FINAL

Report on Carcinogens Background Document for

Captafol

June 20, 2008

U.S. Department of Health and Human Services
Public Health Servces
National Toxicology Program
Research Triangle Park, NC 27709

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of identified substances (i) that either are known to be human carcinogens or are reasonably be anticipated to be human carcinogens and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (HHS), has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP), which prepares the report with assistance from other Federal health and regulatory agencies and nongovernmental institutions.

Nominations for (1) listing a new substance, (2) reclassifying the listing status for a substance already listed, or (3) removing a substance already listed in the RoC are reviewed in a multi-step, scientific review process with multiple opportunities for public comment. The scientific peer-review groups evaluate and make independent recommendations for each nomination according to specific RoC listing criteria. This background document was prepared to assist in the review of captafol. The scientific information used to prepare Sections 3 through 5 of this document must come from publicly available, peer-reviewed sources. Information in Sections 1 and 2, including chemical and physical properties, analytical methods, production, use, and occurrence may come from published and/or unpublished sources. For each study cited in the background document from the peer-reviewed literature, information on funding sources (if available) and the authors' affiliations are provided in the reference section. The draft background document was peer reviewed in a public forum by an *ad hoc* expert panel of scientists from the public and private sectors with relevant expertise and knowledge selected by the NTP in accordance with the Federal Advisory Committee Act and HHS guidelines and regulations. This document has been finalized based on the peer-review recommendations of the expert panel and public comments received on the draft document. Any interpretive conclusions, comments, or statistical calculations made by the authors or peer reviewers of this document that are not contained in the original citation are identified in brackets [ ].

A detailed description of the RoC nomination review process and a list of all substances under consideration for listing in or delisting from the RoC can be obtained by accessing the 12th RoC at http://ntp.niehs.nih.gov/go/9732. The most recent RoC, the 11th Edition (2004), is available at http://ntp.niehs.nih.gov/go/19914.

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PEER REVIEW

The draft background document on Captafol was peer reviewed by the Report on Carcinogens (RoC) expert panel for Captafol and *ortho*-Nitrotoluene. The panel met in a public forum at the Sheraton Chapel Hill Hotel, Chapel Hill, NC on October 15–16, 2007. Members of the expert panel are as follows:

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

The criteria for listing an agent, substance, mixture, or exposure circumstance in the RoC are as follows:

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,

or

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

or

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

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Executive Summary

Introduction

Captafol is a nonsystemic broad-spectrum fungicide (i.e., it is applied topically and works outside the plants to which it is applied). Captafol is categorized as a phthalimide fungicide based on its tetrahydrophthalimide ring structure. Other phthalimide fungicides include captan and folpet.

Captafol was nominated by the National Institute of Environmental Health Sciences (NIEHS) for possible listing in the *Report on Carcinogens* based on a 1991 evaluation by the International Agency for Research on Cancer, which classified captafol as probably carcinogenic to humans (Group 2A) based on sufficient evidence in animals and also because it was genotoxic in a wide range of tests, including the generally insensitive *in vivo* assay for dominant lethal mutations (IARC 1991).

Human Exposure

Captafol was produced and used as a fungicide (on fruits, vegetables, other plants, and timber products) in the United States until 1987, when all registrants of captafol products requested voluntary cancellation of their registrations. Legal use of existing stocks was allowed; however, in 1999, the U.S. Environmental Protection Agency (EPA) further restricted its use, and all captafol tolerances were revoked except those for onions, potatoes, and tomatoes. These remaining tolerances were revoked by the EPA in 2006. Although many countries have now banned its use, it may still be used in some countries, including Mexico. The U.S. Food and Drug Administration (FDA) continues to monitor for captafol residues in domestic and imported food; captafol was detected at low levels in food samples in the United States in the 1980s and 1990s but has not been detected by the FDA in food samples since 1998.

Because of captafol's past high production (14.5 million pounds in 1985) and domestic usage (2 to 3 million pounds per year in the late 1970s and early 1980s), the potential existed for extensive exposure of workers producing captafol and of agricultural workers

applying it to crops. In addition, environmental exposure of the general population may have occurred.

Human Cancer Studies

Captafol has been specifically examined in only one published human study, an ecological case-control study of pancreatic cancer involving mixed exposures to captafol and other organochlorine agents (Clary and Ritz 2003). In this study, an increased risk of pancreatic cancer (odds ratio = 1.73, 95% confidence interval = 0.70 to 4.28) was found for residents who at the time of death had lived for over 20 years in areas with high captafol usage (highest quartile of usage), compared with residents who had lived in areas of lower pesticide usage (lowest three quartiles of usage). [Confounding by co-exposures to other agents, such as smoking, could not be ruled out, and the power to detect an effect was limited by the imprecise measures of exposure and disease.]

Three case-control studies reported an increased risk of non-Hodgkin's lymphoma associated with exposure to the analogue captan (one study) or to phthalimides as a class (two studies). An ecological study reported a significant association between captan exposure and leukemia among Hispanic males and a nonsignificant correlation between captan exposure and prostate cancer among black males. A prospective cohort study found an increased risk of breast cancer associated with indirect exposure to captan via husband's exposure. [However, all of these studies were limited by methodological concerns, and their usefulness for assessing the carcinogenicity of captafol is limited by lack of specificity for exposure to that compound.]

Studies of Cancer in Experimental Animals

Captafol was tested for carcinogenicity in feeding studies in CD-1 mice, $B6C3F_1$ mice, Crl:CD rats, and F344 rats. In CD-1 mice, captafol was associated with increased incidences of hemangiosarcoma (heart, liver, spleen, and subcutaneous tissue) and lymphosarcoma in both sexes, and Harderian gland adenoma in males. Male and female $B6C3F_1$ mice exposed to captafol had increased incidences of hemangiosarcoma (heart), splenic hemangioma, and tumors of the forestomach (papilloma and carcinoma combined), small intestine (adenocarcinoma and adenoma and adenocarcinoma

combined), and liver (hepatocellular carcinoma and neoplastic nodules and hepatocellular carcinoma combined). Female B6C3F_1 mice also had increased incidences of adenoma in the small intestine and neoplastic nodules in the liver. In rats, the primary tumor sites were the liver and kidney. In Crl:CD rats, exposure to captafol was associated with renal-cell carcinoma and renal-cell adenoma and carcinoma combined in both sexes, renal-cell adenoma in males, liver neoplastic nodules and neoplastic nodules and hepatocellular carcinoma combined in females, and mammary-gland fibroadenoma in females. In F344 rats, exposure to captafol was associated with neoplastic nodules of the liver and renal cell adenoma (both sexes), hepatocellular carcinoma (females), and renal-cell carcinoma and renal-cell adenoma and carcinoma combined (males). Captafol also showed significant activity as both an initiator and a promoter of preneoplastic glutathione *S*-transferase placental form positive foci in male rats.

Absorption, Distribution, Metabolism, and Excretion

Captafol is absorbed through the gastrointestinal tract and lungs and, to a lesser extent, through the skin. Following oral administration to animals, captafol appears to be extensively hydrolyzed at the N-S and C-S bonds in the gastrointestinal tract to form tetrahydrophthalimide (THPI, the major metabolite), chloride ion, dichloroacetic acid, and inorganic sulfur. In the presence of sulfhydryl compounds, such as glutathione and cysteine, captafol is rapidly degraded to THPI and chloride ion; this is a much faster reaction than the hydrolytic reaction. Captafol and its metabolites do not accumulate in animal tissues and are excreted rapidly, primarily in the urine.

Mechanistic and Genotoxicity Data

Captafol was shown to be both an initiator and a promoter of carcinogenesis in animal studies, and it induced *in vitro* transformation of BALB/c 3T3 cells. Potential mechanisms of carcinogenicity for captafol include both genotoxic action and epigenetic or indirect mechanisms.

Captafol is an alkylating agent and has produced genotoxic effects in a variety of systems. Captafol caused mutations in *Salmonella typhimurium* strains that detect base-pair change, in *Escherichia coli,* and in non-mammalian *in vivo* systems (the fungus

Aspergillus nidulans and the fruit fly *Drosophila melanogaster*). In *in vitro* studies with cell lines from rodents and other mammals, captafol caused single-strand breaks, sister chromatid exchange, chromosomal aberrations, micronulei, polyploidy (one positive and one negative study), spindle disturbances, cell transformation, and inhibited DNA synthesis. Other reported effects include DNA damage in *S. typhimurium, E. coli*, and *Bacillus subtilis*, and mitotic crossing-over in *A. nidulans.* In human cells *in vitro,* it caused single-strand breaks, sister chromatid exchange, micronuclei, chromosomal aberrations, and inhibited DNA/RNA synthesis, but did not inhibit UV-induced UDS. In mammalian *in vivo* studies, captafol administered to rats caused DNA strand breaks, micronulei (when administered by gavage), and dominant lethal mutations (when administered by intraperitoneal injection or orally) but did not cause mutations in the host-mediated assay. Captafol (administered by intraperitoneal injection) did not cause dominant lethal mutations in albino mice.

In addition to direct genotoxic activity, captafol may also operate through indirect mechanisms, such as cytotoxicity as a result of reduced cellular content of thiol groups (nonprotein and protein), inhibition of enzymes involved in DNA replication (DNA topoisomerases and polymerases), inhibition of DNA and RNA synthesis, and induction of cytochrome P-450 monooxygenases.

Structural analogues of captafol (captan and folpet) also have been shown to cause cancer in experimental animals. Captafol and captan share a common tetrahydrophthalimide ring structure (but have different side chains), and both can give rise to the metabolite THPI. Captan and folpet share identical side chains. The types of tumors produced by the three compounds were generally similar. In mice, all three compounds produced tumors of the gastrointestinal tract, and folpet and captafol produced tumors of the lymphatic system. In rats, captan and captafol produced renal tumors, and there was some evidence that folpet and captafol produced mammary-gland tumors.

Abbreviations

ALT:	alanine aminotransferase
AST:	aspartate aminotransferase
ATPase:	adenosine triphosphatase
BBN:	*N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine
b.w.:	body weight
CAS:	Chemical Abstracts Service
CASRN:	Chemical Abstracts Service Registry Number
CI:	confidence interval
2,4-D:	2,4-dichlorophenoxyacetic acid
DDT:	1,1'-(2,2,2-trichloroethylidene)bis(4-chlorobenzene)
DEN:	diethylnitrosamine
DGA:	D-galactosamine
DHPN:	2,2'-dihydroxy-di-*n*-propylnitrosamine
DMH:	1,2-dimethylhydrazine
DMBA:	7,12-dimethylbenzanthracene
DMBDD:	DEN + MNU + BBN + DMH + DHPN
DNA:	deoxyribonucleic acid
EPA:	Environmental Protection Agency
FDA:	Food and Drug Administration
γ-GT$^+$:	gamma-glutamyl transpeptidase positive
GST-P$^+$:	glutathione *S*-transferase placental form positive
GSTP1-1:	glutathione *S*-transferase pi 1-1
Ha:	hectare
HIV:	human immunodeficiency virus

IARC:	International Agency for Research on Cancer
ICD:	International Classification of Diseases
ICR:	Institute of Cancer Research
i.p.:	intraperitoneal
kkg:	kilokilogram (Mg, or metric ton)
LD_{50}:	lethal dose for 50% of the population
MNU:	*N*-methyl-*N*-nitrosourea
NADH:	nicotinamide adenine dinucleotide
NIEHS:	National Institute of Environmental Health Sciences
NTP:	National Toxicology Program
OR:	odds ratio
r:	correlation coefficient
RNA:	ribonucleic acid
RoC:	Report on Carcinogens
RR:	relative risk (risk ratio or rate ratio)
SCE:	sister chromatid exchange
SD:	standard deviation
SDH:	sorbitol dehydrogenase
SE:	standard error
SHR:	spontaneously hypertensive rats
SMART:	somatic mutation and recombination test
THPI:	tetrahydrophthalimide
TPA:	12-*o*-tetradecanoyl phorbol-13-acetate
WHO:	World Health Organization
WKY:	Wistar Kyoto (the parent strain of SHR) rats

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1 Introduction

1.1 Introduction

Captafol is a broad-spectrum fungicide that was used extensively in the past to control fungal diseases of fruits, vegetables, ornamental plants, and grasses; to control wood rot fungi on logs and wood products in the timber industry; and to control certain seed- and soil-borne organisms. However, all U.S. registrations for food and non-food uses were voluntarily cancelled effective April 30, 1987, halting production of captafol in the United States (see Section 2.1). Captafol was nominated by the National Institute of Environmental Health Sciences for possible listing in the *Report on Carcinogens* based on a 1991 evaluation by the International Agency for Research on Cancer (IARC), which classified captafol as probably carcinogenic to humans (Group 2A) based on sufficient evidence in animals and also because it was genotoxic in a wide range of tests. Captafol was carcinogenic in both rats and mice, inducing tumors at many sites. It was genotoxic in bacterial, mammalian, and human experimental systems, and *in vivo* it induced dominant lethal mutations in rats (IARC 1991).

1.2 Chemical identification

Captafol is a nonsystemic broad-spectrum fungicide (i.e., it is applied topically and works outside the plants to which it is applied). The structure of captafol is illustrated in Figure 1-1. It consists of a partially saturated tetrahydrophthalimide ring with a tetrachloroethylthio side chain. Chemical identification information for captafol is provided in Table 1-1.

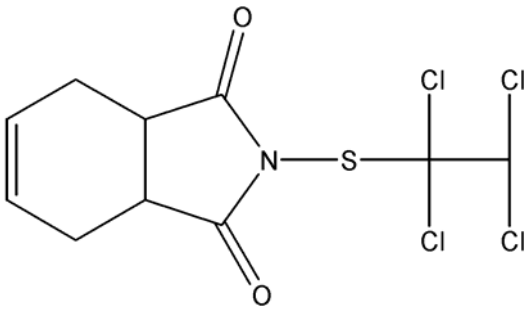


Figure 1-1. Chemical structure of captafol

Table 1-1. Chemical identification of captafol

Characteristic	Information
CAS Registry number	2425-06-1
Molecular formula	$C_{10}H_9Cl_4NO_2S$
Synonyms and trade names	3a,4,7,7a-tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1*H*-isoindole-1,3-(2*H*)-dione (CAS), difolatan (JMAF), 1,2,3,6-tetrahydro-N-(1,1,2,2-tetrachloroethylthio)phthalimide (IUPAC), N-(1,1,2,2-tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide (IUPAC), 3a,4,7,7a,tetrahydro-N-(1,1,2,2-tetrachloroethanesulfenyl)phthalimide (IUPAC), Trade formulations: Alfloc 7020, Alfloc 7046, Arborseal, Captaspor, CS 5623, Difolatan, Difosan, Folcid, Foltaf, Haipen 50, Kenofol, Merpafol, Nalco 7046, Ortho Difolatan 80W, Ortho 5865, Proxel EF, Sanspor, Santar SM, Sulfonimide, Sulpheimide, Terrazol Analytical standard: Captafol PESTANAL

Source: BCPC 2006, ChemIDplus 2006, IARC 1991, O'Neil *et al.* 2006, Saxena *et al.* 1997, SigmaAldrich 2008.

Captafol is categorized as a phthalimide fungicide; however, some classification systems also list captafol as a thiophthalimide fungicide because of the sulfur atom bound to the nitrogen (see Figure 1-2). Other phthalimide fungicides include captan and folpet (see Section 1.4). Captafol, captan, and folpet have also been described as chloroalkylthiodicarboximide fungicides (Quest *et al.* 1993) (see Section 4), and captafol has been grouped with organochlorine pesticides (Clary and Ritz 2003) (see Section 3.1).

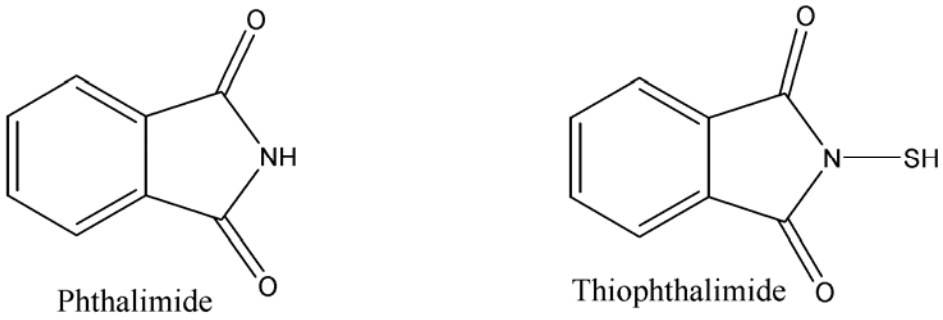


Figure 1-2. Structures of phthalimide and thiophthalimide

1.3 Physical-chemical properties

Captafol exists as white, colorless to pale yellow, or tan (technical captafol) crystals, crystalline solid, or powder with a slight characteristic pungent odor. It is practically insoluble in water but is soluble or slightly soluble in most organic solvents. Captafol reacts with bases, acids, acid vapors, and strong oxidizers (HSDB 2006). Captafol will not burn, but when heated to decomposition, it emits toxic fumes such as nitrogen oxides, sulfur oxides, phosgene, and chlorine (WHO 1993). The physical and chemical properties of captafol are summarized in Table 1-2.

Table 1-2. Physical and chemical properties of captafol

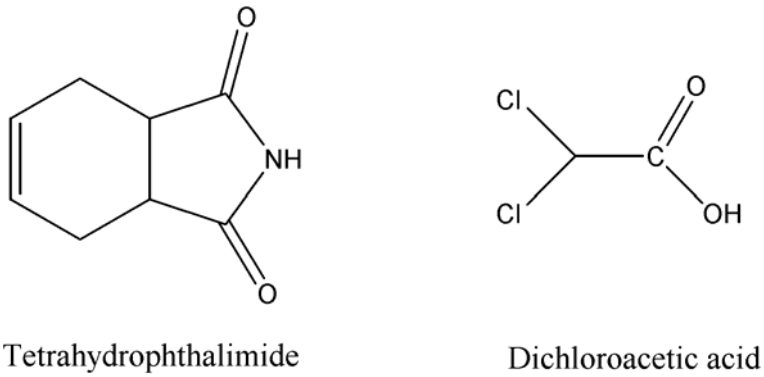
Property	Information
Molecular weight	349.1
Melting point (°C)	160–161 (slow decomposition)[a]
Boiling point (°C)	NF
Specific gravity	NF
Density	[1.64 ± 0.1 g/cm^3 at 20°C[b]] (calculated from molar volume)
Solubility water acetone benzene dimethylsulfoxide isopropanol methyl ethyl ketone toluene xylene slightly soluble in most organic solvents	 1.4 mg/L at 20°C; 2.24 mg/L at 25°C[c] 43 g/kg 25 g/kg 170 g/kg 13 g/kg 44 g/kg 17 g/kg 100 g/kg
Octanol-water partition coefficient (log K_{ow})	3.8 [3.183] at 25°C[c]
Dissociation constant (pK_a)	–2.67 ± 0.20 at 25°C (calculated)[b]
Hydrolysis	slowly hydrolyzed in aqueous emulsions or suspensions, but rapidly in acidic and basic aqueous alkaline media[a]
Vapor pressure (mm Hg)	8.27×10^{-9} at 20°C (calculated)[c]
Vapor density relative to air	12[d]
Henry's law constant	2.79×10^{-9} atm-m^3/mol

Source: HSDB 2006, unless otherwise noted.
NF = not found.
[a] Source: BCPC 2006
[b] Source: CAS 2008
[c] Source: Kim *et al.* 1997b
[d] Source: UAkron 2004.

1.4 Identification of metabolites and analogues

Although the metabolism of captafol has not been extensively studied, tetrahydrophthalimide (also known as THPI or 4-cyclohexene-1,2-dicarboximide) has been identified as the major metabolite of captafol in blood, urine, and feces (HSDB 2006). Additional information on captafol metabolism is provided in Section 5.2. Dichloroacetic acid, a liver carcinogen in experimental animals (see Section 5.6.4) also has been identified as a minor captafol metabolite. The chemical structures of THPI and dichloroacetic acid are shown in Figure 1-3. Additional metabolites of captafol found in animal tissues are listed below (EPA 1988b, HSDB 2006, WHO 1970, 1990a) (the data published by the World Health Organization [WHO] were based on their peer review of unpublished data that were otherwise unavailable for the preparation of this background document):

- 2-chloro-2-methyl-thioethylene sulfonic acid
- 3-hydroxy-delta^{4}-tetrahydrophthalimide
- 4,5-dihydroxyhexahydrophthalimide
- 4,5-epoxyhexahydrophthalimide
- 5-hydroxy-delta^{3}-tetrahydrophthalimide
- delta^{4}-tetrahydrophthalamic acid
- delta^{4}-tetrahydrophthalimide
- delta^{4}-tetrahydrophthalic acid
- dichloroacetic acid
- phthalic acid
- phthalimide
- tetrachloroethylmercaptan
- tetrahydrophthalamidic acid
- tetrahydrophthalic acid



Tetrahydrophthalimide Dichloroacetic acid

Figure 1-3. Structures of captafol metabolites tetrahydrophthalimide and dichloroacetic acid

The chloroalkylthiodicarboximide compounds constitute a group of agents with fungicidal activity. The three most prominent members of this group are (1) captan (CASRN 133-06-2) (*cis*-*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide), (2) folpet (CASRN 133-07-3) (*N*-[(trichloromethyl)thio]phthalimide), and (3) captafol. The structures of captan and folpet are shown in Figure 1-4. Captafol and captan share the same phthalimide ring structure but differ in their side chains, while captan and folpet share identical side chains.

