ROBUST STUDY SUMMARIES:
Critical Studies Cited in the 1,2-Dibromoethane (1,2-DBE) Targeted Assessment Profile for Human Health but Not Referenced in IARC (1999; Vol. 71: 641-699)

Table of Contents

1.0 Genotoxicity 2

1.0 Genotoxicity

CHEMICAL:

Name (CAS No.): 1,2-Dibromoethane (CAS RN 106-93-4)
Purity: Not stated (obtained from Sigma-Aldrich, St. Louis, Missouri, USA).

METHOD:

Method/Guideline: Other.
Type of study: *lacZ* reversion mutagenicity assay..
GLP: Not stated.
Year: ≤ 2006
Species and strain: Bacterium, *Escherichia coli lac*Z
Bacterial strain construction: 1,2-DBE is an Ames test mutagen in strains that revert by base-pair substitutions but not in strains that revert by frameshifts. The characteristic event dominating 1,2-DBE mutational spectra is the G:C → A:T transition, which is also commonly seen with alkylating agents. The episomal *lacZ* allele of strain CC102 reverts by a G:C → A:T transition (GGG, encoding glycine, to GAG, encoding glutamic acid) . The mutagenicity of 1,2-DBE in E. coli is reduced in the presence of an active DNA excision repair system but does not require the activity of Y-class DNA polymerases such as UmuDC or MucAB. Therefore, to establish an E. coli *lacZ* tester strain suitable for the detection of 1,2-DBE mutagenicity, the episome of strain CC102 was moved (by conjugation) into a previously constructed E. coli strain that lacks DNA nucleotide excision repair.
All plasmids for recombinant GST expression were derivatives of pKK-D and were transferred into E. coli XL1-Blue cells or tester strains by electroporation. Plasmid DNA was prepared using the Wizard1 Plus mini-prep DNA purification system (Promega, Madison, WI). Ampicillin (100 μg/mL) was used for selection of plasmid-bearing cells.
***lacZ* reversion assay:** The T1/T2 library was previously created by DNA shuffling of the cDNAs encoding human GST T1-1 and rat GST T2-2 . The lacZ reversion assay was used to screen this library for clones exhibiting mutagenic responses to 1,2-DBE (0.5 μg/plate) differing from that of the parental strains (expressing wild-type human GST T1-1 or rat GST T2-2). Plates were grown for approximately 40 hr at 378C (longer for strains expressing hexa-histidine-tagged GSTs).
Hexa-histidine tagging: A hexa-histidine tag was added to the N-terminus of several recombinant GSTs. The entire plasmid containing the GST insert was amplified by PCR and the resulting linear DNA was then self-ligated to form a circular molecule. Primers were designed specifically for each clone as follows: hT1 forward Eco His (50-ATAT GAA TTC ATG CAT CAC CAT CAT CAT CAC ATG GGC CTT GAG CTG-30) was the forward primer used for clones 3 and 89. rT2 forward Eco His (50-ATAT GAA TTC ATG CAT CAC CAT CAT CAT CAC ATG GGT TTG GAG CTC-30) was the forward primer used for clone 57. hT1 reverse HindSalBam (50-ATAT AAG CTT GTC GAC GGA TCC TTA TTA ACG GAT CAT GGC CAG-30) was the reverse primer used for all clones except clone 3, which required hT1 reverse HindSalBam short (50-ATAT AAG CTT GTC GAC GGA TCC TTA TTA ACG GAT CAT GGC-30) as its reverse primer.
The PCR mixture consisted of mini-prep DNA, dNTPs, primers, 10X Pfu buffer, and Pfu DNA polymerase, as recommended by the manufacturer and H2O; total reaction volume = 50 μL. The PCR was initiated with 3 min at 95°C; followed by 35 cycles consisting of 1 min at 94°C, 2 min at 50°C, and 2 min at 72°C; and concluded with 10 min at 72°C. PCR products were purified by gel extraction from a 1% agarose gel, digested with *Eco*RI and *Hind*III to remove primer overhangs, repurified, and ligated into the pKK-D vector. Reactions were incubated overnight at room temperature. The entire open reading frame of each variant was sequenced.
GST Expression and purification of hexa-histidine-tagged recombinant GST enzymes: Cell lysates of selected clones were prepared and recombinant GST protein expression levels in the lysates were determined by SDS-PAGE gel analysis. The hexa histidine-tagged recombinant GST enzymes were purified from cell lysates by immobilized metal ion affinity chromatography

