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In vitro and *in vivo* mutagenicity of the butadiene metabolites butadiene diolepoxide, butadiene monoepoxide and diepoxybutane

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Three metabolites of 1,3-butadiene, namely butadiene diolepoxide, butadiene monoepoxide and diepoxybutane, were tested in the bacterial mutation assay using *Salmonella typhimurium* strain TA100 with and without metabolic activation (S9 mix). All three compounds showed a mutagenic response. The bifunctional epoxide was more effective than the diolepoxide which was more effective than the monoepoxide. Toxicity appeared to follow the ranking of the chemicals for their mutagenic potency. The monoepoxide and the diolepoxide were also tested for induction of micronuclei in mouse bone marrow erythrocytes and for dominant lethal mutation induction in postmeiotic male mouse germ cells. The effects of the diepoxide in both *in vivo* tests have been published earlier. In the micronucleus assay, the three metabolites gave a positive response whereby the diepoxide was more effective than the monoepoxide which was more effective than the diolepoxide. In contrast to the diepoxide which was positive at a dose as low as 36 mg/kg, the monoepoxide and the diol did not show an induction of dominant lethal effects up to doses of 120 and 240 mg/kg, respectively. It is concluded that the metabolites were mutagenic in bacteria without metabolic activation and clastogenic in mouse bone marrow; only the bifunctional diepoxide, however, was active in postmeiotic male mouse germ cells.

Introduction

1,3-Butadiene is a widely occurring monomer in the petrol industry, tyre plants and synthetic polymer production. Low level urban exposure may occur through inhalation of gasoline vapours, automobile exhaust and cigarette smoke. On the basis of cancer bioassay data, the International Agency for Research on Cancer (IARC, 1992) has upgraded 1,3-butadiene into category 2A 'probably carcinogenic to humans'. Butadiene is genotoxic and clastogenic in somatic and germinal cells of mice (Adler *et al.*, 1994, 1995a; Anderson *et al.*, 1993; Cunningham *et al.*, 1986; Irons *et al.*, 1987; Jauhar *et al.*, 1988; Morrissey *et al.*, 1990; Tice *et al.*, 1987; Victorin *et al.*, 1990)

1,3-Butadiene undergoes a complex pathway of metabolic transformation and forms two main intermediates with potential reactivity towards DNA. The compound is metabolized by cytochrome P450-dependent monooxygenases to the primary metabolite 1,2-epoxy-3-butene (butadiene monoepoxide, BMO). BMO is subjected to further metabolism: oxidation to 1,2:3,4-diepoxybutane (DEB), hydrolysis to 3-butene-1,2-diol and conjugation to glutathione. Butenediol can be oxidized by P450 enzymes to 3,4-epoxy-1,2-butanediol (butadiene diolepoxide, BDE) which can also be formed by hydrolysis of DEB (Adler *et al.*, 1995b). Metabolic pathways are qualitatively similar but quantitatively different in mice, rats, monkeys and humans (Henderson *et al.*, 1993).

The present experiments were performed in order to study and compare the mutagenic effects in bacteria and the clastogenic effects in somatic and germinal cells of mice of the major epoxymetabolites of butadiene. The micronucleus induction and the dominant lethal effects caused by DEB have already been descibed (Adler *et al.*, 1995c).

Materials and methods

Chemicals

Butadiene monoepoxide (BMO) and diepoxybutane (DEB) were purchased from Aldrich Chemie, Steinheim, Germany, and were used as such. Butadiene diolepoxide (BDE) was synthesized from 2-butene-1,4-diol that was also purchased from Aldrich. To synthesize BDE, a mixture of 2-butene-1,4-diol (0.68 mol), water (25 ml), concentrated sulphuric acid (0.35 ml) and mercuric sulphate (0.25 g) was heated under reflux. After 1.5 h, the reaction mixture was cooled to 0°C and neutralized with 10% sodium hydroxide to pH 7.0. The content of the flask was distilled by using a Vigreux fractionating column. The second fraction collected contained 3-butene-1,2-diol as a colourless liquid. Purified 3-butene-1,2-diol (0.1 mol) was subsequently epoxidated by m-Cl-perbenzoic acid (0.25 mol) in dichloromethane (25 ml) at room temperature. The formed diolepoxide was isolated by using flash chromatography. The identification and purity of 3-butene-1,2-diol and the corresponding diolepoxide were determined by nuclear magnetic resonance (NMR). Both of the chemicals showed >96% purity. Proton and carbon NMR spectra are available if requested.

