N87-25897

GENETIC TOXICITY STUDIES OF ORGANIC CHEMICALS FOUND AS CONTAMINANTS IN SPACECRAFT CABIN ATMOSPHERES

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ABSTRACT

Astronauts can be exposed during spaceflight to organic chemical contaminants in the spacecraft cabin atmosphere. Toxic exposures may cause lesions in the cellular DNA which are subsequently expressed as sister-chromatid exchanges (SCE). Analysis of SCE is a sensitive short-term assay technique to detect and quantitate exposures to DNA-damaging (mutagenic) substances. The increase in SCE incidence over baseline (control) levels is generally proportional to the concentration of the mutagen and to the duration of exposure.

The BHK-21 baby hamster kidney cell line was the in vitro test system used for this study. Test organics were added to the culture media for 18 hours, in concentrations ranging from one to 20 parts per million (ppm). Acetaldehyde and carbon disulfide were chosen for this study since they have occurred as atmospheric contaminants in many of the STS flights, and have been reported to have toxic and mutagenic effects in various test systems. Glutaraldehyde was chosen because few data are available on the mutagenicity of this common fixative, which is carried on STS flights for use in biological experiments. Acetaldehyde was a very strong inducer of SCE at concentrations of 2 ppm and above. Glutaraldehyde and carbon disulfide failed to induce SCE.

INTRODUCTION

Control of potentially toxic exposures to organic chemical contaminants in spacecraft cabin atmospheres is a vital concern to astronauts, who are exposed during spaceflight to a recirculating atmosphere containing trace amounts of numerous organic chemicals.

Analysis of sister-chromatid exchanges (SCE) is a sensitive short-term assay technique to detect and quantitate exposures to mutagenic substances (Perry & Evans, 1975; Latt & Schreck, 1980). The increase in SCE over baseline (control) levels is generally proportional to the concentration of the mutagen and to the duration of exposure. BHK-21, an established baby hamster kidney cell line, was chosen as the in vitro test system. BHK-21 cells are readily maintained in culture, are well-known in the biological literature, and are highly sensitive to toxic or mutagenic substances present in their growth media. BHK-21 cells are also capable of metabolically activating promutagens into mutagenically active compounds.

Acetaldehyde and carbon disufide were selected from a list of more than 70 volatile organics detected during STS flights since they have occurred as contaminants in about half of the STS flights, and have been reported to have toxic or mutagenic effects in various test systems. Glutaraldehyde was chosen because relatively few data are available on the mutagenicity of this common biological fixative, which is carried on STS flights for use in biological experiments. This study proposed to test the genotoxic effects of acetaldehyde, glutaraldehyde,

and carbon disulfide -- i.e., their ability to alter or damage cellular DNA or chromosomes. Discovery of significant genetic toxicity of any of these compounds would be an important factor in determining the appropriate spacecraft maximum allowable concentration (SMAC) limit.

MATERIALS AND METHODS

BHK-21 baby hamster kidney cells were obtained from the Biochemistry Department of the M.D. Anderson Hospital, Houtson, Texas. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and maintained in a 37°C incubator in a humidified atmosphere of 5% carbon dioxide and 95% air.

Cell population doubling times were calculated from cell counts done on a Coulter model Zf electronic particle counter.

The following SCE studies were carried out: (1) Negative controls, to determine the baseline SCE levels and the optimum exposure period for the test chemicals, (2) Positive controls, using 10 to 500 micromolar concentrations of cyclophosphamide, a known mutagen which requires metabolic activation in order to produce SCE, and (3) Tests with acetaldehyde, glutaraldehyde, and carbon disulfide, in 1 to 20 parts per million (ppm) concentrations, to test for induction of SCE.

SCE staining was done by the standard "fluorescence-plus-Giemsa" techniques currently used to resolve SCE for light microscopy (Kato, 1974; Perry & Wolff, 1974). Cells were exposed to bromodeoxyuridine (BrdU) for two cell cycles (approximately 18 hours for BHK-21 cells), followed by metaphase arrest of the mitotic cells with colcemid. Exposure to BrdU

for two S-phases results in metaphase chromosomes in which one sister chromatid contains DNA with BrdU bifilarly substituted for thymidine, the other chromatid unifilarly substituted. This condition is the basis for differential staining techniques in which one chromatid is lightly stained (unifilar BrdU) and the other darkly stained (bifilar BrdU).

