資料５

ジクロロメタンに関する文献調査結果の概要について

１　新たに収集した文献（主なもの）

文献１　U.S.EPA(2011), Toxicological Review of Dichloromethane(MethyleneChloride) (CAS No.75-09-2) In Support of Summary Information on the Integrated Risk Information System (IRIS) November 2011

（EPA：U.S.Environmental Protection Agency アメリカ環境保護庁）

文献２　SCOEL (2009), Recommendation from the Scientific Committee on Occupational Exposure Limits for methylene chloride(dichloromethane) SCOEL/SUM/130 June 2009（SCOEL：職業ばく露限界に関する科学委員会。EU の欧州委員会が設置した委員会）

２　収集文献から得られた情報

（１）CYP 経路のヒトでの飽和濃度（下線は事務局で付したもの。カッコ内は、原著の頁数）

ア　文献１　In human subjects exposed to dichloromethane in the workplace, saturation of CYP metabolism appears to be approached at the 400–500 ppm range (Ott et al., 1983c). (P12)

Plots of percent COHb against TWA exposure concentrations showed the appearance of saturation at around 400 ppm, with the beginning of the plateauing occurring around 300 ppm. (P13)

イ　文献２　At higher levels of exposure (> 250 ppm) the oxidative metabolic pathway becomes gradually saturated, the proportionate increase in COHb becomes smaller, and an increasing proportion of the received dose is exhaled unchanged. (P7)

（２）文献１におけるジクロロメタンの発がん性（下線は事務局で付したもの。カッコ内は、原著の頁数）

ア　In summary, the relative amount of dichloromethane metabolized via the GST pathway increases with increasing exposure concentrations. As the high affinity CYP pathway becomes saturated (either from high exposure levels or from genetic or other factors that decrease CYP2E1 activity), the GST pathway increases in relative importance as a dispositional pathway for dichloromethane. Two reactive metabolites (S-(chloromethyl)glutathione and formaldehyde) resulting from this pathway have been identified. GST-T1 is the GST isozyme that catalyzes conjugation of dichloromethane with GST. (P19)

Comparisons of mice, rats, humans, and hamsters for the ability to metabolize dichloromethane via the GST pathway in liver (based on measurement of tissue-specific

enzyme activity) indicate the following rank order: mice > rats > or ≈ humans > hamsters. In mouse liver tissue, GST-T1 appears to be localized in the nuclei of hepatocytes and bile-duct epithelium, but rat liver does not show preferential nuclear localization of GST-T1. In human liver tissue, some hepatocytes show nuclear localization of GST-T1 and others show localization in cytoplasm, as well as in bile duct epithelial cells. The apparent species differences in intracellular localization of GST-T1 may play a role in species differences in susceptibility to dichloromethane carcinogenicity if nuclear production of S-(chloromethyl) glutathione is more likely to lead to DNA alkylation than cytoplasmic production. (P20)。

イ The cohort study with the higher exposures, the Rock Hill triacetate fiber production plant, suggested an increased risk of liver cancer (Lanes et al., 1993; Lanes et al., 1990). The SMR for liver and biliary duct cancer was 2.98 (95% CI 0.81–7.63) in the latest update of this cohort. This observation was based on four cases; three of these cases were biliary duct cancers. The authors estimated a total of 0.15 expected cases of biliary tract cancer in the first of the follow-up studies (Lanes et al., 1990); this subset of cancers may represent a particularly relevant form of cancer with respect to dichloromethane exposure. As the follow-up period has increased, the strength of this association has decreased, although it is relatively strong (albeit with wide CIs). The decrease in the SMR with increasing follow-up reflects the increase in number of expected cases because the four observed cases were seen earlier in the follow-up period. No other cohort 65 study has reported an increased risk of liver cancer mortality, although it should be noted that there is no other inception cohort study of a population with exposure levels similar to those of the Rock Hill plant, and no data from a case-control study of liver cancer are available pertaining to dichloromethane exposure. The available epidemiologic studies, with biological plausibility inferred from the localization of GST-T1 in the nuclei of bile duct epithelial cells in human samples (Sherratt et al., 2002), provide some evidence of an association between dichloromethane and liver and biliary duct cancer, although it should be noted that this evidence is based on limited epidemiologic data in that these observations were based on one study. (P64～P65)

ウ With respect to epidemiologic studies of liver and biliary duct cancer, the highest exposure cohort, based in the Rock Hill, South Carolina, triacetate fiber production plant, suggested an increased risk of liver and biliary tract cancer with an SMR of 2.98 (95% CI 0.81–7.63) in the latest study update (Lanes et al., 1993). This observation was based on four cases (three of which were biliary tract cancers); an earlier analysis in this cohort reported an SMR of 5.75 (95% CI 1.82–13.8), based on these same four cases but with a shorter follow-up period (and thus a lower number of expected cases) (Lanes et al., 1990). The authors estimated a total of