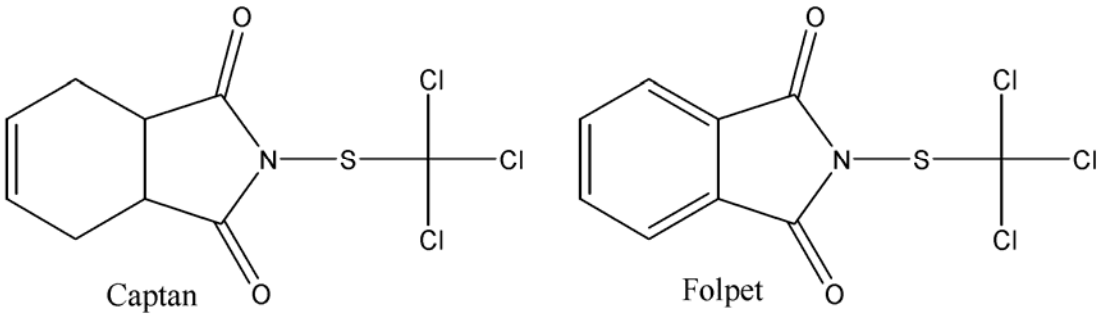


Figure 1-4. Structures of captan and folpet

Folpet also shares some structural features with the teratogen thalidomide (2-(2,6-dioxo-3-piperidyl)isoindole-1,3-dione) (Figure 1-5).

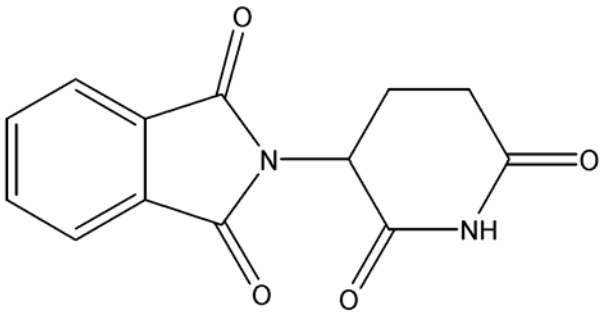


Figure 1-5. Structure of thalidomide

2 Human Exposure

Before the mid 1980s, captafol was widely used in the United States on fruits, vegetables, and other plants, as well as on timber products. Although many countries have now banned its use, it may still be used in some countries, including Mexico, and imports of fruits and vegetables from these countries could contain some captafol residues. However, the revocation of all tolerances by the EPA effectively has made it illegal to import or introduce into commerce in the United States any food with any level of captafol residue. Because of the production and use of millions of pounds of captafol in the past, the potential existed for extensive occupational exposure to this fungicide by workers producing the chemical and agricultural workers applying it to crops and from exposure to workers on reentry after spraying. In addition, environmental exposure may have occurred due to leakage into groundwater from hazardous waste sites, landfills, or contaminated soil. Exposure to captafol residues on foods also may have occurred, given that the U.S. Food and Drug Administration (FDA) reported the presence of captafol in small numbers of food samples analyzed between 1978 and 1998. This section discusses the past and current uses and production of captafol, its environmental occurrence, human exposure, and the primary regulations that control or limit exposure.

2.1 Use

Captafol is a protective nonsystemic fungicide that has been used to control fungal diseases of fruits, vegetables, ornamental plants, and grasses and as a seed treatment. It also has been used in the timber industry to control wood-rot fungi on logs and wood products (IARC 1991, WHO 1990a). Methods of application have included dusting, spraying, misting, and, for wood products, pressure treatment.

Annual use of captafol in the United States from 1979 to 1981 was approximately 500 metric tons (1.1 million pounds) for apples and cherries combined, 410 metric tons (0.9 million pounds) for citrus fruits, 240 metric tons (0.5 million pounds) for potatoes, 200 metric tons (0.4 million pounds) for tomatoes, 110 metric tons (0.2 million pounds) for sweet maize, 60 metric tons (0.1 million pounds) for plums, 10 metric tons (0.02 million pounds) for watermelons, and 110 metric tons (0.2 million pounds) for other crops, for a total of 1,640 metric tons (3.42 million pounds) (IARC 1991). Another source estimated

annual use of captafol in the United States as 2 million pounds in 1980 and 2.2 million pounds in 1982 (SRI 1984).

In January 1985, the U.S. Environmental Protection Agency (EPA) issued a notice in the *Federal Register* initiating a Special Review of captafol, based on concerns over data showing that captafol caused carcinogenic effects in laboratory animals and acute and chronic toxic effects in wildlife. Following the initiation of this Special Review, all registrants of captafol products requested voluntary cancellation of their registrations. All cancellations were effective April 30, 1987 (for food and non-food uses), thereby halting all production of captafol in the United States, although legal use of existing stocks was allowed (EPA 1988a). EPA issued a Final Rule on July 21, 1999, that revoked all tolerances for captafol except those for onions, potatoes, and tomatoes. (Tolerances are maximum limits of the amount of pesticide residue allowed to remain in or on each treated domestically produced or imported food commodity. The tolerance is the residue level that triggers enforcement actions.) The FDA tests food produced in the United States and food imported from other countries for compliance with these residue limits. The tolerances for captafol, which were in effect until 2006, were 0.1 ppm for onions, 0.5 ppm for potatoes, and 15 ppm for tomatoes. In 2006, EPA revoked specific tolerances and tolerance exemptions for captafol, and stakeholders withdrew their support for import tolerances (FR 2006). This action effectively has made it illegal to import or introduce into commerce any food with any level of captafol residue.

Small amounts of captafol (range = 0.04 to 80 lb per application for 27 reported applications) were reported to be applied in California throughout most of the 1990s and also in 2001 and 2003 (CDPR 2006). The highest yearly total application of captafol reported in California was 109 lb in 1991; the yearly totals reported for the other years between 1990 and 2003 ranged from 0 (in four separate years) to 6 lb. The reported uses were for landscape maintenance, pruning, and structural pest control; no uses on agricultural food products were reported. These values reflect amounts of captafol (active ingredient) applied rather than amounts of the captafol-containing products.

The Pesticide Action Network pesticides database identified seven countries where captafol is registered for use, with varying levels of restrictions (from no restrictions to severely restricted): Nigeria, Zimbabwe, India, Japan, Brazil, Mexico, and Suriname (PANNA 2006). The database also listed 25 countries in which use of captafol is currently banned, including 3 in the African region, 7 in Asia and the Pacific region, 11 in Europe and the Central Asian region, 3 in Latin America and the Caribbean region, and 1 in the Middle East region.

2.2 Production

Captafol is produced by the reaction of tetrahydrophthalimide and 1,1,2,2-tetrachloroethylsulfenyl chloride in the presence of aqueous sodium hydroxide (IARC 1991). It was first registered and produced commercially in the United States in 1961 by Chevron Chemical Company as Code Number Ortho-5865 under the trade name Difolatan (WHO 1993). The technical-grade product was required to contain at least 97% captafol as the sole active ingredient. It was formulated as dusts, emulsifiable concentrates, flowable suspensions, wettable powders, and water-dispersible granules (IARC 1991).

From 1979 to 1981, U.S. production of captafol was estimated to be 3,600 to 4,500 metric tons (8 to 10 million pounds) (active ingredient) per year, of which approximately half was exported (IARC 1991). As of 1983, captafol was reported to be produced by one company in the United States, with a production capacity of 12 million pounds per year (SRI 1984). The amount produced in 1985 was estimated at 6,600 metric tons (14.5 million pounds) (IARC 1991).

As discussed in Section 2.1, all captafol registrations were voluntarily cancelled in 1987, halting all production of captafol in the United States as of 1988 (SRI 1989). However, captafol still is produced internationally; Farm Chemicals Handbook (2002) listed 11 overseas suppliers of the fungicide. Additionally, Chem Sources (2006a) reported that in 2006, there were three suppliers of difolatan (captafol synonym) in the United States, one in France, two in India, and one in South Africa. Chem Sources (2006b) also reported that in 2006 there were four suppliers of captafol PESTANAL (a registered trademark for an analytical standard) in the United States and one in Germany. [Chem Sources lists all

chemical firms that have registered that they can supply the chemical for all needs, including small amounts for research purposes.] Currently, only one plant in India was identified as producing captafol internationally (SRI 2006).

2.3 Occurrence and exposure

Limited information is available on environmental occurrence of captafol or on exposure to this compound. Hydrolysis appears to be the major pathway for degradation of captafol in water, with half-lives ranging from approximately 1 to 80 hours, depending on the pH of the water. Captafol's overall half-life in soil has been reported at levels ranging from less than 3 days to around 11 days, and in a laboratory experiment, half-lives based on biodegradation alone ranged from 23 to 55 days. Captafol's half-life when sprayed on crops has been reported to be less than five days, although it may persist for a longer period of time under commercial storage conditions. It is extensively hydrolyzed during thermal processing.

Based on the most recent data available, the FDA and the U.S. Department of Agriculture (USDA) continue to monitor for captafol residues in domestic and imported food; captafol was detected at low levels in food samples in the United States in the 1980s and 1990s, but has not been detected by the FDA or USDA in food samples since 1998. THPI, a metabolite of both captafol and captan, also is monitored for by both agencies and has been detected by the USDA as recently as 2006 (the most recent data available).

Captafol has been found as an impurity in the fungicide Ridomil 25 WP, a commercial formulation of metalaxyl; thus, exposures to captafol could occur as a result of using Ridomil 25 WP (Ziogas and Georgopoulos 1987).

2.3.1 Environmental occurrence, fate, and exposure

Air

Based on its vapor pressure, captafol has been predicted to exist solely in the particulate phase in the atmosphere, with wet and dry deposition being the major removal processes (HSDB 2006); however, some reports suggest that captafol might be present in air or might act through the vapor phase. Captafol was detected in air spray-drift during high-pressure spray boom and aerial field-applications in Ontario, Canada (Frank *et al.* 1994).

Under experimental conditions, captafol was reported to act through the vapor phase to cause inhibition of growth of *Drechslera nodulosa* (Reddy 1988) and *Aspergillus nidulans* (Ziogas and Georgopoulus 1987), and the latter study also reported that crossing-over was induced in *A. nidulans*. Captafol was physically separated from the fungal culture by an air space in both the Reddy study (sterilized soil wetted with difolatan [captafol] in the bottom of a 10-cm bottle) and the Ziogas and Georgopoulos study (a captafol-impregnated filter paper disk in the lid of an inverted Petri dish).

Water

In water, captafol is expected to adsorb to sediment and suspended solids. Based on its Henry's Law constant, little volatilization from water surfaces is expected to occur (HSDB 2006). Hydrolysis appears to be the major pathway for degradation in water, with half-lives for hydrolysis of 77.8, 6.54, and 0.72 hours reported at pH 3, 7, and 8, respectively. A bioconcentration factor of 170 was calculated for captafol, suggesting a high potential for bioaccumulation in aquatic organisms. However, no data were found on detection of captafol in fish or exposure of humans to captafol through consumption of aquatic organisms. No captafol was detected in 34 wells in groundwater analyses performed in two California counties from 1994 to 1995. In addition, a study of 11 wells and 2 rivers in France (Legrand *et al.* 1992) and another of 4 farm wells in Ontario, Canada (Frank *et al.* 1990) reported that captafol was tested for but was below the detection limit (50 ng/L and 500 ng/L, respectively) in all samples. In a study of the Valencia, Spain region that monitored pesticide levels in various surface waters (surface river, irrigation channel, and lake water that originated from various points of the Valencia Community), captafol was found in one of forty samples at a concentration of 0.008 μg/mL (specific type of surface water sampled not reported) (Picó *et al.* 1994).

Other authors have reported detection of captafol in surface waters in Italy (Readman *et al.* 1997) and Spain (Vioque-Fernandez *et al.* 2007). In a study of pesticide runoff from the soil surface, Kim *et al.* (1996) reported that runoff losses of captafol with natural rainfall totaled less than 0.1% of the amount applied. The maximum concentration of captafol in the runoff was 180 ppb, which was observed when the rainfall occurred within

24 hours after the application of captafol; concentrations for other sampling periods were < 20 ppb.

Soil

Based on its soil organic carbon-water partition coefficient (K_{oc}) values, captafol is expected to have slight mobility in soil (HSDB 2006). Volatilization from soil is not expected to be an important fate process. Reported values for soil half-life vary among sources. HSDB (2006) reported that the overall half-life in soil has been shown to be around 11 days, independent of soil type or initial concentration. However, Extonet (1995) reported that captafol's half-life has been shown to be less than three days in nonsterile organic soil, five days in sandy soils, and eight days in clay-loam soils. In one laboratory experiment, based on biodegradation alone, captafol had a half-life in three different types of soil that ranged from 23 to 55 days (HSDB 2006). An Indian study showed that captafol persisted in 4 soil types for up to 60 days (Venkatramesh and Agnihothrudu 1988). In a field study, Garcia *et al*. (1990) reported that after nine years of application, there was no evidence that captafol residues were enriched in the soil.

Food

Exposure to captafol can result from ingestion of foods sprayed with captafol. When used for control of fungal disease associated with foods, captafol is applied directly to plants, fruits, or soil, or is used as a seed treatment. Application methods have included dusting, misting, and spraying (IARC 1991). Half-lives for captafol sprayed on most crops have been reported to be less than five days; however, captafol residues on fruit have been reported to be very stable under commercial storage conditions (UN 1996). A joint report of the FAO and WHO (WHO 1970) proposed that because of the nature of captafol residues on fruit, the residues would be easily removed by washing, blanching, or peeling. Captafol would be extensively hydrolyzed during cooking or other processing. Metabolism is similar in plants and animals, with captafol being metabolized to THPI and dichloroacetic acid (Extoxnet 1995). (See Section 5.2 for further discussion of captafol metabolism in animals.)

As discussed above, captafol is no longer produced or used in the United States (see Section 2.4). It has been used in other countries, such as Mexico, that export agricultural

commodities to the United States, including tomatoes, potatoes, and onions, for which the United States had tolerances established for captafol until 2006 (FR 2006). Imports of tomatoes from Mexico to the United States averaged 762,000 metric tons (1.7 billion pounds) for the time period 2002 to 2004 (the last year for which data were available). During the same time period, imports of fresh onions from Mexico were 172,000 metric tons (0.4 billion pounds) (USDA 2005a). No data were found for potato imports.

Under the Pesticide Residue Monitoring Program, samples of both U.S.-produced and imported foods are collected and analyzed for pesticide residues by the FDA in order to enforce the EPA tolerances (see Section 2.1). Based on these analyses, captafol was detected in domestic apples in only 5 of 2,464 samples (highest level 0.13 ppm [below the EPA tolerance level of 0.25 ppm]) analyzed between 1985 and 1991 (Yess *et al.* 1993). Captafol was not found in numerous other domestic foods analyzed during this period and was not found in any imported foods, including apples. In 1996, detectable levels of captafol were found in only 3 of over 5,000 samples (FDA 1998a), and in 1998, only 1 of over 4,000 samples had detectable captafol residues (2.2 ppm, below the tolerance level for that product) (FDA 1999a). (All four detections were in berries imported from Guatemala.) Captafol residues were detected in unspecified foods in the United States in 1978 to 1982 (Yess *et al.* 1991b) and 1983 to 1986 (Yess *et al.* 1991a). Based on annual reports summarizing results of the FDA's Pesticide Residue Monitoring Program, captafol was detected in foods in 1989, 1990, 1993, 1994, 1996, and 1998 (FDA 1990, 1994, 1995, 1998b, 1999b, Yess 1991). No captafol residues were found in domestic or imported pears or tomatoes from 1992 to 1993 (Roy *et al.* 1995). No other sample-specific data were available. The FDA reported that no residues of captafol were detected in food samples analyzed in each of the years 1995, 1997, and 1999 to 2003 (the latest year for which FDA monitoring data were available) (FDA 1996, 1998c, 2000, 2002, 2003, 2004, 2005). Also, no captafol was detected in state monitoring programs for fiscal years 1988 and 1989 (Minyard Jr. and Roberts 1991). THPI has been monitored for in food samples by the FDA since 1996, and detected in the years 1996 through 1999, and in 2001 and 2003 (the last year for which data were available) (FDA 1998b, 1998c, 1999b, 2000, 2003, 2005). [THPI, however, is not specific to captafol, but also may come from captan degradation or metabolism.]

The U.S. Department of Agriculture (USDA) Pesticide Data Program, managed by the Agricultural Marketing Service, is another U.S. government food residue monitoring program. The program began in 1991, and since 1998 captafol has been monitored for but never detected in various fresh, frozen, or canned fruits or vegetables (USDA 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002b, 2003, 2004, 2005b, 2006). THPI has been monitored for since 1996, and has been detected in various foods in the years 1996 through 1998, and 2002 through 2006.

In addition to monitoring foods for human consumption, FDA also samples and analyzes domestic and imported animal feeds for pesticide residues. For the time-period 1993 to 2003, captafol was detected once in animal feed: in 1999 at a level of 0.036 ppm for a barley sample from Maryland. This was considered to have exceeded regulatory guidance because no tolerance was established for captafol on barley (FDA 1994, 1995, 1996, 1998b, 1998c, 1999a, 2000, 2002, 2003, 2004, 2005).

Data on captafol residue on various crops have been reported from field trials in the United States, South Africa, and the Netherlands. In field trials on peanuts in the United States during 1973 and 1974, captafol was applied at the recommended rate of 1.5 kg/ha and then the residue was measured after harvest and drying. Maximum levels were 0.46 mg/kg on whole mature pods, 1.3 mg/kg for hulls, and below the limit of detection (0.01 mg/kg) for shelled nuts, oil, peanut meal, and peanut butter (WHO 1976). Other field trials in the United States that were reported in the late 1960s to mid-1970s showed maximum concentrations (all in units of mg/kg) of 6.33 for cranberries, 17.5 for apples, 0.2 for apricots, 1.4 for sweet cherries, 0.2 for plums, 9.0 for sour cherries, 14.0 for peaches, 1.8 for melons, 0.4 for cucumbers, and 3.8 for tomatoes (WHO 1969, 1977). WHO (1976) reported data on South African field trials for pineapple with residues ranging from a minimum of < 0.3 mg/kg in the pulp to a maximum of 55.6 mg/kg in the rind. The maximum level was seen seven days after application with concentrations dropping thereafter. Field trials also were performed for potatoes and tomatoes in South Africa with all potato levels reported at < 0.5 mg/kg and tomato levels ranging from 2.4 to 4.7 mg/kg. Field trials were performed on wheat (both grain and straw) during 1974

and 1975 in the Netherlands with maximum concentrations in straw of 4.8 mg/kg and in grain of 0.14 mg/kg (WHO 1976).

General population exposure

In the past, the general public was potentially exposed to captafol through application in nearby agricultural settings or through ingestion of foods that had been treated with captafol. The ingestion of imported foods treated with captafol remains as a potential source of exposure to the general population.

The general population could also be exposed from drinking groundwater that has been contaminated from landfills containing captafol wastes, or from topsoils that have been sprayed with captafol.

The use of captafol in three California counties (Fresno, Kern, and Tulare) was determined by Clary and Ritz (2003) from the California Department of Pesticide Regulation pesticide-use reporting database, and application of a total of 238.93 tons of the fungicide between 1972 and 1989 was documented for 35 of the 102 ZIP Codes in the three counties. Although Clary and Ritz did not estimate the total number of people exposed, they reported the population of these three counties to be almost 1.9 million in 2001, suggesting that there was potential exposure in these three counties. According to U.S. Census estimates, the population of these three counties was approximately 966,000 in 1972 and 1,483,000 in 1989.

The Total Diet Study (TDS) is an element of the FDA's Pesticide Residue Monitoring Program (Section 2.3.1.4) that determines levels of various contaminants and nutrients in table-ready foods. Captafol was included in the list of organic pesticide residues monitored in the TDS (Pennington and Gunderson 1987); however, no reports of captafol above the detection limit were identified in published data on TDS foods (FDA 1988, 1989, 1993, Gunderson 1995a, Yess *et al.* 1993).

The National Research Council (NRC) estimated food ingestion risks for a number of pesticides, including captafol, based on exposure data using EPA Theoretical Maximum Residue Contribution (TMRC) (NRC 1987). The TMRC for captafol was 23.8 μg/kg per

day. The TMRC estimate is a theoretical maximum exposure that assumes that all crops with an EPA residue tolerance actually have the tolerance level of pesticide residue upon consumption. In a study examining risk assessment disparities between methodologies that utilize either TDS or TMRC exposure estimates, Gold *et al.* (2001) noted that the TMRC method generally gives much higher exposure estimates than the TDS method.

THPI levels in plasma have been used to estimate exposure of mothers and their newborn children to captafol and captan (Whyatt *et al.* 2003). (THPI is a metabolite common to both fungicides; see Sections 5.2 and 5.6.1.) In 180 paired maternal and cord blood samples collected from urban minority mothers and newborns at the Columbia (NY) Center for Children's Environmental Health from 1998 to 2001 (more than 10 years after captafol was last produced in the United States), THPI concentrations were 2.1 ± 3.8 pg/g (mean ± SD) in maternal blood and 1.9 ± 3.8 pg/g in cord blood. This study provided no specific information on the source of exposure to captan or captafol. [THPI in both plasma and urine reflects exposure from all routes of exposures.]

The toxicity potential in the nested multi-media fate, exposure and effects model USES-LCA has been estimated for captafol using 6 environmental impacts after initial emission to the 5 compartments air, freshwater, seawater, industrial soil, and agricultural soil (Huijbregts *et al.* 2000).

