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QUARTERLY

The effects of captan and captafol on different bacterial strains and on c-mitosis in V79 Chinese hamster fibroblasts*

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The mutagenic activity of captan and captafol was tested using Ames strains and strains showing an SOS response. Captafol was mutagenic in *S. typhimurium* strain TA102 (*uvr*$^+$) and captan in strain TA104 (*uvrB*). Both captan and captafol elicit damages in DNA recognized by correndonuclease II, as shown by the repair test, and induced the SOS repair system in *E. coli* PQ37 (*uvrA*) strain. Only captafol induced the SOS system in PQ35 (*uvr*$^+$). The lack of induction of β-galactosidase at nonpermissive temperature in *E. coli* MD332 (*dnaC*$_s$ *uvrA*) strain showed that neither chemical was able to produce DNA breaks. In V79 Chinese hamster fibroblasts higher induction of c-mitosis by captafol than by captan (22% and 15% over the control, respectively) was accompanied by a higher decrease in nonprotein sulfhydryl groups, mainly GSH (41% and 77%, respectively). The content of protein sulfhydryl groups was decreased by either fungicide to a similar extent.

Captan and captafol are structurally related phtalimide compounds (Table 1) widely used as nonpersistent fungicides with a broad spectrum of activity. Their agricultural applications include control of fungal diseases; being nonpersistent fungicides, they are applied repeatedly to maintain antifungal effect. Captan and captafol are applied directly on harvested seeds, fruits and vegetable crops [1], but are also used as fungicides in paints, plastics and leather [2]. Thus their use might cause long-term exposure of humans.

Captan and captafol are classified by Lewis as questionable (class 3) carcinogens [3] due to contradicting results obtained in carcinogenicity tests with eukaryotes. It seems however, that the carcinogenic activity of captafol is better documented than that of captan, since carcinogenicity of captan has been demonstrated only in B6C3F$_1$ mice [4], but not in Osborne-Mendel rats [5] or in CD1 mice [6]. On the other hand a broad-spectrum carcinogenic activity of captafol has been proved in tests with mice and rats [4, 7, 8]. The ability of the two compounds to induce sister chromatid exchanges (SCEs)[1] and chromosomal aberrations are similar. In the tests performed on Chinese hamster cells captafol induced three times more SCEs and ten times more chromosomal aberrations than did captan at the same concentration. More-

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[1]Abbreviations: DMSO, dimethyl sulfoxide; DTNB, 5,5'-dithio-bis-(2-nitrobenzoic acid); GSH, reduced glutathione; GSSH, oxidized glutathione; NPSH, nonprotein sulfhydryl groups; PSH, protein sulfhydryl groups; SCEs, sister chromatid exchanges; SDS, sodium dodecyl sulphate.

over, only captafol is able to increase frequency of polyploidisation in Chinese hamster cells [9].

In contrast to eukaryotes, in numerous prokaryotic tests captan appeared mutagenic, whereas the mutagenicity of captafol was revealed only in a few tests [4, 10–13]. Also captan, but not captafol, was classified as mutagenic in a lethal mutagenicity test in mice and in a host-mediated assay in rats [10, 14–16]. Looking for a reason of the different behaviour of captafol in chromosomal and mutagenic tests one may expect that (i) the tests applied so far were not specific enough to reveal the mutagenic activity of captafol and/or (ii), sister chromatid exchanges and chromosomal aberrations induced by captafol did not result from its mutagenic activity. The latter possibility is still more worth considering as both captan and captafol affect the status of thiol groups in the cell [17, 18], which, in turn, might influence the integrity and functions of the mitotic spindle [19, 20]. c-Mitosis is a cytological sign signaling inhibition or disturbances of the spindle function and the c-mitosis agents can give rise to abnormal chromosome numbers in both mitotic [21–23] and meiotic cells [24–26] in experimental systems. The abnormal chromosome number is a potential source of spontaneous abortion in humans [27] and might contribute to carcinogenesis [28–30].

In our complementary study on the mechanism of carcinogenic action of captan and captafol we have applied two different approaches: (i) mutagenic activity of the two fungicides was tested using new bacterial strains showing an SOS response, (ii) the effect of both compounds on sulhydryl levels, and c-mitosis was studied in V79 Chinese hamster fibroblasts.