Ames tests

The bacterial mutagenicity tests were performed according to the revised methods of Maron and Ames (1983) using *Salmonella typhimurium* strain TA100 with and without metabolic activation (S9 mix). Rat liver S9 was prepared from male Wistar rats, pretreated with Aroclor 1254. The Aroclor 1254 induced S9 lot used contained 41.2 mg/ml protein and the total cytochrome P450 content was 20 nmol/ml. S9 mix for bacterial tests contained 0.1 ml/ml S9. Each dose was tested in duplicate and the numbers of revertant colonies on the test plates were counted after 48 h of incubation at 37°C. Solvent controls were tested on six plates per assay. Average numbers (± SD) of revertants per plate were calculated from the plate. Mutagenic activities were calculated using linear regression computations from the positive slopes of the dose–response curves. The mutagenic activity calculations were based on regression coefficient values (B, when $y = A + Bx$) and expressed as numbers of revertant colonies/μmol/plate. Methyl methanesulphonate (MMS, 1 μl/plate) and 2-aminoanthracene (2-AA, 1 μg/plate) were used as positive controls without and with S9 mix respectively.

Animals

The experiments were conducted with (102/El×C3H/El)F_1 and NMRI mice bred at GSF, Neuherberg, Germany. Throughout the experiment, the animals were kept in Macrolon® cages type 2 and fed with pellet food and chlorinated water *ad libitum*. Animal quarters were maintained at 20°C and 50% relative humidity with a 12 h light/dark cycle.

Bone marrow micronucleus test

The BMO experiment consisted of groups of five treated (102/El×C3H/El)F_1 males and five treated (102/El×C3H/El)F_1 females per dose. Control animals were injected i.p. with the solvent (saline). The tested doses were 20, 40 and 80 mg/kg of BMO.

The BDE experiment consisted of groups of six treated (102/El×C3H/El)F_1 males which received 60, 120 or 240 mg/kg of BDE. Control animals were injected i.p. with the solvent (PBS).

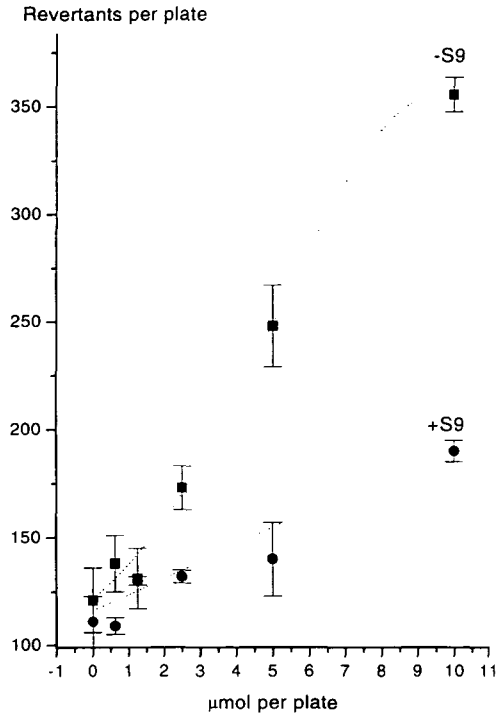

Fig. 1. Mutagenicity of butadiene monoepoxide (BMO) in the Ames test with and without S9 mix using the *Salmonella* strain TA100.

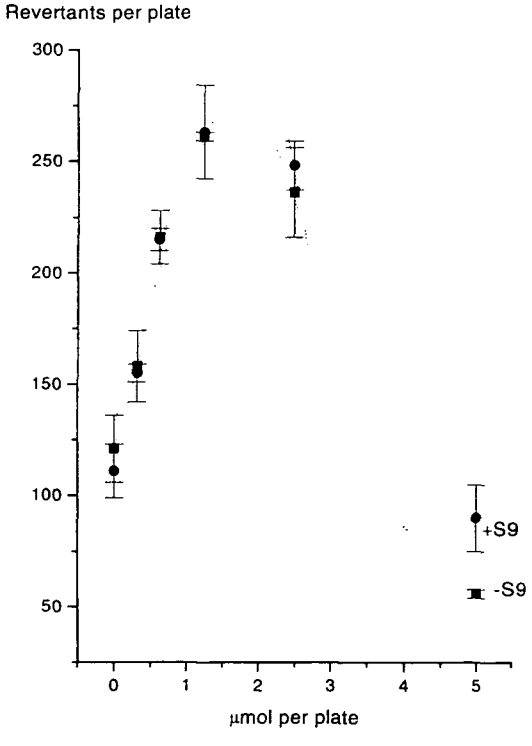

Fig. 2. Mutagenicity of diepoxybutane (DEB) in the Ames test with and without S9 mix using the *Salmonella* strain TA100.