Occupational exposure

Exposure to captafol may have occurred through occupational exposure at workplaces where captafol was produced or used, by agricultural workers involved in formulating or applying the fungicide, or after reentry of a sprayed field (HSDB 2006, WHO 1993). Peoples *et al*. (1978) presented brief case reports of exposures to captafol that were reported to the California Department of Food and Agriculture for the years 1974 through 1976. The reports reflected toxic outcomes of possible captafol exposure that were reported by physicians. Of the 37 cases reported, 7 were systemic illnesses, 22 were skin illnesses, 3 were related to the eye, and 5 were categorized as eye and skin illness. The cases were also presented by job category. Of the specific job categories presented, flaggers had the most reported cases with six. Other job categories and their associated number of reported illnesses include ground applicator (5), field worker (4),

cleaner/repairer (3), mixer/loader (3), aerial applicator (2), irrigator (2), manufacturing (2), tractor driver (2), truck loader (1), exposed to drift (1), and other (6). In a study by Woodruff *et al*. (1994), daily absorbed doses for mixers, loaders, and applicators of captafol were compared with acute human LD_{50} values, and lifetime absorbed daily doses were compared with reference doses and carcinogenic thresholds developed by EPA.

The mechanisms underlying the various sources of exposure due to application of Difolatan 80 Sprills (80% captafol) in central Florida orange groves were assessed by Popendorf (1988). Aerosolized captafol concentrations averaged 56 $\mu g/m^3$ for mixer-loaders and 34 $\mu g/m^3$ for spray applicators. Dermal exposure levels were approximately 1 to 10 $\mu g/h$ per cm^2 for the hands, legs, and arms; however, the authors noted that levels up to 20 $\mu g/h$ per cm^2 were seen when direct contact with captafol solution was evident. Whole-body exposures had a mean of 40 mg/h and ranged from 15 to 116 mg/h, with the hands accounting for approximately 40% of total exposure. Skin protection by coveralls reduced dermal exposure by approximately one to two orders of magnitude compared with unprotected skin.

Positive patch tests for captafol have been reported in studies of workers who packed captafol (Camarasa 1975), agricultural workers and former agricultural workers (Guo *et al.* 1996, Lisi *et al.* 1986, 1987, Rademaker 1998), floral shop workers (Thiboutot *et al.* 1990), and laboratory chemists (Brown 1984); and Stoke (1979) report a history suggestive of occupationally induced dermatitis in 30 of 133 (23%) of workers exposed to captafol in timber treatment plants in New Zealand (see Section 5.3). Also, Royce *et al*. (1993) described a case report of an asthma patient who twelve years before onset of his asthma had started working a captafol bag room where there was visible dust in the air; three personal air samples in the bag room taken in 1986 exceeded the TLV of 0.1 mg/m^3 (actual levels were not presented). See Section 5.3 for additional discussion of captafol-induced dermatitis, asthma, and other toxic effects.

Valcke *et al.* (2005) reported that a total of 200 metric tons of captafol were used in Costa Rica between 1977 and 2000. Monge *et al*. (2005) estimated from these use data an

application rate of 0.6 to 6.0 liters/hectare, which served as a surrogate for exposure intensity for agricultural.

No additional information was found on the number of plants producing captafol or on the number of employees potentially exposed through the production process. It is reasonable, however, to assume that the potential for exposure to captafol existed through occupational activities, including production and use.

2.4 Regulations and guidelines

2.4.1 Regulations

U.S. EPA

Clean Water Act

Effluent Limitations:

Daily discharge maximum = 4.24×10^{-6} kg/kkg (kg/metric ton)

Monthly average discharge maximum = 1.31×10^{-6} kg/kkg

Federal Food, Drug, and Cosmetic Act

Tolerance levels have been revoked for all foods, thereby making it illegal to import or introduce into commerce any foods with captafol residue

2.4.2 Guidelines

American Conference of Governmental and Industrial Hygienists

Threshold limit value–time-weighted average (TLV-TWA) limit = 0.1 mg/m^3 (skin; not classifiable as a human carcinogen)

National Institute for Occupational Safety and Health

Listed as a potential occupational carcinogen

Recommended exposure limit (REL) = 0.1 mg/m^3 (skin)

2.5 Summary

Captafol was produced and used in the United States as a fungicide until 1987, when all registrants of captafol products requested voluntary cancellation of their registrations; however, legal use of existing stocks was allowed. EPA further restricted the use of

captafol in 1999, when all tolerances were revoked except those for onions, potatoes, and tomatoes. In 2006, these remaining tolerances were revoked, making it illegal to import or introduce into commerce any foods with captafol residue. Limited information is available on environmental exposure to captafol, but it has been detected in air, water, and soil. The FDA and USDA continue to monitor for captafol residues in domestic and imported food; captafol was detected at low levels in food samples in the United States in the 1980s and 1990s, but it has not been detected by the FDA or USDA in food samples since 1998. THPI (a metabolite of captafol and captan) is also monitored for in foods by FDA and USDA and has been detected as recently as 2006 by USDA. Occupational exposure to captafol may have occurred through exposure at workplaces where captafol was produced or used, by agricultural workers involved in formulating or applying the fungicide, or after reentry of a sprayed field.

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3 Human Cancer Studies

Captafol belongs to a subgroup of the class of phthalimide fungicides that also includes captan and folpet. Captafol is not persistent in the environment (see Section 2.3.1). Captafol has been reviewed by IARC (1991) and classified as probably carcinogenic to humans (Group 2A). No human data were available for review by IARC at the time of its evaluation (1991).

To date, captafol has been specifically examined in only one published human study, an ecological case-control study of pancreatic cancer involving mixed exposures to captafol and other organochlorine agents (Clary and Ritz 2003) (Section 3.1). Studies on captan also are reviewed (Section 3.2), as well as studies on phthalimides as a class that involve mixed exposure to captafol (Section 3.3). These studies are not reviewed in the same detail as the human study on captafol, because they provide less information for the evaluation of the carcinogenicity of captafol. Section 3.4 discusses the major issues and summarizes the findings.

3.1 Human exposure to captafol

Clary and Ritz (2003) conducted a case-control study of deaths from pancreatic cancer from 1989 to 1996 among residents of three adjacent California counties (Fresno, Kern, and Tulare) in relation to organochlorine pesticide use.

3.1.1 Study design and methodology

State pesticide use data were available for these counties dating back to 1972. The rationale for selecting pancreatic cancer as the outcome of interest was the finding of an association between exposure to organochlorine pesticides (DDT, ethylan, and chloropropylate) and pancreatic cancer (Fryzek *et al.* 1997, Garabrant *et al.* 1992). The authors selected 18 chlorinated pesticides for study, based on usage of greater than 5 tons in the three counties (102 ZIP Codes) in 1972. Total pesticide usage per ZIP Code was estimated based on tons of active ingredient applied from 1972 to 1989. These estimates were divided into quartiles of pesticide usage. Captafol was applied in 35 of the 102 ZIP Codes in the three counties. The bulk of the usage occurred between 1972 and 1982, when usage fell to less than 5 tons per year.

3.1.2 Study subjects

Eligibility was restricted to subjects who died in one of the three target counties between 1989 and 1996 and for whom race and education level were included on death certificates. Controls were randomly selected in a ratio of approximately 10:1 from non-cancer deaths occurring in the same county during the same period. A total of 950 cases of pancreatic cancer (ICD-9 code 157) and 9,435 controls were included in the final sample.

3.1.3 Statistical analysis

Logistic regression analysis was used to calculate crude and adjusted mortality odds ratios (ORs) of death from pancreatic cancer in relation to the quartiles of total tonnage for each of the 18 pesticides applied over the period 1972 to 1989. Odds ratios were adjusted for race, age, gender, education, year of death, years of residence in county, urban residence, and exposure to the 17 other pesticides. Of the study sample, 67% (697 cases plus 6,259 controls) had lived in the county of death for over 20 years. Odds ratios were compared between residents living in areas with the lowest three quartiles of pesticide use and those living in areas with the highest quartile of pesticide use.

3.1.4 Results

The main statistically significant finding was for 1,3-dichloropropene, for which the OR was 1.89 (95% CI = 1.13 to 3.15, 107 deaths for residence in the county for over 20 years). The OR for residence for any length of time in a ZIP Code with captafol use in the highest quartile and pancreatic cancer mortality, in comparison with living in a lower-use area, was not significantly elevated (OR = 0.96, 95% CI = 0.51 to 1.82, 950 deaths, adjusted for gender, age, race, year of death, years of residence in county, urban residence, and 17 other pesticides). For residence over 20 years, the adjusted OR was higher but still not significantly elevated (adjusted OR = 1.73, 95% CI = 0.70 to 4.28, 697 deaths). The first three quartiles of captafol usage were combined as the reference category. [The dose-response relationship between pancreatic cancer mortality incidence and captafol potential exposure was not evaluated.]

3.1.5 Strengths and limitations

The authors noted that given the lethality of this cancer and the comparatively short time between diagnosis and death, it is likely that mortality data reflect cancer incidence with reasonable accuracy. [A strength of this study was the large number of cases and controls.]

The authors also discussed a number of potential limitations, including the incompleteness of pesticide usage data, the lack of complete residential histories, and potential exposure misclassification for urban residents who might live in close proximity to agricultural fields. They suggested that exposure misclassification is most likely nondifferential for cases and controls and consequently would result in bias toward the null.

[In addition to the considerable limitations inherent in ecological studies as noted by the authors, the potential existed for exposure to multiple agents or mixtures of agents, several of which may be known or suspected human carcinogens. Eighteen compounds were studied, and there was no accounting for multiple statistical comparisons; it is unclear whether the method of analysis could adequately control for the effects of the other 17 pesticides in calculation of ORs for individual agents. Given the ecological study design, the direction of bias due to misclassification of exposure is not predictable, since so many exposure data are lacking, for example, correlation data between pesticides usage were not presented. Specifically, the authors did not examine correlations between captafol and each of the three compounds that showed elevated ORs (1,3-dichoropropene, dieldrin, and pentachloronitrobenzene). Correlations between one or more of these compounds could contribute to the elevated ORs for captafol.]

[Comparison of cancer mortality among residents in the highest usage quartile with that of those in the lower three usage quartiles may also underestimate the effect of pesticide exposure. The group of residents with exposure in the lower three quartiles (assigned a relative risk of pancreatic cancer of 1.0) had some, albeit lower, potential exposure to captafol. (The reported upper cut point of estimated captafol use was 4.47 tons for the third quartile and 54.99 tons for the fourth quartile. Missing information about captafol usage and failure to specify actual distributions within quartiles do not permit evaluation

of this potential effect. It is not always clear when levels are zero, or whether the information is missing.) One other variable is that captafol does not appear to persist in soils or on crops, having a half-life of only a few days in most soils, so that exposure via dust, soil, or contaminated food would likely be less than for the more persistent organochlorine compounds, such as DDT.]

The authors acknowledged that possible bias might have resulted from the exclusion of residents who died outside the three target counties (i.e., if the probability of a subject's dying outside of these counties were related to the cause of death), and that there was also a possibility of nondifferential misclassification of cancer cases. Misdiagnosis of some pancreatic cancer cases was considered possible because of a lack of histological confirmation of cases and possible inclusion of cancers not originating in the pancreas. The authors believed that the direction of distortion of odds ratios for misdiagnosis would be toward the null, while failure to adjust for smoking could have led to bias away from the null. However, they proposed that controlling for factors such as period, ethnicity, sex, education, and age, which are determinants of smoking in the U.S. population, could have indirectly controlled for differences in smoking.

3.2 Human exposure to captan

Data on captan are less informative than studies of captafol itself but are included here because captan is closely related chemically to captafol, and because this information could help in understanding some studies of phthalimides as a class that are reviewed in Section 3.3 and which include exposure to captan.

McDuffie *et al.* (2001) conducted a population-based incident, case-control study of non-Hodgkin's lymphoma among men in six Canadian provinces and occupational or nonoccupational lifetime exposure (10 or more hours a year) to a range of herbicides, pesticides, and fungicides, including the captafol analogue captan. Subjects exposed specifically to captan included 20 lymphoma patients and 24 controls. Odds ratios were adjusted for statistically significant medical variables, age, and province of residence. A significant association between captan exposure and non-Hodgkin's lymphoma was reported (OR = 2.51, 95% CI = 1.32 to 4.76). In comparison with zero exposure, an increase in risk was reported for exposure both for more than 2 days per year (OR = 2.80,

95% CI = 1.13 to 6.90) and for more than 0 but less than 2 days per year (OR = 2.69, 95% CI = 1.17 to 6.19). [The findings of multiple elevated ORs for various pesticides and the exposure of subjects to multiple pesticides suggest that the finding for captan could be non-specific.] The authors noted that the limitations of this study were the potential for recall bias and for misclassification of pesticide exposure (both of which they considered as inherent to the case-control design) and low overall response rates, [Further, the lower response rates among controls than cases could have contributed to recall bias. No dose-response relationships were identified, and the authors also did not address multiple comparisons.]

An ecological correlational study of age-, sex-, and race/ethnicity-adjusted cancer incidence rates in relation to county-level pesticide usage data in California was conducted by Mills (1998). Correlation coefficients were calculated for the pesticides captan, atrazine, 2,4-D, diazinon, docofol, and trifluraline, based on pesticide use data from 1993, and six types of cancer diagnosed between 1988 and 1992 (non-Hodgkin's lymphoma, leukemia, soft-tissue sarcoma, and prostate, brain, and testicular cancer). A significant correlation (r = 0.46, 95% CI = 0.01 to 0.76) was noted between potential exposure to captan and leukemia among Hispanic males, and a nonsignificant correlation was observed between potential exposure to captan and prostate cancer among black males (r = 0.49; CI not specified). The author noted that Hispanic males might have been the most highly exposed. [Sample sizes were not given, but differences in population size probably explained why the correlation coefficient of 0.46 was statistically significant while 0.49 was not.] Captan was also associated with a statistically significant decrease in testicular cancer (r = –0.43; "95% confidence interval did not include 0"). No other significant correlations between captan and cancer sites were observed. The authors noted several limitations of their findings, notably imprecision of exposure estimates, lack of control for multiple pesticide exposures, and the possibility that pesticide usage in 1993 did not adequately reflect usage during earlier years (particularly if a latency period of several years for most cancers is taken into account).

Engel *et al.* (2005) examined the association between breast cancer and pesticide use in a large prospective cohort study, conducted between 1993 and 1997, of the wives of

pesticide applicators (primarily farmers) in Iowa and North Carolina. Potential occupational and environmental pesticide exposures were ascertained by self-administered questionnaires regarding ever/never use and duration of use of 50 selected pesticides, including captan, by husbands and their wives. Pesticide use data from husbands were used to estimate wives' indirect exposure, and women reported on their direct exposure via either domestic use or field mixing or application of pesticides through their spouse's license. (Female licensed pesticide applicators were excluded from this study because of the small number of breast cancer cases [N = 15]). Exposures to specific pesticides, including captan, were also examined in this study. Incident breast cancer cases (ICD codes C50.0–C50.9) occurring after cohort enrollment (N = 309; 146,653 person-years at risk) were ascertained and verified via state cancer registries.

With respect to captan exposure, a significantly increased rate ratio (i.e., relative risk, RR) of breast cancer, adjusted for age, race, and state of residence, was observed among women whose husbands had ever used captan but who had never used it themselves (RR = 2.7, 95% CI = 1.7 to 4.3, 23 cases). Among wives who had ever used captan, no association was observed; however, the number of exposed cases was small (RR = 0.5, 95% CI = 0.2 to 1.2, 4 cases). The highest risk ratio (RR = 3.6, 95% CI = 2.1 to 6.1) occurred among 17 postmenopausal women whose husbands had ever used captan but who had never used it themselves. The data from husbands' exposures were insufficient for evaluation of exposure-response relationships for breast cancer and captan exposure. As noted by the authors, the principal strengths of this study are the large cohort size and use of cancer registry data to accurately ascertain cancer incidence, but the authors also noted several limitations of the study. First, there is a likelihood of nondifferential misclassification of exposure due to potential inaccuracies in self-reporting of past exposures. Second, the study did not have the power to examine dose-response relationships. Third, overall response rates were low, and were not reported for cases and controls separately. Fourth, there was a considerable amount of missing data, both on the primary exposures and covariates. [In addition, no simultaneous controlling for other pesticide exposures was reported in the published analysis, so the observed associations could be due to confounding by exposure to other pesticides.]

3.3 Human exposure to phthalimides as a class

A population-based, case-control study of exposure to pesticides, including thiophthalimides, was conducted by Miligi *et al*. (2003) for 1,145 cases of non-Hodgkin's lymphoma and 430 cases of leukemia. A total of 1,232 sex- and age-stratified controls was randomly selected from among residents of the same geographical areas. In addition to pesticide type, ORs also were computed for the various crops to which cases and controls were exposed. No attempt to evaluate the potential risk from the ingestion of specific food items was made, however. Nonsignificant increases in non-Hodgkin's lymphoma in men were observed for thiophthalimides as a group (OR = 1.2, 95% CI = 0.4 to 3.7). [It is not clear whether this population was potentially exposed to captafol.] A significant increase in leukemia also was observed among women exposed to fungicides in general but not to thiophthalimides as a group.

In a case-control study of farming men aged 30 years or older, Schroeder *et al.* (2001) found an increase that approached statistical significance in the risk of non-Hodgkin's lymphoma subtypes defined by the t(14:18) translocation in association with estimated fungicide exposure (OR = 1.8, 95% CI = 0.9 to 3.6). Of potential importance is the finding of a significant increase in the risk of t(14:18)-positive but not t(14:18)-negative non-Hodgkin's lymphoma associated with potential exposure to phthalimides, which included captafol and captan (OR = 2.9, 95% CI = 1.1 to 7.5). The ORs were adjusted for age, state, and vital status. Only a small percentage of cases (29%) was evaluated for the molecular marker, and ORs could not be estimated for phthalimides and translocation-negative non-Hodgkin's lymphoma. The authors noted that a number of potentially confounding variables were not taken into account; however, they considered these unlikely to explain the overall results.

3.4 Discussion and summary

In the ecological case-control study of captafol and pancreatic cancer by Clary and Ritz (2003), the OR was nonsignificantly increased for residence at the time of death in an area where captafol use was in the highest quartile, compared with residence in an area where captafol use was in the three lowest quartiles. This study is the only attempt to date to link residential exposure to captafol with pancreatic cancer. Although several other

studies have suggested associations between exposure to pesticides (including organochlorines) and pancreatic cancer, its etiology is poorly understood; smoking has been implicated, but few other environmental agents or lifestyle factors have been clearly associated with the disease (Weiderpass *et al.* 1998). In a related case-control study of pancreatic cancer in association with agricultural occupations that entailed exposure to fungicides as a class (Ji *et al.* 2001), a marginally significant increase in risk was observed for low fungicide exposure compared with no probable exposure (OR = 1.5, 95% CI = 1.1 to 1.9). For moderate or high estimated exposure, the OR was 1.5 (95% CI = 0.3 to 7.6). However, it is not known whether subjects were exposed to captafol or phthalimide fungicides. Several earlier studies (cited by Ji *et al*.) found associations between pancreatic cancer and occupations with potential or actual exposure to pesticides, but others did not. Pancreatic cancer has not been observed in any of the animal studies of captafol or its analogues thus far conducted.

Three case-control studies reported an increased risk of non-Hodgkin's lymphoma associated with exposure to the captafol analogue captan (one study) or to phthalimides as a class (two studies). [There are three main sources of potential bias in these studies. First, the exposure assessments are generally imprecise (e.g., due to indirect estimates of exposure, problems with recall of past exposures, and the use of proxies for some subjects), which would tend to bias findings toward the null. Second, there may be residual confounding due to other exposures or risk factors, which would tend to bias the findings away from the null. Third, other exposures might be correlated with the exposure of interest, which could bias the findings toward or away from the null, depending on the direction of the correlation. In addition, the studies had small numbers of exposed cases, leading to imprecise risk estimates. It is possible that the risk of non-Hodgkin's lymphoma could be significant. However, it is also possible that the observed increase was due to confounding by other exposures or risk factors that were not taken into account.] Risk factors for non-Hodgkin's lymphoma include hereditary factors, acquired viral infections (e.g., HIV or Epstein-Barr virus), and autoimmune factors, in addition to environmental factors. An ecological study also reported a significant association between captan exposure and leukemia among Hispanic males. [Whether exposure to captafol *per se* occurred in the populations under study could not be readily

ascertained.] No case-control study of captafol in relation to non-Hodgkin's lymphoma has been reported to date.

The study by Engel *et al.* (2005) reported that captan may be associated with a significant increase in breast cancer incidence among women whose husbands used captan in agricultural pesticide applications; [however, this study was limited by possible misclassification of exposure and potential confounding by exposure to other pesticides].

Table 3-1. Human cancer studies of exposure to captafol

Reference and location	Study design and cancer site	Study population	Exposure	Effects OR (95% CI)	Comments
Clary and Ritz 2003 California, USA	Ecological case-control study Pancreatic cancer	*Cases* = 950 cases identified between 1989 and 1996, including 88 exposed to captafol *Controls* = 9,435 (~10 controls/case) randomly selected from all non-cancer deaths between 1989 and 1996	Residential exposure to 18 chlorinated organic pesticides data (tons of active ingredient applied from 1972 to 1989) was obtained from the CA Dept. of Pesticide Regulation.	*Captafol use in 4th quartile of exposure vs. use in quartiles 1 to 3* *All subjects:* 0.96 (0.51–1.82) *> 20 years in county:* 1.73 (0.70–4.28)	[Sufficient sample size] [Potential misclassification of exposure] [Possible misdiagnosis of cancer] ORs adjusted for race, age, gender, education, year of death, years of residence, urban residence and other pesticides [Confounding by smoking]

OR = odds ratio; CI = confidence interval.

