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Cytogenetic effects of benzene: dosimetric studies on rats exposed to benzene vapour

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Summary

Rats were exposed to benzene vapour at nominal concentrations in air of 1, 10, 100 and 1000 ppm acutely for 6 h. Bone marrow cells from each animal were examined for chromosomal abnormalities 24 h after the end of the exposure period. This analysis was carried out on 250 metaphases per animal where possible and showed a significant increase in the percentage of cells with chromosomal abnormalities, excluding gaps, in the groups of animals exposed to 100 and 1000 ppm benzene. In the 10-ppm and 1-ppm exposure groups there were elevated levels of cells with abnormalities which showed evidence of being dose-related, although they were not statistically significant.

Benzene is non-mutagenic in all of the point mutational assay systems in which it has so far been tested (Lyon, 1975; Jerina et al., 1968; Dean, 1978; Shahin and Fournier, 1978; Cotruvo et al., 1978; Tanooka, 1977; Rosenkranz and Leifer, 1980). It can induce chromosome damage in plants and mammalian somatic cells in vitro (Gomez-Arroyo and Villalobos-Pietrini, 1981; Koizumi et al., 1974; Morimoto, 1976; Diaz et al., 1979; Morimoto and Wolff, 1980; Gerner Smidt and Friedrich, 1978), mammalian cells in vivo (Lyon, 1975; Zhong et al., 1980; Diaz et al., 1980; Hite et al., 1980; Siou et al., 1980a; Meyne and Legator, 1980; Siou et al., 1980b; Dean, 1969; Philip and Krogh Jensen, 1970; Lyapkalo, 1973; Dobrokhotov and Enikeev, 1977; Anderson and Richardson, 1979) and also been shown to induce SCE (Tice et al., 1979, 1981). In the chromosome studies in animals benzene was administered by i.p. injection with the exception of the investigations of Anderson and Richardson (1979) and Tice et al. (1979, 1981) where exposure was by inhala-

tion. Numerous cytogenetic studies have been done on bone-marrow cells or peripheral lymphocytes from human subjects exposed to benzene vapour (Dean, 1978; Pollini and Colombi, 1964a,b; Pollini et al., 1964, 1969; Pollini and Bicaldi, 1976, 1977; Fomi et al., 1971a,b; Forni and Moreo, 1967, 1969; Hartwich and Schwanitz, 1972; Sellyei and Kelemen, 1971; Erdogan and Aksoy, 1973; Hudak and Gombosi, 1977; Van den Berghe et al., 1979; Tough and Court-Brown, 1965; Tough et al., 1970; Funes-Cravioto et al., 1977; Picciano, 1979; Khan and Khan, 1973; Fredga et al., 1979; Watanabe et al., 1980). The cytogenetic investigations indicated that at high exposure levels benzene is a human and animal clastogen but the available data from inhalation studies were insufficiently consistent to establish a dose-response relationship and determine whether the present TLV (current OSHA standard is the 8 h time weighted average of 10 ppm) was adequate. The investigation reported here was designed to examine the cytogenetic effects of a range of concentrations of benzene

vapour on rat bone marrow cells following inhalation and to define concentrations of benzene vapour that would be non-clastogenic under conditions of acute exposure.

Materials and methods

Benzene. The benzene (AnalaR) used in this study was supplied by BDH Chemicals Limited, Poole, Dorset.

Reagents for slide processing. The following reagents were used: Hank's basic salt solution obtained from Gibco Biocult, Paisley. **CK:** potassium chloride (AnalaR) from BDH Chemicals Limited, Poole, Dorset, UK; glacial acetic acid (AnalaR) and methanol (AnalaR) from Hopkin and Williams, Chadwell Heath, Essex, UK; colchicine from Sigma (London) Chemical Company, Poole, Dorset, U.K. and Giemsa stain (Gurr's R66) from Searle Diagnostics, High Wycombe, Bucks, UK.

