Mutagenesis vol.14 no.5 pp.479–482, 1999

Induction of genetic damage in human lymphocytes and mutations in *Salmonella* by trihalomethanes: role of red blood cells and *GSTT1-1* polymorphism

Stefano Landi[1,3], Nancy M.Hanley[1], Sarah H.Warren[1], Rex A.Pegram[2] and David M.DeMarini[1]

[1]Environmental Carcinogenesis Division (MD-68) and [2]Experimental Toxicology Division (MD-74), US Environmental Protection Agency, Research Triangle Park, NC 27711, USA

The brominated trihalomethanes (THMs) are mutagenic and carcinogenic disinfection by-products frequently found in chlorinated drinking water. They can be activated to mutagens by the product of the glutathione *S*-transferase-θ (*GSTT1-1*) gene in *Salmonella* RSJ100, which has been transfected with this gene. To evaluate this phenomenon in humans, we have examined the genotoxicity of a brominated THM, bromoform (BF), using the Comet assay in human whole blood cultures exposed *in vitro*. No differences were found in the comet tail length between cultures from *GSTT1-1*$^+$ versus *GSTT1-1*$^-$ individuals (1.67 ± 0.40 and 0.74 ± 0.54 μm/mM, respectively, $P = 0.28$). The high variability was due to the relatively weak induction of comets by BF. Combining the data from both genotypic groups, the genotoxic potency of BF was 1.20 ± 0.34 μm/mM ($P = 0.003$). *GSTT1-1* is expressed in red blood cells but not in the target cells (lymphocytes), and expression within the target cell (as in *Salmonella* RSJ100) may be necessary for enhanced mutagenesis in *GSTT1-1*$^+$ relative to *GSTT1-1*$^-$ cultures. To examine this, we exposed *Salmonella* RSJ100 and a control strain not expressing the gene (TPT100) to the most mutagenic brominated THM detected in *Salmonella*, dibromochloromethane (DBCM), either in the presence or absence of S9 or red blood cells from *GSTT1-1*$^+$ or *GSTT1-1*$^-$ individuals. S9 did not activate DBCM in the non-expressing strain TPT100, and it did not affect the ability of the expressing strain RSJ100 to activate DBCM. As with S9, red cells from either genotypic group were unable to activate DBCM in TPT100. However, red cells (whole or lysed) from both genotypic groups completely repressed the ability of the expressing strain RSJ100 to activate DBCM to a mutagen. Such results suggest a model in which exposure to brominated THMs may pose an excess genotoxic risk in *GSTT1-1*$^+$ individuals to those organs and tissues that both express this gene and come into direct contact with the brominated THM, such as the colon. In contrast, those organs to which brominated THMs would be transported via the blood might be protected by erythrocytes. Such a proposal is reasonably consistent with the organ specificity of drinking water-associated cancer in humans, which shows slightly elevated risks for cancer of the colon and bladder but not of the liver.

Introduction

Bromoform (BF) and dibromochloromethane (DBCM) are brominated trihalomethanes (THMs) that are frequently found as disinfection by-products in chlorinated drinking water (International Agency for Research on Cancer, 1991). The brominated THMs are carcinogenic in rodents at high doses, causing colon, kidney and liver tumors, and they are mutagenic in a variety of systems (Marimoto and Koizumi, 1983; International Agency for Research on Cancer, 1991). Epidemiological studies have demonstrated slightly elevated risks for cancer of the bladder and colon as well as for developmental problems among people who drink chlorinated water versus those who do not (International Agency for Research on Cancer, 1991; Morris *et al.*, 1992; Reif *et al.*, 1996; Koivusalo and Vartiainen, 1997; Hildesheim *et al.*, 1998; Waller *et al.*, 1998).