(IMAC) on ProfinityTM IMAC Ni-charged resin. The volume of lysate added to the column was 0.75 mL. The column was washed with binding/wash buffer to remove unbound protein and GST was eluted with buffer containing 500 mM imidazole. The eluted protein was dialyzed overnight against 10 mM Tris-HCl buffer, pH 7.8, supplemented with 1.0 mM b-mercaptoethanol, 20% (v/v) glycerol, and 0.02% (w/v) sodium azide, and stored at -20°C.

GST Assays: 1,2-Epoxy-3-(4-nitrophenoxy)-propane (EPNP) conjugation: Activities of lysates were measured at room temperature (22°C) . The reaction mixture consisted of EPNP, GSH, lysate, and sodium phosphate buffer, pH 6.5; 10–100 µg protein; total reaction volume = 1 mL. Michaelis constant (Km) determinations were carried out with the same protocol. Three trials were done on separate days and the data were averaged. Km and Vmax analysis was done using a nonlinear curve fitting algorithm .

Ethylene diiodide (EDI) conjugation was measured by the absorbance change at 226 nm . The incubations contained diiodoethane and GSH in sodium phosphate buffer, pH 7.0; 10–100 µg protein, total reaction volume= 1 mL; extinction coefficient of product = 4.5 mM^{-1} $cm^{-1.}$

Statistical analysis: Means and standard variations for revertant yield and specific activities in the GST assays provided. Statistical significance not provided.

RESULTS:

E. coli strain suitable for detection of 1,2-DBE mutagenicity: Construction of an E. coli *lacZ* tester strain that detects 1,2-DBE mutagenicity was accomplished by expressing recombinant class Theta GSTs in a strain that is UvrABC deficient and incorporates the G:C → A:T transition mutation *lacZ* target (JM106 F0102). The presence of the GST expression plasmids was confirmed by restriction digest analysis and DNA sequencing of mini-preps. Strain JM106 F0102 was sensitive to the positive control mutagens N-ethyl-N0-nitro-N-nitrosoguanidine, N-methyl-N0-nitro-N-nitrosoguanidine, 4-NQO, and UV light.

Activation of 1,2-DBE to a mutagen by human GST T1-1: Expression of human GST T1-1 resulted in a strong mutational response to 1,2-DBE (*lac*Z revertants per plate were > 10-fold higher than background at 50 ng/plate and increased with dose to > 100-fold background), whereas only a slight response was observed with expression of rat GST T2-2.

Activation of 1,2-DBE by GST sequence variants: Plasmid DNA containing pools of human GST T1-1 and rat GST T2-2 were transformed into the *lacZ* tester strain and tested for 1,2-DBE mutagenicity. A broad range of responses was observed, indicating that the pool contains variant enzymes showing activities intermediate between those of rat GST T2-2 and human GST T1-1. GST enzyme expression was low. To facilitate purification, it was decided to attach an N-terminal hexa-histidine tag to the recombinant enzyme and this was conducted for wild-type human GST T1-1 and 3 sequence variants obtained from the screening study, chosen on the basis of differing 1,2-DBE responses compared to the wild-type enzyme.

