BACTERIAL MUTAGENICITY AND TOXICITY OF CYCLOALIPHATIC EPOXIDES

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Summary

The mutagenicity of 12 cycloaliphatic epoxides was investigated using the Ames Salmonella assay without the addition of liver homogenate fractions. Base-pair substitution mutagenic activity was detected for 8 members of this series of compounds, confirming our laboratory's previous observations of weak mutagenic response at high dose levels for *cis*-1,2-disubstituted epoxides. While mutagenicity decreased with expanding ring size, inhibition of bacterial growth increased for increases in ring size. Toxicity accompanying the required high doses for the demonstration of mutagenicity for these compounds prevented the establishment of meaningful dose—response ranges for the remaining epoxides tested. The volatility observed with these oxiranes also made dose—response establishment difficult but was countered by the use of petri dish sealing bands during incubation. Mutagenicity in this series was found to be generally more pronounced in TA1535 while toxicity was detected with greater sensitivity by TA100. The use of the less permeable strains TA92, TA1950 and TA2410, all having normal lipopolysaccharide cell wall coatings, failed to reduce this marked toxicity. The repair test compared results in TA1535 (repair-deficient strain) with TA1975 (repair-proficient strain) and demonstrated that the bacteria were not being killed by damage to DNA since toxicity was not reduced in TA1975.

References in the literature to the mutagenicity of cycloaliphatic epoxides are limited **. Thus, while it is well established that biological arene oxide transformation can lead to cyclic aliphatic epoxides such as the 7,8-diol-9,10-epoxide metabolite of benzo[a]pyrene that are highly mutagenic (Gelboin and Ts'o,

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** Note added in proof: See Jung et al., Mutation Res., 81 (1981) 11—19, for a report on the mutagenicity of 17 cycloaliphatic epoxides.

1978; Lehr and Jerina, 1977; Newbold and Brooks, 1976; Thakker et al., 1976; Wislocki et al., 1976; Yagi et al., 1977a,b) little has been reported concerning the mutagenicity of less complex cycloaliphatic epoxides. Previous work in these laboratories on the mutagenicity of aliphatic epoxides indicated that among the limited number of 1,2-disubstituted epoxides tested only structures having a *cis* configuration about the oxirane ring were mutagenic (D.R. Wade et al., 1978). Cyclohexane oxide was one of the 1,2-disubstituted compounds found to be a weak mutagen in the Ames test for TA100. Watabe et al. (1980) also reported this compound to be a weak mutagen in the Ames test for TA100. In addition, the latter study found vinylcyclohexane dioxide to be weakly mutagenic in TA100. Other investigations have shown this diepoxide to be mutagenic for both TA100 and TA1535 in the plate incorporation test (El-Tantawy and Hammock, 1980) and mutagenic for TA100 but not for TA98 in the spot-test version of the Ames test (M.J. Wade et al., 1979). The mutagenicity of 1,2,5,6-diepoxycyclooctane was assessed by the use of Chinese hamster lung-cell culture and found to be totally inactive (Huang et al., 1978). The carcinogenicity of vinylcyclohexane dioxide (Christensen, 1974; Van Duuren et al., 1966) and the non-carcinogenicity of 1,2,5,6-diepoxycyclooctane (Van Duuren et al., 1967; Van Duuren, 1969) in rodent systems has been firmly established.

The present investigation focuses on the expansion of Ames mutagenicity testing with Salmonella for cycloaliphatic oxiranes as examples of *cis*-1,2-disubstituted aliphatic epoxides. The series has been expanded to include 9 cycloalkane and 3 cycloalkene epoxides. The role of toxic interference in the establishment of mutagenic dose—response for these compounds is discussed.

Materials and methods

Cyclopentane oxide (97% purity), cyclohexane oxide (98% purity), cyclooctane oxide (99% purity), cyclododecane oxide (97% purity), *exo*-2,3-epoxynorbornane (98% purity), 4-vinylcyclohexane dioxide (99% purity) and 1,2,5,6-diepoxycyclooctane (97% purity) were obtained from Aldrich Chemical Company (Milwaukee, WI). Cycloheptane oxide and 1-oxaspiro[2.5]octane were acquired from the ABC Rare Chemical Division of Aldrich (purity unspecified).

