Mutagenic evaluation of the pesticides captan, folpet, captafol, dichlofluanid and related compounds with the mutants TA102 and TA104 of *Salmonella typhimurium*

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A mutagenic evaluation of captan, folpet, captafol, dichlofluanid and related compounds was carried out using the *Salmonella*/mammalian microsome test using strains TA102 and TA104. These strains contain A-T base pairs at the site of the mutation in contrast to the other *Salmonella* tester strains that detect mutagens damaging G-C base pairs. In addition, the excision repair system of the TA102 strain is still intact. Captan and folpet were mutagenic in strain TA104, captafol was mutagenic in strain TA102, whereas the remaining test compounds (dichlofluanid, tetrahydrophthalimide and thiazolidine-4-carboxylic acid) were not mutagenic in either strain. In conclusion, we consider it of value to add these two strains to those already used in the Ames test in order to increase confidence in our ability to detect mutagens and to shed further light on their mechanism of action.

Introduction

Many authors have evaluated the mutagenicity of captan and other related pesticides (folpet, captafol and dichlofluanid) using the *Salmonella*/mammalian microsome or Ames test. In most cases, the tests were carried out with the strains initially described by Ames, i.e. his G-46, TA1535, TA1530, TA1950, TA1536, TA1537, TA1538 (Ames *et al.*, 1973), TA98 and TA100 (Ames *et al.*, 1975). Captan and folpet were mutagenic in those strains which detect base-pair substitution mutations and in those that detect frameshift mutations (Bridges, 1975; McCann and Ames, 1975; Ficsor *et al.*, 1977; Carere *et al.*, 1978; de Flora, 1978; Moriya *et al.*, 1978; Hollstein and McCann, 1979; Rinkus and Legator, 1979; de Flora and Boido, 1980; Shiau *et al.*, 1981; Waters *et al.*, 1982; IARC, 1983; de Flora *et al.*, 1984; Xu *et al.*, 1984). With captafol and dichlofluanid, the most sensitive strain was TA100 although the weak mutagenic effect caused by

these products was not always observed (Shirasu *et al.*, 1977; Kawachi *et al.*, 1980; Schuphan *et al.*, 1981; Moriya *et al.*, 1983; Schuphan and Casida, 1983).

At present, the strains recommended by Maron and Ames (1983) for general mutagenicity tests are TA100, TA98, TA97 and TA102. However, there is no published information on the mutagenicity of these products using TA102 strain. In this study we provide information on the mutagenicity of captan and folpet in TA102 and TA104 strains. In contrast to the other *Salmonella* tester strains which detect mutagens damaging G-C base pairs, these strains contain A-T base pairs at the site of the mutation and TA102 is excision repair proficient.

Materials and methods

Chemicals

Captan, folpet, captafol and dichlofluanid were supplied by the US Environmental Protection Agency (EPA).

Tetrahydrophthalimide (a captan metabolite) and thiazolidine-4-carboxylic acid (related to a captan metabolite) were supplied by Fluka and Aldrich respectively. Table I gives names, chemical structure, mol. wt, purity and source of chemicals evaluated. These compounds were dissolved in dimethyl sulphoxide (DMSO) supplied by Merck.

Methylglyoxal, methyl methane sulphonate and 2-aminoanthracene (standard mutagens) were supplied by Aldrich, Merck and Sigma respectively.

The concentration range for each tested compound was selected according to preliminary study results.

Bacterial strains

Salmonella typhimurium strains TA102 and TA104 were obtained from Dr B.N. Ames, University of California at Berkeley. Their genotype has been described previously (Levin *et al.*, 1982; Maron and Ames, 1983).

Metabolic activation system

The supernatant of the post-mitochondrial liver fraction (S9) from Wistar rats (250–500 g body wt) was used as metabolic activation system. A week before rats were killed, their drinking water was made 0.1% in sodium phenobarbital.

Immediately before the mutagenicity tests, S9 mix was prepared, i.e. S9 with NADP and glucose-6-phosphate cofactors.

S9 and S9 mix fractions were prepared according to the method described by Maron and Ames (1983).

Salmonella/mammalian-microsome test

The standard plate incorporation test was used (Ames *et al.*, 1975; Maron and Ames, 1983).

Table I.

Common name	Trade name	Chemical name	Chemical structure	Mol. wt	Purity	Source
Captan	Orthocide	N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide		301	99.8%	EPA 1020
Folpet	Phaltan	N-(trichloromethylthio)phthalimide		297	99.8%	EPA 3660
Captafol	Difolatan	N-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide		349	99.7%	EPA 1000
Dichlofluanid	Euparen	N-[(dichlorofluoromethyl)thio]-N',N'-dimethyl-N-phenylsulphamide		333.2	99%	EPA E0331
Tetrahydrophthalimide		4-cyclohexene-1,2-dicarboximide		151.17	Pure	Fluka 87453
		L-thiazolidine-4-carboxylic acid		133.17	98%	Aldrich T 2, 750-2

Table II. Mutagenic evaluation with TA104

	Captan			Folpet			Captafol			Dichlorofluanid		Tetrahydrophthalimide		Thiazolidine-4-carboxylic acid	
	I ^a	I ^b	I ^{c,d} III ^d IV ^d V	I ^a	I ^b	I ^c	I ^a	I ^b	I ^c	I ^d III ^d	I ^a	I ^b	I ^c III ^d	I ^d III ^d	I ^d III ^d
Range of [I] evaluated in µg/plate + S9+	- S9+ 1.25-10	1.25-10	2.5-20 2.5-20 2.5-20	2.5-2.5	2.5-40	2.5-40	0.62-2.5	0.16-0.62	0.16-0.62	0.16-0.62	0.62-2.5	80-160	80-160	80-500	80-500
Range of mutagenic concentrations according to the mutation index in µg/plate	- S9+ 5	5-10	2.5-5 5	1.25-2.5	5-10	5-10	1.25-2.5	5-10	5-10	2.5-40 2.5-20 2.5-20	0.62-2.5	80-160	80-160	80-500	80-500
ANOVA I	- S9+ ***	***	***	***	***	***	***	***	***	***	NS	NS	NS	NS	NS
Regression analysis	- S9+ *	***	NS	NS	***	***	NS	***	***	NS	NS	NS	NS	NS	NS
Slope of regression line	- S9+ 108.21	348.32			367.13	60.21	44.15	541.99							
Mutagenic potency in rev/nmol						86.99									
Two-way ANOVA with replication		106.63					156.91								
			***	***	***	***	***	***	***	***	***	***	***	***	***
			***	***	***	***	***	***	***	NS	NS	NS	NS	NS	NS
			***	***	***	***	***	***	***	NS	NS	NS	NS	NS	NS

I-V are the different assays used.

