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Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment

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ABSTRACT

1,4-Dioxane is found in consumer products and is used as a solvent in manufacturing. Studies in rodents show liver tumors to be consistently reported after chronic oral exposure. However, there were differences in the reporting of non-neoplastic lesions in the livers of rats and mice. In order to clarify these differences, a reread of mouse liver slides from the 1978 NCI bioassay on 1,4-dioxane in drinking water was conducted. This reread clearly identified dose-related non-neoplastic changes in the liver; specifically, a dose-related increase in the hypertrophic response of hepatocytes, followed by necrosis, inflammation and hyperplastic hepatocellular foci. 1,4-Dioxane does not cause point mutations, DNA repair, or initiation. However, it appears to promote tumors and stimulate DNA synthesis. Using EPA Guidelines (2005), the weight of the evidence suggests that 1,4-dioxane causes liver tumors in rats and mice through cytotoxicity followed by regenerative hyperplasia. Specific key events in this mode of action are identified. A Reference Dose (RfD) of 0.05 mg/kg day is proposed to protect against regenerative liver hyperplasia based on a benchmark dose (BMD) approach. Based on this RfD, a maximum contaminant level goal of 350 μg/L is proposed using a default relative source contribution for water of 20%.

1. Introduction

1,4-Dioxane is a contaminant of personal care products and cosmetics, and is also used as a solvent for organic products, lacquers, paints, varnishes, oils, waxes, inks, and animal and vegetable oils, among other uses (US EPA, 2013). Recent evaluations of 1,4-dioxane's toxicity are available and general information on its physical properties can be obtained from documents developed by the US EPA (2013), Health Canada (2010), NICNAS (1998) and the Sapphire Group (2007).

Based on weight of evidence assessments, several regulatory bodies have concluded that 1,4-dioxane is not a mutagen and that there is evidence of a threshold dose for the formation of liver tumors (Health Canada, 2010; NICNAS, 1998). NICNAS, 1998 did note there are several possible epigenetic mechanisms (including tumor promotion, cell proliferation, etc.) but inconsistent data from mechanistic studies do not support a clear mechanism. Similarly, the US Environmental Protection Agency (US EPA, 2013) in its recent review of 1,4-dioxane based on the oral route of exposure, alluded to several possible modes of action (MOA) including a regenerative hyperplasia, especially for liver tumors, but then concluded that the available evidence was inadequate to establish a mode of action (MOA) by which 1,4-dioxane or a transient or terminal metabolite induces liver tumors in rats and mice. EPA's (2013) conclusion is based, in part, on apparent uncertainty in the toxic moiety for 1,4-dioxane and the apparent lack of noncancer toxicity data from several mouse bioassays at doses that evoke tumors, or that otherwise appear to have conflicting information concerning non-neoplastic lesions in the liver of rodents exposed orally to 1,4-dioxane. Although there is general agreement among studies on 1,4-dioxane with regard to neoplastic changes, there are substantial differences in the reporting of non-neoplastic lesions in the liver from various repeated exposure studies and carcinogenesis studies. One study (NCI, 1978) reported no non-neoplastic lesions in the livers of mice, at least at the high dose, while other studies reported swelling of the centrilobular hepatocytes, necrosis, and hyperplasia at comparable or lower doses (Kano et al., 2008, 2009; Yamazaki et al., 1994). Since these studies span 3 decades, differences in histologic approaches for

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quantifying and reporting non-neoplastic changes may have been responsible for the differences noted across the studies.

No epidemiology studies, case reports, or clinical trials exist that examine potential adverse health effects of 1,4-dioxane following oral or dermal exposure. However, multiple studies examined the effects of acute inhalation exposure in healthy volunteers (Yant et al., 1930; Fairley et al., 1934; Wirth and Klimmer, 1936; Silverman et al. 1946; Young et al., 1977; Ernstgard et al., 2006). Each of these studies is limited by small sample size and some exposures were very high (i.e., up to 5500 ppm in Yant et al., 1930). Although limited by small sample sizes and limited latency periods, these studies found no increased risk of cancer-related mortality among workers exposed to 1,4-dioxane, Theiss et al. (1976) also found no evidence of liver or kidney disease among a small sample of retired workers. Furthermore, two case reports show that high acute occupational exposure to 1,4-dioxane can result in liver, kidney, and central nervous system toxicity (Barber, 1934; Johnstone, 1959). Exposure estimates are not available from Barber (1934), but Johnstone (1959) reported that the worker was exposed to 208–650 ppm (mean 470 ppm) for 1 week in addition to an unknown dermal exposure.