BHK-21 cells were exposed in darkness (in aluminum foil-wrapped flasks) for 18 hours to either BrdU (5 ug/ml) alone (controls) or to BrdU plus either 1 ppm, 2 ppm, 5 ppm, 10 ppm, or 20 ppm of one of the test organics. Colcemid (0.1 ug/ml) was added for the final two hours. The cells were harvested by mitotic shake-off and centrifuged (5 minutes at 200 x g); then washed once in phosphate-buffered saline (PBS) and re-centrifuged. The cells were incubated in hypotonic solution (20% FCS) for 15 minutes at 37°C. The cells were finally centrifuged, resuspended in about 0.5 ml of hypotonic solution, and fixed with a 3:1 mixture of methanol:acetic acid (4°C). Chromosome spreads were prepared by dropping the cell suspension onto cleaned, wet microscope slides. The slides were air-dried and then stained with Hoechst 33258 (5 ug/ml in pH 6.8 phosphate buffer) for 20 minutes. They were then exposed for 1 hour to ultraviolet light (the germicidal UV lamp in the tissue culture hood), incubated in a 62°C water bath for 2 hours, and stained with 3% Giemsa in pH 6.8 buffer for 25 minutes. Slide preparations were air-dried and coverslipped.

SCE were scored by counting the number of times the polarity of light- vs. dark-staining segments was reversed, each marking a symmetrical interchange of material between sister chromatids. The number of SCE in 30 well-differentiated spreads was counted, averaged, and expressed as

the number of SCE per cell. Statistical significance was determined using a t-test ($p < .05$, with 58 degrees of freedom).

RESULTS

Control BHK-21 cells grew rapidly in culture, with population doubling times of 9 to 10 hours. Exposure to BrdU for 18 hours gave the best proportion of completely-differentiated SCE spreads, and this exposure time was used throughout the study. BHK-21 metaphase spreads contained complements of 44 chromosomes (metacentric, submetacentric, and telocentric, with a single large metacentric X chromosome), consistent with published karyotypes of the BHK-21 cell line.

Exposure of BHK-21 cells to cyclophosphamide (Table 1; Fig. 1) showed a very strong exponential dose-response to CP, with 10 uM and higher concentrations inducing significant numbers of SCE. CP was used as a positive control to confirm the ability of the BHK-21 cells to metabolically activate a known mutagen.

Exposure of BHK-21 cells to acetaldehyde (Table 2; Fig. 2) also showed a strong exponential dose-response. Concentrations of 2 ppm (17.7 uM) acetaldehyde and above induced significant numbers of SCE. Cells exposed to 20 ppm (177 uM) acetaldehyde had too many SCE to accurately count (about 65 SCE per cell).

BHK-21 cells exposed to 1 to 10 ppm glutaraldehyde (14.5 to 145 uM) did not show increased SCE rates (Table 3). Neither did cells exposed to 1 to 20 ppm carbon disufide (16.6 to 166 uM) (Table 4). However, BHK-21 cells exposed to 20 ppm glutaraldehyde (290 uM) were severely inhibited in cell division.

TABLE 1: SISTER-CHROMATID EXCHANGES IN BHK-21 CELLS EXPOSED TO CYCLOPHOSPHAMIDE FOR 18 HOURS IN VITRO

Cyclophosphamide concentration (uM)	SCE per cell (mean ± s.d.)	t-test versus Control	
		t-value	significance
0 (Control)	4.47 ± 2.01		
10	5.93 ± 2.19	2.69	n.s.
50	7.16 ± 2.10	4.97	$p < .01$
100	8.07 ± 2.05	6.86	$p < .0001$
250	13.97 ± 4.68	10.21	$p < .0001$
500	20.93 ± 5.01	16.19	$p < .0001$