*0.1 ml of the overnight culture.

*1 ml of the overnight culture.

*Plates made the day before.

*Revertants were counted after 5 or 6 days.

*Plates made 1 week before performing the test.

***P<0.01.

**P<0.025.

*P<0.05.

Table III. Mutagenic evaluation of captan with TA104

(a) Without S9 mix Assay I					Assay II ^a				
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
1.25	178 $\pm$ 101.82	0	NM	4×10^8	2.5	1432 $\pm$ 452.55	4.8	M ²⁺	3×10^8
2.5	327 $\pm$ 18.38	0.3	NM	4×10^8	5	1904	6.7	M ³⁺	10^8
5	668 $\pm$ 96.17	2.5	M ⁺	4×10^8	10	1692	5.9	M ³⁺ /T	$< 10^7$
10	199 $\pm$ 72.12	0	NM/T	2×10^7	20	352	0.3	NM/T	2×10^6
SM	153 $\pm$ 66.47			6×10^8	SM	238			3×10^8
DMSO	278 $\pm$ 43.84	1.8	NM	6×10^8	DMSO	286	1.2	NM	3×10^8
MG	5000	30.8	M ⁴⁺	5×10^8	MG	4500	17.7	M ⁴⁺	2×10^8
Assay IV ^a					Assay V				
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	139 $\pm$ 12.90	0.3	NM	10^7	0.62	675 $\pm$ 108.19	1.2	NM	2×10^9
5	435 $\pm$ 23.35	2.3	NM	10^7	1.25	2132 $\pm$ 62.22	5.9	M ³⁺	2×10^9
10	268 $\pm$ 93.21	1.2	NM/T	$< 10^7$	2.5	2560 $\pm$ 90.51	7.3	M ³⁺	2×10^9
20	46 $\pm$ 6.43(s)		T	2×10^6					
SM	150 $\pm$ 13.50			10^7	SM	306 $\pm$ 0.71			2×10^9
DMSO	90 $\pm$ 37.29	0.6	NM	10^7	DMSO	312 $\pm$ 0.00	1	NM	2×10^9
MG	2300	14.7	M ⁴⁺	10^7	MG	4250	12.8	M ⁴⁺	2×10^9
(b) With S9 mix Assay III ^a					Assay IV ^a				
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	583 $\pm$ 46.01	1.4	NM	5×10^9	2.5	174 $\pm$ 33.94	0.3	NM	10^8
5	595 $\pm$ 24.44	1.5	NM	3×10^9	5	143 $\pm$ 12.02	0.1	NM	$< 10^8$
10	663 $\pm$ 29.70	1.8	NM	8×10^8	10	367 $\pm$ 114.55	1.6	NM	$< 10^8$
20	253 $\pm$ 61.07	0	NM/T	6×10^6	20	136 $\pm$ 11.31(s)		T	$< 10^7$
SM	226 $\pm$ 23.11			6×10^9	SM	148 $\pm$ 19.09			10^8
DMSO	259 $\pm$ 52.56	1.1	NM	6×10^9	DMSO	126 $\pm$ 5.66	0.8	NM	10^8
2-AA	2500	9.9	M ³⁺	4×10^9	2-AA	1400	8.6	M ⁴⁺	10^8

^aRevertants were counted after 5 or 6 days.R, revertants expressed as the $\bar{x} \pm \text{SD}$; MI, mutation index; Q, quality; V, number of viables/ml; s, small colonies; SM, spontaneous mutation; DMSO, mutation in response to DMSO; MG mutation in response to methylglucose; 2-AA, mutation in response to 2-aminoanthracene.NM, non-mutagenic (MI < 2.5);M⁺, very weak mutagenic (MI = 2.5–3.5);M²⁺, weak mutagenic (MI = 3.6–5.5);M³⁺, mutagenic (MI = 5.6–10.5);M⁴⁺, highly mutagenic (MI > 10.5);

T, toxic effect.

To 2 ml of top agar supplemented with 0.2 ml of a 0.5 mM histidine/biotin solution were added: 100 μl of the test chemical solution, 100 or 1000 μl of an overnight culture of the tester strain (corresponding to a 14-h incubation) and 500 μl of S9 mix (containing 4% of S9). After shaking, the mixture was spread onto a fresh or 1-week-old minimal glucose agar plate. Plates were placed in a 37°C incubator for at least 48 h or even more, after which revertants were counted. All the experiments were carried out in triplicate and results were expressed as the average number of revertants per plate. For each manipulation, negative (spontaneous reversion and mutation in response to DMSO) and positive (response to the standard mutagen) controls were included to ensure the validity of the test.

Most mutagens are also toxic to some extent and consequently the number of revertants usually decreases at higher concentrations. This decrease sometimes leads to the misinterpretation of the mutagenicity data, especially if the toxic chemical is only weakly mutagenic. Waleh *et al.* (1982) developed an assay for measuring toxicity quantitatively. They have constructed two sets of strains, isogenic with the Ames tester strains except for their his character. One set consists of the his⁺ derivatives of the tester strains which are used for measuring the

survival of the inoculum cells after exposure to the chemical. The other set is the stable his⁻ derivatives of the tester strains which are used for simulating the background growth in the Ames mutagenicity plate test. The percentage survival of the his⁺ cells in the inoculum in the presence of the 'filler cells' is used as a measure of the toxic effect of the chemical. Lacking these two sets of strains, we decided to determine the number of viables after treatment with the test compound in order to obtain an approximation of its toxic effect. The procedure is similar to that of mutagenicity. To 2 ml of top agar supplemented with 0.2 ml of a 25 mM histidine/0.5 mM biotin solution were added: 100 μl of the test chemical solution, 100 μl of 10⁻⁶ or 10⁻⁵ dilution from an overnight culture of the tester strain and 500 μl of S9 mix. After shaking, the mixture was spread onto a nutrient agar plate. Plates were incubated at 37°C for 24 h after which viables were counted. As in the mutagenicity test, positive and negative controls were included to ensure the validity of the test. A test compound concentration is considered toxic when it produces a 10% survival or less.

