資料４

1,2－ジクロロプロパンに関する文献調査結果の概要について（第 3 回検討会）

１　新たに収集・参照した文献（主なもの）

（１）1,2-ジクロロエタンに関するもの

文献１　Cheever KL,Cholakis JM,el-Hawari AM,et al.(1990), Ethylene Dichloride:The Influence of Disulfiram or Ethanol on Oncogenicity,Metabolism,and DNA Covalent Binding in Rats.

文献２　NIOSH(1976), Criteria for a recommended standard ….occupational exposure to ethylene dichloride(1,2-dichloroethane):U.S.Department of Health, Education, and Welfare.

文献３　Igwe O,Que Hee SS,and Wanger WD(1986), Interaction between 1,2-Dichloroethane and Tetraethythiuram Disulfide(Disulfiram).

文献４　IARC(1999), 1,2-dichloroethane (from:IARC Monographs on the Evaluation of Carcinogenic Risks to Humans volume 71 Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide)

文献５　ATSDR(2001), Toxicological Profile for 1,2-dichloroethane.

（２）1,2-ジブロモエタンに関するもの

文献６　Kim DH and Guengerich FP(1990), Formation of the DNA adduct S-[2-(N^7-guanyl)ethyl]glutathione from ethylene dibromide: effects of formation of glutathione and glutathione S-transferase levels and lack of a role for sulfation.

文献７　IARC(1999), 1,2-dibromoethane (from:IARC Monographs on the Evaluation of Carcinogenic Risks to Humans volume 71)

文献８　U.S.EPA(2004), Toxicological Review of 1,2-dibromoethane(CAS No.106-93-4)

文献９　SCOEL(2011), Recommendation from the Scientific Committee on Occupational Exposure Limits for 1,2-dibromoethane(ethylene dibromide) SCOEL/SUM/166

（３）ジクロロプロパン及びジブロモプロパンに関するもの

文献１０　Bartels MJ and Timchalk C(1990), 1,2-Dichloropropane:investigation of the mechanism of mercapturic acid formation in the rat.

文献１１　Tornero-Velez R,Ross MK,Granville C,et al.(2004), Metabolism and mutagenicity of source water contaminants 1,3-dichloropropane and 2,2-dichloropropane.

文献１２　Zoetemelk CEM,Oei IHB,Van Meeteren-Walchli,et al.(1986),

Biotransformation of 1,2-dibromopropane in rats into four mercapturic acid derivatives.

文献１３　Zoetemelk CE, Mohn GR,et al.(1987),1,2-Dibromo compounds.Their mutagenicity in Salmonella strains differing in glutathione content and their alkylating potential.

文献１４　Onkenhout W,Van Bergen EJC,et al.(1986), Identification and quantitative determination of four different mercapturic acid formed from 1,3-dibromopropane and its 1,1,3,3-tetradeutero analogie by the rat.

文献１５　Guengerich FP(2003), Activation of Dihaloalkanes by Thiol-dependent Mechanisms.

（４）代謝酵素に関するもの

文献１６　Oda Y,Yamazaki H,Their R,et al.(1996), A new *Salmonella typhimurium* NM5004 strain expressing rat glutathione S-transferase 5-5:use in detection of genotoxicity of dihalokanes using an SOS/*umu* test system.

文献１７　Landi S(2000), Mammalian class theta GST and differential susceptibility to carcinogens : a review.

文献１８　Lakehal F,Wendum D,Barbu V,et al.(1999), Phase Ⅰ and Phase Ⅱ Drug-Metabolizing Enzymes Are Expressed and Heterogeneously Distributed in the Biliary Epithelium.

文献１９　Mainwaring GW,Williams SM,Foster JR,et al.(1996), The distribution of Theta-class glutathione S-transferases in the liver and lung of mouse,rat and human.

文献２０　Sherratt PJ,Manson MM,Thomson AM,et al.(1998), Increased bioactivation of dihaloalkanes in rat liver due to induction of class Theta glutathione S-transferase T1-1.

２　収集・参照した文献の関係部分

（１）1,2-ジクロロエタンに関するもの

文献１

[Abstract]

Male and female Sprague-Dawley rats were exposed to 50 ppm ethylene dichloride (EDC) for 7 hr/day, 5 days/week, for 2 years by inhalation. Additional rats were exposed to 50 ppm EDC either with 0.05% disulfiram in the diet or with 5% ethanol in the drinking water. Histopathologic lesions related to the combination of inhaled EDC and dietary disulfiram were observed in the liver, mammary, and testicular tissues of rats. This combined exposure resulted in a significant increase in the incidence of

intrahepatic bile duct cholangiomas in both male and female rats. Male rats exposed to both EDC and disulfiram also had an increased incidence of subcutaneous fibromas, neoplastic nodules, and interstitial cell tumors in the testes. The female rats exposed to EDC and disulfiram also had a higher incidence of mammary adenocarcinomas. No significant increase in the number of any tumor type was observed in rats exposed to only EDC, disulfiram, or ethanol. Similarly, no significant increase in the number of tumors was observed in rats exposed to inhaled EDC and ethanol in water. At the end of the 2-year period animals from each group were evaluated for EDC metabolism and DNA binding. Blood levels of EDC at the end of a 7-hr exposure period were significantly higher for rats exposed to both EDC and disulfiram than for rats exposed to EDC alone. In addition, the elimination of a single oral dose of radiolabeled EDC was affected. The urinary excretion of 14C from control rats was 47 to 55% of the administered dose with 28 to 30% detected as unchanged EDC in the breath. In disulfiram-treated rats, only 35 to 36% of the administered 14C was eliminated in the urine with 41 to 55% as unchanged EDC in the breath. The urinary metabolite HPLC profile was qualitatively unchanged by long-term EDC, disulfiram, or ethanol treatment, either alone or in combination, and consisted primarily of thiodiglycolic acid, thiodiglycolic acid sulfoxide, and chloroacetic acid.

[Results]
(P248-249)

Male rats in certain groups were found to have increased liver masses (32% for the EDC/DS group vs 8% for controls) which were primarily related to bile duct cysts, kidney lesions including cronic nephropathy, calculi of the renal pelvis and associated hyperplasia of the pelvic epithelium (30% for the EDC/DS group vs 8% for controls), or testicular lesions (24% for the EDC group vs 10% for controls). For the female rats the most noteworthy observation was an increased incidence of liver masses including bile duct cysts (46% for the EDC/DS group vs 6% for controls). At termination the body weights of male and female rats exposed to EDC/DS were significantly decreased while the relative liver weights were increased in comparison with control values (Tables 3, 4).

(P249-P250)

For the liver, the incidence of intrahepatic bile duct cholangiomas and cysts was significantly increased for male and female rats exposed to EDC/DS. The male rats in that exposure group also had increased numbers of neoplastic nodules. The cholangiomas did not appear to be locally invasive and consisted of a proliferation of bile ducts lined by flattened epithelium. Although no metastases were observed for this lesion the pathology was reflected by an increased relative liver weight.