3,4-Epoxycyclopentene, 3,4-epoxycyclohexene and 4,5-epoxycyclohexene were synthesized by the method of Crandall et al. (1968) from the respective cyclodienes. 1,3-Cyclohexadiene (96% purity) and 1,4-cyclohexadiene (97% purity) were purchased from Aldrich while 1,3-cyclopentadiene was freshly prepared (Moffett, 1963) from its dimer, dicyclopentadiene (Phillips Chemical, Bartlesville, OK).

All chemicals were analyzed for the absence of trace alkylating impurities using 4-(*p*-nitrobenzyl)-pyridine (Aldrich) by the method of Hammock, et al. (1974) in 2 TLC systems: normal phase using dichloromethane on 250-micron Silicar 7GF plates (Analtech, Newark, DA) and reverse phase with methanol on 250-micron C18 RPS fluorescent plates (Analtech). The structures for the commercially available and synthesized epoxides were confirmed by NMR and IR spectrometry.

Bacteria used for this testing were the specially constructed histidine-dependent mutants of *Salmonella typhimurium*. The mutagenesis assays were accom-

plished by a modified version of the Ames method (Ames et al., 1975). The cultures were grown up overnight (20-ml inoculum volumes of nutrient broth, Difco, Detroit, MI) in Nephalo culture flasks containing spectrophotometric sidearms (Bellco Biological Glassware, Vineland, NJ). The cultures were grown to the absorbance range of 0.78—0.83 at 560 nm (Spectronic 20, Bausch and Lomb, Rochester, NY) which produced spontaneous reversion counts of 15-25 for TA1535 and 70—120 for TA100. Compounds were dissolved in dimethylsulfoxide on the day of the test and 0.1-ml portions were added to 2 ml of molten (45°C) top agar (Ames et al., 1973) together with 0.1 ml of bacterial suspension (which under our conditions resulted in 3—6 × 10^8 cells for TA100 and 8—11 × 10^8 cells for TA1535) and plated onto minimal-glucose (1.5% BBL agar [Becton, Dickenson and Co., Cockeysville, MD] in Vogel—Bonner Medium E with 2% glucose) as described (Ames et al., 1975). The test-dose levels were done in triplicate (Falcon "Muta-Assay" plates, Oxnard, CA) and control plates were treated in the same manner but without test compound.

In addition, strains were also used for this study which contained a normal lipopolysaccharide cell-wall coating [TA92 (hisG46/pKM101), TA1950 (hisG46/ΔuvrB) and TA2410 (TA1950/pKM101) as well as a strain which contained the normal complement of enzyme-repair systems [TA1975 (hisG46/rfa)]. Test cultures for these strains were prepared as described by Ames et al. (1975) using overnight (15 h) cultures.

Positive mutagen controls were routinely included in test protocols. Sodium azide (Pfaltz and Bauer, Stamford, CN) was used for both TA100 and TA1535. Bacterial survival was determined by combining mutagenicity test doses of the compounds with 10^{-5} dilutions of the bacterial cultures (300—600 cells/plate for TA100 and 800—1100 cells/plate for TA1535) on nutrient agar (BBL, Becton, Dickenson and Co., Cockeysville, MD) plates. The use of petri dish sealing bands (Fisher Scientific, Pittsburgh, PA) was routinely employed to prevent the loss of volatile compounds during the 48-h incubation period.

Results and discussion

The cycloaliphatic epoxides tested in this investigation are listed in Table 1. In general, these alicyclic oxiranes possess a *cis*-1,2-disubstituted stereochemistry about the epoxide ring. One exception is compound 9, oxaspirooctane, a 1,1-disubstituted oxirane which was of interest because the hydrocarbon ring moiety represents restricted rotation for the cyclohexane ring carbons analogous to that for the 1,2-disubstituted cycloaliphatic epoxides.