In order to determine the existence of a mutagenic effect caused by the test compound and quantify it, two methods were used: (i) calculation of the mutation index, and (ii) statistical method based on least-squares linear regression.

Table IV. Mutagenic evaluation of folpet with TA104

(a) Without S9 mix									
Assay I					Assay II ^a				
Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	580 $\pm$ 159.10	2	NM	6 $\times$ 10 ⁸	2.5	1012	3	M ⁺	3 $\times$ 10 ⁸
5	711 $\pm$ 247.49	2.8	M ⁺	6 $\times$ 10 ⁸	5	1188	3.8	M ²⁺	10 ⁸
10	726 $\pm$ 542.35	2.9	M ⁺	6 $\times$ 10 ⁸	10	1272	4.1	M ²⁺	10 ⁸
20	415 $\pm$ 352.14	0.9	NM/T	4 $\times$ 10 ⁷	20	1783 $\pm$ 474.27	6.3	M ³⁺ /T	3 $\times$ 10 ⁷
40	267 $\pm$ 166.17	0	NM/T	10 ⁷					
SM	153 $\pm$ 66.47			6 $\times$ 10 ⁸	SM	238			3 $\times$ 10 ⁸
DMSO	278 $\pm$ 43.84	1.8	NM	6 $\times$ 10 ⁸	DMSO	286	1.2	NM	3 $\times$ 10 ⁸
MG	5000	30.8	M ⁴⁺	5 $\times$ 10 ⁸	MG	4500	17.7	M ⁴⁺	2 $\times$ 10 ⁸
Assay IV ^a					Assay V				
Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	285 $\pm$ 53.71	1.3	NM	10 ⁷	0.62	617 $\pm$ 111.01	1	NM	2 $\times$ 10 ⁹
5	367 $\pm$ 46.70	1.8	NM	10 ⁷	1.25	1208 $\pm$ 101.82	2.9	M ⁺	2 $\times$ 10 ⁹
10	600 $\pm$ 136.47	3.4	M ⁺	10 ⁷	2.5	1628 $\pm$ 28.28	4.3	M ²⁺	2 $\times$ 10 ⁹
20	464 $\pm$ 128.00	2.5	M ⁺ /T	2 $\times$ 10 ⁶					
SM	150 $\pm$ 13.50			10 ⁷	SM	306 $\pm$ 0.71			2 $\times$ 10 ⁹
DMSO	90 $\pm$ 37.29	0.6	NM	10 ⁷	DMSO	312 $\pm$ 0.00	1	NM	2 $\times$ 10 ⁹
MG	2300	14.7	M ⁴⁺	10 ⁷	MG	4250	12.8	M ⁴⁺	2 $\times$ 10 ⁹
(b) With S9 mix									
Assay III ^a					Assay IV ^a				
Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	445 $\pm$ 51.74	0.8	NM	5 $\times$ 10 ⁹	2.5	172 $\pm$ 2.83	0.3	NM	10 ⁸
5	787 $\pm$ 12.22	2.3	NM	5 $\times$ 10 ⁹	5	156 $\pm$ 22.63	0.2	NM	10 ⁸
10	1083 $\pm$ 138.58	3.6	M ²⁺	3 $\times$ 10 ⁹	10	196 $\pm$ 8.48	0.5	NM	10 ⁸
20	811 $\pm$ 92.72	2.4	NM/T	1.5 $\times$ 10 ⁸	20	142 $\pm$ 2.83	0.1	NM/T	< 10 ⁷
SM	226 $\pm$ 23.11			6 $\times$ 10 ⁹	SM	148 $\pm$ 19.09			10 ⁸
DMSO	259 $\pm$ 52.56	1.1	NM	6 $\times$ 10 ⁹	DMSO	126 $\pm$ 5.66	0.8	NM	10 ⁸
2-AA	2500	9.9	M ³⁺	4 $\times$ 10 ⁹	2-AA	1400	8.6	M ³⁺	10 ⁸

Legend as Table III.

(i) *Calculation of the mutation index.* The mutation index is defined as the ratio between induced and spontaneous revertants (Mattern, 1981).

$$MI = \frac{T-C}{C}$$

MI = mutation index
T = revertants/treated plate
C = revertants/control plate

A concentration of the test compound is considered mutagenic when the mutation index is 2.5.

(ii) *Statistical method based on least squares linear regression.* The method used is based on the one described by Moore and Felton (1983) and the calculations were made following Sokal and Rohlf's model (Sokal and Rohlf, 1980). The viables count as well as the decrease in the number of revertants give an idea of the test compound concentrations which are toxic for the bacterium and which must therefore be eliminated in order to avoid any mutagenicity/toxicity interaction. At first, a model I analysis of variance (ANOVA) of data is carried out. If ANOVA is not significant, the tested compound is considered non-mutagenic, but if ANOVA is significant, an analysis of regression is carried out in order to prove—in the case of significance—that there is a linear relationship between dose and response, and then the slope is calculated and used as a measure of the mutagenic effect.

If the analysis of regression is not significant, the mutagenic effect cannot be calculated and in this case another method would have to be used.

The mutagenic potency has been expressed as number of induced revertants/nmol of product, taking the number of revertants from the top level of the linear part of dose-response curves. When there were several assays with the same product and with the same strain adjusting to a line, the assay choice always was the assay with the greatest slope.

Finally, a two-way ANOVA with replication (Sokal and Rohlf, 1980) was used to determine the influence of S9 mix on the mutagenic activity observed.

Results

TA104

Results are summarized in Table II. According to the mutation index, only captan and folpet were mutagenic. Table III shows the mutagenic evaluation of captan and Table IV that of folpet.

Four assays have been performed in the absence of S9 mix with both compounds. The range of concentrations evaluated was from 0.62 to 20 $\mu\text{g}/\text{plate}$ for captan, and from 0.62 to 40 $\mu\text{g}/\text{plate}$ for folpet. In all the assays performed with both compounds, there was a dose-related increase in mutant colonies at non-toxic doses used. The toxic concentration of captan was 10 $\mu\text{g}/\text{plate}$ and that

Fig. 1. Dose-effect relationship for captan with *Salmonella typhimurium* TA104.

Fig. 2. Dose-effect relationship for folpet with *Salmonella typhimurium* TA104.

Fig. 3. Dose-effect relationship for captan with *Salmonella typhimurium* TA104 corresponding to Assay IV.