(P253)

The presence of relatively high amounts of covalent binding, amounting to 36 to 44 micromolar equivalents per mole of DNA, was detected in unpretreated rats (Table 14). However, no significant exposure-related differences were noted.

(P247)

DNA covalent binding. The hepatic DNA covalent binding in 2-year-old rats was evaluated for Control, DS, ET, EDC, EDC/DS, and EDC/ET groups. Radiolabeled EDC was administered to three male and three female rats from each group by gavage at a dose of 150 mg/kg body wt 10 to 14 days after cessation of 103 weeks of EDC exposure. The animals were anesthetized with ether 6 hr after dosing and euthanized by exsanguination. The livers were immediately removed, rinsed with 0.1 M Tris/0.01 M EDTA, weighed, frozen in liquid nitrogen, and stored at －70℃ until isolation of the DNA. For the isolation of hepatic DNA, livers were homogenized in 2 vol of 0.1 M Tris/0.01 M EDTA (pH 7.7) and adjusted to 1.5% with 0.1 vol of 15% SDS.

[Discussion]

(P255)

Although DS alone resulted in an increase in the incidence of tumor formation in this and other studies (NCI, 1979) at levels consistent with those used therapeutically, the combined EDC/DS treatment resulted in significant levels of intrahepatic bile duct cholangiomas and cysts in both male and female rats. The cholangiomas, an unusual lesion, consisted of a poorly circumscribed proliferation of bile ducts lined by a flattened or low cuboidal epithelium. These lesions did not appear to be locally invasive and there were no metastases.

(P258-259)

In summary, the results of the present study indicate that the exposure of rats to EDC at 50 ppm (7 hr/day) with DS in the diet resulted in the formation of increased numbers of tumors when compared with rats exposed to only filtered air and standard diet. The combined EDC/DS treatment resulted in a high incidence of hepatic, testicular, and mammary tumors in the rat. No increased incidence, however, was noted for the EDC, EDC/ET, DS, or ET groups. Although the levels of hepatic DNA covalent binding by orally administered [^{14}C]EDC were significant those levels did not appear to be altered by DS treatment, and the metabolism of EDC was qualitatively the same as that of corresponding controls. However, a reduced rate of elimination for EDC, along with sustained blood levels of the unchanged compound, could be related to the carcinogenic effects observed for the combined treatment.

文献２

(P54-55)

The health of workers chronically exposed to ethylene dichloride in the Russian aircraft industry was studied for the years 1951-1955 and reported by Kozik.

(P55)

During application of the glue to the large rubber sheets, ethylene dichloride, the solvent for the glue, was emitted to the air.

During application of the glue to the rubber sheets, concentrations of 5-40 ppm were reported.

(P57)

Eighty-three of the gluers were examined by the Department of Occupational Disease of the Central Institute for Postgraduate Medicine. Diseases of the liver and bile ducts were found in 19 of the workers, neurotic conditions were found in 13, autonomic dystonia in 11, asthenic conditions in 5, and goiter and hyperthyroidism in 10 workers.

文献３

[Abstract]

The synergistic hepatotoxicity of dietary disulfiram (DSF) with 1,2-dichloroethane (DCE) subchronically administered by inhalation at three concentration levels (150, 300, and 450 ppm) was studied. The criteria for hepatotoxicity were treatment-related increases in serum activities of sorbitol dehydrogenase, 5'-nucleotidase, and alkaline phosphatase, and in liver-to-body weight ratios. DSF alone did not elicit these responses while DCE at the highest concentration level increased liver-to-body weight ratios and the activity of 5'-nucleotidase. Exposure to DSF alone decreased cytochrome P450 levels, but in combination with DCE, the decrement of cytochrome P450 was additive in a DCE concentration-dependent manner. However, depression of cytochrome P450 by DCE alone was not concentration dependent. Although DSF and DSF/DCE combination increased the activity of glutathione S-transferases (GSTs), both DSF and DCE singly and in combination increased the tissue levels of reduced glutathione (GSH). Evidence is presented showing that the potentiation of the hepatotoxicity of DCE observed in the presence of DSF may be due to an inhibition of microsomal mixed-function oxidase-mediated metabolism of DCE and to a compensatory increase in DCE metabolism to reactive metabolites generated by GST-mediated conjugation of DCE with GSH.

文献４

(P510-511)

Metabolism appears to occur via two principal pathways, catalysed by cytochrome P450 and by glutathione *S*-transferase (Figure 1). Cytochrome P450 enzymes catalyse oxidative transformation of 1,2-dichloroethane to 2-chloroacetaldehyde, 2-chloroacetic acid and 2-chloroethanol (Guengerich *et al.*, 1980), which are conjugated both enzymatically and non-enzymatically with glutathione (GSH). The other pathway involves direct conjugation with GSH to form *S*-(2-chloroethyl)glutathione, which is a sulfur half mustard (Schasteen & Reed, 1983; Foureman & Reed, 1987). A non-enzymatic reaction of the half mustard gives a putative alkylating agent (episulfonium ion) which may react with water to form *S*-(2-hydroxyethyl)glutathione, with thiols such as GSH to form ethene bis-glutathione, or with DNA to form adducts. With the exception of *S*-(2-chloroethyl) glutathione which forms DNA adducts, the reaction products are considered nontoxic and undergo further metabolism.

Although some DNA damage has been induced via the P450 pathway *in vitro*(Banerjee *et al.*, 1980; Guengerich *et al.*, 1980; Lin *et al.*, 1985), several lines of evidence suggest that the GSH conjugation pathway is probably the major route for DNA damage (Guengerich *et al.*, 1980; Rannug, 1980; Guengerich *et al.*, 1981; Van Bladeren *et al.*,1981; Sundheimer *et al.*, 1982; Crespi *et al.*, 1985; Storer & Conolly, 1985; Inskeep *et al.*, 1986; Koga *et al.*, 1986; Cheever *et al.*, 1990).

文献５

(P91)

Mutagenicity was increased in TA100 strain Salmonella typhimurium expressing the alpha class of human glutathione S-transferase via regulatable tac promoter expression, but not in bacteria expressing the pi class of human glutathione S-transferase (Simula et al. 1993).

(P94)

The primary metabolic pathways for this chemical are MFO and glutathione conjugation. Oxidation products include chloroacetaldehyde, 2-chloroethanol, and 2-chloroacetic acid. MFO metabolism of 1,2-dichloroethane appears to be saturable at oral gavage doses 25 mg/kg and inhalation concentrations of 150 ppm (.500 mg/kg), both of which correspond to blood levels of 5–10 μg/mL. Glutathione conjugation becomes relatively more important at larger doses, and increased metabolism by this pathway may be responsible for the toxic effects noted at these high doses.

