

Overview of Mechanistic Research on
Perfluorinated Alkyl Acids with
Emphasis on the Potential Value of New
Technologies and Bio-Informatics in
Elucidating Modes of Action

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Presentation Overview

- PFOA as a “biochemical”
 - Effects on intermediary metabolism
 - Biochemical/bioinformatic examination of hepatic and pancreatic effects
- Mode of action studies on pancreatic acinar cell tumourigenesis
- Characterisation of PFOA-induced hepatomegaly

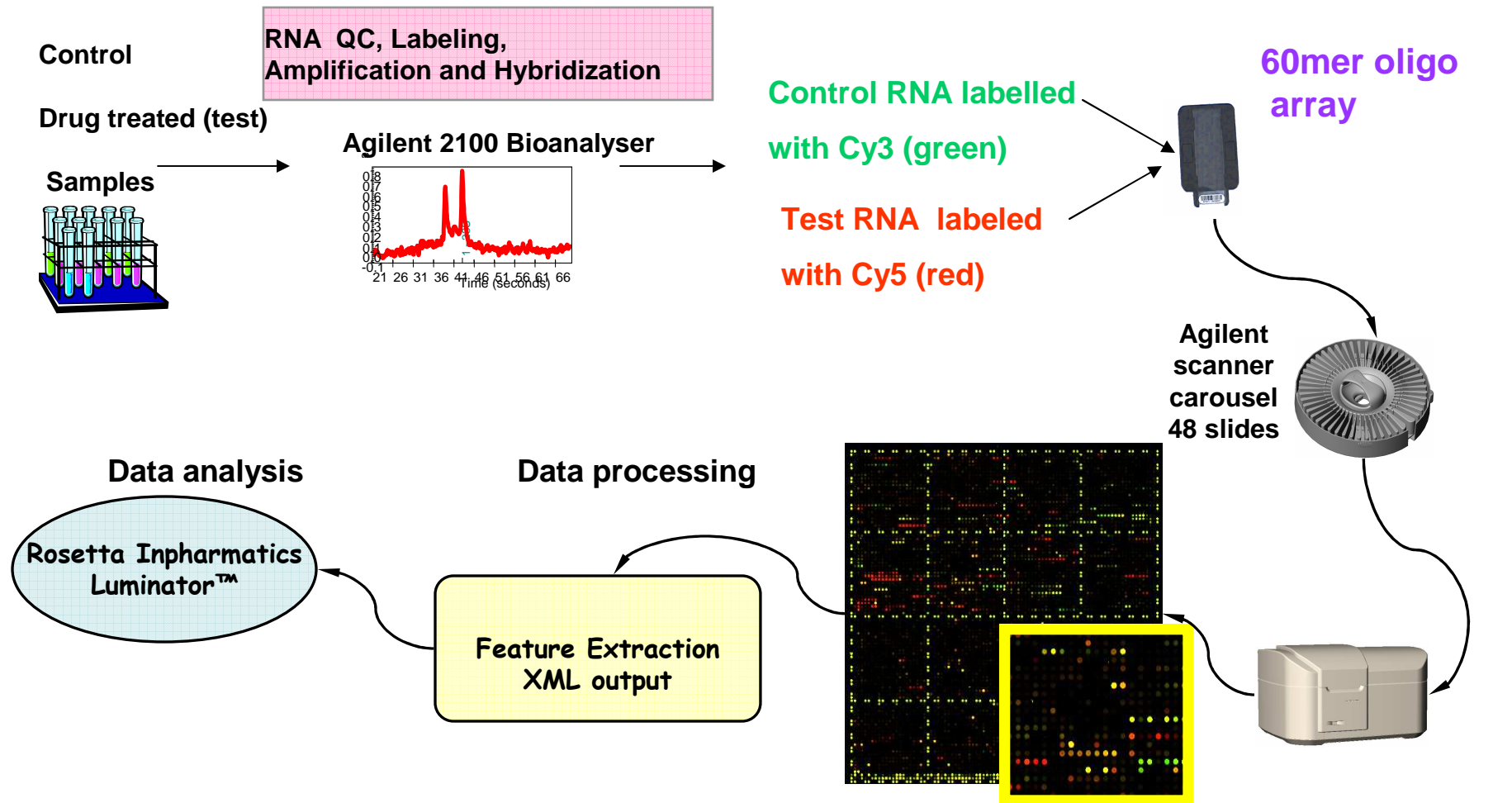
PFOA As a “Biochemical”

- Not overtly cytotoxic
 - Male rats 28 days, 300ppm (20-25mg/kg), plasma concentration 250 $\mu\text{g/ml}$ (600 μM), no increase in serum transaminases, no hepatic necrosis
- However, marked biochemical changes
 - Loss of white adipose tissue
 - Increased food consumption/g body weight
 - hepatomegaly
 - Decreased plasma triglycerides, cholesterol, glucose and insulin; increased leptin

PFOA As a “Biochemical”

- Subtle effects on cellular regulation and homeostasis
- Non-metabolisable fatty acid response?
- Nuclear Receptor involvement
 - Not all peroxisome proliferators show same effects

