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Comparison of Alkylation Rates and Mutagenicity of Directly Acting Industrial and Laboratory Chemicals

Epoxides, Glycidyl Ethers, Methylating and Ethylating Agents, Halogenated Hydrocarbons, Hydrazine Derivatives, Aldehydes, Thiuram and Dithiocarbamate Derivatives

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Abstract. Groups of industrial and laboratory chemicals were tested for their alkylation activity using 4-(p-nitrobenzyl)-pyridine and deoxyguanosine as nucleophiles. The alkylation activity was compared with mutagenicity of the chemicals to *E. coli* WP2 uvrA without metabolic activation. All the epoxide-containing compounds including simple epoxides and glycidyl ethers elicited alkylation activity and mutagenicity. Furthermore there was a reasonable correlation between the rate of alkylation and the mutagenic potency. All the methylating and ethylating compounds tested were active but no correlation was observed between the rate of alkylation and the mutagenic potency, apparently due to the different types of alkylation products formed. The other compounds tested including halogenated hydrocarbons, hydrazine derivatives, aldehydes, thiuram and dithiocarbamate derivatives elicited a slow or no alkylation activity while many of the compounds were mutagenic. There was no evidence among the chemicals tested of an alkylating non-mutagen. Thus evidence of alkylation activity appears to indicate mutagenic risk.

Key words: Alkylation – 4-(p-nitrobenzyl)-pyridine – Deoxyguanosine – Mutagenicity – Industrial chemicals.

Introduction

It is well established that a number of mutagens and carcinogens react covalently with DNA (Heidelberger et al. 1975). It is becoming increasingly clear with many types of mutagens that the chemical reactivity as well as the kind of the adducts formed contribute to the mutagenic and probably also to the carcinogenic potency (Velemínský et al. 1970; Lawley 1976; Hemminki 1979; Bartsch et al. 1979). However, it is not established whether a chemical capable of covalent binding to DNA is in fact mutagenic or carcinogenic. Increased

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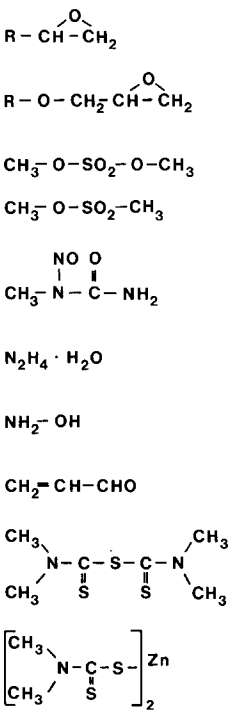

Fig. 1. Structures of the compounds tested

knowledge on such relationships would be valuable in the evaluation of mutagenic and carcinogenic risks by chemicals based on chemical reactivity.

Screening methods are available for the determination of the alkylation rates of chemicals, providing an approximate indication of the reactivity of chemicals towards DNA. Alkylation test using 4-(p-nitrobenzyl)-pyridine (NBP) has been used by several investigators; another alkylation test using a biological nucleophile, deoxyguanosine has been recently developed (Hemminki 1979). In this study we apply the two alkylation tests to correlate the chemical reactivity with mutagenicity in *E. coli* WP2 uvrA. All the chemicals used are thought to be reactive directly without metabolic activation, and thus the assays for mutations are also carried out without microsomal activating enzymes. Many of the chemicals tested are widely used in industry; other chemicals are used in laboratories and they are of interest because of their selective reactivity. The structures of the groups of chemicals tested are shown in Fig. 1.

Materials and Methods

The compounds tested were of analytical grade except for glycidyl ethers of bisphenol A (Epikote 828, 1001, 1004 Shell Chemical Co.) and the chemicals used in rubber industry (thiuram and

dithiocarbamate derivatives), which were of technical grade. The stock solutions of the compounds were prepared immediately prior to the use in dimethyl sulfoxide.

The 4-(p-nitrobenzyl)-pyridine (NBP) reaction was carried out at 37° C as described (Barbin et al. 1975; Hemminki and Falck 1979) using the test compound at 0.286 μM concentration. Aliquots of the reaction mixture were assayed for the alkylation products at 0 min, 20 min, 1 h, 3 h and 5 h. The deoxyguanosine (dG) alkylation was carried out at 37° C as described (Hemminki 1979) using the test compound at 0.1 M concentration. Aliquots of the reaction mixture were assayed for fluorescence at 0 min, 20 min, 1 h, 3 h and 5 h. The reactions with propylene oxide were carried out at 28° C. Epichlorohydrin was used as a reference compound in each assay. The reaction rates were determined from the initial rates. The results of the different assays were calculated using epichlorohydrin as a reference.

