0	3.48	0.199	1.36	0.271
control	6	100.0	61.9	48.8	34.4	16.1	5.1	2.83	0.245	0.89	0.217
AVERAGE		100.0	69.7	56.2	38.6	20.3	4.9	3.26	0.21	1.11	0.23
SD		0.0	6.6	4.3	2.5	2.5	1.8	0.25	0.02	0.25	0.04
fenofibrate	8	100.0	78.6	67.0	49.5	32.8	9.5	4.61	0.150	1.70	0.255
fenofibrate	9	100.0	54.5	54.7	45.4	31.3	8.7	4.13	0.168	1.94	0.326
fenofibrate	10	100.0	87.6	55.2	44.5	33.7	10.8	4.45	0.156	1.78	0.278
fenofibrate	13	100.0	63.3	63.9	48.5	30.3	8.1	4.27	0.163	1.96	0.318
fenofibrate	14	100.0	54.5	59.4	45.8	30.6	9.9	4.14	0.168	2.22	0.371
AVERAGE		100.0	67.7	60.0	46.7	31.7	9.4	4.32	0.16	1.92	0.31
SD		0.0	14.9	5.4	2.1	1.5	1.0	0.21	0.01	0.20	0.05
PFBS	16	100.0	63.1	55.6	33.6	14.6	2.5	2.75	0.252	1.26	0.318
PFBS	17	100.0	65.3	60.3	31.1	16.1	2.1	2.84	0.244	0.74	0.182
PFBS	18	100.0	59.6	49.4	31.2	13.1	2.1	2.58	0.269	0.84	0.226
PFBS	19	100.0	59.4	39.6	29.9	17.9	2.2	2.81	0.247	0.66	0.163
PFBS	20	100.0	73.9	56.0	38.4	23.4	3.9	3.48	0.199	1.80	0.357
AVERAGE		100.0	64.3	52.2	32.8	17.0	2.6	2.89	0.24	1.06	0.25
SD		0.0	5.9	8.0	3.4	4.0	0.8	0.35	0.03	0.47	0.09
PFHS	22	100.0	47.9	30.7	15.8	6.2	0.9	1.80	0.385	0.13	0.049
PFHS	23	100.0	53.2	38.7	23.5	9.6	1.1	2.17	0.319	0.21	0.067
PFHS	25	100.0	62.2	41.0	24.8	9.5	1.1	2.21	0.314	0.48	0.152
PFHS	26	100.0	56.0	43.2	27.8	14.1	2.5	2.56	0.271	0.61	0.166
PFHS	27	100.0	53.6	40.4	26.4	9.3	1.5	2.20	0.315	0.57	0.178
AVERAGE		100.0	54.6	38.8	23.6	9.7	1.4	2.19	0.32	0.40	0.12
SD		0.0	5.2	4.8	4.7	2.8	0.7	0.27	0.04	0.22	0.06
PFOS	29	100.0	58.6	40.4	24.4	10.1	1.1	2.24	0.309	0.31	0.095
PFOS	31	100.0	48.3	39.8	21.9	8.4	1.6	2.07	0.334	0.37	0.124
PFOS	32	100.0	60.1	40.6	24.9	9.4	0.9	2.20	0.315	0.05	0.015
PFOS	33	100.0	58.7	45.5	26.7	10.2	1.8	2.30	0.302	0.34	0.102
PFOS	35	100.0	51.2	33.7	19.8	5.5	1.0	1.81	0.382	0.21	0.080
AVERAGE		100.0	55.4	40.0	23.5	8.7	1.3	2.12	0.33	0.25	0.08
SD		0.0	5.3	4.2	2.7	1.9	0.4	0.19	0.03	0.13	0.04

