



[www.stratoxon.com](http://www.stratoxon.com)

James A. Popp D.V.M., Ph.D.

Stratoxon LLC  
1853 William Penn Way  
Lancaster, PA 17601

Telephone: 717-735-3646  
Fax: 717-293-4470  
Email: [popp@stratoxon.com](mailto:popp@stratoxon.com)

October 15, 2013

Lori White  
NTP Designated Federal Official  
Office of Liaison, Policy and Review  
NIEHS  
P.O. Box 12233, MD K2-03  
Research Triangle Park, NC 27709  
Phone: 919.541.9834  
Mail: [whiteld@niehs.nih.gov](mailto:whiteld@niehs.nih.gov)

**RE:** Comments on the *NTP TECHNICAL REPORT ON THE TOXICOLOGY STUDIES OF TETRABROMOBISPHENOL A (CAS NO. 79-94-7) IN F344/NTac RATS AND B6C3F1/N MICE AND TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRABROMOBISPHENOL A IN WISTAR HAN [CrI:WI(Han)] RATS AND B6C3F1/N MICE (NTP TR587)*  
**Male Mice Hepatoblastomas**

#### Summary and Conclusion

The “Level of evidence of carcinogenic activity” for male mice receiving Tetrabromobisphenol A should be “Equivocal evidence” and not “Some evidence” as indicated in the draft Technical Report released for Peer Review on October 29, 2013. This conclusion is based on a full consideration of multiple aspects of the histopathology and biology of hepatoblastomas as outlined in the comments below.

#### Background

The National Toxicology Program performed a 2- year toxicology and carcinogenicity study on tetrabromobisphenol A in B6C3F1/N mice using doses of 250, 500 and 1000 mg/kg/day administered by gavage. In the draft Technical Report (NTP TR 587) scheduled for Peer Review on

October 29, 2013, hepatoblastomas in male mice are listed as the only basis for a proposed category of “Some evidence” under the “Level of evidence of carcinogenic activity”. This proposed level of evidence is apparently based on the incidence of hepatoblastomas of 2/50, 11/50 and 8/50 in the control, 250 and 500 mg/kg groups respectively, as noted in the “Summary” (page 12). This summary does not acknowledge the incidence of 3/50 in the high dose group that is rationalized in the later sections of the report as due to reduced survival in this group. The incidence of hepatoblastoma in the 250 and 500 mg/kg/day groups is statistically significant compared to the control group and is also above the historical incidence of this tumor in other NTP studies. The points addressed below provide a basis for the “Level of evidence of carcinogenicity” being “Equivocal evidence” rather than “Some evidence”.

### Comments

A full consideration of all aspects of the tumor type (hepatoblastomas) and occurrence within this study should be included in the final determination of the level of evidence designation as outlined in the “Explanation of Levels of Carcinogenic Activity” (page 14).

1. **Hepatoblastomas should not be considered as a separate tumor type.** While the morphologic appearance of these tumors is very interesting to pathologists, the origin and biological relationship to other liver tumors are essential points for determining how these tumors are considered in an assessment of a carcinogenic signal. Multiple publications (Turusov et al, 1973; Turusov et al, 2002; Thoolen et al, 2010; Cattley et al, 2013) have noted a very frequent if not nearly uniform association of hepatoblastomas with hepatocellular adenomas and/or carcinomas. In the instances where this association has not been noted, one must always remember that histology is a 2 dimensional representation of 3 dimensional structures in which not every hepatoblastoma associated with another hepatocellular tumor would be evident on the 2 dimensional section. This close association of hepatoblastomas to other hepatocellular neoplasms is an indication that the hepatoblastomas are arising from within the other hepatocellular tumors (noted in the NTP report on page 77) and therefore simply represents a morphologically altered area of the hepatocellular adenoma or carcinoma and not an independently derived tumor.
2. **Hepatoblastoma incidence should not be used as an independent or sole basis for determination of level of evidence of carcinogenicity.** As indicated in several publications (Turusov et al, 2002; Cattley et al, 2013) on hepatoblastomas, these neoplasms should be considered in conjunction with other neoplasms of hepatocellular origin in assessing a hepatocellular tumor response due to the relationship with other hepatocellular tumors as outlined above in point 1. It should be noted that other morphological variants of hepatocellular adenomas and carcinomas are routinely observed in all sets of these tumors and are NOT separately identified nor used as an independent indicator of a liver tumor response. In the current study, it is important to note that the data indicate that a statistically increased hepatocellular tumor incidence compared to control is identified when hepatoblastomas are combined with hepatocellular carcinomas BUT only in the low dose group and not the mid- dose group without significance in the dose response.