Animals

Male, Wistar-derived rats, 6-8 weeks old, were supplied from the specific pathogen-free colony at Alderley Park, Cheshire, UK. The rats were randomly assigned to cages using a shuffle card method and housed 4 per cage. Animals were given food and water ad libitum except during exposure periods. Male animals were used because they appear to be more susceptible to the clastogenic effects of benzene than females (Meyne and Legator, 1980). Exposure in humans is predominantly in males.

Exposure to benzene

Groups of rats were separately exposed to atmospheres containing benzene at 0, 1, 10, 100 and 1000 ppm for a single 6-h period. Each group of animals at each exposure level consisted of 8 rats except for the controls (0 ppm) which contained 12 animals. The animals were exposed to clean dry air in whole body chambers (Gage, 1959). The air was supplied at a rate of 9 l/min during the exposure period. Animals were generally caged individually but some were housed in pairs.

Atmosphere generation

The test atmospheres were generated by direct

injection of benzene liquid into the air stream entering the chambers. The benzene was delivered from a Hamilton-Bonaduz gas-tight syringe (Hamilton, Bonaduz AG, CM7402 Bonaduz, Switzerland) of the appropriate capacity at an injection rate governed by a Sage Controlled Injection Pump, Model 355 (Sage Instruments Division, Orion Research Inc., Cambridge, MA, U.S.A.). The injection rate was adjusted to bring the concentration range within $\pm 10\%$ of the desired level.

Atmosphere analysis

Benzene at the 1000 ppm level was monitored using a Wilks Miran 1A infra red analyzer (Foxboro Analytical, Milton Keynes, Bucks, UK) and at the lower concentrations by gas chromatography after trapping the test substance on Poropak Q (80-100 mesh) (Waters Associates Inc., Milford, MA, U.S.A.).

Processing of rat bone marrow

Animals were killed 24 h after the end of exposure as recommended by the ad hoc committee report on mutation testing (1972). Legator et al. (1973) and Sugiyama (1971).

The bone-marrow samples were prepared according to the method of Sugiyama (1971) but with slight modifications. The method used was as follows: (1) Each rat received 3 mg colchicine/kg bodyweight intraperitoneally 2 h prior to sacrifice to arrest dividing cells in c-metaphase. This was administered to each of the randomly housed groups working across the rack. This sampling order was maintained throughout further processing until fixation occurred. (2) The rats were killed by cervical dislocation, both femurs from each animal were removed and the bone marrow harvested by aspiration with Hank's basic salt solution. (3) Cells were treated with hypotonic solution (0.075 M potassium chloride at room temperature for 20 min) followed by fixation in glacial acetic acid and methanol in the ratio 1:3. (4) Slides were prepared by air-drying and stained with Giemsa (Gurr's R66). (5) The slides were coded to avoid observer bias while being scored and were read in a random order which was computer-generated. 250 cells from each animal were examined where possible and any abnormality was assigned to one of the following categories:

TABLE 1

GROUP MEAN PERCENTAGE OF CELLS WITH ABNORMALITIES

Acute 6-h exposure, 24 h kill

Abnormalities	Dose level of benzene (ppm)				
	0 Control	1	10	100	1000
Including gaps	154	2.00	3.03	4.45 **	7.81 **
Excluding gaps	0.25 (12)	0.41 (8)	0.91 (8)	1.23 ** (8)	4.49 ** (8)

The number in brackets is the number of animals which were examined.

** Statistically significantly greater than the control group mean at the 1% level (t-test, one-sided on transformed data)

(a) chromatid or chromosome gaps: (b) chromatid breaks: (c) fragments: (d) minutes: (e) any other complex abnormality such as Robertsonian translocations.

The criteria for the above classification is given by Anderson and Richardson (1981).