Transcriptional Profiling Workflow

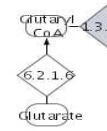
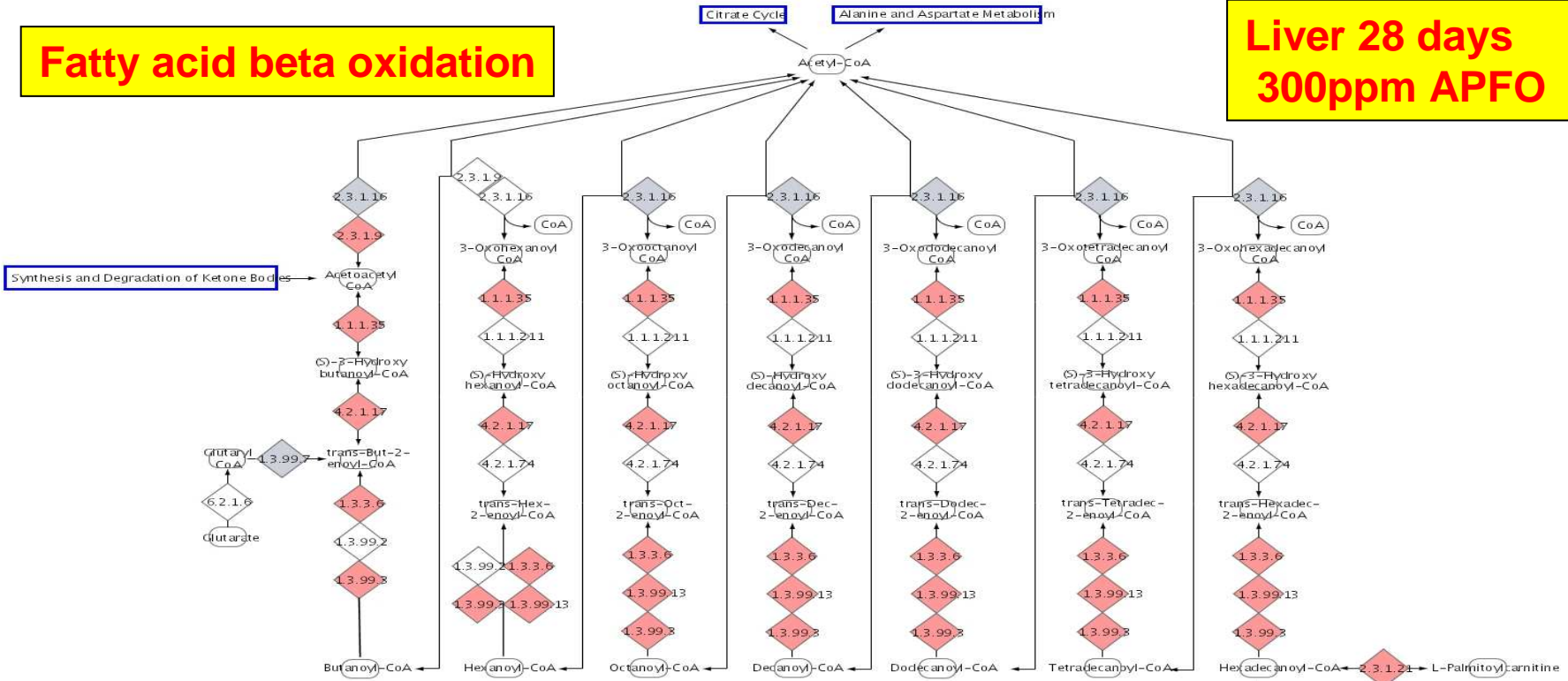


Bioinformatic Analysis

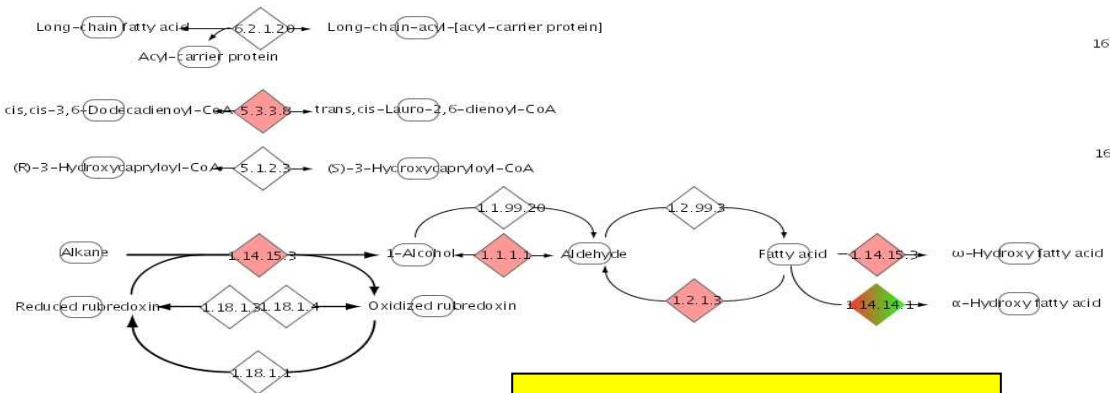
- Generation of a 'signature' list of regulated genes from replicates (e.g. Rosetta Luminator™ software)
- Biological/functional classification (e.g. Dragon Annotate)
- Clustering analysis
 - hierarchical clustering
 - Finds patterns in data sets by grouping similar objects (genes) together – may reveal functional associations between genes
 - Trend analysis
 - Plots relative expression of signature genes across set of conditions
 - Pathways analysis (e.g. Ingenuity™)

