Table 1: Dose Response, Temporality Concordance Table for Dioxane-induced Liver Tumors in Rats.*

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
Stott et al., 1981[b] (11 weeks; oral)	10	3.3	–	–	–	–	–
	1000	330	+	+	+	+	–
Kano et al., 2008 & JBRC, 1990[c] (13 weeks; oral)	52 (m)/83 (f) (640 ppm)	17/28	–	–	–	–	–
	126 (m)/185 (f) (1600 ppm)	42/62	+	+[d]	–	–	–
	274 (m)/427 (f) (4000 ppm)	91/142	+	+	+	–	–
	657 (m)/756 (f) (10000 ppm)	219/252	+	+	**– (m)**/+(f)[e]	–	–
	1554 (m)/1614 (f) (25000 ppm)	518/538	+	+	+	–	–
Kasai et al., 2008[f] (13 week; Inhalation)	584 (800 ppm)	195	+[g]	+	–	–	–
	1168 (1600 ppm)	389	+	+	+/– **(m)**/– (f)	–(m)/+**(f)**[h]	–
	2336 (3200 ppm)[i]	779	+	+	+	+	–
Kano et al., 2009[j] & JBRC, 1990 (2 year; oral)	11 (m)/18 (f) (200 ppm)	11/18	–	–	–	–	–
	55 (m)/83 (f) (1000 ppm)	55/83	+	+**(m)**/ –(f)	–	+[k]	–
	274 (m)/429 (f) (5000 ppm)	274/429	+	+	+[l]	+	+
Kociba et al., 1974 & Kociba et al., 1971[m] (2 year; oral)	9.6 (m)/19 (f) (0.01%)	9.6/19	–	–	+/– **(m)**/– (f)	–	–
	94 (m)/148 (f) (0.1%)	94/148	+[n]	–	+[o]	–	–
	1015 (m)/1078 (f) (1%)	1015/1078	+	+	+	**– (m)**/+**(f)**[p]	+
Kasai et al., 2009[q] (2 year; inhalation)	36 (50 ppm)	36	–/+	–	–	–	–
	181 (250 ppm)	181	+	–	–	–	–
	909 (1250 ppm)	909	+	+	+	+	+
NCI, 1978[r] (2 year; oral)	240 (m)/ 350 (f)	240/350	+	nd	nd	–(m)/+**(f)**[s]	**– (m)**/+**(f)**[t]
	550 (m)/ 640 (f)	550/640	+	nd	nd	+	**– (m)**/+**(f)**

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 & JBRC, 1990[u] (13 weeks; oral)	86 (m)/170 (f) (640 ppm)	29/57	–	–	**– (m)/+/–(f)**	–	–
	231 (m)/387 (f) (1600 ppm)	71/129	–	–	**– (m)/+/– (f)**	–	–
	585 (m)/898 (f) (4000 ppm)	195/299	+	+[v]	+[w]	–	–
	882 (m)/1620 (f) (10,000 ppm)	294/540	+	+	+	–	–
	1570 (m)/2669 (f) (25,000 ppm)	523/890	+	+	+	–	–
Kano et al., 2009 & JBRC, 1990[x] (2 year; oral)	49 (m)/66 (f) (500 ppm)	49/66	–	–	–	Unable[y]	**–(m)/+(f)**
	191 (m)/287 (f) (2000 ppm)	191/287	+	**+(m)/ –(f)**	+[y]	to	+
	677 (m)/964 (f) (8000 ppm)	677/964	+	+	+	determine	+
NCI, 1978 and re-read (2 year; oral)[z]	720[aa] (m)/380 (f)	720/380	+	+	**+(m)/ –(f)[bb]**	+	+
	830 (m)/860 (f)	830/860	+	+	+	+	+

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

[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 to100 mg/kg-day (Young et al., 1978 and Kociba et al., 1975). For mouse studies this saturation appears to start at ~200 mg/kg-day (Sweeney et al., 2008).

[b] 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.

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

[d] The most sensitive sign of toxicity was centrilobular swelling of hepatocytes in male rats given 1,600 ppm for 13 weeks. No foci were observed at any dose levels.

[e] 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.

[f] 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.

[g] 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.

[h]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.

[i] 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.
[j]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.

[k] 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 ≧ 1,000 ppm male groups and the 5,000 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.

[l] 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.

[m] 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 914-1229mg/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.

[n] 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.

[o] 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.

[p] 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.

[q] 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%.

[r] 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.

[s] 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.

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

[u] 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.

[v] Mouse hepatic lesions were characterized by centrilobular swelling of hepatocytes occurring at 4,000 ppm and above.

[w] 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.

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

[y] Japan Bioassay Research Center, 1990. Increased plasma GOT (~115 to 690%) & GPT (124 to 470%) in male mice, and GOT (~700 to 1400%) & GPT (600 to 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.

[z] 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.

[aa] 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.

[bb] 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).