MATERIALS AND METHODS

Chemicals. The following chemicals were obtained from the sources listed: captan (CAS No 133-06-2) purity 99.8% and captafol (CAS No 2425-06-1) purity 99.8% were from Organika-Azot (Jaworzno, Poland); Aroclor 1254 was from Analabs Inc.; mitomycin C, 2-aminoanthracene, Tris, trichloroacetic acid and 5,5′-dithiobis(2-nitrobenzoic acid) (DNTB, Ellman's reagent) were from Sigma; methylglyoxal from Aldrich; 2-aminofluorene from Koch-Light Lab. Ltd.; 1-oxide-4-nitroquinoline (NQO) and dimethyl sulfoxide (DMSO) were from Fluka AG; aflatoxin B1, L-tryptophan from Calbiochem; *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and D-biotin were from Serva; sodium dodecyl sulphate (SDS), Bacto tryptone, Bacto yeast extract and Casamino acids were from Difco; uracil and l-histidine were from Ciech (Gliwice, Poland). All chemicals for cell cultures were from Pharmacia.

Table 1 gives names, chemical structure and relative molecular mass of chemicals evaluated.

Bacterial strains. *Salmonella typhimurium* strains: TA102, *hisG*428 *rfa* pQA1; TA104, *hisG*428 *uvrB rfa* pkM101; TA1538, *hisD*3052 *rfa uvrB*, and TA1978, *hisD*3052 *rfa* were kindly supplied by Professor B.N. Ames, University of California, Berkeley, U.S.A.

Escherichia coli K12 strains PQ37 (F⁻, *thr, leu, pyrD, thi, trp: :Muc*$^+$, *srl*300::Tn10, *rpsL, rpoB, sfi::Mud/Aplac/cts, lac*AU169, *uvrA*6, *galE, galY, phoC, rfa*); PQ35 as PQ37 but *uvr*$^+$ used in the β-galactosidase assay were gifts from Drs. P. Quillardet and M.Hofnung, U.B.M.T.G. Institut Pasteur, Paris, France.

Escherichia coli MD332 (*dnaC*28, *uvrA*6, *serB*, *sfiA::Mud/Aplac*) was a gift from Dr. B. Salles, CNRS, Toulouse, France.

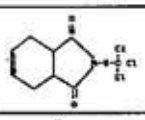
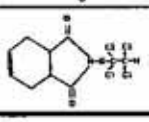
Bacterial tests. a) *Ames test* was performed with *S. typhimurium* strain TA102 and liver homogenate fraction (S9) which was prepared according to Maron & Ames [31]. For determination of mutagenic effect the mutation index (MI) was calculated [32].

The concentration of the compound tested is considered mutagenic when the mutation index is 2.5 and toxic when survival is below 10%.

b) *The DNA repair test* was performed as described by Ames *et al.* [33]. The culture media used for the assays with *Salmonella* were as described by Ames *et al.* [34].

c) *β-galactosidase assays.* The induction of the SOS response by captan and captafol was measured in *E.coli* PQ35 and PQ37 strains by means of an *sfiA::lacZ* operon fusion according to the principle of the SOS Chromotest [35, 36]. Strain *E.coli* MD332 was grown in M9 minimal medium supplemented with 0.5% casamino acids and 1 μg/ml thiamine [37]. Uracil, tryptophan and ampicillin were added at 50 μg/ml, 40 μg/ml and 50 μg/ml, respectively. β-Galactosidase assays were carried out with the cul-

Table 1
Chemical structures of captan and captafol

Common name	Trade name	Chemical name	Chemical structure	Mol. wt.
Captan	Orthocide	*N*-[(trichloromethyl) -thio]-4-cyclohexene- -1,2-dicarboximide		301
Captafol	Difolatan	*N*-[(1,1,2,2-tetrachlo- roethyl)thio]-4-cyclo- hexene-1,2-dicarboximide		349

tures treated with fungicides for 2 or 3 h at nonpermissive (42°C) and permissive (30°C) temperature.

β-Galactosidase activity was measured at 30°C and calculated in conventional units [37] referred to cell density measured at 600 nm.