The injected volumes were 0.01 ml/g body weight of freshly prepared solutions of the test chemicals BMO and BDE. The time between preparation of the solution and injection was kept short (maximum 10 min) in order to minimize hydrolysis of the test compounds.

Bone marrow was sampled 24 h after treatment. Bone marrow preparation, staining and scoring were performed as described before (Adler, 1984). Per animal, 2000 polychromatic erythrocytes (PCE) were scored microscopically

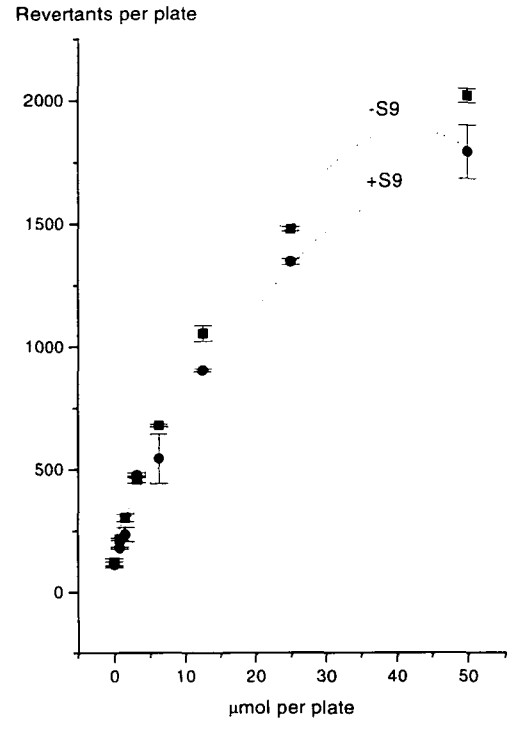

Fig. 3. Mutagenicity of butadiene butadiene diolepoxide (BDE) in the Ames test with and without S9 mix using the *Salmonella* strain TA100.

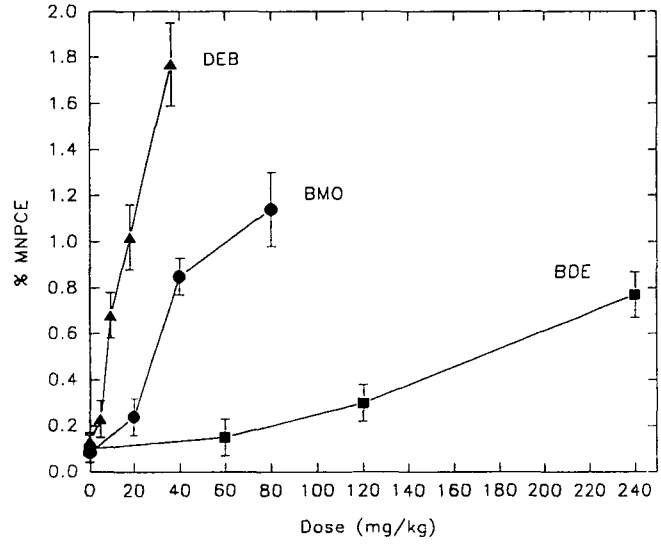

Fig. 4. Results of the mouse bone marrow micronucleus test with butadiene monoepoxide (BMO) and butadiene diolepoxide (BDE). The data for diepoxybitane (DEB) were published by Adler *et al.* (1995c).

for the presence of micronuclei and the means were expressed as micronucleated PCE (MPE) per 1000 PCE. To determine a shift in erythroblast proliferation the number of PCE was counted in the microscope fields that contained 2000 normochromatic erythrocytes (NCE) and the mean values were expressed as percentage PCE of the total erythrocyte counts. Micronucleated NCE were also recorded. The significance of differences between exposure groups were determined by the Mann–Whitney test (Sachs, 1974). The dose response was determined by regression analysis.