TABLE 2: SISTER-CHROMATID EXCHANGES IN BHK-21 CELLS EXPOSED TO ACETALDEHYDE FOR 18 HOURS IN VITRO

Acetaldehyde concentration ppm (uM)	SCE per cell (mean ± s.d.)	t-test versus Control	
		t-value	significance
0 (Control)	7.16 ± 2.36		
1 (17.77)	6.73 ± 1.91	0.776	n.s.
2 (35.54)	9.90 ± 3.33	3.667	$p < .0005$
5 (88.85)	14.87 ± 4.29	8.625	$p < .0001$
10 (177.7)	26.06 ± 7.68	12.877	$p < .0001$

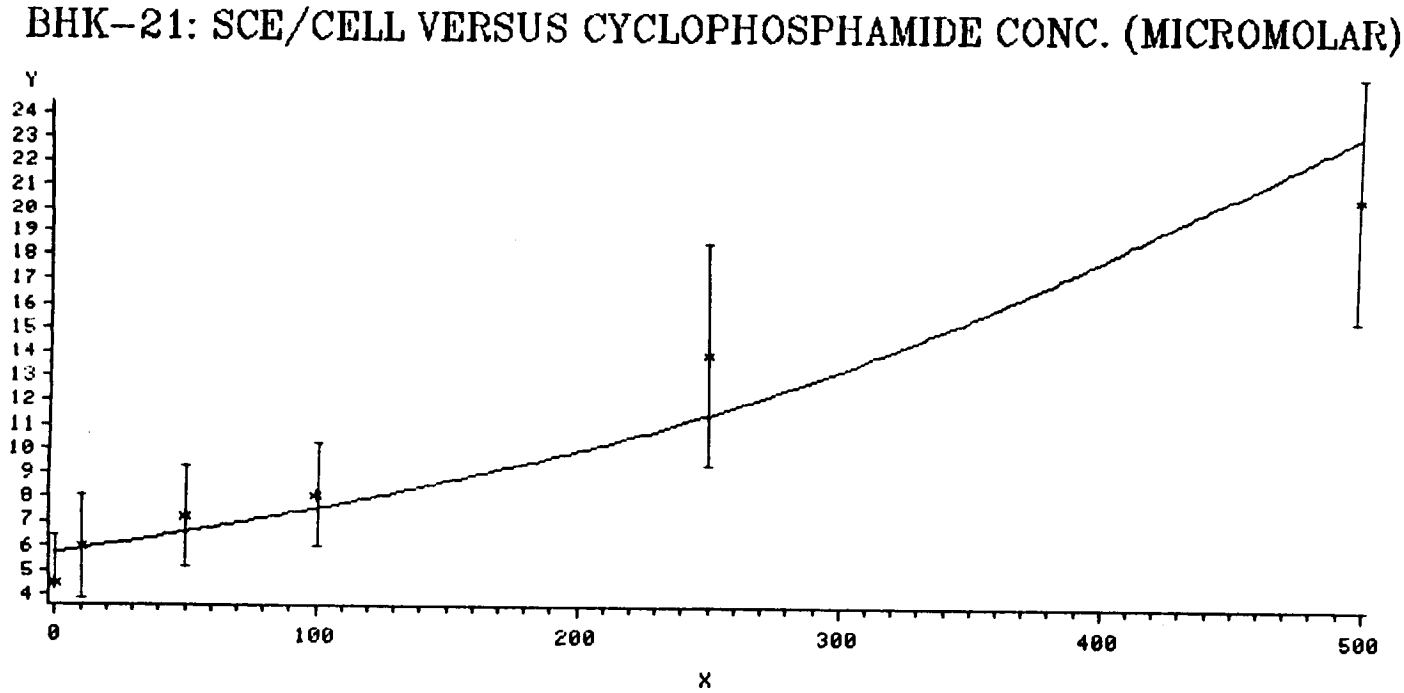

Figure 1: BHK-21: SCE induction by cyclophosphamide, 18 hours in vitro. SCE/cell versus concentration (micromolar).