The metabolism of the brominated THMs is not completely understood. The primary route of metabolism may involve an oxidation reaction that leads to production of the dihalocarbonyl and CO_2 (International Agency for Research on Cancer, 1991). However, mono- and dihalogenated ethanes or methanes, such as dichloromethane, are chemically similar to THMs and are known to undergo an oxidation reaction that results in the formation of formaldehyde. Glutathione *S*-transferase-θ (GSTT1-1) mediates this activation step in which the reduced glutathione is not consumed (Ahmed *et al.*, 1980; Abdel-Rahman *et al.*, 1984; Andersen *et al.*, 1987; Thier *et al.*, 1993, 1996; Graves *et al.*, 1994; Hallier *et al.*, 1994; Graves and Green, 1996). The brominated THMs likely share some of the same pathways because recent studies have shown that BF and DBCM are activated to mutagens in a transgenic strain of *Salmonella* (RSJ100) containing the rat homolog of the *GSTT1-1* gene (DeMarini *et al.*, 1997; Pegram *et al.*, 1997).

GSTT1-1 is polymorphic in humans, with 20–25% of Caucasians and 50% of Asians having a homozygous deletion of this gene, resulting in a null genotype (*GSTT1-1*$^-$) (Pemble *et al.*, 1994; Kelsey *et al.*, 1995; Wiencke *et al.*, 1995). The results in *Salmonella* suggest that people carrying at least one copy of the gene (*GSTT1-1*$^+$) might be more susceptible to the genotoxic effects of the brominated THMs than those missing the gene (*GSTT1-1*$^-$). In a previous study, we tried to verify this hypothesis by evaluating the ability of BF to induce sister chromatid exchanges (SCEs) *in vitro* in whole blood cultures from the two groups of people (Landi *et al.*, 1999). Although we found no enhanced induction of SCEs among the *GSTT1-1*$^+$ individuals, SCEs may not have been a suitable end-point and/or the experimental design may have prevented detection of increased mutagenicity in *GSTT1-1*$^+$ individuals because the enzyme is expressed in red blood cells, which were present in the cultures, but the enzyme is not expressed in the target cells (lymphocytes).

In the present study we have again evaluated the genotoxicity of BF in whole blood cultures from *GSTT1-1*$^+$ and *GSTT1-1*$^-$ individuals, but we have used the single cell gel electrophoresis (SCGE or Comet) assay to score for a different end-point, DNA breaks or alkali-labile sites. A previous study (Thier *et al.*, 1993) exposed liquid suspension cultures of *Salmonella*

[3]To whom correspondence should be addressed. Tel: +1 919 541 1309; Fax: +1 919 541 0694; Email: landi.stefano@epa.gov

RSJ100 to dihalomethanes and showed that the glutathione *S*-transferase-mediated metabolites had to be generated inside the cell in order to be mutagenic. Therefore, we have examined this issue relative to THMs by exposing *Salmonella* RSJ100 to vapor of the most mutagenic brominated THM detected in *Salmonella* (DeMarini *et al.*, 1997), DBCM, either in the presence or absence of rat liver S9 or red blood cells from *GSTT1-1*$^+$ or *GSTT1-1*$^-$ individuals. The inability of rat liver S9 or red blood cells from *GSTT1-1*$^+$ individuals to activate DBCM to a mutagen would indicate that, as with dihalomethanes, the THMs must be activated via *GSTT1-1* within the target cell.

Materials and methods

Blood donors and preparation

Blood was collected from four anonymous individuals after they had completed a questionnaire regarding their medical history and signed a consent form. Blood was collected by venipuncture into heparinized tubes and kept at room temperature for 1–4 h until use. For whole blood suspensions, 0.6 ml of heparinized blood was added to 9.4 ml of RPMI 1640 medium with glutamax (Gibco BRL, Rockville, MD). To obtain isolated red cells, whole blood was centrifuged in a Ficoll Isopaque (Sigma, St Louis, MO) density gradient according to the manufacturer's instructions. The red cell pellet was washed twice in RPMI 1640 medium with glutamax, concentrated by centrifugation, counted, and diluted appropriately in RPMI 1640 with glutamax. Red cells were lysed by adding an equal volume of 0.075 M KCl to the cell pellet (10^{10} cells/ml) and mixing vigorously using a vortex mixer. The lysed cells were then diluted in RPMI 1640 with glutamax to the appropriate concentrations.

