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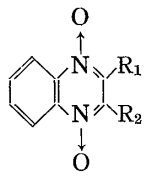
Mutagenicity of Carbadox and Several Quinoxaline 1,4-Dioxide Derivatives

Carbadox (2-formylquinoxaline 1,4-dioxide carbomethoxyhydrazone), a feed additive for swine, showed mutagenicity against *Salmonella typhimurium* TA 98 and TA 100 both in the absence and presence of S-9 mix. The mutagenic activity was stronger than that of quinoxaline 1,4-dioxide and was close to that of benzo[*a*]pyrene.

Keywords——carbadox; quinoxaline 1,4-dioxide; Ames test; mutagenicity; *Salmonella typhimurium*; S-9 mix; near-UV light

We have shown that quinoxaline 1,4-dioxide (**1**) is mutagenic toward *Salmonella typhimurium* strains TA 98 and TA 100 both in the absence and presence of microsomal metabolic activation system (S-9 mix).[1] Carbadox (2-formylquinoxaline 1,4-dioxide carbomethoxyhydrazone, **2**), a bactericidal agent currently being used as a feed additive for swine to promote growth, was now found to have much stronger mutagenicity than quinoxaline 1,4-dioxide. This paper describes the mutagenicities of carbadox and of several other related compounds.

Carbadox was prepared from Mecadox (Pfizer) by extraction with dimethylformamide followed by concentration of the extract and precipitation with addition of water. This material was further purified by reprecipitation from dimethylformamide and water, and was used in this study. It was analytically pure and gave a single ultraviolet-absorbing spot on silica gel thin-layer chromatography (solvent, chloroform–methanol, 10: 1). Benzo[*a*]-pyrene was obtained from Wako Pure Chemicals. Quinoxaline 1,4-dioxide (**1**) and 2,3-dimethylquinoxaline 1,4-dioxide (**3**) were gifts from Drs. S. Hayakawa and T. Namba of this Faculty, and 2,3-diphenylquinoxaline 1,4-dioxide (**4**) and pyrazine 1,4-dioxide were those from Dr. C. Kaneko of Kanazawa University. Mutagenicity assay was done as described previously[1] by using the *Salmonella*/S-9 system of Ames *et al.*[2] with the modification of Yahagi *et al.*[3] The room where the assays were carried out was dimly lighted. Liver S-9 fraction was prepared from rats induced with polychlorinated biphenyl.



1: $R_1=R_2=-H$
2: $R_1=-CH=NNHCOOCH_3$, $R_2=-H$
3: $R_1=R_2=-CH_3$
4: $R_1=R_2=-C_6H_5$

Chart 1

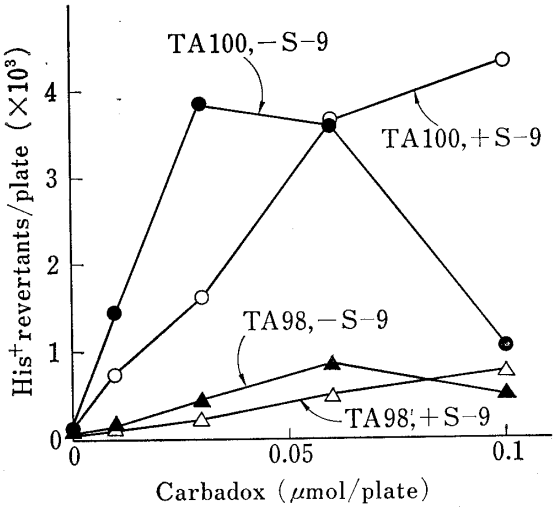


Fig. 1. Mutagenicity of Carbadox

1) T. Hashimoto, T. Negishi, T. Namba, S. Hayakawa, and H. Hayatsu, *Chem. Pharm. Bull.*, **27**, 1954 (1979).
2) B.N. Ames, J. McCann, and E. Yamasaki, *Mutation Res.*, **31**, 347 (1975).
3) T. Yahagi, M. Nagao, Y. Seino, T. Matsushima, T. Sugimura, and M. Okada, *Mutation Res.*, **48**, 121 (1977).