Appendix XVIII Liver microsomal DGAT activity

Group	Mouse#	protein microsomes (mg/ml)	DGAT-1 activity (nmol/min/mg protein)	DGAT-2 activity (nmol/min/mg protein)
control	1	4.9	1.73	1.29
control	2	7.4	3.53	1.33
control	3	6.7	1.62	1.85
control	4	7.1	1.77	2.08
control	5	sacrificed for HDL isolation		
control	6	7.8	2.42	1.43
control	7	used for bile canulation		
AVERAGE			2.21	1.59
SD			0.80	0.35
fenofibrate	8	9.8	1.86	3.35
fenofibrate	9	10.5	1.90	1.79
fenofibrate	10	7.6	2.20	4.13
fenofibrate	11	sacrificed for HDL isolation		
fenofibrate	12	used for bile canulation		
fenofibrate	13	9.3	1.65	2.07
fenofibrate	14	7.1	2.81	2.48
AVERAGE			2.08	2.76
SD			0.45	0.96
PFBS	15	sacrificed for HDL isolation		
PFBS	16	7.5	3.64	1.92
PFBS	17	6.1	2.45	2.58
PFBS	18	9.8	2.14	1.91
PFBS	19	8.5	2.09	0.98
PFBS	20	9.6	2.49	0.91
PFBS	21	used for bile canulation		
AVERAGE			2.56	1.66
SD			0.63	0.71
PFHS	22	8.3	2.43	2.26
PFHS	23	7.6	1.30	1.27
PFHS	24	used for bile canulation		
PFHS	25	6.8	2.60	2.46
PFHS	26	8.0	3.68	3.52
PFHS	27	9.0	3.02	2.51
PFHS	28	sacrificed for HDL isolation		
AVERAGE			2.60	2.40
SD			0.88	0.80
PFOS	29	7.3	3.55	2.41
PFOS	30	sacrificed for HDL isolation		
PFOS	31	6.8	3.32	4.36
PFOS	32	5.1	3.29	2.89
PFOS	33	5.6	4.05	2.04
PFOS	34	used for bile canulation		
PFOS	35	6.8	2.77	2.27
AVERAGE			3.40	2.79
SD			0.46	0.93

mouse 1 Total 14C TAG based on average efficiency
mouse 16 DGAT-2 is based on 1 measurement

Appendix XIX Liver lipids

Group	Mouse#	Cage#	Eartag		Liver lipids (µg/mg protein)			
			L	R	FC	CE	TC	TG
[redacted]	1	1	0	1	13.0	20.4	33.5	77.9
[redacted]	2	1	1	2	16.2	26.0	42.3	106.0
[redacted]	3	2	0	1	14.2	29.9	44.1	72.2
[redacted]	4	2	2	0	15.3	23.9	39.3	70.1
[redacted]	5	2	0	2	12.5	25.9	38.4	53.0
[redacted]	6	3	0	0	14.7	21.4	36.0	67.7
[redacted]					14.3	24.6	38.9	74.5
[redacted]					1.4	3.5	3.9	17.5
fenofibrate	8	4	2	1	11.6	12.1	23.7	50.9
fenofibrate	9	4	1	2	11.7	11.9	23.6	60.5
fenofibrate	10	5	2	0	10.7	9.9	20.6	39.2
fenofibrate	11	5	1	0	10.8	10.5	21.3	51.8
fenofibrate	13	6	0	1	12.5	13.0	25.5	76.6
fenofibrate	14	6	2	1	12.7	13.9	26.6	80.2
AVERAGE					11.7	11.9	23.6	59.9
SD					0.8	1.5	2.3	15.9
[redacted]	15	1	1	7	10.7	14.1	24.7	35.4
[redacted]	16	2	1	7	12.7	22.2	34.9	96.7
[redacted]	17	1	0	7	15.0	24.0	39.0	104.0
[redacted]	18	1	0	8	11.4	16.9	28.3	47.8
[redacted]	19	2	1	8	10.4	8.5	18.9	41.5
[redacted]	20	0	1	9	9.5	9.4	18.9	45.7
[redacted]					11.6	15.9	27.5	61.8
[redacted]					2.0	6.4	8.3	30.2
[redacted]	22	0	2	10	14.8	31.0	45.8	140.1
[redacted]	23	2	1	10	13.5	22.9	36.4	134.8
[redacted]	25	0	2	11	14.0	28.6	42.6	122.0
[redacted]	26	1	1	12	10.3	16.6	26.9	75.3
[redacted]	27	2	0	12	12.8	19.1	31.9	94.1
[redacted]	28	0	2	12	12.8	27.2	40.0	114.4
[redacted]					13.1	24.2	37.3	113.5
[redacted]					1.6	5.7	7.0	24.8
PFOS	29	0	0	13	16.0	39.9	55.9	171.9
PFOS	30	1	0	13	15.1	43.4	58.5	186.2
PFOS	31	0	2	13	15.3	39.9	55.2	195.9
PFOS	32	0	1	14	17.8	64.3	82.1	275.7
PFOS	33	1	2	14	16.3	41.5	57.9	193.8
PFOS	35	0	2	15	18.8	56.4	75.1	279.4
AVERAGE					16.6	47.6	64.1	217.2
SD					1.5	10.3	11.5	47.5