Comet assay

BF (purity >99%; Aldrich, Milwaukee, WI) was diluted in dimethyl sulfoxide (DMSO; Burdick and Jackson, Muskegon, MI) and 50 µl aliquots of various concentrations were added to 10 ml whole blood cultures prepared as described above in glass tubes; control cultures received 50 µl of DMSO. After incubating on a roller wheel for 3 h at 37°C, the cells were prepared for the Comet assay according to standard procedures (Singh *et al.*, 1988). Briefly, 0.5% normal melting point agarose was prepared in calcium/magnesium-free phosphate-buffered saline and used to make the first layer on the slides; 0.7% low melting point agarose prepared similarly together with 3×10^5 cells was used to make the second layer. After cells were lysed with lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Triton X-100, and 10% DMSO, pH 10), the slides were placed for 20 min in a horizontal electrophoresis unit containing buffer composed of 1 mM Na_2EDTA and 300 mM NaOH, pH 13. Cells were then electrophoresed for 30 min at 25 V and 300 mA, after which the cells were neutralized with 0.4 M Tris–HCl (pH 7.5), dried in ethanol and stored in the dark at room temperature. After staining with ethidium bromide, the cells were assessed for DNA breaks by fluorescence microscopy by measuring the lengths of the comet tails with an ocular micrometer; 100 nuclei were scored per dose, each in duplicate.

Salmonella *assay*

Salmonella typhimurium strains RSJ100 and TPT100 were kindly provided by Dr F.P. Guengerich (Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN). Strain RSJ100 was constructed by transfecting the base substitution strain TA1535 (*hisG46, rfa,* Δ*uvrB*) with the rat glutathione *S*-transferase gene *GSTT1-1*; strain TPT100 is identical to RSJ100 except that the *GSTT1-1* gene is in the opposite orientation, making it non-functional (Thier *et al.*, 1993). The standard plate-incorporation assay was used with modifications for testing volatile chemicals (Hughes *et al.*, 1987) as described previously (Pegram *et al.*, 1997). S9 was from aroclor 1254-induced Sprague–Dawley rat liver.

Briefly, DBCM vapor (Pegram *et al.*, 1997) was injected into sealed 4 l Tedlar bags containing: (i) a volume of sterile air necessary to achieve the desired final chemical concentration; (ii) six glass Petri dishes in which the bacteria and red blood cells (whole or lysed, prepared as described above) or Sprague–Dawley aroclor 1254-induced rat liver S9 had been plated just prior to exposure. The six plates/bag represented one plate of each S9 or red cell concentration. A duplicate set of six plates was placed in a second bag and these bags were exposed in parallel. Thus, the experiment was performed once, but duplicate sets of plates (each set in separate bags) were used to generate replicated data. A Hewlett Packard 5890A gas chromatograph equipped as described previously (Pegram *et al.*, 1997) was used to quantify the vapor concentrations in the bags. After 24 h of exposure to DBCM vapor at 37°C, the plates were removed from the bags, incubated for an additional 48 h at 37°C, and the revertant (rev) colonies counted.

Table I. Sex, age and genotypes of subjects

Subject	Sex	Age	*GSTM1-1*	*GSTT1-1*
0	Female	40	–	–
1	Male	27	–	–
3	Male	31	–	+
5	Male	48	+	+
14	Female	34	+	+
32	Male	26	+	–

Table II. Tail length (µm ± SE) of control and BF-exposed lymphocyte DNA

Subject	BF concentration (M)			
	0	10^{-4}	10^{-3}	10^{-2}
0	42.7 ± 1.7	38.3 ± 1.6	40.5 ± 1.7	50.3 ± 2.4
1	45.2 ± 2.4	46.5 ± 2.0	58.4 ± 3.0	53.6 ± 2.6
3	42.7 ± 2.0	38.8 ± 1.9	48.8 ± 2.5	52.3 ± 2.7
5	36.6 ± 1.8	37.1 ± 1.5	39.9 ± 1.9	60.3 ± 2.4