Fig. 4. Dose-effect relationship for folpet with *Salmonella typhimurium* TA104 corresponding to Assay IV.

Table V. Influence of the inoculum size in the mutagenicity of captan and folpet to TA104

(a) 0.1 ml of the overnight culture									
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
1.25	178 $\pm$ 101.82	0	NM	4×10^8	2.5	580 $\pm$ 159.10	2	NM	6×10^8
2.5	327 $\pm$ 18.38	0.3	NM	4×10^8	5	711 $\pm$ 247.49	2.8	M ⁺	6×10^8
5	668 $\pm$ 96.17	2.5	M ⁺	4×10^8	10	726 $\pm$ 542.35	2.9	M ⁺	6×10^8
10	199 $\pm$ 72.12	0	NM/T	2×10^7	20	415 $\pm$ 352.14	0.9	NM/T	4×10^7
					40	267 $\pm$ 166.17	0	NM/T	10^7
SM	153 $\pm$ 66.47			6×10^8	SM	153 $\pm$ 66.47			6×10^8
DMSO	278 $\pm$ 43.84	1.8	NM	6×10^8	DMSO	278 $\pm$ 43.84	1.8	NM	6×10^8
MG	5000	30.8	M ⁴⁺	5×10^8	MG	5000	30.8	M ⁴⁺	5×10^8
(b) 1 ml of the overnight culture									
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Folpet ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
1.25	1310	0.6	NM	4×10^8	2.5	1584	1.1	NM	6×10^8
2.5	1448	0.8	NM	4×10^8	5	2496	2.8	M ⁺	6×10^8
5	2704	3.2	M ⁺	4×10^8	10	4240	6	M ³⁺	6×10^8
10	4080	5.7	M ³⁺ /T	2×10^7	20	2856	3.4	M ⁺ /T	4×10^7
					40	3200	4.1	M ²⁺ /T	10^7
SM	540			6×10^8	SM	540			6×10^8
DMSO	996	1.8	NM	6×10^8	DMSO	996	1.8	NM	6×10^8
MG	15000	25.9	M ⁴⁺	5×10^8	MG	15000	25.9	M ⁴⁺	5×10^8

Legend as Table III.

Table VI. Influence of the minimal glucosa agar plates used in the mutagenicity of captan to TA104

(a) Fresh plates					(b) Old plates				
Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captan ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
2.5	1432 $\pm$ 452.55	4.8	M ²⁺	3×10^8	2.5	624	2.1	NM	3×10^8
5	1904	6.7	M ³⁺	10^8	5	812	3	M ⁺	10^8
10	1692	5.9	M ³⁺ /T	$< 10^7$	10	284	0.4	NM/T	$< 10^7$
20	352	0.3	NM/T	2×10^6	20	0(s)		T	2×10^6
SM	238			3×10^8	SM	203			3×10^8
DMSO	286	1.2	NM	3×10^8	DMSO	196	1	NM	3×10^8
MG	4500	17.7	M ⁴⁺	2×10^8	MG	3200	14.7	M ⁴⁺	2×10^8

Legend as Table III.

MMS = mutation in response to methyl methane sulphonate.

of folpet was 20 $\mu\text{g}/\text{plate}$ since these concentrations produced a survival of $\leq 10\%$ in most assays. However, there was variability probably due to differences in spontaneous reversion and the number of bacteria plated. The higher the spontaneous reversion, then the higher the captan- and folpet-induced reversion; when spontaneous reversion was similar the higher the number of bacteria plated, then the higher captan- and folpet-induced reversion (Figures 1 and 2; Tables III and IV).

Two assays have been performed in the presence of S9 mix for both compounds and the range of concentrations evaluated was from 2.5 to 20 $\mu\text{g}/\text{plate}$. The toxic concentration of both captan and folpet was 20 $\mu\text{g}/\text{plate}$. The differences from one assay to another again seem to be due both spontaneous reversion and the number of bacteria plated (Tables III and IV). The mutagenic activity of both compounds decreased in the presence of S9 mix, so they are considered direct mutagens (Figures 3 and 4).

The influence of the inoculum size in the mutagenicity of both captan and folpet is shown in Table V. When 1 ml of the

overnight culture was used to perform the test, we did not get a better mutagenic response than when 0.1 ml of the overnight culture was used but a clearer dose-response relationship was observed. However, the minimum level of detection of mutagenicity was the same, irrespective of the inoculum size.

On the other hand, when 'old' minimal glucose agar plates were used to perform the test, the mutagenic effect caused by captan decreased (Table VI).

The minimum mutagenic concentration was 1.25 $\mu\text{g}/\text{plate}$ for both captan and folpet. A concentration range could be established with dose-response ranging from 1.25 to 5 $\mu\text{g}/\text{plate}$ for captan and from 1.25 to 10 $\mu\text{g}/\text{plate}$ for folpet, the difference being due to the higher toxicity of captan. When there were at least three concentrations of compound that were non-toxic to bacteria, the statistical analysis was performed on data (Table II). The mutagenic potency calculated for the assay with the greatest slope was established at 106.63 revertants/nmol for captan and 156.91 revertants/nmol for folpet.

Table VII. Mutagenic evaluation of captafol with TA104

(a) Without S9 mix Assay I					Assay II ^a					(b) With S9 mix Assay III ^a				
Captafol ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captafol ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Captafol ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
0.16	288 $\pm$ 57.98	0.06	NM	6×10^8	0.16	383 $\pm$ 118.09	0.4	NM	2×10^8	0.16	267 $\pm$ 12.12	0.03	NM	5×10^9
0.31	276 $\pm$ 69.30	0	NM	3×10^8	0.31	373 $\pm$ 78.94	0.4	NM	6×10^7	0.31	215 $\pm$ 27.23	0	NM	4×10^9
0.62	284 $\pm$ 96.17	0.03	NM/T	9×10^6	0.62	414 $\pm$ 37.48	0.5	NM/T	5×10^6	0.62	255 $\pm$ 18.90	0	NM	7×10^8
SM	153 $\pm$ 66.47			6×10^8	SM	238			3×10^8	SM	226 $\pm$ 23.11			6×10^9
DMSO	278 $\pm$ 43.84	1.8	NM	6×10^8	DMSO	286	1.2	NM	3×10^8	DMSO	259 $\pm$ 52.56	1.1	NM	6×10^9
MG	5000	30.8	M ⁴⁺	5×10^8	MG	4500	17.7	M ⁴⁺	2×10^8	2-AA	2500	9.9	M ³⁺	4×10^9

Legend as Table III.