Appendix XX Liver microarray analysis

TNO Quality of Life

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TNO report

Transcriptome analysis of effects of 3M compounds in liver of ApoE3L.CETP mice

Date	5 June 2009
Author(s)	Marjan van Erk
Copy no	copy no
No. of copies	no of copies
Number of pages	10
Number of appendices	number of appendices
Customer	3M
Projectname	Projectname
Projectnumber	Projectnumber

Contents

1 Introduction

VMD (Vascular and Metabolic Diseases) team is conducting a study for 3M, investigating the effects of 3 compounds in ApoE3L CETP mice and comparing these effects to the effects of fenofibrate (FF), a PPARalpha activator. Study compounds (PFBS, PFHS, PFOS) are per-fluoro-alkylsulfonates. These compounds accumulate in humans and in nature. Two of the study compounds were taken off the market, one is still in use. 3M aims to investigate effects of these compounds in light of potential health risks. Furthermore, this knowledge will help them to develop compounds that are less harmful.

Transcriptome analysis of the liver will be performed to investigate effects of the compounds on a pre-selected set of genes and on lipid and lipoprotein metabolism pathways, and to compare these effects to the effects of PPAR-alpha activator fenofibrate.

2 Methods

2.1 RNA isolation and microarray hybridization

RNA was isolated from livers of ApoE3L.CETP mice. After quality control of the RNA, microarray analysis was carried out at Service XS B.V. (Leiden, the Netherlands) using the Affymetrix technology platform and Affimetrix GeneChip® mouse genome MOE430-2.0 arrays. Data were sent to TNO.

2.2 Gene expression data analysis

Quality control of microarray data was performed using BioConductor packages (a.o. simpleaffy and affyplm). All samples passed the QC. Raw signal intensities (from CEL-files) were normalized using the GCRMA algorithm (gc-rma slow). For annotation of probes and summarization of signals from probes representing one gene the custom MNBI CDF-file was used (based on EntrezGene, version 11.0.2) (http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/cdfreadme.htm). This resulted in expression values for 16331 genes, represented by unique Entrez gene identifiers.
Genes were filtered for expression above 5 in 3 or more samples, resulting in a set of 11587 genes that was used for further analysis. Gene expression data were log-transformed (base 2). Statistical analysis was performed using the moderated t-test (Limma http://bioinf.wehi.edu.au/limma/) with correction for multiple testing. Cut-off for statistically significant changes was set at q-value <0.05 (q-value = p-value corrected for multiple testing).
Transcription factor analysis was performed in Bibliosphere software v7.21 (Genomatix Software GmbH, Munich, Germany). For each compound, genes were selected that were significantly differentially expressed and that were involved in lipid metabolism. Involvement in lipid metabolism was determined based on Gene Ontology annotation in at least one of the following biological processes: lipid biosynthesis, lipid catabolism, lipid biosynthesis, fatty acid biosynthesis, fatty acid metabolism, fatty acid beta oxidation, cholesterol metabolism, cholesterol biosynthesis, cholesterol catabolism, lipoprotein metabolism, lipid transport, cholesterol transport.
In addition, T-profiler analysis was performed using expression values corrected for mean expression in the control group. This analysis resulted in scores (t-scores) and significance values for pathways and biological processes. A positive score means that the majority of the genes in the pathway are up-regulated; a negative score means that the majority of the genes in the pathway are down-regulated. Pathways and biological processes with significant scores (>4 or <-4) in 5 or 6 animals per group were selected. A hierarchical clustering of these pathways and biological processes and their scores in all samples was generated in GenePattern (Broad Institute, MIT, USA).