Table 3-2. Human cancer studies of exposure to captan

Reference and location	Study design and cancer site	Study population	Exposure	Effects	Comments
McDuffie *et al.* 2001 6 provinces in Canada	Population-based case-control study Non-Hodgkin's lymphoma	*Cases* = 517 men diagnosed between 1991 and 1994 (incident cases) *Controls* = 1,506 men randomly selected from Provincial Health Insurance records, telephone listings, or voters lists Captan exposure: 20 cases, 24 controls	Self-reported occupational or non-occupational exposure (10 hours or more) was obtained from questionnaires and telephone interviews.	OR (95% CI) adjusted for statistically significant medical variables and with strata for age and province of residence *Captan exposure:* 2.51 (1.32–4.76)	[Potential misclassification of exposure] ORs not significant after controlling for exposure to other pesticide agents

Reference and location	Study design and cancer site	Study population	Exposure	Effects	Comments
Mills 1998 California, USA	Ecological study Non-Hodgkin's lymphoma, leukemia, soft-tissue sarcoma, and prostate, brain, and testicular cancer	County-specific (58 counties) cancer-incidence rates (average and age-adjusted) by sex (male and female) and race/ethnicity (non-Hispanic white, Hispanic, black, and Asian/other) 1988–1992, California Cancer Registry	Residential exposure to six pesticides (pounds of active ingredient applied per county) was obtained from the CA Dept. of Pesticide Regulation.	Correlation (Pearson) *r* (95% CI) *Leukemia* *Hispanic males:* 0.46 (0.01–0.76) *Prostate cancer* *Black males:* 0.49 (CI not given) *Testicular cancer* *White males:* –0.43 ("95% confidence interval did not include 0")	Correlation design [Potential misclassification of exposure]
Engel *et al.* 2005 Iowa and North Carolina, USA	Prospective cohort study Agricultural health study Breast cancer	*Cohort* = 30,454 women without breast cancer prior to enrollment in 1993–1997 who were the wives of private pesticides applicators Average duration of follow-up = 4.8 years; total duration of follow-up = 146,653 person-years Cases were identified from population cancer registries 309 cases occurred among all wives in the cohort; 157 cases occurred among wives who did not use pesticides but husbands did *Non-cases* = 30,145 (all wives), 13,297 (wives who did not use pesticides but husbands did)	Pesticide exposure information was obtained at enrollment using self-administered questionnaires regarding ever/never use and duration and/or frequency of use of 50 pesticides, including captan. Information obtained from farmers was used as a measure of possible indirect exposure for their wives, while the information from the women themselves was used to assess direct exposure.	RR (95% CI); no. of cases/non-cases adjusted for age, race, and state of residence *Indirect exposure (women who had never used captan but husband had used it)* 2.7 (1.7–4.3); 23/1,233 *Postmenopausal women* 3.6 (2.1–6.1); 17/335 *Direct exposure (women who had used captan)* 0.5 (0.2–1.2); 4/634	[Likelihood of nondifferential misclassification of exposure] [Potential confounding from exposure to other pesticides] Risk factors for breast cancer such as body mass index, reproductive factors (e.g., parity, etc.), physical activity, lifestyle (smoking, diet, etc.) and education were examined as potential confounders but did not change risk estimates

OR = odds ratio; CI = confidence interval; RR = rate ratio.

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4 Studies of Cancer in Experimental Animals

The carcinogenicity of captafol has been reviewed by IARC (1991) and Quest *et al.* (1993). IARC reviewed one long-term study in mice (Ito *et al.* 1984), two long-term studies in rats (Nyska *et al.* 1989, Tamano *et al.* 1990), and one medium-term, two-stage (initiation-promotion) study in rats (Ito *et al.* 1988) and concluded that there was sufficient evidence in experimental animals for the carcinogenicity of captafol. IARC also reviewed the carcinogenicity of captan (IARC 1983) and dichloroacetic acid (IARC 1995), a metabolite of captafol (see Sections 1 and 5). Quest *et al.* reported the results of a consensus peer-review process for captafol, captan, and folpet conducted by the Health Effects Division of the Office of Pesticide Programs of the U.S. EPA, based on both published and unpublished studies.

This section describes the studies reviewed by IARC (1991) and Quest *et al.* (1993). In addition, several medium-term studies of captafol carcinogenicity in rats are reviewed. Two long-term studies in mice (96 to 111 weeks) are presented in Section 4.1. Section 4.2 describes three long-term studies (104 weeks), one medium-term study (32 weeks), and six initiation-promotion studies (8 to 28 weeks) in rats. Captafol was administered in the diet in all studies reviewed.

[In the studies by Ito *et al.* (1984) and Tamano *et al.* (1990) summarized below, neoplastic lesions in the liver are described as "hyperplastic nodules" or "hyperplastic (neoplastic) nodules." As noted by Maronpot *et al.* (1986), the use of these terms may result in some uncertainty about the nature of the lesion. However, Ito and Tamano and coworkers used the term "hyperplastic nodules" to describe nodular hepatocellular lesions equivalent to "hepatocellular adenoma" (the term adopted by the National Toxicology Program [NTP] in the mid 1980s to describe this type of hepatoproliferative lesion) (Shirai 2005, personal communication). This equivalence is noted below.]

4.1 Mice

Quest *et al.* (1993) reviewed a study (unpublished study submitted to EPA's Office of Pesticide Programs in 1981 and peer reviewed by EPA) in which captafol [purity not reported] was administered in the diet to groups of Institute of Cancer Research (ICR)–

derived CD-1 mice [age not reported] at a concentration of 300, 1,000, or 3,000 ppm (equivalent to 45, 150, or 450 mg/kg of body weight [b.w.] per day) for 110 to 111 weeks. The control group included 52 mice of each sex, and the exposed groups included 80 mice of each sex. Excessive toxicity was indicated by poor survival [survival and body weight data not reported] in all exposed groups except low-dose females; most of the early deaths were attributed to lymphosarcoma. [This does not impact on the overall evaluation/interpretation of the carcinogenicity results for this study. Significantly increased lymphosarcoma incidences were not seen in all dosed groups with reduced survival, so there were factors other than lymphosarcoma contributing to the reduced survival.] The study authors did not report their statistical methods, but significantly increased incidences of lymphosarcoma and hemangiosarcoma (in high-dose females) were reported. The authors also reported increased incidences of Harderian gland adenoma in mid-dose males and a significant dose-related trend in the incidence of hemangiosarcoma in male mice. Hemangiosarcomas occurred in the heart, liver, spleen, and subcutaneous tissue [site-specific tumor incidences were not reported]. [The Fisher's exact test (one-tailed) was used to check the results reported for pairwise comparisons, and the Cochran-Armitage exact test was used to evaluate dose-response trends when they were not reported by the authors. In some instances, the results from the reanalysis did not match the results reported by the study authors. These results are noted with footnotes in Table 4-1. The NTP did not have access to the individual animal data with time of observations or survival recorded; therefore, a survival-adjusted statistical analysis could not be conducted. Although not reported as significant by the study authors, incidences of lymphosarcoma in high-dose males, and Harderian gland adenoma in low-dose males were significant and there were significant dose-related trends in incidences of lymphosarcoma (both sexes) and hemangiosarcoma in females.]. Quest *et al.* (1993) reported that for all the tumor types with increased incidences in male and female mice, the incidences also exceeded the historical control ranges. [Historical control ranges were not reported.] The results are summarized in Table 4-1.

Table 4-1. Neoplastic lesions observed in CD-1 mice exposed to captafol in the diet for 110 to 111 weeks

Sex	Conc. (ppm)	No. mice	Tumor incidence (%)		
			Lymphatic: lymphosarcoma	Vascular: hemangiosarcoma[a]	Harderian gland: adenoma
Male	0	52	0/52 (0)	1/52 (2)	0/52 (0)
	300	80	3/80 (4)	0/80 (0)	8/80 (10)[*[b]]
	1,000	80	4/80 (5)	5/80 (6)	19/80 (24)**
	3,000	80	13/80 (16)[***[b]]	6/80 (8)	2/80 (3)
	trend[c]	–	[$P < 0.001$]	$P < 0.01$	[$P = 0.134$]
Female	0	52	6/52 (12)	0/52 (0)	NR
	300	80	8/80 (10)	1/80 (1)	NR
	1,000	80	10/80 (13)	3/80 (4)	NR
	3,000	80	21/80 (26)**[d]	6/80 (8)**[d]	NR
	trend[c]	–	[$P = 0.001$]	[$P = 0.007$]	–

Source: Quest *et al.* 1993.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared with controls, statistical test was not reported by study authors).
NR = not reported.
[a] Occurred in heart, liver, spleen, and subcutaneous tissue [site-specific tumor incidences were not reported].
[b] [Not reported as significant by the study authors even though the reported incidence represents a greater numerical difference than reported for hemangiosarcoma in high-dose females, which was reported to be highly significant. These values were confirmed as significant by the Fisher's exact test].
[c] Calculated by NTP using the Cochran-Armitage exact test.
[d] [P value reported as < 0.01 but is actually < 0.05 by one-sided Fisher's exact test.]

In a similar study, Ito *et al.* (1984) fed groups of 50 to 51 male and 50 to 51 female $B6C3F_1$ mice diets containing captafol (purity 94.9%; impurities not identified) at a concentration of 750, 1,500, or 3,000 ppm for 96 weeks, followed by a return to the basal diet for 8 weeks. The mice were 6 weeks old at the beginning of the study. Calculated average intakes of captafol were 120, 240, and 520 mg/kg b.w. per day for males and 140, 270, and 610 mg/kg b.w. per day for females. There was a dose-related decrease in body-weight gain in both sexes and a dose-related trend in mortality in female mice. The decrease in body weight gain exceeded 10% for all dose-groups in both sexes. Mortality increased rapidly in the high-dose groups after 78 weeks (males) or 58 weeks (females), and none of the mice in the high-dose groups survived until the end of the study. Survival at 104 weeks was 66% for males and 70% for females in the control groups, compared with 67% for low- and mid-dose males, 76.5% for low-dose females, and 45% for mid-dose females.

Tumor incidences were based on the number of mice surviving 42 weeks or longer. Significantly increased incidences were reported for heart hemangioendothelioma

[equivalent to hemangiosarcoma], adenoma and adenocarcinoma of the small intestine, liver hyperplastic nodules [considered equivalent to hepatocellular adenoma], hepatocellular carcinoma, splenic hemangioma, forestomach papilloma, and forestomach papilloma combined with squamous-cell carcinoma. [Since the authors reported using the Fisher's exact test for their pairwise comparisons, the NTP checked the *P* values reported as less than 0.05 and found some of them to be be slightly greater than 0.05 (0.056 to 0.066). Based on the recalculated *P* values, incidences of forestomach papillomas in female mice, small intestine adenoma in male mice, and hemangioma of the spleen in both sexes were not significantly increased.] Lung metastases were associated with heart hemangioendothelioma [hemangiosarcoma], a rare tumor in mice. Neoplasms of the forestomach and small intestines also are rare in $B6C3F_1$ mice. The authors suggested that the lower incidences of tumors in the liver and small intestines in the high-dose groups than in the mid-dose groups were likely due to early deaths attributable to hemangioendothelioma [hemangiosarcoma]. Other significant effects included increased heart weight (in low- and mid-dose males and females; not examined in high-dose animals), increased liver and kidney weights (in low- and mid-dose females; not examined in high-dose animals), hemangioendothelial hyperplasia in the heart (in mid-dose females), and forestomach hyperplasia (in low- and high-dose males). [Although, incidences of hyperplasia in the small intestine were not statistically significant, this lesion may be relevant to the neoplastic effect because it was observed in 3 high-dose males, 1 low-dose female, and 2 high-dose females and was not observed in controls.] The results for gastrointestinal tumors are summarized in Table 4-2a, and other neoplasms are summarized in Table 4-2b. [Ito *et al.* did not report *P* values for the combined incidences of forestomach papilloma and squamous-cell carcinoma, small intestine adenoma and adenocarcinoma, or liver hyperplastic nodules (hepatocellular adenoma) and hepatocellular carcinoma; however, these data were reported in the Carcinogenic Potency Database (CPDB 2008). along with a statistical evaluation of dose-response trends and are included in the tables. Pairwise comparisons for the combined gastrointestinal tumors were calculated by the NTP using Fisher's exact test and significant results are enclosed in brackets.]

Table 4-2a. Gastrointestinal tumors observed in B6C3F$_1$ mice exposed to captafol in the diet and surviving at least 42 weeks

Sex	Conc. (ppm)	Effective no. of mice	Tumor incidence (%)					
			Forestomach			Small intestine		
			Papilloma	Squamous-cell carcinoma	Combined[a]	Adenoma	Adenocarcinoma	Combined[a]
Male	0	47	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	750	51	2 (3.9)	0 (0)	2 (3.9)	3 (5.9)	7 (13.7)**	10 (19.6)[***]
	1,500	46	3 (6.5)	1 (2.2)	4 (8.7)*[b]	0 (0)	32 (69.6)***	32 (69.6)[***]
	3,000	47	2 (4.3)	2 (4.3)	4 (8.5)*[b]	4 (8.5)*[b]	22 (46.8)***	26 (55.3)[***]
	trend[a]	–	NR	NS	$P < 0.05$	NR	$P < 0.001$	$P < 0.001$
Female	0	48	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	750	50	1 (2.0)	0 (0)	1 (2.0)	3 (6.0)	3 (6.0)	6 (12)[*]
	1,500	49	1 (2.0)	0 (0)	1 (2.0)	3 (6.1)	13 (26.5)***	16 (32.7)[***]
	3,000	51	4 (7.8)*[b]	1 (2)	5 (9.8)*	5 (9.8)*	7 (13.7)**	12 (23.5)[***]
	trend[a]	–	NR	NS	$P < 0.01$	NR	$P < 0.001$	$P < 0.001$

Table 4-2b. Other neoplastic lesions observed in B6C3F$_1$ mice exposed to captafol in the diet and surviving at least 42 weeks

Sex	Conc. (ppm)	Effective no. of mice	Tumor incidence (%)				
			Heart	Spleen	Liver[d]		
			Hemangioendo-thelioma	Hemangioma	Hyperplastic nodules	Hepatocellular carcinoma	Combined[a]
Male	0	47	0 (0)	0 (0)	11 (23.4)	8 (17)	19 (40.4)
	750	51	1 (2)	0 (0)	18 (35.3)	23 (45.1)**	41 (80.4)***
	1,500	46	4 (8.7)*[b]	5 (10.9)*	15 (32.6)	15 (32.6)	30 (65.2)*
	3,000	47	20 (42.6)***	0 (0)	2 (4.3)	1 (2.1)	3 (6.4)
	trend[a]	–	$P < 0.001$	$P < 0.01$	NR	$P < 0.01$	$P < 0.01$
Female	0	48	0 (0)	0 (0)	2 (4.2)	2 (4.2)	4 (8.3)
	750	50	2 (4.0)	2 (4.0)	14 (28)**	13 (26)**	27 (54.0)***
	1,500	49	2 (4.1)	4 (8.2)*[b]	10 (20.4)*	12 (24.5)**	22 (44.9)***
	3,000	51	11 (21.6)***	0 (0)	0 (0)	0 (0)	0 (0)
	trend[a]	–	$P < 0.001$	$P < 0.05$	NR	$P < 0.01$	$P < 0.001$

Source: Ito *et al.* 1984.

* $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$ (compared with the control group by Fisher's exact test, one-sided; P values calculated by NTP are enclosed in brackets).

NR = not reported by CPDB, NTP chose to not calculate a Cochran-Armitage trend analysis because of reported dose-related mortality.

NS = not significant.

[a] Data reported in CPDB 2008; combined tumor incidence data were not reported by study authors.

[b] Reported by Ito *et al.* as $P < 0.05$ [recalculated P values ranged from 0.056 to 0.066].

4.2 Rats

The carcinogenicity of captafol in rats has been investigated in several long-term and medium-term studies. These studies indicate that captafol causes kidney and liver tumors in rats and is an effective promoter of tumors induced by the known carcinogens *N*-methyl-*N*-nitrosourea (MNU), diethylnitrosamine (DEN), 1,2-dimethylhydrazine (DMH), *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN), and 2,2'-dihydroxy-di-*n*-propylnitrosamine (DHPN). Long-term carcinogenicity studies are reviewed in Section 4.2.1, one medium-term (32-week) study is reviewed in Section 4.2.2, and initiation-promotion studies are reviewed in Section 4.2.3. [The NTP supplemented the statistical analyses reported in the following studies by conducting additional pairwise and trend analyses (Fisher's exact test and Cochran-Armitage test), or by adding data reported in the Carcinogenic Potency Database (2008) for combined tumor incidences that were not reported by the study authors.]

4.2.1 Long-term studies

Quest *et al.* (1993) reviewed a study (unpublished study submitted to EPA's Office of Pesticide Programs in 1983 and peer reviewed by EPA) in which captafol [purity not reported] was administered in the diet to groups of 50 male and 50 female Crl:CD rats [age not reported] at an initial concentration of 75, 300, or 1,200 ppm for two years. Average exposure concentrations for the study were reported as 56, 241, and 1,096 ppm. Body-weight gain was reduced by 10% to 12% in the high-dose groups. Survival data were not reported; however, the authors stated that the highest dose tested was not overly toxic. Significantly increased incidences of combined renal tubular adenoma and carcinoma were observed in high-dose males, and incidences of liver neoplastic nodules, neoplastic nodules and hepatocellular carcinoma combined, and mammary-gland fibroadenoma were significantly increased in high-dose females. No liver tumor data were reported for males. [In addition, the NTP's trend analyses indicated that there were significant dose-related trends for kidney, liver, and mammary tumors.] Reported non-neoplastic lesions included renal tubular epithelial-cell hyperplasia, renal megalocytic cells, and stomach lesions (hemorrhage, ulcers, hyperkeratosis/acanthosis, and dilated gastric pits). Quest *et al.* (1993) reported that for all tumor types with increased

incidences in male and female rats, the incidences also exceeded the historical control ranges.

Nyska *et al.* (1989) fed groups of 50 male and 50 female Fischer 344 (F344) rats captafol (purity 97%; impurities not identified) at a concentration of 500, 2,000, or 5,000 ppm in their diet for up to two years. The rats were 4 weeks old when received. Mortality in the high-dose group was 78% for males and 60% for females at 96 weeks; therefore, all remaining animals in these groups were sacrificed at 98 weeks. Mortality data were not reported for other groups. No pairwise comparisons were made, but there was a significant positive dose-related trend for renal-cell carcinoma in male rats. [Pairwise comparisons conducted by the NTP indicated that renal-cell carcinoma in the high-dose males, and renal-cell adenoma and renal-cell carcinoma combined in the mid- and high-dose males were significantly increased compared with controls. The incidences for renal-cell adenoma and renal-cell carcinoma combined were reported in the Carcinogenic Potency Database (CPDB, 2008).] No renal tumors were observed in female rats. Dose-related increases in non-neoplastic renal lesions were observed in both sexes. Cortical tubular cysts were the most common renal lesion and were observed in almost all animals in the mid- and high-dose groups of both sexes. Tubular epithelial nodular hyperplasia occurred primarily in males in the mid- and high-dose groups. The authors concluded that these findings support the assumption that epithelial hyperplastic foci arise from cortical tubular cysts, and subsequently lead to neoplastic formations.

Tamano *et al.* (1990) fed groups of F344 rats (50 per sex per group) diets containing captafol (purity 97.5%; impurities not identified) at a concentration of 750 or 1,500 ppm for 104 weeks. The rats were 6 weeks old at the beginning of the experiment. The high dose was identified as the maximum tolerated dose in a 13-week oral toxicity test. Survival in the exposed groups (62% and 58% for low- and high-dose males and 62% and 68% for low- and high-dose females) was not significantly different from that in the control groups (58% for males and 76% for females). Compared with controls, high-dose males and both low- and high-dose females had consistently lower mean body weights. The incidence of renal-cell adenoma was significantly increased in all exposed groups, and the incidence of carcinoma was significantly increased in high-dose males.

Incidences of hyperplastic (neoplastic) nodules in the liver [considered to be equivalent to hepatocellular adenoma] also were significantly increased in all exposed groups, and foci of cellular alteration were increased in high-dose males and low- and high-dose females. A few hepatocellular carcinomas occurred in the male control group and in high-dose males and females, but the increased incidences in the high-dose groups were not statistically significant. [The NTP's trend analysis indicated a significant dose-related trend for hepatocellular carcinoma in female rats.] Significant non-neoplastic effects included increased heart weight (high-dose females), liver weight (low- and high-dose females), kidney weight (high-dose groups of both sexes), testes weight (low- and high-dose males), kidney lesions (karyocytomegaly, infarction, and altered tubules), liver lesions (nuclear pleomorphism, oval-cell proliferation, and foci of cellular alteration), and forestomach lesions (basal-cell and squamous-cell hyperplasia). [A high incidence of chronic progressive nephrotoxicity occurred in male rats but did not appear to be related to tumor findings because it also occurred in more than half of the controls.] The results of long-term carcinogenicity studies of captafol in rats are summarized in Table 4-3.