Preliminary screening tests with the 5 conventional tester strains, TA98, TA100, TA1535, TA1537 and TA1538, demonstrated mutagenic activity only in the base-pair indicator strains, TA100 and TA1535. The results of nonactivated mutagenicity assays in these 2 strains for the cycloalkane epoxides (1—2, 6—9) and the cycloalkene oxiranes (11, 12) which are not complicated by toxic inhibition are shown in Table 2. Duplicate runs of these tests, to confirm the reproducibility of the data, are presented in Table 2 as the second result under each dose level and are consistent with day-to-day variation under standardized experimental conditions observed for the Ames technique (Cheli et al., 1980; Peak et al., 1980). Compounds 1, 2, 6, 8, 11 and 12 were found to be muta-

TABLE 1

COMPOUNDS TESTED

No.	Name	Molecular weight	Structure
1	Cyclopentane oxide (6-oxabicyclo[3.1.0]hexane)	84.12	
2	Cyclohexane oxide (7-oxabicyclo[4.1.0]heptane)	98.15	
3	Cycloheptane oxide (8-oxabicyclo[5.1.0]octane)	112.17	
4	Cyclooctane oxide (9-oxabicyclo[6.1.0]nonane)	126.20	
5	Cyclododecane oxide (13-oxabicyclo[10.1.0]tridecane)	182.31	
6	Norbornane oxide, *exo*-2,3- (3-oxatricyclo[3.2.1.0^{2,4}]octane)	110.16	
7	Diepoxycyclooctane, 1,2,5,6- (5,10-dioxatricyclo[7.1.0.0^{4,6}]decane)	140.18	
8	Vinylcyclohexane dioxide (3-oxiranyl-7-oxabicyclo[4.1.0]heptane)	140.18	
9	Oxaspirooctane (1-oxaspiro[2.5]octane)	112.17	
10	Cyclopentene-3,4,epoxide (6-oxabicyclo[3.1.0]hex-2-ene)	82.12	
11	Cyclohexene-3,4-epoxide (7-oxabicyclo[4.1.0]hept-2-ene)	96.15	
12	Cyclohexene-4,5-epoxide (7-oxabicyclo[4.1.0]hept-3-ene)	96.15	

genic for both strains. Oxaspirooctane (9) was weakly mutagenic only for TA100 and diepoxycyclooctane (7) was inactive in both strains. The order of positive mutagenic response in TA100 is: vinylcyclohexane dioxide (8) ≫ cyclopentane oxide (1) > 4,5-epoxycyclohexene (12) ≈ cyclohexane oxide (2) > norbornane oxide (6) > 3,4-epoxycyclohexene (11) > oxaspirooctane (9). However, for TA1535 the order is: 4,5-epoxycyclohexene (12) > cyclopentane oxide (1) > cyclohexane oxide (2) ≈ norbornane oxide (6) > vinylcyclohexane oxide (8) > 3,4-epoxycyclohexene (11). In both strains, mutagen-