3 Results

3.1 Differentially expressed genes

In the statistical analysis, the fenofibrate (FF), PFBS, PFHS and PFOS groups were compared to the control group. FF resulted in 2924 differentially expressed gene. PFBS resulted in 438 diffferentially expressed genes. PFHS resulted in 4230 differentially expressed genes and PFOS resulted in 3986 differentially expressed genes (figure 1).

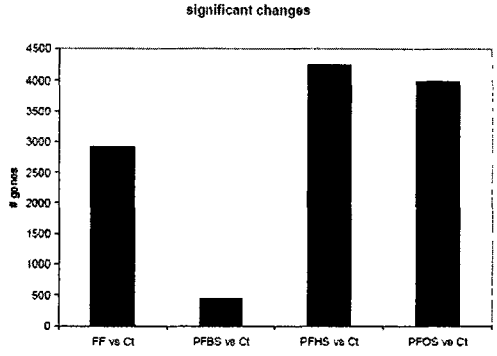

Figure 1. Number of differentially expressed genes in each of the interventions compared to control

Figure 2 indicates the overlap in differentially expressed genes for FF and each of the three 3M compounds. Overall, 43-54% of the genes differentially expressed in response to the 3M compounds are also regulated by FF.
Furthermore, there is considerable overlap in genes regulated by PFHS and PFOS, as shown in figure 3. In total, 2945 genes are regulated by both compounds, in addition, 262 of these are also regulated by PFBS.

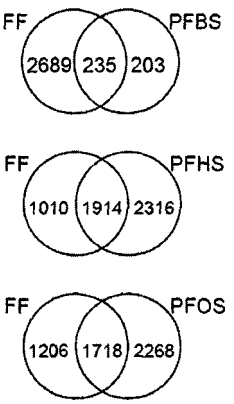

Figure 2. Venn diagrams showing overlap in sets of differentially expressed genes in response to FF and PFBS, PFHS and PFOS respectively.

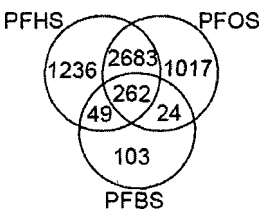

Figure 3 Venn diagram showing overlap between genes regulated by PFHS, PFOS and PFBS.

3.2 Lipid metabolism

3.2.1 *Pre-selected list of genes*

A set of genes of interest was defined, with a focus on PPARα metabolism and cholesterol metabolism. The expression changes as a result of the 4 interventions are listed in the excel file added to this report. Of the 87 genes in the list, 72 were expressed and the majority was differentially expressed in response to one or more of the interventions. Expression of seven genes was not measured (genes were not present on the microarray) and expression of 10 genes was below detection. FF intervention resulted in 31 up-regulated genes and 7 down-regulated pre-selected genes. Of the 31 genes up-regulated by FF, 25 were also significantly up-regulated by PFHS, 18 were also up-regulated by PFOS and 3 were also up-regulated by PFBS. The selected set of

genes was grouped into smaller sets of genes related to triglyceride metabolism (lipolysis, fatty acid/triglyceride synthesis, beta oxidation, fatty acid uptake/transport/binding, VLDL assemblage/formation, PL excretion), cholesterol (cholesterol synthesis, storage, uptake, metabolism, excretion) and HDL metabolism (HDL formation, HDL maturation, HDL modelling/destabilisation, HDL uptake).

