EXHIBIT 104

Regulatory Toxicology and Pharmacology 88 (2017) 45–55

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

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

Michael L. Dourson [a], Jeri Higginbotham [b], Jeff Crum [c], Heather Burleigh-Flayer [d], Patricia Nance [a, *], Norman D. Forsberg [e], Mark Lafranconi [f], John Reichard [a]

[a] University of Cincinnati, College of Medicine, Cincinnati, OH, United States
[b] Kentucky Department for Environmental Protection, Frankfort, KY, United States
[c] Hamp, Mathews & Associates, Inc., Bath, MI, United States
[d] PPG, Monroeville, PA, United States
[e] Arcadis U.S., Inc., Chelmsford, MA, United States
[f] Environmental Resources Management, Cincinnati, OH, United States

ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form
20 February 2017
Accepted 23 February 2017
Available online 30 March 2017

Keywords:
Dioxane
Regenerative hyperplasia
Liver toxicity
Mode of action
Risk assessment

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)

* Corresponding author. University of Cincinnati, College of Medicine, Cincinnati, OH, United States.
E-mail address: patricia.nance@uc.edu (P. Nance).

http://dx.doi.org/10.1016/j.yrtph.2017.02.025

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.

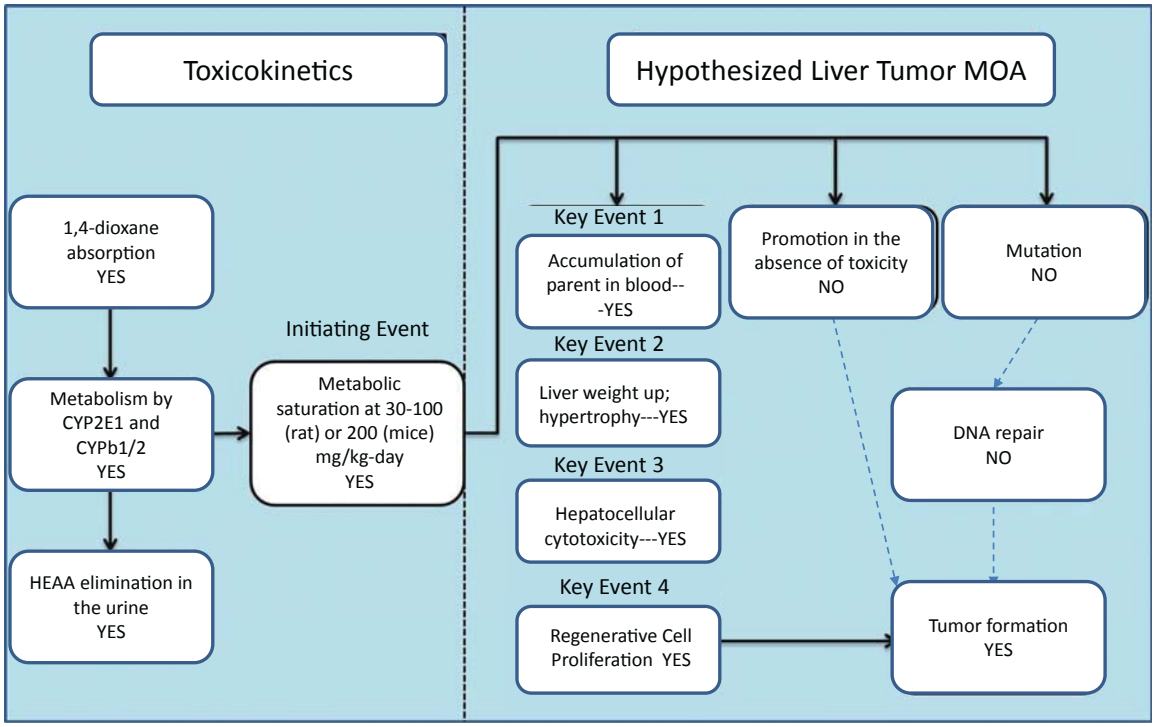

Fig. 1. Mode of Action (MOA) for 1,4-dioxane induced liver tumors.

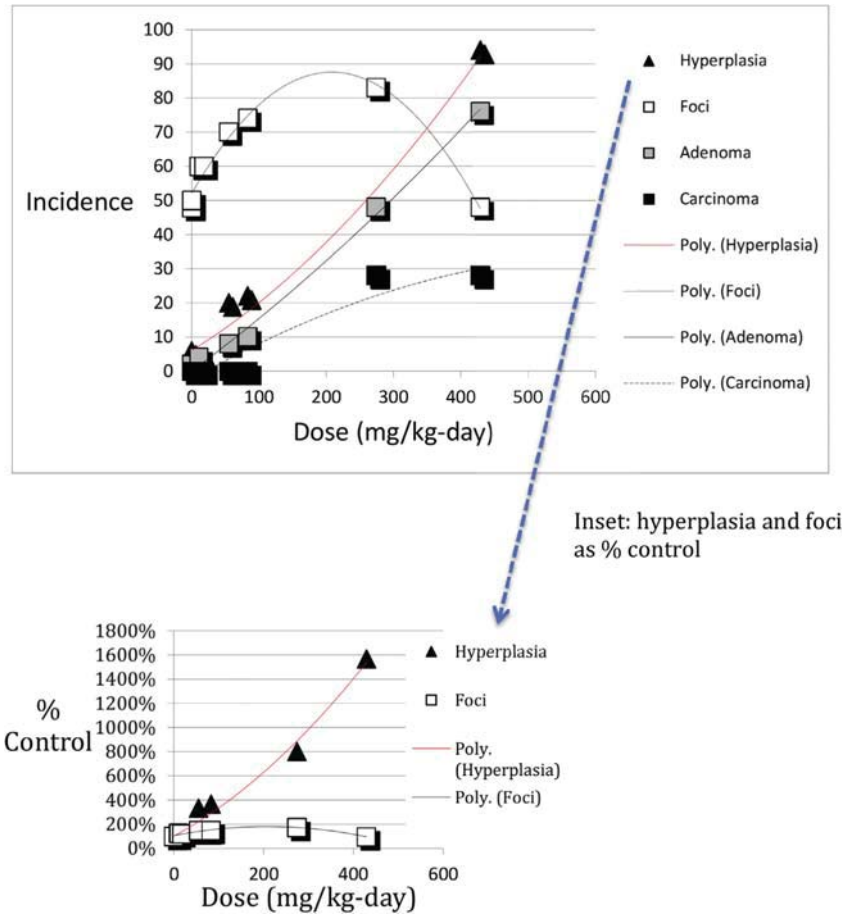

Fig. 2. Pooled incidence for 4 effects in fischer 344 male and female rats given 1,4-Dioxane orally for 2 years (JBRC, 1990a,b). Inset shows % of control for 2 effects.