Statistical analysis

The proportion of cells with any abnormalities and the proportion of cells with any abnormalities other than gaps were transformed to stabilise the variance using the double Arcsine transformation (Freeman and Tukey, 1950). The transformed data were then considered by analysis of variance. The

analysis allowed for the 3 different cytogeneticists who examined the slides. The group means were adjusted for reader differences (and therefore do not correspond to a straight arithmetic mean of the data) before the mean transformed proportion for each benzene group was compared with the mean transformed proportion in the control group using a one-sided Student's *t*-test. The test was based on the error mean square in the analysis of variance.

Results**Test atmosphere analysis**

The test atmospheres generated were: 0.8 ± 0.2

TABLE 2

MEAN PERCENTAGE INCIDENCE ($\pm$ SD) OF CELLS WITH DIFFERENT CATEGORIES OF CHROMOSOMAL DAMAGE FOLLOWING EXPOSURE TO DIFFERING ATMOSPHERIC CONCENTRATIONS OF BENZENE

Group	Number of animals	Number of cells analysed per group	Mean % cells with chromosome or chromatid gaps	Mean % cells with chromatid breaks	Mean % cells with chromosome breaks or fragments	Mean % cells with minutes	Mean % cells with interchanges
Control	12	2641	1.40 ± 1.22	0.04 ± 0.13	0.34 ± 0.80	0.08 ± 0.18	0
1 ppm benzene	8	1846	1.88 ± 0.72	0.05 ± 0.15	0.37 ± 0.48	0	0
10 ppm benzene	8	1193	1.68 ± 1.48	0.08 ± 0.24	0.34 ± 0.51	0.17 ± 0.48	0.08 ± 0.24
100 ppm benzene	8	1429	3.16 ± 2.40	0.21 ± 0.29	0.49 ± 0.76	0.14 ± 0.26	0
1000 ppm benzene	8	2039	2.40 ± 1.03	0.15 ± 0.20	3.53 ± 1.66	0.20 ± 0.55	0.10 ± 0.18

135 ± 36 ppm: 1093 ± 110

Discussion

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from other exposure levels

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Human exposure to benzene is predominantly
by inhalation, hence the results of this study sug-
gest that an acute exposure level not exceeding 10
ppm is unlikely to cause clastogenic effects in man
provided that rat and man are pharmacodynami-
cally similar with respect to benzene. This conclu-
sion is supported by data from chronic exposure
studies in humans where concentrations of ben-
zene were estimated. Fomi et al. (1971a,b) ob-
served chromosome abnormalities in lymphocytes
from people exposed to 125-532 ppm benzene:
Tough et al. (1965,1970) found that exposure of
workers to 25-150 ppm benzene caused a signifi-
cant increase only in the unstable aberrations in
lymphocytes whereas levels of 12 ppm did not
induce any significant increase in chromosomal
abnormalities. Picciano (1979) has reported that in
subjects exposed to < 10 ppm benzene there was
an increase in the incidence of certain categories of
chromosomal damage compared with controls, al-
though the percentage of aberrant cells was not
significantly different. This study has been criti-
cised by Dabney (1981) on the following grounds:
first, there was inadequate information on extra-

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LS WITH DIFFERENT CATEGORIES OF CHROMOSOMAL DAMAGE PER CELL
RING ATMOSPHERIC CONCENTRATIONS OF BESZESE

an dence of romosome or matid gaps ell × 10 ⁻²	Man incidence of chromatid breaks per cell × 10 ⁻²	Mean incidence of chromosome breaks or fragments per cell × 10 ⁻²	Mean incidence of minutes per cell × 10 ⁻²	Mean incidence of interchanges per cell × 10 ⁻²
0.038 ± 0.13	0.38 ± 0.89	0.076 ± 0.18	0	0
0.054 ± 0.16	0.37 ± 0.49	0	0	0
0.084 ± 0.23	0.42 ± 0.61	0.168 ± 0.47	0.084 ± 0.24	0
0.21 ± 0.28	0.49 ± 0.77	0.14 ± 0.25	0	0
0.147 ± 0.20	4.12 ± 1.94	0.29 ± 0.80	0.147 ± 0.26	0

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