(P103)

Based on the data of these 2 groups of investigators, it appears that saturation of 1,2-dichloroethane metabolism occurs when blood levels reach 5–10 μg/mL blood or

after exposure to 150–250 ppm 1,2-dichloroethane. When blood concentrations of 1,2-dichloroethane exceed these levels (i.e., at exposure concentrations 150 ppm), manifestations of toxicity became more apparent. For example, Maltoni et al. (1980) reported that most of the toxicity associated with inhalation exposure to 250 ppm 1,2-dichloroethane in rats and mice was alleviated when exposure levels were reduced to 150 ppm, and no treatment-related effects were noted at 50 ppm. These findings suggest that 1,2-dichloroethane-induced toxicity occurs once a threshold blood level has been exceeded.

(P105)

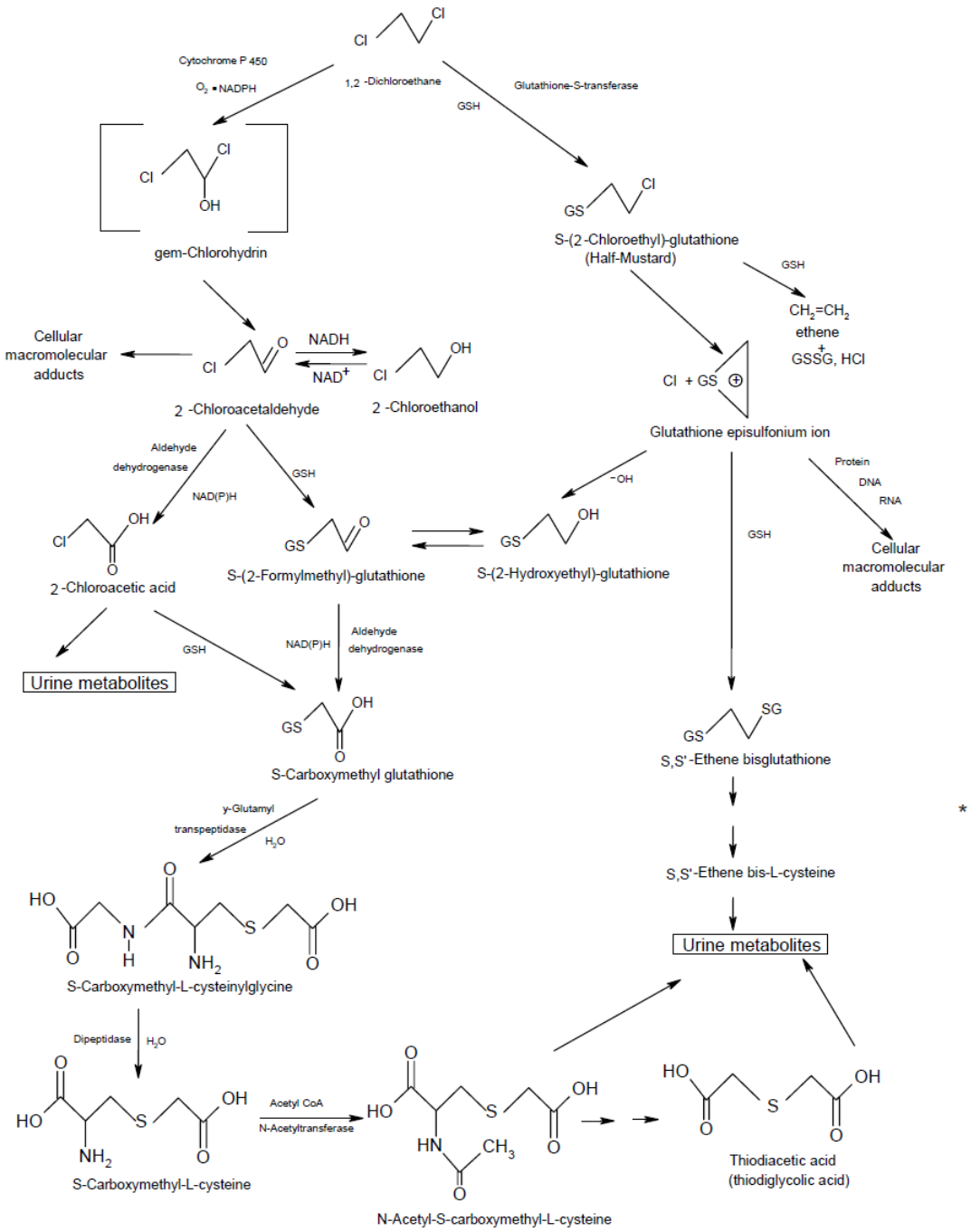
Guengerich et al. (1991) demonstrated that cytochrome P-450 2E1 is the primary oxidation catalyst of 1,2-dichloroethane in humans.

(P111-112)

There is compelling evidence that the toxicity and carcinogenicity of 1,2-dichloroethane are associated with its metabolism to active intermediates. Studies in rats and mice indicate that 1,2-dichloroethane is metabolized to 2-chloroacetaldehyde, S-(2-chloroethyl)glutathione, and other putative reactive intermediates capable of binding covalently to cellular macromolecules (Fabricant and Chalmers 1980; Jean and Reed 1989). The ability of a chemical to bind covalently to cellular macromolecules is often correlated with the induction of toxic and carcinogenic effects. In addition, 1,2-dichloroethane has been shown to promote lipid peroxidation in vitro (Sano and Tappel 1990; Tse et al. 1990). Lipid peroxidation is also associated with tissue damage. The lag time between inhalation exposure and onset of effects reported by Nouchi et al. (1984) in an occupationally exposed 51-year-old male may have been a reflection, in part, of the time required to metabolize 1,2-dichloroethane to active intermediates.

The level of glutathione present in the liver appears to modulate effects of 1,2-dichloroethane in animals. Glutathione is believed to be heavily involved in the biotransformation of 1,2-dichloroethane (Anders and Livesey 1980; Yllner 1971b). The metabolic pathway of 1,2-dichloroethane is linear at low doses, but at higher concentrations, as the P-450 enzymes become saturated, the amount of glutathione conjugate produced rises disproportionately with increasing administered dose; at very high doses, the GSH pathway is also saturated, and the glutathione conjugate produced declines disproportionately with increasing dose (D'Souza et al. 1987). It has been suggested that 1,2-dichloroethane-induced toxicity occurs when the biotransformation processes are saturated, thereby allowing higher levels of 1,2-dichloroethane to circulate throughout the body and conjugate with glutathione instead of being detoxified and eliminated (D'Souza et al. 1987; Reitz et al. 1982).

Figure 3-3. Proposed Pathways for 1,2-Dichloroethane Metabolism*



Derived from NTP 1991a

（２）1, 2−ジブロモエタンに関するもの

文献６

[Abstract]

Hepatic S-[2-(N7-guanyl)ethyl]glutathione DNA adducts were determined in several strains of rats and mice after i.p. injection of a dose of 37 mg ethylene dibromide/kg

body wt.

Disulfiram, a known cytochrome P450 inhibitor, significantly increased the formation of DNA adducts whereas it did not affect GSH S-transferase activity.