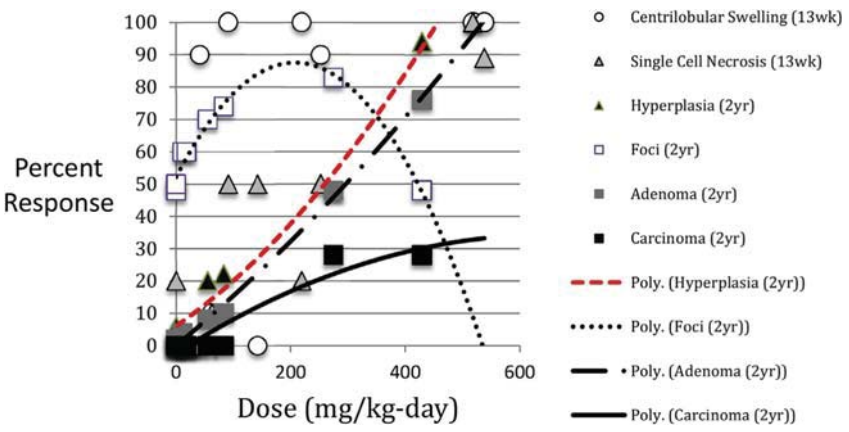

Fig. 3. Pooled Incidence of 6 effects in F344 Male and Female Rats Given 1,4-Dioxane for either 13 Weeks or 2 Years. 13 Week doses have been adjusted to chronic equivalents (JBRC, 1990a,b).

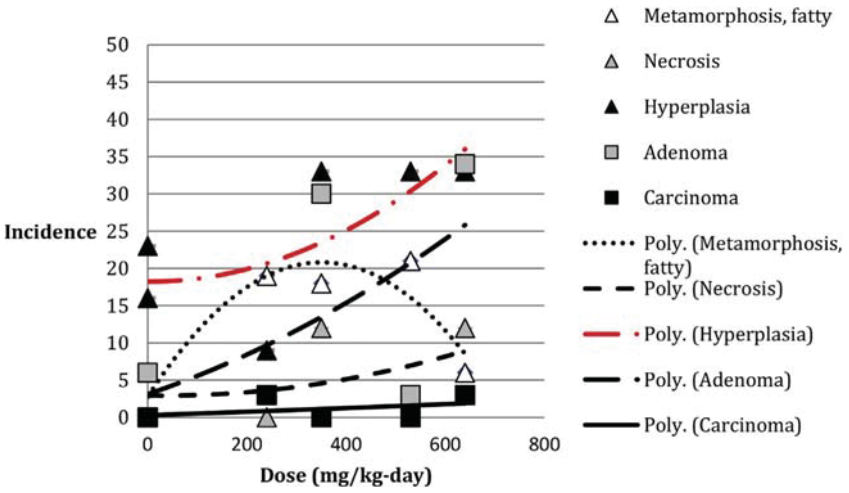

Fig. 4. Pooled incidence for 5 effects in fischer 344 male and female rats given 1,4-dioxane orally for 2 years (NCI, 1978).

week studies overlaid on Fig. 2.[4] These two effects precede the development of other effects in both dose and time. Liver enzyme changes in the blood of rats shown in Supplemental Fig. 1 as ALT and AST pattern the histology shown in Figs. 2 and 3 with slight increases at lower doses followed by dramatically larger increases in these enzymes at doses above 200 mg/kg-day.[5] Fig. 4 shows the histopathology results from the NCI (1978) study in rats (note scale change in the y-axis; corresponding liver enzyme changes were not monitored in this study). Although the overall incidences of the various effects are lower in the NCI (1978) rat bioassay, the pattern of these results match the findings in rats from the JBRC (1990).

All of these findings in rats (including some not shown in these figures) show the expected changes in the liver due to a regenerative cell proliferation to promote liver tumors. That is, liver cell swelling, hypertrophy and liver weight increase occur at doses of 42–55 mg/kg-day; this precedes necrosis at doses of 94–219 mg/kg-day; which has a lower overlapping range of hyperplasia and foci development found at 55–389 mg/kg-day; which precedes in dose the development of adenomas and carcinomas at doses of 274–1015 mg/kg-day. Changes in liver AST and ALT enzymes also follow the expected pattern with increases seen at doses in excess of about 200 mg/kg-day. Importantly, the observed effects are in the expected dose sequence, and several of these effects occur in the expected time sequence (the data are limited in this respect because only two time points were monitored). This sequence in dose also matches the findings from the laboratory study report of Kociba et al. (1971), which was subsequently published by Kociba et al. (1974) (see Supplemental Figs. 2 and 3 based on the laboratory report that is available by request from The Dow Chemical Company).

3.2. *Review of the Japanese translations and integration with other findings: mice*

The information from the Japanese translated study reports and

[4] Here, the doses from the 13-week studies have been reduced by a 3-fold factor to address the well-known differences in effect level among durations (Dourson and Stara, 1983). Some might argue that a 10-fold uncertainty factor would be more appropriate here. If so, the use of this factor would shift the data points for centrilobular swelling and single cell liver necrosis to the left, making the pattern of noncancer effect proceeding the development of tumors more apparent. Perhaps more appropriately, the use of the area under the curve might be able to better adjust doses among studies of different durations. We are open to doing this given sufficient data.

[5] Here, the doses from the 13-week studies have been divided by a 10-fold uncertainty factor; caveats as in the previous footnote still apply.

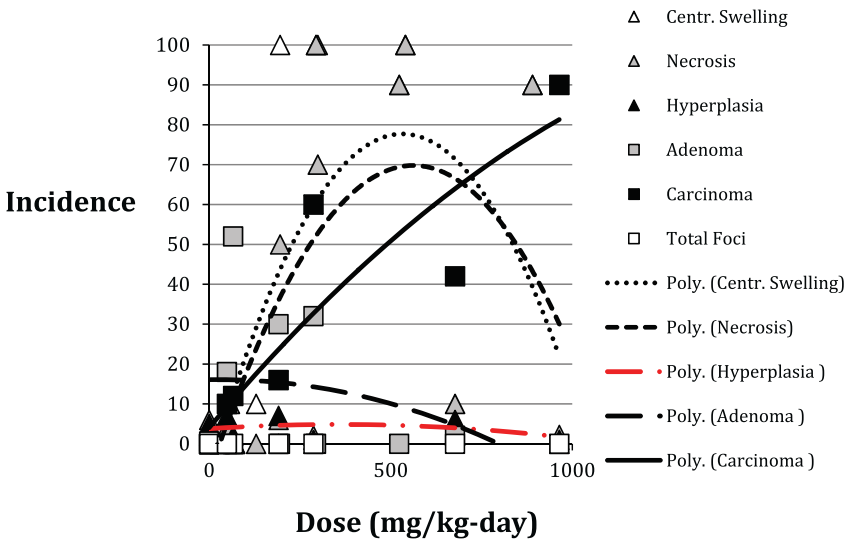

Fig. 5. Incidence of 6 effects in Male and Female Mice Given 1,4-Dioxane for either 13 Weeks (doses adjusted to chronic equivalent) or 2 years; variable control tumor incidence subtracted out (JBRC, 1990a,b).

publications on mice are found in Fig. 5, with information from the 13-week studies also plotted as adjusted by 3-fold uncertainty factor.[4]; Centrilobular liver cell swelling, hypertrophy and liver weight increase appear between 190 and 200 mg/kg-day, and overlap necrosis in the same dose range, but hyperplasia and foci are nearly absent and adenomas and carcinomas appear early in the dose sequence, as low as at doses of 66 mg/kg-day in females. The corresponding changes in mouse liver enzymes from the Japanese work occur at or around 200 mg/kg-day, where the 13-week doses are adjusted by 10-fold uncertainty factor, and appear to follow the pattern of liver cell swelling and necrosis, but not the adenoma and carcinoma sequence (see Supplemental Fig. 4). Specifically, the lack of noncancer histopathology in the chronic mouse study is not consistent with the changes in liver enzymes in this same chronic study, nor is this lack of noncancer findings expected based on the histopathology of the precursor 13-week study. Nor does the tumor response in the low dose female mice of JBRC (1990a) match the tumors findings in the McConnell (2013) re-read of NCI (1978).