The bacterial mutagenicity assay, using *E. coli* WP2 uvrA (Green and Muriel 1976), was carried out as described earlier (Hemminki and Falck 1979). Six different concentrations, usually in the range, 20–10,000 μM, based on a toxicity determination, were tested for each chemical. The incubation with the chemicals was at 37° C (28° C for propylene oxide) for 18 h, after which aliquots were plated either onto DM-minimal agar (for prototrophs) or onto nutrient agar containing tryptophan (for total number of cells). The cells were incubated at 37° C for 2 days and the colonies in each population were determined. The results are given as the ratio of the total number of prototrophs/total number of viable cells in each sample (*RF* of the compound – *RF* of the control/concentration μM, where *RF* is revertant fraction). Epichlorohydrin (mutagenicity 100%) was included as a reference in all assays.

Results

The alkylation rates and mutagenicity of a group of aliphatic epoxides was determined (Table 1). Among the halogenated epoxides, epichlorohydrin and epibromohydrin were almost similar in their alkylation rates and mutagenicity, exceeding those of epifluorohydrin. The other epoxides tested, propylene oxide, butylene oxide and butadiene monooxide were markedly less reactive and mutagenic than the halogenated compounds.

Glycidyl ethers, including diglycidyl ethers of bisphenol A and aliphatic monoglycidyl ethers, were tested (Table 2). The reactivity of the bisphenol A derivatives decreased with increasing molecular weight. The same appeared to be true for mutagenicity, although the poor solubility of the high molecular weight epoxy resing interfered with assay. Anyway it was of interest that the epoxy resins were at least as mutagenic on the molar basis as epichlorohydrin.

Table 1. Alkylation rates and mutagenicity of epoxides

Compound	Rate of alkylation				Mutagenicity	
	NBP (ΔA_{560}/h)	%[a]	dG (ΔI/h)	%[a]	WP2 uvrA ($\Delta RF \times 10^{-11}/\mu M$)	%[a]
Epichlorohydrin	405	100	205	100	200	100
Epibromohydrin	425	105	280	137	190	95
Epifluorohydrin	360	89	175	85	40	20
Propylene oxide	90	22	95	46	20	10
Butylene oxide	80	20	75	37	20	10
Butadiene monoxide	125	31	28	14	40	20

[a] As per cent in relation to epichlorohydrin

Table 2. Alkylation rates and mutagenicity of glycidyl ethers

Compound	Rate of alkylation				Mutagenicity	
	NBP (ΔA_{560}/h)	%[a]	dG (ΔI/h)	%[a]	WP2 uvrA (ΔRF × 10^{-11}/μM)	%[a]
Epikote[b] 828, mw. 370	230	57	100	49	298	149
Epikote 1001, mw. 1,000	60	15	45	22	204	102
Epikote 1004, mw. 1,300	20	5	20	10	...[c]	–
Phenyl glycidyl ether	240	59	290	141	190	95
Butyl glycidyl ether	145	36	135	66	80	40
Allyl glycidyl ether	130	32	130	63	150	75
Isoprophyl glycidyl ether	160	40	90	44	44	22

[a] As per cent in relation of epichlorohydrin
[b] Epikote is diglycidyl ether of bisphenol A
[c] Poorly soluble in standard assay conditions

Table 3. Alkylation rates and mutagenicity of methylating and ethylating agents

Compound	Rate of alkylation				Mutagenicity	
	NBP (ΔA_{560}/h)	%[a]	dG (ΔI/h)	%[a]	WP2 uvrA (ΔRF × 10^{-11}/μM)	%[a]
Methyl methanesulfonate	670	165	250	112	190	95
Ethyl methanesulfonate	16	4	145	71	30	15
Dimethyl sulfate	2,450	605	7,590	3,702	100	50
Diethyl sulfate	455	112	240	117	30	15
Methyl nitrosourea	8	2	41	20	180	90
Ethyl nitrosourea	1	0	32	16	100	50
Methyl iodide	110	27	56	27	180	90
Ethyl iodide	55	14	17	9	170	85

[a] As per cent in relation to epichlorohydrin

The aliphatic glycidyl ethers were also quite active. Phenyl glycidyl ether was more active than the butyl, alkyl and isopropyl derivatives.

A number of methylating and ethylating agents were tested (Table 3). NBP and deoxyguanosine alkylation rates of dimethyl sulfate were higher by far than those of any other compound tested. Methyl methanesulfonate and diethyl sulfate were active, too. There was a discrepancy between the alkylation rates and mutagenicity in this group of compounds. The alkyl nitrosoureas and the alkyl iodides were relatively much more potent mutagens than their alkylation activity would indicate.