3. **Evolving increase in incidence of spontaneous hepatoblastomas makes comparison of incidence in the treated groups in the current study of questionable relevance.** The substantial increasing incidence of spontaneous hepatoblastomas in control groups in the NTP program has been documented (Turusov et al, 2002). While all toxicologists agree that concurrent controls are generally the most appropriate comparator for incidence in treated groups, the vagaries of concurrent data are well known to all toxicologists resulting in the consideration of historical control data as done by NTP in this and other studies. While great attempts are made to identify the appropriate historical control data sets for comparison as done in the current study, the relevance of historical control data is reduced if the historical control data are changing over time. Specifically, an incidence in either control or treated groups noted in the most recent study may not represent the latest increase in background tumor incidence as this incidence is increasing. It is unclear whether this incidence of spontaneous hepatoblastomas has continued to increase in more recent years. In summary, the value of historical control data is of reduced value when the spontaneous tumor incidence is changing.
  
4. **Data from all groups should be considered in the evaluation of a carcinogenic response.** Data from the 1000 mg/kg/day group has not been considered in the determination of a liver tumor response as indicated by exclusion of this group in the Summary Table (Page 12) and statements in the text. While reduced survival can certainly limit the identification of a tumor response, consideration of the tumor response should not be made based on the exclusion of a data set (high dose group) from consideration when these data can provide some perspective. In the current study, 3 hepatoblastomas were identified in the 1000 mg/kg/day group, a number that appears to be less than might be expected if the test agent were causing a hepatoblastoma tumor response. While the survival is substantially reduced (due to gastrointestinal toxicity and not related to tumors at any site) in the male mice receiving 1000 mg/kg/day, it should be noted that a survival of 44% is present at 21.5 months after administration. If the administered agent were indeed causing a hepatoblastoma response, a greater incidence might be expected in the animals dosed through 21.5 months of study particularly considering that the higher dose would be expected to cause a greater tumor response in the high dose than in lower dose groups. It is also important to note the lack of a dose response in hepatoblastomas between the 250 and 500 mg/kg/day groups. Indeed the 500 mg/kg/day groups has a lower incidence of hepatoblastomas than the 250 mg/kg/day group despite the 2 fold higher dose.

#### References

Cattley RC, Popp JA, Vonderfect SL Chapter 10 Liver Gall Bladder and Exocrine Pancreas. IN Toxicologic Pathology Nonclinical Safety Assessment. Eds. Pritam S Sahota, James A Popp, Jerry F Hardisty, Chirukandath Gopinath. CRC Press Taylor & Francis Group, Boca Raton FL, 2013

Thoolen B, Maronpot R, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufman W, Kuttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F, Ward JM Proliferative and Nonproliferative Lesions of the rat and Mouse Hepatobiliary System. Toxicologic Pathology 38: 5S-81S, 2010

Turusov VS, Deringer MK, Dunn TB, Stewart HL Malignant Mouse-Liver Tumors Resembling Human Hepatoblastomas. Journal National Cancer Institute 51: 1689-1695, 1973

Turusov VS, Torii M, Sills RC, Willson GA, Herbert RA, Hailey JR, Haseman JK, Boorman GA Hepatoblastoma in Mice in the US National Toxicology Program (NTP) Studies. Toxicologic Pathology 30: 580-591, 2002

**These comments are being submitted on behalf of the American Chemistry Council's North American Flame Retardant Alliance (NAFRA). We thank the members of the NTP Technical Report Peer Review Panel in advance for their consideration of these comments.**

[Redacted]

James A. Popp D.V.M., Ph.D.  
Diplomate, American College of Veterinary Pathologists  
Fellow, Academy of Toxicological Sciences  
Fellow, International Academy of Toxicologic Pathology