Table VIII. Mutagenic evaluation of dichlofluanid with TA104

(a) Without S9 mix Assay II ^a					(b) With S9 mix Assay III ^a				
Dichlofluanid ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Dichlofluanid ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
0.62	258 $\pm$ 11.53	0	NM	3×10^8	0.62	263 $\pm$ 4.16	0.02	NM	5×10^9
1.25	306 $\pm$ 36.94	0.08	NM	2×10^8	1.25	227 $\pm$ 30.61	0	NM	4×10^9
2.5	397 $\pm$ 115.31	0.5	NM	6×10^7	2.5	231 $\pm$ 26.56	0	NM	4×10^9
SM	238			3×10^8	SM	226 $\pm$ 23.11			6×10^9
DMSO	286	1.2	NM	3×10^8	DMSO	259 $\pm$ 52.56	1.1	NM	6×10^9
MG	4500	17.7	M ⁴⁺	2×10^8	2-AA	2500	9.9	M ³⁺	4×10^9

Legend as Table III.

Table IX. Mutagenic evaluation of tetrahydrophthalimide with TA104

(a) Without S9 mix Assay I					Assay II ^a					(b) With S9 mix Assay III ^a				
Tetrahydro- phthalimide ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Tetrahydro- phthalimide ($\mu\text{g}/\text{plate}$)	R	MI	Q	V	Tetrahydro- phthalimide ($\mu\text{g}/\text{plate}$)	R	MI	Q	V
80	457 $\pm$ 29.70	1.2	NM	6×10^8	80	314 $\pm$ 31.18	0.1	NM	3×10^8	80	166 $\pm$ 48.12	0	NM	6×10^9
160	413 $\pm$ 103.94	0.9	NM	6×10^8	160	373 $\pm$ 48.22	0.4	NM	3×10^8	160	186 $\pm$ 27.78	0	NM	6×10^9
					320	259 $\pm$ 39.89	0	NM	3×10^8	320	201 $\pm$ 20.13	0	NM	6×10^9
					500	238 $\pm$ 87.68	0	NM	2×10^8	500	227 $\pm$ 20.43	0	NM	5×10^9
SM	153 $\pm$ 66.47			6×10^8	SM	238			3×10^8	SM	226 $\pm$ 23.11			6×10^9
DMSO	278 $\pm$ 43.84	1.8	NM	6×10^8	DMSO	286	1.2	NM	3×10^8	DMSO	259 $\pm$ 52.56	1.1	NM	6×10^9
MG	5000	30.8	M ⁴⁺	5×10^8	MG	4500	17.7	M ⁴⁺	2×10^8	2-AA	2500	9.9	M ³⁺	4×10^9

Legend as Table III.

The remaining chemicals were non-mutagenic according to the mutation index (Tables VII–X). ANOVA was not significant in most assays. In one case, however, there was a significance but the variations due to the dose did not adjust to a regression line; therefore the only method used to determine the mutagenic effect was the mutation index calculation which showed the non-mutagenicity of these compounds (Table II).

As regards toxicity, the following sequence can be seen: thiazolidine-4-carboxylic acid = tetrahydrophthalimide < folpet < captan < dichlofluanid < captafol.

TA102

The results are summarized in Table XI. Captafol was the only clearly mutagenic compound. Table XII shows the mutagenic evaluation of captafol. Three concentrations were evaluated: 0.16,

0.31 and 0.62 $\mu\text{g}/\text{plate}$. In the two assays without S9 mix, there was a dose-related increase in the number of mutant colonies; however, there were differences between assays. The lower spontaneous reversion showed the higher captafol-induced reversion (Figure 5). In the presence of S9 mix, the mutagenic activity of captafol decreased, so captafol is considered a direct mutagen (Figure 6).

The minimum mutagenic concentration was 0.31 $\mu\text{g}/\text{plate}$. A concentration range could be established with dose–response ranging from 0.31 to 0.62 $\mu\text{g}/\text{plate}$. The statistical analysis was performed on data (Table XII) and the mutagenic potency calculated for the assay with the greatest slope was established at 537.64 revertants/nmol (Table XI).

The remaining chemicals were non-mutagenic according to the mutation index (Tables XIII–XVII) and the variations due to

Table X. Mutagenic evaluation of thiazolidine-4-carboxylic acid

(a) Without S9 mix Assay I ^a					(b) With S9 mix Assay II ^a				
Thiazolidine-4-carboxylic acid (μg/plate)	R	MI	Q	V	Thiazolidine-4-carboxylic acid (μg/plate)	R	MI	Q	V
80	370 ± 71.00	0.3	NM	3 × 10 ⁸	80	226	0	NM	6 × 10 ⁹
160	350 ± 2.83	0.3	NM	3 × 10 ⁸	160	166 ± 41.01	0	NM	6 × 10 ⁹
320	341 ± 34.08	0.2	NM	3 × 10 ⁸	320	202 ± 31.43	0	NM	6 × 10 ⁹
500	332 ± 22.63	0.2	NM	2 × 10 ⁸	500	181 ± 12.22	0	NM	5 × 10 ⁹
SM	238			3 × 10 ⁸	SM	226 ± 23.11			6 × 10 ⁹
DMSO	286	1.2	NM	3 × 10 ⁸	DMSO	259 ± 52.56	1.1	NM	6 × 10 ⁹
MG	4500	17.7	M ⁴⁺	2 × 10 ⁸	2-AA	2500	9.9	M ³⁺	4 × 10 ⁹

Legend as Table III.

Table XI. Mutagenic evaluation with TA102

		Captan		Folpet		Captafol		Dichlofluanid		Tetrahydrophthalimide		Thiazolidine-4-carboxylic acid	
		I	II	I	II	I	II	I	II	I	II	I	II
Range of [I] evaluated in μg/plate	- S9+	2.5-20	2.5-20	2.5-20	2.5-20	0.16-0.62	0.16-0.62	0.62-2.5	0.62-2.5	80-500	80-500	80-500	80-500
Range of mutagenic concentrations according to the mutation index in μg/plate	+ S9+		2.5-20		2.5-20		0.16-0.62		0.62-2.5		80-500		80-500
	- S9+					0.62	0.31-0.62						
	+ S9+						0.62						
ANOVA I	- S9+			NS	***	**	***			**	NS	NS	***
	+ S9+		NS		***		***				***		*
Regression analysis	- S9+				NS	***	**			NS			*
	+ S9+				NS		**				NS		NS
Slope of regression line	- S9+					1039.36	1617.65						0.17
	+ S9+						774.01						
Mutagenic potency in rev/nmol							537.64						
Two-way ANOVA with replication					NS		***				NS		NS
					***		***				NS		NS
					***		***				***		***

I and II refer to different assays.