Table 4. Alkylation rates and mutagenicity of halogenated hydrocarbons, hydrazine and hydroxylamine derivatives and aldehydes

Compound	Rate of alkylation				Mutagenicity	
	NBP (ΔA_{560}/h)	%[a]	dG (ΔI/h)	%[a]	WP2 uvrA ($\Delta RF \times 10^{-11}/\mu M$)	%[a]
Ethylene dichloride	0	0	0	0	4	2
Ethylene dibromide	3	1	0	0	156	78
Hydrazine hydrate	0	0	0	0	440	220
1,1-dimethyl hydrazine	14	3	0	0	150	75
Hydroxylamine	0	0	0	0	230	115
Formaldehyde	0	0	0	0	0	0
Acetaldehyde	0	0	0	0	0	0
Glutaraldehyde	0	0	0	0	0	0
Acrolein	8	2	95	46	4	2

[a] As per cent in relation to epichlorohydrin

Table 5. Alkylation rates and mutagenicity of thiuram and dithiocarbamate derivatives

Compound %[a]	Rate of alkylation				Mutagenicity[b] *S. typhimurin*
	NBP (ΔA_{560}/h)	%[a]	dG (ΔI/h)	%[a]	
Tetramethylthiuram monosulfide	0	0	0	0	++
Tetramethylthiuram disulfide	0	0	0	0	+++
Tetraethylthiuram disulfide	0	0	0	0	–
Zinc dimethyl-dithiocarbamate	0	0	0	0	+++
Copper dimethyl-dithiocarbamate	. . [c]	–	2	1	+
Zinc diethyl dithiocarbamate	0	0	0	0	++
Cadmium diethyl dithiocarbamate	0	0	10	5	++
Tellur diethyl dithiocarbamate	2	0	1	0	–
Zinc dibutyl dithiocarbamate	1	0	1	0	–

[a] As per cent in relation to epichlorohydrin
[d] The data is taken from Hedenstedt et al. (1979) using *S. typhimurium* TA 100 without the S9 mixture: – negative, + positive with > 100 µg, ++ 10–100 µg, +++ < 10 µg/plate
[c] Color prevented the assay

Three groups of industrial chemicals were tested as reported in Table 4. Both ethylene dichloride and ethylene dibromide were mutagenic to *E. coli,* although the latter very weakly. Only ethylene dibromide was positive in the NBP alkylation test and the other alkylation results were negative. Except for dimethyl hydrazine, the alkylation tests were negative with the hydrazine derivatives and hydroxylamine, all of which were potent mutagens. The aldehydes tested were negative in the alkylation tests and in the mutagenicity test, with the exception of acrolein.

A group of sulphur-containing compounds of industrial use were also tested (Table 5). The thiuram derivatives did not alkylate NBP nor deoxyguanosine. The alkylation potency of the dithiocarbamate derivatives was very low but for most compounds above the background. The low level of alkylation was in contrast to the mutagenic potency of these chemicals reported recently by Hedenstedt and coworkers (1979).

Discussion

In this study alkylation rates and mutagenicity of a number of directly acting mutagens were determined. The compounds selected included structural analogues and groups of chemicals with selective reactivity. Both a synthetic (4-(p-nitrobenzyl)-pyridine) and a biological (deoxyguanosine) nucleophile were used in the alkylation reactions. It is likely that the compounds that react with deoxyguanosine also react with DNA; however, some compounds binding to DNA may fail to react with nucleosides and nucleotides (Kadlubar et al. 1978).

The compounds used were of analytical or of technical grade. We cannot be sure that in all cases the main component was responsible for the reactions measured. Thus caution has to be exerted particularly with the compounds showing slow alkylation rates (e.g., ethylene dibromide, 1,1-dimethylhydrazine and dithiocarbamates).

The epoxides included halogenated compounds (epichlorohydrin, epibromohydrin and epifluorohydrin) and unsubstituted compounds (propylene oxide, butylene oxide and butadiene monoxide). The halogenated derivatives were several times more reactive and mutagenic than the unsubstituted compounds indicating that the withdrawal of electrons by halogen atoms from the oxirane ring makes the molecules more reactive in accordance with previous studies (Hemminki and Falck 1979; Wade et al. 1978). By contrast, the type of the halogen atom and the length of the hydrocarbon chain failed to change appreciably the reactivity of the compounds, although the electrophilicity of the carbonium ion formed from the epoxide could be expected to vary. This may be explained by the reactivity of the halomethyl group.