Hexa-Histidine-Tagged Human GST T1-1: The plasmid vector for expression of the hexa-histidine-tagged human GST T1-1 was introduced into the *lacZ* background strain. This strain was very sensitive to 1,2-DBE mutagenicity. The revertants yield was > 10-fold higher relative to spontaneous background at 1 ng 1,2-DBE per plate (24.5 vs. 1.7 revertants/plate). The N-terminal hexa-histidine tag was also attached to the three variant enzyme clones (3, 57, and 89). The variant clones were identical to the human GST T1-1 sequence except for point mutations leading to the following amino acid substitutions: variant 89, L89P and R94C; variant 57, A180T; variant 3, V169I, and L236P. Relative to the wild-type enzyme, variant 89 gave a similar level of expression and yield of purified protein. Variant 57 was significantly less effective than the wild-type, in terms of 1,2-DBE mutagenicity, and both the yield of purified GST protein and its specific activity were also much reduced. Variant 3 gave a very slight 1,2-DBE mutagenicity response and both protein expression and enzyme activity were below the limits of detection. Results are shown in table 1 below.

TABLE 1. Hexa-Histidine-Tagged Human GST Theta 1-1 Variants: Expression and Activity

			Affinity-purified protein		
				Sp. act.	
GST	Expression	1,2-DBE Mutagenicity	Yield (mg)	ethylene diiodide (1,2-diiodoethane); (EDI)	1,2-epoxy-3-(40-nitrophenoxy)-propane (EPNP)
None	N	0	Not done	Not done	Not done
GST	N	++	Not done	Not done	Not done
His6GST	Y	+++	2.13 (0.6)	1.10 (0.26)	2.97 (0.61)
Variant 3 (V169I, L236P)	N	+	0.084 (4%)	Not done	<0.2
Variant 57 (A180T)	N	++	0.17 (0.05) (8%)	0.58 (0.10) (53%)	0.31 (0.14) (10.5%)
Variant 89 (L89P, R94C)	Y	+++	2.10 (0.2) (99%)	0.97 (0.13) (88.5%)	1.47 (0.38) (49.4%)

Bacterial strains: The first row corresponds to the E. coli background strain that carries the *lacZ* mutational target but does not carry the GST expression plasmid. The second and third rows correspond to strains carrying plasmids for expression of wild-type human GST Theta 1-1 with and without the N-terminal hexa-histidine tag, respectively. Rows 4–6 correspond to strains carrying plasmids for expression of hexa-histidine-tagged variant GST enzymes, identified by the screening strategy. The sequence changes, relative to the wild-type human GST Theta 1-1 sequence, are stated in parentheses. GST expression: Column 2 indicates the levels of recombinant GST protein expression, as measured by SDS-PAGE gel. "Y" indicates that the protein band was detectable on the gel, and "N" indicates that it was not detectable. Column 3 provides a semi-quantitative ranking of the EDB mutagenicity response of the strain carrying the indicated GST expression.. Column 4 indicates the yield of purified GST enzyme following affinity purification (see Methods). The final two columns indicate the enzyme activity of the purified GST with respect to conjugation of EDI and EPNP, respectively (units: µmol per min per mg protein). All data represent three independent trials (separate extractions/protein purifications from cultures grown on different days). Quantitative data indicate means, with the standard deviation (n = 3) given in parentheses (except for variant 3, for which data were not replicated). For the variants, the percent yield and specific activities relative to the wild-type (100%) are also given (parentheses). For variant 3, EPNP activity was below the limit of detection.

CONCLUSIONS:

The *lacZ* reversion mutagenicity assay was positive in *E. coli* containing GST-expression plasmids Also, a good correlation was found between the results of the 1,2-DBE mutagenicity assay and the results of the enzyme activity measurements.

RELIABILITY:

(2) Valid with restrictions.

GENERAL REMARKS:

The authors used a screening strategy called detection of active variant enzymes by reversion assay/mutagen activation (DAVERAMA). In this strategy, pools of sequence-variant enzymes (generated, for example, by random mutagenesis of the open reading frame) can be screened rapidly for clones with altered activity, using the mutagenicity response as an endpoint. The authors stated, "This correlation demonstrates the power of the DAVERAMA screening approach, which allows the detection of enzymes with variant properties, in terms of expression level or catalytic properties."