Table 4-3. Neoplastic lesions observed in rats exposed to captafol in the diet for two years

Reference	Strain	Sex	Conc. (ppm)	Tumor incidence (%)						
				Kidney			Liver			Mammary gland
				Renal-cell adenoma	Renal-cell carcinoma	Combined	Neoplastic nodule	Hepatocellular carcinoma	Combined	Fibro-adenoma
Quest *et al.* 1993[a]	Crl:CD	M	0	1/50 (2)	0/50 (0)	1/50 (2)	NR	NR	NR	NR
			56	0/50 (0)	1/50 (2)	1/50 (2)	NR	NR	NR	NR
			241	0/50 (0)	0/50 (0)	0/50 (0)	NR	NR	NR	NR
			1,096	3/50 (6)	4/50 (8)	7/50 (14)*	NR	NR	NR	NR
			trend[b]	[$P = 0.049$]	[$P < 0.01$]	[$P < 0.001$]	–	–	–	–
		F	0	1/50 (2)	0/50 (0)	1/50 (2)	4/50 (8)	0/50 (0)	4/50 (8)	18/49 (37)
			56	0/50 (0)	0/50 (0)	0/50 (0)	2/49 (4)	0/49 (0)	2/49 (4)	26/49 (53)
			241	0/50 (0)	0/50 (0)	0/50 (0)	2/50 (4)	1/50 (2)	3/50 (6)	28/50 (56)
			1,096	0/50 (0)	3/50 (6)[c]	3/50 (6)	17/50 (34)***	2/50 (4)	17/50 (34)***	33/50 (66)**
			trend[b]	[$P = 0.25$]	[$P = 0.015$]	[$P = 0.049$]	[$P < 0.001$]	[$P = 0.06$]	[$P < 0.001$]	[$P < 0.01$]
Nyska *et al.* 1989[d]	F344	M	0	0/50 (0)	0/50 (0)	0/50 (0)[f]	NR	NR	NR	NR
			500	0/49 (0)	1/49 (2)	1/49 (2)[f]	NR	NR	NR	NR
			2,000	2/49 (4)	3/49 (6)	5/49 (10.2)[*][f]	NR	NR	NR	NR
			5,000[e]	0/49 (0)	12/49 (24)[***]	12/49 (24)[***][f]	NR	NR	NR	NR
			trend[b]	[$P = 0.5$]	$P < 0.001$	$P < 0.001$[a]	–	–	–	–
Tamano *et al.* 1990[g]	F344	M	0	0/50 (0)	0/50 (0)	NR	2/50 (4)	2/50 (4)	NR	5/50 (10)
			750	26/49 (53)***	1/49 (2)	NR	8/50 (16)*	0/50 (0)	NR	2/50 (4)
			1,500	38/50 (76)***	8/50 (16)**	NR	21/50 (42)***	1/50 (2)	NR	2/50 (4)
			trend[b]	[$P < 0.001$]	[$P < 0.001$]	–	[$P < 0.001$]	[$P = 0.37$]	–	[$P = 0.15$]
		F	0	0/50 (0)	0/50 (0)	NR	3/50 (6)	0/50 (0)	NR	10/50 (20)
			750	8/50 (16)**	0/50 (0)	NR	14/50 (28)**	0/50 (0)	NR	12/50 (24)
			1,500	6/50 (12)*	0/50 (0)	NR	34/50 (68)***	4/50 (8)	NR	5/50 (10)
			trend[b]	[$P = 0.028$]	–	–	[$P < 0.001$]	[$P = 0.01$]	–	[$P = 0.12$]

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared with the control group).
NR = not reported.
[a] Statistical test and survival data were not reported.
[b] Results in brackets wre calculated by the NTP using the Cochran-Armitage test.
[c] Incorrectly reported as significant in Quest *et al.* 1993 (personal communication from Dr. Kerry Dearfield, EPA, March 24, 2005).
[d] No tumors in female rats, significant positive dose-related trend for renal-cell carcinoma (Peto test); no pairwise comparisons reported, Fisher's exact test conducted by NTP.
[e] Due to high mortality, animals in the high dose groups were sacrificed at 98 weeks.
[f] Data reported in CPDB 2008.
[g] Pairwise comparisons based on one-sided Fisher's exact probability test.

4.2.2 Thirty-two week studies

Captafol (purity not specified) fed to groups of 16 male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY, the parent strain of SHR) at 1,500 ppm for 32 weeks did not increase the incidence of hemangiosarcoma or neoplastic nodules in the liver, and no histopathological lesions were observed in the other organs (i.e., heart, spleen, kidney, lung, and mesentery) examined (Futakuchi *et al.* 1996). The rats were 5 weeks old when received. One hemangiosarcoma occurred in WKY rats exposed to captafol. The authors attributed the low incidence of tumors to the short experimental period.

4.2.3 Initiation-promotion studies

Several studies investigated the promoting effects of captafol in medium-term, two-stage assays using various initiation protocols, and one study investigated captafol as an initiator. Both the number and size of pre-neoplastic glutathione *S*-transferase placental form positive ($GST\text{-}P^+$) foci were significantly increased in the livers of male F344 rats when captafol was used as a promoter (Ito *et al.* 1996, Ito *et al.* 1988, Kim *et al.* 1997, Uwagawa *et al.* 1991) or as an initiator (Tsuda *et al.* 1993). The study protocols and results are summarized in Table 4-4. The rats were 6 or 7 weeks old at the beginning of the studies. Control groups in the promotion studies were administered the initiators, followed by the basal diet. The control group in the initiation study received only the promotion protocol. In addition to the $GST\text{-}P^+$ foci, promotion with captafol significantly increased the incidences of forestomach hyperplasia and small intestinal adenoma (Uwagawa *et al.* 1991), thyroid follicular adenoma (Ito *et al.* 1996), and expression of the proliferating cell nuclear antigen in the kidney (Kim *et al.* 1997).

Table 4-4. Occurrence of GST-P$^+$ foci in male F344 rats in initiation-promotion studies of captafol

Reference	N	Exposure	Study duration (wk)	Study protocol	GST-P$^+$ foci[a]	
					No. foci/cm^2	Area (mm^2/cm^2)
Ito *et al.* 1988	18 19	DEN DEN + captafol	8	initiated with DEN by intraperitoneal injection (i.p.) at 200 mg/kg b.w., partial hepatectomy at week 3, then captafol in diet at 3,000 ppm weeks 3–8	11.60 ± 3.19 19.75 ± 4.87***	1.23 ± 0.59 1.66 ± 0.48**
Uwagawa *et al.* 1991	23 25	MNU MNU + captafol	20	initiated with MNU (i.p.) at 20 mg/kg b.w. twice weekly for 4 weeks, then captafol in diet at 1,500 ppm weeks 5–20	0.115 ± 0.284 0.357 ± 0.416*	0.001 ± 0.004 0.004 ± 0.005*
Tsuda *et al.* 1993	14 9	corn oil + promotion captafol + promotion	10	partial hepatectomy; after 12 hours, captafol by gavage at 300 mg/kg b.w.; after 2 weeks, phenobarbitol in the diet (0.05%) for 8 weeks and DGA by gavage at 300 mg/kg b.w. at week 3	0.13 ± 0.13 0.75 ± 0.57*[b]	0.002 ± 0.002 0.006 ± 0.005*
Ito *et al.* 1996	20 19	DMBDD DMBDD + captafol	28	initiated with DEN (i.p.) at 100 mg/kg; MNU (i.p.) at 20 mg/kg b.w. on days 2, 5, 8, and 11; DMH by subcutaneous injection at 40 mg/kg b.w. on days 14, 17, 20, and 23; BBN in drinking water at 500 mg/L weeks 1 and 2; and DHPN in drinking water at 1,000 mg/L weeks 3 and 4; then captafol in diet at 1,500 ppm weeks 5–28	[3.9 ± 2.1][c] [9.0 ± 3.4**][c]	[0.2 ± 0.1][c] [0.6 ± 0.3**][c]
Kim *et al.* 1997	10 10	DEN + DGA DEN + DGA + captafol	8	initiated with DEN (i.p.) at 200 mg/kg b.w. and DGA (i.p.) at 300 mg/kg b.w. at ends of weeks 2 and 5, then captafol in diet at 1,500 ppm or captafol + L-cysteine in drinking water at 1,500 ppm weeks 3–8	3.68 ± 1.33 12.9 ± 2.37**	0.05 ± 0.02 0.29 ± 0.05**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.00$ (compared with the control group by Student's *t* test).
BBN = *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine, DEN = diethylnitrosamine, DGA = D-galactosamine, DHPN = 2,2'-dihydroxy-di-*n*-propylnitrosamine, DMH = 1,2-dimethylhydrazine, MNU = *N*-methyl-*N*-nitrosourea, DMBDD = DEN + MNU + BBN + DMH + DHPN.
[a]Data are means ± SD except that Kim *et al.* (1997) reported SE, and Tsuda *et al.* (1993) did not identify values as SD or SE.
[b][Stated to be significant in the text but not marked as significant in Table 1 in Tsuda *et al.* (1993).]
[c][Values estimated from figure; measured values were not presented.]

Tsuda *et al.* (1984) investigated the effects of captafol on the frequency of gamma-glutamyl transpeptidase-positive (γ-GT$^+$) foci in rat liver. Captafol was tested along with 30 other compounds for promoting activity in groups of 25 male F344 rats. An initial dose of DEN at 200 mg/kg b.w. was followed after two weeks with administration of captafol for six weeks. Animals had a partial hepatectomy at week 3 and were sacrificed at week 8. In rats given captafol as a promoter, there was a slight but statistically significant ($P < 0.05$) increase in the area of γ-GT$^+$ foci (0.67 ± 0.33 vs. 0.53 ± 0.20 mm^2/cm^2) but not in the number of foci (11.18 ± 3.57 vs. 9.65 ± 3.55 per cm^2), compared with initiated controls. A third group exposed to captafol but not initiated with DEN had only a few foci (0.01 ± 0.06 per cm^2), very small in area (< 0.01 mm^2/cm^2). These results were considered equivocal.

4.3 Summary

Captafol was tested for carcinogenicity in feeding studies in CD-1 mice, B6C3F$_1$ mice, Crl:CD rats, and F344 rats. Captafol induced Harderian gland adenoma in male CD-1 mice, and hemangiosarcoma and lymphosarcoma in male and female CD-1 mice; and heart hemangioendothelioma [hemangiosarcoma], splenic hemangioma, and tumors of the forestomach, small intestine, and liver in male and female B6C3F$_1$ mice. In rats, the kidney and liver were the primary organs affected. Female Crl:CD rats had significantly increased incidences of liver neoplastic nodules, neoplastic nodules and hepatocellular carcinoma combined, and mammary-gland fibroadenoma. Kidney tumors (renal-cell adenoma or carcinoma) were not significantly increased in female Crl:CD rats but there were significant dose-related trends for renal-cell carcinoma and renal-cell adenoma and carcinoma combined. Male Crl:CD rats had significantly increased incidences of renal-cell adenoma and carcinoma combined, but the trend analysis was significant for kidney tumors when analyzed separately or combined. Male F344 rats had significantly increased incidences of liver neoplastic nodules, renal-cell adenoma, renal-cell carcinoma, and renal-cell adenoma and carcinoma combined. Female F344 rats had significantly increased incidences of liver neoplastic nodules, renal cell adenoma, and a significant dose-related trend for hepatocellular carcinoma. Captafol also showed significant activity as both an initiator and a promoter of preneoplastic GST-P$^+$ foci in

male rats. Table 4-5 summarizes the neoplastic lesions found in mice and rats exposed to captafol.

Table 4-5. Summary of neoplastic lesions in mice and rats exposed to captafol in the diet

System or organ	Tumor type	Mice				Rats			
		CD-1		$B6C3F_1$		Crl:CD		F344	
		M	F	M	F	M	F	M	F
Lymphatic	lymphosarcoma	✓	✓						
Vascular	hemangiosarcoma[a] (heart)			✓	✓				
	hemangiosarcoma[a] (heart, liver, spleen, subcutaneous tissue)	**T**	✓						
	hemangioma (spleen)			✓	**T**[b]				
Gastrointestinal	papilloma (forestomach)			×	×[b]				
	squamous-cell carcinoma (forestomach)			×	×[c]				
	papilloma and squamous-cell carcinoma combined (forestomach)			**T**[b]	✓				
	adenoma (small intestine)			×[b]	✓[d]				
	adenocarcinoma (small intestine)			✓	✓				
	adenoma and adenocarcinoma combined (small intestine)			✓	✓				
Liver	neoplastic nodules[e]				✓[e]		✓	✓	✓
	hepatocellular carcinoma			✓	✓		×		**T**
	neoplastic nodules and hepatocellular carcinoma combined			✓	✓		✓	**NR**	**NR**
Kidney	renal-cell adenoma					**T**		✓	✓
	renal-cell carcinoma					**T**	**T**	✓	
	renal-cell adenoma and carcinoma combined					✓	**T**	✓	**NR**
Other	fibroadenoma (mammary gland)						✓		
	adenoma (Harderian gland)	✓[f]							

✓ = significantly increased compared with controls and a significant positive dose-response trend, $P < 0.0$ ✓ .
× = higher incidence than observed in controls, but not statistically significant.
T = significant positive dose-response trend, $P < 0.05$.
NR = combined incidences were not reported.
[a] Called hemangioendothelioma by Ito *et al.* 1984.
[b] Reported as significant by study authors, but *P* values calculated by NTP ranged from 0.056 to 0.066.
[c] One squamous-cell carcinoma was found in 51 high-dose females vs. 0 of 48 in control females.
[d] Trend analysis was not reported.
[e] Or hyperplastic nodules; considered equivalent to hepatocellular adenoma.
[f] Trend analysis was not significant.

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5 Other Relevant Data

Limited information was available on the absorption, distribution, metabolism, and excretion of captafol in experimental animals, and no specific data in humans were identified. Most of the available data were jointly published by the Food and Agriculture Organization of the United Nations and WHO, based on their peer review of several unpublished reports: this section provides a summary of this information. In addition, this section summarizes information on captafol toxicity, genetic and related effects, potential mechanisms of carcinogenicity, and the metabolism, mutagenicity, and carcinogenicity of captafol analogues and metabolites.

5.1 Absorption, distribution, and excretion

Captafol is absorbed through the gastrointestinal tract and lungs and, to a very limited extent, through the skin (WHO 1970, 1977). The available data indicate that captafol and its metabolites do not accumulate in the tissues of animals but are metabolized and eliminated, primarily in the urine. After 36 hours, the liver, heart, kidneys, blood, muscle, and fat of rats, dogs, and monkeys were found to contain less than 0.5% of the dose of ^{14}C-carbonyl-labeled captafol, and tissues of lactating Holstein cows contained less than 0.01 mg/kg of ^{14}C-captafol equivalents, except for liver (0.01 mg/kg) and kidney (0.014 mg/kg) in one of three cows 24 hours after oral administration for 30 days. Both equilibration and elimination of captafol by the cows were reported to be rapid. Hayes (1982) reported that THPI, the major metabolite of captafol, was present in blood along with other more soluble (but unidentified) metabolites.

No absorption studies of captafol in humans were identified. One study, by Whyatt *et al.* (2003), analyzed maternal and cord plasma samples collected from mother and newborn pairs in New York City between 1998 and 2001 as part of a study of pesticide use during pregnancy in an urban minority population (see also Section 2.3.2). The authors reported that THPI (the major metabolite of both captafol and captan) was present in 99 of 199 maternal plasma samples and in 92 of 211 cord plasma samples. [Because this study took place after U.S. production of captafol had ceased and all registrations had been cancelled (see Section 2), it is likely that the THPI resulted from exposure to captan, rather than to captafol.]

Excretion of ^{14}C-carbonyl-labeled captafol was measured in urine, feces, and expired carbon dioxide in rats, dogs, and monkeys (Hayes Jr. 1982, WHO 1970). Excretion was mainly via the urine, with almost 80% of the dose excreted within 36 hours, and the rate of excretion was almost identical for all three species. Smaller amounts were found in the feces and none in expired carbon dioxide. The radioactivity in the feces consisted primarily of unchanged, most likely unabsorbed, captafol. THPI was detected in feces and urine, but other, more water-soluble (but unidentified), metabolites of captafol accounted for the majority of radioactivity in blood, feces, and urine. When lactating Holstein cows were administered 5.7 or 11.4 mg of ^{14}C-captafol orally for 30 days, the major route of excretion was in the urine (~90%) with a lesser, but significant, amount in the feces (~10%) (WHO 1977). Milk from the cows contained no detectable captafol, and the maximum concentration of ^{14}C-containing metabolites (calculated as captafol equivalents) was 0.006 mg/kg in the milk from cows given the higher dose. Two days after the last dose of captafol, no residues were detected in the milk.

5.2 Metabolism

The N-S and C-S bonds in captafol are easily broken by hydrolysis or nucleophilic attack by sulfhydryl compounds (see Figure 5-1). In animals, following oral administration, captafol appears to be extensively hydrolyzed in the gastrointestinal tract to THPI, chloride ion, dichloroacetic acid, and inorganic sulfur (WHO 1970). THPI is the major metabolite of captafol in both animals and plants (WHO 1990a) and the major degradation product in water hydrolysis and from heating (see Figure 5-1 and Table 1-2). Further metabolism of THPI results in formation of tetrahydrophthalic acid, with the chemically unstable tetrahydrophthalamic acid as an intermediate. Epoxidation of captafol is not believed to be a metabolic route, as no epoxide was detected in blood, urine, or feces (Hayes Jr. 1982).

In the presence of sulfhydryl compounds, such as glutathione and cysteine, captafol is rapidly degraded to THPI and chloride ion (Bridges 1975, WHO 1970). Because this reaction in the presence of sulfhydryl compounds is much faster than the hydrolytic reaction, it may be the dominant reaction in biological systems, where sulfhydryl groups

are present. The half-life of captafol at 25°C and pH 7 for the sulfhydryl reaction was 4 minutes, compared with a half-life of 1,000 minutes for the hydrolytic reaction.

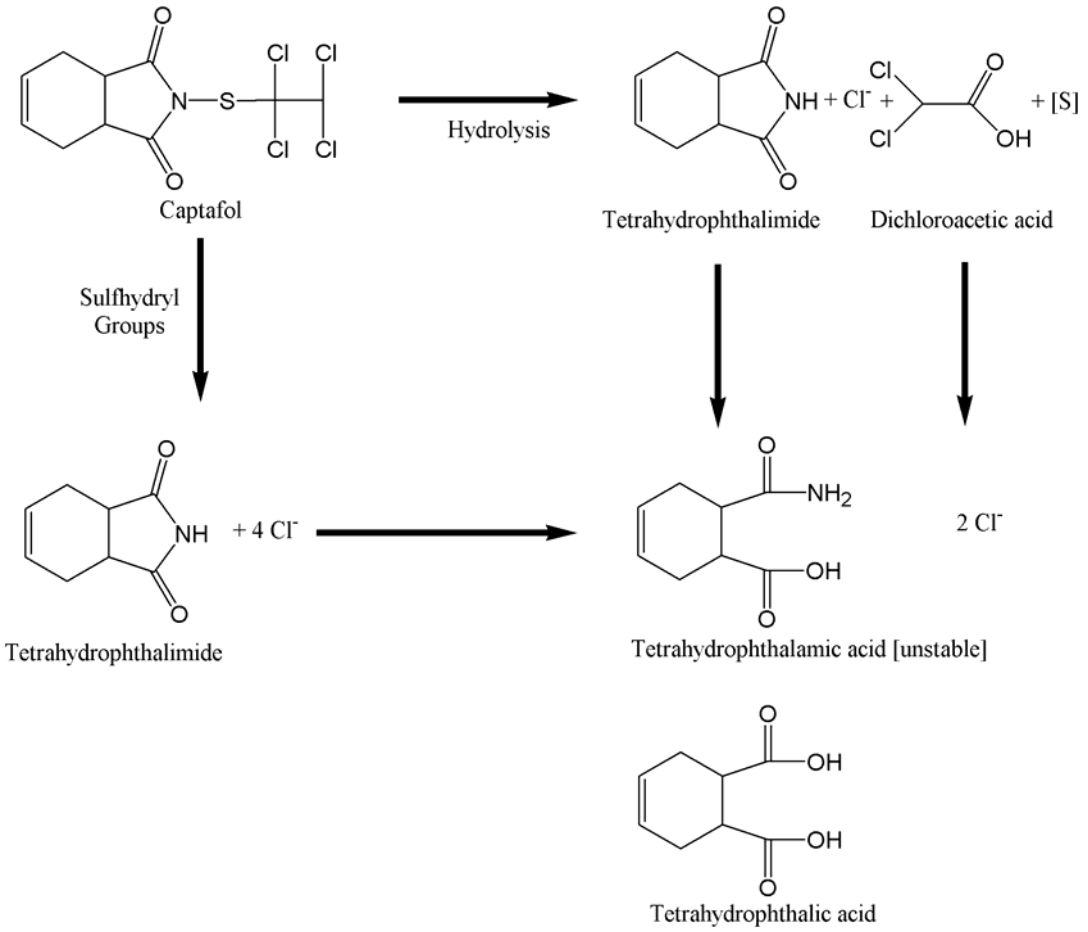

Figure 5-1. Metabolism of captafol

Source: WHO 1970.

Alternate routes of metabolism for captafol are shown resulting from breaking of the N-S bond by either a hydrolytic mechanism (horizontal arrow from captafol) or by a nucleophilic attack by sulfhydryl groups to form THPI (vertical arrow below captafol).

Another reported metabolite of captafol is tetrachloroethylmercaptan (the side chain of captafol), which is further metabolized to 2-chloro-2-methylthioethylene sulfonic acid (WHO 1990a). Metabolism of the side chain of captafol to tetrachloroethylmercaptan is proposed to form a transient intermediate, a cyclic sulfonium ion, which is a potential alkylating agent and has been proposed to be responsible for the toxic and carcinogenic actions of captafol. Bernard and Gordon (2000) studied the structure of captafol and concluded that the tetrachloroethylthio side chain of captafol is able to form an

episulfonium ion (Figure 5-2), which is considered to be a carcinogenic electrophile (Williams 1992). However, no direct evidence has been reported for the formation of this metabolite from captafol.

