

Compound C - Mechanisms of Non-Genotoxic Carcinogenesis – Role in Safety Evaluation and Human Risk Assessment

Compound C has low mammalian toxicity and is non-genotoxic in conventional assays for mutagenicity and clastogenicity. However, when administered to Fischer 344 rats in a two year bioassay Compound C induced liver tumours and thyroid follicular cell neoplasms in male and female rats and renal adenocarcinomas in male rats only. Two 90-day rat experiments, with Compound C administered in the diet for one study and by gavage in corn oil for the other study, revealed similar pathological trends to those seen in the two-year study. There was marked hepatomegaly accompanied by hepatocellular hypertrophy in both sexes, chronic nephritis in male rats and thyroid follicular cell hypertrophy and hyperplasia in both sexes.

Significant human exposure to Compound C occurs and the discovery of its carcinogenicity led to concerns over potential human health problems. Without an understanding of the mechanisms for the induction of these tumours, one must take the precautionary approach and assume that these tumours are relevant to human safety evaluation and risk assessment. This necessitated the initiation of research aimed at understanding the mechanism of carcinogenicity in rats, in order to evaluate the potential hazard of exposure of humans to Compound C.

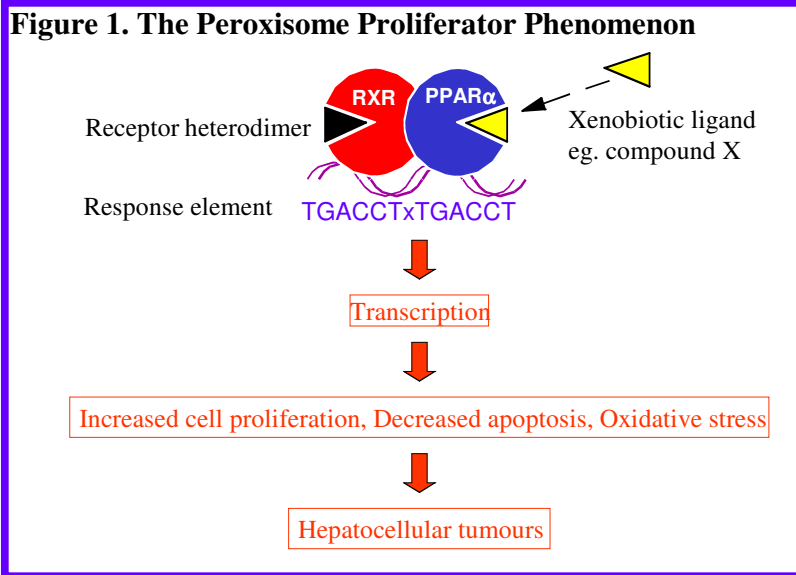
In light of the non-genotoxicity of Compound C and the spectrum of tumours induced in rats and mice it was hypothesised that the tumours were induced *via* three relatively well-characterised mechanisms as described below: -

Liver Tumours – The Peroxisome Proliferation Phenomenon

Chemicals inducing liver tumours in rodents *via* this mechanism are termed peroxisome proliferators. The compound interacts with the peroxisome proliferator activated receptor (PPAR α) and initiates transcription. This in turn leads to a characteristic pleiotropic response involving hepatomegaly that is characterised by proliferation of peroxisomes, inhibition of apoptosis and stimulation of replicative DNA synthesis and cell proliferation (Figure 1). This sustained hepatomegaly ultimately leads to the development of hepatocellular carcinoma.