TABLE 2

MUTAGENIC DOSE RESPONSE FOR CYCLOALIPHATIC EPOXIDES

No.	Compound	Mutagenicity [a]							
		TA100				TA1535			
	Dose (µmoles)	15	30	45	60	15	30	45	60
Cycloalkane epoxides									
1	Cyclopentane oxide	338 ± 17	567 ± 56	1013 ± 30	1132 ± 230	467 ± 54	897 ± 17	1013 ± 60	1321 ± 136
		384 ± 25	688 ± 39	867 ± 28	970 ± 19	419 ± 183	764 ± 93	1008 ± 90	1387 ± 210
2	Cyclohexane oxide	287 ± 22	573 ± 24	576 ± 84	808 ± 84	366 ± 81	604 ± 37	1004 ± 57	1293 ± 17
		312 ± 26	555 ± 5	809 ± 46	761 ± 206	331 ± 104	608 ± 107	831 ± 0	1125 ± 145
6	Norbornane oxide	260 ± 48	514 ± 90	724 ± 110	752 ± 128	184 ± 39	326 ± 79	591 ± 132	1306 ± 125
		241 ± 29	417 ± 10	646 ± 16	805 ± 144	252 ± 59	368 ± 11	601 ± 94	1063 ± 172
7	Diepoxycyclooctane	82 ± 10	96 ± 3	85 ± 22	103 ± 4	19 ± 6	19 ± 2	16 ± 7	19 ± 2
		102 ± 3	108 ± 5	97 ± 9	109 ± 10	19 ± 3	21 ± 7	14 ± 2	14 ± 1
8	Vinylcyclohexane dioxide	621 ± 47	1134 ± 183	2071 ± 269	2429 ± 547	107 ± 14	141 ± 38	355 ± 52	600 ± 95
		592 ± 118	1045 ± 31	1947 ± 280	1963 ± 372	217 ± 25	357 ± 76	487 ± 57	669 ± 106
9	Oxaspirooctane	152 ± 21	241 ± 44	279 ± 62	276 ± 88	29 ± 8	27 ± 3	32 ± 2	39 ± 4
		165 ± 21	243 ± 20	263 ± 25	320 ± 66	16 ± 9	38 ± 2	28 ± 9	34 ± 10
	Spontaneous reversion counts [b]			70 ± 13				24 ± 0.5	
				77 ± 2				15 ± 3	
	Cell populations [c] (×10^{8} cells/plate)			6.34				11.65	
				2.76				8.87	
Cycloalkene epoxides									
11	Cyclohexene-3,4-epoxide	191 ± 10	522 ± 107	233 ± 32	413 ± 68	83 ± 35	188 ± 13	173 ± 29	123 ± 35
		232 ± 47	382 ± 90	407 ± 24	374 ± 42	119 ± 28	205 ± 4	225 ± 15	235 ± 14
12	Cyclohexene4,5-epoxide	247 ± 17	364 ± 30	543 ± 68	840 ± 57	415 ± 56	550 ± 140	1091 ± 83	1594 ± 226
		311 ± 31	520 ± 46	649 ± 90	877 ± 199	527 ± 49	748 ± 107	776 ± 36	1670 ± 93
	Spontaneous reversion counts [b]			94 ± 2				15 ± 5	
				94 ± 12				21 ± 4	
	Cell populations [c] (×10^{8} cells/plate)			3.85				9.59	
				3.29				11.60	

[a] Mutagenicity for 2 determinations of 3 replicate plates per dose level is reported as mean counts ± standard deviation. The results of a second set of determinations are presented as the second entry under each dose level measured.

[b] Background counts are the result of adding 0.1 ml dimethylsulfoxide per plate to determine the spontaneous reversion rate. The counts have not been subtracted from the data for mutagenicity counts.

[c] Cell populations represent average cell numbers plated onto minimal agar plates in the mutagenicity test. These populations were determined by plating nutrient agar with 0.1 ml of a 10^{-5} serial dilution of the same culture used for mutagenicity testing.

icity could only be demonstrated for the compounds with 5- or 6-membered rings.

Under the conditions of this test, mutagenicity for these compounds was generally found to be more pronounced in TA1535 than in TA100, with the exceptions of vinylcyclohexane dioxide (8) and oxaspirooctane (9). The influence of toxic inhibition varied with compounds and strains, complicating a comparison of mutagenicity between the 2 strains. The findings of this study are in agreement with previous investigations (El-Tantaway and Hammock, 1980; M.J. Wade et al., 1979; Watabe et al., 1980) of mutagenicity in the Ames test for vinylcyclohexane dioxide (8). The higher dose levels used for cyclohexane oxide (2) in the present study extended the literature reports (D.R. Wade et al., 1978; M.J. Wade et al., 1979) of mutagenicity in TA100 only by demonstrating greater mutagenicity for TA1535 than for TA100. The weak mutagenic dose—response found in TA100 for oxaspirooctane (9) with its