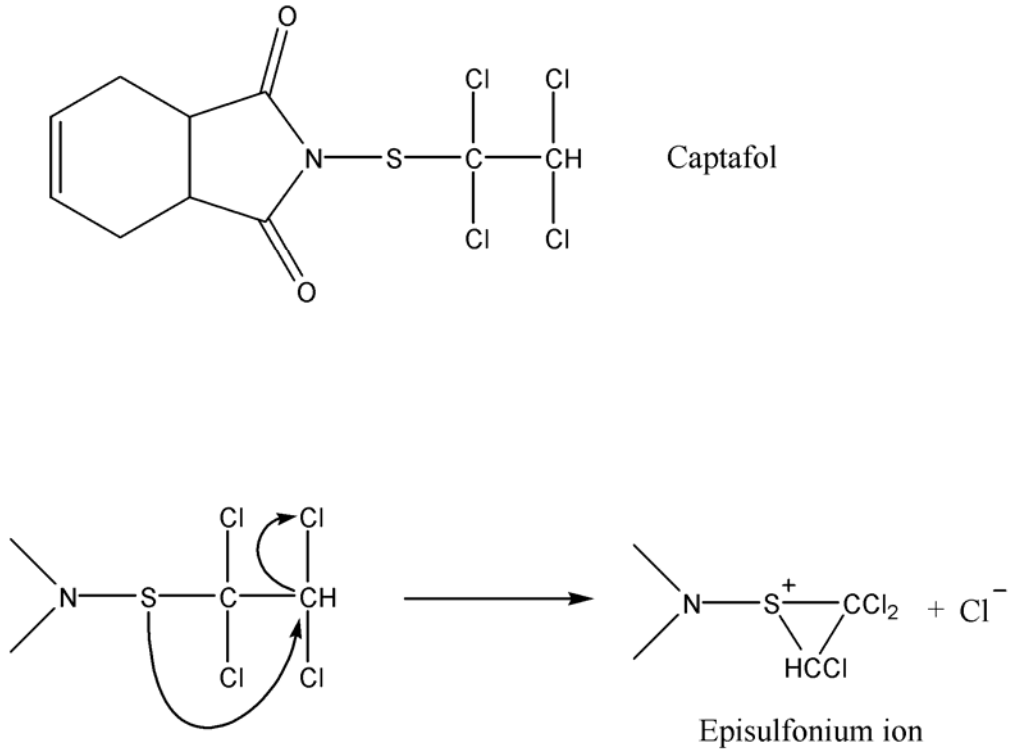

Figure 5-2. Proposed mechanism for formation of the polar episulfonium ion from captafol

Source: Bernard and Gordon 2000.

Note that the side-chain is shown as still attached to the tetrahydrophthalamide ring structure in this diagram, whereas other sources (WHO 1990a) suggest that a cyclic sulfonium ion could be formed from the tetrachloroethylmercaptan side chain after it is cleaved from captafol.

5.3 Toxicity

Although the liver is a primary target organ in animals administered captafol by injection or in the diet, the major toxic effects reported in humans exposed to captafol are dermatitis and asthma. A number of studies have reported contact dermatitis in humans following dermal exposure to captafol. Groundwater (1977) reviewed a case of skin and respiratory irritation in a welder who was employed by a maintenance company that serviced plants distributing captafol. After about 1.5 years working in various plants, and frequently contacting large bags of captafol, he suddenly developed marked vesiculation and edema of the face and hands, and wheezing. Subsequent patch testing with a 0.1%

test solution of captafol was positive, and systemic steroids were required to suppress the patch test reaction. Hayes (1982) reviewed several studies of skin irritation in Japanese farmers. One study reported high incidences (about 25% to 41%) of skin irritation among more than 1,400 farmers that used captafol in tangerine orchards. Erythematous dermatitis of the eyelids with local edema usually appeared within 1 to 3 days after exposure and persisted for about a week. Irritation was usually limited to the conjuctiva or to skin areas with direct contact to captafol and included mild to severe cases. Mark *et al.* (1999) reported positive patch test reactions to captafol in 4 of 26 patients, while Rademaker (1998) reported 2 positive patch test reactions to captafol in 46 New Zealand farmers. Lisi *et al.* (1986, 1987) conducted a series of patch tests in 200 (1986) or 652 (1987) subjects and reported that allergic reactions to the thiophthalimide fungicides, including captafol, were relatively common. In a survey of 14 timber treatment plants in New Zealand, 23% of 133 workers exposed to captafol reported a history suggestive of occupationally induced dermatitis (Stoke 1979). Thiboutot *et al.* (1990) reported that 1 of 16 floral workers had a positive patch test to captafol. Several case reports also have reported dermatitis after contact with captafol (Brown 1984, Camarasa 1975, Cushman *et al.* 1990, Guo *et al.* 1996, Matsushita *et al.* 1980). Occupational asthma was reported in a pesticides manufacturing worker after several years of exposure to captafol, but improved symptoms and pulmonary function were seen after cessation of exposure (Royce *et al.* 1993).

The hepatotoxic effects of captafol metabolism were investigated in rats (Dalvi and Mutinga 1990). Captafol was injected i.p. at 5 mg/kg b.w., and its effects were compared with those of captan and folpet (see Section 5.6), which were injected i.p. at 20 mg/kg b.w. Activities of serum sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in the blood to assess the extent of liver injury. The activities of SDH, ALT, and AST in serum samples were significantly increased in captafol-exposed groups. Captafol also caused a significant loss of cytochrome P-450 protein and NADH-cytochrome c reductase activity. Captafol, captan, and folpet caused similar hepatotoxicity, but the dose of captafol was one-fourth that of the other two fungicides. These authors demonstrated that a small amount of i.p.-administered captafol (5 mg/kg) can cause severe hepatotoxic effects. Liver injury was

characterized by inhibition of hepatic microsomal enzymes and elevation of serum enzymes that are markers of liver dysfunction. **[**The liver toxicity of captafol may be attributed, at least in part, to its interaction with and metabolism by liver microsomal enzymes.]

In addition to the tumorigenic effects in $B6C3F_1$ mice reported by Ito *et al.* (1984) (see Section 4.1), a significant increase in chronic nephropathies was reported in both sexes fed diets containing 3,000-ppm captafol for 96 weeks. [No further details were reported.]

The effects of subchronic administration of captafol also were studied in $B6C3F_1$ mice (Tamano *et al.* 1993). Captafol in the diet for 12 weeks at a concentration of 0, 0.3%, 0.625%, or 1.25% resulted in a dose-related decrease in body-weight gain and decreased body weight in both male and female mice in the high-dose group. Relative liver weights showed a tendency toward a dose-dependent increase. Light-microscopic examination revealed cytoplasmic vacuolar degeneration in the livers of mice of both sexes; the severity was dose related. The authors concluded that the liver was the primary target organ for captafol.

Other toxic effects of captafol have been demonstrated in *in vitro* systems. Exposure of human erythrocytes to captafol *in vitro* resulted in a 50% reduction in Ca^{+2}-transport-ATPase activity (IC_{50}) at a concentration of 2 μmol/L (Janik and Wolf 1992). Di Ilio *et al.* (1996) investigated the interaction of glutathione transferase P1-1 (GSTP1-1) activity (purified from human placenta) with captan and captafol. These authors reported that GSTP1-1 activity was strongly inhibited by both pesticides with IC_{50} values of 5.8 μM for captan and and 1.5 μM for captafol. This inactivation involved the formation of disulfide bonds between the four cysteinyl groups of the enzymes. Captafol also affected sulfhydryl groups in cultured cells. In V79 Chinese hamster fibroblasts, captafol reduced the content of nonprotein sulfhydryl groups (particularly those of reduced glutathione) to 41.5% and protein sulfhydryl groups to 58.5% of control levels (Rahden-Staroń *et al.* 1994). The activity of purified glutathione *S*-transferase pi 1-1 (GSTP1-1) isolated from human placenta was inhibited by captafol in a time- and concentration-dependent manner

(Di Ilio *et al.* 1996). The authors concluded that captafol inactivated GSTP1-1 through formation of disulfide bonds between the four cysteinyl groups of the enzyme.

5.4 Genetic damage and related effects

Captafol has been tested for genetic and related effects in a number of *in vitro* and *in vivo* test systems. In a review by IARC (1991), the reported genetic and related effects of captafol included DNA damage and gene mutation in bacteria; mitotic recombination and gene mutation in yeast; sister chromatid exchange, micronucleus formation, and chromosomal aberration in cultured mammalian and human cell lines; and a small but significant trend toward increased numbers of early deaths per pregnancy (dominant lethal effect) in rats. No data were available on DNA adducts. This section summarizes the studies reviewed by IARC (1991) and relevant studies published since that review.

5.4.1 Prokaryotic systems

The genetic effects of captafol have been investigated in *Salmonella typhimurium*, *Escherichia coli,* and *Bacillus subtilis*, and the results are summarized below.

Salmonella typhimurium

Captafol induced reverse mutations in some *S. typhimurium* strains (Barrueco and de la Peña 1988, Rahden-Staroń *et al.* 1994, Ruiz and Marzin 1997, Saxena *et al.* 1997, Seiler 1973) (Table 5-1). In general, positive or weakly positive results occurred in some strains used to detect point mutations at G·C base pairs (*his* G46, TA1530) or A·T base pairs (TA102, TA100) while negative results occurred with strains used to detect frameshift mutations (TA98, TA1531, TA1532, TA1534, TA1536, TA1537, and TA1538). Exceptions included TA1535 (negative for point mutations at G·C base pairs), and TA97a (positive for frameshift mutations). Studies in TA100 (A:T base-pair mutations) were conflicting.

One forward mutation study with *S. typhimurium* strain SV3 was reviewed (Ruiz-Vázquez *et al.* 1978). Captafol was mutagenic in this assay, which detects a change from arabinose sensitivity to arabinose resistance.

Table 5-1. Results of genotoxicity testing of captafol in *S. typhimurium*

End point	Test strain	Conc. (µg/plate)	Results without S9 (LEC)	Results with S9 (LEC)	Reference
Reverse mutation	*his* G46	NR	+ (NR)	NR	Seiler 1973
(G·C base pairs)	TA100	NR[a]	(+) (NR)	–	Moriya *et al.* 1983
		0.5–2.5	(+) (0.5)	NR	Saxena *et al.* 1997
		0.1–20	(+) (0.3) [b]	(+) (1.0) [b]	Ruiz and Marzin 1997
	TA1530	NR	+ (NR)	NR	Seiler 1973
	TA1535	10–100	–	NR	Kada *et al.* 1974
		50	–	NR	Shirasu *et al.* 1976
		200	–	–	Carere *et al.* 1978
		NR[a]	–	–	Moriya *et al.* 1983
		0.1–10	–	–	Ruiz and Marzin 1997
Reverse mutation	TA102	0.16–0.62	+ (0.31)	+ (0.62)	Barrueco and de la Peña 1988
(A·T base pairs)		0.5–2.5	+ (0.5)	NR	Saxena *et al.* 1997
		0.5–50	+ (1.25)[c]	+ (0.5)[c]	Ruiz and Marzin 1997
		0.25–5	+ (0.25)	–	Rahden-Staroń *et al.* 1994
	TA104	0.16–0.62	–	–	Barrueco and de la Peña 1988
		0.5–2.5	(+) (0.5)	NR	Saxena *et al.* 1997
		0.25–5	?[d]	–	Rahden-Staroń *et al.* 1994
Reverse mutation	TA97a	0.5–2.5	+ (0.5)	NR	Saxena *et al.* 1997
(frameshift)	TA98	0.5–2.5	–	NR	Saxena *et al.* 1997
		0.05–30	–	–	Ruiz and Marzin 1997
		NR[a]	–	–	Moriya *et al.* 1983
	TA1531	NR	–	NR	Seiler 1973
	TA1532	NR	–	NR	Seiler 1973
	TA1534	NR	–	NR	Seiler 1973
	TA1536	10–100	–	NR	Kada *et al.* 1974
		50	–	NR	Shirasu *et al.* 1976
		200	–	–	Carere *et al.* 1978
	TA1537	10–100	–	NR	Kada *et al.* 1974
		50	–	NR	Shirasu *et al.* 1976
		200	–	–	Carere *et al.* 1978
		NR[a]	–	–	Moriya *et al.* 1983
		0.03–10	–	–	Ruiz and Marzin 1997
	TA1538	10–100	–	NR	Kada *et al.* 1974
		50	–	NR	Shirasu *et al.* 1976
		200	–	–	Carere *et al.* 1978
		NR[a]	–	–	Moriya *et al.* 1983
Arabinose resistance	SV3	0.01–100	+ (0.3)	NR	Ruiz-Vázquez *et al.* 1978
DNA repair test	TA1538	0.25–5	+ (0.5)	?	Rahden-Staroń *et al.* 1994
	TA1978	0.25–5	+ (1.25)	?	

+ = positive result; – = negative result; (+) = weakly positive; ? = No clear interpretation, or contradictory interpretations given by the study authors; LEC = lowest effective concentration; NR = not reported.

[a]Authors tested 50 pesticides at concentrations of up to 5,000 µg/plate but did not identify specific levels for each pesticide.

[b]Different dose range tested without S9 (0.3 to 10 µg/plate) and with S9 (0.1 to 50 µg/plate).

[c]Different dose range tested without S9 (1.25 to 20 µg/plate) and with S9 (0.5 to 50 µg/plate).

[d] [Significantly different from control ($P < 0.01$) by the Student's *t* test (consistent with the authors' methodology), but apparently considered to be negative by the study authors.]

S. typhimurium strains TA1538 (*uvrB*) and TA1978 (uvr^+) were used in the DNA repair test to determine whether captafol damaged DNA (Rahden-Staroń *et al.* 1994). TA1978 has excision repair, and TA1538 does not. The zone of inhibition was greater for TA1538 than for TA1978, particularly in the absence of metabolic activation (the results are summarized in Table 5-2); however, no statistical comparisons between strains were reported. When the strain without excision repair is more sensitive (i.e., shows a greater zone of inhibition, indicating greater killing), this is evidence that the test compound kills through a covalent reaction with DNA (Ames *et al.* 1973).

Table 5-2. Results of DNA repair tests with captafol in *S. typhimurium*

Concentration (μg/plate)	Diameter of growth inhibition zone (mm)[a]			
	Without S9		With S9	
	TA1538	TA1978	TA1538	TA1978
0.25	7.5 ± 0.7	6.0 ± 0	6.0 ± 0	6.0 ± 0
0.50	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

Source: Rahden-Staroń *et al.* 1994.

[a]Mean values from 9 plates ± SD; diameters < 6.0 mm could not be measured and were recorded as 6.0. The authors described an "appreciable difference in the zones of growth inhibition" between the strains, but no statistical comparisons between strains were reported.

Escherichia coli *and* Bacillus subtilis

Studies with *E. coli* and *B. subtilis* (rec-assay) are summarized below and in Table 5-3.

All studies of reverse mutation in *E. coli* strain WP2 exposed to captafol gave positive results without metabolic activation (Kada *et al.* 1974, Moriya *et al.* 1978, Moriya *et al.* 1983, Shirasu *et al.* 1976). Captafol was not mutagenic in this strain after incubation with S9 fraction, S9 mix, cysteine, or rat blood in one study (Moriya *et al.* 1978) but was positive in another study with metabolic activation at higher test concentrations (Moriya *et al.* 1983). The number of revertants per plate in the WP2 *hcr* strain tested at 0.15 μmole/plate was 158 (without S9) but decreased to 21 to 31 after incubation with S9, cysteine, or rat blood. Spontaneous revertant levels were reported as less than 30 for this strain. The authors concluded that the mutagenic activity of captafol and its

analogues (captan and folpet, tested similarly) was eliminated by interaction with sulfhydryl compounds, which also would possibly be expected to occur *in vivo*.

The SOS chromotest was used by several investigators to assess DNA damage in *E. coli* following captafol exposure (Ohta *et al.* 1984, Rahden-Staroń *et al.* 1994, Ruiz and Marzin 1997). Mersch-Sundermann *et al*. (1994) compared the results of the SOS chromotest with those of the *S. typhimurium* assay for 330 chemicals and reported a concordance of 86.4%. All three SOS chromotest studies indicated that captafol caused DNA damage in *E. coli* strain PQ37 without metabolic activation. Ruiz and Marzin (1997) found DNA damage in the presence of S9 mix, albeit at a higher concentration. Captafol also induced the SOS repair system in PQ35 (uvr^+), an excision-repair-proficient strain (maximum induction factor = 2.5). The effect was less pronounced than in PQ37, an excision-repair-deficient strain (maximum induction factor = 5) (Rahden-Staroń *et al.* 1994).

E. coli MD332 ($dnaC_s$ *uvrA*), derived from the commonly used SOS chromotest tester strain PQ37, harbors the *uvrA* mutation and a temperature-sensitive mutation in the *dnaC* gene involved in initiation of DNA replication. In this strain, DNA replication is blocked at the nonpermissive temperature (42°C), and therefore the SOS system cannot be induced by typical SOS genotoxins. However, exposure of this strain to an agent that produces single-strand breaks restores induction of the SOS system. Rahden-Staroń *et al.* (1994) reported that captafol did not induce single-strand breaks under these test conditions.

Saxena *et al.* (1997) studied the genotoxic effects of captafol on DNA-repair-deficient mutants of *E. coli* K-12. The mutants $polA^-$, rec^-, and $lexA^-$ showed significantly lower survival on exposure to captafol than did their wild-type counterparts. The authors concluded that captafol damages DNA and initiates the error-prone SOS response, thus causing mutations in bacterial DNA.

Shirasu *et al.* (1976) used *B. subtilis* strains M45 (rec^-) and H17 (rec^+) in a rec-assay to screen 166 pesticides, including captafol, for further testing in reversion assays. M45 was derived from H17 through introduction of a recombination-deficient gene, *rec45*. In this

assay, differential killing of the repair-deficient strain (measured by zones of growth inhibition) indicates DNA damage. M45 was sensitive to captafol, and H17 was not.

Table 5-3. Results of genotoxicity testing of captafol in *E. coli* and *B. subtilis*

Test system	End point	Concentration range	Results without S9 (LEC)	Results with S9 (LEC)	Reference
Escherichia coli					
WP2	reverse mutation	10–100 μg/plate	+ (50)	NR	Kada *et al.* 1974
WP2	reverse mutation	50 μg/plate	+ (50)	NR	Shirasu *et al.* 1976
WP2	reverse mutation	5–200[a] μg/plate	+ (5)	+ (50)	Moriya *et al.* 1983
WP2	reverse mutation	0.15 μmol/plate	+ (0.15)	–	Moriya *et al.* 1978
PQ37 (*uvrA*)	SOS induction	0.2–1 μg/mL	+ (0.2)	NR	Ohta *et al.* 1984
PQ37 (*uvrA*) PQ35 (*uvr*$^+$)	SOS induction	0.5–6 μg/mL	+ (0.5) + (0.5)	– –	Rahden-Staroń *et al.* 1994
PQ37 (*uvrA*)	SOS induction	0.01–100 μg/mL	+ (0.1)[b]	+ (10)[b]	Ruiz and Marzin 1997
MD332 (*dnaC*$_s$ *uvrA*)	single-strand breaks	0.5–10 μg/mL	–	–	Rahden-Staroń *et al.* 1994
K-12 (*recA*$^-$ *rec*$^-$ *lexA*$^-$ *polA*$^-$)	DNA damage	5–25 μg/mL	+ (5)	NR	Saxena *et al.* 1997
Bacillus subtilis					
M45 (rec$^-$) H17 (rec$^+$)	rec-assay differential toxicity	0.1 μg/disk	+ (0.1) –	NR NR	Shirasu *et al.* 1976

– = negative result; + = positive result; LEC = lowest effective concentration; NR = not reported.

[a] Concentrations estimated from graph (log scale): maximum concentration without S9 was 100 μg/plate.

[b] Different dose range tested (0.01 to 10 μg/plate without S9 and 5 to 100 μg/plate with S9).

Summary of genetic effects in prokaryotes

Genotoxicity studies in bacteria demonstrated that captafol is a weak base-change mutagen. The mutagenicity of captafol is generally decreased in the presence of S9 metabolic activation, indicating that captafol does not require metabolic activation and damages DNA directly through covalent binding.

5.4.2 Non-mammalian eukaryotic systems

Captafol was mutagenic in the fungus *Aspergillus nidulans* and the fruit fly *Drosophila melanogaster*. The results are summarized in Table 5-4.

When *A. nidulans* grown on agar plates was exposed to 20 to 2,000 μg of captafol on paper triangles (3 cm × 5 cm), point mutations were induced resulting in 8-azaguanine resistance; mitotic crossing-over, but not mitotic nondisjunction, was induced when the paper triangles contained 0.2 to 2,000 μg captafol (Bignami *et al.* 1977). Ziogas and Georgopoulos (1987) reported that a commercial formulation of the fungicide metalaxyl (Ridomil 25 WP) increased the frequency of mitotic segregation in diploid colonies of *A. nidulans*. The genetic activity was attributed to captafol that was present in the formulation as an impurity. Mitotic crossing-over was also reported to be induced by vapor-phase action of captafol and captan.

The somatic mutation and recombination test (SMART) was used in wing cells of *D. melanogaster* (wing spot test) to check a possible mechanism of captafol action (Rahden-Staroń 2002). In this assay, captafol was fed to three-day-old larvae for 3 hours at concentrations of 10 to 100 mM (acute study) or 48 hours at 0.25 to 10 mM (chronic study). In the acute feeding studies, captafol was positive for small single spots and total spots at all concentrations tested but was inconclusive for large single spots and twin spots. Twin spots are produced only by recombination, but single spots may be produced by other mechanisms, such as gene mutation or deletion. Chronic feeding studies were inconclusive or negative. The author concluded that the overall evidence for mutagenic activity of captafol was weak.