3.2.2 Transcription factor analysis of genes involved in lipid metabolism

In addition to focusing on the pre-selected list of genes, we aimed to identify transcription factors relevant for regulating the expression of genes involved in lipid metabolism in response to the different compounds

Genes involved in lipid metabolism were identified from Gene Ontology annotation in at least one of the following biological processes: lipid biosynthesis, lipid catabolism, lipid biosynthesis, fatty acid biosynthesis, fatty acid metabolism, fatty acid beta oxidation, cholesterol metabolism, cholesterol biosynthesis, cholesterol catabolism, lipoprotein metabolism, lipid transport, cholesterol transport. Next, for each compound, the significantly differentially expressed genes involved in lipid metabolism were selected: 143 genes for fenofibrate, 172 genes for PFHS, 162 genes for PFOS and 23 genes for PFBS. These sets of genes were submitted to transcription factor analysis in the Bibliosphere software by Genomatix. An excel file containing the results of this analysis is added to the report. The results file lists transcription factors that are expected to play in role in regulating expression of the significantly differentially expressed genes involved in lipid metabolism, based on presence of a binding site in the promoter region combined with co-citation in literature, or based on co-citation in literature only (for transcription cofactors). The top three of transcription factors is equal for PFHS, PFOS and PFBS: PPAR-alpha, PPAR-gamma and LXR-beta.

3.3 Pathway analysis

Figure 4 gives an overview of functional gene sets (based on gene ontology annotation) regulated by one or more interventions. Selected functional gene sets were significantly regulated in at least 5 animals of one or more treatment groups.

The heatmap shows a number of groups of gene sets that show a similar profile of response. The first cluster consists of gene sets related to transcription, which are most strongly down-regulated by PFHS and PFOS. The second cluster consists of gene sets related to inflammation. FF results in down-regulation of these gene sets. However, PFBS results in up-regulation of some gene sets, most clearly of acute-phase response and inflammatory response. The third cluster consists of gene sets related to lipid metabolism and energy metabolism (mitochondrion). FF most strongly up-regulates lipid metabolism, the effect of PFHS and PFOS is similar but less pronounced. A small set of gene sets related to Acyl-CoA hydrolysis or metabolism are strongly up-regulated by FF, PFHS and PFOS. These effects could be related to PPARα activation. Lastly, gene sets related to glutathione transferase and monooxygenase activity are specifically down-regulated by PFHS and PFOS. The effects in these clusters of gene sets are also visualized in figure 5.

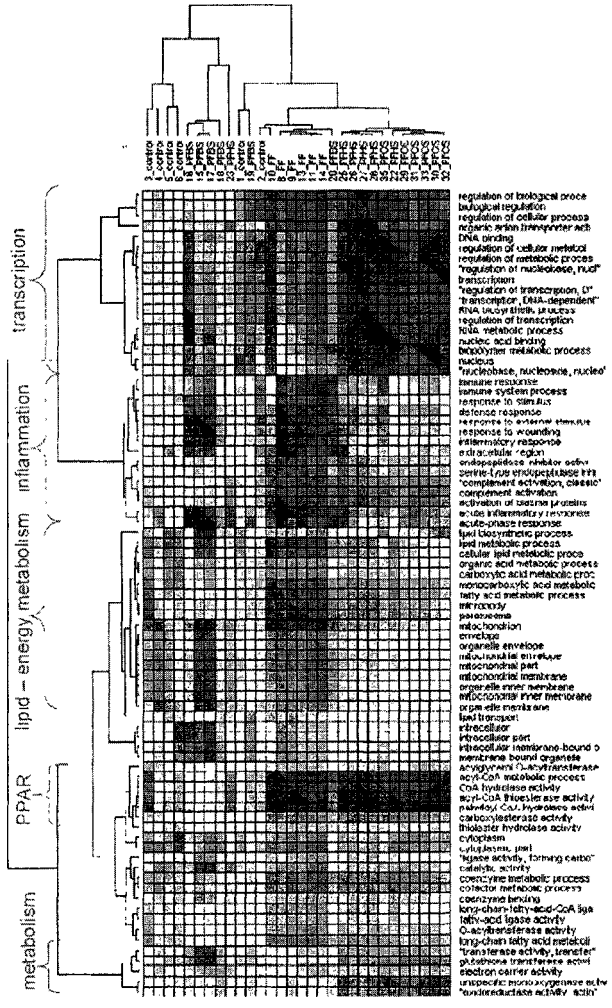

Figure 4. Cluster of scores for functional gene groups (based on Gene Ontology) that are significantly affected in at least 5 animals of a treatment group Red indicates positive score (i.e. majority of genes up-regulated) and blue indicates negative score (i.e. majority of genes down-regulated)