Genotyping

A multiplex PCR was performed as described previously except that 6.2 instead of 3.3 mM $MgCl_2$ was the final concentration in the reaction (Bell and Pittman, 1998). Briefly, genomic DNA was isolated by standard methods from a portion of the blood samples and a multiplex PCR was performed in which *GSTM1-1* and *GSTT1-1* were co-amplified together with β-globin as an internal positive control. Although *GSTM1* is not known to be involved in THM metabolism, our laboratory performs the multiplex genotype assay routinely. Consequently, data regarding a possible influence of the *GSTM1* gene on THM metabolism were also available to us for consideration.

Statistical analysis

The genotoxic potencies of BF in the Comet assays were calculated initially as the slope of the dose–response curves using linear regression analysis. However, because the concentration–response relationships were generally not linear, the response was recalculated as the net increase in average comet tail lengths above the spontaneous value at the top dose. Student's *t*-test was used to compare the responses between the different genotypes. In the *Salmonella* experiments, data for each dose of DBCM and each concentration of red cells were pooled. The comparisons between genotypes or among groups of plates with or without red cells were done using Student's *t*-test.

Results and discussion

Table I shows the age, sex, and genotype of the subjects whose blood was used for the Comet assay. As shown previously with SCEs (Landi *et al.*, 1999), the DNA damaging potency of BF was not significantly different between *GSTT1-1*$^+$ and *GSTT1-1*$^-$ subjects, with the genotoxic potency being 1.67 ± 0.40 versus 0.74 ± 0.54 µm/mM, respectively ($P = 0.28$). Nonetheless, as with SCE induction (Landi *et al.*, 1999), BF also induced DNA damage, as evidenced by a weak but significant increase in DNA tail length (1.20 ± 0.34 µm/mM, $P = 0.003$), based on combining the data from both genotypic groups (Table II). SCEs and the Comet assay are highly sensitive assays for DNA damage, and it seemed unlikely to us that if there were a different genotoxic response between the two groups of people that we would not have detected it using one or other of these end-points.

Although BF was not more genotoxic (for either SCEs or DNA damage) in lymphocytes cultured in the presence of red blood cells from *GSTT1-1*$^+$ versus *GSTT1-1*$^-$ individuals, BF and the other brominated THMs are activated to potent

Table III. Mutagenicity of DBCM in *Salmonella* ± red blood cells

Dose (p.p.m.)	Red cells		Revertants/plate ± SE[a]			
	Type	No./plate	TPT100		RSJ100	
			GSTT1-1^{-b}	*GSTT1-1*$^{+b}$	*GSTT1-1*$^{-b}$	*GSTT1-1*$^{+b}$
0	Whole	10^5–10^6	18.5 ± 3.5	17.0 ± 0.7	16.5 ± 6.4	24.0 ± 4.2
		10^7–10^9	4.0 ± 1.4	8.0 ± 1.4	7.0 ± 1.4	9.0 ± 8.5
800		10^5–10^6	17.0 ± 4.2	28.0 ± 1.4	18.5 ± 0.7	22.5 ± 6.4
		10^7–10^9	14.5 ± 3.5	13.5 ± 0.7	8.0 ± 0.7	16.0 ± 14.1
0	Lysed	10^5–10^6	20.0 ± 1.4	16.0 ± 0.7	18.5 ± 3.5	21.5 ± 7.8
		10^7–10^9	10.5 ± 6.4	8.5 ± 4.9	13.0 ± 1.4	9.0 ± 0.7
800		10^5–10^6	24.0 ± 5.7	23.5 ± 5.0	18.5 ± 0.7	21.5 ± 5.0
		10^7–10^9	12.5 ± 5.0	10.0 ± 2.8	16.5 ± 2.1	8.0 ± 0.7