The results are expressed as a ratio of the activities in the treated to non-treated bacteria at each time point (Induction factor). Each assay was accompanied by the controls: NQO (30 ng/ml) was used for the estimation without, and aflatoxin B_1 (30 ng/ml) for the estimations with, metabolic activation.

Liver homogenate fraction (S9). The liver homogenate fraction was prepared according to Maron & Ames [31] using Aroclor 1254-induced Wistar male rats.

S9 mix. The S9 mix used in the β-galactosidase assays was prepared according to Quillardet & Hofnung [36]. The S9 mix used in the Ames test was prepared after Maron & Ames [31].

Cell culture. V79 Chinese hamster fibroblasts were grown in flasks or on microscopic slides. They were incubated for 24 h in Eagle's minimum essential medium supplemented with 1.8 mM L-glutamine, 90 units penicillin per ml, 90 μg/ml streptomycin, 45 μg/ml kanamycin and 10% heat-inactivated fetal calf serum, in the atmosphere containing 5% CO_2 at 37°C.

Cells from the exponential phase of growth in Hanks' balanced salt solution were transfered (10^6 per flask) to the same medium supplemented with fungicides (from 5×10^{-5} M to 1×10^{-8} M) dissolved in DMSO and incubated for 30 min. The experiment was run in duplicate. The amount of DMSO added to the medium never exceeded 0.2%, a concentration which *per se* did not affect the cells.

Survival. After the treatment the cells were trypsinized, counted in a Coulter counter, seeded (100 cells per one Petri dish — 5 cm in diameter) and incubated in Eagle's medium supplemented as above. After 1 week the cells were fixed with methanol and scored.

c-Mitosis. Cells growing directly on microscopic slides were treated with the fungicides, then fixed with a mixture of acetic acid:methanol (1:3, v/v) and dehydrated with ethanol (70%). The cells were stained with 2% Giemsa in phosphate buffer. Normal and abnormal metaphases, anaphases and telophases were scored. A hundred mitotic cells at these stages per slide and 2 slides per each fungicide concentration were scored in each of three experiments.

TCA-soluble sulfhydryl (NPSH) and protein sulfhydryl groups (PSH). The assay was carried out with DTNB according to Söderpalm-Berndes & Önfelt [22]. Absorbance was read at 412 nm. The amount of sulfhydryl groups was related to the protein content in the sample.

Reduced glutathione and protein determinations. Glutathione was measured according to Moron *et al.* [38]. Protein was determined according to Lowry *et al.* [39] with bovine serum albumin as a standard.

Statistical evaluation. Each result was compared to the corresponding pooled control value by the Student's *t* test. All changes were found significant at $P < 0.05$.

RESULTS

Bacterial assays

In order to reveal possible damages in DNA induced by captan and captafol we have used the following bacterial strains:

(I) *Salmonella typhimurium* TA102 which enabled detection of oxidative and cross-linking

mutagens, and TA104 sensitive only towards oxidative mutagens [40], whereas the use of some other *Salmonella* strains can give negative results [41]. Both strains used contain A-T base pairs at the site of the mutation, while the other *Salmonella* tester strains detect mutagens damaging G-C base pairs. In addition, the excision repair system of the TA102 strain remains intact.

The results of the mutagenic activity of captan and captafol are summarized in Table 2 and Table 3. Mutation index (see Methods), show that captan was mutagenic under the test conditions applied only in TA104, whereas captafol only in TA102. In all assays without S9 mix, there was a dose-related increase in the number of mutant colonies. Since in the absence of S9

Table 2

Mutagenic evaluation of captan and captafol with S. typhimurium TA102 strain in the absence and presence of the metabolic activation system

The mutagenicity assays were carried out in triplicate and the number of *his*$^+$ revertants was scored after incubation for 48 h at 37° C. The number of revertants per plate is an average number from 3 separate experiments ± S.D.; where indicated, 50 µl of S9/plate was added; NT, not tested.

