Reviewer’s report

Title: Effects of chronic carbon monoxide exposure on fetal growth and development in mice.

Version: 1 Date: 31 October 2011

Reviewer: Megan Probyn

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Major compulsory revisions:
1. COHb dissociation curves are sigmoidal in shape and not linear. Is a linear regression analyses of maternal/regal %COHb and Hb therefore appropriate?

2. Figures 3 and 4 indicate that up to 15 litters were exposed to between 0 and 60 ppm CO but only 6-7 litters were exposed to CO concentrations >100 ppm. Why were twice as many litters studied at the lower CO concentrations than the higher CO concentrations? Was this due to a decrease in successful maintenance of pregnancy (ie, presence of seminal plug at mating but no later indication of a successful pregnancy)? If so, this information should be included.

3. Increasing the number of litters studied at 400ppm may reduce the error associated with CO concentrations in maternal organs, especially when the error (SD?) seen for the liver and lung data at 400ppm is almost as large as the mean. This casts doubt on whether CO concentrations in these organs at 400ppm is actually significantly different to control.

4. While I agree that the number of live fetuses per litter at 400ppm CO may be significantly reduced compared to control, what evidence is there that ‘Total live fetuses/litter were negatively correlated with CO concentrations’? Looking at the values in Table 2, I doubt that a linear regression analysis would indicate this to be true (control = 12.6, 150ppm = 13.5, 250ppm = 12.4, 400ppm = 8.2 live fetuses per litter). I would also suggest the correlation between EGD/LGD and maternal CO exposure would be weak. Were these three correlations significant?

5. Analyses indicated in the ‘Statistical Analysis’ section ( ‘The EGD and LGD data was analysed with a chi squared test...’) do not match that indicated in the ‘Results’ section (implication that a linear regression analyses were performed for EGD and LGD data). Please clarify which form of analyses were actually used.

6. For 4% PFA perfused fetuses, were the six embryos used for each CO concentration from different litters?

7. What is meant by ‘in each case the abnormal fetus number was compared to implantation total subtract EDG or LGD’?

8. Was fetal to placental size altered by maternal CO exposure? This parameter indicates whether the placenta is able to appropriately transfer nutrients to the
fetus for growth and development.

Minor Essential Revisions
1. Page 7, line 12: a word (existed?) is missing between the words ‘distinction’ and ‘between’.

2. When embryos were sectioned, how many sections were collected from each embryo? How thick were the sections? Where were the sections taken from within the embryo? Were the placentas that were used for histological analyses taken from the same embryos that were used for histological analyses? That is, were they embryo and placental pairs? If the placentas were photographed whole, how were the images taken randomly? What was meant by ‘random pictures’?

3. Page 8, line 12: Analysis is singular; please change to analyses.

4. Page 9, last sentence: please change to ‘these organs are shown separately as they contained…’

5. Table 1 is of little value and should be removed.

6. Table 2 includes a CO concentration of 330ppm. Such a group is not included anywhere else in the manuscript. Is this an error? Should this not be 300ppm?

7. Do the errors in the figures represent SD or SEM?

8. Figure 1: While all panels show a scale bar, the images are of different sizes. Please make them all the same size so the structures in images A and B can be seen more clearly.

9. Figure 4 provides the same information as that in Table 2 (Total live fetuses/litter column) and therefore is unnecessary.

10. Does figure 5 represent CO concentrations in the maternal or fetal spleen?

11. Page 14, line 13-14: ‘…fetal/placental abnormalities were insignificantly increased.’ What abnormalities were these? The results indicate that placental size, dimensions and histomorphology were not different between groups and apart from fetal weight and survival, no other fetal measurements were reported.

12. The placenta is not a maternal tissue. Therefore all references to the ‘maternal placenta’ should be replaced with ‘placenta’.

Discretionary Revisions
1. A brief statement/introduction as to why CO may be therapeutically administered to the pregnant women (as given in the last paragraph of the discussion) would be of value.

2. A stronger link between maternal cigarette smoking, and resulting maternal CO concentrations, and the developmental effects fetal hypoxia would also be of value.
3. What is the relevance of a chronic exposure model to humans?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.