文献７

(P646)

Human liver preparations metabolize ethylene dibromide to water-soluble and irreversibly protein- and DNA-bound metabolites by both cytochrome P450 and glutathione *S*-transferase (GST) enzymes (Wiersma *et al*., 1986). DNA adduct formation occurs also in isolated human hepatocytes (Cmarik *et al*., 1990). There is convincing evidence that CYP2E1 is a major enzyme metabolizing ethylene dibromide. Among heterologously expressed human cytochromes P450, only CYP2E1(low *K*m enzyme), CYP2B6 and CYP2A6 (high *K*m enzymes) metabolized ethylene dibromide to 2-bromoacetaldehyde (Wormhoudt *et al*., 1996), CYP2E1 having the highest intrinsic clearance. Interindividual variation in P450-catalysed microsomal metabolism, reflecting presumably variable amounts of CYP2E1 enzyme, was almost 50-fold. Human fetal liver cytosol and several GST forms from human fetal liver catalyse the conjugation of ethylene dibromide (Kulkarni *et al*., 1992; Mitra *et al*., 1992). The α-class GST enzymes from human liver are especially active in the conjugation of ethylene dibromide(Cmarik *et al*., 1990).

文献８

(P5)

1,2-Dibromoethane is metabolized by two major pathways, cytochrome-P450-monooxygenase and glutathione (GSH) conjugation via glutathione-S-transferase (GST).

(P6)

1,2-Dibromoethane can also conjugate directly with GSH by a GST-mediated reaction to form S-(2-bromoethyl)GSH (Jean and Reed, 1992), a half-mustard that can spontaneously rearrange to an episulfonium ion, thiiranium, and is further hydrolyzed to S-(**$**- hydroxyethyl)GSH; or it can bind to DNA (Hodgson and Levi, 1994; Jean and Reed, 1992; Peterson et al., 1988). S-(2-bromoethyl)GSH can also undergo further GSH conjugation to form S,S-1,2-ethanediyl-bis-GSH (Jean and Reed, 1992). Formation of these GSH-containing metabolites correlated with a 71% depletion of intracellular GSH. In vitro studies have shown that approximately 60% of the episulfonium ion is trapped as S,S-ethanediyl-bis-GSH with the remainder reacting with water to form S-hydroxyethylGSH (Cmarik et al., 1990). The episulfonium ion is believed to be responsible for the genotoxicity of 1,2-dibromoethane. The major adduct derived from

the episulfonium pathway is S-[2-N^7-guanyl)ethyl]GSH (Koga et al., 1986).

(P7)

Van Bladeren et al. (1981) reported that the oxidative route compared to the conjugative route occurred in a ratio of about 4:1 in rats. This would mean that 1,2-dibromoethane is preferentially metabolized to 2-bromoacetaldehyde and then conjugated with GSH. The cytochrome-P450 isozyme responsible for the oxidation of 1,2-dibromoethane to 2- bromoacetaldehyde appears to be CYP2E1.

(P7-8)

The results of several experiments suggest that of the several mammalian GSH transferases that can catalyze conjugation with GSH (Cmarik et al., 1990), theta-class GSH transferase (GSTT) may be most important for conjugation of 1,2-dibromoethane. Investigation of human erythrocyte cytosol from 12 people not exposed to 1,2-dibromoethane revealed that two of the cytosols did not catalyze GSH conjugation with 1,2-dibromoethane (Ploemen et al., 1995).

In vitro experiments found *Salmonella typhimurium* expressing human GST-θ had greater genotoxicity following 1,2-dibromoethane exposure than strains that did not express this enzyme (Thier et al., 1996). Simula et al. (1993) reported that GST-α expression increased the mutagenicity of 1,2-dibromoethane in an *S. typhimurium* assay but GST-π did not. The authors did not investigate the role of GST-θ.

文献９

(P4)

There is convincing evidence that CYP2E1 is a major enzyme in the oxidative metabolism of 1,2-dibromoethane.

The alpha-class (Cmarik et al., 1990) and theta class (Thier et al., 1996) GST enzymes from human liver are especially active in the conjugation of 1,2-dibromoethane.

(P14)

Thus, 1,2-dibromoethane presents itself as a both a local and systemic experimental carcinogen. Its mode of action is clearly genotoxic. The biological activation pathway is mediated by glutathione-S-transferases, leading to formation of a very reactive episulfonium (thiiranium) ion. As these enzymes is also active in humans, a carcinogenic effect in humans, qualitatively similar to that in rodents, appears likely.

文献１０

[Discussion]

(P1040-1041)

The identification of these three mercapturic acid metabolites of DCP in the Fischer 344 rat is consistent with previous work for this class of compounds. Jones and Gibson (1980) reported the 2-hydroxypropyl mercapturate Ⅰ as a major urinary metabolite of DCP in Sprague-Dawley rats (100 mg/kg, orally). The same three mercapturates Ⅰ-Ⅲ were observed as metabolites of 1,2-dibromopropane in the male Wistar rat (Zoetemelk *et al*. 1986). In studies with 1-chloro-, 1-bromo- and 1-iodopropane in the rat, Ⅰ was isolated as a common urinary metabolite (Barnsley 1966). Barnsley also identified Ⅰ as a metabolite of 1,2-epoxypropane and 1-chloro-2-hydroxypropane.

The proposed metabolic scheme for the formation of these DCP metabolites is shown in figue 4. Oxidation of the 1-position of the parent compound and subsequent GSH conjugation would give rise to the 1-carboxyethyl mercapturate Ⅲ. This pathway was also proposed by Zoetemelk *et al*. (1986), for the formation of Ⅲ from 1,2-dibromopropane. Similar conjugation at the terminal carbon of DCP and oxidation would result in Ⅱ. Reduction of this mercapturate could then give rise to the 2-hydroxypropyl mercapturate Ⅰ.

An alternative route for the formation of Ⅰ would be through an episulphonium ion intermediate. This reactive metabolite could possibly form via GSH displacement of both chlorides of the parent compound. Subsequent hydrolysis would afford Ⅰ, with possible further oxidation to the keto-metabolite Ⅱ.