Thyroid Follicular Cell Tumours – Perturbation of Thyroid Hormone Homeostasis

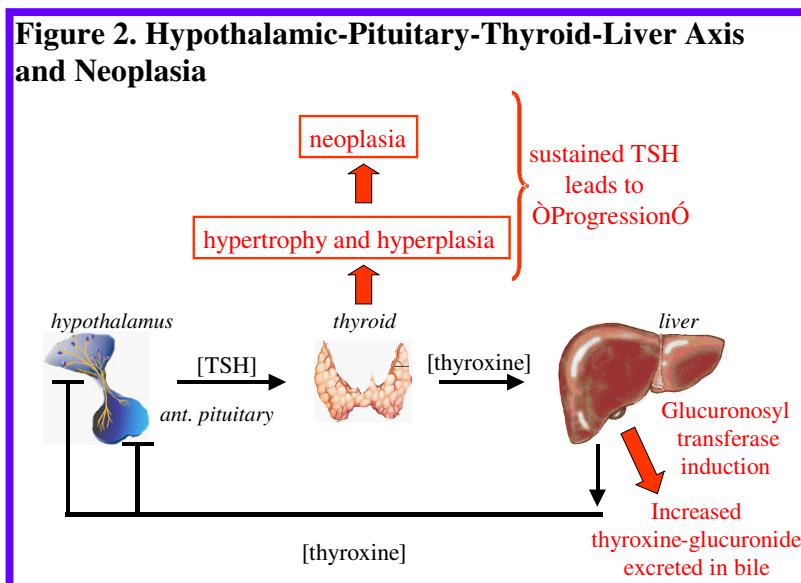
A variety of non-genotoxic chemicals perturb thyroid function and lead to follicular cell tumours. The liver has been shown to play a major role in the metabolism of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Compounds which have



the ability to induce hepatic microsomal enzymes have been shown to increase the clearance of T4 by hepatic glucuronidation with a concomitant lowering of plasma T3 and T4 levels. This decrease in plasma T4 leads to release of the pituitary from its usual negative feedback inhibition, thus causing an increase in plasma thyroid stimulating hormone (TSH).

The depression in plasma thyroid hormones with a concomitant increase in TSH is the classical response of the thyroid to a thyroid hormone imbalance. The lowering of plasma thyroid hormones triggers the pituitary to increase TSH in order that the thyroid will release and/or synthesise more thyroid hormones in order to maintain thyroid hormone homeostasis. However, sustained increases of TSH lead to follicular cell hypertrophy and hyperplasia and eventual formation of follicular cell carcinoma.

It has been implied that the induction of hepatic microsomal enzymes (e.g. glucuronosyl transferase), which increase thyroid hormone clearance and thus alter thyroid function in rats, is part of the process that will eventually lead to thyroid gland neoplasia (Figure 2).



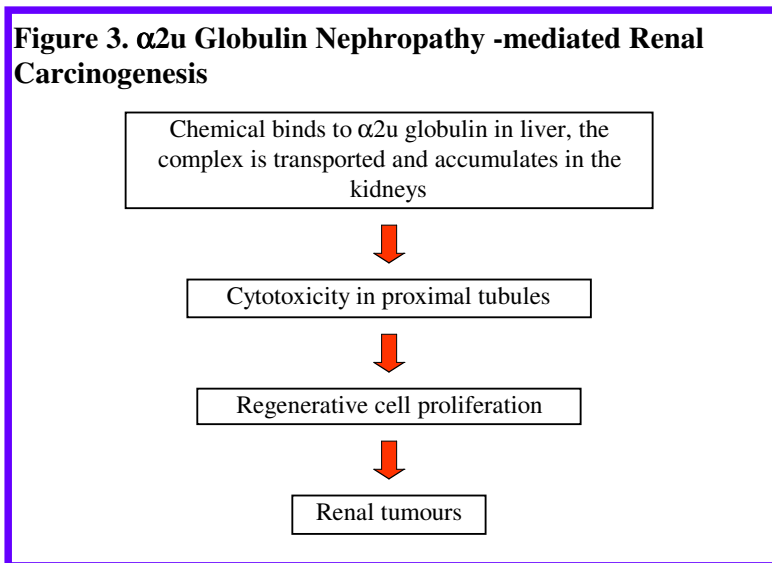
Kidney Tumours - α 2u globulin nephropathy

The induction of male rat-specific kidney tumours has been observed with a number of chemicals including 1,4-dichlorobenzene (DCB) and *d*-limonene (DL). These chemicals act *via* the α 2u globulin (α 2u) mechanism. α 2u is a rat-specific protein that is expressed in male rat liver under androgenic control. The female rat, mice and humans do not synthesise α 2u in the liver. The male rat is a 100 to 300 times more proteinuric than the female rat due to the large amount of α 2u secreted in male rat urine. In the mature male rat about 50mg of α 2u is filtered per day, 40% is excreted in the urine and 60% undergoes reabsorption and catabolism in the proximal tubule cells of the kidney.