Dose (µg/plate)	Number of revertants/plate			
	Captan		Captafol	
	–S9	+S9	–S9	+S9
0	340 ± 12	343 ± 53	340 ± 12	343 ± 53
0.25	371 ± 54	328 ± 64	836 ± 264	356 ± 72
0.50	386 ± 74	354 ± 72	1494 ± 328	378 ± 68
1.25	453 ± 68	336 ± 68	2360 ± 983	320 ± 96
2.50	594 ± 221	347 ± 112	3820 ± 1200	396 ± 84
5.00	966 ± 357	350 ± 96	3780 ± 1100	368 ± 75
Mitomycin C (0.5 µg/plate)	2660 ± 260	NT		
2-Aminoanthracene (5 µg/plate)	NT	1400 ± 75		

Table 3

Mutagenic evaluation of captan and captafol with S. typhimurium TA104 strain in the absence and presence of the metabolic activation system.

For details see Table 2.

Dose (µg/plate)	Number of revertants/plate			
	Captan		Captafol	
	–S9	+S9	–S9	+S9
0	340 ± 60	370 ± 50	340 ± 60	370 ± 50
0.25	453 ± 83	395 ± 75	439 ± 85	388 ± 89
0.50	838 ± 67	453 ± 82	533 ± 139	410 ± 74
1.25	1076 ± 95	582 ± 93	642 ± 94	396 ± 86
2.5	1360 ± 90	439 ± 88	797 ± 79	439 ± 142
5.0	1592 ± 85	539 ± 97	859 ± 140	393 ± 79
Methylglyoxal (25 µg/plate)	4600 ± 300	NT		
2-Aminoanthracene (5 µg/plate)	NT	2380 ± 120		

mix both fungicides were mutagenic, they should be considered as direct mutagens.

As regards toxicity, captan was less toxic than captafol.

(II) *Salmonella typhimurium* TA1538 (*uvrB*) and TA1978 (*uvr*$^+$) which, when used in the repair test, allowed to detect the chemicals bound covalently to DNA [34]. The results of the repair test are shown in Table 4. As can be seen there was an appreciable difference in the zones of growth inhibition, produced both by captan and captafol, between the two strains tested, but this inter-strain difference was observed only in the absence of metabolic activation. The extent of changes was similar for both fungicides. According to Ames *et al.* [34], the difference in susceptibility of TA1538 and TA1978 might be ascribed to a covalent reaction with DNA.

(III) *Escherichia coli* PQ35 (*uvr*$^+$) and PQ37 (*uvrA*) which were used to reveal the induction of the SOS response [42–44]. The expression of one of the SOS genes, *sfiA* gene [45] was monitored in bacterial SOS Chromotest to reveal induction of the SOS-response. In the bacterial strains used, the *sfiA* gene is fused with *lacZ*, a structural gene for β-galactosidase [35, 36].

Captan and captafol were used at the nontoxic concentration range, i.e. they did not affect the alkaline phosphatase activity. Induction of β-galactosidase by either chemical was observed only without metabolic activation. The results presented in Fig. 1A indicate that both captan and captafol induced the SOS repair system in PQ37 (*uvrA*). The effect of captan was more pronounced than that of captafol. In contrast, captan did not induce an SOS response in the PQ35, an excision repair proficient strain, whereas captafol still induced β-galactosidase (max. induction factor 2.5) (Fig. 1B), although the effect was less pronounced that for PQ37, an excision repair deficient strain (max. induction factor 5).

(IV) *Escherichia coli* MD332 (*dnaC*$_s$ *uvrA*), derived from the commonly used SOS Chromotest tester strain (PQ37), harbouring the *uvrA* mutation and a temperature sensitive mutation in the *dnaC* gene involved in initiation of DNA replication. In this strain, at nonpermissive temperature (42°C), DNA replication is blocked and therefore the SOS system cannot be induced by typical SOS genotoxins. However, treatment of this strain with an agent producing single-strand breaks restores induction of the SOS system [46]. Thus, it is possible

Table 4

The zones of growth inhibition produced by captan and captafol in TA1538 (uvrB) and TA1978 (uvr$^+$*) strains of S. typhimurium in the absence and presence of the metabolic activation system*

Data are mean values from 9 plates (± S.D.); NT, not tested; where indicated 200 µl of S9 mix was added per plate.

