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Update: Mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane

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ABSTRACT

Previous work has shown that the weight of evidence supports the hypothesis that 1,4-dioxane causes liver tumors in rodents through cytotoxicity and subsequent regenerative hyperplasia. Questions regarding a lack of concordant findings for this mode of action (MOA) in mice have not been resolved, however. In the current work, a reanalysis of data from two chronic mouse cancer bioassays on 1,4-dioxane, one 13-week mouse study, seven rat cancer bioassays, coupled with other data such as 1,4-dioxane's negative mutagenicity, its lack of up-regulated DNA repair, and the appearance of liver tumors with a high background incidence, support the conclusion that rodent liver tumors, including those in mice, are evoked by a regenerative hyperplasia MOA. The initiating event for this MOA is metabolic saturation of 1,4-dioxane. Above metabolic saturation, higher doses of the parent compound cause an ever increasing toxicity in the rodent liver as evidenced by higher blood levels of enzymes indicative of liver cell damage and associated histopathology that occurs in a dose and time related manner. Importantly, alternative modes of action can be excluded. The observed liver toxicity has a threshold in the dose scale at or below levels that saturate metabolism, and generally in the range of 9.6–42 mg/kg-day for rats and 57 to 66 mg/kg-day for mice. It follows that threshold approaches to the assessment of this chemical's toxicity are supported by the non-mutagenic, metabolic saturation kinetics, and cytotoxicity-generated regenerative repair information available for 1,4-dioxane promoted rodent liver tumors.

1. Introduction

Differences in the evaluation and interpretation of toxicological data for 1,4-dioxane (CAS number 123-91-1) has led to contrasting approaches for extrapolating results from experimental animals to humans for assessment of cancer risk. Some investigators, such as Health Canada (2005), Neumann et al. (1997), NICNAS (1998), Netherlands (1999), and Stickney et al. (2003), rely on a threshold approach for this extrapolation, while others, such as the U.S. Environmental Protection Agency (U.S. EPA, 2013) and Office of Environmental Health Hazard Assessment (2002), default to a non-threshold or linear low-dose extrapolation approach for their toxicological assessment. Despite these differences, however, none of these groups consider 1,4-dioxane to be mutagenic, a hallmark of a non-threshold approach (US EPA, 2005), nor to cause DNA repair. Importantly, all groups describe data that support alternative modes of action (MOA), such as a regenerative hyperplasia.

The source of this inconsistency stems from apparently conflicting data from rat and mouse bioassays, specifically, in findings for dose-related non-neoplastic liver lesions in rats from multiple studies that support a cytotoxicity, regenerative repair, in contrast to the general lack of non-neoplastic (or noncancer)

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histopathology findings in the livers of mice from two chronic studies. As one step to resolve this apparent conflict, U.S. EPA's external peer review panel for 1,4-dioxane suggested a re-read of liver slides from the first mouse study, by the National Cancer Institute (NCI, 1978).[1] This suggestion was based on the fact that NCI pathologists in 1978 generally recorded the most severe pathology for individual experimental animals, and when tumors were found, did not always record, or otherwise were not able to record available non-neoplastic toxicity (McConnell, 2011). Evidence of this practice is found in the NCI (1978) report, where female mice are shown to have liver hyperplasia in the low dose group, but do not have this effect at the high dose where most animals had liver tumors. Thus, because of early practices to ignore histological findings in the presence of liver tumors, important histology data went unreported in the original reports with these histological data providing critical information for establishing the MOA.

Based on this suggestion, we previously worked with scientists from the National Toxicology Program to re-read the 1978 NCI slides (Dourson et al., 2014). The older mouse liver slides were re-stained and then re-read in a blinded protocol. The findings from the re-read were in stark contrast to the minimal noncancer liver findings in the original NCI report. Specifically, noncancer toxicity was evident at all doses and in a manner (i.e., hypertrophy, necrosis, inflammation, foci, adenoma, carcinoma) that was consistent with a regenerative hyperplasia MOA for the development of liver tumors. This published reanalysis of the NCI (1978) mouse slides was supported by the pathology report by McConnell (2013).

The second long-term oral mouse bioassay and a 13-week precursor were conducted by the Japan Bioassay Research Center (JBRC, 1990a,b) and subsequently published as Kano et al. (2008, 2009). Like the NCI (1978) bioassay, the Japanese work reported little noncancer toxicity in the mouse liver after long-term exposure. The lack of reported noncancer toxicity is perhaps not surprising given a similar underreporting in the NCI (1978) bioassay. However, noncancer liver toxicity was reported in the 13-week study.