TABLE 3

COMPARATIVE TOXICITY FOR CYCLOALIPHATIC EPOXIDES

No.	Compounds	Dose (μmoles/plate)	Relative toxicity (% inhibition) [a]	
			TA100	TA1535
1	Cyclopentane oxide	48	0	2
		60	10	7
2	Cyclohexane oxide	48	40	50
		60	44	58
3	Cycloheptane oxide	48	88	73
		60	90	85
4	Cyclooctane oxide	48	96	89
		60	99	97
5	Cyclododecane oxide	48	100	100
		60	100	100
6	Norbornane oxide	48	49	17
		60	64	30
7	Diepoxycyclooctane	48	9	0
		60	10	0
8	Vinylcyclohexane dioxide	48	6	8
		60	12	15
9	Oxaspriooctane	48	46	57
		60	60	76
Cycloalkene epoxides				
10	Cyclopentene-3,4-epoxide	45	100	100
		60	100	100
11	Cyclohexene-3,4-epoxide	45	2	12
		60	17	23
12	Cyclohexene-4,5-epoxide	45	4	5
		60	0	10

[a] Toxic inhibition of bacterial growth is reported as the average % growth inhibition for at least 2 determinations of 3 replicate plates per dose level tested as compared to a positive growth control group in which only 0.1 ml dimethylsulfoxide per plate is added. Test compound doses in 0.1 ml dimethylsulfoxide were plated onto nutrient agar plates with 0.1 ml of a 10^{-5} serial dilution of the culture used for mutagenesis testing. Only the high dose levels were tested.

TABLE 4

COMPARATIVE MUTAGENICITY FOR THE EXPANDING RING CYCLOALKANE EPOXIDES

No.	Compound	Dose [a] (μmole/plate)	Revertants per plate [b]	
			TA1535	TA100
1	Cyclopentane oxide	12	678 ± 101	234 ± 24
			$P < 0.05$ [c]	
2	Cyclohexane oxide	12	534 ± 74	187 ± 47
3	Cycloheptane oxide	12 [d]	29 ± 7 $P < 0.05$ [e]	67 ± 17
4	Cyclooctane oxide	12	22 ± 5	53 ± 12
Spontaneous reversion counts [f]			20 ± 4	77 ± 9

[a] Test compound is dissolved in 0.1 ml dimethylsulfoxide per plate.

[b] Mutagenicity for 6 replicates per compound is reported as mean counts ± standard deviation. Background counts are not subtracted.

[c] Statistical comparison of results for compounds 1 and 2 in TA1535 using a one-sided Student's t-test.

[d] This compound was only partially soluble in dimethylsulfoxide and was delivered to the plates as a suspension rather than a solution.

[e] Statistical comparison of results for compound 3 with spontaneous reversion counts in TA1535 using a one-sided Student's t-test.

[f] See note b, Table 2.

restricted rotation at the oxirane ring is another exception to the general rule that 1,1-disubstituted epoxides are inactive as mutagens (D.R. Wade et al., 1978). The atypical lack of mutagenicity for diepoxycyclooctane (7) observed in this investigation was also found by Huang and coworkers (1978) in a mammalian cell culture. The view that diepoxides of this type are frequently more carcinogenic than monoepoxide compounds (Van Duuren, 1969; Weil, 1963) cannot be extended for mutagenicity with diepoxycyclooctane in the Ames system.

The toxicity observed for cycloaliphatic epoxides made the establishment of meaningful dose—response relationships difficult. The assessment of toxicity at high dose levels for all compounds tested is summarized in Table 3. Toxic inhibition is very pronounced for cycloheptane oxide (3), cyclooctane oxide (4), cyclododecane oxide (5), as well as 3,4-epoxycyclopentane (10) and none of these compounds demonstrated a mutagenic dose—response. This was evidenced by a reduction in the number of revertant colonies observed to be well below background and a disappearance of the background lawn normally present. In addition, compounds 5 and 10 were lethal to the bacteria at concentrations larger than 12 and 15 μmoles respectively in both strains. Toxic inhibition of bacterial growth made it impossible to correlate the mutagenic activity of cycloaliphatic epoxides to structural features such as ring size, degree of ring strain and degree of planar conformation.