DCB and DL are thought to bind to α 2u and slow down its degradation leading to the accumulation of α 2u in large protein droplets known as hyaline droplets. Protein overload leads to cytotoxicity and necrosis of the tubule epithelium. Sustained regenerative cell proliferation gives rise to foci of hyperplasia and eventually leads to the formation of renal tubule tumours (Figure 3).

Thus, in the absence of α 2u globulin (i.e. in female rats and humans) this chain of events leading to renal tumours will not occur.

Figure 3. α 2u Globulin Nephropathy -mediated Renal Carcinogenesis



Study Objective

These studies were designed to characterise some of the early (pre-neoplastic) events that occur in the livers, kidneys and thyroids of male and female Fischer 344 rats administered Compound C. In this manner the hypothesis can be examined that Compound C elicits tumours in rats by three distinct non-genotoxic mechanisms; namely, the phenomenon of peroxisome proliferation in the liver, the perturbation of thyroid hormone homeostasis, and α_2 u globulin accumulation and chronic stimulation of cell replication in the kidney.

With this information an assessment can be made of the relevance of the animal carcinogenicity data to human safety assessment.

Results

Administration of Compound C to male and female rats resulted in:

Hepatic Parameters

- Increased liver weight
- Peroxisome proliferation, as evidenced by induction of CN-insensitive palmitoyl CoA oxidation (a peroxisomal enzyme marker)
- Increased replicative DNA synthesis in liver
- Decreased apoptotic index in liver

Thyroid Parameters

- Increased thyroid weight
- Thyroid follicular cell hypertrophy and hyperplasia
- Increased follicular cell replicative DNA synthesis
- Decreased plasma [thyroxine]
- Increased plasma [TSH]
- Increased hepatic UDPGA-glucuronosyl transferase activity.

Kidney Parameters

- Accumulation of α_2 u globulin in the proximal tubules
- A chronic protein nephropathy, associated with a regenerative hyperplasia and increased S-phase (replicative DNA synthesis) in the proximal tubules
- These effects were observed in the kidneys of male, but not female, rats.

These changes are consistent and indeed diagnostic for the three non-genotoxic mechanisms of carcinogenicity described earlier.

Conclusions - Relevance to Humans

Liver Tumours – The Peroxisome Proliferation Phenomenon

Activation of the PPAR α receptor is a prerequisite for the induction of liver tumours *via* this mechanism. The lack of hepatocarcinogenicity of peroxisome proliferators in PPAR α null mice substantiates this contention. In the human liver, PPAR α is

expressed at levels of 1-10% of that found in rat and mouse liver. This level of expression appears too low to support the receptor-mediated mechanism of hepatocarcinogenesis observed in rodents. Hence, non-genotoxic peroxisome proliferators are not considered generally to pose a hepatocarcinogenic hazard to humans.

Thyroid Follicular Cell Tumours – *Perturbation of Thyroid Hormone Homeostasis*

The regulation of the hypothalamic-pituitary-thyroid-liver axis is similar in mice, rats and humans. Hence, a decrease of plasma thyroxine concentrations in humans would potentially lead to an overcoming of the negative feedback control of TSH release from the pituitary gland leading to thyroid hypertrophy and hyperplasia (i.e. goitre). While goitre does not usually progress to thyroid cancer in humans, it may be implied that sustained thyroid growth may predispose to carcinogenesis.

However, using primary cultures of human hepatocytes, we were able to demonstrate that Compound C was not an inducer of the human glucuronosyl transferase responsible for conjugating thyroxine.

Hence, Compound C will not perturb plasma concentrations of thyroxine in humans and the consequential thyroid gland growth will not occur. Hence, this chemical does not pose a thyroid cancer hazard to humans.

Kidney Tumours - *α₂u globulin nephropathy*

The α₂u globulin-mediated mechanism of renal tubular carcinogenesis is dependent ultimately upon the synthesis of α₂u globulin in the liver. This is specific to the male rat. Female rats do not express the protein and do not develop nephropathy or renal tumours. Similarly, other studies with Compound C in mice (non-expressers of α₂u globulin) did not demonstrate nephropathy or kidney tumour induction.

Therefore, as humans do not express α₂u globulin, it is reasonable to conclude that Compound C will not elicit kidney tumours in humans.

Summary

Such marked species differences in response underline the need for caution when using rodent toxicity data to assess potential hazard and risk to humans. However, based on this work, Compound C was not classified as a human carcinogen.