Compound tested (µg/plate)		Diameter of zone of growth inhibition (mm)			
		–S9		+S9	
		TA1538	TA1978	TA1538	TA1978
Captan	0.25	6.6 ± 0.8	6.0 ± 0	6.0 ± 0	6.0 ± 0
	0.50	8.2 ± 0.8	6.6 ± 0.7	6.0 ± 0	6.0 ± 0
	1.25	12.2 ± 0.9	7.7 ± 1.4	6.0 ± 0	6.0 ± 0
	2.50	13.9 ± 0.9	9.1 ± 1.6	7.9 ± 1.1	6.0 ± 0
	5.0	15.9 ± 1.5	10.7 ± 1.6	8.6 ± 1.0	6.4 ± 0.5
Captafol	0.25	7.5 ± 0.7	6.0 ± 0	6.0 ± 0	6.0 ± 0
	0.5	10.4 ± 0.8	6.9 ± 1.0	6.2 ± 0.4	6.0 ± 0
	1.25	12.2 ± 0.9	9.3 ± 1.1	6.6 ± 0.7	6.0 ± 0
	2.50	14.3 ± 1.4	11.5 ± 2.7	7.6 ± 0.5	6.2 ± 0.7
	5.0	13.7 ± 0.8	11.8 ± 1.8	8.3 ± 0.5	7.4 ± 0.9
Mitomycin C	1.0	21.4 ± 2.5	15.4 ± 2.2	NT	NT
2-Aminofluorene	50.0	NT	NT	10.9 ± 2.1	7.2 ± 0.4

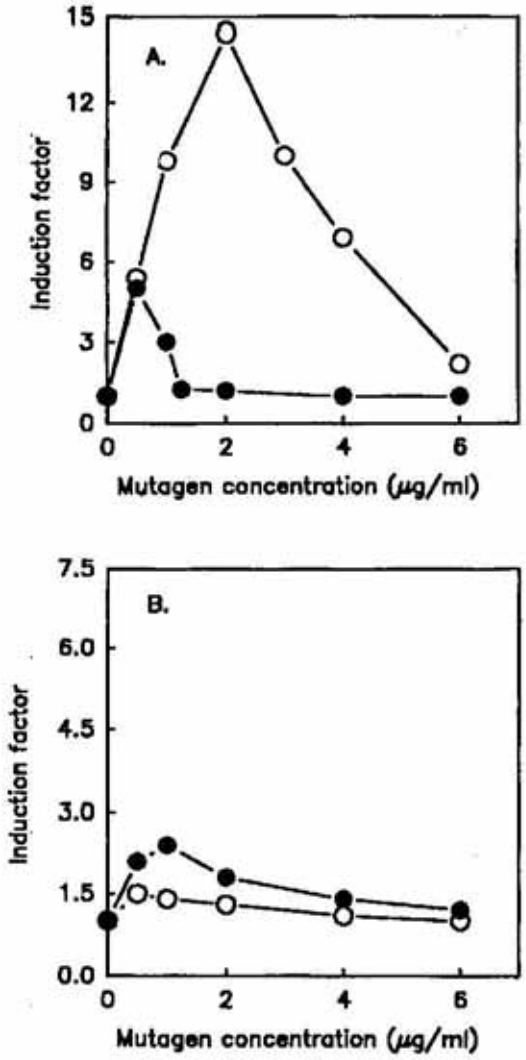


Fig. 1. Induction of β-galactosidase resulting from sfi::lacZ fusion in E. coli strains PQ37 uvrA (A) and PQ35 uvr⁺ (B) after treatment with increasing concentrations of captan (○) and captafol (●).
Bacteria (5×10^7/ml) were incubated in the culture medium (see Methods) till A_{600} reached about 0.2, then diluted with 10 vol. of the same medium and treated with the fungicide indicated for 2 h at 30°C. The activities of β-galactosidase in PQ35 and PQ37 strains in the absence of mutagens were: 10 ± 2 and 42 ± 2 units, respectively. The induction factor was calculated as the ratio of the activity in the treated bacteria to that in untreated bacteria at each time point.

to check by this method whether a genotoxic compound is able to create single strand breaks in DNA.