Dominant lethal test

The experiments were performed by treating (102/El×C3H/El)F_1 males. In the BMO experiment 30 males were injected i.p. with 120 mg/kg of BMO and 20 males were injected i.p. with the solvent (saline). In the BDE experiment, 30 males were injected i.p. with 240 mg/kg of BDE and 30 males were injected i.p. with the solvent (PBS). Each male was caged with one (102/El×C3H/El)F_1 and one NMRI female 6 h after treatment. Every 4 days

Table I. Results of the bone marrow micronucleus test with BMO and BDE

Dose (mg/kg)	MPE/2000 PE males	MPE/2000 PE females	MPE/1000 PE mean ± SE	Percentage PE
BMO				
0	0, 1, 2, 2, 3	0, 0, 2, 3, 4	0.9 ± 0.4	49.3
20	2, 4, 5, 6, 7	2, 3, 4, 6, 9	2.4 ± 0.4[a]	49.3
40	14, 17, 19, 21, 22	14, 15, 15, 16, 17	8.5 ± 0.7	49.5
80	22, 25, 27, 29, 33	12, 18, 18, 21, 22	11.4 ± 0.8	49.5
BDE				
0	0, 1, 1, 2, 3, 5		1.0 ± 0.3	48.7
60	2, 2, 2, 3, 3, 6		1.5 ± 0.4	48.2
120	4, 4, 5, 5, 7, 11		3.0 ± 0.4[a]	47.7
240	14, 14, 15, 15, 16, 18		7.7 ± 0.5	48.1

[a]$P < 0.05$ (Mann–Whitney test).

Table II. Results of the dominant lethal test with BMO, 120 mg/kg i.p. (102/El×C3H/El)F_1 females

Mating intervals	Dose (mg/kg)	Pregnant females		Total implants		Live implants (LI)		Total dead implants (DI)		Early DI per animal	Late DI per animal	Percentage DI	Dominant lethals[a]
		n	%	no.	per animal	no.	per animal	no.	per animal				
I	120	25	83.3	276	11.0	249	10.0	27	1.08	0.96	0.12	9.8	0
	0	17	85.0	183	10.8	170	10.0	14	0.82	0.59	0.24	7.6	–
II	120	28	93.3	300	10.7	266	9.5	34	1.21	1.07	0.14	11.1	2.1
	0	15	75.0	158	10.5	146	9.7	12	0.8	0.67	0.13	7.6	–
III	120	24	80.0	263	11.1	236	9.8	27	1.1	0.88	0.25	10.3	0
	0	19	95.0	209	11.0	187	9.8	22	1.2	0.90	0.26	10.5	–
IV	120	23	76.7	256	11.1	236	10.3	20	0.9	0.87	0	7.8	–10.7
	0	16	80.0	166	10.4	149	9.3	17	1.1	1.00	0.06	10.2	–
V	120	28	93.3	295	10.5	270	9.6	25	0.9	0.79	0.11	8.5	–2.1
	0	19	95.0	198	10.4	178	9.4	20	1.1	0.89	0.16	10.1	–
VI	120	22	73.3	242	11.0	218	9.9	24	1.1	0.96	0.14	9.9	–0.9
	0	14	70.0	152	10.9	140	10.0	12	0.86	0.86	0	7.9	–
VII	120	23	76.7	246	10.7	219	9.5	27	1.2	1.09	0.09	11.0	6.9
	0	16	80.0	176	11.2	163	10.2	16	1.0	0.94	0.06	8.9	–

[a]Dominant lethals (%) = [1 – (LI exp./LI contr.)]×100.

Table III. Results of the dominant lethal test with BMO, 120 mg/kg i.p., NMRI females