In contrast, Fig. 6 shows the results of a sequence of effects in mice found in the McConnell (2013) reread of NCI (1978) and as reported in Dourson et al. (2014). Here, hypertrophy and necrosis at doses between control and less than 400 mg/kg-day precede in dose the development of fewer foci (of various types) at similar and higher doses, which appears to precedes in dose the development of tumors at higher doses. These findings are similar to the pattern found in the rat data.

When the data for mice from both chronic bioassays are overlaid, the results are mixed (see Supplemental Fig. 5). Centrilobular liver cell swelling, hypertrophy and necrosis more clearly precede tumor development in mice from the NCI (1978) study as re-read by McConnell (2013), and these results in mice are consistent with the sequence observed in rat studies. In contrast, the Japanese histopathology findings in mice (JBRC, 1990a,b) are not consistent in sequence with either McConnell (2013) or rat studies. This difference may be due to a change in histopathological analysis, as stated by Kano et al. (2009, page 2777):

> "The hepatic hyperplasia of rats and mice diagnosed in the previous report (Yamazaki et al., 1994) [*authors note: which was a presentation of the* JBRC, 1990a] was re-examined histopathologically and changed to hepatocellular adenomas and altered hepatocellular foci including acidophilic, basophilic and clear cell foci in the present studies, according to the current diagnostic criteria of liver lesions in rats and mice."

This statement suggests that results from the JBRC (1990a) study report were modified prior to publication as Kano et al. (2009). However, the translation of this Japanese laboratory report does not show any dose-related hepatic hyperplasia in mice. Specifically, the report shows hyperplasia of 5, 7, 5, 6 out of 50 males at each dose, and of 2, 2, 1, 1, out of 50 females, for control, low, medium and high doses, respectively. Foci are likewise nearly absent in mice in the observations included in this report and in the publication (Kano et al., 2009). Therefore, it is uncertain from reading this report as to what has been specifically changed in the mouse findings from the original JBRC report compared to the later publication. Additional pictures of a sufficient number of mouse liver slides to solve this dilemma were not available.

3.3. Saturation kinetics

Metabolism of 1,4-dioxane in humans and experimental animals is well characterized and extensive. Workers exposed to 1,4-dioxane at low concentrations (~2 ppm) showed a metabolite to parent ratio in the urine of 118:1 (Young et al., 1976). Higher concentrations (~50 ppm) by Young et al. (1977) also showed a linear elimination of 1,4-dioxane in both plasma and urine indicating that, at low levels, 1,4-dioxane metabolism is a nonsaturated, first-order process, leading to the principle metabolite β-hydroxyethoxy acetic acid with a pH dependent reversal to 1,4-dioxane-2-one.

However, higher doses of 1,4-dioxane in experimental animals show that the metabolism of 1,4-dioxane is saturable. For example, rats given *i.v.* exposures demonstrated a dose-related shift from linear, first-order metabolism to nonlinear, saturable metabolism of 1,4-dioxane in the range of 30–100 mg/kg (Young et al., 1978a,b). Similarly, rats given gavage doses of 10, 100, or 1000 mg/kg singly showed that the percent urinary excretion of the radiolabel decreased significantly with dose while radiolabel in expired air increased, again indicating saturable kinetics in the dose range of 100 mg/kg. For mice this saturation appears to start at 200 mg/kg (Sweeney et al., 2008). The point of saturation for rats is consistent with effects being caused by the accumulation of the parent compound; the point of saturation found in mice is mostly consistent

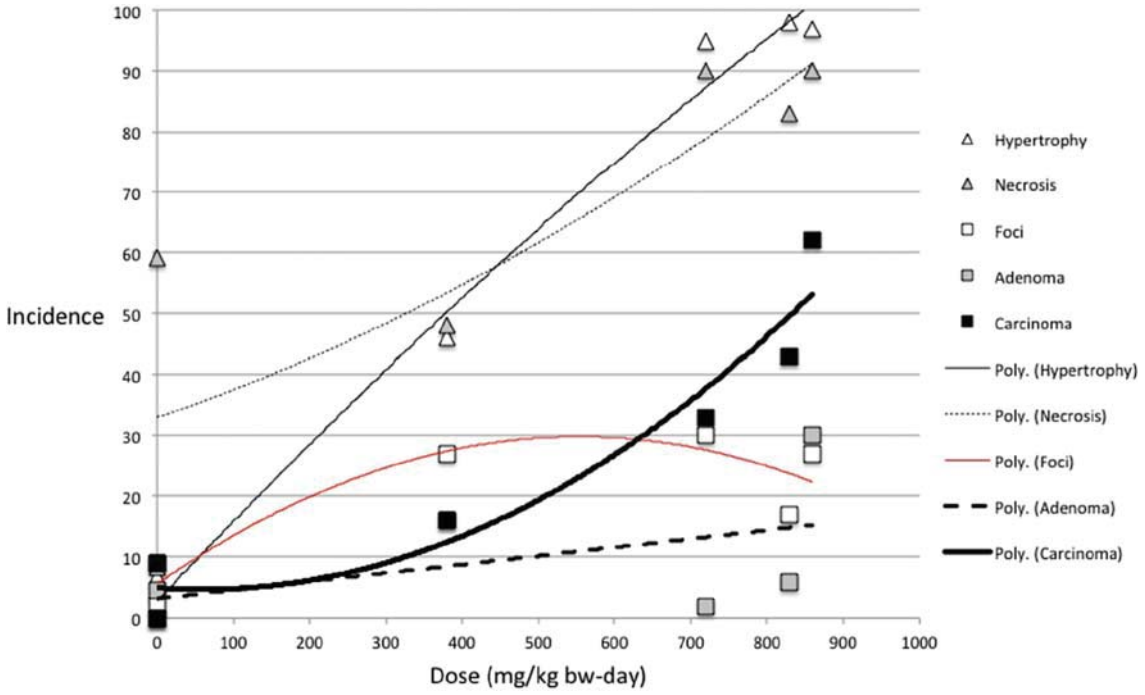

Fig. 6. Pooled Incidence for 4 Effects in B6C3F1 Male and Female Mice Given 1,4-Dioxane (McConnell reread of NTP, 1978).

with effects caused by the accumulation of the parent compound (tumors found in the low dose female mice of the Japanese study being the exception).

Studies on whether 1,4-dioxane or one or more of its metabolites was the toxic moiety were pursued. Nannelli et al. (2005) investigated the role of CYP450 isozymes in the liver toxicity of 1,4-dioxane by inducing hepatic CYPB1/2 and CYP2E1 levels with phenobarbital or fasting. No change in glutathione content or ALT activity was observed when compared with control, suggesting that potentially highly reactive and toxic intermediates did not play a role in the liver toxicity of 1,4-dioxane. Pretreatment with inducers of mixed-function oxidases also did not significantly change the extent of covalent binding in subcellular fractions (Woo et al., 1977), again indicating that metabolites were not toxicologically active. Furthermore, a comparison of the pharmacokinetic profile of 1,4-dioxane with the toxicology data from a chronic drinking water study (Kociba et al., 1975) showed that liver toxicity did not occur unless clearance pathways were saturated and elimination of 1,4-dioxane from the blood was reduced. Koissi et al. (2012) also found that a major metabolite of 1,4-dioxane, namely 1,4-dioxane-2-one, fails to induce pre-neoplastic hepatic foci in orally treated rodents. Taken together, these data collectively support the hypothesis that the parent compound, 1,4-dioxane, and not a metabolite, is the toxic moiety. After metabolic saturation, when more of the parent chemical is available, liver toxicity occurs with sufficient frequency to be recorded. Such saturation occurs at an oral dose in the range 30–100 mg/kg in rats and at approximately 200 mg/kg in mice, although after induction, these saturation doses may be higher.