A structure—toxicity relationship, however, did emerge from this work in that toxicity increased with expanding ring size for the homologous epoxide series composed of compounds 1—5 (Table 3). Conversely, a decrease in mutagenicity with expanding ring size is observed for these same compounds as expressed in Table 4 with multiple tests at the same dose. Cyclododecane oxide (5) was tested in the same manner but demonstrated no viable colonies even at the 12-μmole dose level selected to minimize toxic inhibition of growth. The

TABLE 5

MUTAGENICITY OF CYCLOALKENE EPOXIDES IN THE SPOT TEST

No.	Compound	Dose [a] (μmoles)	TA100		TA1535	
			Revertants per plate	Toxic zone (mm) [b]	Revertants per plate	Toxic zone (mm) [b]
Expanding ring series						
1	Cyclopentane oxide	30	352	0.5	1000	0.5
		50	679	0.5	1246	0.5
2	Cyclohexane oxide	30	338	0	646	0.5
		50	552	0	1006	0.5
3	Cycloheptane oxide	30	73	2.0	20	2.0
		50	65	3.5	36	3.0
4	Cyclooctane oxide	30 [c]	39	2.5	10	1.5
		50 [c]	32	4.0	16	1.5
5	Cyclododecane oxide	30	0 [d]	z [e]	0 [d]	z [e]
		50	0	z	0	z
Background [f]		0	71	0	18	0
Control [g]		30	52	1.5	19	0
		50	40	1.5	13	0.5

[a] Dose levels reported were spotted directly as liquid samples based on volume equivalents to μmole amounts using density values at 25°C.

[b] Toxicity is expressed as the width (mm) of the zone of inhibition of the background lawn of bacterial growth around the spot.

[c] This compound is the only solid in the group and μmole amounts reported were spotted per 10 μl dimethylsulfoxide.

[d] Indicates killing of bacterial lawn by the test compound.

[e] The zone of toxicity extends to the edge of the plate.

[f] Dimethylsulfoxide (10 μl) was added per plate to determine spontaneous reversion rate.

[g] Cyclohexanol was used as a nonepoxide control compound of similar structure to the alicyclic epoxides and was spotted directly as a liquid sample.

result for compound 3 in TA1535 shows a statistically significant difference ($P < 0.05$) from background. The observed toxic interference makes it difficult to assess mutagenicity of these examples by this testing method.

The spot test version of the Ames assay (Ames et al., 1975) was run for compounds 1—5 in an attempt to gain a better balance between doses that produced toxicity and those required for mutagenicity so that clearly identifiable rings of mutagenic growth might be demonstrated. However, this did not occur as the distribution of compounds was not controlled by diffusion alone but was also influenced by the volatility of the compounds. Nevertheless, the data presented in Table 5 further demonstrates the same trend for decreased mutagenicity with expanding ring size as determined by total plate counts. There is also the previously demonstrated increase in toxicity with increasing ring size indicated by the radius of the toxic zone around the initial spot.

The use of very high dose levels is required for cycloaliphatic epoxides to demonstrate only weak mutagenic dose—response and it is, therefore, not surprising that toxic inhibition is observed. These toxic effects have also reduced the reproducibility of measurements for mutagenic activity for the cycloaliphatic epoxides. There is an additional problem observed for the volatile toxic

TABLE 6

MUTAGENICITY AND TOXICITY IN STRAINS POSSESSING A NORMAL LIPOPOLYSACCHARIDE CELL-WALL COATING

No.	Compound	Dose (μmoles)	Mutagenicity [a]			Relative toxicity [b] (% inhibition)		
			TA92	TA1950	TA2410	TA92	TA1950	TA2410
		24	47 ± 3	350 ± 35	515 ± 91	0	7	10
		48	53 ± 3	254 ± 3	436 ± 100			
2	Cyclohexane oxide	24	44 ± 8	320 ± 35	470 ± 210	0	10	22
		48	54 ± 2	371 ± 6	726 ± 131			
		24	33 ± 7	18 ± 4	105 ± 12	12	23	24
		48	32 ± 4	39 ± 2	165 ± 23			
4	Cyclooctane oxide	24	18 ± 6	13 ± 2	121 ± 17	23	33	42
		48	19 ± 6	33 ± 0	140 ± 31			
		24	33 ± 8	16 ± 3	88 ± 27	84	96	98
		48	42 ± 7	32 ± 5	132 ± 10			
5	Cyclododecane oxide	24	39 ± 3	19 ± 6	89 ± 38	84	97	99
		48	44 ± 8	40 ± 12	127 ± 29			
			44 ± 4	26 ± 4	128 ± 19			
			35 ± 0	39 ± 4	158 ± 10			
Spontaneous reversion counts [c]			1.97	3.17	1.54			
			3.16	2.70	1.45			
Cell population [d] (×10^8 cells/plate)								
Cells/control plate [e]						316 ± 28	294 ± 28	150 ± 19