Fatty acid beta oxidation

Liver 28 days
300ppm APFO



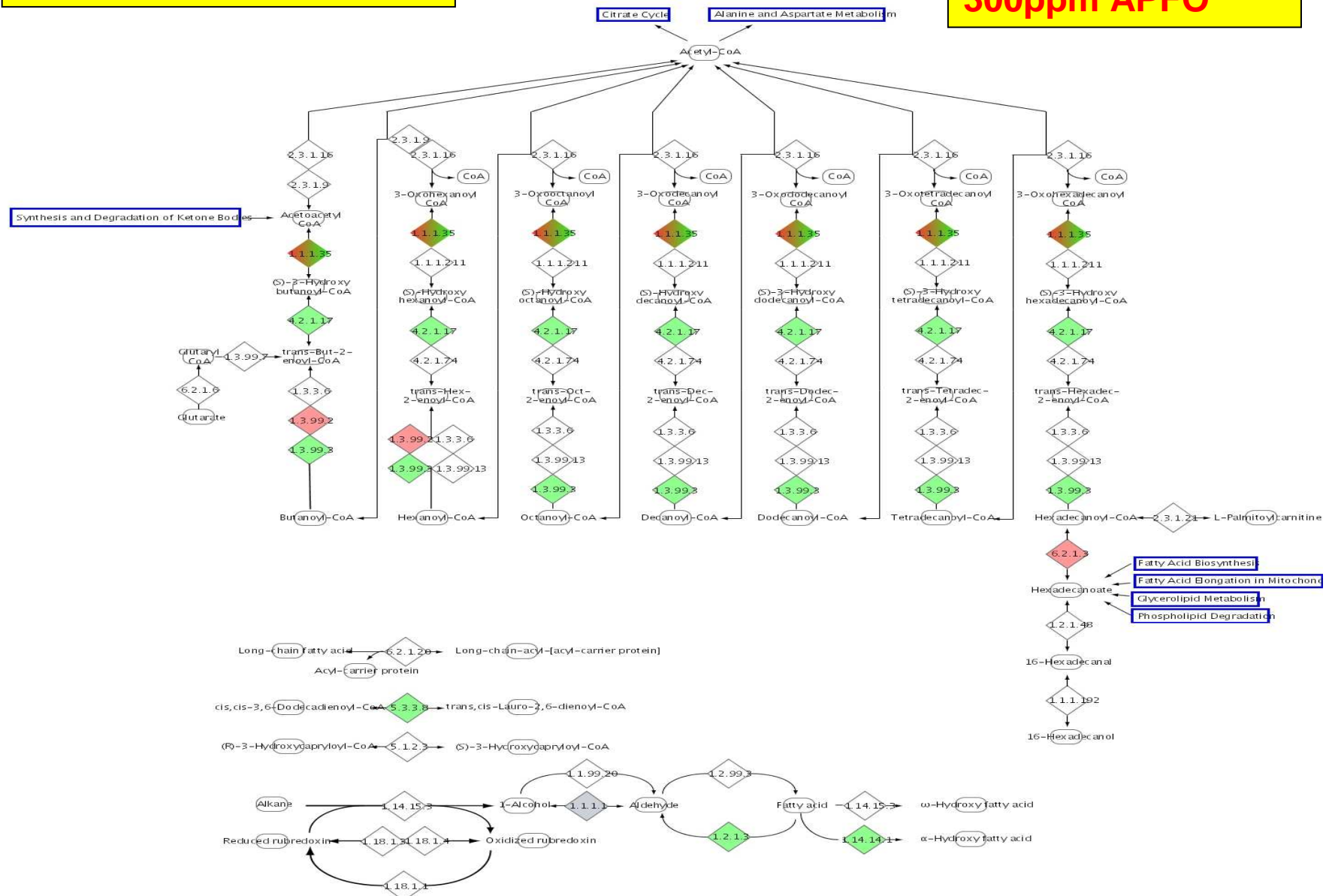
- Fatty Acid Biosynthesis
- Fatty Acid Elongation in Mitochondria
- Glycerolipid Metabolism
- Phospholipid Degradation



Fatty acid transport

Fatty acid beta oxidation

Pancreas 28 days, 300ppm APFO



Fatty acid transport

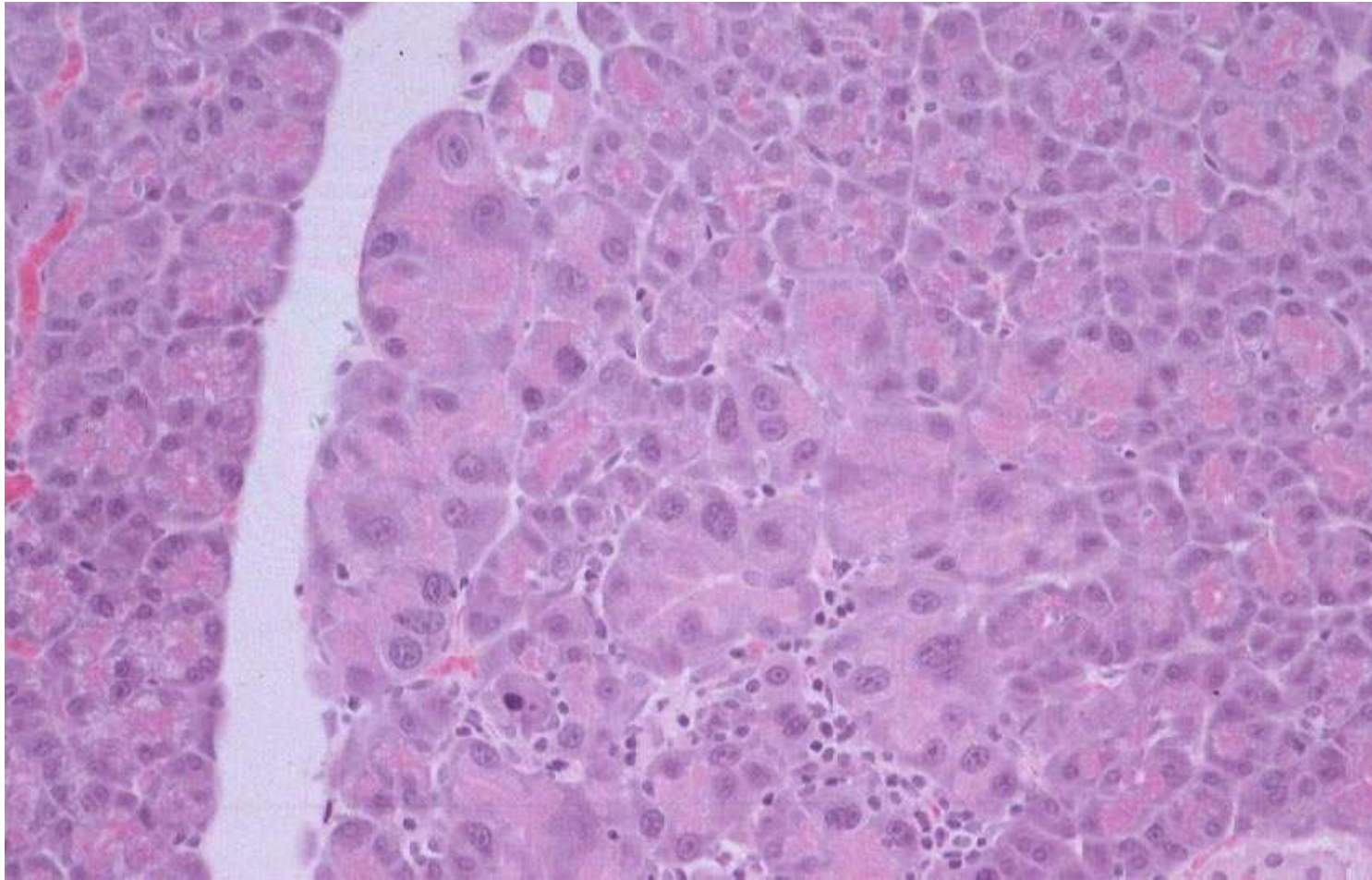
Mode of Action Studies on Pancreatic Acinar Cell Tumourigenesis

Effect of APFO on Rat Pancreas- Preliminary Study

- Rats administered APFO (300ppm) for up to 1 year
 - Pancreas H&E sections and DNA synthesis/cell proliferation (BrdU osmotic pumps)
 - Gene expression profiles (Agilent Rat 22K Oligo Microarray slides)
- Histopathology
 - a diffuse decrease in amount of zymogen in pancreas
 - an increased incidence of altered acinar cell foci.
 - the foci were hypertrophic, generally basophilic, exhibited increased cell replication rates and exhibited nuclear pleomorphism.