[a]Revertants/plate in the absence of red cells with and without DBCM are reported in Table IV; the data in Table IV are the control data with which the data in Table III should be compared. Data are the averages ± SE from duplicate plates (one set of plates in one bag and another set in another bag) from one experiment.
[b]*GSTT1-1*$^-$ is subject no. 32; *GSTT1-1*$^+$ is subject no. 14.

mutagens by the product of the *GSTT1-1* gene when the gene is expressed inside the target cells (*Salmonella* RSJ100) (DeMarini *et al.*, 1997; Pegram *et al.*, 1997). A previous study with dihalomethanes in *Salmonella* showed that activation by *GSTT1-1* outside the cell would not produce any mutagenesis (Thier *et al.*, 1993). Thus, instead of the end-points (SCEs and DNA damage) being inappropriate, we suspected that the problem in detecting increased genotoxicity in the presence of *GSTT1-1* may have been due to the fact that the target cells (lymphocytes) did not express *GSTT1-1*, which was expressed, instead, by the co-cultured red cells.

Therefore, to explore the effect of the presence of red cells in the culture, we performed the following studies in *Salmonella*. *Salmonella* cells were exposed to DBCM in the presence of whole or lysed red cells from donors who were either *GSTT1-1*$^+$ or *GSTT1-1*$^-$. This permitted us to evaluate the influence of extracellular GSTT1-1 (supplied by red cells from *GSTT1-1*$^+$ donors) on the mutagenicity of DBCM in strains of *Salmonella* that can (RSJ100) or cannot (TPT100) activate DBCM to a mutagen.

Red cells from either genotypic group were not able to activate DBCM in the non-expressing strain TPT100 (Table III). However, red cells from both genotypic groups completely repressed or abrogated the ability of the expressing strain RSJ100 to activate DBCM to a mutagen (Table III). This effect was observed irrespective of the genotype through a range of concentrations of either whole or lysed red cells. Only the highest concentrations of whole or lysed cells (10^7–10^9/plate) appeared to be toxic to *Salmonella*, as evidenced by an ~50% reduction in the spontaneous mutant yield relative to plates with low concentrations of red cells or no red cells (Table III).

The fact that red cells from even *GSTT1-1*$^-$ individuals could totally inhibit the mutagenicity of DBCM in strain RSJ100 indicated that erythrocytes can sequester or inactivate DBCM, preventing the chemical from reaching the *Salmonella* cells and that *GSTT1-1* does not exert any role in this mechanism. Indeed, the efficient interference exhibited by red cells in this study in *Salmonella* may explain the relatively low frequencies of BF-induced SCEs in lymphocytes in the presence of red cells (Landi *et al.*, 1999).

To further examine this inhibition of DCMB mutagenicity by red cells, we replaced red cells with rat liver S9. The results showed that rat liver S9 was not able to activate DBCM in the non-expressing strain TPT100 and that S9 had no effect on the ability of the expressing strain RSJ100 to activate DBCM (Table IV). Thus, even the presence of S9 protein did not result in any significant non-specific binding of the DBCM molecule to significantly reduce the mutagenic effect of this THM in RSJ100.

Table IV. Mutagenicity of DBCM in *Salmonella* ± rat liver S9

Dose (p.p.m.)	Revertants/plate ± SE			
	TPT100		RSJ100	
	–S9	+S9	–S9	+S9
0	22.5 ± 14.4	12.3 ± 6.8	16.3 ± 2.2	11.5 ± 6.3
800	18.5 ± 0.7	18.0 ± 0.3	1110.0 ± 28.0	1018.0 ± 41.0

Considering these results and other data (DeMarini *et al.*, 1997; Pegram *et al.*, 1997; Landi *et al.*, 1999), THMs are clearly activated to mutagens in exposed cells that express *GSTT1-1* (*Salmonella* RSJ100), but human erythrocytes from either *GSTT1-1*$^+$ or *GSTT1-1*$^-$ donors appear to sequester and/or inactivate THMs, preventing the mutagenicity of THMs in cells that either express *GSTT1-1* (*Salmonella* RSJ100) or do not express this enzyme (*Salmonella* TPT100 and human lymphocytes).