a See note a, Table 2.
b This data was determined as described in footnote a of Table 3 and represents comparison of means for 3 replicate values per dose in TA92 and 6 replicate values per dose in TA1950 and TA2410 with means of positive growth control groups.
c See note b, Table 2.
d See note c, Table 2.
e This is a positive growth control used as the basis for assessment of results for toxic inhibition of growth. It is determined by adding only 0.1 ml dimethylsulfoxide per control plate.

members of this epoxide group (compounds 1—5, 9—12) in that reproducibility is further complicated by uneven distribution and uneven loss of the compounds from replicate plates at a given dose level. Use of rubber-band petri-dish sealers prevented loss of the test compound and decreased replicate plate variation for the less toxic alicyclic epoxides tested (compounds 1—2, 11—12) but were found to increase toxicity of the more toxic compounds (3—5, 10).

The possibility of failing to detect a given mutagen in the Ames test because the compound is toxic to bacteria for reasons unrelated to its mutagenic potential has been raised by McCann and Ames (1977). The repair test as described by Ames et al. (1975) was employed to determine if the mechanism of toxicity was related to that of mutagenicity for the compounds under study. Although this test will not detect mutagenicity, it can indicate whether a compound is killing bacteria through damage to DNA. DNA damage can be repaired by the *uvrB* excision-repair enzyme system which is present in these strains but has been deleted from conventional strains. Cyclooctane oxide (4) was tested with TA1975 (a repair-proficient strain) in comparison with TA1535 (a repair-deficient strain) and showed that toxicity for this epoxide was not reduced, indicating that the 2 effects do not have DNA damage as a common mechanism.

Table 6 summarizes the results of one approach to separate toxicity from mutagenicity through the use of specially constructed, base-pair mutagen detector strains, TA92, TA1950 and TA2410. Since these strains possess the normal lipopolysaccharide cell-wall coating which has been altered in TA100 and TA1535 to make the cells more permeable, such a test might reduce toxicity by diminishing entry of the epoxides into the bacterial cell while still allowing mutagenic activity to occur. The results indicate, however, that this approach has failed for compounds 2, 4 and 5 to reduce the ratio of mutagenicity to toxicity in comparison to that of the conventional strains (Table 6 compared to Tables 2 and 3).

Another approach to test the presence of dose—response mutagenicity for this cycloalkane epoxide series was made based on the observation that some colonies of both TA100 and TA1535 survived the toxicity observed previously for compound 3 (Table 3). This suggested the possibility of resistance by these colonies to toxic effects of compound 3 and if so, a chance to subculture the colonies for use in a test for a dose—response demonstration. However, a check for the genotypes of these suspected colonies in histidine-depleted media revealed that the colonies were in fact histidine-independent bacteria unsuitable for further testing and is in agreement with a toxic effect described by de Serres and Shelby (1979) in which surviving bacteria give rise to small colonies which appear to be revertants.

The mutagenic response at high dose levels for compounds 1, 2, 6—9, 11 and 12 establishes the mutagenicity for cycloaliphatic epoxides and confirms the hypothesis that *cis*-1,2-disubstituted oxiranes are mutagenic. More cyclic epoxide compounds containing substituent groups should be tested to determine how such groups on reactive ring structures can alter the mutagenic potential of the oxirane ring. Such information is needed to expand our knowledge of the toxicity of cycloaliphatic epoxides per se and as metabolites in biological systems.

Acknowledgements

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