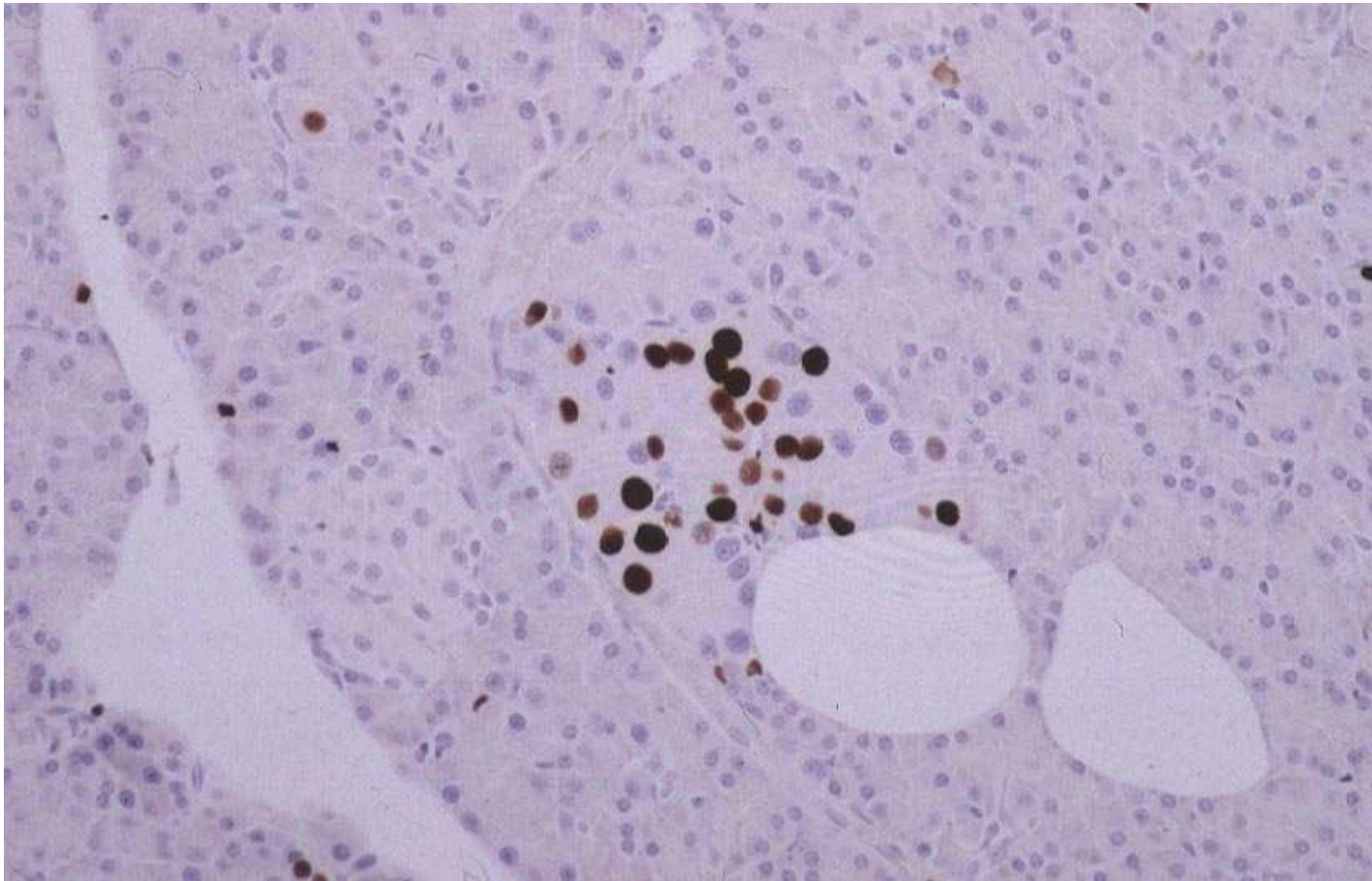
APFO Rat Study

Pancreatic Basophilic focus No 188 (300 ppm APFO)



APFO Rat Study

Pancreatic Basophilic focus No 193 (300 ppm APFO) BrdU



Preliminary Gene Expression Profiling

- Concentrated on genes that are plausibly related to carcinogenesis
 - GADD45 α , up regulated – a marker of DNA damage
 - EGR1 (early growth response 1), up-regulated
 - TISII (TPA-inducible sequence II), up-regulated
 - thymine DNA glycosylase, down regulated
- Non-cancer genes regulated
 - Gluconeogenesis, up-regulated
 - Pancreatitis, up-regulated

Main Study – Aims

- To examine the gene expression changes elicited by chemicals of varying potencies that have been reported to induce pancreatic acinar cell tumours.
 - Pancreatic acinar tumour induction potency at selected dose levels : Wy14,643 > APFO > DEHP
- To identify a battery of gene expression changes which may serve as indicators (biomarkers) of pro-carcinogenic potential in the pancreas with agents that fall into this class (peroxisome proliferators).