Table 5-4. Results of genotoxicity testing of captafol in *Aspergillus* and *Drosophila*

Test system	End point	Concentration range	Results (LEC)	Reference
A. nidulans	point mutation crossing-over nondisjunction	20–2,000 μg/plate 0.2–2,000 μg/plate 0.2–2,000 μg/plate	+ (20) + (0.2) –	Bignami *et al.* 1977
	mitotic segregation/ crossing-over	0.05–0.25 μg/mL	+ (0.05)	Ziogas and Georgopoulos 1987
D. melanogaster	mutation recombination	10–100 mM (3 h)	+ (10) ?	Rahden-Staroń 2002

+ = positive result; – = negative result; ? = the author reported that these results were inconclusive.
LEC = lowest effective concentration.

5.4.3 *Mammalian* in vitro *assays*

End points investigated in mammalian *in vitro* studies included SCE, chromosomal aberrations, micronucleus formation, single-strand breaks, polyploidy, spindle disturbances (c-mitosis), unscheduled DNA synthesis (UDS), inhibition of RNA and DNA synthesis, and cell transformation. Results are summarized in Table 5-5 by end point.

Sasaki *et al.* (1980) reported that captafol at 3.5 μg/mL caused SCE, chromosomal aberrations, and micronucleus formation in an *in vitro* study with human HE 2144 cells without metabolic activation (cited by IARC 1991).

In a study by Robbiano *et al.* (2004), captafol was shown to cause a dose-dependent increase in single-strand breaks and micronuclei in Sprague-Dawley rat and human kidney cells isolated from the kidney cortex and found by light microscopy to contain a large majority of proximal tubular cells. The comet assay (alkaline single-cell gel electrophoresis) was used to measure DNA fragmentation after a 20-hour exposure to captafol at concentrations of 0.5 to 2 μM. The concentrations were the same for the micronucleus assay (measured after 48 hours) in rat cells, but were increased to 1 to 4 μM for the assay in human cells [the authors did not state whether they used cytochalasin B in the study]. The DNA-damaging potency determined with the comet assay (measured as the tail length in exposed cells minus the tail length in control cells divided by the concentration) was higher in human than in rat cells, while the micronucleus-inducing potencies were about the same in both human and rat cells.

Tezuka *et al.* (1980) reported a significant dose-related increase in the frequency of SCE and chromosomal aberrations in cultures of Chinese hamster V79 cells exposed to captafol (at concentrations of 2×10^{-6} to 2×10^{-5} M) without metabolic activation. A significant increase in the frequency of polyploid cells was observed in some of the captafol-exposed cultures, but the frequency was not dose related. Captafol produced a doubling of the SCE frequency over the control level at 5×10^{-6} M and a threefold increase at 2×10^{-5} M.

Captafol caused significant increases in SCE and chromosomal aberrations in cells of red muntjac (a species of deer, *Muntiacus muntjac*, found throughout Asia) (He *et al.* 1982). Of seven pesticides tested, captafol induced the strongest response.

Mitotic Chinese hamster V79 fibroblasts exhibited spindle disturbances after exposure to captafol at a concentration of 0.01 μM (Rahden-Staroń *et al.* 1994). At 0.01 μM, mitosis was significantly affected, with induced alterations 22% above the control value; however, increasing the concentration did not increase the percentage of induced c-mitotic cells. Chromosomal aberrations increased in Chinese hamster CHL cells exposed to captafol without metabolic activation at a concentration of 4 or 8 μg/mL (Ishidate 1983). No increase in the frequency of polyploids was observed. Incubation of captafol-exposed cultures with S9 decreased the frequency of chromosomal aberrations to the control level.

Captafol induced *in vitro* transformation of BALB/c 3T3 cells (Perocco *et al.* 1995). Transforming activity of captafol was apparent after S9-mix-induced activation in level-II (amplification) transformation cultures. In the presence of S9, captafol showed strong activity as a cell-transforming agent, significantly increasing the number of transformed foci per plate at concentrations of 0.01 to 0.1 μg/mL. In the absence of bioactivation, only the highest concentration significantly increased the number of transformed foci.

The effect of captafol on UDS in human lymphocytes after ultraviolet irradiation (UV) and in the presence or absence of hydroxyurea was examined as part of a study of 17 pesticides by Rocchi *et al.* (1980). The authors concluded that neither captafol (0% inhibition) nor the related fungicides captan (4% inhibition) and folpet (0% inhibition) inhibited UV-induced UDS.

Captafol at concentrations of 0.25, 0.5, 0.75, and 1 μg/mL inhibited the growth of pig kidney IB-RS-2 cells (Rodrigues and D'Angelo 1994). The highest concentration caused complete suppression of cell growth after 24 hours and cell death at 48 hours. Synthesis of DNA and RNA was inhibited in parallel by increasing concentrations of the chemical. Captafol also inhibited DNA synthesis in human lymphocytes by 61% at a concentration of 5 μg/mL [14.3 μM] (Rocchi *et al.* 1980) and in bovine liver nuclei with an ID_{50} of

approximately 50 μM (Dillwith and Lewis 1980). Both Rocchi *et al.* and Dillwith and Lewis reported similar results with the related fungicides captan and folpet (see Section 5.5 for a discussion of the possible mechanism of inhibition).

Table 5-5. Results of genotoxicity testing of captafol in mammalian *in vitro* systems

End point	Test system	Concentration range	Results (LEC)	Reference
SCE	Chinese hamster V79 cells red muntjac cells[a] human HE 2144 cells	2–20 μM 0.35–3.5 μg/mL 3.5 μg/mL	+ (2) + (0.35) + (3.5)	Tezuka *et al.* 1980 He *et al.* 1982 Sasaki *et al.* 1980[b]
Chromosomal aberrations	Chinese hamster V79 cells Chinese hamster CHL cells red muntjac cells[a] human HE 2144 cells	2–20 μM 4.0–8.0 μg/mL 0.35–3.5 μg/mL 3.5 μg/mL	+ (10) + (4) + (3.5) + (3.5)	Tezuka *et al.* 1980 Ishidate 1983 He *et al.* 1982 Sasaki *et al.* 1980
Micronuclei	rat kidney cells human kidney cells human HE 2144 cells	0.5–2.0 μM 1.0–4.0 μM 3.5 μg/mL	+ (1.0) + (2.0) + (3.5)	Robbiano *et al.* 2004 Robbiano *et al.* 2004 Sasaki *et al.* 1980
Single-strand breaks	rat kidney cells human kidney cells	0.5–2.0 μM 0.5–2.0 μM	+ (0.5) + (0.5)	Robbiano *et al.* 2004
Polyploidy	Chinese hamster V79 cells Chinese hamster CHL cells	2–20 μM 4.0–8.0 μg/mL	+ (2[c]) –	Tezuka *et al.* 1980 Ishidate 1983
C-mitosis	Chinese hamster V79 cells	0.01–10 μM	+ (0.01)	Rahden-Staroń *et al.* 1994
Cell transformation	mouse BALB/c 3T3 cells	0.01–5 μg/mL	+ (0.1)	Perocco *et al.* 1995
UDS inhibition	human lymphocytes	5 μg/mL	–[d]	Rocchi *et al.* 1980
Inhibition of RNA/DNA synthesis	pig kidney IB-RS-2 cells bovine liver nuclei human lympocytes	0.12–1 μg/mL NR 5 μg/mL	+ (0.12) + (NR) + (5)	Rodrigues and D'Angelo 1994 Dillwith and Lewis 1980 Rocchi *et al.* 1980

+ = positive result; – = negative result; LEC = lowest effective concentration.
[a]The cells used by He *et al.* were described as diploid, but the tissue of origin was not identified.
[b]Cited in IARC 1991.
[c]Results were not dose related.
[d]UV-induced UDS was not inhibited.

5.4.4 Mammalian in vivo *assays*

End points investigated in mammalian *in vivo* studies included dominant lethality (germ-cell mutations), DNA breaks, and micronucleus formation. Results are summarized in Table 5-6.

Three male Sprague-Dawley rats were given a single oral dose of captafol at 1,250 mg/kg b.w. (half the LD_{50}), and the kidneys were examined for DNA breaks and micronuclei two days later (Robbiano *et al.* 2004). DNA breaks and/or alkali-labile sites and micronuclei in exposed animals were significantly more frequent than in controls.

The dominant lethal assay was used to investigate mutagenic effects in germ cells in rats and mice exposed to captafol by gavage or i.p. injection (Collins 1972b, Kennedy *et al.* 1975). Collins (1972b) administered captafol to male rats at 2.5, 5.0, or 10 mg/kg b.w. per day (i.p.) or 50, 100, or 200 mg/kg b.w. per day (orally) for five days and mated each male with one unexposed female for each of the following 10 weeks. The incidence of pregnancy and the number of implants were not affected. Mean early deaths per pregnancy were higher than in the control group in 6 of 10 litters in the low- and mid-dose i.p. exposure groups and in all 10 litters of the high-dose group. The difference was statistically significant only for the week 3 litters in the high-dose group. In the gavage studies, mean early deaths per pregnancy were higher in all litters in the exposed groups except the week 9 litters in the low- and mid-dose groups. The differences were statistically significant for the week 1, 2, and 4 litters in the high-dose group. A significant dose-related trend was reported for week 3 in the i.p. study and for the first three weeks of the gavage study. When litters with two or more early deaths in the gavage study were evaluated, significant increases were reported for all exposed groups for week 2, the high-dose group for week 3, and the mid-dose group for week 6. IARC (1991) considered the positive results in this study as important supporting information, because of the generally insensitive nature of the dominant lethal assay.

In another dominant lethal study, male mice were administered a single i.p. injection of captafol and mated weekly with separate groups of three nonexposed virgin females for six consecutive weeks (Kennedy *et al.* 1975). This study did not show an increase in early embryonic deaths; [however, only two relatively low dose levels (1.5 and 3.0 mg/kg b.w. per day) were used].

Kennedy *et al.* (1975) also used the host-mediated assay in rats to test for mutagenicity of captafol. Groups of male rats were administered captafol by gavage for 15 days at 125 or 250 mg/kg b.w. per day. Indicator microorganisms (*S. typhimurium*) recovered from the peritoneal cavity of the exposed male rats after a three-hour residence showed no increase in numbers of revertants. Although the host-mediated assay was a favored *in vivo* procedure in the 1970s, it is no longer considered appropriate, because of low sensitivity (WHO 1990b).

Table 5-6. Results of genotoxicity testing of captafol in mammalian *in vivo* systems

Test system	End point	Dose	Results (LEC)	Reference
Sprague-Dawley rats (male), kidney cells	DNA breaks micronuclei	1,250 mg/kg (gavage)	+ (1,250) + (1,250)	Robbiano *et al.* 2004
Osborne-Mendel rats (male), dominant lethal mutation	Early fetal deaths per pregnancy	2.5, 5.0, or 10 mg/kg per day (i.p. for 5 days) 50, 100, 200 mg/kg per day (gavage for 5 days)	(+) (10) (+) (200)	Collins 1972b
Albino mice, dominant lethal mutation	Early embryonic deaths per pregnancy	1.5 or 3.0 mg/kg (i.p.)	–	Kennedy *et al.* 1975
Albino rats + *S. typhimurium* (host-mediated assay)	mutation in *S. typhimurium*	125 or 250 mg/kg per day for 15 days (gavage)	–	Kennedy *et al.* 1975

+ = positive result; (+) = weakly positive; – = negative result; LEC = lowest effective concentration.

Results for all genotoxicity studies of captafol are summarized in Table 5-7.

Table 5-7. Summary of the genotoxic effects of captafol

Effect	Prokaryotes	Lower eukaryotes	Mammalian systems	
			In vitro	*In vivo*
Somatic mutations	+	+	NT	NT
Germ-cell mutations	NT	NT	NT	+
Sister chromatid exchange	NT	NT	++	NT
Chromosomal aberrations	NT	NT	++	NT
Micronucleus formation	NT	NT	++	+
DNA damage	+	NT	NT	NT
Single-strand breaks	–	NT	++	+
Polyploidy	NT	NT	+	NT
Mitotic crossing over	NT	+	NT	NT
Cell transformation	NT	NT	+	NT
Spindle disturbances (c-mitosis)	NT	NT	+	NT
Inhibition of UV-induced UDS	NT	NT	–	NT
Inhibition of RNA or DNA synthesis	NT	NT	+	NT

++ = positive result in all studies (2 or more); + = positive result in at least one study or in the only study reviewed; – = negative result (only one study reviewed); NT = not tested.

5.5 Mechanistic studies and considerations

Captafol was shown to be both an initiator and a promoter of carcinogenesis in animal studies (see Section 4.2.3). Captafol also induced *in vitro* transformation of BALB/c 3T3

cells (Perocco *et al.* 1995), showing strong transforming activity at concentrations of 0.01 to 0.1 μg/mL with S9 metabolic activation and at 0.1 μg/mL in the absence of S9.

Potential mechanisms of carcinogenicity for captafol include both genotoxic action and epigenetic or indirect mechanisms. Potential indirect mechanisms include cytotoxicity from the effects of captafol on cellular thiol groups (both nonprotein and protein), inhibition of enzymes involved in DNA replication (DNA topoisomerases and polymerases), inhibition of DNA and RNA synthesis, induction of cytochrome P-450 monooxygenases, and promotion. These potential mechanisms are discussed below.

Captafol exhibited mutagenic activity in a variety of *in vitro* short-term tests and in mammalian *in vivo* studies (see Section 5.4). The genetic lesions measured by a defining set of short-term tests are quite relevant to the events now known to be involved in human cancer (mutation at specific loci, chromosomal aberrations, and loss of heterozygosity) (Heddle and Swiger 1996).

Captafol is a potent hepatotoxic agent in rats (Dalvi and Mutinga 1990). The liver toxicity of captafol may be attributed, at least in part, to its interaction with and metabolism by liver microsomal enzymes. Captafol reacts both with nonprotein thiols (mainly glutathione) and protein thiols to reduce the number of cellular sulfhydryl groups (see Section 5.3) (Kumar *et al.* 1975, Rahden-Staroń *et al.* 1994).

As noted in Section 5.4.3, captafol induced a significant dose-related increase in the frequency of SCE in Chinese hamster V79 cells (Tezuka *et al.* 1980). Inhibition of topoisomerases has been reported to have the potential to cause SCE, DNA strand breaks, chromosomal aberrations, and other genotoxic effects (Anderson and Berger 1994). When Rahden-Staroń (2002) investigated the effect of captafol on topoisomerase activity in nuclear extracts from mouse lymphoma cells, captafol inhibited DNA topoisomerase I by 10% to 20% at 10 to 100 μM and topoisomerase II by 50% at 1 μM. However, Rahden-Staroń (2002) concluded that the specific effect of inhibition of topoisomerase II did not seem to be a major event in captafol mutagenicity and carcinogenicity because only a weak response was obtained in an *in vivo* test for mitotic recombination using *Drosophila* (SMART test; see Section 5.4.2), which was reported in the same publication.

Rodrigues and D'Angelo (1994) reported dose-dependent cytotoxic effects and inhibition of DNA and RNA synthesis in pig kidney cells exposed to varying concentrations of captafol for 72 hours (see Section 5.4.3). The cytotoxic effects were only partially reversible, even at the lowest concentration. Inhibition of DNA synthesis is mediated through direct interaction of captafol with the DNA polymerase and is irreversible. The authors concluded that the effects on nucleic acid synthesis could account for the cytotoxic and genotoxic effects of captafol. Dillwith and Lewis (1980) reported comparable inhibitory effects of 100 μM concentrations of captafol (62%), captan (65%), folpet (66%), and trichloromethylsulfenyl chloride (65%), an analogue for the side chain of captan and folpet, on DNA polymerase β activity in isolated bovine liver nuclei (see Section 5.4.3). Inhibition of the polymerase was observed when captan was incubated separately with the DNA polymerase before adding the DNA template but not when the incubation was with the DNA alone before adding the polymerase. Based on these results and the lack of an inhibitory effect of phthalimide or tetrahydrophthalimide in the same system, the authors concluded that captan irreversibly inhibits the DNA polymerase and proposed that transfer of the side chain of the fungicide molecules to amino, hydroxyl, or thio groups was responsible for the inhibition. They also noted that the inhibition of DNA polymerase β by captafol, which contains a tetrachloroethylthio group as a side chain, was equal to the inhibition by captan. Thiophosgene, which is a potential metabolite of captan and folpet, but not of captafol, was not considered by the authors to be an important intermediate in the inhibitory effect.

Captafol also was shown to induce cytochrome P-450 activity in the S9 fraction prepared from the livers of rats given a single i.p. injection of captafol at 80 mg/kg b.w. (Rahden-Staroń *et al.* 2001). The ability of this S9 fraction to activate ethidium bromide (CYP1A isoenzyme) or cyclophosphamide (CYP2B isoenzyme) in the *S. typhimurium* reverse mutation assay was determined. At the single dose tested, captafol was much more effective as an inducer of CYP2B than of CYP1A in rats.

Although no direct link has been established between the effects of captafol summarized above and its ability to induce genotoxic or carcinogenic effects, these effects do provide potential areas for further investigation. For example, it has been speculated that a

decrease in nonprotein sulfhydryl groups (particularly glutathione) might influence the integrity and functions of the mitotic spindle (Rahden-Staroń *et al.* 1994). C-mitosis is a cytological sign indicating inhibition or disturbances of the spindle function, and c-mitotic agents can give rise to abnormal chromosome numbers in both mitotic and meiotic cells in experimental systems. The abnormal chromosome number can contribute to carcinogenesis (Önfelt 1983). Also, it is generally accepted that the induction of cytochrome P-450 monooxygenases, as noted above for captafol, may have toxicological consequences such as initiation and promotion of cancer and tissue necrosis (Rahden-Staroń *et al.* 2001).

5.6 Metabolism, genotoxic effects, and carcinogenicity of structural analogues and metabolites

As noted in Section 1, captafol is one of a group of three structurally related chloroalkylthiodicarboximide compounds with fungicidal activity. The other two compounds are captan and folpet (see Figure 1-4). Captan shares structural similarities with each of the other two fungicide molecules, as shown in Figure 5-3. Captan and captafol both have partially saturated tetrahydrophthalimide rings, but folpet has an unsaturated aromatic phthalimide ring. Conversely, captafol has a tetrachloroethylthio side chain, while captan and folpet have identical trichloromethylthio side chains.

5.6.1 Metabolism of captafol analogues

Studies in several animal species have shown that captan and folpet are rapidly absorbed from the gastrointestinal tract and are rapidly metabolized (IARC 1983, WHO 1992). Captan and folpet are rapidly hydrolyzed at the N-S bond in the gastrointestinal tract and in the blood to THPI and to derivatives of the trichloromethylthio side chain. One proposed metabolic scheme is that the side-chain moiety of these two analogues of captafol is converted initially to thiophosgene. Degradation in the gut appears to play a major role in the metabolism of folpet; here, the reactive intermediate thiophosgene is generated and further metabolized (EPA 1986, Owens 1969). Because the trichloromethylthio moiety is the same in both captan and folpet (the only difference between the two compounds being that the ring portion of folpet is aromatic), it has been assumed that all metabolic data for captan relative to the trichloromethylthio portion of the molecule will also be applicable to folpet.

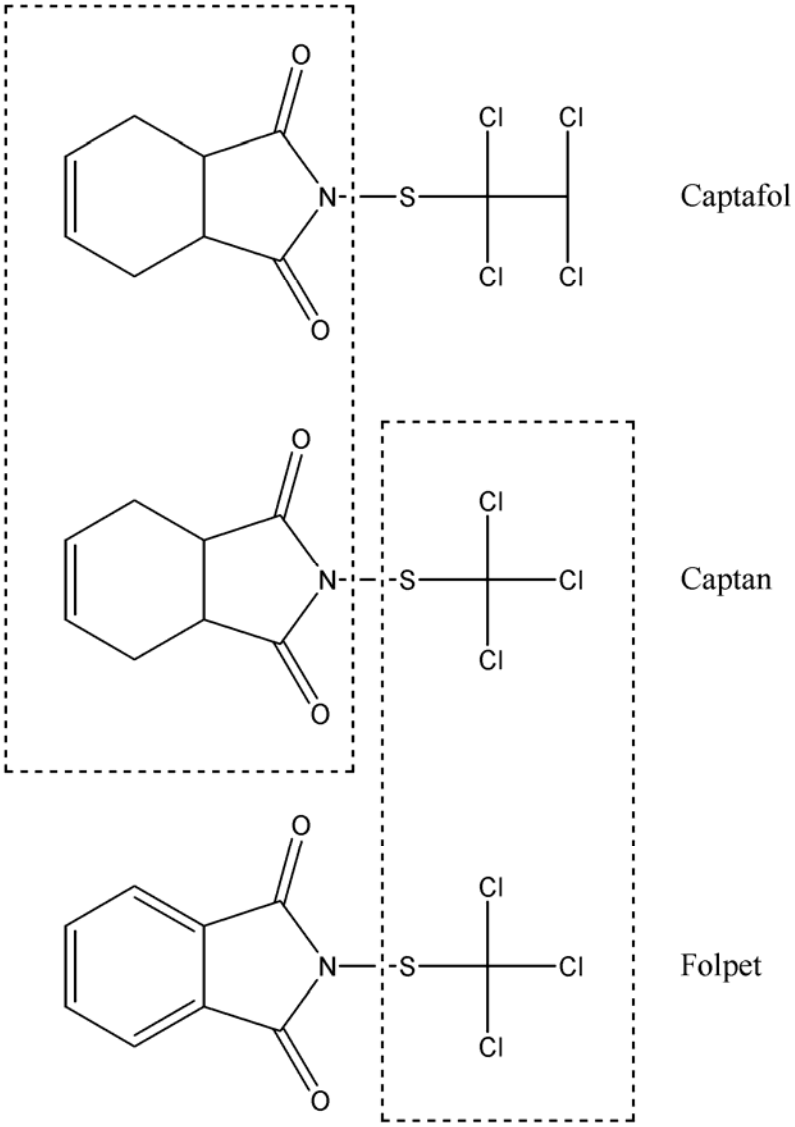

Figure 5-3. Similarities in structures of captafol, captan, and folpet

As illustrated above, captan shares features in common with both captafol and folpet. The tetrahydrophthalimide ring structure is shared by both captafol and captan (box on left), while the trichloromethylthio side chains of captan and folpet are identical (box on right).