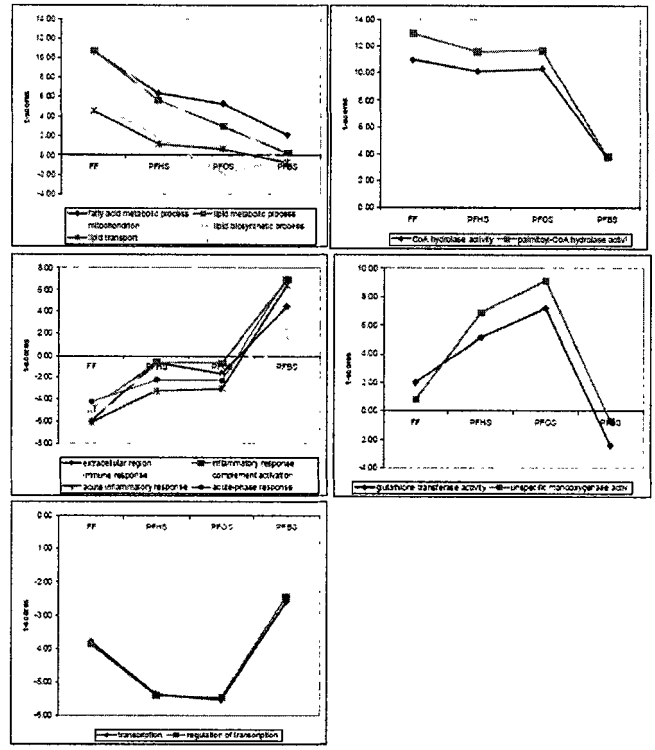

Figure 5. Response profiles of clusters of gene sets that show a similar response to the treatments.

4 Conclusions

- PFBS has a more limited effect on transcriptome of liver in ApoE3L.CETP mice than PFHS and PFOS
- There is a considerable overlap in genes regulated by FF and those regulated by PFHS or PFOS
- Overall gene set analysis indicates that fatty acid metabolism, CoA hydrolase activity and lipid metabolism are most strongly up-regulated by FF, followed by PFHS and PFOS. FF up-regulates lipid biosynthesis and lipid transport, these gene sets are not significantly affected by the 3M compounds. FF down-regulates inflammation and immune response. PFBS resulted in up-regulation of acute inflammatory response. Specific effects of PFHS and PFOS are up-regulation of glutathione transferase and monooxygenase activity and down-regulation of transcription.