0.15 expected cases of biliary tract cancer in the first of the follow-up studies (Lanes et al., 1990); this subset of cancers may represent a particularly relevant form of cancer with respect to dichloromethane exposure based on localization of GST-T1 in the nuclei of bile duct epithelial cells seen in human samples (Sherratt et al., 2002). （P143）

エ *Is the hypothesized mode of action sufficiently supported in test animals*? The mode of action for dichloromethane is hypothesized to involve mutagenicity via reactive metabolites. The extensive body of research examining the proposed mode of action was summarized in the previous section. Mechanistic evidence indicates that dichloromethane-induced DNA damage in cancer target tissues of mice involves DNA-reactive metabolites produced via a metabolic pathway initially catalyzed by GST. Although mutational events in critical genes leading to tumor initiation have not been established, evidence supporting a mutagenic mode of action includes the identification of mutagenic response (reverse mutations) in short-term bacterial assays (with microsomal activation) and induced DNA-protein cross-links and DNA SSBs in mammalian cell assays. There are numerous positive in vivo mutagenicity and genotoxicity studies specifically examining responses in the liver and/or lung; these studies included evidence of chromosomal aberrations, SSBs, sister chromatid exchanges, and DNA-protein cross-links. The negative assays are generally those that were either micronucleus tests using mouse bone marrow, which is expected, as halogenated hydrocarbons (such as dichloromethane) are not very effective in this type of assay (Dearfield and Moore, 2005; Crebelli et al., 1999)), or unscheduled DNA synthesis, a relatively insensitive indicator of DNA damage. In conclusion, there is sufficient evidence supporting a mutagenic mode of action and indicating the involvement of GST metabolism in the lung and liver carcinogenicity of dichloromethane in mice.

オ *Is the hypothesized mode of action relevant to humans*? The postulated mode of action that dichloromethane is metabolized by GST to reactive metabolites that induce mutations in DNA leading to carcinogenicity is possible in humans. Mutagenicity as a mode of action for carcinogenicity in humans is generally accepted and is a biologically plausible mechanism for tumor induction. The toxicokinetic and toxicodynamic processes that would enable reactive metabolites to produce mutations in animal models are biologically plausible in humans. Furthermore, the detection of the GST pathway in human tissues indicates that the hypothesized mode of action involving reactive metabolites from this pathway, S-(chloromethyl)glutathione and formaldehyde, is relevant to humans.

Some investigators question the relevance of the proposed mode of action to humans in low-exposure scenarios given the high exposure conditions of the genotoxicity and bioassay

studies in mice and the relatively high GST activity in this species (Green, 1997). Comparisons in mice, rats, humans, and hamsters of GST enzyme activity in liver and lung tissues have indicated the following rank order: mice > rats > or ≈ humans > hamsters (Thier et al., 1998b; Reitz et al., 1989a).

Underlying questions of human relevance of dichloromethane-induced mouse tumors is the assumption that, at very low exposures, the amount metabolized through the GST activity in humans is effectively zero, and so any risk to humans would thus effectively be zero. EPA considered this line of reasoning, but found that it was not supported by several pieces of evidence. As discussed in Section 3.3, based on enzyme kinetics, the rate of reaction below ~20% of the Km becomes indistinguishable from a first-order reaction, as it depends on the probability that a substrate molecule collides with an unoccupied active site on the enzyme. Thus, at low concentrations (i.e., [substrate] << Km) the rate of enzyme-catalyzed reactions becomes proportional to the concentration of the substrate(s) and enzyme, and the reaction will proceed at a non-zero rate as long as GSH, GST, and dichloromethane are present at non-zero concentrations. The linearity of this metabolism at very low concentrations is discussed in the section on uncertainties in low-dose extrapolation (Section 5.4.5). At very low exposures, the amount of dichloromethane metabolized in humans through the GST pathway, while very low, is not zero.（P162～163）

カ　Another factor noted by Green (1997) that may play a role in the apparent species differences in carcinogenicity resulting from dichloromethane exposure is species differences in intracellular localization of GST-T1. Nuclear production of S-(chloromethyl)glutathione catalyzed by GST-T1 in the nucleus is more likely than cytoplasmic production to lead to DNA alkylation. Using immunostaining techniques, Mainwaring et al. (1996) demonstrated that in mouse liver tissue, GST-T1 was localized in the nuclei of hepatocytes and bile-duct epithelium, whereas the rat and human liver did not show preferential nuclear localization of GST-T1. A later study by Sherratt et al. (2002) reported that in human tissue samples, bile duct epithelial cells and some hepatocytes showed nuclear localization of GST-T1, and other hepatocytes showed localization in cytoplasm. Although the degree of GST-T1 localization in the mouse is greater than in humans, the finding of some nuclear localization of GST-T1 in human liver tissue and in the nuclei of bile duct epithelial cells, and the observation of three biliary tract cancers, a very rare cancer, in a small cohort of dichloromethane exposed workers (Lanes et al., 1993; Lanes et al., 1990) support the relevance of the hypothesized mode of action to humans.（P163）