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Mode of action of butoxyethanol-induced mouse liver hemangiosarcomas and hepatocellular carcinomas

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Abstract

Chronic exposure to 2-butoxyethanol resulted in an increase in liver hemangiosarcomas and hepatic carcinomas in male mouse liver. No increase in liver neoplasia was observed in similarly exposed male and female rats or female mice. We have proposed that the production of liver neoplasia in the male mouse is the result of oxidative damage secondary to the hemolytic deposition of iron in the liver. Our working hypothesis is that the mode of action of butoxyethanol-induced mouse liver hemangiosarcomas and hepatic neoplasia involves the metabolism of 2-butoxyethanol to butoxyacetic acid which results in the induction of RBC hemolysis. This hemolytic response is translated into the accumulation of iron in both liver hepatocytes and Kupffer cells. The Kupffer cell response to this insult is two-fold: (1) the production of oxidative species—through both Kupffer cell activation and through the Fenton reaction involving iron and (2) the production of cytokines (for example TNF alpha). The induction of reactive oxygen species can, if not scavenged, produce oxidative DNA damage (the formation of OH8dG), as well as increase cell growth through modulation of gene expression. While the reactive oxygen species generation would occur in the both rats and mice, the ability of the rat to detoxify the reactive oxygen species would preclude the remaining steps from occurring. In contrast, in the mouse, the reactive oxygen species would override antioxidant defense mechanisms and allow the proposed mode of action to move forward. Our results to date in male B6C3F1 mice and male F344 rats treated with 2-butoxyethanol (via daily gavage; five times per week) at doses of 0, 225, 450, and 900 mg/kg/day (mice) and 0, 225, 450 mg/kg/day (rats), respectively, showed: an increase in hemolysis in 2-butoxyethanol treated rats and mice in a dose-dependent manner, in addition, an increase in the percent of iron stained Kupffer cells in the liver was observed following treatment with 450 and 900 mg/kg of 2-butoxyethanol in mice and 225 and 450 mg/kg of 2-butoxyethanol in rat. With the iron deposition, a biphasic increase in oxidative damage (OH8dG and malondialdehyde) was seen in mouse liver after treatment with 2-butoxyethanol. In contrast, no increase in oxidative damage was observed in the rat liver at any of the doses examined. Concomitant with the increase in oxidative damage, Vitamin E levels were similarly reduced by 2-butoxyethanol in both mice and rat liver. However, the basal level of Vitamin E in rat liver was 2.5-fold greater than in mouse liver. A biphasic induction of DNA synthesis was seen following 2-butoxyethanol in the mouse. In mouse liver, increased DNA synthesis was observed in hepatocytes at 90 days and in endothelial cells at 7 and 14 days at all doses. No change in DNA synthesis was seen in 2-butoxyethanol treated rat liver. No apparent differences in apoptosis and

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mitosis in the liver were observed in mouse and rat liver between 2-butoxyethanol treatment groups and untreated controls. These results suggest that the induction of DNA synthesis, possibly from oxidative stress and/or Kupffer cell activation, occurs selectively in the mouse liver, in endothelial cells and in hepatocytes following exposure to 2-butoxyethanol, and support the hypothesis proposed above.

Keywords: Butoxyethanol; Hemangiosarcomas; Oxidative stress; Hepatocellular carcinomas

1. Introduction

The understanding of the mechanism by which chemicals induce neoplasia is an important and required component of scientifically based risk assessment. The utilization of a mode of action framework for understanding components of this mechanism allows for a peer reviewed, transparent, literature supported approach to understanding the induction of a specific neoplasm by a specific chemical agent. The defining of the key molecular and cellular events necessary for the progression of a normal cell to a neoplastic cell allows both investigators and regulators a clear understanding of the strengths and weaknesses of the available experimental evidence in support of the proposed mode of action. The use of the mode of action for the assessment of carcinogens is a salient feature of the current US EPA guidelines for carcinogen Risk Assessment (US EPA, 1986, 1999). The use of the mode of action approach also provides for the identification of data gaps that through subsequent investigation may help solidify our understanding of the mode of action. Utilization of this process also helps define the plausibility of the mode of action in rodents and the plausibility of the same mode of action to humans. Using a step-wise approach, the weight of experimental evidence, identification of causal events versus associative toxicological changes, and the specificity for each key event to the proposed mode of action can be defined.

This manuscript will provide an analysis of the proposed mode of action for mouse liver tumors (hemangiosarcomas and hepatocellular carcinomas) induced by ethylene glycol monobutyl ether (2-butoxyethanol) using the US EPA framework for carcinogen risk assessment (US EPA, 1986, 1999). The two modes of actions are presented (Tables 1 and 2) for hemangiosarcomas and hepatocellular carcinomas, respectively. A discussion of the scientific literature supporting each step of the proposed key events for each tumor type is presented. This manuscript reflects the summation of the key evidence in support of the postulated modes of action for both hemangiosarcomas and hepatocellular carcinomas.

Chronic exposure of rats and mice to 2-butoxyethanol resulted in an increase in hepatocellular carcinomas as well as liver hemangiosarcomas in B6C3F1 mice (NTP, 2000) (Table 3). The induction of these liver tumors was seen selectively in male mice for hemangiosarcomas and male and female mice for hepatocellular neoplasia. 2-Butoxyethanol has been shown to be negative in bacterial mutagenesis and other standard genotoxicity assays (Elliott and Ashby, 1997; Park et al., 2002a, 2002b), thus, an epigenetic or non-genotoxic mode of action appears to be responsible for the liver neoplasms produced by this compound. Hemolysis is another prominent toxic effect associated with 2-butoxyethanol exposure in rodents (Ghanayem and Sullivan, 1993; Udden and Patton, 1994; Udden, 2000). Associated with 2-butoxyethanol-induced hemolysis in rodents was an increase in hemosiderin (iron deposition) in Kupffer cells in the liver (NTP, 2000; Seisky et al., 2002). These findings, along with the induction of hepatic lesions, has led our laboratory to hypothesize that the induction of liver hemangiosarcomas as well as the increase in hepatocellular carcinomas, may be attributable to activation of Kupffer cells due to phagocytosis of hemolysed red blood cells and iron deposition in Kupffer cells. Iron, via Fenton reactions and/or Haber–Weiss reactions and activation of Kupffer cells can produce reactive oxygen species including hydroxyl radicals, that in turn may produce oxidative DNA damage, lipid peroxidation and/or protein modifications (Seisky et al., 2002; Imlay and Linn, 1988; Park et al., 2002a, 2002b). In addition, oxidative radical formation has been shown to contribute to the carcinogenesis process through the induction of oxida-