Revertants were counted after 6 days.

Table XII. Mutagenic evaluation of captafol with TA102

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Captafol (μg/plate)	R	MI	Q	V	Captafol (μg/plate)	R	MI	Q	V	Captafol (μg/plate)	R	MI	Q	V
0.16	341 ± 6.00	1.4	NM	10 ⁷	0.16	156 ± 3.21	1	NM	10 ⁷	0.16	195 ± 13.05	0.3	NM	2 × 10 ⁹
0.31	403 ± 96.87	1.9	NM	10 ⁷	0.31	457 ± 95.00	4.8	M ²⁺	10 ⁷	0.31	324 ± 112.43	1.2	NM	2 × 10 ⁹
0.62	790 ± 262.66	5.1	M ²⁺	5 × 10 ⁶	0.62	1037 ± 103.25	12	M ⁴⁺ /T	10 ⁶	0.62	611 ± 107.04	3	M ⁺	5 × 10 ⁸
SM	122 ± 6.36			10 ⁷	SM	80 ± 19.52			10 ⁷	SM	151 ± 23.43			2 × 10 ⁹
DMSO	164 ± 8.48	1.3	NM	10 ⁷	DMSO	74 ± 18.45	0.9	NM	10 ⁷	DMSO	147 ± 26.50	1	NM	2 × 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 × 10 ⁶	MMS	2080	25	M ⁴⁺ /T	<10 ⁶	2-AA	473	2.1	NM	3 × 10 ⁸

Legend as Table III.

MMS = mutation in response to methyl methane sulphonate.

the dose, if present, either did not adjust to a regression line or, if they did, the slope was too small (Table XI) and it may be considered that captan, folpet, dichlofluanid, tetrahydro-

phthalimide and thiazolidine-4-carboxylic acid were non-mutagenic to TA102 under own test conditions.

As regards toxicity, the following sequence can be seen:

Fig. 5. Dose-effect relationship for captafol with *Salmonella typhimurium* TA102.

Fig. 6. Dose-effect relationship for captafol with *Salmonella typhimurium* TA102 corresponding to Assay II.

Table XIII. Mutagenic evaluation of dichlofluanid with TA104

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Dichlofluanid R (μ g/plate)	MI	Q	V		Dichlofluanid R (μ g/plate)	MI	Q	V		Dichlofluanid R (μ g/plate)	MI	Q	V	
0.62	311 $\pm$ 2.83	1.2	NM	<10 ⁷	0.62	133 $\pm$ 10.07	0.7	NM	10 ⁷	0.62	181 $\pm$ 29.28	0.2	NM	2 $\times$ 10 ⁹
1.25	303	1.1	NM	<10 ⁷	1.25	134 $\pm$ 2.83	0.2	NM	10 ⁷	1.25	188 $\pm$ 48.87	0.3	NM	2 $\times$ 10 ⁹
2.5	173	0.07	NM/T	10 ⁶	2.5	100	0	NM	3 $\times$ 10 ⁶	2.5	106 $\pm$ 5.66	0	NM/T	2 $\times$ 10 ⁸
SM	122 $\pm$ 6.36			10 ⁷	SM	80 $\pm$ 19.52			10 ⁷	SM	151 $\pm$ 23.43			2 $\times$ 10 ⁹
DMSO	164 $\pm$ 8.48	1.3	NM	10 ⁷	DMSO	74 $\pm$ 18.45	0.9	NM	10 ⁷	DMSO	147 $\pm$ 26.50	1	NM	2 $\times$ 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 $\times$ 10 ⁶	MMS	2080	25	M ⁴⁺ /T	<10 ⁶	2-AA	473	2.1	NM	3 $\times$ 10 ⁸

Legend as Table III.

MMS = mutation in response to methyl methane sulphonate.

Table XIV. Mutagenic evaluation of captan with TA102

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Captan R (μ g/plate)	MI	Q	V		Captan R (μ g/plate)	MI	Q	V		Captan R (μ g/plate)	MI	Q	V	
2.5	139	0	NM	10 ⁷	2.5	196 $\pm$ 7.81	1.5	NM	10 ⁷	2.5	351 $\pm$ 72.99	1.3	NM	2 $\times$ 10 ⁹
5	100 $\pm$ 24.75	0	NM	10 ⁷	5	199 $\pm$ 6.03	1.6	NM	10 ⁷	5	186 $\pm$ 96.17	0.2	NM	2 $\times$ 10 ⁹
10	41 $\pm$ 1.00(s)		T	<10 ⁷	10	69 $\pm$ 45.54	0	NM/T	<10 ⁷	10	310 $\pm$ 112.93	1.1	NM	2 $\times$ 10 ⁹
20	20 $\pm$ 13.00(s)		T	10 ⁶	20	37 $\pm$ 5.51(s)		T	10 ⁶	20	16 $\pm$ 18.15(s)		T	5 $\times$ 10 ⁷
SM	122 $\pm$ 6.36			10 ⁷	SM	80 $\pm$ 19.52			10 ⁷	SM	151 $\pm$ 23.43			2 $\times$ 10 ⁹
DMSO	164 $\pm$ 8.48	1.3	NM	10 ⁷	DMSO	74 $\pm$ 18.45	0.9	NM	10 ⁷	DMSO	147 $\pm$ 26.50	1	NM	2 $\times$ 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 $\times$ 10 ⁶	MMS	2080	25	M ⁴⁺ /T	<10 ⁶	2-AA	473	2.1	NM	3 $\times$ 10 ⁸

Legend as Table III.

MMS = mutation in response to methyl methane sulphonate.