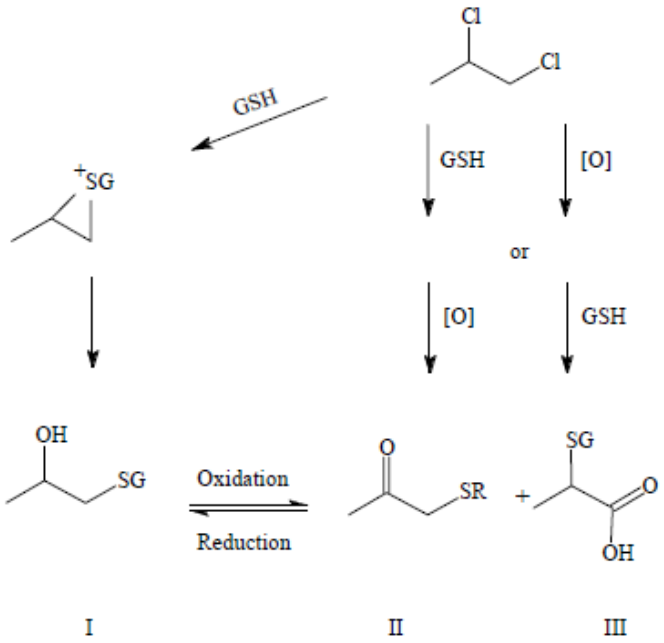
Several groups have investigated episulphonium ion metabolites as reactive intermediates in the biotransformation of similar compounds. Evidence of this type of intermediate was found in the metabolism of D_4-1,2-dibromoethane to N-acetyl-S-(2-hydroxyethyl)-L-cysteine in the rat (van Bladeren *et al*. 1981). This group reports complete deuterium retention in approximately 20% of this mercapturate in the rat, indicating at least partial conversion to this metabolite via an apisulphonium ion intermediate. In another study Onkenhout *et al*. (1986) found nearly completeisotope retention in the N-acetyl-S-(3-hydroxypropyl)-L-cysteine metabolite arising from 1,3-dibromopropane (1,1,3,3-D_4analogue) in the rat. These results were interpreted as supporting either a sulphonium ion intermediate, or alternatively, a deirect hydrolysis of the parent compound followed by conjugation with GSH.

In contrast, the results of the current study with D_6-DCP suggest that no episulphonium ion formation, or equivalent mechanism, occurs in rat. The 2-hydroxypropyl-mercapturate Ⅰ retained primarily three deuterium atoms, with less than 1% containing all six deuterium atoms of the original test material. The 2-oxopropyl-mercapturate Ⅱ had a similar isotope retention. These results do not support an episulphonium ion intermediate in the formation of Ⅰ. Instead, the conjugation/oxidation mechanism describe above is thought to give rise to Ⅱ, which is then further reduced to Ⅰ. While a suphonium ion intermediate would not be observed

if Ⅰ was rapidly oxidaized to Ⅱ, the mass spectral results indicate only trace levels of D_6- Ⅰ. This assumption is further supported by the experiments with D_4-1,3-dibromopropane that show nearly complete retention of the deuterium atoms geminal to the hydroxyl moiety of the analogous hydroxypropylmercapturate (Onkenhout *et al*. 1986).

The results of this mechanistic study, vs. the related studies described above (van Bladeren *et al*. 1981, Onkenhout *et al*. 1986), correlate with the known mutagenic/carcinogenic potential of these compounds. Both 1,2-dibromoethane and 1,3-dibromopropane have been shown to be mutagenic *in vitro* (Stolzenberg and Hine 1980). In addition, 1,2-dibromoethane has been reported to be a potent carcinogen, inducing multiple tumours in rats (both sexes) after oral or inhalation exposure (Chu and Milman 1981, Weisburger 1977). In contrast, little to no mutagenicity has been observed for DCP (Stolzenberg and Hine 1980, NTP 1986).Although female rats dosed with DCP (orally) had a marginal increase in mammary gland adenocarcinomas, no carcinogenicity was observed in male rats (NTP 1986).These biological deta correlate well with the mechanistic studies described above, and therefore support the possible role of episulphonium ion intermediates in the mutagenic and/or carcinogenic potential for this class of compounds.

In conclusion, three mercapturic acid metabolies of DCP (Ⅰ-Ⅲ) have been identified in the male and female Fischer 344 rat. These metabolites were found in the urine of these animals following both oral administration and inhalation exposure. The same three compounds were identified as metabolites of D_6-DCP in the female rat. An episulphonium ion intermediate in the formation of Ⅰ is not supported. Instead, this metabolites is thought to arise via direct oxidation of DCP, eiher prior to or following conjugation with GSH.



SG, glutathionyl; I, *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; II, *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine; III, *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine
From Bartels and Timchalk (1990)

文献１１

[ABSTRACT]

Cytochrome P450-dependent oxidation and glutathione (GSH)-dependent conjugation are the primary routes of metabolism of haloalkanes. Using rat liver microsomes and cytosol, we investigated the metabolism of two halopropanes found on the U.S. Environmental Protection Agency Contaminant Candidate List, 1,3-dichloropropane (1,3-DCP) and 2,2-dichloropropane (2,2-DCP). An automated headspace technique using gas chromatography was developed to determine rates of metabolism. Additional dihaloalkanes (1,2-dichloroethane, 1,2-dichloropropane, 1,4-dichlorobutane, 1,2-dibromoethane, 1,2-dibromopropane, 1,4-dibromobutane) were evaluated to assess structure-activity relationships. In general, brominated dihaloalkanes were eliminated from rat cytosol faster than chlorinated dihaloalkanes, reflecting the expected halide order of reactivity (Br > Cl). Furthermore, the rate of GSH conjugation was proportional to α, ω-haloalkane chain length. The clearance of 1,3-DCP via the GSH conjugation pathway (1.6×10^{-4} l/h/mg cytosol protein) was minor relative to the P450 pathway (2.8×10^{-2} l/h/mg microsomal protein). In contrast, we did not observe metabolism of 2,2-DCP via the GSH-dependent conjugation pathway and observed only a minor level of clearance via the P450 pathway (7×10^{-4} l/h/mg microsomal protein). Neither compound was mutagenic in various strains of

Salmonella, including those containing *GSTT1–1*, indicating that GSTT1–1 does not metabolize 1,3-DCP or 2,2-DCP to mutagens. Analysis of the reaction products of 1,3-DCP and GSH in cytosol by liquid chromatography/mass spectrometry revealed significant production of *S*-(3-chloropropyl) glutathione conjugate, indicating that the conjugate halfmustard does not rearrange to form a sulfonium ion, as typically occurs with vicinal dihaloalkanes.

文献１２

[Abstract]

1,2-Dibromopropane was administered orally in doses of 50-350 mg/kg to male Wistar rats. Four mercapturic acids were identified in urine by GC/MS, viz. N-acetyl-S-(2-oxopropyl)-L-cysteine (Ⅰ), N-acetyl-S-(2-hydroxypropyl)-L-cysteine (Ⅱ), N-acetyl-S-(1-carboxyethyl)-L-cysteine (Ⅲ), and N-acetyl-S-(2-bromo-2-propenyl)-L-cysteine (Ⅳ). Mercapturic acid IV was a minor metabolite which could only be measured at doses of 200 mg/kg or higher. In 24 hr, urinary excretion of mercapturic acids amounted to about 36% of the dose (11% I, 21% II, 4% III, 0.2% IV). No dose dependency was found up to the highest dose. A unified scheme is proposed for the metabolism of 1,2-dibromopropane in the rat, which accounts for the identified mercapturic acids. The role of direct glutathione conjugation in the route leading to the major metabolite II, presumably involving thiiranium ion formation, is discussed. This route probably is biologically not very important because of the absence of detectable activity of 1,2-dibromopropane toward glutathione S-transferases in vitro, the very low mutagenicity of 1,2-dibromopropane, and the high mutagenic activity of N-acetyl-S-(2-bromopropyl)-L-cysteine methyl ester which was studied as a model compound for direct conjugation.