REFERENCES:

Josephy PD, Taylor PL, Vervaet G, Mannervik B. 2006. Screening and characterization of variant theta-class glutathione transferases catalyzing the activation of ethylene dibromide to a mutagen. Environ Mol Mutagen 47:657–665.

CHEMICAL:

Name: 1,2-Dibromoethane (CAS RN 106-93-4)
Purity: Not stated (obtained from Wako, Osaka, Japan)

METHOD:

Method/Guideline: Comet assay = Single cell gel (SCG) electrophoresis assay
Type of study: DNA damage study.
GLP: Not stated.
Year: ≤ 1998
Species and strain: Mouse, CD-1
No. /sex: 4 males/group/sampling time. The animals were killed at 0 (zero-time control), 3, and 24 h after treatment.
Analyses conducted: 1,2-DBE was injected intraperitoneally at 100 mg/kg bw. From shortly after treatment until just before they were killed, the animals were observed for pharmacotoxic signs. At each sacrifice, 7 organs were collected: stomach, liver, kidney, urinary bladder, lung, brain, and bone marrow. Necropsies were performed, and the organs were examined for changes in size, color, and texture. A small portion of each organ was fixed by 10% formaldehyde solution and embedded in paraffin. Sections were cut, stained with hematoxylin-eosin, and histopathological observation was conducted when positive results were obtained in the alkaline SCG assay. Slides for the alkaline SCG assay prepared using nuclei isolated by homogenization were placed in a chilled lysing solution and kept at 0°C in the dark for 60 mm. After the slides were left in chilled alkaline solution for 10 min in the dark at 0°C, electrophoresis was conducted at 0°C in the dark for 15 min at 25 V and approximately 250 mA. The neutralized slides were stained with 50 µl of 20 µg/ml ethidium bromide. Fifty nuclei were examined per slide per organ at 200 X magnification. The length of the whole comet (‘length’) and the diameter of the head (‘diameter’) were measured for 50 nuclei per organ per mouse. Migration was calculated as the difference between length and diameter.

Statistical analysis: The average of 4 mean migrations of 50 nuclei was analyzed by one-way ANOVA and Dunnett t-test. A P-value < 0.05 was considered significant.

RESULTS:

1,2-DBE caused DNA damage in the stomach, liver, kidney, lung, and bladder. Migration of nuclear DNA was significantly different in the lungs at 3 h after dosing ($P < 0.05$), in the liver and kidney at 3 and 24 h after treatment ($P < 0.05$ or < 0.01 for kidney; $P < 0.001$ for liver), and in the stomach and bladder at 24 h after dosing ($P < 0.001$ for stomach; $P < 0.05$ for bladder)...Non-significant results were observed in the brain and bone marrow.

No deaths, morbidity distinctive clinical signs or gross pathology were observed. No microscopic signs of necrosis were detected in the organs in which DNA damage was observed. Therefore, the DNA damage observed was not due to the toxic cell death.

CONCLUSIONS:

The alkaline Comet assay showed in vivo positive results for 1,2-DBE in several organ tissues in the mouse.

RELIABILITY:

(2) Valid with restrictions.

GENERAL REMARKS:

The authors also tested the following compounds in mice: l,2-dibromo-3-chloropropane (DBCP), 1,3-dichloropropene (mixture of *cis* and *trans*) (DCP), 1,2-dichloroethane (EDC), vinyl bromide, dichloromethane, and carbon tetrachloride. The authors stated that "The halogenated carcinogens studied here, except for the non-genotoxic carcinogen carbon tetrachloride, were genotoxic in mouse organs at non-necrogenic doses. Our present results, therefore, suggest that the in vivo alkaline SCG assay can compensate for the limitations of the micronucleus assay."