In experimental animals, 1,4-dioxane from oral exposures caused liver toxicity as evidenced by several histological and/or biochemical changes (e.g., liver enzyme changes, centrilobular swelling, and/or necrosis) at all time points as early as 13 weeks of treatment (Kano et al., 2008), and as nicely summarized by EPA (2013) in a dose-related manner in both sexes of rats and mice after both oral and inhalation exposures.[1] EPA (2013) also showed that this liver toxicity and nasal toxicity (e.g., nuclear enlargement; vacuolar change, and/or squamous cell hyperplasia); precedes tumors in time in both sexes of rats and mice[2] with liver histopathology preceding tumors in dose in both sexes of rats[3] and liver toxicity *as evidenced by biochemistry* occurring at doses similar to those that evoke tumors in either sex of mice.[4] Liver toxicity *as evidenced by histopathology* does not consistently appear to either precede tumors in either sex of mice or necessarily even occur at tumorigenic doses. For example, liver hyperplasia (evidence of a regenerative cell proliferation) is not recorded in high dose female mice (NCI, 1978), although it is shown at 15% in the low dose females. Hepatic cytomegaly and necrosis was also observed for low dose females and for males from both exposure groups, although this latter evidence for male mice is equivocal. Single cell necrosis and hepatocellular hypertrophy were increased in male and female mice given 4000 ppm and above in drinking water for 13 weeks (Kano et al., 2008) and hyperplasia was noted in rats and mice following exposure to 1,4-dioxane in drinking water for 2 years (Yamazaki et al., 1994). The hyperplasia originally reported in the Yamazaki et al. study (1994) was later changed to altered hepatocellular foci including acidophilic, basophilic, and clear cell foci based on certain diagnostic criteria (Kano et al., 2009).

In reviewing this information, the lack of appearance (or consistency) of liver toxicity in mice before tumor occurrence, in terms of dose, seemed at odds with the appearance of liver toxicity before tumor occurrence in both rats and mice at all time points, and the appearance of this toxicity before tumors in dose in rats. For the NCI study, we suspected that the lack of evidence for non-neoplastic lesions had more to do with the common practice of pathologists at this period of time (1978) to record only the most severe endpoint within an organ of an experimental animal (e.g., a tumor) despite the presence of noncancer toxicity. Our suspicion was confirmed by McConnell (2011) who stated that a common practice in the late 1970's was to identify the most severe endpoint, and specifically for these bioassays, to identify and score tumors.

In order to explore these differences in liver histopathology for mice observed across studies and to better understand the sequence of events that may have contributed to the MOA of the observed liver tumors, a blinded reread of the NCI (1978) liver slides for mice was conducted since this study had no reported non-neoplastic lesions in the liver at the high dose. Following the completion of this work, a review of all non-neoplastic lesions in the liver observed in the repeated exposure studies was conducted. In addition, a thorough review of genotoxicity studies was conducted which included DNA replication and promotion bioassays as well as mutation, initiation, and DNA repair studies. Based on the histopathology data as well as the genotoxicity information, a hypothesis concerning the MOA was developed, as shown in Fig. 1, and an MOA analysis was performed.

The EPA (2005) cancer guidelines were utilized to conduct the MOA analysis. This analysis was then used to conduct a dose response assessment, which was similar in many respects to that derived by EPA (2013), but sufficiently different in outcome and direction to warrant additional discussion and review by the scientific community. In brief, our proposed MOA for liver tumors accounts for nearly all of the findings, to the point where other tumor MOAs, such as mutagenicity, can be credibly excluded. This new information and analysis may be valuable to address the potential environmental risk from oral exposures to 1,4-dioxane, and specifically for the development of safe concentrations of this chemical in drinking water. A similar MOA analysis may be needed for other tumors evoked by 1,4-dioxane.

2. Methods

2.1. National Cancer Institute (NCI) data review

Because terminology and practices for reporting liver lesions has changed since the time of the NCI study (1978), and because EPA (2005) is focusing more on an understanding of a chemical's Mode of Action (MOA) prior to any determination of its dose response, a re-review of the liver slides of mice from the NCI study (1978) was performed. This reanalysis was performed at the Experimental Pathology Laboratories (EPL), Research Triangle Park, NC during September through November 2012. The objective of the slide review was to determine if any non-neoplastic lesions in the liver were present in an effort to understand the sequence of events that may have contributed to the MOA of the observed liver tumors in mice. Another reason for the slide review was because at the time of the original slide review (i.e., 1978) the NCI typically recorded only the most severe diagnosis on a given slide, (e.g., adenoma or carcinoma). During this timeframe, the focus of cancer bioassays was to determine the potential carcinogenic activity of the chemical, not its potential chronic toxicity. For example, if an adenoma, carcinoma, and evidence of chronic toxicity (e.g., hepatocellular hypertrophy), were all present on a given slide, only the tumor response was typically recorded. Thus, it was unclear whether non-neoplastic lesions were present in the livers of mice but were not recorded in the NCI carcinogenicity study. An initial review of the livers from five control male mice was conducted followed by five male mice from the high dose. The reason for the initial review was to obtain a baseline for the livers of control animals and to understand the spectrum of lesions that occurred in the high dose

[1] See EPA (2013) Table 4-2 (p. 32), Table 4-5 (p. 41), Table 4-8 (p. 47), Table 4-9 (p. 48), and Table 4-12 (p. 52).

[2] See EPA (2013) Table 4-2 (p. 32), Table 4-16 (p. 58), Table 4-17 (p. 59), and Table 4-22 (p. 69).

[3] Compare EPA (2013) Table 4-5 (p. 41) to its Table 4-6 (p. 42); compare Tables 4-8 & 4-9 (p. 47-48) to Tables 4-10 & 4-11 (p. 50); compare Table 4-20 (p. 64) to Table 4-21 (p. 65).

[4] See EPA (2013) Table 4-25 (p. 85).