[Discussion]

(P603-606)

A major objective of our investigations was to study the influence of substituents on the biotransformation and bioactivation of vicinal dibromo compounds. For 1,2-DBE it has clearly been demonstrated that the major metabolite in urine of rats is N-acetyl-S-(2-hydroxyethyl)-L-cysteine (10). This metabolite can be formed *via* two routes of biotransformation: 1) oxidation to 2-bromoacetaldehyde followed by GSH conjugation and reduction of the aldehyde, and 2) direct conjugation with GSH, giving rise to a reactive half-mustard (S-2-bromethyl-glutatione) which, after further reaction with water *via* an intermediate thiirauim ion, leads to the 2-hydroxyethyl-adduct (14). These two routes occur in a ratio of about 4:1 (8, 9). Other investigations have indicated that mainly the second route is responsible for the mutagenicity (10,25) and DNA

binding (16) of 1,2-DBE *in vitro*.

We wished to carry out similar studies with 1,2-DBP in order to investigate the effect of the introduction of a methyl substituent at one of the reaction centers. 1,2-DBP had been shown to be weakly mutagenic in *Drosophila* (17) and in *Salmonella typhimurium* (3), but nothing was known about its biotransformation pathways *in vivo*. Its choro-analgue, 1,2-dichloropropane, studied in rats by Jones and Gibson (26), gave N-acetyl-S-(2-hydroxypropyl)-L-cysteine as the major metabolite. On this basis we expected to find this metabolites also upon biotransformation of the chemically more reactive 1,2-DBP. Indeed, this compound (metabolite Ⅱ), was found to be excreted in urine of rats as the most abundant metabolite. In addition, two other metabolites (Ⅰ and Ⅲ) were present in considerable amounts, and a fourth, minor, metabolite (Ⅳ) could be identified at higher doses. In 24hr, urinary excretion of mercapturic acids amounted to 36% of the dose. In the second 24 hr about 6% of the dose was excreted, bringing the total amount to 42%. The remaining 58% of the dose was not excreted in the form of mercapturic acids. A similar result was obtained by Jones and Gibson (26), who established that mercapturic acid excretion in urin accounted for only 25-38% of the dose of administered 1,2- dichloropropane. Furthermore, they detected approximately 10% of the dose unchanged in the expired air of rats. Like 1,2-dichloropropane, 1,2-DBP could be excreted unchanged in expired air, but most likely only as a small part of the remaining dose. A larger amount of 1,2-DBP is expected to be further metabolized by oxidative pathways to debrominated metabolites (27), which can enter the tricargoxylic acid cycle and be converted to carbon dioxide. Experiments with carbon-labeled 1,2-DBP are needed to obtain additional information on these routes.

Fig. 4 shows the metabolic pathways that are proposed to account for the formation of the identified mercapturic acids. It is suggested that metabolites Ⅰ, Ⅲ, and Ⅳ are mainly formed *via* oxidative pathways, with subsequent GSH conjugation and mercapturic acid formation. Metabolite Ⅱ, which accouts for 60% of the total excreted mercapturic acids, can arise by either or both of the two pathways mentioned above: oxidation followed by a GSH conjugation and a reduction step or direct conjugation to GSH followed by hydrolysis. Like 1,2-DBE, the second pathway presumably involves a reactive thiiranium ion. Terefore, its possible presence could be an important factor in relation to the bioactivation of 1,2-DBP. 1,2-DBP, in contrast with 1,2-DBE, is a very weak mutaagen in bacterial systems (3, 17), even on the addition of GSH and GSH S-transferases. This was confirmed in our own experiments, a preincubation test of 1,2-DBP and *Salmonella typhimurium* TA 100 in the absence and presence of rat liver cytosol and added GSH. The synthetically derived N-acetyl-L-cysteine methyl ester conjugate of 1,2-DBP, in contrast, a half-mustard precursor of the major 2-hydropropyl

mercapturic acid by the direct conjugation route, induced, in the same incubation system with TA 100, a number of revertants,[2] which were similar to that induced by the N-acetyl-L-cysteine methyl ester derived from 1,2-DBE (10).

[2] Unpublished results.

On the basis of these results, we suggest that the role of direct GSH conjugation in the metabolism of 1,2-DBP is very limited, if it exists at all. This is also in agreement with experiments in which the activity of GSH S-transferases toward 1,2-DBP was determined according to the method of Baars *et al*. (28). We used a large range of concentrations (1-20 mM 1,2-DBP and GSH) and different quantities of cytosolic protein but, apart from a small amount of spontaneous conjugation, no activity was found.

In principle it should be possible to distinguish between the two biotransformation pathways described above with the aid of the 2-deutero analogue of 1,2-DBP, by determining the percentage of deuterium preserved in mercapturic acid Ⅱ, formed *in vivo*. Only derect conjugation will result in retention of the deuterium atom. Such experiments are in progress and the first results indeed suggest a minor role of direct GSH conjugation.

In conclusion, it can be stated that substitution of one hydrogen of a methyl group (1,2-DBE *vs*. 1,2-DBP) has a pronounced effect on biotransformation. The metabolic profile of 1,2-DBP is similar to that of a normal alkyl halide, and side chain oxidation plays a major role. Although both bromo compounds (1,2-DBP and 1,2-DBE) give rise to analogous hydroxylated metabolites in considerable amounts, the ratio of the two passible leading to their formation is likely to be quite different. This is also expressed in the striking difference in mutagenic potency of these two vicinal dibromo compounds.

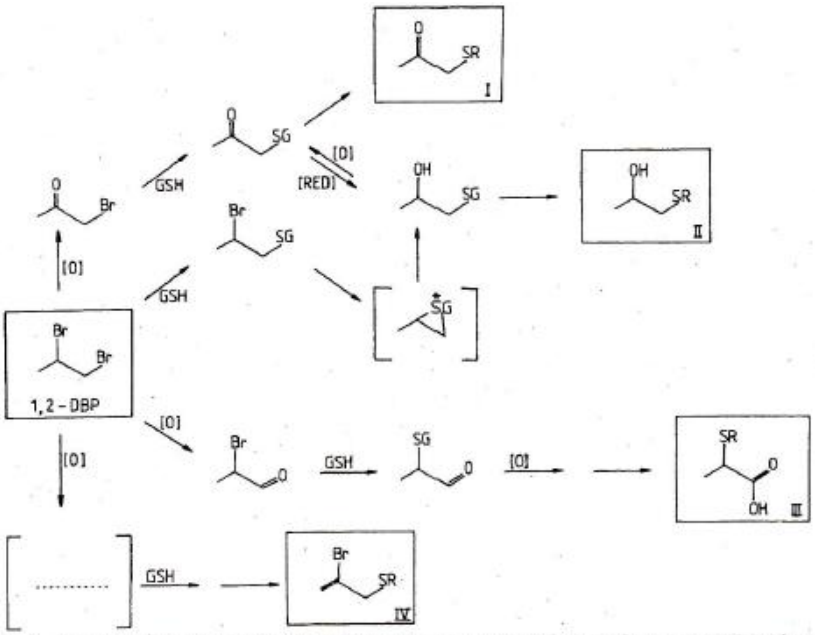


FIG. 4. *Proposed pathways for the formation of mercapturic acids I–IV from 1,2-DBP in the rat.*

SR, SCH_2 $CH(COOH)(NHCOCH_3)$.

