

**EXPERT REPORT ON KIDNEY-RELATED FINDINGS IN
TOXICITY/CARCINOGENICITY STUDIES WITH
DI(ISONONYL) PHTHALATE (DINP) IN F344 RATS
AND B6C3F1 MICE**

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I have been asked by the Phthalate Esters Panel of the American Chemistry Council to comment on kidney-related findings in 2-year studies with di(isononyl) phthalate (DINP) by Moore (1998a, 1998b) and Lington et al (1997). I am a Fellow of the Royal College of Pathologists, a Fellow of the Royal College of Veterinary Surgeons, and a Fellow of the Academy of Toxicological Sciences. I have specialized in, and conducted research on, renal toxicologic pathology, toxicology, and carcinogenesis over the past 35 years. I am now an independent consultant, but the positions I have held in previous years include Associate Director of the Fels Research Institute, Temple University, Philadelphia, PA (1978-1985); Director and CEO of the British Industrial Biological Research Association (BIBRA), Carshalton, Surrey, UK (1985-1988); and Director of Administration and Senior Pathologist and Toxicologist, American Health Foundation, Valhalla, NY (1991-2001). Amongst the special assignments that I have had are participation in several Monograph Working Groups for IARC, participation on expert panels for ILSI and JECFA, and preparation of documents used in hazard assessment by US EPA on alpha₂u-globulin related kidney carcinogenesis and thyroid carcinogenesis. At various times, I have been Editor-in-Chief of *Food and Chemical Toxicology*, *Toxicology In Vitro*, and *Toxicologic Pathology*. My curriculum vita is attached to this report.

Introduction

In 2-year studies of di(isononyl) phthalate (DINP) conducted in Fischer 344 rats and B6C3F1 mice (Moore, 1998a, 1998b), the main kidney-related cancer finding was an increased incidence of renal tubule tumors in male rats but not in female rats or mice of either sex. The US Environmental Protection Agency (EPA) has accepted that the weight of evidence, including hyaline droplets in subchronic studies and linear papillary mineralization at chronic time-points, supports an alpha₂u-globulin (α_2 u-g) nephropathy mode-of-action as the underlying basis for the kidney tumor incidence (USEPA, 2005). However, based in part on non-neoplastic findings in the kidney, and certain clinical parameters, EPA has proposed to add a DINP category to the list of chemicals under Section 313 of the Emergency Planning and Community Right-to-Know Act, (USEPA, 2005). Accordingly, in this report, I have focused on, and attempted to address the points from the Moore et al (1998a, 1998b) and Lington et al (1997) studies, which EPA has identified as indicative of possible kidney toxicity of human relevance associated with DINP. These points are:

1. Increased relative kidney weights in male and female rats.

2. Increased urine output in male and female rats and mice, interpreted by EPA as indicative of treatment-related, compromised ability to concentrate urine.
3. Increased incidence and severity of nephropathy in female mice.

In addition, I addressed two other kidney-related end-points discussed in the hazard assessment. These were:

4. Increased pigment in renal tubules of male and female rats.
 5. Increase in serum urea nitrogen levels in male rats
1. **Increased relative kidney weights in male and female rats.** Increased relative kidney weights were recorded in males above 359 mg/kg/d, and in females above 442 mg/kg/d. Contrary to the statement by EPA in the revised technical review (USEPA, 2005), linear papillary mineralization certainly will have a positive effect on relative kidney weights as it represents retention of cell debris that has become calcified. In addition, a major cause of death in this study was mononuclear cell leukemia (MCL). The infiltration of MCL cells into the kidney, which occurred to greater extent in the higher dose groups of both sexes, would also lead to an increase in relative kidney weight in affected animals. These factors would be expected to influence the group mean.
 2. **Increased urine output in male and female rats and mice, interpreted by EPA as indicative of treatment-related, compromised ability to concentrate urine.** Increased urinary volume, along with a decrease in osmolality and urinary electrolytes was observed in treated animals of groups 4 and 5 in male rats and both sexes of mice. The greatest effect occurred in male rats, where a possible contributor was the α 2u-g nephropathy. A reduction in the concentrating ability of the male rat kidney has been demonstrated with light hydrocarbons that produce this syndrome (Phillips and Egan, 1984; Phillips and Cockrell, 1984). However, in the absence of any other observed tubule histopathology (other than that associated with spontaneous chronic nephropathy) it is probable that the observation of increased urine output in each species was unrelated to kidney involvement. Some studies have shown that

urine concentrating ability is not impaired until at least 50% of the nephrons have become involved in a pathologic process (Osborne et al, 1983). As there was no histological evidence for this, the cause of the alteration is very likely non-renal. The most probable explanation would be an increase in water intake (polydipsia), but as these animals were on an automated watering system, water intake was not measured. Polydipsia is often due to the taste (e.g. saltiness) and/or gustatory perception of the administered compound.