Mating intervals	Dose (mg/kg)	Pregnant females		Total implants		Live implants (LI)		Total dead implants (DI)		Early DI per animal	Late DI per animal	Percentage DI	Dominant lethals[a]
		n	%	no.	per animal	no.	per animal	no.	per animal				
I	120	24	80.0	371	15.5	359	15.0	12	0.5	0.5	0	3.2	5.1
	0	16	80.0	265	16.6	253	15.8	12	0.8	0.56	0.19	4.5	–
II	120	20	66.7	304	15.2	285	14.3	19	1.0	0.80	0.15	6.3	4.7
	0	11	55.0	174	15.8	165	15.0	9	0.8	0.8	0	5.2	–
III	120	26	86.7	403	15.5	382	14.7	21	0.8	0.73	0.08	5.2	–9.7
	0	16	80.0	227	14.2	214	13.4	13	0.8	0.69	0.13	5.7	–
IV	120	18	60.0	243	13.5	215	11.9	28	1.6	1.44	0.11	11.5	18.5
	0	14	70.0	214	15.3	205	14.6	9	0.6	0.57	0.07	4.2	–
V	120	28	93.0	420	15.0	381	13.6	39	1.4	1.29	0.11	9.3	8.1
	0	15	75.0	239	15.9	222	14.8	17	1.1	1.00	0.13	7.1	–
VI	120	24	80.0	369	15.4	351	14.6	18	0.8	0.67	0.08	4.9	–0.7
	0	16	80.0	241	15.1	232	14.5	9	0.6	0.56	0	3.7	–
VII	120	25	83.0	387	15.5	376	15.0	11	0.4	0.40	0.04	2.7	–4.9
	0	15	75.0	240	16.0	215	14.3	25	1.7	1.66	0	10.4	–

[a]Dominant lethals (%) = [1 – (LI exp./LI contr.)]×100.

males were mated again to new females for a total of seven mating intervals. Females were inspected for the presence of vaginal plugs every morning.

The females were killed on pregnancy days 14–16 and the contents of the uterus were inspected for live and dead implants (Bateman and Epstein, 1971; Ehling *et al.*, 1978). The numbers of dead implants were compared between control and treated mating groups using the χ^2 test (Sachs, 1974). Dominant lethality (DL) was expressed as %DL = [1 – (live implants per female in the experimental group/live implants per female in the control group)]×100.

Table IV. Results of the dominant lethal test with BDE, 240 mg/kg i.p. (102/El×C3H/El)F_1 females

Mating intervals	Dose (mg/kg)	Pregnant females		Total implants		Live implants (LI)		Total dead implants (DI)		Early DI per animal	Late DI per animal	Percentage DI	Dominant lethals[a]
		n	%	no.	per animal	no.	per animal	no.	per animal				
I	240	30	100.0	325	10.8	290	9.7	35	1.17	0.93	0.23	10.8	−1.0
	0	29	96.7	307	10.6	275	9.5	32	1.10	0.97	0.14	10.4	–
II	240	27	90.0	296	11.0	267	9.9	29	1.07	0.89	0.19	9.8	2.9
	0	25	83.3	279	11.2	254	10.2	25	1.00	0.88	0.12	9.0	–
III	240	26	86.7	284	10.9	257	9.9	27	1.04	0.85	0.19	9.5	5.7
	0	25	83.3	282	11.3	263	10.5	19	0.76	0.68	0.08	6.7	–
IV	240	28	93.3	314	11.2	283	10.1	31	1.11	0.89	0.21	9.9	−9.8
	0	23	76.7	236	10.3	212	9.2	24	1.04	0.87	0.17	10.2	–
V	240	23	76.7	252	11.0	230	10.0	22	0.96	0.74	0.22	8.7	−12.4
	0	23	76.7	236	10.3	205	8.9	31	1.35	1.26	0.09	13.1	–
VI	240	24	80.0	257	10.7	235	9.8	22	0.92	0.79	0.13	8.5	3.9
	0	26	86.7	293	11.3	266	10.2	27	1.04	0.81	0.24	9.2	–
VII	240	25	83.3	276	11.0	253	10.1	23	0.92	0.72	0.20	8.3	−3.1
	0	20	66.7	214	10.7	196	9.8	18	0.90	0.75	0.15	8.4	–

[a]Dominant lethals (%) = [1 − (LI exp./LI contr.)]×100.

Table V. Results of the dominant lethal test with BDE, 240 mg/kg i.p., NMRI females