Neither captan nor captafol induced the SOS response at the nonpermissive temperature (42°C) over the whole range of concentrations tested (Fig. 2A, 2B). This indicates that neither compound introduced single strand breaks under test conditions. It is also interesting that, at the permissive temperature (30°C), the induction of β-galactosidase was time dependent both with captan and captafol. This means that, in bacterial liquid medium test, it was possible to evaluate mutagenic activity of chemicals even those readily decomposed in water (half life of captan is 2.5 h at pH 7.0 [47]) and/or the activity of their degradation products [18].

V79 Chinese hamster fibroblasts

In the experiments performed the mitotic index was 3%–5% and was in no case affected by the treatment. The spontaneous frequency of c-mitotic cells was 6.2 ± 2.9%.

Survival. The toxicity of captan and captafol is presented in Fig. 3; 80% of the cells survived at 2 μM concentration of captan and 0.2 μM concentration of captafol. At 50 μM concentration of captan and at 10 μM concentration of captafol no cells survived.

c-Mitosis. Mitotic cells exhibited spindle disturbances after treatment with 0.01 μM captan or captafol (Fig. 3). At this concentration mitosis was significantly affected, the number of induced alterations increased above the control value up to 15% and 22% for captan and captafol, respectively. An increase of the concentration did not enhance the percentage of induced c-mitotic cells over this initial level.

Sulfhydryl groups. The levels of nonprotein sulfhydryl groups (NPSH, practically those of reduced glutathione) [20] and protein sulfhydryl groups (PSH) in non-treated cells were: 38.0 ± 5.3 and 87.8 ± 15.7 nmol/mg protein, respectively. The treatment of cells with 0.01–0.2 μM concentration of captan resulted in an increase in both PSH and NPSH content, which at the 50 μM concentration decreased to 40.8% and 75.9% of control value, respectively (Table 5). The increase was not observed with captafol. Instead, over its whole concentration range the content of PSH and NPSH gradually decreased, to 58.5% and 41.5%, respectively, at 10 μM concentration. Thus, captafol reduced the content NPSH to a significantly lower extent than did captan (Table 5).

DISCUSSION

The results presented in this work demonstrate the mutagenic activity of captan and captafol in *S. typhimurium* TA1538, TA1978, TA102, TA104 and *E. coli* PQ35, PQ37, MD332 strains.

