CHLORINATED PARAFFINS: MECHANISMS OF NON-GENOTOXIC CARCINOGENESIS

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Introduction

Chlorinated paraffins (CPs) are a group of industrially important chemicals ranging in chain length from 10 to 30 carbon atoms (C_{10-13} short-, C_{14-17} medium-, and C_{20-30} long-chain) and chlorinated from 40-70% by weight The CPs are chemically relatively inert and have a low mammalian toxicity[1,2]. Despite having been shown to be non-genotoxic in conventional assays[1,3]; in a 2 year bioassay, a short chain CP (60% chlorinated) administered to male and female B6C3F1 mice and Fischer 344 rats induced liver tumours in both sexes of both species, follicular cell neoplasms in the thyroid gland of females of both species, and renal adenocarcinomas in male rats[4]. Two 90-day rat experiments with a similar short chain CP (58% chlorinated) 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 hepatocellular hypertrophy in both sexes, chronic nephritis in male rats, and thyroid follicular cell hypertrophy and hyperplasia in both sexes[2].

CPs are hepatic enzyme inducers (CYP2B1/2 and CYP4A) and peroxisome proliferators in rats and mice[5,6]. Mice were more responsive than rats, and short chain CPs were more effective than the medium chain. Despite suggestions that CPs share induction profiles with dioxins, our recent experiments have furnished no evidence for the induction of CYP1A1 in rats or mice (no increase in ethoxyresorufin *O*-deethylation and no induction of CYP1A1 protein following SDS-PAGE and western blotting). Thus, clearly demonstrating the marked differences in the biological activities of the CPs and dioxins. Earlier studies have demonstrated the stimulation of replicative DNA synthesis (S-phase) in the livers of rats exposed to CPs[3]. Together, these data suggest that certain CPs may elicit liver tumours by a non-genotoxic mechanism, such as the peroxisome proliferation phenomenon[7].

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). Similar claims and observations have been made for several hepatic peroxisome proliferators, for example, nafenopin[8], phthalic acid esters[9] and CPs[6].

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 leads to follicular cell hypertrophy and hyperplasia and eventual formation of follicular cell carcinoma[10]. It has been implied that the induction of hepatic microsomal enzymes (e.g., glucuronosyl transferase) which effect thyroid hormone clearance and thus alter thyroid function in rats might be part of the process that will eventually lead to thyroid gland neoplasia. This hypothesis has been proposed for CPs in a previous study[6].

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)[11-13]. These chemicals act *via* the α2u globulin (α2u) mechanism. α2u is a rat-specific protein expressed in male rat liver under androgenic control. The female rat does not synthesise α2u in the liver. The male rat is a 100 to 300 times more proteinuric than the female rat due to the large amount of α2u secreted in male rat urine. In the mature male rat about 50mg of α2u 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 α2u and slow down its degradation leading to the accumulation of α2u 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. It is plausible that CPs elicit renal tumours *via* this non-genotoxic, male rat-specific mechanism.

The objective of the present studies was to characterise some of the early events that occur in the liver, kidneys and thyroid of male and female Fischer 344 rats administered Chlorowax 500C (C_{10-13} short chain CP with 58% chlorination) and Chlorparaffin 40G (C_{14-17} medium chain CP with 40% chlorination). Thereby, examining the hypotheses that CPs elicit 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 α2u globulin accumulation and chronic stimulation of cell replication in the kidney.

Methods and Materials

Male and female Fischer 344 rats (approximately 6-7 weeks of age at the start of the studies), were administered the chlorinated paraffins, by gavage, for up to 90 days at doses of 312 and 625 mg/kg body weight (these are the doses used in the NTP carcinogenicity bioassay). Control animals received corn oil vehicle alone (5ml/kg body weight). Seven days prior to sacrifice osmotic pumps containing BrdU (a DNA precursor) were implanted subcutaneously to allow the measurement of replicative DNA synthesis (S-phase, labelling index) in target tissues. At sacrifice, blood was taken by cardiac puncture and the livers, thyroids and kidneys harvested for examination.

Marked species differences have been reported in non-genotoxic carcinogenesis. Hence, we have examined the effects of a chlorinated paraffin (Chlorowax 500C) on putative early markers of carcinogenesis in guinea pigs. Male Dunkin Hartley Guinea Pigs (initial weights, 350-450g) were administered Chlorowax 500C (C500C; 500 and 1000 mg/kg body weight),

dissolved in corn oil (5ml/kg body weight) daily by oral gavage for 14 consecutive days.

Results and Discussion

Fischer 344 Rats

Both CPs stimulated liver growth and peroxisome proliferation, as evidenced by induction of CN^-- insensitive palmitoyl CoA oxidation, in male and female rats.