The reanalysis of rodent data on 1,4-dioxane that we highlight here can be used to evaluate the strength of the hypothesized MOA as suggested by (U.S. US EPA, 2005; Boobis et al., 2008; Meek et al., 2013). Tables 1 and 2 show these data arranged in dose, time, and severity of effect, following the hypothesized regenerative hyperplasia MOA shown in Fig. 1.

3.4. Rat toxicity data

Table 1 shows the key event sequence for the available rat data. The hypothesized key event 1 is metabolic saturation resulting in accumulation of parent compound. Key event 2 is shown to be cellular swelling, hypertrophy and liver weight increases. These occur at administered 13-week doses as low as 126 mg/kg-day (chronic dose equivalent of 42 mg/kg-day), or 2-year doses as low as 55 mg/kg-day. Key event 3, necrosis and/or inflammation, is shown to occur at administered 13-week doses as low as 657 mg/kg-day (chronic dose equivalent of 219 mg/kg-day), or 2-year doses as low as 94 mg/kg-day. Key event 4a, increased DNA synthesis as measured by [3H]-thymidine incorporation, is shown to occur at administered 11-week doses as low as 1000 mg/kg-day (chronic dose equivalent of 330 mg/kg-day). Key event 4b, hyperplasia, is also shown to occur at administered 11-week doses as low as 1000 mg/kg-day (chronic dose equivalent of 330 mg/kg-day, and is seen at administered 2-year doses as low as 55 mg/kg-day. Key event 4c, pre-neoplastic foci, is seen at administered 13-week doses as low as 1168 mg/kg-day (chronic dose equivalent of 389 mg/kg-day), or 2-year doses as low as 55 mg/kg-day. Finally, the apical effect, adenomas and/or carcinomas is not seen at 13 weeks, but does occur after two years at doses as low as 274 mg/kg-day.

Thus, the dose sequence of these key events is:

- Key event 1, metabolic saturation at 30 to 100 mg/kg;
- Key event 2, cellular swelling, hypertrophy and liver weight increases at 42–55 mg/kg-day;
- Key event 3, necrosis and/or inflammation at 94–219 mg/kg-day;
- Key event 4
  a. increased DNA synthesis at 330 mg/kg-day;
  b. hyperplasia development at 55–330 mg/kg-day; and
  c. basophilic and mixed cell (when measured) foci development at 55–389 mg/kg-day

Table 1
Dose response, temporality concordance table for dioxane-induced liver tumors in rats.[a]

Study	Dose in mg/kg-day (Author reported)	Chronic Dose Equivalent (dose ÷ 3)	Key event 1	Key event 2	Key event 3	Key event 4	APICAL EVENT
			Saturated metabolism[b]	Liver weight increase or hypertrophy	Necrosis/Inflammation	DNA synthesis, hyperplasia or foci	Liver adenomas/carcinomas
Stott et al., 1981 [c]	10	3.3	–	–	–	–	–
(11 weeks; oral)	1000	330	+	+	+	+	–
Kano et al., 2008 & JBRC, 1990 [d]	52 (m)/83 (f)	17/28	–	–	–	–	–
(13 weeks; oral)	(640 ppm)						
	126 (m)/185 (f) (1600 ppm)	42/62	+	+[e]	–	–	–
	274 (m)/427 (f) (4000 ppm)	91/142	+	+	+	–	–
	657 (m)/756 (f) (10000 ppm)	219/252	+	+	– **(m)**/+(f)[f]	–	–
	1554 (m)/1614 (f) (25000 ppm)	518/538	+	+	+	–	–
Kasai et al., 2008 [g]	584 (800 ppm)	195	+[h]	+	–	–	–
(13 week; Inhalation)	1168 (1600 ppm)	389	+	+	+/– **(m)**/– (f)	–(m)/+**(f)**[i]	–
	2336 (3200 ppm)[j]	779	+	+	+	+	–
Kano et al., 2009 [k] & JBRC, 1990	11 (m)/18 (f)	11/18	–	–	–	–	–
(2 year; oral)	(200 ppm)						
	55 (m)/83 (f) (1000 ppm)	55/83	+	+**(m)**/–(f)	–	+[l]	–
	274 (m)/429 (f) (5000 ppm)	274/429	+	+	+[m]	+	+
Kociba et al., 1974 &	9.6 (m)/19 (f) (0.01%)	9.6/19	–	–	+/– **(m)**/– (f)	–	–
Kociba et al., 1971 [n] (2 year; oral)	94 (m)/148 (f) (0.1%)	94/148	+[o]	–	+[p]	–	–
	1015 (m)/1078 (f) (1%)	1015/1078	+	+	+	– **(m)**/+**(f)**[q]	+
Kasai et al., 2009 [r]	36 (50 ppm)	36	–/+	–	–	–	–
(2 year; inhalation)	181 (250 ppm)	181	+	–	–	–	–
	909 (1250 ppm)	909	+	+	+	+	+
NCI, 1978 [s]	240 (m)/350 (f)	240/350	+	nd	nd	–(m)/+**(f)**[t]	– **(m)**/+**(f)**[u]
(2 year; oral)							
	550 (m)/640 (f)	550/640	+	nd	nd	+	– **(m)**/+**(f)**

[a] Abbreviations and symbols: +, key event observed; -, key event not present; +/–, equivocal; nd, not determined/reported; m, male only; f, female only.

[b] Metabolic saturation is found generally in kinetic studies and not in hazard identification bioassays. For rats, saturation appears to start at oral doses of 30–100 mg/kg-day (Young et al., 1978a,b; Kociba et al., 1975). For mouse studies this saturation appears to start at ~200 mg/kg-day (Sweeney et al., 2008).

[c] Stott et al., 1981. Sprague-Dawley rats were dosed daily for 11 weeks (7 days/week) via drinking water with 10 or 1000 mg dioxane/kg body weight. DNA synthesis was measured by [3H]-thymidine incorporation.

[d] Kano et al., 2008. Fifty male and female Fisher 344 rats were administered 1,4-dioxane in drinking water for 13 weeks.

[e] The most sensitive sign of toxicity was centrilobular swelling of hepatocytes in male rats given 1600 ppm for 13 weeks. No foci were observed at any dose levels.

[f] In the ≧ 10,000 ppm male groups and the 25,000 ppm female group, increased incidences of centrilobular hepatic vacuolar degeneration were noted, which were consistent with increased plasma AST/ALT levels (male rats) and AST (females) at the high dose.

[g] Kasai et al., 2008. Thirteen-week Inhalation of 1,4-Dioxane in male and female F344 rats vapor for 6 h/day and 5 days/wk. Inhalation exposures were mg/kg doses assuming a minute volume as 561 ml/min/kg body weight for rats and an uptake ratio of 1,4-dioxane of 100%. Authors included dose groups ranging from 3200 ppm to 100 ppm with doubling dilutions, but since the lower three groups were negative for the occurrence of key events they have not been included in the table.

