

## INTRODUCTION

Ammonium perfluorooctanoic acid is non-genotoxic but has induced liver tumours when administered to rats. APFO is a peroxisome proliferator activated receptor (PPAR $\alpha$  and PPAR $\gamma$ ) ligand, and has previously been shown to elicit hepatomegaly in rats. Peroxisome proliferators have been shown to generally increase hepatocellular replicative DNA synthesis (S-phase) and to inhibit apoptosis. **The objective of this study was to characterise APFO-induced hepatomegaly.**

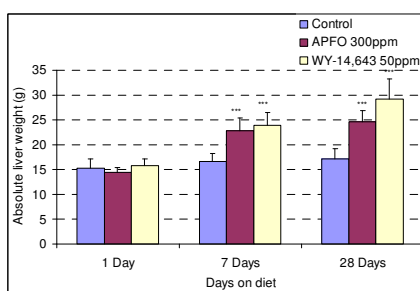
## METHODS

APFO (300ppm) was administered in the diet to male Sprague Dawley rats (7-8 weeks old) for 1, 7 and 28 days. Wy14,643 (50ppm) was administered to other groups of rats as a positive control. Rats were implanted with osmotic pumps containing BrdU five days before termination. Rats exposed to diet for one day were administered BrdU (sc) two hours prior to sacrifice. On days 2, 8 and 29 rats were killed by exposure to a rising concentration of CO<sub>2</sub>. The livers were excised, weighed and samples fixed and processed for H&E staining, BrdU IHC and TUNEL. The remaining liver was homogenised and differentially centrifuged to prepare a 13,000g "heavy pellet" fraction and a microsomal fraction. CN-insensitive palmitoyl CoA oxidation (peroxisomal  $\beta$ -oxidation) was measured in the "heavy pellets". Microsomes were submitted to SDS-PAGE and Western blotting with antibodies to specific rat cytochrome P450s.

## RESULTS

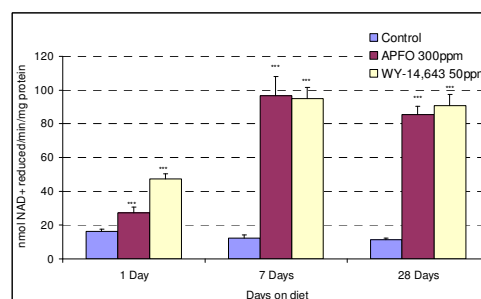
Administration of APFO decreased body weights after 7 and 28 days of exposure (90% and 85% of controls respectively). This effect was not observed in rats receiving Wy14,643. No adverse clinical observations were made. The mean ingested doses of APFO and Wy14,643 were 23.25  $\pm$  0.11 and 3.66  $\pm$  0.02 mg / kg bwt / day respectively. Absolute liver weights were increased with time following exposure to both APFO and Wy14,643 (Figure 1). Administration of APFO or Wy14,643 increased  $\beta$ -oxidation at all times (Fig. 2)

Fig. 1. Absolute Liver Weights



Values are Mean  $\pm$  SD (n = 10)  
\*statistically different from control p<0.05; \*\* p<0.01; \*\*\* p<0.001.

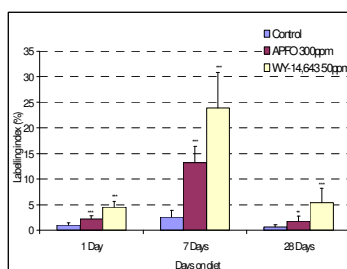
Fig. 2. Hepatic Peroxisomal  $\beta$ -oxidation



Values are Mean  $\pm$  SD (n = 10)  
\*statistically different from control p<0.05; \*\* p<0.01; \*\*\* p<0.001.

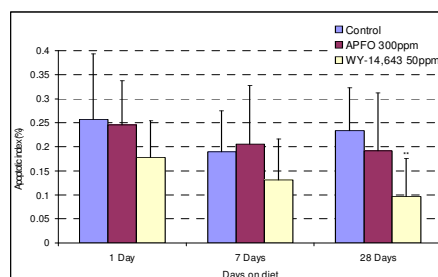
Wy14,643 and APFO increased the labelling index (S-phase) by 4- and 2- fold respectively after one day of treatment. At day 8, S-phase activity was increased to 9- and 5- fold respectively. After 28 days of treatment S-phase remained elevated in Wy14,643-treated rats, but appeared to be returning to control levels in APFO-treated rats (Fig. 3). Apoptosis was decreased with time following exposure to Wy14,643, but APFO had no effect upon apoptotic index (Fig. 4). The administration of Wy14,643 or APFO resulted in a depletion of glycogen, hepatocellular hypertrophy and hyperplasia (an increase in the number of hepatocyte nuclei per unit area and increased mitotic figures). The intensity of these observations was greater for Wy14,643 and increased with time of treatment. SDS-PAGE and Western blotting demonstrated that APFO induced CYP2B2, CYP3A4 and CYP4A1. Wy14,643 only induced CYP4A1 (Fig. 5).

Fig. 3. Hepatic Labelling Index (S-Phase)



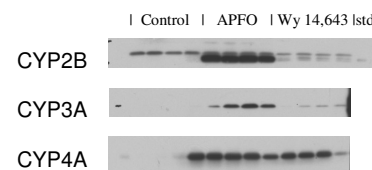
Values are Mean  $\pm$  SD (n = 10)  
\*statistically different from control p<0.05; \*\* p<0.01; \*\*\* p<0.001

Fig. 4. Hepatic Apoptotic Index



Values are Mean  $\pm$  SD (n = 10)  
\*statistically different from control p<0.05; \*\* p<0.01; \*\*\* p<0.001.

Fig. 5. Western Blotting of Hepatic Microsomes



## CONCLUSIONS

**APFO-induced hepatomegaly is characterised by hypertrophy (proliferation of peroxisomes and SER) and hyperplasia (increased S-phase and hepatocyte proliferation).**

Unlike other peroxisome proliferators APFO appears not to inhibit apoptosis. This observation may be confounded due the simultaneous activation of PPAR $\alpha$  (decreased apoptosis) and PPAR $\gamma$  (increased apoptosis). Unlike most peroxisome proliferators that are ligands for PPAR $\alpha$  only, APFO is a ligand for both receptors. There is evidence to suggest that, in the rat liver, activation of PPAR $\alpha$  and PPAR $\gamma$  decreases and increases apoptosis respectively.

APFO is a promiscuous ligand. The study presented suggests that APFO interacts with other receptors such as CAR (CYP2B induction, phenobarbitone-like) and PXR (CYP3A induction, dexamethasone-like) in addition to the PPARs.