- 3. Increased incidence and severity of nephropathy in female mice.** Chronic progressive nephropathy (CPN) is an age-related disease of spontaneous origin commonly observed in laboratory rats, with males affected more than females (Gray, 1977; Barthold, 1979; Hard and Khan, 2004). This disease commences at a relatively early age of 2 to 3 months, and progresses relentlessly to affect virtually 100% of male rats (Fischer 344 or Sprague-Dawley) in 2-year studies (Hard and Khan, 2004). Mice are also spontaneously affected by an age-related CPN, which is very similar to the rat disease in respect of histopathology, and appears to follow the same clinical course (Wolfe and Hard, 1996). CPN (also referred to as chronic glomerulopathy and glomerulosclerosis in mice by some authors), has been reported as the most frequent non-neoplastic lesion leading to early death in mouse studies, affecting males and females to a similar extent (Ettlin et al, 1994), although in the CD-1 mouse, females were found to be more susceptible to this naturally occurring disease than males (Maita et al, 1988). In rats, it is well known that CPN can be exacerbated by some chemicals after chronic exposure (Hard and Khan, 2004), and chemical enhancement of this disease can also occur in mice. For example, CPN was enhanced in both sexes of B6C3F1 mice administered quinapril, an ACE inhibitor (Gough et al, 1993), and both incidence and severity were exacerbated in female B6C3F1 mice exposed to di(2-ethylhexyl) phthalate, another phthalate ester (David et al, 2000). Although the etiology is unknown, much evidence exists in rats to show that CPN is influenced by dietary and hormonal factors, including variations in the quantity or quality of dietary protein (Iwasaki et al, 1988; Masoro and Yu, 1989; Masoro et al, 1989; Rao et al, 1993), in caloric intake (Keenan et al, 2000), and by sex hormone manipulation (Baylis,

1994). Because the disease can be modified by such physiological factors, chemically exacerbated CPN, by itself, should not be considered indicative of a toxic effect of the chemical, although it does represent an adverse effect. Rodent CPN is clinically and histopathologically distinct from the common forms of chronic renal disease in humans, and it has been concluded that there is no strict counterpart of CPN in humans (Hard and Khan, 2004). Therefore, a chemical exacerbation of this spontaneous disease in rodents appears to have no relevance for extrapolation to humans

4. **Increased pigment in renal tubules of male and female rats.** Over the past 2 years, I have had the opportunity to review the renal histopathology induced by chemicals accepted as causing renal tubule tumors through an α 2u-g mechanism in male rats, including d-limonene and decalin. It has been my personal observation that, at the chronic time-point, there is an increase in aggregates of golden-brown pigment in the second segment of proximal tubule, probably reflecting earlier tubule involvement in the α 2u-g nephropathy process. At least in male rats, this long-term accompaniment of α 2u-g nephropathy might explain, in part, the increased pigment seen with DINP exposure in groups 4 to 6. In addition, it is known that MCL also causes the accumulation of pigment in proximal tubule cells (Ward and Reznik-Schuller, 1980; Hard and Snowden, 1989). As noted above, MCL was a major cause of death in this study in both male and female rats, and the trend for increased pigment recorded for the unscheduled deaths, appears to match the increase in MCL, for both sexes. In my opinion, MCL would be a sufficient explanation for the observation of increased pigment in the higher male and female dose groups, with α 2u-g nephropathy itself contributing to pigment accumulation in the males.
5. **Increase in serum urea nitrogen levels in male rats.** It has been suggested that clinical chemistry observations are not the most sensitive indicators of toxicity (Irausquin, 1992). Furthermore, compound-induced renal histopathology has occurred more reliably than changes in blood urea nitrogen (BUN) or creatinine concentrations (Travlos et al, 1996). It is known that increased BUN can be due to causes other than those

emanating from the kidney, for example, dehydration, level of protein in the diet, liver function, and animal health and nutritional status (Finco, 1989; Travlos et al, 1996). When due to renal involvement, at least 75% of the nephrons need to be compromised before nitrogen levels in the blood begin to increase (Finco, 1989; Osborne and Stevens, 1998). There was no histological evidence for this (other than chronic progressive nephropathy and linear papillary mineralization) in the male rats at 2 years. Furthermore, when reflecting kidney damage, an increase in BUN is invariably accompanied by a change in serum creatinine. Creatinine is not as affected by extrarenal factors as BUN (Ragan, 1989), and increases in serum creatinine concentration have a greater predictive value for morphological change in rat kidneys than BUN (Travlos et al, 1996). Creatinine levels were unaffected in these chronic studies, supporting the view that the mild increase in BUN in male rats was not related to kidney toxicity.

In conclusion, it is my view that, if DINP was causing renal toxicity, it would be reflected by observation *in the parenchyma that was not affected by CPN*, of at least one of the following changes: tubule basophilia, vacuolation, evidence of cell degeneration (single cell death) and regeneration (increase in mitotic figures), or some other morphological abnormality such as tubule dilation, interstitial nephritis, crystal formation, etc. From the pathology reports of the Moore (1998a, 1998b) studies, and the Lington et al (1997) report, it appears that this was not the case other than for changes that can be explained by α 2u-g nephropathy, MCL development, or CPN. In my expert judgment, the kidney-related findings discussed above are not a consequence of toxicity of DINP to the kidney, except for the association between α 2u-g and pigment and linear papillary mineralization. Therefore, in my view, there is not sufficient evidence to establish that DINP can reasonably be anticipated to cause serious or irreversible renal effects in humans.



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