[h] Kasai et al., 2008. Demonstrate steady-state proportionality between dose and plasma blood levels for the top 4 exposure levels (≥400 ppm). Based on the pharmacokinetics of 1,4-dioxane, these plasma concentrations are predicted to be associated with saturation-limited metabolism, although Sweeney et al. (2008) suggests that at such doses 1,4-dioxane might induce its own metabolism.

[i] GST-P-positive liver foci were observed in 3/10 males exposed to 3200-ppm; 2/10 females exposed to 3200-ppm; and 4/10 females exposed to 1600-ppm; no GST-P-positive foci could be found in any of the 800- and 1600-ppm-exposed males and 800-ppm-exposed females and control groups of both sexes.

[j] A 6400-ppm exposure was also tested but is not relevant to this mode of action analysis because all animals in this group died at the first week of the 13-wk exposure period.

[k] Kano et al., 2009. 1,4-dioxane was administered in drinking-water to F344/DuCrj rats (50 of each sex/treatment group) for 2 years.

[l] From JBRC, 1990, translated, page 2: "There were increased incidences of hyperplasia or cell focus in the livers which could be considered as a preneoplastic change in the ≧ 1000 ppm male groups and the 5000 ppm female group." However, a review of the rest of this lab report indicates that the mid dose for females is biologically significant for hyperplasia. See also JBCR (1990), Appendix 2, PDF pages 36 and 58.

[m] Statistically significant increased plasma GOT & GPT and some histological evidence of necrosis in dead, moribund and sacrificed rats. Note that the terms GOT or GPT are outdated nomenclature and have been replaced with ALT and AST, respectively.

[n] Kociba et al., 1974. Sixty male and female Sherman strain rats, 6–8 weeks old, were administered 1,4-dioxane in their drinking water for up to 716 days. Female rats during days 114–198 consumed a dose of 1,4-dioxane ranging from 914-1229 mg/kg/day, but consumed less (1019–1176 mg/kg/day) days 446–460. Male rats receiving the 1% exposure has similar consumption during the same exposure periods.

[o] Kociba et al., 1975. 1,4-dioxane: correlation of the results of chronic ingestion and inhalation studies with its dose-dependent fate in rats. In proceedings of the 6th Annual Conference on Environmental Toxicology (pp. 345–354). Wright-Patterson Air Force Base, OH: Wright-Patterson Air Force Base, Air Force Systems Command, Aerospace Medical Division, Aerospace Medical Research Laboratory.

[p] Kociba et al., 1974. Reported that the occurrence of hepatocellular degeneration and necrosis, as well as hyperplastic nodule formation, are significantly increased by doses of 1,4-dioxane ≥ 0.1%; the incidence for these changes are provided in Kociba et al., 1971.

[q] Kociba et al., 1971. Describes this lesion as "Hepatocellular Hyperplastic Nodule Formation." Thus, it is uncertain to which category this lesion applies, and so both hyperplasia and foci formation are marked.

[r] Kasai et al., 2009. 2-year inhalation exposure of male fisher 344 rats (50 animals per dose group. Internal exposure from 6-hr inhalation exposure was approximated by the authors assuming the minute volume as 561 ml/min/kg body weight for rats and an uptake ratio of 1,4-dioxane of 100%.

[s] NCI, 1978. Groups of 35 rats of each sex administered 1,4-dioxane at concentrations of either 0.5% or 1.0% (v/v) in the drinking water for 110 weeks.

[t] Hyperplasia in female rat liver was 7/31 (23%), 11/33 (33%) and 17/53% (53%) for the control, low- and high-dose groups, respectively. In male rats the incidents were 5/31 (16%), 3/32 (9%)and 11/33 (33%) for control, low- and high-dose groups, respectively.

[u] Adenoma only in female rats and no tumors in male rats.

Table 2
Dose response, temporality concordance table for dioxane-induced liver tumors in mice.

Study	Dose in mg/kg-day (Author reported)	Chronic Dose Equivalent (dose ÷ 3)	Key event 1	Key event 2	Key event 3	Key event 4	APICAL EVENT
			Saturated metabolism[a]	Liver weight increase or hypertrophy	Necrosis/Inflammation	DNA synthesis, hyperplasia or foci	Liver adenomas/carcinomas
Kano et al., 2008	86 (m)/170 (f) (640 ppm)	29/57	–	–	– **(m)**/+/–**(f)**	–	–
& JBRC, 1990 [b]	231 (m)/387 (f) (1600 ppm)	71/129	–	–	– **(m)**/+/– **(f)**	–	–
(13 weeks; oral)	585 (m)/898 (f) (4000 ppm)	195/299	+	+[c]	+[d]	–	–
	882 (m)/1620 (f) (10,000 ppm)	294/540	+	+	+	–	–
	1570 (m)/2669 (f) (25,000 ppm)	523/890	+	+	+	–	–
Kano et al., 2009	49 (m)/66 (f) (500 ppm)	49/66	–	–	–	Unable[f]	–**(m)**/+**(f)**
& JBRC, 1990 [e]	191 (m)/287 (f) (2000 ppm)	191/287	+	+**(m)**/–**(f)**	+[f]	to	+
(2 year; oral)	677 (m)/964 (f) (8000 ppm)	677/964	+	+	+	determine	+
NCI, 1978 and	720[h] (m)/380 (f)	720/380	+	+	+**(m)**/–**(f)**[i]	+	+
re-read	830 (m)/860 (f)	830/860	+	+	+	+	+
(2 year; oral)[g]							

[a] Metabolic saturation is found generally in kinetic studies and not in hazard identification bioassays. For rats, saturation appears to start at oral doses of 30–100 mg/kg-day (Young et al., 1978a,b; Kociba et al., 1975). For mouse studies this saturation appears to start at ~200 mg/kg-day (Sweeney et al., 2008).

[b] Kano et al., 2008. Four-week-old Crj:BDF_1 mice of both sexes (n = 60, 10 animals per control or treatment group) were administered 1,4-dioxane in drinking water for 13 weeks.

[c] Mouse hepatic lesions were characterized by centrilobular swelling of hepatocytes occurring at 4000 ppm and above.

[d] Hepatocellular damage indicated by dose related single cell necrosis in both sexes and increases in plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in male and female mice dose with 25,000 ppm dioxane; ALT was increased in female mice at 10,000 ppm.

[e] Kano et al., 2009. 1,4-dioxane was administered to 50 Crj:BDF1 mice of each sex in the drinking-water for 2 years.

[f] Japan Bioassay Research Center, 1990. Increased plasma GOT (~115–690%) & GPT (124–470%) in male mice, and GOT (~700–1400%) & GPT (600–1500%) in female mice. Appendices F3 & F4. The terms GOT or GPT are outdated nomenclature and have been replaced with ALT and AST, respectively. AST/ALT elevations instead of ALP elevations favor liver cell necrosis as a mechanism. When AST and ALT are both over 1000 IU/L, the differential can include acetaminophen toxicity, shock, or fulminant liver failure. When AST and ALT are greater than three times normal but not greater than 1000 IU/L, the differential can include alcohol toxicity, viral hepatitis, drug-induced level, liver cancer, sepsis, Wilson's disease. Some histopathology findings also support this clinical work, specifically increases of necrosis/degeneration/fatty change are found in both sexes at the two middle doses (Appendix volume 2, e-pages 70 and 94 for males and e-pages 82, 83 and 104 for females). The highest dose does not show this histopathology, however, possibly due to masking by the tumor response.