Table XV. Mutagenic evaluation of folpet with TA102

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Folpet R (μ g/plate)	MI	Q	V		Folpet R (μ g/plate)	MI	Q	V		Folpet R (μ g/plate)	MI	Q	V	
2.5	331 $\pm$ 89.32	1.4	NM	10 ⁷	2.5	208 $\pm$ 2.00	1.7	NM	10 ⁷	2.5	271 $\pm$ 24.44	0.8	NM	2 $\times$ 10 ⁹
5	320 $\pm$ 11.31	1.3	NM	10 ⁷	5	225 $\pm$ 30.00	1.9	NM	10 ⁷	5	185 $\pm$ 30.55	0.2	NM	2 $\times$ 10 ⁹
10	339 $\pm$ 94.85	1.4	NM	10 ⁷	10	243 $\pm$ 60.14	2.1	NM	10 ⁷	10	198 $\pm$ 12.16	0.3	NM	2 $\times$ 10 ⁹
20	94 $\pm$ 20.50	0	NM/T	10 ⁶	20	152 $\pm$ 18.33	1	NM/T	2 $\times$ 10 ⁶	20	251 $\pm$ 14.05	0.7	NM/T	2 $\times$ 10 ⁸
SM	122 $\pm$ 6.36			10 ⁷	SM	80 $\pm$ 19.52			10 ⁷	SM	151 $\pm$ 23.43			2 $\times$ 10 ⁹
DMSO	164 $\pm$ 8.48	1.3	NM	10 ⁷	DMSO	74 $\pm$ 18.45	0.9	NM	10 ⁷	DMSO	147 $\pm$ 26.50	1	NM	2 $\times$ 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 $\times$ 10 ⁶	MMS	2080	25	M ⁴⁺ /T	<10 ⁶	2-AA	473	2.1	NM	3 $\times$ 10 ⁸

Legend as Table III.

MMS = mutation in response to methyl methane sulphonate.

thiazolidine-4-carboxylic acid = tetrahydrophthalimide < folpet
< captan < dichlofluanid < captafol.

Discussion

Three of the compounds evaluated showed mutagenic activity with TA104 and TA102 strains. These were captan and folpet with TA104 and captafol with TA102. S9 mix decreased the mutagenic activity of these compounds and the results obtained confirm those obtained by different authors for captan (Ficsor *et al.*, 1977; Shirasu *et al.*, 1977; Carere *et al.*, 1978; de Flora, 1978; Moriya *et al.*, 1978, 1983; Schuphan *et al.*, 1981; Shiao *et al.*, 1981; IARC, 1983; Schuphan and Casida, 1983; de Flora *et al.*, 1984) and for folpet (Shirasu *et al.*, 1977; Moriya *et al.*,

1978, 1983; Shiao *et al.*, 1981; Schuphan and Casida, 1983) in the Salmonella/mammalian microsome test and for captafol (Shirasu *et al.*, 1977; Moriya *et al.*, 1983) in the test carried out with *Escherichia coli*. The remaining compounds—dichlofluanid, tetrahydrophthalimide and thiazolidine-4-carboxylic acid—were non-mutagenic with these strains.

In all the assays performed with a mutagenic compound, there was a dose-related increase in the number of mutant colonies. However, there were differences from one assay to another. Many factors can have an influence on these differences (Grafe *et al.*, 1981; Friederich and Würzler, 1983; Anderson *et al.*, 1984). In this case, the main factors seem to be the spontaneous reversion and the number of bacteria plated.

Table XVI. Mutagenic evaluation of tetrahydrophthalimide with TA102

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Tetrahydro- phthalimide (µg/plate)	R	MI	Q	V	Tetrahydro- phthalimide (µg/plate)	R	MI	Q	V	Tetrahydro- phthalimide (µg/plate)	R	MI	Q	V
80	247 ± 25.46	0.7	NM	10 ⁷	80	101 ± 36.02	0.3	NM	10 ⁷	80	73 ± 22.54	0	NM	2 × 10 ⁹
160	269 ± 51.78	0.8	NM	10 ⁷	160	129 ± 33.17	0.7	NM	10 ⁷	160	67 ± 7.55	0	NM	2 × 10 ⁹
320	285 ± 58.69	1	NM	10 ⁷	320	118 ± 8.72	0.5	NM	10 ⁷	320	75 ± 13.58	0	NM	2 × 10 ⁹
500	300 ± 7.07	1.1	NM	< 10 ⁷	500	100 ± 12.02	0.3	NM	10 ⁷	500	86 ± 7.78	0	NM	10 ⁹
SM	122 ± 6.36			10 ⁷	SM	80 ± 19.52			10 ⁷	SM	151 ± 23.43			2 × 10 ⁹
DMSO	164 ± 8.46	1.3	NM	10 ⁷	DMSO	74 ± 18.45	0.9	NM	10 ⁷	DMSO	147 ± 26.50	1	NM	2 × 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 × 10 ⁶	MMS	2080	25	M ⁴⁺ /T	< 10 ⁶	2-AA	473	2.1	MN	3 × 10 ⁸

Legend as Table III.
MMS = mutation in response to methyl methane sulphonate.

Table XVII. Mutagenic evaluation of thiazolidine-4-carboxylic acid with TA102

(a) Without S9 mix Assay I ^a					Assay II ^a					(b) With S9 mix Assay II ^a				
Thiazolidine-4- carboxylic acid (µg/plate)	R	MI	Q	V	Thiazolidine-4- carboxylic acid (µg/plate)	R	MI	Q	V	Thiazolidine-4- carboxylic acid (µg/plate)	R	MI	Q	V
80	263 ± 39.60	0.8	NM	10 ⁷	80	99 ± 5.57	0.3	NM	10 ⁷	80	101 ± 26.03	0	NM	2 × 10 ⁹
160	359 ± 111.04	1.6	NM	10 ⁷	160	131 ± 17.01	0.7	NM	10 ⁷	160	109 ± 4.62	0	NM	2 × 10 ⁹
320	340 ± 66.47	1.4	NM	10 ⁷	320	163 ± 36.55	1.1	NM	10 ⁷	320	101 ± 19.73	0	NM	2 × 10 ⁹
500	292 ± 106.29	1	NM	10 ⁷	500	155 ± 7.07	1	NM	10 ⁷	500	87 ± 20.53	0	NM	2 × 10 ⁹
SM	122 ± 6.36			10 ⁷	SM	80 ± 19.52			10 ⁷	SM	151 ± 23.43			2 × 10 ⁹
DMSO	164 ± 8.48	1.3	NM	10 ⁷	DMSO	74 ± 18.45	0.9	NM	10 ⁷	DMSO	147 ± 26.50	1	NM	2 × 10 ⁹
MMS	2704	20.7	M ⁴⁺	2 × 10 ⁶	MMS	2080	25	M ⁴⁺ /T	< 10 ⁶	2-AA	473	2.1	NM	3 × 10 ⁸

Legend as Table III.
MMS = mutation in response to methyl methane sulphonate.