REFERENCE:

Sasaki YF, Saga A, Akasaka M, Ishibashi S, Yoshida K, Su YQ, Matsusaka N, Tsuda S. 1998. Detection of *in vivo* genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs. Mutat Res 419:13–20.

CHEMICAL:

Name: 1,2-Dribromoethane (CAS RN 106-93-4)
Purity: > 95% purity

METHOD:

Method/Guideline: Reverse mutation assay (following Proposal for replacement of OECD guidelines 471 and 472. ENV/MC/C1.M, 18, 1996).
Type of study: Gene mutation
GLP: Not stated.
Year: ≤ 1998.
Species and strain: *Salmonella typhimurium* TA102 and TA2638 (without S9 activation). *Escherichia coli* WP2/pKM101 and WP2 *uvr*A/pKM101 (without S9 activation).
Dose/Concentrations: Each strain subjected to 7 different doses up to 5000 µg/plate (0, 156, 313, 625, 1250, 2500, or 5000 µg/plate).
Analyses conducted: This was an interlaboratory collaborative study of chemically-induced mutation involving up to 20 different laboratories. The plates were incubated at 37°C for 48 h and colonies were scored by automatic colony counters (nine laboratories) or manually (five laboratories) or both counting methods (six laboratories). 1,2-Dibromoethane was tested in at least two replicate experiments and three plates per dose were conducted. 1,2-Dibromoethane was tested in two laboratories to assess reproducibility. The positive controls, mitomycin C (MMC) for TA 102 and TA2638, and 2-(2-furyl)-3-(5-nitro-2-furyl) acry] amide (AF-2) for WP2/pKM101 and WP2 uvrA/pICM101 were used in each experiment with out metabolic activation.
Statistical analysis: Statistical significance was determined using a linear regression test. This statistical analysis recommended by Mahon et al. (1989) was conducted based on the dose-response relationship and carried out at the 1% significance level. Doses with observed cytotoxicity, which was judged by a toxicity to the background level and/or a reduction in the number of revertant colonies, were excluded from the statistical analysis.

RESULTS:

Positive results were observed for all strains of *S. typhimurium* and *E. coli* by both laboratories and for all replicate experiments tested. Over the dose range of 0 to 2500 µg/plate, the number of revertants/plate increased in a dose-related manner from 326 to 504 for *S. typhimurium* TA102, 25 to 69 for *S. typhimurium* TA2638, 44 to 102 for *E. coli* WP2/pKM101, and 93 to 428 for *E. coli uvr*A/pKM101. At 5000 µg/plate, the numbers of revertants/plate were decreased but normally remained above control levels, and sometimes toxicity was observed.

Increases in the number of revertant colonies were also observed with the positive controls.

CONCLUSIONS:

1,2-Dibromoethane was positive for gene mutation in *Salmonella typhimurium* TA102 and TA2638 and *Escherichia coli* WP2/pKM101 and WP2 *uvr*A/pKM101 without metabolic activation..

RELIABILITY:

(2) Valid with restrictions.

GENERAL REMARKS:

This was a large collaborative study of chemically-induced mutation using the four bacterial strains *Salmonella typhimurium* TA 102 and TA2638 and *Escherichia coli* WP2/pKM101 and WP2 uvrA/pKM101 in order to compare the specific spectrum of response to chemicals and to evaluate the usefulness (sensitivity) of each strain. In addition to 1,2-dibromoethane, 10 non-mutagenic compounds (butylated hydroxytoluene, butylated hydroxy-anisole, clofibrate, dehydroepiandrosterone, di(2-ethythexyl)phthalate, ethyl carbamate, methyl carbamate, safrole, tannic acid and thioacetamide) and 11 other chemicals (benzoin, p-pbenylenediamine 1,1-dibromoethane, dichloromethane,diiodomethane 5-fluorouracil, nitrofurantoin, 3,4-Dichlorobutene-1, vinyl acetate, nalidixic acid and oxolinic acid) were tested.