Such results suggest a model in which exposure to THMs (e.g. from chlorinated drinking water) may pose an excess genotoxic risk in *GSTT1-1*$^+$ individuals to those organs and tissues that both express this gene and come into direct contact with the THM. This could include the gastrointestinal tract, such as the colon (Juronen *et al.*, 1996). In contrast, organs to which THMs would be transported via the blood, such as the liver, might be considered to be protected by erythrocytes. This proposal is in reasonable agreement with the organ specificity of drinking water-associated cancer in humans, which shows slightly elevated risks for cancer of the rectum and bladder but not of the liver (International Agency for Research on Cancer, 1991; Morris *et al.*, 1992). Although some portion of ingested THMs would contact the bladder after filtration through the blood (and contact with red cells), it may be possible that another portion may arrive at the bladder without prior contact with or binding to red cells. In this form, THMs could then enter bladder cells and be activated

in situ. *GSTT1-1*$^{-}$ people might be at less risk than *GSTT1-1*$^{+}$ individuals; however, THMs may be activated by other pathways, presenting a risk regardless of *GSTT1-1* status (DeMarini *et al.*, 1997).

Acknowledgements

We thank the staff at Integrated Laboratory Systems for providing the training and facilities to perform the Comet assay. S.L. acknowledges the support of a Research Associateship Award from the National Research Council, US National Academy of Sciences. This manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Abdel-Rahman,M.S., Suh,D.H. and Bull,R.J. (1984) Pharmacodynamics and toxicity of chlorine in drinking water in the rat. *J. Appl. Toxicol.*, **4**, 82–86.

Ahmed,A.E., Kubic,V.L., Stevens,J.L. and Anders,M.W. (1980) Halogenated methanes: metabolism and toxicity. *Fed. Proc.*, **39**, 3150–3155.

Andersen,M.E., Clewell,H.J., Gargas,M.L., Smith,F.A. and Reitz,R.H. (1987) Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.*, **87**, 185–205.

Bell,D. and Pittman,G. (1998) Genotype analysis. In Vanden Heuvel,L.P. (ed.), *PCR Protocols in Molecular Toxicology.* CRC Press, Boca Raton, FL, pp. 163–176.

DeMarini,D.M., Shelton,M.L., Warren,S.H., Ross,T.M., Shim,J.-Y., Richard, A.M. and Pegram,R.A. (1997) Glutathione *S*-transferase-mediated induction of GC→AT transitions by halomethanes in *Salmonella*. *Environ. Mol. Mutagen.*, **30**, 440–447.

Graves,R.J. and Green,T. (1996) Mouse liver glutathione *S*-transferase-mediated metabolism of methylene chloride to a mutagen in the CHO/HPRT assay. *Mutat. Res.*, **367**, 143–150.

Graves,R.J., Callander,R.D. and Green,T. (1994) The role of formaldehyde and *S*-chloromethylglutathione in the bacterial mutagenicity of methylene chloride. *Mutat. Res.*, **320**, 235–243.

Hallier,E., Schroder,K.R., Asmuth,K., Dommermuth,A., Aust,B. and Goergens, H.W. (1994) Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influence of polymorphism of glutathione transferase theta (GSTT1–1). *Arch. Toxicol.*, **68**, 423–427.

Hildesheim,M.E., Cantor,K.P., Lynch,C.F., Dosemeci,M., Lubin,J., Alavanja,M. and Craun,G. (1998) Drinking water source and chlorination byproducts. II. Risk of colon and rectal cancers. *Epidemiology*, **9**, 29–35.

Hughes,T.J., Simmons,D.M., Monteith,L.G. and Claxton,L.D. (1987) Vaporization technique to measure mutagenic activity of volatile organic chemicals in the Ames/*Salmonella* assay. *Environ. Mol. Mutagen.*, **9**, 421–441.

International Agency for Research on Cancer (1991) *IARC Monographs on the Evaluation of Carcinogenic risks to Humans. Chlorinated Drinking-Water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*. IARC Scientific Publications no. 52, IARC, Lyon.