TA102 and TA104 strains do not reach the stationary phase after a 14-h incubation period (Barrueco, 1986) and in some cases it was necessary to count revertants after 5 or 6 days, instead of 2 days, to observe the mutagenic effect, indicating that these strains grow slowly in a liquid as well as in a solid medium.

We used cultures from 14 h for both strains and in spite of the culture conditions being the same, the bacteria titre changed in different assays for TA104 (10⁷–2 × 10⁹ viables/ml) and remained constant for TA102 (10⁷ viables/ml).

The number of spontaneous revertants is dependent on the histidine concentration (Green, 1981; Salmeen and Durison, 1981; Aeschbacher *et al.*, 1983; Anderson *et al.*, 1984); however, in this study, although the concentration was always the same, spontaneous reversion varied from one assay to another. This variation may also be influenced by the copy number of plasmids in individual cell populations (Levin *et al.*, 1982; Maron and Ames, 1983).

As regards strain TA104, the higher the spontaneous reversion, the higher the captan- and folpet-induced reversion; when spontaneous reversion was similar, the higher the number of bacteria plated, the higher the captan- and folpet-induced reversion. On the other hand, with TA102 strain, the lower the spontaneous reversion, the higher the captan-induced reversion.

In order to quantify the mutagenic effect caused by test chemicals we used mutation index and mutagenic potency. The maximum mutation index was 12 for captan, 7.3 for captan

and 6.3 for folpet. The mutagenic potency, in revertants/nmol, was 537.64 for captan, 106.63 for captan and 156.91 for folpet. Captan was the most mutagenic compound. Captan and folpet were very similar.

Captan and folpet were mutagenic with strains initially described by Ames that detect mutagens damaging G-C base pairs (Bridges, 1975; McCann *et al.*, 1975; Ficsor *et al.*, 1977; Carere *et al.*, 1978; de Flora, 1978; Moriya *et al.*, 1978; Hollstein *et al.*, 1979; Rinkus and Legator, 1979; de Flora and Boido, 1980; Shiao *et al.*, 1981; Waters *et al.*, 1982; IARC, 1983; de Flora *et al.*, 1984; Xu *et al.*, 1984). This effect was confirmed in our laboratory (Barrueco, 1986). On the other hand, captan and folpet had positive results with TA104, thus these compounds also behave like mutagens which induce base-pair substitutions affecting A-T base pairs. Both compounds were non-mutagenic with TA102—the only strain with an intact excision repair system—indicating that the mutagenic activity of these compounds involves the production of a DNA lesion which is detectable and repairable by the excision repair system, according to results of different authors (Bridges *et al.*, 1972; Ficsor *et al.*, 1978).

Captan and folpet behave similarly, the only difference being the cyclic group: an aromatic ring (phthalimide) in the case of folpet and tetrahydrophthalimide in captan. Tetrahydrophthalimide was non-mutagenic. Therefore, the trichloromethylthio moiety seems to be the one responsible for the attack on the DNA.

Captan is, however, more toxic than folpet, indicating that the rest of the molecule, i.e. tetrahydrophthalimide or phthalimide, influences the activity of trichloromethylthio moiety.

Captafol showed only a very weak mutagenic effect and this was not always observed with TA100 (Kawachi *et al.*, 1980; Moriya *et al.*, 1983). This effect was not confirmed in our laboratory (Barrueco, 1986). By using TA102 strain the mutagenicity of captafol was clearly shown. Captafol was non-mutagenic with TA104. If the structures of captan and captafol are compared, their cyclic groups are seen to be identical, i.e. tetrahydrophthalimide, and the compounds differ in the reactive moiety, which is trichloromethylthio for captan and tetrachloroethylthio for captafol. Both compounds were mutagenic, so both reactive groups seem to be responsible for the attack on the DNA, although their mechanism of action seems to be different. Captafol was the most toxic compound of all those evaluated, and it therefore seems probable that captafol causes more serious damage to DNA than captan or folpet by acting as a crosslinking agent. Thus, the presence of an intact excision repair system in TA102 decreases the toxicity of captafol, allowing the detection of its mutagenic effect.

Dichlofluanid showed only a very weak mutagenic effect which was not always observed with TA100 (Shirasu *et al.*, 1977; Schuphan *et al.*, 1981; Moriya *et al.*, 1983; Schuphan and Casida, 1983). This effect was not confirmed in our laboratory (Barrueco, 1986) and there was no mutagenic response with either TA102 or TA104 strains. Dichlofluanid has a reactive group similar to that of captan and folpet in which a chlorine has been substituted by a fluorine. Schuphan *et al.* (1981) found that dichlofluanid and two of its metabolites—thiazolidine-2-thione-4-carboxylic acid and bis-(fluorodichloromethyl)disulfide—were non-mutagenic to TA100. However, the chlorinated derivative of dichlofluanid was mutagenic to TA100 (Schuphan and Casida, 1983). Everything, therefore, seems to point to the fluorine atom as the cause of the absence of mutagenicity of dichlofluanid. In addition, dichlofluanid was the second most toxic compound after captafol and it is again the fluorine atom which appears as the cause of this toxicity which would prevent the detection of any possible mutagenic effect.

This study has shown that it is necessary to include strains TA104 and TA102 together with those strains initially used in the Ames test in order to increase the confidence of detecting mutagens and shedding light on their mechanism of action.

The mutagenicity of these compounds has also been evaluated in different derivatives of *Escherichia coli* WP2 (Bridges, 1975). These strains carry an ochre mutation at a trp locus and can revert by structural gene mutations that include transitions at an A-T base pair or by the formation of ochre suppressors that include transitions at a G-C base pair. It was found that both types of mutation were induced in these strains although with differing proportions and it was necessary to perform another assay with T4 ochre mutant phage in order to establish the mutational specificity of these compounds. However, using a set of *Salmonella typhimurium* strains we can establish the mutational specificity of these compounds without performing another complementary assay. We thus conclude that *Salmonella typhimurium* strains are preferable to *Escherichia coli* strains for the detection of chemicals in this class.

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