Perturbations of thyroid homeostasis, evidenced as decreases in plasma thyroxine and increases in plasma TSH concentrations, were generally noted, as was thyroid follicular cell hypertrophy and hyperplasia. These histopathological changes were reflected in an increased labelling index of the thyroid follicular cells.

Both CPs produced a chronic protein nephropathy, associated with a regenerative hyperplasia and increased S-phase in the proximal tubules, in the male but not female rats. There was some limited evidence for an involvement of α2u globulin in the male rat-specific nephropathy.

Similar effects were seen with the medium and short chain chlorinated paraffins although, in general, the short chain CP was more potent than the medium chain CP. The major toxicological changes observed are summarised in Table 1.

Table 1. Effects of Short and Medium Chain Chlorinated Paraffins in Fischer 344 Rats.

Parameter	Male Rat	Female Rat
Liver weight	Increased	Increased
Liver PCO[1]	Increased	Increased
Liver T4 - GT[2]	Increased	Increased
Plasma thyroxine	Decreased	Decreased
Plasma TSH	Increased	Increased
Thyroid hypertrophy	Yes	Yes
Thyroid S-phase[3]	Increased	Increased
Kidney S-phase[3]	Increased	No change
Chronic nephropathy	Yes	No

[1] CN^- insensitive palmitoyl CoA oxidation

[2] Thyroxine-UDPG glucuronosyl transferase

[3] Percentage of cells having undergone replicative DNA synthesis

These data suggest that, in rats, the chlorinated paraffins may induce liver, thyroid, and renal tumours in rats by well studied non-genotoxic mechanisms.

Guinea Pigs

There was no effect on liver weights and no observable hepatic histopathology. In addition, no histological changes were seen in the kidneys or the thyroids of the C500C-treated guinea pigs. C500C had no effect on any of the plasma thyroid hormones measured (Total T4, Free T4, Total T3 and Free T3), or on plasma concentrations of the pituitary hormone TSH (Table 2). Hepatic T4-glucuronosyl transferase activity was not affected at either dose level.

Table 2. Effects of a Short Chain (Chlorowax 500C) Chlorinated Paraffin in Guinea Pigs.

Liver weight	Liver PCO	Liver T4-GT	Plasma T4	Plasma TSH	Thyroid histology	Kidney histology
No effect	No effect	No effect	No effect	No effect	No effect	No effect

The present findings in guinea pigs are in sharp contrast to those of the previous studies in rats. Specifically, the early hepatic effects (peroxisome proliferation, induction of T4-glucuronosyl transferase) and thyroid-related changes (alterations in thyroxine and TSH) seen in the rat were not reproduced in the guinea pig. In addition the protein nephropathy seen in rat kidney, was not seen in the guinea pig.

Such marked species differences in response underline the need for caution when using rodent toxicity data to assess potential hazard and risk to humans.

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1. Birtley RDN, Conning DM, Daniel JW, Ferguson DM, Longstaff E. and Swann AAB. (1980) Toxicol. Appl. Pharmacol. 54, 514.
2. Serrone DM, Birtley RDN, Weigand W. and Millischer R. (1987) Food Chem Toxicol 25, 553.
3. Ashby J, Lefevre PA. and Elcombe CR. (1990) Mutagenesis 5, 515.
4. National Toxicology Program (NTP). (1986) Technical Report 308, US Department of Health and Human Services, Public Health Service, National Institutes of Health.
5. Nilsen OG, Toftgard R. and Glaumann H. (1981) Arch Toxicol 49, 1.
6. Wyatt I, Coutts CT. and Elcombe CR. (1993). Toxicology 77, 81.
7. Ashby J, Brady AM, Elcombe CR, Elliott BM, Ishmael J, Odum J, Tugwood JD, Kettle S. and Purhase IFH. (1994) Human Expt. Toxicol. 13, supp. 2, 1.
8. Kaiser CA, Seydoux J, Giacobino J-P, Girardier L. and Burger AG. (1988) Endocrinol 122, 1087.
9. Hinton RH, Mitchell FE, Mann A, Chescoe D, Price SC, Nunn A, Grasso P. and Bridges JW. (1986) Environ Health Perspec. 70, 195.
10. Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL. and Wilkinson CF. (1989) Fundam. Appl. Toxicol 12, 629.
11. Eldridge SR, Goldsworthy TL, Popp JA. and Butterworth BE. (1990) Carcinogenesis 13, 409.
12. Lehman-McKeeman LD, Rodriquez PA, Takigiki R, Caudil D. and Fey ML. (1989) Toxicol. Appl. Pharmacol. 99, 250.
13. Lehman-McKeeman LD, Rivera-Torres MI. and Caudil D. (1990) Toxicol. Appl. Pharmacol. 103, 539.