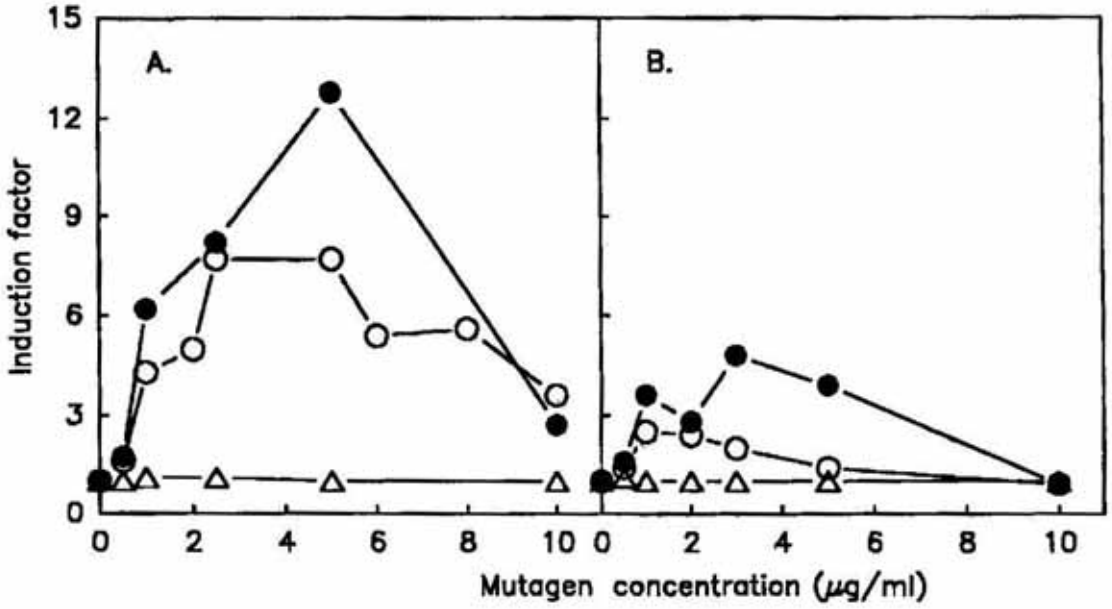


Fig. 2. Induction of β-galactosidase resulting from sfiA::lacZ fusion in E. coli MD332 dnaC$_s$ uvrA after treatment with increasing concentrations of captan (A) and captafol (B) at 30°C or 42°C.
Bacteria (5×10^7/ml) were incubated for 70 min at 30° or 42°C, then treated with the fungicide indicated at 30°C for 2 h (○) or 3 h (●), and at 42°C for 2 h (Δ), and kept at either temperature throughout the experiment. The results represent the average of at least 3 experiments. The activity of β-galactosidase at 30°C in the absence of mutagens was 49 ± 13 units. The induction factor was calculated as the ratio of the activity in the treated bacteria to that in untreated bacteria at each time point.

We found, also that both fungicides act as inducers of c-mitosis and lower the level of sulfhydryl groups in eukaryotic cells. All these effects were significantly more pronounced with captafol than with captan.

Both captan and captafol are direct mutagens. We found that incubation of these compounds with S9 mix did not increase but, instead, decreased their mutagenic activity in all the bacterial strains tested. This observation is in

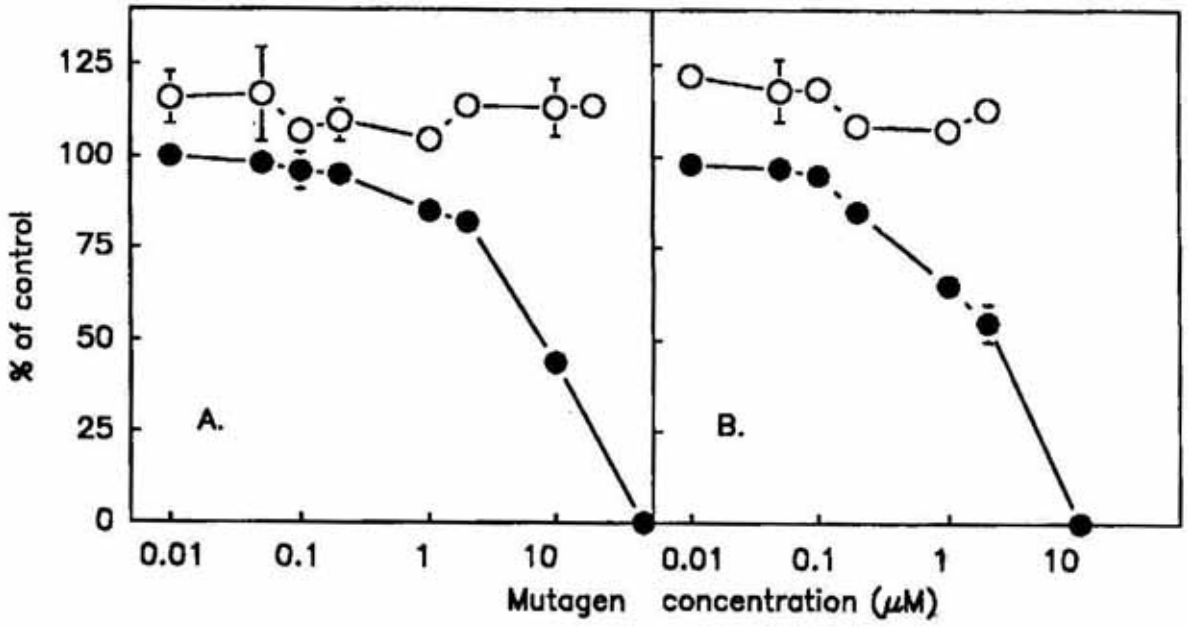


Fig. 3. Effects of captan (A) and captafol (B) on survival (●), and on induced frequency of c-mitosis (○), in Chinese hamster fibroblasts.
Treatment time 30 min. The data are expressed as percentage of corresponding pooled control value ± S.E.