Mating intervals	Dose (mg/kg)	Pregnant females		Total implants		Live implants (LI)		Total dead implants (DI)		Early DI per animal	Late DI per animal	Percentage DI	Dominant lethals[a]
		n	%	no.	per animal	no.	per animal	no.	per animal				
I	240	22	73.3	325	14.8	301	13.7	24	1.09	1.0	0.09	7.4	9.7
	0	25	83.3	394	15.8	375	15.0	19	0.76	0.68	0.08	4.8	–
II	240	22	73.3	342	15.5	315	14.3	27	1.23	1.23	0	7.9	0
	0	21	70.0	327	15.6	300	14.3	27	1.29	1.14	0.14	8.3	–
III	240	24	80.0	374	15.6	345	14.4	29	1.21	1.08	0.13	7.8	−0.7
	0	23	76.7	355	15.4	328	14.3	27	1.17	1.04	0.13	7.6	–
IV	240	25	83.3	406	16.2	381	15.2	25	1.00	1.00	0	6.2	−1.3
	0	23	76.7	376	16.4	344	15.0	32	1.39	1.26	0.13	8.5	–
V	240	27	90.0	441	16.3	406	15.0	35	1.30	1.22	0.07	7.9	0.7
	0	25	83.3	409	16.4	379	15.1	30	1.20	1.04	0.16	7.3	–
VI	240	29	96.7	495	17.1	461	15.9	34	1.17	1.07	0.10	6.9	−13.5
	0	24	80.0	361	15.0	337	14.0	24	1.00	0.92	0.08	6.7	–
VII	240	23	76.7	360	15.7	328	14.3	32	1.39	1.30	0.09	8.9	−1.4
	0	21	70.0	318	15.1	297	14.1	21	1.00	0.90	0.10	6.6	–

[a]Dominant lethals (%) = [1 − (LI exp./LI contr.)]×100.

Dominant lethality was compared on a male to male basis by the Mann–Whitney test using average values of live implants from all females per male (Chanter *et al.*, 1989).

Results

Ames test

All three epoxy metabolites of 1,3-butadiene were mutagenic when tested in Ames test using the *S.typhimurium* strain TA100. BMO showed a clear dose response without activation with rat liver S9 mix and started to be toxic at a concentration of 10 µmol/plate (Figure 1). The mutagenic activity of BMO without metabolic activation was 27 revertants/µmol/plate. The addition of S9 mix to the test plates lowered the mutagenic response to eight revertants/µmol/plate. The reduction in mutagenicity indicates that BMO is not converted to DEB by S9 mix, but rather that it is metabolized to a non-reactive form by the S9 mix. Binding to proteins in S9 mix, resulting in a reduction of the concentration of free BMO on the test plates, may also explain the observed reduction in mutagenic activity. However, this explanation is not supported by the data obtained from Ames tests with DEB and BDE. The mutagenic activity of DEB (Figure 2) was about two times higher than that of BDE (Figure 3) and 4–12 times higher than the activity of BMO, with and without S9 mix. The mutagenic activities of DEB were 98 revertants/µmol/plate (−S9) and 103 revertants/µmol/plate (+S9). The activities of BDE were 67 revertants/µmol/plate (−S9) and 56 revertants/µmol/plate (+S9). BDE was much less toxic than DEB which started to decrease sharply in mutagenicity at concentrations >1.3 µmol/plate. The use of S9 mix did not appreciably alter the mutagenicity of either DEB or BDE, only a slight decrease in the toxicity of DEB was noted.

Bone marrow micronucleus test

Data on the induction of micronuclei are shown in Table I and Figure 4. BMO significantly increased the micronucleus frequency at the lowest tested dose of 20 mg/kg ($P < 0.05$). The dose response can be described by the linear equation $Y = 0.09 + 0.01D$. At the two higher doses, the response in male mice was significantly higher than in female mice ($P < 0.05$). Therefore, the micronucleus test with BDE was only performed with male mice. The lowest effective dose was 120