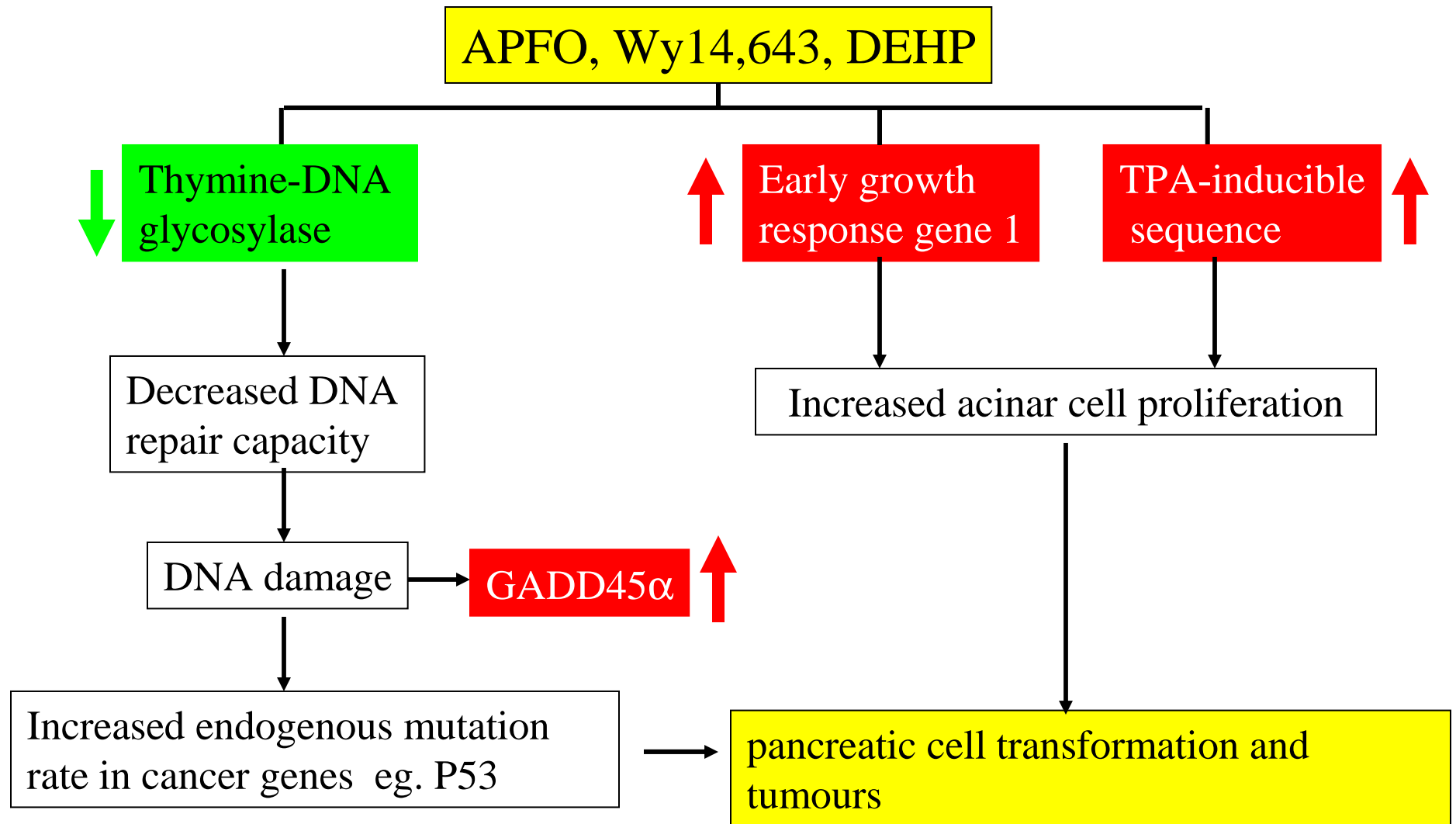
Experimental Design

- Male Sprague Dawley rats
- Wy14,643, 50 ppm in diet
- DEHP, 12000 ppm in diet
- APFO, 300 ppm in diet
- Time points; 1, 7 and 28 days

Summary of Gene Expression Profiles

- Time-dependent regulation in pancreas of genes associated with carcinogenesis were observed:
 - Wy14,643
 - EGR1 (early growth response 1) – up-regulated
 - TIS11 (TPA-inducible sequence II) – up-regulated
 - GADD45 α (growth arrest and DNA damage 45 α) – up-regulated
 - thymine-DNA glycosylase – down-regulated
 - APFO
 - Similar observations but to a lesser extent
 - DEHP
 - Similar observations but even smaller changes

Pancreatic Acinar Cell Tumours – An Hypothesis



Initiation

Promotion/progression

Red boxes, up-regulated ; green boxes, down-regulated

Conclusions

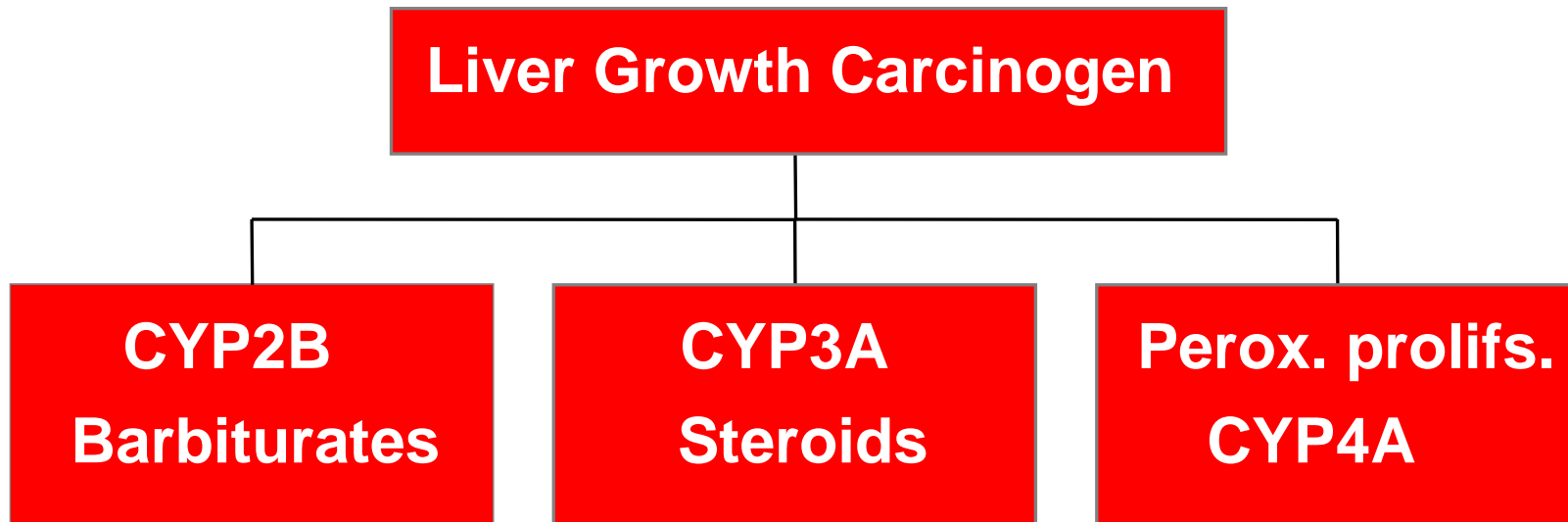
- These results suggest that there are similar carcinogenesis-associated processes occurring in the pancreas of rats exposed to the three compounds.
- Differences in the magnitude and duration of the alterations in gene expression may account for the differences in the tumourigenic potency of the compounds in the pancreas.
- Several genes have been identified that may be biomarkers of response in the pancreas