5.6.2 Genetic effects of captafol analogues

IARC (1983) reviewed the mutagenicity of captan and reported that there was sufficient evidence of mutagenicity in cellular systems; however, the data were considered insufficient to establish mutagenicity in mammals. Garrett *et al.* (1986) reported on the genetic profiles of 65 pesticides tested in short-term assays, including captan and folpet. The metabolic profiles for captan and folpet were very similar, yielding more than three times as many positive as negative test results, and both fungicides caused gene mutation

in prokaryotic and eukaryotic systems and DNA damage in eukaryotes. Perocco *et al.* (1995) demonstrated that both captan and folpet caused transformation of BALB/c 3T3 cells. Rocchi *et al.* (1980) investigated the effect of 17 pesticides (including captan and folpet) on scheduled and unscheduled DNA synthesis in rat thymocytes and human lymphocytes. Both captan and folpet inhibited DNA synthesis in rat thymocytes and human lymphocytes but did not inhibit UDS in human lymphocytes. Dillwith and Lewis (1980) reported that both captan and fopet inhibited DNA synthesis in isolated bovine liver nuclei. The genotoxic effects of captan and folpet are summarized in Table 5-8.

Table 5-8. Genotoxic effects of captan and folpet

End point	Test system	Captan	Folpet	Reference
Prokaryotes				
Reverse mutation	*S. typhimurium* G46	+	NR	Quest *et al.* 1993
	S. typhimurium TA98	+	+	Garrett *et al.* 1986
	S. typhimurium TA98	+	NT	Ruiz and Marzin 1997
	S. typhimurium TA1537	–	+	Garrett *et al.* 1986
	S. typhimurium TA1537	+	NT	Ruiz and Marzin 1997
	S. typhimurium TA1538	+	NR	IARC 1983
	S. typhimurium TA100	+	+	Garrett *et al.* 1986
	S. typhimurium TA100	+	NT	Ruiz and Marzin 1997
	S. typhimurium TA102	+	NT	Ruiz and Marzin 1997
	S. typhimurium TA1535	+	NT	Ruiz and Marzin 1997
	S. typhimurium TA1950	+	NR	Quest *et al.* 1993
	S. typhimurium JK947	+	+	Hour *et al.* 1998
	S. typhimurium JK3	(+)	(+)	Hour *et al.* 1998
	E. coli WP2 *uvrA*	+	+	Garrett *et al.* 1986
	E. coli lacZ mutants	+	NT	Lu *et al.* 1995
DNA damage				
SOS chromotest	*E. coli* PQ37	+	NT	Ruiz and Marzin 1997
Differential toxicity	*E. coli polA*	+	+	Garrett *et al.* 1986
Differential toxicity	*B. subtilis rec*	+	+	Garrett *et al.* 1986
Differential toxicity	*S. typhimurium uvrB,* rec	+	+	Garrett *et al.* 1986
Eukaryotes				
Recessive lethal mutation	*D. melanogaster*	+	+	Garrett *et al.* 1986
Wing-spot assay	*D. melanogaster*	(+)	NT	Rahden-Staroń 2002
Sex-linked mutation	*D. melanogaster*	– (+)	NR	IARC 1983
Mutation	*A. nidulans*	+	NR	Quest *et al.* 1993
Mutation	*Neurospora crassa*	+	NR	IARC 1983
Mutation at the TK locus	mouse L51784 cells	+	+	Garrett *et al.* 1986
Mutation (spot test)	mice *(in vivo)*	–	NR	Quest *et al.* 1993
Mutation	hamster V79 cells	+	NR	Quest *et al.* 1993
Mutation (host-mediated)	mice/*S. typhimurium*	±	NR	IARC 1983
Dominant lethal mutation	mice	(+)	NT	Collins 1972a
Dominant lethal mutation	rats	(+)	NT	Collins 1972a
Urine mutagenesis	human (*in vivo*)[a]	+	NT	Lebailly *et al.* 2003
Mitotic recombination	*Saccharomyces cerevisiae*	+	+	Garrett *et al.* 1986
DNA repair induction	*S. cerevisiae*	+	NR	Quest *et al.* 1993

End point	Test system	Captan	Folpet	Reference
DNA repair induction	*A. nidulans*	+	NR	Quest *et al.* 1993
DNA repair induction	human fibroblasts	+	NR	Quest *et al.* 1993
DNA repair induction	hamster V79 cells	+	NR	Quest *et al.* 1993
Cell transformation	mouse BALB/c 3TC cells	+	+	Perocco *et al.* 1995
Unscheduled DNA synthesis	human SV-40 VA-4 cells	+	NR	IARC 1983
Unscheduled DNA synthesis	human lung fibroblasts	–	–	Garrett *et al.* 1986
Unscheduled DNA synthesis	human lymphocytes	–	–	Rocchi *et al.* 1980
Inhibition of DNA synthesis	human lymphocytes	+	+	Rocchi *et al.* 1980
Inhibition of DNA synthesis	rat thymocytes	+	+	Rocchi *et al.* 1980
Inhibition of DNA synthesis	bovine liver nuclei	+	+	Dillwith and Lewis 1980
DNA damage (comet assay)	human (*in vivo*)	–	NR	Lebailly *et al.* 2003
Sister chromatid exchange	Chinese hamster cells	+	NT	IARC 1983
Sister chromatid exchange	human fibroblasts	–	NR	IARC 1983
Micronucleus formation	mouse bone-marrow cells	–	NR	IARC 1983
Chromosomal aberrations	hamster V79 cells	+	NR	Quest *et al.* 1993
Chromosomal aberrations	kangaroo rat cells	+	NR	Quest *et al.* 1993
Chromosomal aberrations	human embryo lung cells	+	NR	Quest *et al.* 1993
Chromosomal aberrations	Chinese hamster cells	+	NT	IARC 1983
Chromosomal aberrations	human fibroblasts	–	NR	IARC 1983
Chromosomal aberrations	mice (*in vivo*)	–	NR	Quest *et al.* 1993

+ = positive result; (+) = weakly positive result; ± = both positive and negative results, – (+) = negative to weakly positive result; – = negative result; NR = not reported; NT = not tested.
[a]Tested in *S. typhimurium* TA102; urine collected from fruit growers one day after spraying of captan.

5.6.3 Carcinogenicity and toxicity of captafol analogues

The National Cancer Institute (1977) conducted a two-year bioassay of captan and reported negative results in Osborne-Mendel rats and positive results in $B6C3F_1$ mice (tumors of the duodenum). IARC reviewed the carcinogenicity of captan in 1983 and concluded that there was limited evidence of carcinogenicity in experimental animals. The carcinogenicity of folpet has not been investigated by the NTP, nor has it been reviewed by IARC. Bernard and Gordon (2000) reported that captan and folpet exert their carcinogenic effects through an epigenetic mechanism as evidenced by the necessity of large sustained doses for tumor development. Gordon (2007) also reported that captan is a potential carcinogen only at prolonged high doses that result in cytotoxicity and regenerative cell hyperplasia. The carcinogenicity of captafol in animals is reviewed in Section 4 and is compared with the carcinogenicity of captan and folpet in this section. In 1993, Quest *et al.* published the results of unpublished studies conducted in mice and rats that had been submitted to the U.S. EPA Health Effects Division, Office of Pesticide Programs.

Captan and folpet were tested for carcinogenicity in mice and rats (unpublished studies peer reviewed by EPA; the peer review was not available for the IARC review) when administered in the diet (Quest *et al.* 1993), and captan was tested for tumor initiating, tumor promoting, and complete carcinogenic (initiation and promotion) activity following topical administration to mice (Antony *et al.* 1994). The gastrointestinal tract was a target organ for benign or malignant tumor formation following exposure to captan, folpet, or captafol in mice (Table 5-9). Both captafol and folpet caused tumors of the lymph system in mice, and captafol also induced tumors in the vascular system. Only captafol was associated with forestomach tumors. Renal tumors (captan and captafol) and mammary-gland tumors (captafol and folpet) were observed in rats. In male and/or female rats of the CD, Wistar, or F344 strains, tumors were induced in the kidney (renal carcinoma or adenoma and carcinoma combined) by captan and captafol, in the uterus by captan, in the thyroid by folpet, and in the mammary gland and liver by captafol. Positive trends for thyroid, testicular, and mammary-gland tumors and malignant lymphoma also were observed for folpet in these rats.

Antony *et al.* (1994) tested captan for carcinogenic and cocarcinogenic activity following topical exposure in groups of 20 female Swiss albino mice. All 16 animals in the positive control group (7,12-dimethylbenzanthracene [DMBA] plus 12-*o*-tetradecanoyl phorbol-13-acetate [TPA]) developed tumors within 10 weeks. Captan showed some tumor-initiating activity (with TPA as the promoter), causing benign squamous-cell papilloma in 3 of 14 mice in the single-application group and 12 of 18 in the multiple-application group at the end of 52 weeks. Captan did not demonstrate any tumor-promoting activity (with DMBA as the initiator) or complete carcinogenic activity (initiation and promotion) in these experiments.

Table 5-9. Comparison of carcinogenic effects of captan, folpet, and captafol administered in the diet of mice and rats

Tumor site	Test animal	Captan		Folpet		Captafol	
		Male	Female	Male	Female	Male	Female
Small intestine (duodenum)	CD-1 mice	✓	✓	✓	✓		
	$B6C3F_1$ mice	✓	**T**	✓	✓	✓	✓
Vascular system	CD-1 mice					**T**	✓
	$B6C3F_1$ mice					✓	✓
Forestomach	$B6C3F_1$ mice					**T**	✓
Lymphatic system	CD-1 mice					✓	✓
	$B6C3F_1$ mice				✓		
	F344 rats			**T** [a]	**T** [a]		
Kidney	CD rats	**T**				✓	**T**
	F344 rats					✓	✓
Liver	$B6C3F_1$ mice					✓	✓
	CD rats						✓
	F344 rats					✓	✓
Thyroid gland	CD rats			✓			
	F344 rats				**T**		
Mammary gland	CD rats						✓
	F344 rats			**T** [a]	**T** [a]		
Uterus	Wistar rats		✓				
Testes	CD rats			**T**			
Harderian gland	CD-1 mice					✓	

✓ = significantly increased compared with controls and a significant positive dose-related trend, $P < 0.05$.

T = a significant dose-related trend ($P < 0.05$), but no significant pairwise comparisons.

[a] Significant trend only when data for males and females were combined.

Captafol, folpet, and captan caused similar toxic effects in the gastrointestinal tract of mice (Quest *et al.* 1993). The effects included glandular proliferative changes, hyperkeratosis/acanthosis, and hyperplasia in animals with gastrointestinal tumors. Folpet and captafol induced similar toxicity in the esophagus and stomach of rats, although no gastrointestinal tumors were observed in rats administered captafol. Captan and captafol produced similar changes in the kidney, including increased kidney weight, the presence of megalocytic cells, enlarged nuclei, cystic and dilated tubules, glomerulopathy, and hyperplasia of the renal tubular epithelium.

Quest *et al.* (1993) discussed a proposed metabolic pathway for the carcinogenicity of captan and folpet based on the formation of thiophosgene, a highly reactive intermediate (see Section 5.6.1). Because both captan and folpet were associated with gastrointestinal

tumors, the formation of thiophosgene in the gut could be part of the mechanism of carcinogenicity. However, thiophosgene is not a metabolite of captafol, which also caused a significant increase in gastrointestinal tract tumors in $B6C3F_1$ mice. In addition, a possible common mechanism for renal tumor formation observed in rats might involve the common ring structure of captafol and captan, or metabolites derived from the ring.

5.6.4 Carcinogenicity of captafol metabolites

Quest *et al.* (1993) suggested that the ring structure of THPI (the major metabolite of captafol) or metabolites derived from the ring might be associated with tumors caused by captafol; however, no carcinogenicity studies of this compound in experimental animals were found. Dichloroacetic acid, which has been identified as a minor metabolite of captafol (see Sections 1.4 and 5.2), was tested for potential carcinogenicity in four drinking-water studies in $B6C3F_1$ mice (IARC 1995). Mice were exposed for 37 to 104 weeks to dichloroacetic acid at concentrations of 0.05 to 5 g/L. Significantly increased incidences of hyperplastic nodules, hepatocellular adenoma, and hepatocellular carcinoma were reported in each study. However, dichloroacetic acid has not been proposed as an active metabolite of captafol in tumor formation, probably because it is not formed in the dominant metabolic pathway, involving interaction with sulfhydryl groups, but only in the hydrolytic pathway, which is a much slower reaction *in vivo* (see Section 5.2 and Figure 5-1).

5.7 Summary

5.7.1 Absorption, distribution, and excretion

Captafol is absorbed through the gastrointestinal tract and lungs and, to a lesser extent, through the skin. It distributes to tissues, including liver and kidneys, but neither captafol nor its metabolites have been found to accumulate in animal tissues and excretion is rapid, primarily via the urine.

5.7.2 Metabolism

Following oral administration to animals, captafol appears to be extensively hydrolyzed at the N-S bond in the gastrointestinal tract to form THPI, and the C-S bond also hydrolyzes easily. This reaction is much more rapid in the presence of sulfhydryl

compounds, such as glutathione and cysteine. Cleavage of the side chain results in formation of another metabolite, tetrachloroethylmercaptan.

5.7.3 Toxicity

The major toxic effects of captafol in humans are dermatitis and asthma; however, the liver is a primary target organ in animals exposed to captafol. Captafol also causes several toxic effects in *in vitro* systems, including reductions in the content of protein and nonprotein sulfhydryl groups in cultured cells and inhibition of the activity of purified glutathione *S*-transferase pi 1-1.

5.7.4 Genetic damage and related effects

Captafol is an alkylating agent and has produced genotoxic effects in a variety of systems. Captafol caused mutations in *S. typhimurium* strains that detect base-pair change, in *E. coli,* and in non-mammalian *in vivo* systems (the fungus *Aspergillus nidulans* and the fruit fly *Drosophila melanogaster*). Other reported effects include DNA damage in *S. typhimurium, E. coli*, and *B. subtilis*, and mitotic crossing over in *A. nidulans.* [In general, higher concentrations of captafol were needed to induce genotoxicity in the presence of S9 metabolic activation, suggesting that captafol is a direct mutagen.] In *in vitro* studies with cell lines from rodents and other mammals, captafol induced single-strand breaks, SCE, chromosomal aberrations, micronuclei, polyploidy, spindle disturbances, cell transformation, and inhibitied DNA/RNA synthesis. It also induced SCE, micronuclei, and chromosomal aberrations, and inhibited DNA/RNA synthesis in human cells *in vitro,* but did not inhibit UV-induced UDS. In mammalian *in vivo* studies, captafol caused DNA strand breaks, micronuclei (when administered by gavage) and dominant lethal mutations (when administered i.p. or orally) in rats but did not cause mutations in the host-mediated assay. No dominant lethal effect was observed in albino mice administered captafol by i.p. injection.

5.7.5 Mechanistic studies and considerations

In addition to direct genotoxic activity, captafol also may operate through indirect mechanisms, such as cytotoxicity as a result of reduced cellular levels of thiol groups (nonprotein and protein), inhibition of enzymes involved in DNA replication (DNA

topoisomerases and polymerases), inhibition of DNA and RNA synthesis, and induction of cytochrome P-450 monooxygenases.

5.7.6 Metabolism, genotoxic effects, and carcinogenicity of structural analogues and metabolites

The chloroalkylthiodicarboximide group of fungicides also includes captan and folpet. Captan shares some similarities in structure with both captafol and folpet: captan and captafol share a common tetrahydrophthalimide ring structure, and captan and folpet have identical side chains. Captafol and captan have some similarity in metabolism, as both can give rise to the metabolite THPI. However, the side chain of captafol differs from that of either captan or folpet; thus, the metabolism of this part of the captafol molecule differs from that of the side chains of the other two compounds. The types of tumors produced by the three compounds are generally similar. In mice, all three compounds produced tumors of the gastrointestinal tract, and folpet and captafol produced tumors of the lymphatic system. Captan and folpet are believed to exert their carcinogenic effects through cytotoxicity at high sustained doses followed by regenerative hyperplasia. In rats, captan and captafol produced renal tumors, although for captan, only a significant dose-related trend in males was observed. There was some evidence that folpet and captafol caused mammary-gland tumors in rats. A significant dose-related trend was reported for folpet when data for male and female F344 rats were combined, and an increased incidence of mammary-gland tumors was observed in female CD rats exposed to captafol. Only folpet was associated with thyroid tumors in both sexes of F344 rats and testicular tumors in CD rats (significant trends).

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Glossary of Terms

Boiling point: The boiling point of the anhydrous substance at atmospheric pressure (101.3 kPa) unless a different pressure is stated. If the substance decomposes below or at the boiling point, this is noted (dec). The temperature is rounded off to the nearest °C.

C-mitosis: A cytological sign indicating inhibition or disturbances of the spindle function (named for the effect of colchicine). C-mitotic agents can give rise to abnormal chromosome numbers in both mitotic and meiotic cells in experimental systems.

Density: The density for solids and liquids is expressed in grams per cubic centimeter (g/cm^3) and is generally assumed to refer to temperatures near room temperature unless otherwise stated. Values for gases are generally the calculated ideal gas densities in grams per liter at 25°C and 101.325 kPa (atmospheric pressure).

Exogenous: Due to an external cause; not arising within the organism.

HE 2144 cells: Human diploid embryonic fibroblasts.

Hemangiosarcoma (also, hemangioendothelioma): A malignant tumor characterized by rapidly proliferating cells derived from the blood vessels and lining irregular blood-filled spaces.

Henry's Law constant at 25°C: The ratio of the aqueous-phase concentration of a chemical to its equilibrium partial pressure in the gas phase. The larger the Henry's law constant the less soluble it is (greater tendency for vapor phase).

K_{oc}: Soil organic adsorption coefficient, which is calculated as the ratio of the concentration of a chemical adsorbed to the organic matter component of soil or sediment to that in the aqueous phase at equilibrium.

Lipophilic: Having a strong affinity for fats.

Log octanol-water partition coefficient (log K_{ow}): The ratio of concentrations of a substance in octanol and in water, when dissolved in a mixture of octanol and water. For

convenience, the logarithm of K_{ow} is used. The octanol/water partition coefficient of a substance is useful as a means to predict soil adsorption, biological uptake, lipophilic storage, and bioconcentration.

Melting point: The melting point of the substance at atmospheric pressure (101.3 kPa). When there is a significant difference between the melting point and the freezing point, a range is given. In case of hydrated substances (i.e., those with crystal water), the apparent melting point is given. If the substance decomposes at or below its melting point, this is noted (dec). The temperature is rounded off to the nearest °C.

Molecular weight: The molecular weight of a substance is the weight in atomic mass units of all the atoms in a given formula. The value is rounded to the nearest tenth.

Neoplasm: Tumor.

Negative log acid dissociation constant (pK_a): A measure of the degree to which an acid dissociates in water (a measurement of acid strength). The pKa is the negative logarithm (to the base 10) of the acid dissociation constant (Ka); the lower the pKa, the stronger the acid.

Pesticide field trials: Controlled testing of a pesticide in a field under normal agricultural operating conditions. Pesticide field trials are carried out principally for residue analysis of crop or soil samples and to evaluate the efficacy and crop tolerance of crop protection products.

Physical state: Substances may either be gases, liquids, or solids according to their melting and boiling points. Solids may be described variously as amorphous, powders, pellets, flakes, lumps, or crystalline; and the shape of the crystals is specified if available. Solids also may be described as hygroscopic or deliquescent depending upon their affinity for water.

Red muntjac: A species of deer (*Muntiacus muntjac*) found throughout Asia**.**

S9: The post-mitochondrial supernatant fraction, which is prepared by subjecting tissue homogenate to centrifugation at 12,000 g. This subcellular fraction contains both cytosol and microsomes.

Solubility: The ability of a substance to dissolve in another substance and form a solution.

SOS chromotest: A bacterial test for detecting DNA-damaging agents consisting of a colorimetric assay based on the induction by these agents of the SOS function sfiA, whose level of expression is monitored by means of a sfiA::lacZ operon fusion. The name SOS for this repair process is based on its nature as a response to distress (analogous to the SOS signal in Morse code).

t(14:18) translocation: A translocation that joins the *bcl-2* gene on chromosome 18 to the immunoglobulin heavy chain gene (*IgH*) on chromosome 14, resulting in increased production of bcl-2 protein, a potent inhibitor of apoptosis.

Vapor density, relative: A value that indicates how many times a gas (or vapor) is heavier than air at the same temperature. If the substance is a liquid or solid, the value applies only to the vapor formed from the boiling liquid.

Vapor pressure: The pressure of the vapor over a liquid (and some solids) at equilibrium, usually expressed as mm Hg at a specific temperature (°C).

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