Glycidyl ethers constituted another group of structural analogues widely used in industry presently. Three diglycidyl ethers of bisphenol A, each with different molecular weights, were used in addition to the four monoglycidyl ethers. The reactivity and the mutagenicity of diglycidyl ethers of bisphenol A correlated inversely with the molecular weight. Of the monoglycidyl ethers

phenyl glycidyl ether was the most reactive and mutagenic; butyl, alkyl and isopropyl glycidyl ether did not differ much in their reactivity. Our results on the mutagenicity of glycidyl ethers are in agreement with those of Andersen et al. (1978) and Nishioka and Ohtani (1978) testing the diglycidyl ethers of bisphenol A. By contrast, NIOSH (1978) in its criteria document cited a somewhat different order of mutagenicity for the glycidyl ethers. Wade and coworkers (1979) found alkyl and butyl glycidyl ether positive, and the diglycidyl ethers of bisphenol A negative in *S. typhimurium.*

Large dicrepancies were observed between the rates of alkylation and mutagenicity among the methylating and ethylating agents. Dimethyl sulfate was highly reactive exceeding the rate of alkylation of epichlorohydrin 6- to 37-fold, while it was less mutagenic than epichlorohydrin. The alkyl nitrosoureas and alkyl iodides were, by contrast, relatively potent mutagens as compared to their alkylation rates. It is likely that the low mutagenicity of dimethyl sulfate is in part due to its extreme lability and in part to the type of DNA adducts that it forms: predominantly N7-methylation of guanine (Lawley 1976). Analogously, the relatively high mutagenic potency of the nitrosoureas and the iodides may be attributable to the type of DNA-adducts formed [O^6-guanine alkylation; (Singer 1975)].

It has been shown previously that ethylene dichloride and ethylene dibromide can cause mutations in *S. typhimurium* without an added tissue preparation (Fischbein 1979) although metabolic activation routes have been suggested for these compounds (Rannug and Beije 1979). In our hands the halogenated hydrocarbons were mutagenic but only ethylene dibromide was weakly positive in the alkylation tests.

The hydrazine derivatives and hydroxylamine are known mutagens (Fishbein 1979) and they were also quite active with *E. coli* used here. The interest in using these compounds was their reported high base specificity as pyrimidines, and particularly cytosine residues, are attackted (Shugar et al. 1976; Kimpel 1977). Accordingly the compounds did not alkylate deoxyguanosine nor NBP.

Most aldehydes tested were negative in the alkylation as well as in the mutagenicity assay. Acrolein was an exception showing a weak NBP alkylation and mutagenicity.

The thiuram and dithiocarbamate derivatives tested are widely used in rubber, plastics and pesticide production. Additionally tetraethyl thiuram disulfide is used as a drug in the treatment of alcoholism. The compound have recently been tested for mutagenicity in bacterial systems (Hedenstedt et al. 1979). We found that the thiurams were inactive and most of the dithiocarbamate compounds reacted very slowly with NBP and deoxyguanosine. There appeared to be no clear correlation between the alkylation activity and mutagenicity of these compounds.

The present study showed that there was a reasonable correlation between the alkylation activity and mutagenicity among epoxide-containing compounds (simple epoxides and glycidyl ethers). It may be assumed that the DNA-adducts formed by these compounds are largely similar as has been shown for a group of epoxides (Hemminki et al. 1980). No clear correlation is found between

chemically unrelated compounds, e.g., different types of methylating and ethylating agents. In such cases the DNA-adducts formed are very different, which may be responsible for the poor correlation. It has been suggested that alkylation at exocyclic O-atoms may be particularly relevent for mutagenesis (Loveless 1969; Singer 1976; Abbott and Saffhill 1979). With some compounds such as halogenated hydrocarbons, hydrazine derivatives, thiurams, dithiocarbamates the alkylation tests gave a marginal or no response at all. In such cases several possibilities exist. Firstly, the compounds may be activated by bacterial enzymes. Secondly, the compounds may not cause alkylations but other types of chemical interactions. Thirdly, the compounds may attack very specific sites in DNA such that cannot be detected with the present type of tests. It is important, for the further application of alkylation tests, to unravel such possibilities.

The present study illustrated some limitations of the alkylation tests in assessing mutagenic risks. The mutagenicity of a chemical did not necessarily quantitatively correlate with its alkylation activity towards the two test nucleophiles. Moreover, evidence of little or no alkylation activity towards the nucleophiles did not always imply lack of mutagenicity. Yet it was of interest, that in the present series there was no compound, clearly positive in the alkylation tests, which was non-mutagenic. Thus evidence of alkylation is also evidence of mutagenic risk. The simple alkylation tests appear helpful, with the above precautions, in the primary screening studies of genotoxic chemicals.

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