Table I. Mutagenic Activities of Carbadox and Related Compounds

	His+ revertants/μmol			
	TA 100		TA 98	
	+S-9	−S-9	+S-9	−S-9
Carbadox (**2**)	71700	143800	11100	15300
2,3-Dimethylquinoxaline 1,4-dioxide (**3**)	200	160	24	23
2,3-Diphenylquinoxaline1,4-dioxide (**4**)	540	550	40	20
Pyrazine 1,4-dioxide	(−)	(−)	(−)	(−)
Quinoxaline 1,4-dioxide (**1**)	9890	10900	1310	1470
Benzo[*a*]pyrene	138600	(−)	24160	(−)

Carbadox showed a strong mutagenicity when assayed on *Salmonella typhimurium* TA 98 and TA 100 both in the absence and presence of S-9 mix (Fig. 1). The results shown in this figure were confirmed by the repeated experiments in which very similar curves were obtained. From the linear portions of the dose-response curves, the numbers of revertants formed per 1 μmol of carbadox were calculated and the values are listed in Table I. The quinoxaline 1,4-dioxide derivatives **3** and **4** were also mutagenic, although their activities were low (Table I). Pyrazine 1,4-dioxide was not mutagenic. In Table I, the activities of quinoxaline 1,4-dioxide[1] and of benzo[*a*]pyrene are also presented. The numbers of revertants found for benzo[*a*]pyrene in our experiments agreed with the values reported in the literature[4] within a range of experimental error.

Table II. Effect of Near-UV Light Irradiation on Mutagenic Activities of Carbadox and Quinoxaline 1,4-Dioxide

Reagent	Concentration (mM) during irradiation[a]	His+ revertants/plate[b] Time of irradiation		
		0	1 hr	3 hr
Carbadox	1	4080	380	10
	10	4750	4170	1810
Quinoxaline 1,4-dioxide	10	4020	410	130
	100	3800	3890	2320

a) The irradiation was done with two 20 W black-light lamps (Toshiba FL 20S BLB; wavelength, 300—400 nm) at a distance of 14 cm from the reagent solutions.
b) The reversion was tested with TA 100, -S-9. The amounts of the reagents withdrawn at each period of irradiation were 0.06 μmol/plate for carbadox and 0.6 μmol/plate for quinoxaline 1,4-dioxide.

Since it is known that quinoxaline 1,4-dioxide undergoes rearrangement on photo-irradiation to give 2-hydroxyquinoxaline 4-oxide,[5] the possibility was considered that the photo-decomposition products or some intermediates might be the mutagenic principle. The solutions of carbadox and quinoxaline 1,4-dioxide (solvent, dimethylsulfoxide) were irradiated with near-ultraviolet light and then the mutagenicity was examined. As Table II shows, the irradiated solution exhibited lower activities than the original solutions; irradiation of 1 mM carbadox for 3 hrs caused an almost complete loss of the activity. The experiments at 10 mM concentrations indicate that carbadox loses the activity less rapidly than quinoxaline 1,4-dioxide. This is obviously a reflection of a lower photo-sensitivity of carbadox than

4) J. McCann, N.E. Spingarn, J. Kobori, and B.N. Ames, *Proc Natl. Acad. Sci., U.S.A.*, **72**, 979 (1975).
5) J.K. Landquist, *J. Chem. Soc.*, **1953**, 2830.

quinoxaline 1,4-dioxide (data not shown). These results clearly indicate that carbadox and quinoxaline 1,4-dioxide themselves, but not the photo-decomposition products, are the mutagens.

Quinoxaline 1,4-dioxide was previously added to feeds of swine as a growth promoting agent. It is carcinogenic to rats,[6] and was withdrawn from the market because of the possible hazard to personnel concerned in the manufacture (M.J. Tucker, personal communication). The mutagenicity of carbadox, which is comparable to that of the potent carcinogen benzo[*a*]pyrene, poses a similar problem concerning its manufacture as the feed additive.

After the present work was completed, we were informed that the mutagenicity of carbadox has been briefly mentioned in a meeting abstract[7] and has been continued to be studied by the investigators of that work.

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6) M.J. Tucker, *J. Natl. Cancer Inst.*, **55**, 137 (1975).
7) Y. Shirasu, M. Moriya, and K. Kato, *Mutation Res.*, **53**, 261 (1978).