REFERENCE:

Watanabe K, Sasaki T, Kawakami K. 1998. Comparisons of chemically-induced mutation among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 *uvrA*/pKM101: collaborative study III and evaluation of the usefulness of these strains. Mutat Res 416:169–181.

Mahon GAT, Green MHL, Middleton B, Mitchell IG, Robinson WD, Tweats DJ. 1989. Analysis of data from microbial colony assays. In: Kirkland DJ. (Ed.). Statistical Evaluation of Mutagenicity Test Data. Cambridge Univ. Press, Cambridge. pp. 26—65.

CHEMICAL:

Name (CAS No.): 1,2-Dibromoethane (CAS RN 106-93-4) (

Purity: 95% radiochemical purity

METHOD:

Method/Guideline: Other

Type of study: DNA binding study

GLP: Not stated.

Year: ≤ 2007

Species and strain: Rats, Fischer 344 and Mice, B6C3F1

No. of animals/sex/dose: Rats: 2 males/dose/timepoint; Mice: 2/sex/timepoint; 24 control animals used but number/species and strain not stated.

Route of exposure: Intratraperitoneal injection

Vehicle: Phosphate buffered saline.

Dose/Concentrations: 5 mg/kg bw (= 69 μCi/kg bw)

Frequency and duration of exposure: Once.

Duration of follow-up: Animals sacrificed at 1 and 8 hrs after administration (method of sacrifice not stated). Kidneys and livers were removed with approximately 100 mg of each used for DNA isolation and analysis.

Analyses conducted: DNA was isolated from tissue and purified using a Puregene DNA Purification Kit. DNA solutions were incubated with porcine spleen DNase II and bovine spleen phosphodiesterase II at 37 °C for 60 min, and acid phosphatise was then added to the reaction; incubation continued for another 120 min. Samples were concentrated to dryness under an N2 stream. After concentration, samples were reconstituted with the HPLC eluent, and whole samples were immediately injected onto the HPLC system. Nucleoside adducts were separated by HPLC using a Beckman ODS Ultrasphere octadecylsilane column. Eluent A was 10 mM NH4CH3CO2 (w/v, pH 4.5), and solvent B was CH3OH. The gradient was as follows: 98% A/2% B (v/v) (0 min), 90% A/10% B (v/v) at 5 min, and 80% A/20% B (v/v) at 40 min. Authentic standards of nucleoside adducts were initially injected to determine their *t*R values. All four nucleoside adduct fractions (2.0 mL aliquots) were collected from each run. Semicarbazide was added to 500 μL aliquots (to 1.0 mM) before concentration to complex any formaldehyde formed after the decomposition of nucleoside adducts and prevent loss by evaporation. Samples were concentrated to dryness using a Labconco vacuum centrifuge for accelerator mass spectrometry (AMS). In this study, nucleoside adduct samples were reconstituted with 50 μL of H2O and analyzed by AMS without further processing. Aliquots (1.5 μL) were absorbed into pellets of packed CuO powder that had previously been exposed for 30 min to an atmosphere of O2 at 700 °C. After brief drying in a vacuum oven, the sample-loaded CuO pellets were transferred to a laser-induced combustion interface for subsequent AMS analysis. Quantitation was performed by integrating peaks produced in the continuous trace of ^{14}C detector count rate vs time generated during operation of the combustion interface, which produces and delivers the CO2 of combustion to the AMS ion source and takes the product of the sample/standard peak area ratio multiplied by the standard concentration as the concentration of the sample. Quantitation standards consisted of aqueous solutions of methylated serum albumin. Negative controls comprising an aqueous solution of serum albumin were included in all runs as a check for contamination.

