Minority opinion regarding the classification on the Carc. 2 classification (GHS) of PHMB

by Helmut Greim, October 18, 2011

Minority vote on the Carc. 2 classification (GHS) of PHMB

In its opinion on the proposal of France RAC concluded that PHMB is not genotoxic *in vitro* and *in vivo*, and the proposed classification is mainly based on the results from one study in one species. Since no mode of action has been identified a classification as carcinogenic category 3; R40 (CLP Carc 2 – H351) is proposed. Although this conclusion may be formally correct, a weight of evidence evaluation does not justify classification when considering the high dose effect, the possible mode of action and the relevance of the observed vascular tumours for humans. This conclusion is based as follows:

First: The RAC opinion correctly states that the vascular tumours occur at the high dose of 4000 ppm in one mouse experiment. This dose led to increased mortalities and reductions in body weight up to 35-42% in males and 22-33% in females although food consumption increased. This is explained by the general effect of biguanides that leads to impaired glucose utilisation so that biguanides are used in the anti-diabetic therapy. The effect is mediated by decreased intestinal glucose absorption and suppression of gluconeogenesis and ATP production.

Second: Biguanide suppression of ATP synthesis results from impairment of mitochondrial oxidative phosphorylation. Similar to cellular hypoxia this impairment leads to insufficient inactivation of HIFs (Heat-Inducible Factors), which activate multiple cellular functions such as transcription of the hormone erythropoietin (EPO) to enhance red blood cell proliferation, vascular endothelial growth factor (VEGF) to promote angiogenesis and glycolytic enzymes to increase glycolysis (Tormos and Chandel 2010). In tumour cells this adaptive response involves modulation of the synthesis of multiple proteins controlling processes such as glucose homeostasis, adjustment to glycolysis, mitochondrial respiration, angiogenesis, vascular permeabilityandinflammation (Marignol*et al.* 2005; Rankin andGiaccia 2008, Hanahanand Weinberg 2011). Thus, the induction of vascular tumours most likely is related to the specific effect of biguanides: impairment of mitochondrial respiration, which among other cellular responses enhances angiogenesis.

Third: RAC noted that the formation of the vascular tumours is a high dose effect. RAC also noted that doses, which induce these effects concur with severe loss of body weight as a result of impaired glucose utilisation. Thus, in humans even single high exposures to PHMB would lead to drastic disturbance of glucose metabolism, which would lead to severe illness, so that neither acute nor long term high exposures to PHMB would be tolerated. Consequently high and long lasting exposures, which lead to vascular tumours in the mouse study, cannot be reached in humans.

Forth: Haemangio-adenomas and –sarcomas, which result from exposure to chemicals are extremely rare in humans and have only been observed in workers exposed to high concentrations of vinylchloride or in patients given thorotrast for diagnostic purposes (Cohen et al 2009).

In conclusion: Most likely the vascular tumours observed at high doses are the result of disturbed glucose metabolism with the consequence of impaired cellular energy supply and among other responses result in enhanced angiogenesis. Due to the anti-hypoglycaemic effect of PHMB such high exposure cannot occur in humans, so that the exposure conditions of the animal studies have no

relevance to humans. Consequently, I cannot support the RAC proposal to classify PHMB as a Cat. 2 (GHS) carcinogen. Instead, no classification is warranted.

References

Cohen SM, Storer RD, Criswell KA, Doerrer NG, Dellarco VL, Pegg DG, Wojcinski ZW, Malarkey DE, Jacobs AC, Klaunig JE, Swenberg JA, Cook JC. Review. Hemangiosarcoma in rodents: Mode-of-action evaluationand human relevance. Toxicol Science 111, 4-18, 2009

Hanahan D, Weinberg RA: Hallmarksofcancer: The nextgeneration. Cell 144, 646-674, 2011

Marignol L, Lawler M, Coffey M, Hollywood D: Achieving hypoxy inducible gene expression in tumors. Cancer BiolTher. 4, 359-364, 2005.

Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell Death Differ. 15, 678-685, 2008.

Tormos KV, Chandel NS: Interconnection between mitochondria and HIFs. J Cell Mol Med 14, 795-804, 2011