[g] NCI, 1978. Groups of 50 mice of each sex administered 1,4-dioxane at concentrations of either 0.5% or 1.0% (v/v) in the drinking water for 90 weeks. Note that McConnell (2013) also re-reviewed mouse liver slides with help from NCI staff for noncancer endpoints. Results from this re-read are included here.

[h] It is noteworthy that the dose of 1,4-dioxane consumed by the high and low doses males in the NCI (1978) study was similar and in the words of the authors, "did not reflect the two-fold difference in concentration between the low and high doses". Thus, histologic pathology between the low and high males is generally similar.

[i] In the re-read of slides by McConnell (2013), the occurrence of necrosis in low-dose female mice was equivocal with an incidence similar to the elevated control level but with increased severity of centrilobular necrosis. Both incidence and severity were increased at the high-dose (Tables 2 and 5 of Dourson et al., 2014).

- Apical effect, adenomas and carcinomas at 274–1015 mg/kg-day.

This sequence of key events from seven rat bioassays, when coupled with 1,4-dioxane's negative mutagenicity, its lack of induction of DNA repair (indicating no DNA damage), and the appearance of background/spontaneous liver tumors (U.S. EPA, 2013), leads to the conclusion that rat liver tumors are evoked by a regenerative hyperplasia. Regenerative hyperplasia is due to nonmutagenic toxicity in the rat liver that occurs in a dose and time related manner throughout the animal lifespan after metabolic saturation of 1,4-dioxane metabolism as shown in Table 1. Findings include similarities in toxicity between shorter term/high dose and longer term/lower dose, which is recognized as typical for other chemicals. Thus, the expectation that the shorter-term higher dose liver toxicity shown in Kano et al. (2008) would occur at lower doses with longer exposures as in Kano et al. (2009) is evident in Fig. 2 for rats. Here the adjustment of the shorter-term exposures by a 3-fold uncertainty factor matches the doses in the chronic study, and shows similar findings.

3.5. Mouse toxicity data

Table 2 shows the key event sequence for the available mouse data. As before, the hypothesized key event 1 is metabolic saturation resulting in accumulation of parent compound. Key event 2 is shown to be cellular swelling, hypertrophy and liver weight increases, which occur at administered 13-week doses as low as 585 mg/kg-day (chronic dose equivalent of 195 mg/kg-day) or 2-year doses as low as 191 mg/kg-day. Key event 3, necrosis and/or inflammation, is also shown to occur at administered 13-week doses as low as 585 mg/kg-day (chronic dose equivalent of 195 mg/kg-day), or 2-year doses as low as 191 mg/kg-day. Information on Key event 4a, DNA synthesis, was not reported in mice. Key event 4b, hyperplasia, is not shown to occur in the sole 13 week study, but is seen in the 2-year dose of 380 mg/kg-day (interestingly this effect is not recorded for the high dose of the NCI bioassay, see previous discussion). Key event 4c, pre-neoplastic foci, was also not reported in the 13-week doses, but is found at administered 2-year doses as low as 380 mg/kg-day in the McConnell re-read of the NCI (1978) bioassay, but was generally not found in JBRC (1990a) nor its publication by Kano et al. (2009). Finally, the apical effects, adenomas and/or carcinomas are not seen at 13-weeks, as expected, but does occur after two years at doses between 66 and 964 mg/kg-day.

Thus, the dose sequence of these key events is:

- Key event 1, metabolic saturation at ~200 mg/kg;
- Key event 2, cellular swelling, hypertrophy and liver weight increase, in the range of 190–200 mg/kg-day;
- Key event 3, necrosis and/or inflammation in the same range of 190–200 mg/kg-day;
- Key event 4,
  a. DNA synthesis has not been evaluated in mice;
  b. hyperplasia at doses as low as 380 mg/kg-day in one study but not the other, and
  c. foci development at doses as low as 380 mg/kg-day in one study but not the other
- Apical effect, adenomas and carcinomas at doses of 66–1015 mg/kg-day

This sequence of key events from two chronic mouse studies and a subchronic mouse study generally support the hypothesized regenerative hyperplasia MOA. The collective results are not any stronger than this, however, due to the varying interpretations of liver lesions in the chronic mouse study of JBRC (1990a) versus that of Kano et al. (2009). Specifically, tumors in female mice from JBRC (1990a) are reported at the lowest dose of 66 mg/kg-day, which is lower than doses from suggested key events. Although it might be appropriate to adjust 13-week mouse study doses by a 10-fold factor to estimate the chronic dose equivalent (rather than a 3-fold factor), which would allow a sequence in doses of the key events in mice to be more similar to that found in the rat studies, the underlying reality is that the results of the two chronic mouse bioassays are simply different. This difference may be due in part to the change in the diagnostic criteria used to record the liver lesions reported by Kano et al. (2009).

4. Discussion

As discussed more extensively by U.S. US EPA (2005) animal tumor findings give important clues in making decisions about potential MOAs. Often, animal cancer bioassays and their supporting sub-chronic and *in-vitro* data provide the only mechanistic/key event insights for a MOA serving to support the application of animal cancer data in risk assessment. Thus, all lines of evidence need to be explored when developing a rodent liver tumor MOA. Some of this evidence includes the number of studies conducted, the similarity of metabolic activation and detoxification among species, the influence of route of exposure on the spectrum of tumors, the effects of high dose exposures on the target organ or systemic toxicity that may not reflect typical physiological conditions, the presence of proliferative lesions, the effect of dose and time on the progression of lesions, the ratio of malignant to benign tumors as a function of dose and time, the time of appearance of tumors, the spectrum of tumors developed, the number and incidence of tumors at organ sites with high or low background historical incidence, the biomarkers in tumor cells, and the shape of the dose-response curve for key events and tumors.

In considering this evidence Dourson et al. (2014) stated that in some respects 1,4-dioxane appears to be a mutagenic carcinogen. It evokes multisite and multispecies tumors that are not restricted to one sex suggesting an influence that is not restricted to gender, strain, or species, and, tumors evoked by 1,4-dioxane are both benign and malignant. However, all but one of the tumor types (i.e., nasal tumors) are at sites with a high historical background incidence, and findings in mutagenicity bioassays, initiation bioassays, and DNA repair bioassays are predominantly negative as described by U.S. EPA (2013). Woo et al. (1977) also found that covalent binding of radiolabeled 1,4-dioxane within hepatocytes was greatest in the cytosolic fraction, followed by the microsomal, mitochondrial, and nuclear fractions, but not to DNA. U.S. EPA (2005) concludes that: "The results from *in vitro* and *in vivo* assays do not provide overwhelming support for the hypothesis of a genotoxic MOA for 1,4-dioxane carcinogenicity." Thus, a MOA involving mutagenicity, which has been addressed by U.S. EPA (2013) and Dourson et al. (2014), is not further analyzed here since new information is not available. Both groups concluded that a mutagenic MOA is not likely.

Alternative MOAs include infection, receptor mediated processes, oxidative damage and cytotoxicity with compensatory hyperplasia. None of the available studies recorded infections, and since all of the studies showed a dose related response in tumors, infection was not the likely MOA. Data for receptor mediated processes or DNA binding are also generally unavailable or otherwise negative (e.g., Woo et al., 1977; U.S. EPA, 2013). Data for oxidative damage as a potential MOA are limited, but otherwise negative, with enhanced metabolism of 1,4-dioxane not showing any greater toxicity as discussed above.