Statistical analysis: Adduct levels were expressed as means ± standard deviations of fmol/mg DNA. For radioactivity, a background value of 0.075 dpm/sample was used, which is the mean of 24 control samples (animals not treated with any radiochemicals; samples processed in parallel). The level of radioactivity in the DNA control samples analyzed was below the limit of statistical detection using a conventional liquid scintillation counter.

RESULTS:

The adduct results were roughly similar between the 1 and the 8 h time points, but none appeared to follow a clear trend in the animals. Thus results were pooled from both time points. The amount of

DNA processed in each sample varied from 136 to 367 μg, and the recovered radioactivity ranged from 0.50 to 2.78 dpm above background.

Table 2. Adducts Derived from 1,2-DBE

species	tissue	adducts [fmol (mg DNA)$^{-1}$]^{a}
rat	liver	1900 ± 540
mouse (male)	liver	840 ± 90
mouse (female)	liver	800 ± 80
Rat	kidney	1490 ± 540
mouse (male)	kidney	390 ± 60
Mouse (female)	kidney	580 ± 90

[a] Expressed as means ± SD (n = 4), using pooled 1 and 8 h timepoint values

As shown in table 2, the level of binding was higher in rats than in mice, both in liver and in kidney. No major difference was observed between male and female mice. The level of liver or kidney *S*-[2-(*N*7-guanyl)ethyl]GSH in rats treated with 1,2-dibromoethane was ∼1 adduct/10^5 DNA bases; in male or female mice, the level was approximately one-half of this.

CONCLUSIONS:

1.2-DBE was positive for *in vivo* DNA binding in the liver and kidney of rats (males) and mice (both sexes).

RELIABILITY:

(2) Valid with restrictions.

GENERAL REMARKS:

In this study, the DNA was digested using a procedure designed to minimize processing time, because some of the potential dihalomethane-derived DNA–glutathione (GSH) adducts are known to be unstable, and the HPLC fractions corresponding to major adduct standards were separated and analyzed for ^{14}C using accelerator mass spectrometry. Other dihalomethanes tested in this study were 1,2-dichloroethane, dibromomethane, and dichloromethane

REFERENCE:

Watanabe K, Liberman RG, Skipper PL, Tannenbaum SR, Guengerich FP. 2007. Analysis of DNA adducts formed *in vivo* in rats and mice from 1,2-dibromoethane, 1,2-dichloroethane, dibromomethane, and dichloromethane using HPLC/accelerator mass spectrometry and relevance to risk estimates. Chem Res Toxicol 20:1594–1600.

CHEMICAL:

Name (CAS No.): 1,2-Dibromoethane (CAS RN 106-93-4)
Purity: Not stated

METHOD:

