Alliance for Risk Assessment's 1,4-Dioxane Reanalysis in Support of a Regenerative Hyperplasia Mode of Action (MOA)

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Abstract

The State of Kentucky petitioned the Alliance for Risk Assessment (ARA) to obtain additional information from Japanese studies to inform 1,4-dioxane's cancer mode of action (MOA) based on a recent reanalysis. Additional information and translations of the Japanese studies are also supportive of a regenerative hyperplasia MOA but with one exception, specifically, the reported findings from the histopathology and clinical chemistry of the mouse liver in the Japanese studies are contradictory. The reanalysis of data leads to the conclusion that these rodent tumors are evoked by a regenerative hyperplasia mode of action (MOA) that stimulates existing background mutations. Regenerative hyperplasia in this context is due to an overwhelming toxicity in the rodent liver as evidenced by an increase in blood levels of enzymes indicative of liver cell damage and associated histopathology due to 1,4-dioxane exposure that occurs in a dose and time related manner throughout the lifespan. This contradiction may be due in part to the investigators changing criteria for liver histopathology scoring during the course of reporting their results. A limited amount of additional information from the Japanese studies, including potentially rereading some of the mouse liver histopathology slides, may be helpful. The intent of this ARA project is to obtain this limited, additional information from the Japanese studies, or other information as appropriate, in order to resolve the hypothesized MOA for 1,4-dioxane's liver tumor formation (and potentially other tumors).

Japanese Studies

2-year carcinogenicity study in rats and mice with drinking water

- 5,000, 1,000, or 200 ppm for rats, and 8,000, 2,000, or 500 ppm for mice was provided ad libitum for 2 years
- Mice in the ≧ 500 ppm groups showed increased incidences of hepatoma
- Indicated that 1,4-dioxane was carcinogenic to both rats and mice.

Two-week carcinogenicity study in rats and mice

- 90,000, 30,000, 10,000, 3,330, 1,110 ppm (2-week)
- Deaths found only in the 90,000 ppm groups; suppression of body weight gain in the ≧ 10,000 ppm groups; kidney effects
- No Observed Effect Concentration (NOEC) ≤ 1,100 ppm

Thirteen-week carcinogenicity study in rats and mice

- 25,000, 10,000, 4,000, 1,600, 640 ppm, 0 ppm (13-week)
- rats had slightly higher sensitivity to 1,4-dioxane than mice.
- 10,000 and 4,000 ppm groups showed decrease in water consumption and changes in the nasal cavity, trachea, lungs, and liver.
- 1,600 ppm groups showed changes in the lungs only
- No significant changes in the 640 ppm groups.

Results

Review of the Japanese Translations and Integration with Other Findings

Rats

- Liver cell swelling and necrosis precede hyperplasia, which precedes the development of foci, which precedes the development of adenomas, which precedes the development of carcinomas.
- Liver enzyme changes in rats shown pattern the histology
- Overall incidences of the various effects are lower in the NCI (1978) rat bioassay, the form of these results match the findings in rats from the JBRC (1990a)
- All of these findings in rats show the expected changes due to a regenerative cell proliferation and stimulation of endogenously mutated DNA, and the observed effects occur in the expected dose sequence

Mice

- Hypertrophy and necrosis precede the development of foci, which precedes the development of tumors, similar to what is found in the rat data.
- Centrilobular liver cell swelling, hypertrophy and necrosis more clearly lead to tumor development in mice and are consistent with the well-established sequence observed in **all** rat studies.
- Japanese histopathology findings in mice are not consistent with **any** of the rat studies.

"The hepatic hyperplasia of rats and mice diagnosed in the previous report (Yamazaki et al., 1994) [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 (Mohr, 1997; Deschl et al., 2001)". – Kano, 2009

Review of the Hypothesized Regenerative Hyperplasia MOA

Rat dose sequence key events show:

- Negative mutagenicity
- Lacks of DNA repair
- Only naturally occurring liver tumors

Conclusion: rat liver tumors are evoked by a regenerative hyperplasia that stimulates existing mutations

	Rat dose sequence	Mouse dose sequence
Key Event 1	Non-linear metabolism, liver weight increase, and/or hypertrophy (cellular swelling) at 42-55 mg/kg-day	Non-linear metabolism, liver weight increase, and/or hypertrophy (cellular swelling) in the range of 190-200 mg/kg-day
Key Event 2	Necrosis and/or inflammation at 94-219 mg/kg-day	Necrosis and/or inflammation in the same range of 190-200 mg/kg-day
Key Event 3	DNA synthesis at 330 mg/kg-day (DNA synthesis was only evaluated at 3.3 mg/kg/day [negative] and at 330 mg/kg/day [positive])	DNA synthesis has not been evaluated
Key Event 4	(a) Hyperplasia and foci development at 55-389 mg/kg-day (b)Apical effect, adenomas and carcinomas at doses of 66-1015 mg/kg-day	(a) Hyperplasia and foci development at doses as low as 380 mg/kg-day in one study but not the other (b) Apical effect, adenomas and carcinomas at doses of 66-1015 mg/kg-day

Mouse dose sequence key events:

- Generally support the hypothesized regenerative hyperplasia MOA
- Collective results are not any stronger than this, however, because tumors in female mice from the JBRC (1990) report are found at the lowest dose of 66 mg/kg-day, which is lower than doses from suggested key events

Timeline

2012

- External peer review of EPA's draft IRIS document on 1,4-dioxane
- TERA presented comments during the meeting where it was recommended by the peer review panel that a re-read of the NCI mouse liver slides would be helpful
- TERA scientists work with Gene McConnell and staff of the NTP to prepare slides for re-reading
- Gene McConnell re-reads the slides blindly and works with TERA staff to prepare a report

2013

- Final report of the review of liver slides from the National Cancer Institute's Bioassay of 1,4-Dioxane for Possible Carcinogenicity conducted in 1978. Report sent to EPA and EPA's external peer review committee.

2014

- Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment – Dourson et al., 2014
- Contacted several state agencies to inquire about their interest in signing a request letter for three studies that were conducted by the Japanese Bioassay Research Center (JBRC) on 1,4-dioxane in mice and rats.
- Collected signatures from 5 state agencies (MN, MO, MI, TX, and KY) to place on a request letter to the Japanese government (Ministry of Health, Labour and Welfare of Tokyo, Japan) for the Japanese studies.
- TERA submitted a request to the Japanese government for copies of the full studies.
- Received the oral studies from the Japanese Ministry of Health, Labour, and Welfare. Submitted a second request for additional missing appendices.
- Japanese studies received and then submitted for translation
- Received English translation of the Japanese studies.

2015

- Began review and QA of the translated studies
- Emailed translated studies to the 5 States that signed the request letter. Asked each to review and submit any comments or questions about translation.
- Draft analysis prepared on the translated Japanese studies. Missing individual experimental animal appendices requested.
- Draft analysis sent to state and industry partners for comment.
- State of Kentucky petitioned the Alliance for Risk Assessment (ARA) to obtain additional information from Japanese studies to inform 1,4-dioxane's cancer mode of action (MOA) based on a recent reanalysis.
- Conference call held with interested groups to form ARA coalition for the 1,4-Dioxane Reanalysis

Alliance for Risk Assessment

Objectives

- Obtain additional information on mouse liver histopathology from the Japanese long term cancer bioassay on 1,4-dioxane
- Determine whether the additional analysis of the mouse liver pathology supports a non-linear (threshold) Mode Of Action (MOA) for cancers caused by 1,4-dioxane

Current Members of the Coalition

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