Exponential regression: $Y = A * e^{BX}$ with A = 5.707, B = 0.003, and correlation coefficient, r = 0.960.

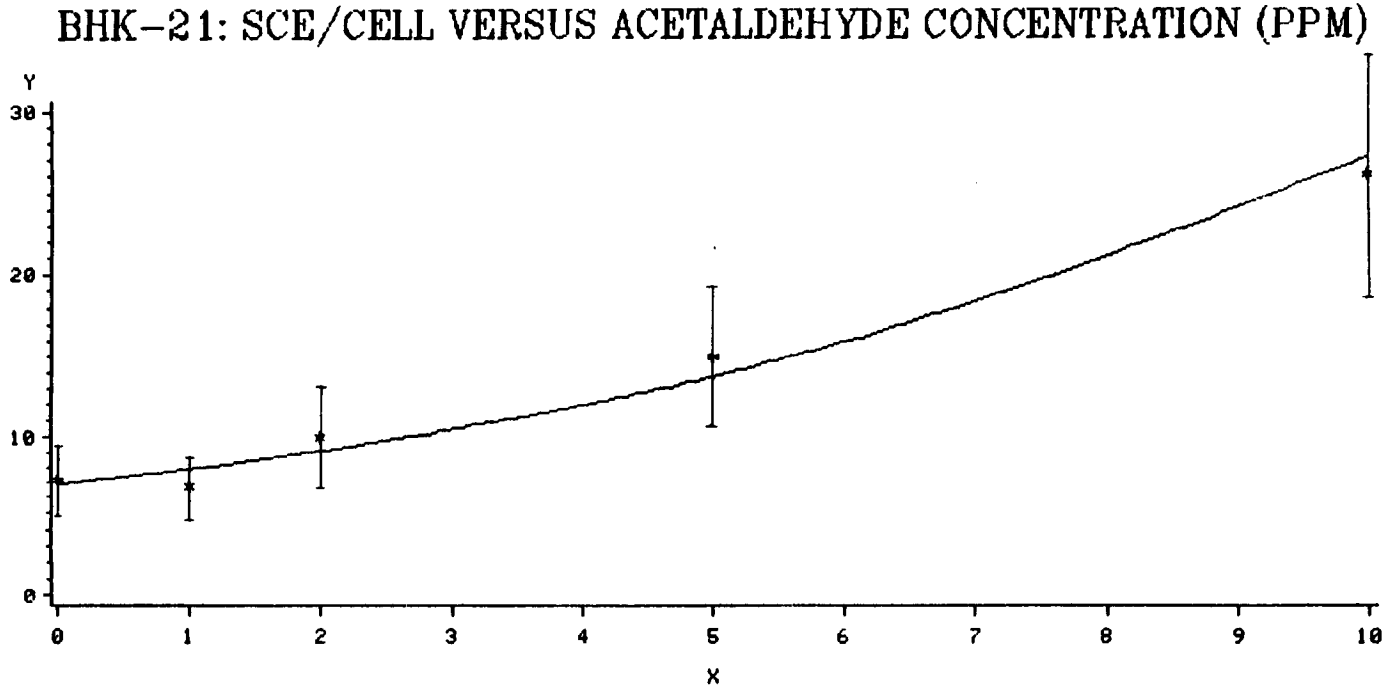

Figure 2: BHK-21: SCE induction by acetaldehyde, 18 hours in vitro. SCE/cell versus concentration (ppm).

Exponential regression: $Y = A * e^{BX}$ with A = 6.932, B = 0.137, and correlation coefficient, r = 0.983.

TABLE 3: SISTER-CHROMATID EXCHANGES IN BHK-21 CELLS EXPOSED TO GLUTARALDEHYDE FOR 18 HOURS IN VITRO

Glutaraldehyde concentration ppm (uM)	SCE per cell (mean ± s.d.)	t-test versus Control	
		t-value	significance
0 (Control)	5.30 ± 1.78		
1 (14.5)	5.70 ± 1.29	0.998	n.s.
2 (29.0)	6.06 ± 2.00	1.554	n.s.
5 (72.5)	5.20 ± 2.00	0.204	n.s.
10 (145.0)	5.90 ± 2.11	1.190	n.s.

