Comments

on

NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Ethylene Glycol
(CERHR Public Common Draft 12/05/02)

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Major concerns:

Unfortunately, the Expert Panel Report had overlooked that the ethylene glycol metabolites glycolic acid and oxalic acid are found in substantial amounts in plasma and urine of healthy humans non-exposed to ethylene glycol. Both compounds are well known products of the intermediary metabolism of proteins and carbohydrates. An evaluation of the risk arising from an ethylene glycol exposure must consider such "background values".

A further problem that I should mention is the absence of human inhalation data. Fortunately, in my laboratory we have performed controlled inhalation exposures to vaporous ethylene glycol. Blood concentrations of ethylene glycol and glycolic acid as well as urinary excretion data of ethylene glycol, glycolic acid and oxalic acid are available now in two male volunteers. The manuscript describing the exposure experiments, the resulting concentration-time courses and background concentrations has been submitted to Archives of Toxicology. A copy of this manuscript is attached for being reviewed by the Expert Panel.

Further comments:

Chapter 1 Chemistry, Use, and human exposure
Page 5, first paragraph: 70 mg/L should be replaced by 7 mg/L.

Chapter 2 General Toxicology and Biological Effects
Page 14, first paragraph: „absorption through the skin and lung appear to be slow and incomplete" Statement is not correct: Absorption will be complete if one waits long enough.
Concerning the lung: Uptake from the alveoli into blood is complete as shown in Carstens et al. 2002 and in Carstens et al. submitted for publication. This has also been demonstrated and treated theoretically on a series of other highly blood soluble gases and vapors (Csanády and Filser 2001).

Page 14, second paragraph: Dermal absorption is given in percent of the dose. Unfortunately, the time period during which this absorption took place is missing.

Page 15, first paragraph: the value of 1.3 ml/min/g bw given for the minute volume of rats is too high. This value was already questioned by Marshall and Cheng, the authors of the study. The minute volume in a 250 g rat is 174 ml/min (Arms and Travis 1988), which corresponds to about 0.7 ml/min/g bw. Using this value, Marshall and Cheng estimated a more than 90% uptake of EG in rats which fits to the findings in humans (Carstens et al., cited above).

Page 15, second paragraph: Was the urine to plasma ratio of 1.0 - 1.4 related to polyuria? Please specify.

Pages 15, 16, 17, 2.1.3 Metabolism: The two toxicologically relevant metabolites, glycolic acid and oxalic acid, are found in substantial amounts in plasma and urine of healthy non-exposed humans. Both are well known products of the intermediary metabolism of proteins and carbohydrates. The endogenous sources of these substances should be mentioned, too.

Page 18, third paragraph, Strengths/Weakness: The data to estimate human burden by ethylene glycol (EG) and glycolic acid (GA) are not appropriate to be used for calculating EG and GA burdens at the much lower exposure concentrations occurring at workplaces because all these data had been collected in poisoned patients who had undergone medical treatment. Considering all the weaknesses summarized on pages 17 and 18, it seems to be impossible to develop a reasonable PBTK model based on these data.

Page 18, last paragraph and Page 19, Table 2-3: The data given for male rats hint to a saturation kinetics of EG metabolism starting at 1000 mg/kg bw.

Pages 19 and 20 (Tables 2-3 and 2-4a): From the AUC in Table 2-3, it becomes obvious that EG metabolism shows saturation kinetics in the female mouse already at a dose of 100 mg/kg.
Consequently, the decrease of the ratio of $^{14}CO_2$ to urinary ^{14}C in urine, which is observed in the female mouse already at 100 mg/kg, can result from saturation of both EG and GA. In the male rat, the corresponding shift becomes evident starting at about 800 mg/kg EG. At the dose of 1000 mg/kg this shift is more pronounced than expected from the just beginning of the EG saturation observed in Table 2-3. Consequently, it can be speculated that the increased urinary ^{14}C-excretion in the high dosed male rats could result mainly from saturation of GA metabolism and to a lesser extent from EG metabolism. In female rats, saturation kinetics of GA metabolism (i.e. leaving the first order kinetics) seems to begin in the dose range between 150 and 500 mg/kg (Pottenger et al. 2001). This might be reflected by the dose dependent ratio of $^{14}CO_2$ to urinary ^{14}C in female rats (Table 2-4).

Page 22, first paragraph: Pottenger did not use "^{14}C-ethylene glycol" but ^{13}C-ethylene glycol.