（参考）Lee SK,Jin et al.(2004), Identification of glutathione conjugates and mercapturic acids of 1,2-dibromopropane in female BALB/c mice by liquid chromatography-electrospray ionization tandem mass spectrometry.

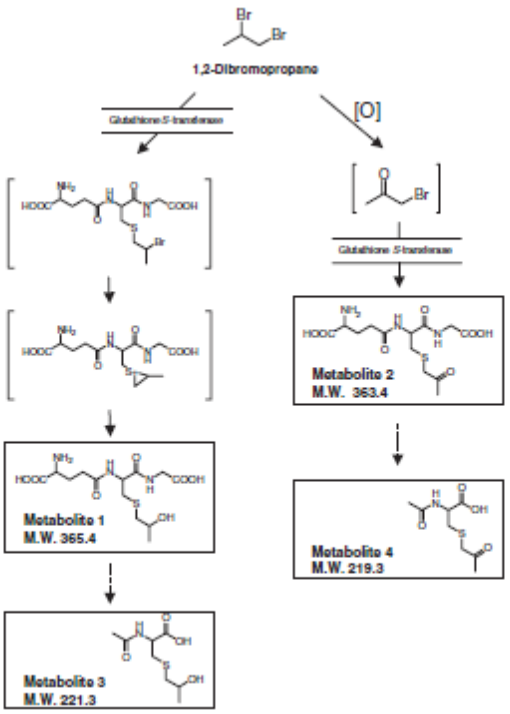


Figure 1. Proposed pathways for the formation of glutathione conjugates from 1,2-DBP. The boxes indicate the glutathione conjugates identified in the present study.

文献１３

[Abstract]

The mutagenic activities of several structurally related dibromo compounds were compared in Salmonella strains sensitive to base substitution mutagenesis (TA1535 and/or TA100) and in the glutathione (GSH)-deficient derivative TA100/NG-57, using a preincubation procedure. The compounds tested were 1,2-dibromoethane (DBE), 1,2-dibromopropane (DBP), 1,2-dibromo-1-phenylethane (DBPE) and model compounds for the half-mustards resulting from their conjugation with GSH, i.e. the N-acetyl-S-2-bromoalkyl-L-cysteine methyl esters SBE, SBP, and SBPE, respectively. The alkylating potential of all compounds was assayed with the 4-(p-nitrobenzyl)pyridine (NBP) alkylation test. Five of the compounds showed a good correlation between relative mutagenic activity in TA100 and electrophilic reactivity in the NBP-test, the order of decreasing potency being SBE greater than SBP greater than DBPE greater than DBP. SBPE displayed the highest reactivity in the NBP-test, but was devoid of mutagenic activity. The mutagenic activity of DBE was substantially decreased in the GSH-deficient strain TA100/NG-57 and could be restored by pretreating the cells with GSH. None of the other chemicals showed different mutagenic activities in TA100 and TA100/NG-57. From the results it can be concluded that 2-bromothioethers possess higher alkylating activities than the 1,2-dibromo compounds. Methyl substitution has a deactivating effect on the mutagenic activity. The results with the phenyl-substituted analogue, DBPE, show that a higher alkylating activity does not always lead to a higher mutagenic activity.

文献１４

[Abstract]

1. 1,3-Dibromopropane (1,3-DBP) was administered i.p. in doses ranging from 5.6 to 54 mg to male Wistar rats. Four different mercapturic acids, viz. N-acetyl-S-3-bromopropyl-(MA Ⅰ), N-acetyl-S-3-chloropropyl-(MA Ⅱ),N-acetyl-S-2-carboxyethyl-(MAⅢ)and N-acetyl-S-3-hydroxypropyl(-1-)cysteine (MA Ⅳ) were synthesized and identified as metabolites in urine by g.l.c.-mass spectrometry.
2. 1,3,3-Tetradeutero-1,3-dibromopropane was used to study the mechanism of formation of the mercapturic acids in more detail. It was found that in the formation of MA Ⅳ a reactive episulphonium ion could be involved.

[Discussion]

(P30)

One of the aims of the present investigation was to study the mechanism of biotransformation in the rat of 1,3-DBP into MA Ⅳ, in particular to elucidate the

possible occurrence of reactive electrophilic intermediates, which might explain the recently observed weak mutagenic activity of 1,3-DBP Salmonella typhimurium TA 100 (Buijs *et al*. 1984).

(P31-32)

As far as the mechanism of formation of MA Ⅳ is concerned, in principle there are three possible metabolic pathways via 3-bromopropionaldehyde, 3-bromopropanol (Jones and Wells 1981) or an episulphonium ion (figure 5). As after dosing rats with 1,1,3,3-tetradeutero-1,3-DBP the d4:d3 content in MA Ⅳ was found to be 10:1, and since only in the metabolic route involving the intermediary 3-bromopropionaldehyde one deuterium atom is lost, it can be concluded that the ratio of this route to the other two possible routes is 1:10. On the basis of the present deuterium-labelling experiment it is not possible to discriminate further between the metabolic routes involving hydrolysis of 1,3-DBP to 3-bromopropanol (Jones and Wells 1981) and the one involving a reactive episulphonium intermediate.