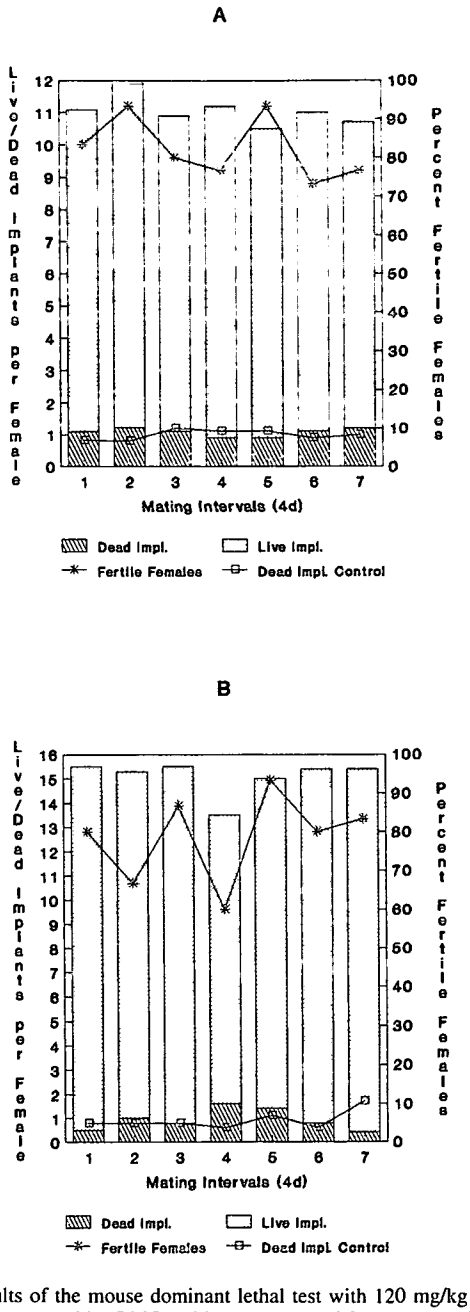

Fig. 5. Results of the mouse dominant lethal test with 120 mg/kg of butadiene monoepoxide (BMO) with two stocks of females mated to the treated $(102\times C3H)F_1$ hybrid males: **(A)** $(102\times C3H)F_1$ hybrid females and **(B)** NMRI females.

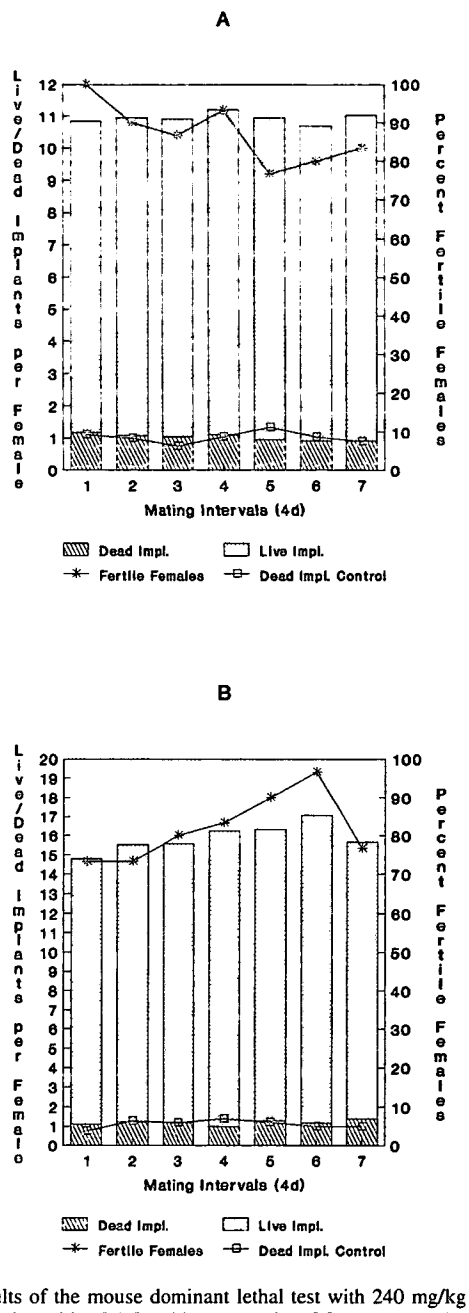

Fig. 6. Results of the mouse dominant lethal test with 240 mg/kg of butadiene diolepoxide (BDE) with two stocks of females mated to the treated $(102\times C3H)F_1$ hybrid males: **(A)** $(102\times C3H)F_1$ hybrid females and **(B)** NMRI females.

mg/kg of BDE ($P < 0.05$). The dose–response can be described by the linear equation $Y = 0.026 + 2.9\times10^{-3}D$. There was no toxicity noticable in the ratio of polychromatic to normochromatic erythrocytes in any of the treated groups.

Dominant lethal test

The dominant lethal test was performed by mating each treated male with two females of different genotypes. The NMRI females have a higher implantation rate (15–16 fetuses) than the hybrid females (10–12 fetuses) and it was expected that this would allow a dominant lethal effect to be detected more readily. However, neither type of female showed an increase in the rate of dead implants per female after mating with BMO- or BDE-treated males at any of the mating intervals (Tables II–V, Figures 5 and 6). The percentages of calculated

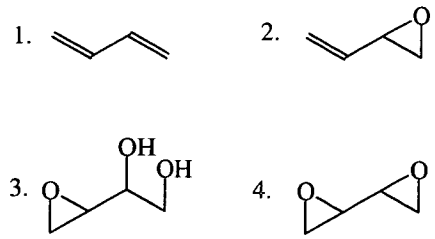

Fig. 7. The chemical structures of: 1. 1,3-butadiene; 2. butadienemonoepoxide (BMO); 3. butadienediolepoxide (BDE); and 4. diepoxybutane (DEB).

dominant lethal effects were not significantly elevated in any of the test groups. Likewise, the pregnancy rates were not reduced in any of the test groups in comparison with the concurrent controls.

