Dr. Thomas A. Gasiewicz
Editorial Office - TAP
Academic Press, Inc.
525 B Street, Suite 1900
San Diego, CA 92101-4495

Kuopio, 2002-11-08

Dear Dr. Gasiewicz:

Please find enclosed our revised manuscript TAAP5, “Dose response analysis on short-term effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in three differentially susceptible rat lines” We are very thankful for the comments and recommendations of the reviewers, which helped us to improve the manuscript.

Sincerely,

Ulla Simanainen

The changes and revisions are marked in following copies:
TAAP5_ReviewedSimanainenText_compared.doc
TAAP5_ReviewedSimanainenFigs_compared.doc,
TAAP5_ReviewedSimanainenTables_compared.doc,
and listed below.

The selection of the time point for measurements has been more carefully discussed. Collection of samples 8 days after TCDD exposure represents a compromise in aim to observe many different short-term effects. Despite the fact that liver EROD peaks earlier and is already affected by liver toxicity at day 8 it does not have a major influence on the conclusion derived from the model. Additionally, for other endpoints day 8 postexposure is considered to be a representative time point for short-term effects.

Liver EROD acitivity:

1. In the time response studies of EROD induction it has been demonstrated that the maximal liver EROD activity occurs in 2-3 days postexposure and it remains maximal up to 7 days or even longer.

   In Sprague-Dawley rats receiving TCDD up to 67 % of their LD50 showed maximal hepatic EROD induction compared to the control from day 1 to 28 after treatment (Håkanson et al., 1994).

   In responsive C57Bl/6 and non-responsive DBA/2 mice receiving TCDD up to 76 % and 64 % of their LD50, respectively, the maximal hepatic EROD induction compared to control occurred 7 days postexposure (Håkansson et al., 1994).

In Swiss [NIH] mice at no overtly toxic TCDD doses (determined by body weight) the hepatic EROD activity was elevated at 48 hr, and maximally induced at 1 week postexposure (Beebe et al., 1990).

2. There is no clear difference in sensitivity to liver EROD induction between H/W and L-E rats 3 days after treatment.

   There was no difference in response to cytochrome P-450-associated enzyme induction at low TCDD dose levels between TCDD-resistant Han/Wistar (Kuopio) and -sensitive Long-Evans (Turku AB) on day 3 postexposure (Pohjanvirta et al., 1988).

Serum ASAT activity and bilirubin concentration:

At day 8 postexposure the increased serum ASAT activity and bilirubin concentration showed highly different efficacy between the line A and C rats. However, Viluksela et al. (2000) showed that in TCDD-resistant H/W and –TCDD-sensitive L-E rats there is little difference in efficacy but about a 100-fold difference in potency for ASAT activity after 20-week exposure to TCDD. Therefore it seems that the toxicity may accumulate in time also in type II endpoints disguising the original efficacy difference. However, this accumulation cannot be considered as a short-term effect, and we have limited this manuscript to short-term effects..

Teeth:

In a recent study we were able to show that both H/W and L-E rats showed similar responses in the impaired formation of the incisor tooth after 20-week exposure to TCDD (Kiukkonen et al., 2002). This implies that selection of a later time point for examination of teeth would have resulted in similar classification (type I) of this effect.

Reviewer No. 1

Title has been changed according to recommendation given.

Page 3, para 3, line 4. Corrected as results in an….

Page 4, para 2, line 7. Corrected as 10000

Page 5, para 3, line 4. Corrected as in the animal….

Page 10, last line. Corrected as log normal

Page 12, para 1, line 7. Corrected as involving a different….

Page 13, para 2, line 1 and line 4. Corrected as TCDD efficacy

Page 14, para 1 lines 2 and 3. Changed to: …of a functionnal AHR conformation that increases promoter accessibility and facilitates promoter….

Page 14, para 1, line 4. Changed to: diverse involving separate…

Reviewer No. 2

1. See above for the discussion concerning the time point selected.

2. More details have been added to figure and table legends to make them self explanatory.

3. In contrast to Fig.1 the data in Fig. 2 is not quantitative in nature and therefore it is presented separately from Fig. 1.

4. For the purpose of keeping the figures more clear for readers we did not insert the error bars for the data points in Figs 1 and 2.

   The data points represent group means (5-6 rats per treatment group) as indicated in the figure legend.

   Large 95% CI values for some endpoints in Table 1 represent uncertainty of values obtained by extrapolation using the model. They do not represent variation of actual measurements. For the information of the reviewers about the actual variation of raw data we have attached a table with averages and standard errors for all doses and endpoints measured.