TABLE 4: SISTER-CHROMATID EXCHANGES IN BHK-21 CELLS EXPOSED TO CARBON DISULFIDE FOR 18 HOURS IN VITRO

Carbon Disulfide concentration ppm (uM)	SCE per cell (mean ± s.d.)	t-test versus Control	
		t-value	significance
0 (Control)	6.57 ± 1.65		
1 (16.59)	6.13 ± 2.01	0.928	n.s.
2 (33.17)	6.20 ± 1.99	0.785	n.s.
5 (82.94)	6.03 ± 1.84	1.199	n.s.
10 (165.9)	6.10 ± 2.29	0.910	n.s.
20 (331.7)	6.13 ± 1.83	0.980	n.s.

DISCUSSION

BHK-21 cells are a rapidly-growing, easily maintained permanent cell line, and are sensitive to the effects of genotoxic agents in their growth medium. BHK-21 cells are able to metabolically activate indirect mutagens such as cyclophosphamide, presumably through the activity of mixed-function oxidase (MFO) enzymes. The exponential dose-response of BHK-21 cells to cyclophosphamide, with a minimum effective concentration of 10 uM, is apparently equal to the dose-response of several hepatoma cell lines, which are known known to possess MFO enzymes (Abe et al., 1983). Cyclophosphamide is a powerful anticancer agent, which acts by alkylating DNA.

Acetaldehyde is a direct-acting mutagen which has been identified as a cross-linker of DNA (Ristow & Obe, 1978). It is an inducer of SCE in relatively low concentrations — about 40 micromolar for human lymphocytes and fibroblasts in culture (Veghelyi & Ostovics, 1978) and about 35 micromolar for BHK-21 cells. Acetaldehyde was a much stronger inducer of SCE than cyclophosphamide (Fig. 3); this effect may be attributable in part to the direct-acting mechanism of acetaldehyde in producing SCE. The mutagenic potential of acetaldehyde should be considered in conjunction with its toxic and irritating properties in setting exposure limits (current SMAC 30 ppm). However, acetaldehyde should not present toxicity problems in spacecraft cabin atmospheres, since the source of acetaldehyde is attributed to offgassing from plastic materials in the cabin, and measured acetaldehyde concentrations