Discussion

The chemical structures of 1,3-butadiene and its three epoxy-metabolites are shown in Figure 7.

All three epoxymetabolites of butadiene were mutagenic in bacteria, however, their mutagenic potential decreased in the order DEB > BDE > BMO.

The enantiomers of styrene oxide showed a chirality dependent mutagenicity if tested in *S.typhimurium* TA100. The *R*-enantiomer of styrene oxide showed a substantially higher mutagenicity than the corresponding *S*-enantiomer (Seiler, 1990). The mutagenic activity of the *R*-enantiomer of styrene oxide was on the same level as the mutagenic activity of the racemic BMO. However, the results with BMO are an average of both enantiomers. At the time of the presently described experiments, the optical enantiomers of BMO were not available for testing.

On the basis of the results shown in Figure 1 it was concluded that an S9 mix extracted from rat liver cannot further oxidize BMO to bifunctional DEB. This conclusion is supported by the lack of increased toxicity of BMO in the presence of S9. The question arises why BMO is not converted to DEB and why it does not show similar toxicity as DEB at higher concentrations. The answer may be that the epoxy-hydrolase activity in rats is high and rat S9 was used in the experiments (Bentley *et al.*, 1985). The high epoxyhydrolase activity in the S9 mix may induce the formation of 1-butene-diol instead of DEB. The activity of epoxyhydrolase has been shown to be species specific. It is about four to five times higher in rat liver than in mouse liver. A sex difference in epoxyhydrolase activity was also reported for mice. The activity of this phase II metabolizing enzyme may therefore explain also the species and sex differences seen *in vivo*.

BDE has obtained a little attention in 1,3-butadiene related research as a potentially genotoxic metabolite. However, BDE is an interesting compound in the sense of chemical character. It has alkylating capacity but the neighbouring hydroxy groups as a result of hydrolysis of the epoxy groups make BDE the most hydrophilic metabolite of 1,3-butadiene. This hydrophilicity may contribute to chemico–physical actions, i.e. distribution and penetration. BDE showed similar mutagenicity as DEB the latter being more toxic. However, more research is needed to allow a risk estimation for this interesting metabolite of 1,3-butadiene.

All three electrophilic epoxymetabolites studied in this exercise can form DNA adducts. Some key adducts of BMO have been characterized *in vitro* and detected also *in vivo* (Koivisto *et al.*, 1995, 1997; Neagu *et al.*, 1995). DNA adduct work related to BDE has not been described in the literature but the adduct formation of DEB has been studied by Leuratti *et al.* (1994). This work was unable to demonstrate the formation of inter- or intra-strand cross-links by this bifunctional alkylating agent. However, as described in their pioneering work, Ehrenberg and Hussein (1981) summarized that a 30 times higher effectivity per primary alkylation is seen when monofunctional ethyleneoxide is compared with bifunctional diepoxybutane.

The clastogenic activity in mouse bone marrow erythrocytes showed a different order of the compounds than in the Ames test, i.e. DEB > BMO > BDE. Compared on a molar basis, DEB was about three times as effective as BMO which was about four times as effective as BDE. The response to BMO in the micronucleus assay indicates that BMO is clastogenic; however, part of the response may be attributed to the formation of DEB by the animals' metabolism. BDE seems to be clastogenic by itself.

It is remarkable that neither the monoepoxide nor the diolepoxide showed a dominant lethal effect in male mouse germ cells when tested at doses that gave a significant induction of micronuclei in bone marrow erythrocytes. The lack of effect of the two monofunctional metabolites BMO and BDE is in contrast to the induction of dominant lethal effects and cytotoxic effects in mouse spermatozoa and late spermatids by the bifunctional metabolite DEB (Adler *et al.*, 1995c). This indicates that the amount of DEB formed from BMO in germ cells or in the liver and transported to the testes is too small to cause an effect measurable with the presently used dominant lethal test protocol.

The results presented here confirm the expected high mutagenicity of bifunctional DEB when compared with its monofunctional analogues.

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