Table 5

Changes in sulfhydryl levels at different concentrations of captan and captafol

Control values (nmol/mg protein): PSH, 87.8 ± 15.7; NPSH, 38.0 ± 5.3. The results are expressed as percentage of control values (± S.D.)

Concentration (μM)	Captan	Captafol
Protein sulfhydryl groups		
Control	100	100
0.01	109.4 ± 4.1	87.1 ± 0.1
0.05	115.1 ± 12.9	78.3 ± 1.6
0.1	104.2 ± 7.2	72.1 ± 4.5
0.2	105.2 ± 8.4	77.8 ± 2.7
2.0	91.8 ± 41.9	73.5 ± 16.8
10.0	48.2 ± 4.3	58.5 ± 3.3
50.0	40.8 ± 1.3	58.5 ± 3.3
Nonprotein sulfhydryl groups		
Control	100	100
0.01	113.5 ± 7.6	99.7 ± 4.8
0.05	122.0 ± 8.9	93.7 ± 2.6
0.1	126.0 ± 15.5	92.2 ± 7.3
0.2	126.2 ± 1.3	93.7 ± 1.8
2.0	99.3 ± 18.6	57.1 ± 12.6
10.0	77.5 ± 0.5	41.5 ± 5.2
50.0	75.9 ± 5.4	–

agreement with the results of other authors for captan and captafol applied in the same and many other bacterial tests [12, 16]. The effect of S9 mix was possibly due to the reaction of captan and captafol with thiol compounds present in liver homogenate, leading to the decrease of the mutagenic activity of the fungicides [17].

Only captan gave positive results with TA104 and was nonmutagenic with TA102, the strain with an intact excision repair system. This indicates that the mutagenic activity of this fungicide involves a DNA lesion which is detectable and reparable by the excision repair system, consistently with the results of other authors [48]. Unlike captan, captafol acted as a very strong mutagenic agent in TA102, but was nonmutagenic in TA104. The TA102 strain is unique among the battery of Ames tester strains, as its introduction increase the ability of the assay system to detect oxidative and cross-linking mutagens. Location of the reverting gene on a multicopy plasmid in *uvr*B$^+$ background served to combine sensitivity of TA2638 (toward crosslinking agents) and of TA104 (toward oxidative mutagens) [40]. Basing on our results with both Ames strains we conclude, that captafol, but not captan, can act as a crosslinking agent. This observation is in agreement with a previous finding of Barrueco & de la Peña on *Salmonella* strains [12].

It seems of interest that, in the SOS Chromotest in *E. coli* strains, with a defective excision repair system (PQ37), both captan and captafol induced similarly SOS response, whereas only captafol was active in the strain with the intact excision repair system (PQ35). The ability to induce the SOS response was earlier reported by Ohta *et al.* [49] only for captafol.

Results of the test with *E. coli* MD332 show that neither captafol nor captan can introduce single strand breaks into DNA. However both fungicides can covalently bind to DNA, as revealed by the repair test.

Crosslinks probably created by captafol might cause chromosome aberrations. Another property of captafol which could contribute to chromosomal aberrations, is its ability to lower the level of sulfhydryl groups, especially of GSH. In this respect the effect of captafol was much more pronounced than that of captan. The cellular GSH/GSSG status has been shown to affect both cytoplasmic microtubules and mitotic spindle [19, 20]. Therefore we think that the ability of captafol to induce polyploids [9] might reflect its reaction with cellular thiols that, in turn, can affect functions of the mitotic spindle. This suggestion is supported by the observation that both captafol and captan induce the number of c-mitoses in V79 Chinese hamster fibroblasts. However, it should be stressed that the ability of captan and captafol to induce c-mitosis did not differ as significantly as their ability to lower the level of GSH.

The results of this work reveal two properties of captafol that might explain its enhanced ability to induce sister chromatid exchanges and raise the frequency of polyploidisation [9]. These are: the ability to crosslink DNA, as was revealed with the Ames tester strains, and the ability to reduce the NPSH (GSH) level. However, these effects suggest that the relationship between these properties and captafol-induced chromosomal aberrations is not simple and that aberrations are rather of multifactorial origin.

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