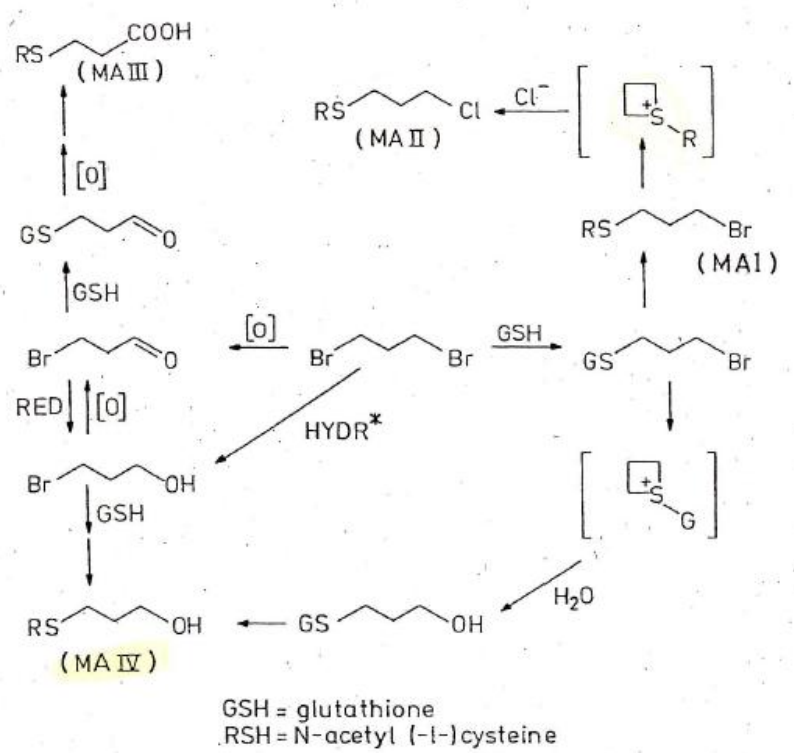


Figure 5. Proposed mechanisms of formation of the MAs I–IV from 1,3-dibromopropane in the rat.
*This route has been proposed by Jones and Wells (1981).

文献１５

(P22)

DNA Adducts Formed with Other Dihaloalkanes(> 1-Carbon)

In early work with the GSH-dependent activation, we reported evidence for

activation of DBCP and *tris*-(2,3-dibromopropyl) phosphate, as well as BrCH2CH2Br (Inskeep and Guengerich,1984). Subsequently we found a far lower level of DNA adduct formation in rat liver with ClCH2CH2Cl than BrCH2CH2Br (Inskeep *et al.*, 1986). This halide order also holds in the activation of these dihaloethanes to mutagens in bacteria expressing mammalian GSH transferases (Wheeler *et al.*, 2001a). Apparently the rate of conjugation to generate the initial half-mustard is an issue. DBCP reacts with GSH to generate a series of DNA adducts (Humphreys *et al*., 1991)(Fig. 4). These adducts are complex because of the potential trifunctional alkylating capability of this compound. Reaction of a DBCP-GSH conjugate with calf thymus DNA yielded the *N*7-guanyl adducts plus the intra-strand crosslinked guanines shown in Fig. 4. We have not further evaluated the capability of the reagent to form interstrand crosslinks. We can summarize the work in this area by stating that most 1,2-bifunctional alkanes are capable of causing genotoxic damage by this GSH-dependent pathway, with the leaving group order playing a major role in the extent of binding and biological activities (Thier *et al.*, 1996; Wheeler *et al.*, 2001b).

文献１６

(P297)

The theta-class rat GST 5-5 enzyme is reported to have dehalogenase activity towards dihalomethanes and has been shown to be effective in the conjugation of dichloromethane with glutathione (GSH) . Recently, it was reported that this enzyme shares 82% sequence identity with human theta-class GST Tl.

(P299-300)

We determined the effects of EDB, BCE, 1,2-DCE, and CH_2C1_2 on the rates of cell growth and induction of *umuC* gene expression in the cells (Figure 3). The cell growth rates were retarded very markedly with EDB, BCE, 1,2-DCE and CH_2C1_2 in NM5004 strain as compared with those in the original TA1535/pSK1002 strain. The expression of *umuC* gene by these compounds was induced in a dose-dependent manner in the NM5004 strain but was not affected in TA1535/pSK1002 strain. The order of induction of *umuC* gene expression in NM5004 strain was found to be EDB>BCE>1,2-DCE>CH_2C1_2.

文献１７

(P248)

The human theta subunits are named “hGSTT1” and “hGSTT2” whereas the rat GST 5 or GST 12 subunits were renamed according to their homologies with the human subunits as “rGSTT1” and “rGSTT2”, respectively. The mouse subunit Yrs is now named “mGSTT2” [7, 9, 10]. When the enzyme, instead of the subunit, are named, they

are named with the repeated number of their subunits (i.e. hGSTT1-1, mGSTT"-2, rGSTT1-1, etc.) according to the homodimeric structures. Finally, the gene coding for each subunit adopt the names of the repective submit in italics; for example *hGSTT1* for hGSTT1, *hGSTT2* for hGSTT2, etc.

(P253)

GST theta shows important differences in the catalytic activity compared with the other GSTs [33]. Theta GSTs poorly metabolize the 1-chloro-2.4-dinitrobenzene (CDNB) compared to alpha, mu and phi [4, 10] and are inactive towards ethacrynic acid [13]. Among mammals, GSTT1 or GSTT2 from human, rat and mouse are very similar, whereas the homology of these subunits with mammalian alpha, mu, and phi is weak (19-23%) [6, 18]. The homology mostly relies on the N-terminus of the proteins and on residues 25-27 which are identical and conserved [34]. As previously described, the theta class shows a specific Ser-11 residue in place of the N-terminal tyrosine found in classes alpha, mu, and phi. The residue in this position has a key-role in GSTs for glutathione deprotonation and activation [29, 35, 36] and accounts for the particular enzymatic behavior of the theta class [4, 37]. Any site-directed mutation towards Ser-11 inactivates the theta enzyme by raising the pK_a of bound of GSH and lowering the turnover number [29, 38]. The serine residue, instead of the tyrosine, can form a hydrogen bond with the glutathionyl sulfur atom and this can be responsible for the very different affinity towards GSH in the theta class [37, 39]. These differences raised the fascinating hypothesis that the classes of GSTs evolved with different logics, in the process of GSH detoxification: theta GSTs process the substrates quickly, use GSH at a higher concentration and easily release the product to be ready for a new cycle. The more recently evolved alpha, mu, and phi developed lower catalytic activity and increased affinities towards conjugate-products ending in a higher capability of sequestering the conjugateproducts.

文献１８

(P1499)

CYP2E1 protein was also constantly identified in BEC (Fig. 3), and was approximately 10-fold less abundant than in hepatocytes, while the transcripts were detected in only four of six preparations (Fig. 1, *BEC*-2). This discrepancy can be explained by a difference in the regulation of CYP2E1 mRNA and protein.

(P1501)

We concluded from these results that gallbladder-derived BEC express genes of the CYP1A, CYP2E1, and CYP3A subfamilies. The expression is 5- to 20-fold lower than in hepatocytes, and further differs by a higher proportion of CYP3A5 over CYP3A4 and by the predominance of CYP1A1 over CYP1A2, at least at mRNA levels.

文献１９

(P300)

In rat liver, mRNA to both Theta-class GSTs was present, but at considerably lower levels than in the mouse. The pattern of distribution was similar to the mouse in that the concentration of mRNA was highest in the central vein hepatocytes and bile-duct epithelial cells (Figure4).

文献２０

(P627)

The biliary epithelium in control rats of both sexes stained positively for GST T1-1, but acharacteristic of rats fed benzyl isothiocyanate in particular, was an apparent increased staining in the bile ducts (Figures 3E and 3F).