Page 23, Strengths/Weakness: The first comment about the weakness "it is difficult to asses how well one might extrapolate these data to the human situation and the authors make no attempt to do so" is not comprehensible, since it was not the goal of the paper in question to extrapolate the rat data to the human situation. The paper deals with the rat solely.
The second negative comment "The study was limited to a narrow window of gestation, i.e., GD 10-11 in the Sprague Dawley rat" is contradicted by the following sentence "The rational for choosing this gestational period was that previous studies

had demonstrated this is a sensitive time-frame for ethylene glycol developmental toxicity". Therefore, the appropriateness of the second comment is questionable.
To the third comment: "…since exhaled breath was not collected, there is no attempt at mass balance." Usually, it is not the aim to make a mass balance if ^{13}C is used in kinetic studies.

Page 24, second paragraph, calculation of the expert panel (bold). This calculation results in a drastic overestimation of the initial body burden (see comment to Page 15, first paragraph). According to the calculation of the expert panel, vapor and aerosol exposures result in inhaled doses of 1.25 mg/kg and 7.1 mg/kg, respectively. Indeed, Marshall and Cheng reported doses of 0.74 mg/kg and 2.4 mg/kg after 30 min and 17 min, respectively. Carrying out a calculation of the absorbed doses using the physiological minute volume of 0.7 ml/min/g bw (see previous comment) for rats of 250 g bw, the amounts taken up are obtained to be 0.67 mg per kg bw (32 mg/m^3 x 0.7 ml/min/g x 250 g x 4 (animals) x 30 min /1000000 ml/m^3) and 2.2 mg per kg bw (184 mg/m^3 x 0.7 ml/min/g x 250 g x 4 (animals) x 17 min /1000000 ml/m^3), respectively. This calculation is in agreement with the data of Marshall and Cheng. Furthermore, it has to be stressed, that the percentages reported by the expert panel in the next sentences are related to the doses given by Marshall and Cheng and not to those calculated by the expert panel.

Page 24, third paragraph: I do not understand the comment that "the radiocarbon data in blood and tissues may be confounded" since a slow formation of $^{14}CO_2$ over several days is a direct hint that the labeled compound or metabolites thereof entered intermediary metabolism. The study was carried out to examine the quantitative fate of ^{14}C-ethylene glycol and not to determine specified metabolites and exactly this was done.

Page 25, first paragraph: see first comment to page 24. Accordingly, it becomes obvious that the panel assumption of a reduced minute volume due to irritating properties of EG is not justified.

Page 25, comments given in bold, sentence "...the long-term cumulative (AUC) dose is the more relevant dose metric." This statement is surprising. Is it justified? Usually, AUC is used as a dose measure for direct alkylating (carcinogenic) compounds. If effects are due to receptor binding, the concentration is more relevant.

Page 33, 6th paragraph, study of Wills et al. A comment by the expert panel that the background ethylene glycol concentrations given by these authors seem to be very high for unexposed controls in the light of the series of other published data would be helpful.

Page 34, third paragraph, LaKind et al. The information of decreased blood cell counts in the light of unknown exposure conditions is not very informative. A comment of the expert panel to these findings would be helpful.

Page 43 (Summary) first paragraph: The statements are not correct. Ethylene glycol uptake is complete by the oral and the inhalation route. Dermal uptake is slow, but one should not say "incomplete" because the dose taken up depends on the time length of exposure. Especially, the statements concerning the inhalation uptake in the Marshall and Cheng work is erroneous. The whole paragraph should be rewritten considering the comments given above.
I have to stress that human inhalation data are now available (Carstens et al. submitted).

Page 43 (Summary) third paragraph: Glycolic acid, glyoxal, glyoxylic acid, oxalic acid and formic acid are all endogenous intermediary metabolites of carbohydrate and amino acid pathways. This has to be considered and natural background levels should be given, since the comparison between endogenous levels and levels resulting from ethylene glycol exposure are very helpful for risk assessment. Unfortunately, the corresponding information is missing completely.

Page 43 (Summary) last paragraph: We have data demonstrating that the half-lives of ethylene glycol and glycolic acid in two humans exposed to low atmospheric concentrations of ethylene glycol (according to the German MAK value) were between 2 and 3 h (Carstens et al. submitted). The much longer half-lives obtained from poisoned patients are not relevant at conditions of normal workplace and environmental exposure.

Page 44, The statements in the section „Exposure route and dose rate effects on metabolic saturation" contain several serious errors and misinterpretations (see detailed comments given above).

References

Arms AD and Travis CC (1988) Reference physiological parameters in pharmacokinetic modeling. EPA/600/021

Carstens J, Csanády GyA, Faller T and Filser JG (submitted) Human inhalation exposure to ethylene glycol.

Csanády GyA and Filser JG (2001) The relevance of physical activity for the kinetics of inhaled gaseous substances. Arch Toxicol 74: 663-672

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