In contrast, extensive toxicity is seen at the primary tumor sites (liver and nose) suggesting a growth-promoting, and specifically, a regenerative cell proliferation MOA. A regenerative hyperplasia MOA is also supported by positive findings in promotion bioassays and DNA replication bioassays suggesting growth stimulation. We re-evaluated the regenerative cell proliferation MOA hypothesis for liver tumors, as reported by Dourson et al. (2014), in light of the translations of JBRC (1990a,b) laboratory reports, and reaffirm that the U.S. EPA (2005) criteria for evaluation are met for strength, consistency, biological plausibility, and coherence. Moreover, dose response and temporal concordance for noncancer precursors to tumors are clearly evident for rats (Table 1), and generally supportive for mice (Table 2). Furthermore, 1,4-dioxane appears to be able to induce its own metabolism via CYP2E1. If so, 1,4-dioxane might share some characteristics with ethanol or phenobarbital-induced liver neoplasia.

The reason that the findings in mice are not more supportive of the regenerative hyperplasia MOA, however, is because the histopathological characterizations of McConnell (2013) and of JBRC (1990a) in mice do not agree. McConnell (2013) found extensive liver noncancer toxicity as demonstrated by histopathology and fewer tumors than JBRC (1990a). JBRC (1990a) reported more tumors and nearly an absence of liver noncancer histopathology in the chronic study. The lack of liver noncancer histopathology in JBRC (1990a) is unexpected, especially since an increase in liver enzymes associated with cell damage is found in this same study. Also, the JBRC (1990b) 13-week study showed extensive liver noncancer histopathology at suitably adjusted-to-chronic doses. Unfortunately, this internal inconsistency is not resolvable because slides or pictures from a sufficient number of experimental animals are not available for the current reanalysis.

During the course of this analysis, we obtained the opinions of several pathologists on the contrasting findings of the chronic mouse bioassays (see Supplemental materials). Collectively these pathology opinions support the hypothesized MOA discussed in U.S. EPA (2013) and Dourson et al. (2014) that the liver tumors from oral exposure to 1,4-dioxane occur after metabolic saturation, accumulation of the parent 1,4-dioxane molecule, liver toxicity and a regenerative hyperplasia. While additional live experimental animal testing might add confirmatory findings, a threshold for these tumors is expected if metabolism of the parent compound is not saturated, since subsequent liver toxicity does not occur.

When the many lines of evidence are taken together, the reevaluation of the Japanese studies show consistent findings in rats and consistent findings in mice other than liver histopathology not being fully recorded in the chronic study. However, based on the number of studies conducted, the well established metabolic saturation of 1,4-dioxane metabolism in humans and experimental animals, the effects of higher dose exposures on target organ toxicity, the presence of proliferative lesions, the effect of dose and time on the progression of lesions, the time of appearance of tumors, the spectrum of tumors developed, the number and incidence of tumors at organ sites with high or low background historical incidence, and the shapes of the dose-response curve for key events and tumors, all lead to the conclusion that a regenerative hyperplasia MOA is operating with 1,4-dioxane induced liver tumors. Furthermore, Tox21 dataset provides additional support for a non-genotoxic mode of action for 1,4-dioxane as more fully described in PubChem Tox21 (2015). These data including inactive outcomes of quantitative high-throughput ELG1-luciferase reporter gene assay that identifies compounds blocking DNA replication, and no activation of any biological pathways in the high-

throughput screening assays.

Thus, the available lines of evidence, the perspectives of erudite pathologists, and the available Tox21 information collectively indicate that a nonlinear approach to dose-response assessment will protect against these tumorigenic effects. It might also be added that mouse liver tumors by themselves are often difficult to match to corresponding human disease and many groups have suggested reliance on other animal models such as the rat (e.g., U.S. FDA, 1997). While this issue does not directly address the likely MOA for 1,4-dioxane induced liver tumors, it suggest that reliance of this MOA in rats is more appropriate, because the MOA is more clear in rats than in mice. Future work along these lines might be to describe this MOA within the emerging AOP framework described earlier.

Acknowledgements

The authors wish to thank the Steering Committee of the Alliance for Risk Assessment (http://allianceforrisk.org/steering-committee/) for their review and endorsement of this collaborative effort, and for the endorsement of the Texas Commission on Environmental Quality, the Commonwealth of Kentucky, ERM, the University of Cincinnati, and Waste Management (see also http://allianceforrisk.org/14-dioxane-analysis/). We also wish to acknowledge our many colleagues who supported this activity through active participation on technical conference calls, and specifically: Chris Prucha, Satish Deshpande, Jonathan D. Garoutte, Sarah Labib, Nadine Weinberg, Shawn Sager, Barbara Sassi, Shannon Ethridge and Dennis Wambuguh. Sources of funding for this activity at the TERA Center at the University of Cincinnati include Hamp, Mathews & Associates, Inc., PPG Corporation, Waste Management, and the University of Cincinnati, College of Medicine.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.02.025.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.02.025.

References

Boobis, Alan R., Doe, John E., Heinrich-Hirsch, Barbara, E Bette Meek, M., Munn, Sharon, Ruchirawat, Mathuros, Schlatter, Josef, Seed, Jennifer, Vickers, Carolyn, 2008. IPCS framework for analyzing the relevance of a non-cancer mode of action for humans (February). Crit. Rev. Toxicol. 38, 87–96.

Dourson, M.L., Stara, J.F., 1983. Regulatory history and experimental support of uncertainty (safety) factors. Regul. Toxicol. Pharmacol. 3, 224–238.

Dourson, M., Reichard, J., Nance, P., Burleigh-Flayer, H., Parker, A., Vincent, M., McConnell, E.E., 2014. Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment. Regul. Toxicol. Pharmacol. 68, 387–401.

Health Canada, 2005. Drinking Water Guidance Value for 1,4-Dioxane. August 23.

Japan Bioassay Research Center (JBRC), 1990a. Report of Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice. Japan Industrial Safety and Health Association. December 28.

Japan Bioassay Research Center (JBRC), 1990b. Report of Preliminary Carcinogenicity Studies (Acute, Two-week, and Thirteen-week Studies) by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice. Japan Industrial Safety and Health Association. December 28.

Kano, H., Umeda, Y., Saito, M., Senoh, H., Ohbayashi, H., Aiso, S., Yamazaki, K., Nagano, K., Fukushima, S., 2008. Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J. Toxicol. Sci. 33, 141–153.

Kano, H., Umeda, Y., Kasai, T., Sasaki, T., Matsumoto, M., Yamazaki, K., Nagano, K., Arito, H., Fukushima, S., 2009. Carcinogenicity of 1,4-dioxane administered in drinking-water to rats and mice for 2 years. Food Chem. Toxicol. 47, 2776–2784.

Kasai, T., Saito, M., Senoh, H., Umeda, Y., Aiso, S., Ohbayashi, H., Nishizawa, T., Nagano, K., Fukushima, S., 2008. Thirteen-week inhalation toxicity study of 1,4-dioxane in rats. Inhal. Toxicol. 20, 961–970.

Kasai, T., Kano, H., Umeda, Y., Sasaki, T., Ikawa, N., Nishizawa, T., Nagano, K., Arito, H., Nagashima, H., Fukushima, S., 2009. Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal. Toxicol. 21 (11), 889–897.