Appendix XXI Summary table

Parameter		Study	week of treatment	Fenofibrate	PFHS	PFOS
Body weight, food intake and tissue weight	Body weight	1	4 and 6	n.s.	n.s.	n.s.
		2	4	n.s.	n.s.	n.s.
		3	4	n.s.	n.s.	↓ (-5%)
	Food intake	1	4 and 6	n.s.	n.s.	n.s.
		2	4	n.s.	n.s.	n.s.
		3	4	n.s.	n.s.	n.s.
		Mean		***n.s.***	***n.s.***	***n.s.***
	Liver weight	1	6	↑ (+44%)	↑ (+162%)	↑ (+155%)
		2	4	↑ (+23%)	↑ (+110%)	↑ (+107%)
		3	4	↑ (+20%)	↑ (+82%)	↑ (+83%)
	Perigonadal fat weight	1	6	n.s.	n.s.	↓ (-39%)
		2	4	n.s.	n.s.	n.s.
		3	4	n.s.	↓ (-36%)	n.s.
Plasma parameters	Plasma cholesterol	1	4	↓ (-38%)	↓ (-60%)	↓ (-63%)
		2	4	↓ (-35%)	↓ (-67%)	↓ (-60%)
		3	4	↓ (-35%)	↓ (-69%)	↓ (-67%)
		Mean		***↓ (-36%)***	***↓ (-65%)***	***↓ (-63%)***
	Plasma triglycerides	1	4	↓ (-60%)	↓ (-69%)	↓ (-61%)
		2	4	↓ (-65%)	↓ (-59%)	↓ (-50%)
		3	4	↓ (-78%)	↓ (-69%)	↓ (-72%)
		Mean		***↓ (-67%)***	***↓ (-67%)***	***↓ (-62%)***
	Plasma HDL-cholesterol	1	4	↑ (+52%)	↓ (-38%)	↓ (-54%)
		2	4	↑ (+97%)	↓ (-34%)	↓ (-54%)
		3	4	↑ (+77%)	↓ (-62%)	↓ (-74%)
	Lipoprotein profiles (pooled)	1	4 and 6	decreased VLDL, LDL, increased HDL1 and HDL	decreased VLDL, LDL and HDL, increased HDL1	decreased VLDL, LDL and HDL
		2	4	decreased VLDL, LDL, increased HDL1 and HDL	decreased VLDL, LDL and HDL, increased HDL1	decreased VLDL, LDL and HDL
		3	4	decreased VLDL, LDL, increased HDL1 and HDL	decreased VLDL, LDL and HDL	decreased VLDL, LDL and HDL
	ALT (pooled)	1	4 and 6	-/↓	↑	↑
		2	4	↓	↑	↑
		3	4	↓	↑	↑
	Plasma free glycerol	1	6	↓ (-28%)	↓ (-52%)	↓ (-54%)
	Plasma free fatty acids	1	6	n.s.	↓ (-37%)	↓ (-60%)
VLDL production & de novo ApoB synthesis	VLDL-TG production rate	1	6	↑ (+46%)	↓ (-74%)	↓ (-86%)
	apo B synthesis rate	1	6	n.s.	↓ (-76%)	↓ (-87%)
	TG production per ApoB	1	6	↑ (+40%)	n.s.	n.s.
	lipid composition Apo B	1	6	CE↓ (-55%), TG↑(+26%)	CE↑(-46%)	FC↑(+164%), TG↑(+38%), PL↑ (+74%)
VLDL clearance	Post heparin LPL activity	1	5	↓ (+110%)	↑ (+74%)	↑ (+54%)
	Post heparin HL activity	1	5	↑ (+67%)	n.s.	↑ (+22%)
	[^{3}H]-Triolein half life	2	4	↓ (-68%)	↓ (-61%)	↓ (-51%)
	[^{3}H]-Triolein uptake in liver	2	4	↑ (+98%)	↑ (+149%)	↑ (+84%)
	[^{14}C]-Cholesteryl oleate half life	2	4	↓ (-80%)	↓ (-54%)	n.s.
	[^{14}C]-Cholesteryl oleate uptake in liver	2	4	↑ (+142%)	↑ (+79%)	n.s.
HDL clearance	Plasma ApoA1	3	4	n.s.	↓ (-76%)	↓ (-81%)
	Plasma CETP mass	3	4	↓(-38%)	↓(-36%)	↓(-38%)
	Plasma CETP activity	3	4	↓(-53%)	n.s.	↓(-20%)
	Fractional catabolic rate HDL	3	4	↓(-25%)	↑ (+50%)	↑ (+54%)
	Catabolic rate HDL	3	4	n.s.	↓(-48%)	↓(-65%)
Fecal lipids	Neutral sterol excretion	1	5	n.s.	n.s.	n.s.
	Phytosterol excretion	1	5	n.s.	n.s.	n.s.
	Bile acid excretion	1	5	↓ (-38%)	↓ (-41%)	↓ (-50%)
	Fatty acid excretion	1	5	n.s.	n.s.	n.s.
Bile flow and biliary lipids	Bile flow	2	4	↑ (+18%)	↑ (+46%)	n.s.
	Bile acid flow	2	4	n.s.	n.s.	n.s.
	Phospholipid flow	2	4	n.s.	↑ (+68%)	n.s.
	Cholesterol flow	2	4	n.s.	n.s.	n.s.
Liver measurements	DGAT activity	3	4	n.s.	n.s.	DGAT-2 ↑(+75%)
	Liver lipids	3	4	FC ↓ (-18%), CE↓ (-52%)	TG↑ (+52%)	FC↑(+16%), CE↑(+93%), TG↑ (+192%)

n.s.: not signficantly different from the control

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