Juronen,E., Tasa,G., Uuskula,M., Pooga,M. and Mikelsaar,A.-V. (1996) Purification, characterization and tissue distribution of human class theta glutathione S-transferase T1-1. *Biochem. Mol. Biol. Int.*, **39**, 21–29.

Kelsey,K.T., Wiencke,J.K., Ward,J., Bechtold,W. and Fajen,J. (1995) Sister-chromatid exchanges, glutathione *S*-transferase theta deletion and cytogenetic sensitivity to diepoxybutane in lymphocytes from butadiene monomer production workers. *Mutat. Res.*, **335**, 267–273.

Koivusalo,M. and Vartiainen,T. (1997) Drinking water chlorination by-products and cancer. *Rev. Environ. Health*, **12**, 81–90.

Landi,S., Ponzanelli,I., Hirvonen,A., Norppa,H. and Barale, R. (1996) Repeated analysis of sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes: effect of glutathione *S*-transferase T1 and M1 genotype. *Mutat. Res.*, **351**, 79–85.

Landi,S., Hanley,N.M., Kligerman,A.D. and DeMarini,D.M. (1999) Induction of sister chromatid exchanges in human peripheral blood lymphocytes by bromoform: investigation of the role of *GSTT1-1* polymorphism. *Mutat. Res.*, in press.

Marimoto,K. and Koizumi,A. (1983) Trihalomethanes induce sister chromatid exchanges in human lymphocytes *in vitro* and mouse bone marrow cells *in vivo*. *Environ. Res.*, **32**, 72–79.

Morris,R.D., Audet,A.M., Angelillo,I.F., Chalmers,T.C. and Mosteller,F. (1992) Chlorination, chlorination by-products, and cancer: a meta-analysis. *Am. J. Public Health*, **82**, 955–963.

Pegram,R.A., Andersen,M.E., Warren,S.H., Ross,T.M. and Claxton,L.D. (1997) Glutathione *S*-transferase-mediated mutagenicity of trihalomethanes in *Salmonella typhimurium*: contrasting results with bromodichloromethane and chloroform. *Toxicol. Appl. Pharmacol.*, **144**, 183–188.

Pemble,S., Schroeder,K.R., Spencer,S.R., Meyer,D.J., Hallier,E., Bolt,H.M., Ketterer,B. and Taylor,J.B. (1994) Human glutathione *S*-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism. *Biochem. J.*, **300**, 271–276.

Reif,J.S., Hatch,M.C., Bracken,M., Holmes,L.B., Schwetz,B.A. and Singer,P.C. (1996) Reproductive and developmental effects of disinfection by-products in drinking water. *Environ. Health Perspect.*, **104**, 1056–1061.

Singh,N.P., McCoy,M.T., Tice,R.R. and Schneider,E.L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.*, **175**, 184–191.

Thier,R., Taylor,J.B, Pemble,S.E., Humphreys,W.G., Persmark,M., Ketterer,B. and Guengerich,F.P. (1993) Expression of mammalian glutathione *S*-transferase 5-5 in *Salmonella typhimurium* TA1535 leads to base-pair mutations upon exposure to dihalomethanes. *Proc. Natl Acad. Sci. USA*, **90**, 8576–8580.

Thier,R., Pemble,S.E., Kramer,H., Taylor,J.B., Guengerich,F.P. and Ketterer,B. (1996) Human glutathione *S*-transferase T1-1 enhances mutagenicity of 1,2-dibromoethane, dibromomethane and 1,2,3,4,-diepoxybutane in *Salmonella typhimurium*. *Carcinogenesis*, **17**, 163–166.

Waller,K., Swan,S.H., DeLorenze,G. and Hopkins,B. (1998) Trihalomethanes in drinking water and spontaneous abortion. *Epidemiology*, **9**, 134–140.

Wiencke,J.K., Pemble,S., Ketterer,B. and Kelsey,K.T. (1995) Gene deletion of glutathione *S*-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 253–259.

Received on January 22, 1999; accepted on May 25, 1999