Characterisation of PFOA- induced Hepatomegaly in Rats

Liver Growth Carcinogens

- Hyperplasia
 - Stimulation of cell proliferation
 - Inhibition of apoptosis
- Hypertrophy
 - Organelle proliferation
 - SER
 - Peroxisomes

Rodent Liver Growth Carcinogens



Characteristics of the “Peroxisome Proliferation Phenomenon” in Rats and Mice

- Hepatomegaly
- Proliferation of **peroxisomes** and smooth endoplasmic reticulum
- **Induction of peroxisomal fatty acid oxidising enzymes**
- Induction of CYP **4A1**
- Stimulation of replicative DNA synthesis
- Inhibition of apoptosis
- Hepatocellular tumours in long term studies

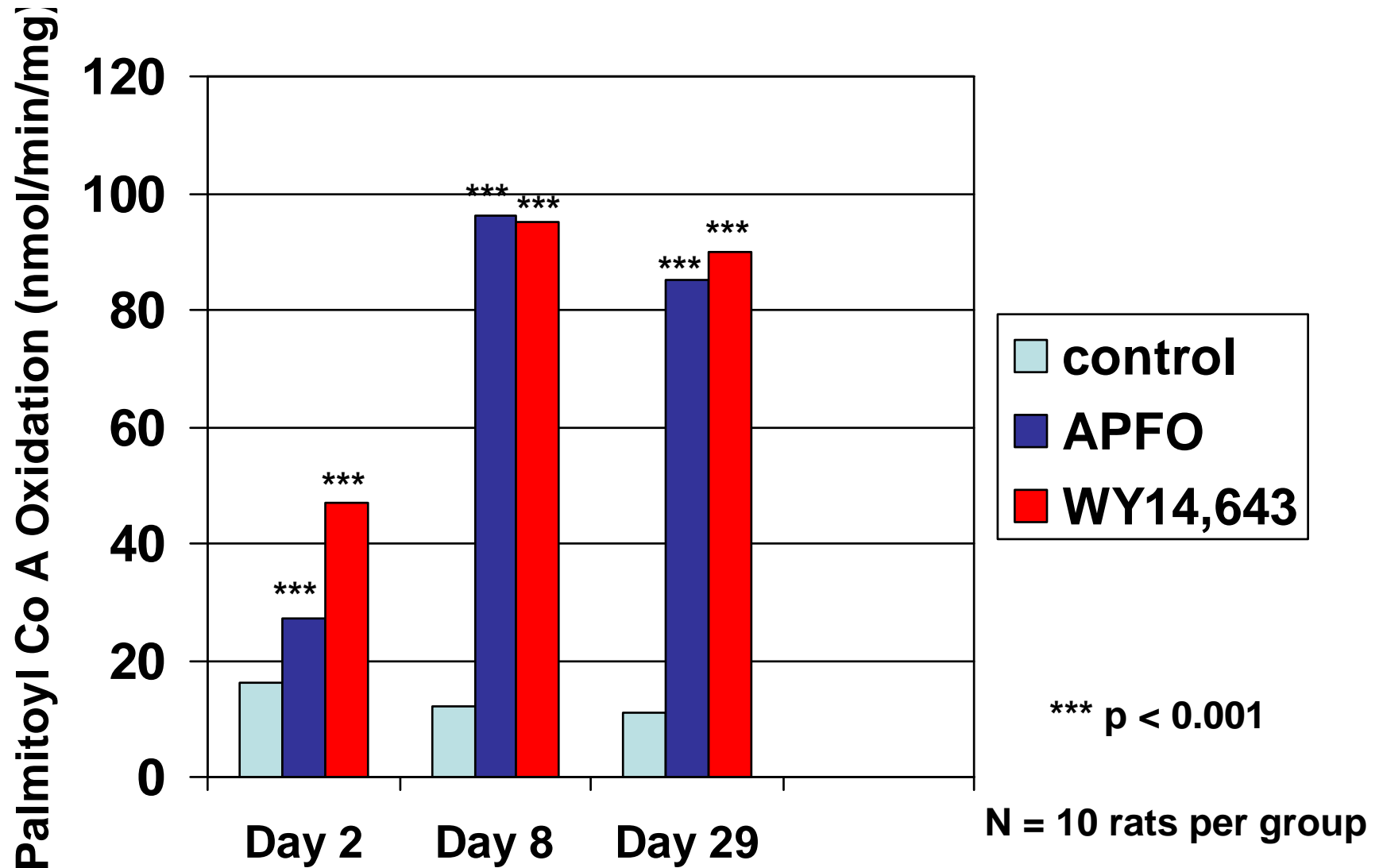
Study Design - Liver

- Male Sprague Dawley (CD) rats
- Wy-14,643 at 50ppm or APFO at 300ppm in the diet for either 1, 7 or 28 days (N=10)
- Implanted with osmotic pumps containing BrdU 5 days before termination (1 day time point - BrdU subcut.)
- Analysis of liver growth
 - H&E, apoptotic index, labelling index (S-phase)
 - Peroxisome proliferation
 - SDS-PAGE/Western blots for P450's
- Clinical chemistry