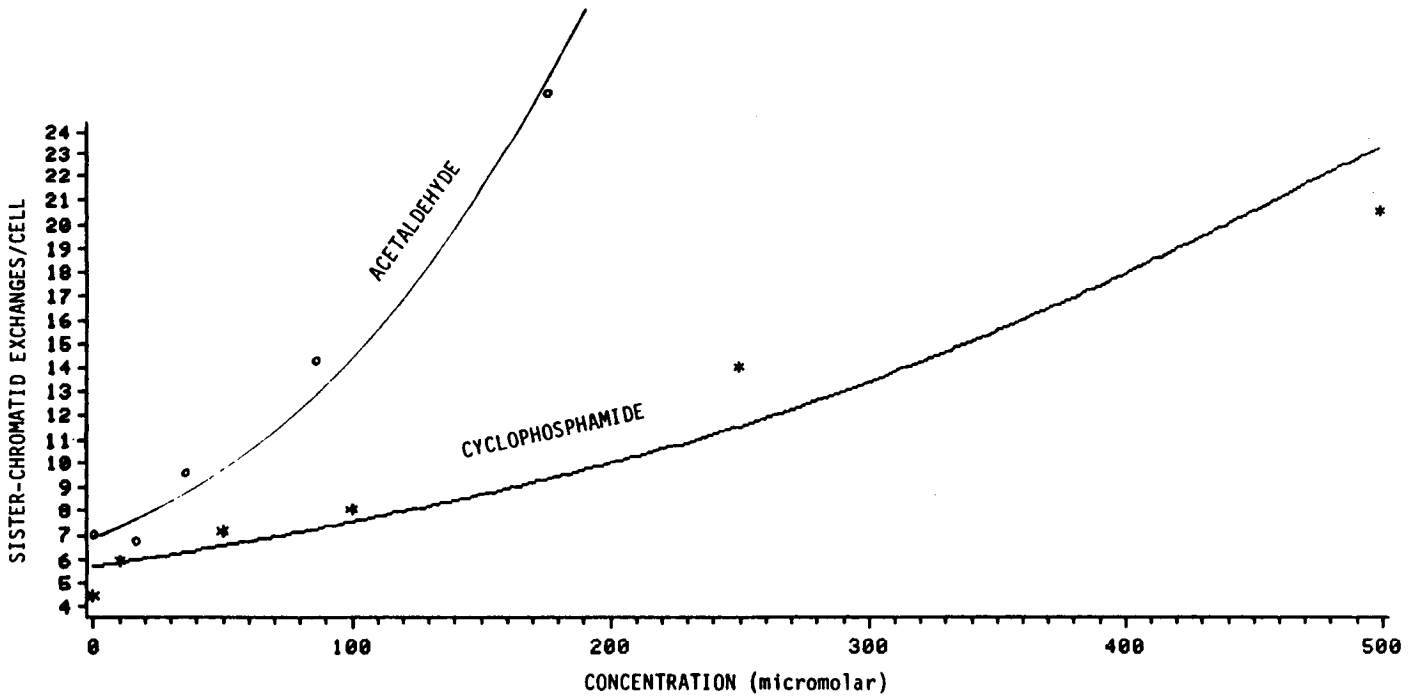

Figure 3: BHK-21: Comparison of SCE induction by acetaldehyde and cyclophosphamide, 18 hours in vitro. SCE/cell versus concentration (micromolar).

have been low (maximum 0.31 ppm, average approximately 0.15 ppm).

Glutaraldehyde is a common fixative which is carried on STS flights for use in biological experiments. It is a highly irritating compound, with low SMAC limits (0.098 ppm), but was negative for induction of SCE in BHK-21 cells exposed for 18 hours to from 1 to 20 ppm glutaraldehyde. Likewise, it was found to be negative for SCE induction in Chinese hamster ovary (CHO) cells in 0.6 to 2.5 uM concentrations, with and without S-9 mix (rat liver microsomal enzymes added to provide metabolic activation) by Slesinski et al. (1983). They found that 5 uM glutaraldehyde produced excessive mitotic inhibition to carry out the SCE test. BHK-21 cells were much less sensitive to the antimitotic effects of glutaraldehyde than were CHO cells; concentrations of 20 ppm (290 uM) halted mitosis and prevented spreading of the chromosomes. Glutaraldehyde is a strong cross-linking agent of proteins and most likely interacts with proteins in the cell membrane and cytoplasm, making interactions with DNA unlikely (Slesinski et al., 1983).

Carbon disulfide has been found in minute concentrations in spacecraft cabin atmospheres (about 0.001 ppm when present) apparently from offgassing from plastic materials in the cabin. It is a potent neurotoxin, with SMAC limits of 0.96 ppm. Carbon disulfide failed to induce SCE in BHK-21 cells following 18-hour exposures to 1 to 20 ppm concentrations (16 to 331 uM).

CONCLUSIONS

The BHK-21 baby hamster kidney cell line is a useful model system for in vitro genetic toxicology research. BHK-21 cells are capable of metabolically activating indirect mutagens such as cyclophosphamide.

Neither glutaraldehyde nor carbon disulfide induced sister-chromatid exchanges during 18-hour exposures to 1 to 20 ppm concentrations of these chemicals. No modification of SMAC limits for glutaraldehyde or carbon disulfide are suggested by these preliminary results.

Acetaldehyde strongly induced sister-chromatid exchanges in BHK-21 cells, in concentrations as low as 2 ppm (35 uM). These findings are consistent with similar SCE studies in the literature which confirm that acetaldehyde is a strong inducer of SCE even at relatively low concentrations. The SMAC limits for acetaldehyde should be considered for toxicological review in light of the significant mutagenic potential of this compound.

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