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Lindane induces nephropathy and renal accumulation of α2u-globulin in male but not in female Fischer 344 rats or male NBR rats

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Lindane, the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane, is a widely used organochlorine pesticide. The application of lindane in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops, its application on livestock, pets, and on agricultural premises, in pharmaceutical preparations, as well as in public health pest control, could lead to the exposure of humans to low concentrations of this compound. Various experiments were carried out in order to assess the toxicity and carcinogenicity of lindane [1,2]. In some of these experiments, male rat specific renal toxicity was observed. This toxicity was characterized by an exacerbated formation of hyaline droplets, enlargement of lysosomes in the proximal tubules, and necrosis of proximal tubule epithelial cells [3,4].

The male rat specific occurrence of hyaline droplet formation and necrosis of proximal tubule epithelial cells are characteristic lesions for the chemically induced male rat renal disease, α2u-globulin nephropathy. This disease occurs only in male rats of strains other than the NBR, the only strain that does not synthesize the androgen-dependent form of α2u-globulin [5]. It is induced by the reversible binding of chemicals or their metabolites, such as 2,2,4-trimethylpentane, *d*-limonene, 1,4-dichlorobenzene, unleaded gasoline, and isophorone to the low-molecular-weight protein α2u-globulin, which leads to the accumulation of this α2u-globulin–chemical complex in the lysosomes of the proximal tubule epithelial cells [6]. This results in lysosomal overload, which leads to degeneration and necrosis of epithelial cells, followed by compensatory regeneration [7].

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The object of this study was to verify whether lindane induces α2u-globulin nephropathy.

Five 11-week-old male and female Fischer 344 (F344) rats, respectively, and five 11-week-old male NBR rats were exposed to 10 mg/kg/d of lindane in corn oil by oral gavage on 4 consecutive days. For the vehicle control, the same number of animals, sex and strain were gavaged simultaneously with corn oil only, using the same protocol. All animals were killed 24 h after the last dose. The left kidney of each animal was perfused with isotonic saline, excised, immersed in cold buffer (67 mOsm K_2PO_4, pH 7.0), homogenized with a Tekmar tissumizer, and stored at –80°C for future analyses. The right kidney was perfused with sodium phosphate buffer (pH 7.2) to remove all blood and then with a sodium-phosphate-buffered saline (pH 7.2) containing 1% glutaraldehyde and 2% paraformaldehyde to fix the kidney for histopathological evaluation. One half of the right kidney was embedded in glycolmethacrylate (GMA) and the other in paraffin. The GMA- and paraffin-embedded kidneys were sectioned to 1–2 μm and 4–5 μm thin slices, respectively. The presence of hyaline droplets was assessed by staining the GMA sections with Lee's Methylene Basic Blue Fuchsin (LMBBF). The paraffin sections were stained with hematoxylin-eosin (H&E) for routine analysis of kidney lesions and immunohistochemically for the presence of α2u-globulin.

The kidney sections of treated F344 male rats exhibited a marked increase in α2u-globulin staining area and intensity in comparison to the control F344 males. No α2u-globulin was detected in the sections of control or treated female F344 and male NBR rats. The pathological changes observed in the H&E stains of the treated male F344 rats were similar to those seen in the LMBBF-stained slides, and occurred in the same regions of the kidney that stained positive for α2u-globulin. These changes were observed exclusively in the proximal tubule epithelial cells, and were characterized by exacerbated accumulation of protein droplets of crystalline appearance, various stages of hypertrophy and necrosis, pyknotic and karyolytic nuclei, exfoliation of epithelial cells, and the presence of regenerative epithelium. No pathological changes or protein droplet accumulation could be detected in the H&E- and LMBBF-stained sections of the control male F344 rats, control and treated female F344 rats, and male NBR rats, respectively.

The lack of protein accumulation and the absence of pathological changes in the lindane-treated, α2u-globulin-deficient [5] male NBR rats indicate that the presence of α2u-globulin is a prerequisite for the lindane-induced renal disease. Furthermore, the exacerbated occurrence of protein droplets and the presence of renal lesions in treated male F344 rats, in conjunction with the positive identification of the accumulating protein as α2u-globulin, and the absence of protein droplets and renal lesions in treated female F344 rats, leads to the assumption that lindane forms a complex with α2u-globulin similar to other nephropathy-inducing compounds. This assumption is, at least in part, corroborated by the observation that ^{14}C-labelled lindane accumulates primarily in the renal cortex of treated rats [4]. In addition, the shape, vol-

ume and lipophilic characteristics of lindane are similar to structure–activity relationships for other chemicals that cause α2u-globulin nephropathy [8; personal communication with Aaron Miller]. It therefore seems feasible to conclude that lindane induces α2u-globulin nephropathy in male F344 but not in female F344 or male NBR rats.

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