The objective of this work was to perform a detailed evaluation of the findings reported in the original Japanese rat and mouse bioassays and to integrate these findings with other lines of evidence to determine whether a regenerative hyperplasia MOA for hepatic tumor formation is supported. Evaluation of these findings expands the scope of our previous work and allows for a more comprehensive MOA analysis.

2. Methods

Because the JBRC reports (1990a,b) were not available in English, a consortium of government and nongovernment scientists requested full access to the lab reports and had them translated.[2] These reports were graciously received during 2014 and then translated in early 2015. Taken together, these translated reports include additional noncancer effects in the liver of rats and mice, which were otherwise not available in the published versions (Kano et al., 2008, 2009). Unfortunately, slides from these studies were not available for a re-reading.

The U.S. US EPA (2005) guidelines for cancer risk assessment state that the MOA should be evaluated in determining the quantitative approach for dose response assessment from positive human or experimental animal tumor data. This evaluation is accomplished by first proposing a MOA, including identification of key events as shown in Fig. 1, which is adapted from U.S. EPA (2013) and Dourson et al. (2014). Data on these key events, including available *in vivo, in vitro*, and mechanistic studies are then evaluated as per U.S. US EPA (2005). When sufficient data are available, a biologically based dose-response (BBDR) model is the preferred method for low dose extrapolation. Absent such data, low dose extrapolation usually proceeds via a linear model if the chemical acts via a direct DNA-reactive MOA or the MOA is not known, or a threshold model based on one or more combinations of relevant tumors for a non-DNA-reactive MOA. Finally, the human equivalent dose is determined from the experimental animal dose by comparing human and experimental animal kinetics or a default procedure (U.S. EPA, 2011). Adverse outcome pathway (AOP) frameworks are also emerging for expanding the use of mechanistic toxicological data for risk assessment and regulatory applications (NRC, 2007). The use of an AOP for 1,4-dioxane might prove useful for future investigations.

These guidelines were followed by Dourson et al. (2014) in their analysis of two potential MOAs for liver tumor development from exposure to 1,4-dioxane: a heritable mutation to liver and/or nasal cell DNA, or liver cytotoxicity followed by regenerative cell proliferation. The analyses reported by Dourson et al. (2014) were performed on the basis of pooled results[3] from both male and female mice for hepatocellular necrosis because the incidences of this effect were similar between the sexes. In the current work, we again utilized a pooled approach for data analysis, and we specifically enhanced the investigation of the MOA on regenerative cell proliferation by performing a detailed evaluation of the translated Japanese study reports (JBRC, 1990a,b).

3. Results

The translated study reports from the JBRC (1990a,b) confirm information found in the publications of Kano et al. (2008, 2009) and add some information relevant to the hypothesized MOA not found in the published articles. From the Japanese studies, the NCI (1978) bioassay, the re-read of the mouse liver slides from the NCI (1978) study by McConnell (2013), and other relevant information, we have further developed the hypothesized regenerative hyperplasia MOA, to the point where we conclude that consistent noncancer effects are observed in both rats and mice preceding tumor development, with the level of documentation of these observations more evident in the rat studies.

3.1. Review of the Japanese translations and integration with other findings: rats

Fig. 2 shows hyperplasia preceding the development of liver foci (generally basophilic and mixed cell) in rats in a dose related fashion, and both of these effects are shown to precede the development of liver adenomas and carcinomas. The inset shows the relationship of hyperplasia and foci more clearly. Fig. 3 shows the pooled incidence of two additional effects in rats, namely centrilobular swelling and single cell liver necrosis from the 13-

[1] Specifically: "The EPA should explore the possibility that slides from the NCI studies on 1,4-dioxane are available and in adequate condition to evaluate possible linkages between toxic effects and tumor outcome in the drinking water carcinogenicity studies in rats and mice." PEER REVIEWER COMMENTS. External Peer Review on the *Toxicological Review of 1,4-Dioxane* (CASRN No. 123-91-1). Versar, Inc. Contract No. EP-C-07-025 Task Order 118 (May 2012).

[2] The full translations of these Japanese findings can be obtained from http://allianceforrisk.org/14-dioxane-analysis/(TERA, 2015).

[3] Data are considered "pooled" when individual group level information is maintained in any analysis, such as the development of a dose response curve. In contrast, data are considered combined, when individual group level information is combined at the same or similar dose for subsequent analysis.