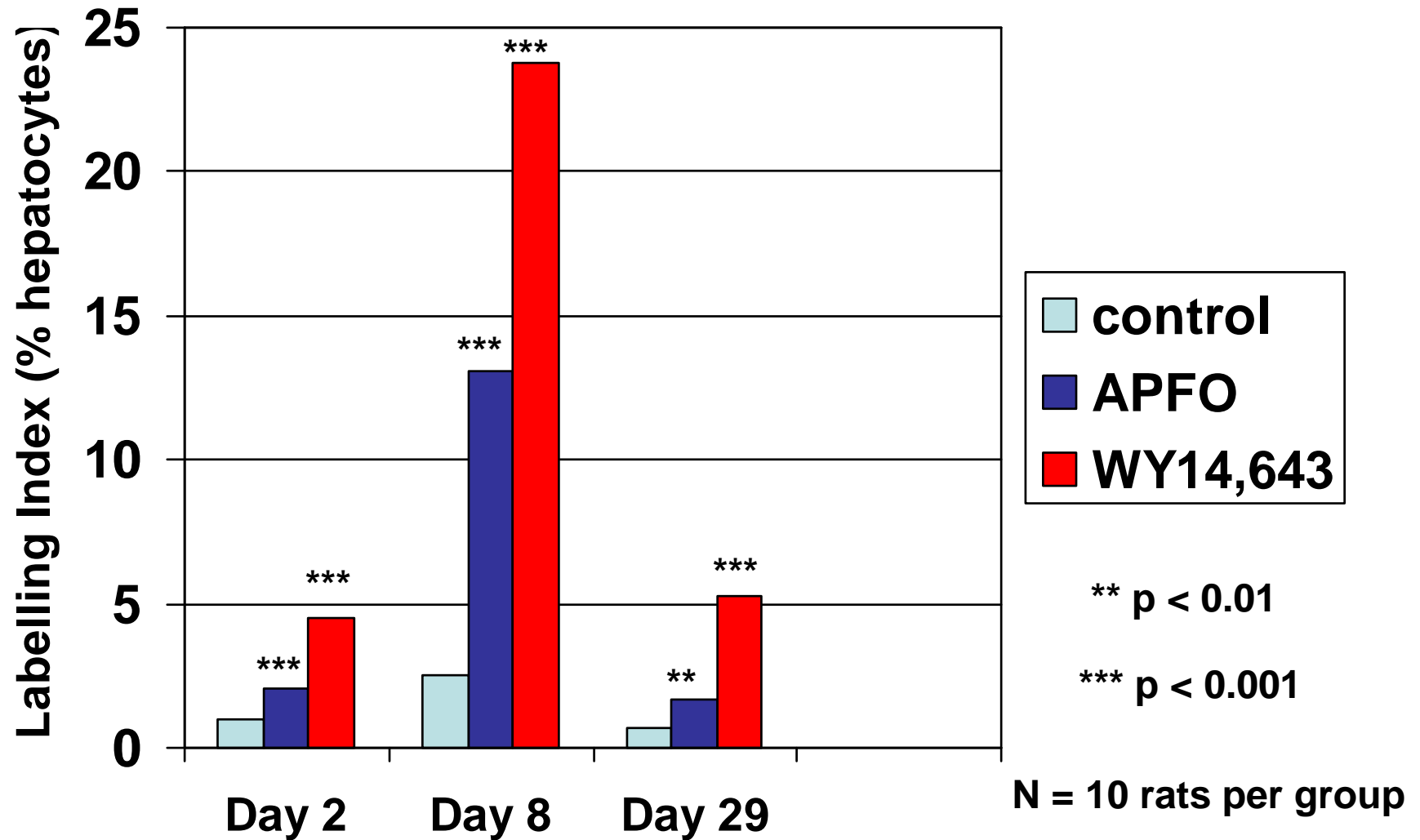
APFO-Induced Hepatomegaly in Rats

- Histopathology
 - Hepatic hypertrophy and hyperplasia
 - More severe in Wy14,643 groups

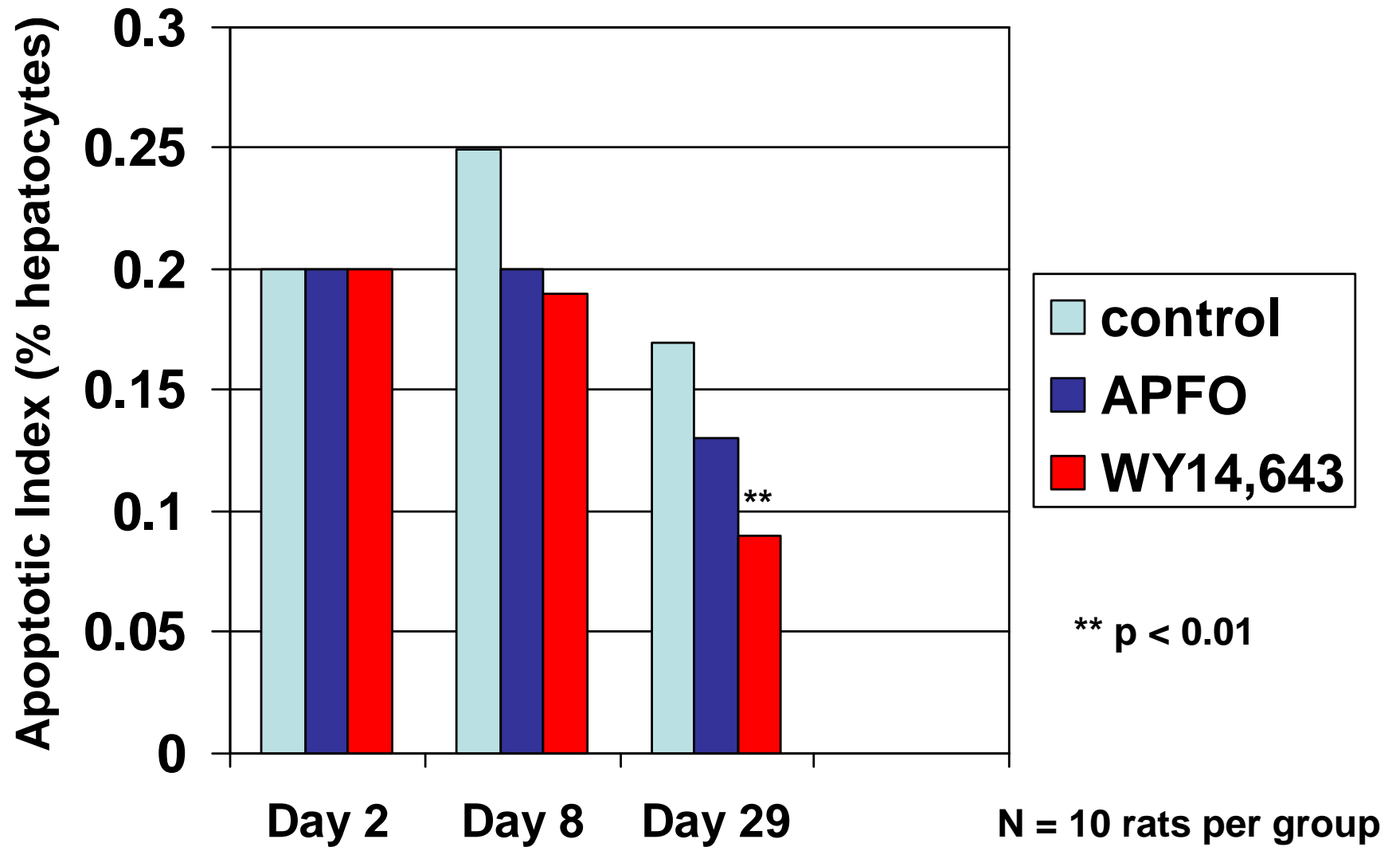
Hepatic Acyl CoA Oxidase in Rats Administered APFO (300ppm) and Wy14,643 (50ppm)