Kociba, R.J., Torkelson, T.R., Clashman, A., McCollister, S., Gehring, P.J., 1971. Continuous Exposure of Sherman Rats to Various Concentrations of 1,4-dioxane in Drinking Water for Two Years. The Dow Chemical Company. Biochemical Research Laboratory. 7901059. March 10.

Kociba, R.J., McCollister, S.B., Park, C., Torkelson, T.R., Gehring, P.J., 1974. 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. Toxicol. Appl. Pharmacol. 30, 275–286.

Koissi, N., Shah, N.H., Ginevan, B., Eck, W.S., Roebuck, B.D., Fishbein, J.C., 2012. Lactone metabolite common to the carcinogens dioxane, diethylene glycol, and N-nitrosomorpholine: aqueous chemistry and failure to mediate liver carcinogenesis in the F344 rat. Chem. Res. Toxicol. 25 (5), 1022–1028.

Kociba, et al., 1975. 1,4-dioxane: correlation of the results of chronic ingestion and inhalation studies with its dose-dependent fate in rats. In: Proceedings of the 6th Annual Conference on Environmental Toxicology. Wright-Patterson Air Force Base, Air Force Systems Command, Aerospace Medical Division, Aerospace Medical Research Laboratory, OH, pp. 345–354.

(Bette) Meek, M.E., Palermo, Christine M., Bachman, Ammie N., North, Colin M., Jeffrey Lewis, R., 2013. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. J. Appl. Toxicol. http://dx.doi.org/10.1002/jat.2984.

McConnell, G., 2011. Personal Communication with M. L. Dourson in October.

McConnell, G., 2013. Report on the Review of Liver Slides from the National Cancer Institute's Bioassay of 1,4-Dioxane for Possible Carcinogenicity Conducted in 1978 (NCI, 1978). Submitted to Toxicology Excellence for Risk Assessment. January/March 2013.

Nannelli, A., De Rubertis, A., Longo, V., Gervasi, P.G., 2005. Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. Arch. Toxicol. 79, 74–82.

NCI (National Cancer Institute), 1978. Bioassay of 1,4-dioxane for possible carcinogenicity. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention. National Cancer Institute. National Institutes of Health, Bethesda, MD. (NIH), pp. 78–1330.

Netherlands, 1999. Risk Assessment: 1, 4–Dioxane. Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and the Environment (RIVM). Chemical Substances Bureau, Ministry of Housing, Spatial Planning and the Environment (VROM), Netherlands. Final Version, 5 November, EINECS-No.: 204-661-8.

Neumann, H.G., Thielmann, H.W., Filser, J.G, Gelbke, H.P., GriemH, H., Kappus, H., Norpoth, K.H., Reuter, U., Vamvakas, S., Wardenbach, P., Wichmann, H.E., 1977. Proposed changes in the classification of carcinogenic chemicals in the work area. Reg. Toxicol. Pharmacol. 26, 288–295.

NICNAS (National Industrial Chemicals Notification and Assessment Scheme), 1998. Priority Existing Chemical Assessment Reports: 1,4-dioxane. Australia Department of Health and Ageing, Sydney, Australia. June. Also available online at: http://www.nicnas.gov.au/Publications/CAR/PEC/PEC7/PEC7index.asp.

NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: a Vision and a Strategy. National Academy of Science, Committee on Toxicity. The National Academic Press, Washington, D.C, ISBN 978-0-309-15173-3.

OEHHA, December 2002. Air Toxics Hot Spots Program. Risk Assessment Guidelines. Part II. Technical Support Document for Describing Available Cancer Potency Factors. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

PubChem Tox21, 2015. Substance Summary for 1,4-dioxane, CAS 123-91-1. SID 144208169. 1–4 dioxane- CID_31275 assay data downloaded on 8/11/2014. Available from: http://pubchem.ncbi.nlm.nih.gov/substance/144208169.

Stickney, J.A.1, Sager, S.L., Clarkson, J.R., Smith, L.A., Locey, B.J., Bock, M.J., Hartung, R., Olp, S.F., 2003. An updated evaluation of the carcinogenic potential of 1,4-dioxane, 2003 Oct Regul. Toxicol. Pharmacol. 38 (2), 183–195.

Stott, W., Quast, J., Watanabe, P., 1981. Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. Toxicol. Appl. Pharmacol. 60, 287–300.

Sweeney, L.M., Thrall, K.D., Poet, T.S., Corley, R.A., Weber, T.J., Locey, B.J., Clarkson, J., Sager, S., Gargas, M.L., 2008. Physiologically Based Pharmacokinetic Modeling of 1,4-Dioxane in Rats, Mice, and Humans. Toxicol. Sci. 101 (1), 32–50.

Toxicology Excellence for Risk Assessment (TERA), 2015. Available at: http://allianceforrisk.org/14-dioxane-analysis/.

US EPA (U.S. Environmental Protection Agency), 2005. Guidelines for Carcinogen Risk Assessment. Washington D.C. EPA/630/P-03/001B.

U.S. EPA, 2011. Recommended Use of Body Weight 3/4 as the Default Method in Derivation of the Oral Reference Dose. Risk Assessment Forum, Washington, D.C.

U.S. EPA (U.S. Environmental Protection Agency), 2013. Toxicological Review of 1,4-Dioxane (With Inhalation Update) (CAS No. 123-91-1) in Support of Summary Information on the Integrated Risk Information System (IRIS) [EPA Report] (EPA-635/R-11/003-F). Washington, DC.

U.S. FDA (Food and Drug Administration), 1997. Guidance for Industry. S1B Testing for Carcinogenicity of Pharmaceuticals. U.S.Department of Health and Human Services (July).

Woo, Y.-T., Argus, M.F., Arcos, J.C., 1977. Metabolism in vivo of dioxane: effect of

inducers and inhibitors of hepatic mixed-function oxidases. Biochem. Pharmacol. 26, 1539–1542.
Yamazaki, K., Ohno, H., Asakura, M., Narumi, A., Ohbayashi, H., Fujita, H., Ohnishi, M., Katagiri, T., Senoh, H., Yamanouchi, K., Nakayama, E., Yamamoto, S., Noguchi, T., Nagano, K., Enomoto, M., Sakabe, H., 1994. Two-year toxicological and carcinogenesis studies of 1,4- dioxane in F344 rats and BDF1 mice. In: Sumino, K., Sato, S., Shinkokai, N.G. (Eds.), Proceedings: Second Asia-Pacific Symposium on Environmental and Occupational Health 22-24 July, 1993: Kobe. Kobe University School of Medicine, International Center for Medical Research, Kobe, Japan, pp. 193–198.
Young, J.D., Braun, W.H., Gehring, P.J., Horvath, B.S., Daniel, R.L., 1976. 1,4-Dioxane and beta-hydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors. Toxicol. Appl. Pharmacol. 38, 643–646.
Young, J.D., Braun, W.H., Rampy, L.W., Chenoweth, M.B., Blau, G.E., 1977. Pharmacokinetics of 1,4-dioxane in humans. J. Toxicol. Environ. Health 3, 507–520.
Young, J.D., Braun, W.H., Gehring, P.J., 1978a. The dose-dependent fate of 1,4-dioxane in rats. J. Environ. Pathol. Toxicol. 2, 263–282.
Young, J.D., Braun, W.H., Gehring, P.J., 1978b. Dose-dependent fate of 1,4-dioxane in rats(b). J. Toxicol. Environ. Health A Curr. Iss 4, 709–726.