Method/Guideline: Erythrocyte Micronucleus assay
Type of study: Determination of micronucleated erythrocytes in peripheral blood samples. NOTE: According to Witt et al. (2000), "The mouse peripheral blood micronucleus (MN) test was performed on samples collected from 20 short-term, 67 subchronic, and 5 chronic toxicity and carcinogenicity studies conducted by the National Toxicology Program (NTP). Data are presented for studies not previously published." Although table 2 of this paper indicates that the 1,2-DBE micronucleus test was based on a 25-week inhalation study conducted in mice and the source report was NTP (1982), the mouse inhalation studies reported in NTP (1982) were of different durations and used different dose levels than those reported below (i.e. 90-days using 0, 3, 15 or 75 ppm; 78-103 weeks using 0, 10 or 40 ppm). It thus appears that the 25-week inhalation study described here was "…not previously published."[1]
GLP: Not stated.
Year: $\leq$ 2000
Species and strain: Mice, B6C3F1 strain.
No. of animals/sex/dose: 10 males/group.
Route of exposure: Inhalation
Vehicle: air
Dose/Concentrations: 0, 10, 20 or 50 ppm.
Frequency and duration of exposure: 25-week inhalation study.
Duration of follow-up: Specific method for 1,2-DBE not stated. According to the authors, "Blood was obtained immediately before or at the time of sacrifice by retro-orbital bleeding; less often, other methods were employed, including heart puncture, puncture of the ventral tail vessels, or tail clip." Sampling times were not stated but appeared to follow US EPA (1996): "If a repeated treatment schedule is used, samples shall be taken at least three times, starting not earlier than 12 h after the last treatment and at appropriate intervals following the first sample, but not extending beyond 72 h."
Analyses conducted: Drops of blood were spread on precleaned standard glass microscope slides, air dried, and fixed in absolute methanol for 5 min. Unstained slides were shipped to the USDA laboratory where they were stained immediately before scoring with either Hoechst 33258/pyroninY or acridine orange. Slides stained with acridine orange or Hoechst 33258/pyronin Y were scored at 630X or 1000X magnification by epifluorescence microscopy. Polychromatic erythrocytes (PCE) were scored by direct manual counting. Normochromatic erythrocytes (NCE) were scored using a semiautomated method, in which cell counts were determined by counting a subfield of approximately 1/16th of the full microscope field. Routine micronucleus frequency scores were based on approximately 10,000 NCE or 1000 PCE per sample.
Statistical analysis: The frequency of micronucleated cells among NCE or PCE was analyzed by a statistical software package that tested for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (chi-square significant at $P \leq 0.025$). Although statistical analyses were used as an important aid in evaluating the test results, statistical significance was not the only determining factor in arriving at an overall call for a chemical. A decision to classify a test as negative, equivocal, or positive for induction of micronuclei in this in vivo assay was based on a broader evaluation of a number of factors that determined the biological relevance of the results, including the appropriateness of the concurrent control data, the magnitude of

[1] One published study was identified in which female A/J mice were exposed to 1,2-DBE for 6 months (= 26 weeks) by inhalation at concentrations of 0, 20 or 50 ppm. All authors, except the primary author, were staff of the National Toxicology Program: Adkins, B, Jr., Van Stee, EW, Simmons, JE, and Eustis, SL. 1986. Oncogenic response of strain A/J mice to inhaled chemicals. J Toxicol Environ Health 17:311-322.

the observed response and the presence of a dose-dependent increase in the frequency of micronucleated cells.

RESULTS:

A statistically significant (*P* = 0.004) increase in the frequency of MN-NCE was observed and the two highest dose groups (20 and 50 ppm) were significantly elevated over the control value. MN-NCE/1000 NCE values were (mean ± standard error of mean) 0.50 ± 0.18, 1.08 ± 0.37, 1.67 ± 0.35, and 1.75 ± 0.53 for 0, 10, 20 and 50 ppm groups, respectively.

subchronic

CONCLUSIONS:

1,2-DBE induced micronuclei in mouse peripheral blood.

RELIABILITY:

(2) Valid with restrictions.

GENERAL REMARKS:

The authors presented results for many chemicals conducted under the US NTP program for studies not previously published (see NOTE in "Type of study" section). In the case of 1.2-DBE, when compared to other chemicals tested, the authors noted that the control value (0.50 MN-NCE per 1000 NCE), although not the lowest negative control value among the tests reported, was unusually low compared to published historical control averages and all treatment group values fell within published control ranges. However, despite the low concurrent control value, the observation of two significantly increased dose groups, along with the significant trend test, resulted in a judgment of a positive result.

REFERENCE:

Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. 2000. Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. Environ Mol Mutagen 36:163–194.

[NTP] National Toxicology Program. 1982. Carcinogenesis studies of 1,2-dibromoethane (CAS No. 106–93-4) in F344/N rats and B6C3F1 mice (inhalation studies): Technical Report Series No. 210. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC

[US EPA] US Environmental Protection Agency. 1996. Health Effects Test Guideline OPPTS 870.5395; In Vivo Mammalian Cytogenetics Tests: Erythrocyte Micronucleus Assay. EPA 712-C-96-226. 7 pp.