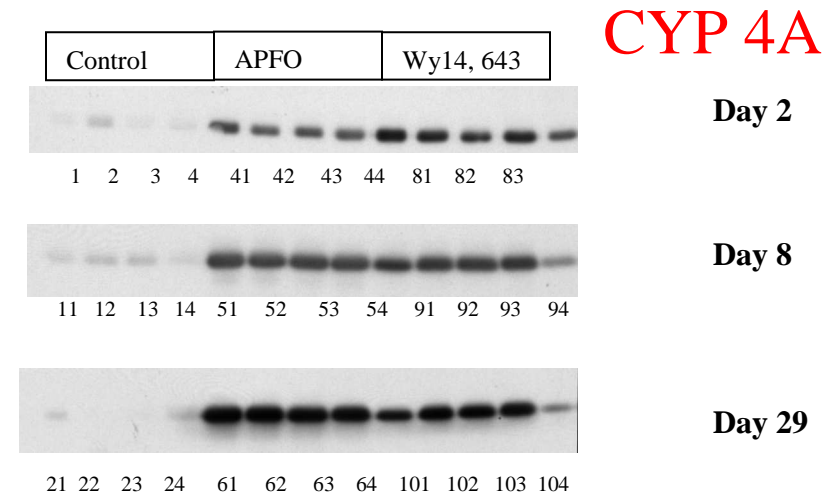
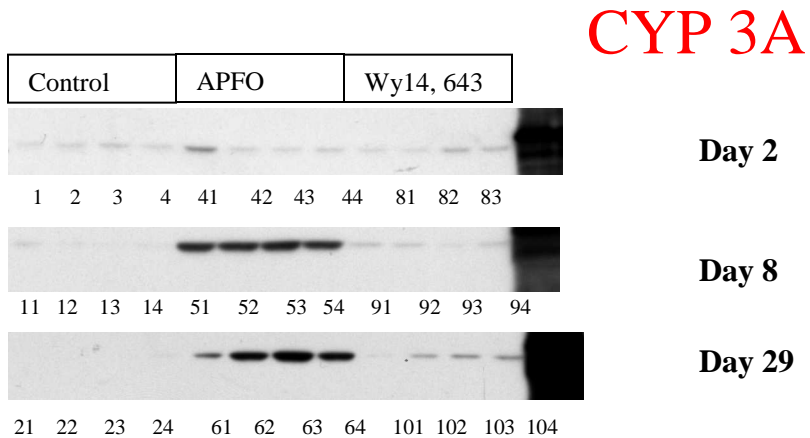
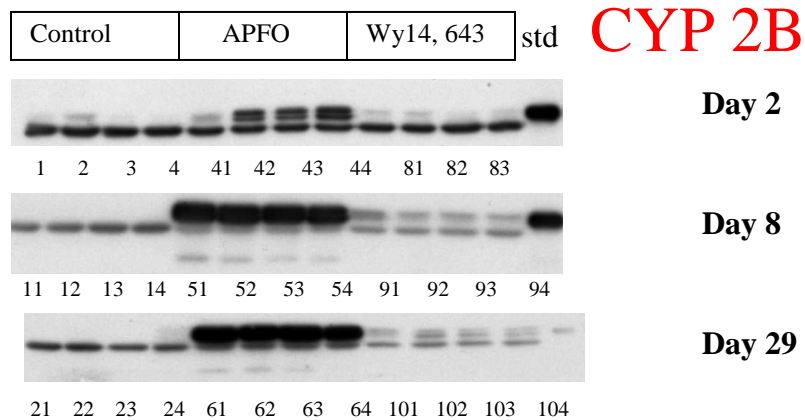
Hepatic DNA Synthesis in Rats Administered APFO (300ppm) and Wy14,643 (50ppm)



Hepatic Apoptosis in Rats Administered APFO (300ppm) and WY14,643 (50ppm)



APFO-Induced Hepatomegaly in Rats. Western Blotting of Hepatic Microsomes



Liver Growth Carcinogens

- Hyperplasia
 - Stimulation of cell proliferation
 - Inhibition of apoptosis
- Hypertrophy
 - Organelle proliferation
 - SER
 - Peroxisomes

Liver Conclusions

- APFO exhibits the characteristics of a rodent peroxisome proliferator.
- Marked species differences are seen in PPAR-mediated mechanisms
 - Quantitative and qualitative species differences
- In addition APFO has characteristics in common with drugs such as phenobarbitone (CYP 2B) and dexamethazone (CYP 3A).
 - qualitative species differences are apparent
- The epidemiology for such drugs appears “clean”

Overall Conclusions

- PFOA toxicity is largely a modulation of homeostasis *via* transcriptional regulation through nuclear hormone receptors.
- It is an oversimplification to relate all effects to the interaction of PFOA with PPAR α .
- PPAR γ , PXR and CAR (and probably other receptors) are also involved.

Overall Conclusions (continued)

- A plausible mechanism for hepatic tumourigenicity involves liver growth mediated by PPAR α , PXR and CAR.
- A working hypothesis involving increased endogenous DNA damage and promotion is presented for the increased incidence of pancreatic acinar cell tumours